THE PONTIFICAL
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SCIENCES

Scripta Varia

111

STEM CELL TECHNOLOGY
and Other
INNOVATIVE THERAPIES

Working Group
10-11 November 2003

VATICAN CITY
2007

STEM CELL TECHNOLOGY AND OTHER INNOVATIVE THERAPIES

Address:
The Pontifical Academy of Sciences
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PONTIFICIAE ACADEMIAE SCIENTIARVM SCRIPTA VARIA
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STEM CELL TECHNOLOGY AND OTHER INNOVATIVE THERAPIES

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EX AEDIBVS ACADEMICIS IN CIVITATE VATICANA

MMVII

The opinions expressed with absolute freedom during the presentation of the papers of this meeting, although published by the Academy, represent only the points of view of the participants and not those of the Academy.

Editors of the Proceedings:

Marcelo Sánchez Sorondo
Nicole M. Le Douarin

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His Holiness Pope Benedict XVI

The Participants of the Working Group of 10-11 November 2003

The Participants of the Working Groups and the Commemorative Session, 10 November 2003

The Academy or *The School of Athens* by Raphael, in the Vatican Palace
'In those people you will have recognised your oldest predecessors in the investigation of both matter and spirit'
(Pius XII, Address to the Plenary Session of the Academy, 3 December 1939)

CONTENTS

Scientific Papers

ADDRESS TO THE HOLY FATHER

NICOLA CABIBBO

Holy Father,

We are grateful to be received in your presence on this occasion when the 25th Anniversary of your accession to the Pontificate happens to coincide with the 400th Anniversary of the foundation in Rome of the Accademia dei Lincei, under the reign of Clement VIII, the Pope Aldobrandini. The Lincei of Federico Cesi was the ancestor of our present Academy – your present Academy – the Pontifical Academy of Sciences, but also of all the subsequently created academies of science, many of whose leaders have joined us in our celebration. Of particular significance is the presence of the Italian Accademia dei Lincei, which shares with us a direct descendance from Federico Cesi, and of the Academy of the Third World, which was conceived a few years ago at the Casina Pio IV and has become the focal point for the discussion of science and development among the leading third world scientists. Central to the conception of Cesi and of Galilei is the disinterested search for the truth and the concern for the human condition. These are still our ideals today.

To celebrate this anniversary the Academy decided to coin a special medal where your figure is ideally associated to that of Galilei, both as a celebration of the 400 years from our foundation, and in grateful recognition of your continued effort, over the first 25 years of your pontificate, for establishing a fruitful collaboration between the world of religion and the world of science. Your effort was crowned in 1992 with the solemn conclusion of the Galilei case, an enterprise that you started in 1979.

On another level, it only remains for me to thank you for offering us, this year, the gift of the restoration of the splendid buildings which have been the headquarters of the Pontifical Academy of Sciences since the great Pontiff Pius XI gave them to this institution in 1923. The completion of the restoration has allowed Casina Pio IV not only to return to its former architectonic splendour, but has also improved its working facili-

ties, particularly in the conference hall. Now we can really say that the Academicians who work in this Pontifical Academy will raise their minds to God through the contemplation of nature, of art, the grace of St. Peter, and their own research and reflections, aided in this by the presence of state-of-the-art technology.

Thank you, Holy Father, for all of this that can help us achieve a future where faith and reason are fully reconciled and cohabitate peacefully.

ADDRESS OF JOHN PAUL II TO THE MEMBERS OF THE PONTIFICAL ACADEMY OF SCIENCES

10 November 2003

Dear Members of the Pontifical Academy of Sciences,

I am especially pleased to greet you today as we celebrate the Four Hundredth Anniversary of the Pontifical Academy of Sciences. I thank the President of the Academy, Professor Nicola Cabibbo, for the kind sentiments expressed on your behalf and I acknowledge with gratitude the thoughtful gesture with which you have wished to commemorate the Silver Jubilee of my Pontificate.

The *Accademia dei Lincei* was founded in Rome in 1603 by Federico Cesi with the encouragement of Pope Clement VIII. In 1847 it was restored by Pius IX and in 1936 re-established by Pius XI. Its history is linked to that of many other Scientific Academies throughout the world. I am happy to welcome the Presidents and representatives of these institutions who have so kindly joined us today, especially the President of the *Accademia dei Lincei*.

I recall with gratitude the many meetings we have had over the past twenty-five years. They have been opportunities for me to express my great esteem for those who work in the various scientific fields. I have carefully listened to you, shared your concerns, and considered your suggestions. In encouraging your work I have emphasized the spiritual dimension always present in the search for truth. I have also affirmed that scientific research must be directed towards the common good of society and the integral development of its individual members.

Our gatherings have also enabled me to clarify important aspects of the Church's doctrine and life relating to scientific research. We are united in our common desire to correct misunderstandings and even more to allow ourselves to be enlightened by the one Truth which governs the world and guides the lives of all men and women. I am more and more convinced that scientific truth, which is itself a participation in divine

Truth, can help philosophy and theology to understand ever more fully the human person and God's Revelation about man, a Revelation that is completed and perfected in Jesus Christ. For this important mutual enrichment in the search for the truth and the benefit of mankind, I am, with the whole Church, profoundly grateful.

The two topics which you have chosen for your meeting concern the life sciences, and in particular the very nature of human life. The first, *Mind, Brain and Education*, draws our attention to the complexity of human life and its pre-eminence over other forms of life. Neuroscience and neurophysiology, through the study of chemical and biological processes in the brain, contribute greatly to an understanding of its workings. But the study of the human mind involves more than the observable data proper to the neurological sciences. Knowledge of the human person is not derived from the level of observation and scientific analysis alone but also from the interconnection between empirical study and reflective understanding.

Scientists themselves perceive in the study of the human mind the mystery of a spiritual dimension which transcends cerebral physiology and appears to direct all our activities as free and autonomous beings, capable of responsibility and love, and marked with dignity. This is seen by the fact that you have decided to expand your research to include aspects of learning and education, which are specifically human activities. Thus your considerations focus not just on the biological life common to all living creatures but also include the interpretive and evaluative work of the human mind.

Scientists today often recognize the need to maintain a distinction between the mind and the brain, or between the person acting with free will and the biological factors which sustain his intellect and capacity to learn. In this distinction, which need not be a separation, we can see the foundation of that spiritual dimension proper to the human person which biblical Revelation explains as a special relationship with God the Creator[1] in whose image and likeness every man and woman is made.[2]

The second topic of your meeting concerns *Stem Cell Technology and Other Innovative Therapies*. Research in this field has understandably grown in importance in recent years because of the hope it offers for the cure of ills affecting many people. I have on other occasions stated that

[1] Cf. *Gen* 2:7.
[2] Cf. *Gen* 1:26-27.

stem cells for purposes of experimentation or treatment cannot come from human embryo tissue. I have instead encouraged research on adult human tissue or tissue superfluous to normal fetal development. Any treatment which claims to save human lives, yet is based upon the destruction of human life in its embryonic state, is logically and morally contradictory, as is any production of human embryos for the direct or indirect purpose of experimentation or eventual destruction.

Distinguished friends, reiterating my thanks for your valued assistance I invoke upon you and your families God's abundant blessing. May your scientific work bear abundant fruit and may the activities of the Pontifical Academy of Sciences continue to promote knowledge of the truth and contribute to the development of all peoples.

PREFACE

As the existence of the Pontifical Academy of Sciences demonstrates, the Church has always promoted scientific research that is in conformity with human dignity. In parallel fashion, it has been sensitive to discoveries in the scientific field in the evolution of its teaching. We find, for example, that the Church has taken into account advances in knowledge about the human embryo in developing the details of its doctrine. In 1827 Karl-Ernst Von Baer discovered the ovum in mammals and humans and the mechanism of fertilisation, as a result of which it was definitively proved that the human being does not begin as a coagulate of blood but as a fertilised ovum. This was the reason why, for most theologians of the time, animation took place at the moment of conception and not subsequently.[1] It is to be noted that in his Constitution *Apostolicae Sedis* of 1869 Pius IX abolished the distinction between the animated foetus and the non-animated foetus. The abolition of this distinction implied above all the adoption of a stance by the Magisterium of the Church that recognised human animation from the first instance of conception, and involved a moral evaluation of, and sanctions on, the elimination of a human embryo at all stages of its development.

A further turning point came with the discovery of DNA (deoxyribonucleic acid), the macromolecule which contains and transfers genetic characteristics in all living organisms beginning with a genetic code that is the same genetic code that the individual will have throughout his/her life. Indeed, as Nicole Le Douarin, the organiser of this meeting, has observed, the point of departure of embryology is the following: 'each one of us began our lives as a cell, an ovum…a tiny corpuscle of living matter'. From this comes the fundamental question of embryology: 'how can it be that from this single *isolated* cell come the parts of the body of an adult, made up of various billions of harmoniously ordered cells to form

[1] J.P. Gury, *Compendium theologiae moralis*, vol. I (Lugduni, 1866), p. 431.

various and complex organs such as the brain, the limbs, the eyes and the face?'[2] A biologist observes a living cell that is all potential and then begins to have quantitative and qualitative changes directed by that specific genetic code. This cellular behaviour of the human being, which for that matter is matched by the cellular behaviour of higher animals, is inscribed, so to speak, and reference is no longer made to the genetic code or to DNA but to the same subject who has an internal principle of development or self-genesis beginning with an active potentiality that reaches a mature reality that is also the same physical and biological subject with the same genetic code during the whole time of his/her existence from the beginning until death. With respect to humans, it is not the case that the embryonic cell is a kind of mini-man. Instead, the genetic code is a project of development, a 'programme', that contains a collection of information which means that the same subject progressively organises himself/herself so as to form, one after the other, the various organs that make him/her up, to the point of arriving at the complete individual who emerges at the moment of birth.

This philosophical relevance of the discovery of DNA was emphasised a few years later by an American biologist of German origins, Max Delbrück (1906-1981), who was awarded the Nobel Prize for medicine for his research in the field of viruses, in an article on Aristotle entitled 'Aristotle-totle-totle' (the reference is to a German jingle centred round the name 'Mariandle').[3] In this article Delbrück argued that if a Nobel Prize could be awarded to great figures of the past, then it should be given to Aristotle for his discovery of the principle implicit in DNA. Indeed, in his biological works Aristotle argued that the germ from which the embryo develops, which for him was only the male semen (Aristotle did not have a microscope with which to see the female ovum), is not a mini-man, as Hippocrates had believed, but a formal principle (Delbrück thus translates the Aristotelian terms *eidos* and *morphê* with 'project of development' and 'programme'). This principle acts as a motorial cause, that is to say that it transmits to matter, which in this case is made up of the menstrual blood supplied by the mother, a series of mechanical impulses,

[2] N. Le Douarin, *Des chimères, des clones et des gènes* (Editions Odile Jacob, Paris, 2000), p. 15.

[3] M. Delbrück, 'Aristotle-totle-totle', in J. Monod and E. Borek (eds.), *Of Microbes and Life* (Columbia University Press, New York-London, 1971) pp. 50-55.

of movements, which ensure that the matter organises itself so as to form the organs one after another, beginning with the heart, ending up with the complete individual who emerges at the moment of birth.[4] Delbrück concludes by affirming that a return to Aristotle the biologist could lead to a clearer understanding of the concepts of form and finality, and perhaps achieve something better than a mere co-existence between natural scientists and their colleagues from other disciplines.

This means, in terms of modern science, that human DNA is present from the outset in the nucleus of the cells that form, first, the zygote (the cell produced by the union of the two – male and female – gametes), then the morula (the set of four cells), then the blastocysts (the set of a number of cells) and, lastly, the embryo proper. And the human genome, that is to say the set of about 25,000 genes that form the chromosomes contained in the zygote, which was recently (in the late 1990s) completely mapped, is made up of human DNA, which is different, albeit very slightly (less than 5%), from that of the higher animals (for example that of the chimpanzee whose DNA was mapped even more recently), that is to say it already contains the programme of the adult individual which, in addition to the vegetative faculties, will also develop the sensitive and intellective faculties.

In terms of modern science one can state, I believe, that human DNA is equal in all the individuals of the human species and different from that of all other animals, but also that the DNA of each human individual is different from that of every other human individual (as is the case, for example, with finger prints), and indeed analysis of DNA is also used today for the establishment of paternity or to identify the person responsible for a crime or for any other action where traces have been left that contain cells of the DNA of that person. This is not 'biologism', that is to say an excessive emphasis on the biological aspect, an accusation that is made against the approach which bases the individuality of biological identity on sources that are often involuntarily spiritualistic, because a human being is a biological reality, namely a living being, albeit with human life. St. Thomas added to this Aristotelian doctrine of the unique substantial form that the human soul is created directly by God. For the Angelic Doctor the human soul (differently from other living forms) is that which has the act of being (*actus essendi*) 'in itself' and keeps it necessarily united to itself. It therefore receives from God the act of being in itself and then communicates it to the

[4] Aristotle, *De generatione animalium*, I, 18 and 21-22.

body which *trahitur ad esse animae*.[5] When the body is no longer able to be its subject and potency, the soul conserves the act of being that it had communicated to the body and continues in being, taking on another kind of life. Today one can affirm that God infuses the intellective soul in the human zygote at the very moment of its conception because the DNA contained in the nucleus of the zygote (of which Aristotle and St. Thomas had no knowledge) already contains all the information required to permit the development of the nervous system, i.e. the matter through which the intellective soul operates.[6]

It is to be observed that we often encounter a certain linguistic dualism in speaking about the same reality which reflects the complexity of the dimensions of the human being. A biologist, perhaps without metaphysical prejudices, refers to a cell of great potentiality, 'totipotent'[7] (employing, one may observe, a term taken from philosophy, namely the concept of potency of Aristotelian origins), which has a dynamism of development beginning with the biological identity of the same genetic code. A realistic philosopher who takes this biological characteristic of human beings seriously, refers to the same ontological subject who, from the first moment, is what he/she is and will be what he/she is, although at

[5] *De Spirit. Creaturis*, a. 2 ad 8.

[6] Even E. Berti maintained recently that Aristotle was misinterpreted regarding the progressive animation of the embryo, while he seems to have affirmed instead the immediate animation of the intellectual soul. Cf. E. Berti, 'Is DNA range a sufficient definition of human nature? Aristotle v/s Thomas Aquinas and Jacques Maritain', in M.S.S. (ed.), *What Is Our Knowledge about the Human Being* (The Pontifical Academy of Sciences), in press.

[7] Biology makes a distinction between the different types of potency of the cells. Totipotent cells have total potential. They specialize into pluripotent cells that can give rise to most, but not all, of the tissues necessary for fetal development. Pluripotent cells undergo further specialization into multipotent cells that are committed to give rise to cells that have a particular function. For example, multipotent blood stem cells give rise to the red cells, white cells and platelets in the blood. Cf. s.v. 'Totipotent', *Wikipedia, The Free Encyclopedia*, http://en.wikipedia.org/wiki/Totipotent. It should be noted that theology also takes the concept of potency to express one of God's attributes with the word 'omnipotent', which indicates the essence of God as active principle of generation both of the divine processions and of creation: 'Quia vero de divinis loquimur secundum modum nostrum, – quem intellectus noster capit ex rebus inferioribus, ex quibus scientiam sumit, – ideo sicut in rebus inferioribus cuicumque attribuitur actio, attribuitur aliquod actionis principium, quod potentia nominatur; ita et in divinis, quamvis in Deo non sit differentia potentiae et actionis, sicut in rebus creatis. Et propter hoc, generatione in Deo posita, quae per modum actionis significatur, oportet ibi concedere potentiam generandi, vel potentiam generativam' (St. Thomas Aquinas, *De Potentia*, q. 2, a. 1).

the outset he/she is not developed, because the physical/biological identity is given by its formal principle which progressively organises the organs and the complete body of that subject. Thus, where for a biologist one is dealing with a human genetic embryonic cell, for a realistic philosopher one is in front of a developing human individual. Therefore, the corollary of an inter-disciplinary anthropological vision, i.e. one that takes into account these two languages (which reflect these different dimensions of the human being) to explain the same reality, is that the human genetic embryonic cell cannot be seen as pure genetic material that can be used as a means or exploited, even for good ends. It cannot be used, for example, for experiments in an attempt to cure illnesses that afflict mankind, because one is dealing with a human being.

The doctrine of the Church, in this field, once again demonstrates the careful attention that the Magisterium pays to the voice of science. When in the 1950s Watson and Crick discovered DNA, the Church with more conviction than ever before affirmed that the embryonic cell is a human being. Indeed, as Cardinal Lehmann observes in the Introduction to this volume, the clearest example of the language used most recently by the Church to this effect is perhaps to be found in the Instruction *Donum vitae* of 1987 of the Congregation for the Doctrine of the Faith. This Declaration, drawing upon advances in scientific discovery, states that 'the conclusions of science regarding the human embryo provide a valuable indication for discerning by the use of reason a personal presence at the moment of the first appearance of a human life: how could a human individual not be a human person?' (I, 1).

We may also observe that after the discovery of DNA there has been the very clear conviction that human life must be absolutely respected and protected from the moment of conception. The Second Vatican Council, for example, clearly stated that it is necessary to protect human life from the very moment of human existence (cf. *Gaudium et spes*, 51); the Declaration on Procured Abortion of 1974 stated that 'from the moment in which the ovum is fertilized, a new life begins that is neither that of the father nor of the mother, but a new human being that develops independently…Recent genetics confirm this fact, which has always been unambiguous…in an impressive way. It has shown that from the very first moment this living being has a fixed structure of its own: namely that of a human being, indeed this particular human individual already containing all its precisely defined characteristics' (Latin text, p. 738); and the Instruction *Donum vitae* of 1987 makes clear that 'The human being

must be respected – as a person from the very first instance of his/her existence' (I, 1). It is clear, therefore, that contemporary scientific research has decisively strengthened the conviction of the Magisterium of the Church that if the embryonic cell contains the genetic code of the individual, then that subject can only be a human subject, a subject that requires respect and protection.

In this context, in addition to the scientific dimension, i.e. that one is dealing with a human embryonic cell with a code, and the philosophical dimension, namely that this cell has a formal identity – the subsistent soul with its own *actus essendi* (Aquinas), Pope Benedict XVI, as the great theologian that he is, has also recently stressed what we may call the theological dimension of the human embryonic cell, namely its relationship with God: 'God's love does not differentiate between the newly conceived infant still in his or her mother's womb and the child or young person, or the adult and the elderly person. God does not distinguish between them because he sees an impression of his own image and likeness (*Gn* 1: 26) in each one. He makes no distinctions because he perceives in all of them a reflection of the face of his Only-begotten Son, whom "he chose...before the foundation of the world.... He destined us in love to be his sons...according to the purpose of his will" (*Eph* 1: 4-6)'.[8]

These three dimensions – the biological, the philosophical and the theological – make up the human embryo, unless we want to reduce everything to the biological dimension alone, with the result that our rational understanding becomes limited to the data of scientific experience. Science, with great generosity, offers us continual discoveries about the structure of the matter of life and thus of human life, but it cannot provide answers to questions about the ultimate origins and finality of the human being, which, instead, belong to the field of theology and philosophy. However, it is clear that the theologian and the philosopher, who study these ultimate origins and finality, must have available direct information on the true results and developments of scientific research. As we have seen, science enriches theology and philosophy not only collaterally but also from within. This does not mean precipitously forgoing (abandoning in this a serious dialogue with the data of science) the great

[8] Address of His Holiness Benedict XVI to the Participants at the 12th General Assembly of the Pontifical Academy for Life and Congress on 'The Human Embryo in the Pre-Implantation Phase', 27 February 2006.

notions of philosophy, such as that of substantive form of Aristotle or of the soul with its own act of being of St. Thomas Aquinas, which, indeed, form a part of the perennial heritage of thought, or those essential messages where the human being recognises his own dignity, to employ the phrase of G.B. Vico who not by chance adopted as the theme of his *Il Diritto Universale* Cicero's reply to the question posed to him by Atticus: '*Non ergo a preatoris Edicto, ut plerique nunc, neque a XII Tabulis, ut superiores, sed penitus ex* intima Philosophia *hauriendum iuris disciplinam putas*?'[9] On the contrary: it means, as we have observed, that these notions are rehabilitated through an attentive reading of the data of science. The facts of contemporary science are not those of Aristotle and St. Thomas but this does not involve a change in the metaphysical horizon of the relationship between matter and form, between form and being, potency and act, and in general between God and the creature. However, this implies a need for particular adaptation in the employment of philosophical categories derived from ancient physics and biology.

In this context, it was in order to acquire detailed knowledge of recent scientific advances in the field of stem cells that we organised the meeting on 'Stem Cell Technology and Other Innovative Therapies' whose proceedings are published in this volume. I take this opportunity to thank Prof. Nicole Le Douarin for her role as the organiser, Prof. Nicola Cabibbo for the support and encouragement he offered as President of the Pontifical Academy of Sciences, and all the participants who with so much expertise and commitment contributed to the high scientific quality of the papers and discussions. Special thanks go to Cardinal Lehmann who in his paper explained the approach of the Magisterium of the Church and of philosophy to the dignity of the human embryo. Lastly, I would like to thank Cardinal Tarcisio Bertone, the Secretary of State of the Holy See, who authorised the publication of these proceedings.

✠ Marcelo Sánchez Sorondo

[9] Cicero, *De Legibus*, I, 5. Cf. G.B. Vico, *Il Diritto Universale*, (F. Nicolini, Bari, 1936), t. I, p. 24.

PROGRAMME

Organising Committee:
N. Le Douarin (PAS, Paris), T. Boon-Falleur (PAS, Brussels)

MONDAY, 10 NOVEMBER

9:00 Prof. Nicole Le Douarin (PAS, Paris)
Introduction

9:30 Card. Karl Lehmann (Mainz, President of the Bishops' Conference)
Human Rights and Bioethics

10:30 Prof. Irving Weissmann (Stanford)
Stem Cells: Overview

11:30 Coffee Break

12:00 Prof. Ronald McKay (Nat. Inst. Neurological Disorder and Stroke, Bethesda)
Comparing the Properties of Embryonic, Fetal and Adult Stem Cells

13:00 Lunch

14:30 Prof. M. Azim Surani (Cambridge, UK)
Germ Cells: The Eternal Link Between Generations

15:30 Prof. Helen Blau (Stanford)
Repair of Adult Tissues by Adult Bone Marrow Derived Stem Cells

16:30 Coffee Break

17:00 Prof. Rudolf Jaenisch (MIT, Cambridge)
Nuclear Cloning and Embryonic Stem Cells

18:00 Prof. Ann McLaren (Cambridge, UK), Chair
General Discussion

TUESDAY, 11 NOVEMBER

9:00 Prof. Thierry Boon-Falleur (PAS, Brussels)
Therapeutic Vaccination of Cancer Patients

10:00 Prof. Alain Fischer (INSERM, Paris)
Gene Transfer in Hematopoietic Stem Cells: Perspectives, Results and Problems

11:00 Coffee Break

11:30 Prof. François Sigaux (INSERM, Paris)
From Genes to Therapy

12:30 Lunch

LIST OF PARTICIPANTS

Academicians:

Prof. Nicola Cabibbo, President
H.E. Msgr. Prof. Marcelo Sánchez Sorondo, Chancellor
Prof. Werner Arber
Prof. Antonio M. Battro
Prof. Paul Berg
Prof. Günter Blobel
Prof. Thierry Boon-Falleur
Prof. Bernardo M. Colombo
Rev. Prof. George V. Coyne, S.J.
Prof. Paul J. Crutzen
Rev. Prof. Raffaele Farina, S.D.B.
Prof. Antonio García-Bellido
Prof. Paul Germain
Prof. Raymond Hide
Prof. Fotis C. Kafatos
Prof. Vladimir I. Keilis-Borok
Rev. Prof. Stanley L. Jaki, O.S.B.
Prof. Paul J.A. Janssen
Prof. Nicola M. Le Douarin
Prof. Pierre J. Léna
Prof. Rita Levi-Montalcini
Rev. Prof. Jean-Michel Maldamé
Prof. Govind Kumar M. Menon
Prof. Jürgen Mittelstrass
Prof. Marcos Moshinsky
Prof. Rudolf Muradian
Prof. Ryoji Noyori
Prof. Michael Sela
Prof. Maxine F. Singer

Prof. Andrzej Szczeklik
Prof. Walter Thirring
Prof. Hans Tuppy
Prof. Rafael Vicuña
Prof. Robert J. White
Prof. Antonino Zichichi

Prof. Nicole M. Le Douarin and Prof. Thierry Boon-Falleur are the organizers of the Working Group.

Experts:

Prof. Helen M. Blau
Stanford University School of Medicine
Department of Microbiology & Immunology
Baxter Laboratory in Genetic Pharmacology
CCSR Room 4215, 269 Campus Drive
Stanford, CA 94305-5175
(USA)

Prof. Alain Fischer
Hôpital Necker-Enfants Malades
Unité d'Immunologie – Hématologie
149, rue de Sèvres
F-75743 Paris Cedex 15
(France)

Prof. Rudolf Jaenisch
Massachusetts Institute of Technology
Whitehead Institute for Biomedical Research
Nine Cambridge Center
Cambridge, MA 02142-1479
(USA)

H.E. Card. Karl Lehmann, President of the Bishops' Conference
Bischofplatz 2a
D-55116 Mainz
(Federal Republic of Germany)

Prof. Ronald D.G. McKay
National Institute of Neurological Disorders and Stroke
Laboratory of Molecular Biology
Building 36, Room 5A29
36 Convent Drive, MCS 4157
Bethesda, MD 20892-4157
(USA)

Prof. Anne McLaren
University of Cambridge
The Wellcome Trust / Cancer Research UK
Institute of Cancer and Developmental Biology
Tennis Court Road
Cambridge CB2 1QR
(United Kingdom)

Prof. François Sigaux
INSERM – Unité 462
IUH (porte 7, Entrée B, 2ème étage) – Hôpital Saint Louis
1, avenue Claude Vellefaux
F-75475 Paris Cedex 10
(France)

Prof. M. Azim Surani
University of Cambridge
Welcome Trust and Cancer Research UK
Institute of Cancer and Developmental Biology
Tennis Court Road
Cambridge CB2 1QR
(United Kingdom)

Prof. Irving L. Weissman
Stanford University School of Medicine
Department of Pathology
B265 Beckman Center
Stanford, CA 94305-5323
(USA)

Observer:

Dr. John M. Templeton, Jr., President
John Templeton Foundation
Five Radnor Corporate Center
Suite 100 – 100 Matsonford Road
Radnor, PA 19087
(USA)

SCIENTIFIC PAPERS

INTRODUCTION

NICOLE M. LE DOUARIN

Since the discovery of the chemical nature of the DNA molecule, unprecedented progress has been made in the Biological Sciences during the past 50 years. One can imagine that this period is only a beginning and that the Golden Age of Biology will continue for a long period of time. The increase in our knowledge is due, in large part, to the advent of molecular biology and the ability to clone and manipulate genes with the goal of exploring their function and mode of action. These new scientific developments made it possible to approach the central question of what characterizes living organisms and how they function. Thus, considerable advances have been made in our understanding of the biology of the cell which is the universal constitutive unit of all organisms, of developmental biology (that is, how a complex organism is built up from a single cell, the fertilized egg), as well as all branches of physiology such as immunology and neuroscience.

All these advances have *benefited medicine* in a major way. I would like to highlight organ transplantation as an example which is particularly relevant to this symposium on stem cells. To replace an organ, or a part of the body which is deteriorated or does not function, with a new one removed from another human being, has been a dream of humanity for a long time. The scene represented in Figure 1 (see page 137), the painting of Fernando del Rincon (1450-1517), illustrates Saint Come and Saint Damien who had exchanged during his sleep the leg (that was being destroyed by gangrene) of one of their servants with the leg of a Maure that had just died.

Unfortunately, at that time, transplantation was only a myth. Now it is possible to graft organs safely, thanks to the discovery of the immunosuppressive effect of a natural compound (cyclosporine), produced by a fungus. For a long time, organ transplantation was considered as unacceptable and unethical, but it is now performed routinely and it has saved many lives.

The aim of the Symposium that Prof. Thierry Boon and I have organized, concerns the possibility that novel therapies may be foreseen due to the remarkable progress that has been made both in developmental and cell biology (see section I of the program), and in immunology and genetics (see section II of the program).

I would like to introduce the topic of Stem Cell Biology that was discussed by experts in the field. First, I wish to thank them warmly for accepting the invitation of the Pontifical Academy of Sciences to participate in this symposium. Since they are among the most renowned leaders in this very active field, they are much solicited. Thus I appreciate their efforts to attend this event. I also want to thank the President and the Chancellor of the Academy who have welcomed our proposition to discuss these topics at the Vatican.

The issue of stem cells has attracted a great deal of interest during the last few years because it provides the hope that stem cells may be used in the future for replacing cells which are deficient or which have been subjected to abnormal and massive death, as it is the case in some degenerative diseases. Grafting cells for therapeutic purposes has been ongoing for a long time in the case of bone marrow or skin transplantation. *But now we are faced with the exciting prospect that cells with large differentiation potential, that are maintained in vitro in a normal proliferative state, could provide a variety of cell types for transplantation and therefore help cure diseases for which there is as yet no other treatment.*

I first discuss how the concept of the stem cell has emerged and second, the different types and sources of stem cells that have been described.

The Stem Cells Concept

The current definition of the term *stem cell* is that of a cell which is pluripotent and, on the one hand yields cells that differentiate into various phenotypes, while on the other hand, it produces cells that do not differentiate, remain pluripotent and similar to the mother cell.

Therefore, stem cells undergo asymmetrical divisions which not only allow them to self renew but also to replace differentiated cells that have died. Stem cells themselves divide slowly but they yield a pool of rapidly proliferating derivatives or tissue-specific precursors.

The first experimental demonstration that stem cells exist goes back to the early 1960s when the hematopoietic system was shown to harbor single cells responsible for the renewal of the circulating blood.

Earlier, Wilson (1925) (1) had introduced the term of stem cells in his description of the development of the *Ascaris* worm. In Wilson's view, the early steps of egg segmentation generate stem cells which undergo asymetric divisions, so that each time they divide, they give rise to both a cell similar to themselves and a cell with a different fate.

This scheme was not based on experimental evidence but on a conceptual view deduced from the careful analysis of the *Ascaris* cell lineage. Experimental data were first provided by studies carried out on hematopoiesis.

The Hematopoietic Stem Cell

It had long been known that cells present in the circulating blood have a short life span and are regularly renewed. The fact that the bone marrow contains a large number of cells that are immature forms of the different cell types present in the blood has designated this tissue as an important site for blood cell production.

During the first half of the 20th century, it was found that living cells are more vulnerable to X irradiation while they are dividing than during interphase. This explains why full body irradiation of mice with a sufficient dose of X-rays is lethal after a few days and that this death is due to the loss of blood cells and to aplasia of the hematopoietic tissues, namely the bone marrow and the spleen which becomes greatly reduced in volume. However, these mice can be rescued by injections of immunocompatible bone marrow cells, which reconstitute the pool of circulating cells as well as the population of precursors in the bone marrow. This process leads to long term reconstitution of the blood cells and restores the spleen to its normal size.

The fact that all types of blood cells can originate from a single cell which is endowed with self-renewal capacity was demonstrated by Till and McCulloch in 1961 (2).

Till and McCulloch rescued lethally irradiated mice with 10^5 bone marrow cells, instead of the usual millions of cells. In this case, the volume of the spleen did not increase globally, as in the previously performed experiments. In contrast, 8 to 10 bulges developed on its surface. These structures corresponded to colonies of hematopoietic cells (HC) that appeared to have developed independently from one another due to the rare seeding of the injected bone marrow cells. An analysis of each colony revealed that each contained all the blood cell types (except lymphocytes). Moreover, the new cells were shown to all be derived not from the host, but from the

injected donor bone marrow. To test if the founder cells were heterogeneous in nature, a few donor cells were labelled with a chromosomal marker and each colony was found to contain cells of one type or the other, but both cell types (with or without the nuclear marker) were never found in the same colony. This finding indicated that each colony was derived from one single progenitor that was designated a colony-forming unit-spleen: CFU-S. A clear demonstration that the CFU-S were cells endowed with self-renewal capacity derived from the following experiment: bone marrow cells were injected into a lethally irradiated mouse in which spleen colonies developed, as described above. These colonies were used as a source of cells to reconstitute a second lethally irradiated mouse. In this manner, the hypothesis was tested that long term reconstitution could occur only if the colony contained, not only dividing progenitors, but also stem cells capable of renewing these progenitors once they were differentiated. Indeed, this was exactly what was observed. The CFU-S had generated not only the differentiating precursors that had developed in the colonies but also another round of CFU-S that could form colonies in the spleen of a third lethally irradiated recipient. In addition, if the cells of these colonies were seeded at low density in a culture dish containing an appropriate culture medium, after 10 to 15 days, some of these cells generated colonies which contained several types of blood cells, as well as CFU-S capable of further propagations. These results were obtained by Don Metcalf in Australia and by Leo Sachs in Israel. Furthermore, the *in vitro* methods for growing HSC led to the discovery of growth factors that favor the differentiation of a given phenotypes of blood cells (3). For example, *erythropoietin* is a critical cytokine for red blood cell development.

After many decades of work the present view is that a pluripotent hematopoietic stem cell is able to generate i) *the stem cell* (SC) for the myeloid lineages: corresponding to the CFU-S which gives rise to erythrocytes and to other types of white blood cells, and ii) *the lymphoid SC* which generates the two types of lymphocytes, T and B.

The pluripotent SC are not actively dividing and, for this reason, they are radioresistant. They yield lineage restricted stem cells which divide actively under the influence of precise mixtures of growth and survival factors. This cellular compartment is destroyed by X-irradiation.

Progress has been made since these pioneering experiments, and although stem cells which divide slowly are rare, they are now well characterized particularly thanks to the work of Prof. Irving Weissman. For example, in the mouse, the HSC are recognizable because they express cell sur-

face molecules designated CD34, ckit, and sca-1 and, at the same time, are devoid of all the antigens that are specifically expressed by cells of the various differentiated blood cell lineages. A chapter by Prof. I. Weissman describes the hematopoietic and other stem cells in greater detail.

Other Types of Stem Cells

Blood cells are not the only cells that are replaced throughout life and stem cells are present in many tissues such as skin, skeletal muscle and intestinal epithelium in which cells are constantly renewed. Their presence has been demonstrated also in the liver and even in the central and peripheral nervous systems (CNS, PNS).

Under normal circumstances, these stem cells give rise to a progeny which is restricted to the cell types of the tissue to which they belong.

Consequently, owing to the presence of their stem cells, mammals are endowed, at least to a certain extent, with tissue regenerative capacity.

Regeneration in the Animal Kingdom

The capacity to regenerate tissues and organs is widespread in the animal kingdom, since it exists in species belonging to virtually all groups of animals. Only in nematodes and cephalocordates has no case been reported, perhaps because the possibility has yet to be investigated.

The strategies developed by nature to regenerate tissues are of these different types. First, in certain animals, like Planaria, which are freshwater flat worms 3-4 cm long, the capacity to regenerate relies on the fact that they possess, even in the adult, pluripotent embryonic-like stem cells, designated *neoblasts*, which are dispersed throughout the body. When the differentiated cells composing their organs have exhausted their normal life span, they are replaced by the progeny of neoblasts (4). Moreover, if the worm is cut into several pieces, each can reconstitute a complete organism due to the capacity of the neoblasts to proliferate, to form a regeneration blastema at the site of wound, and to differentiate those parts of the worm which are lacking in the correct antero-posterior and ventro-dorsal orientation. This regenerative capacity has been termed a 'secondary embryogenesis'.

Second, certain vertebrates, such as Urodeles are also endowed with considerable potential to regenerate including limbs, the jaw, eyes and tail. When the limb of a salamander is cut, the epidermis covers the lesion and a bud, similar to the regeneration blastema of Planarians, forms at the site of

the cut. This blastema grows and is progressively patterned to regenerate the part of the limb that was removed. In constrast to the blastemas which grow in Planarian worms and which are formed by neoblasts that have migrated to the wound site, regeneration in Urodeles results from the dedifferentiation and growth of the tissues located at the site of the cut.

Third, in mammals, removal or destruction of tissues is merely followed by healing: the epidermis lining the lesion covers the exposed internal tissues but this healing process does not involve the replacement of the removed tissue. However, even in higher vertebrates, stem cells, distributed in numerous types of adult tissues, are able to ensure the replacement of differentiated cells when they have reached the end of their normal life span.

In summary, a brief review of the regeneration processes in animals indicates that they rely on three different strategies:

i) mobilisation of embryonic-like pluripotent stem cells (e.g. Planaria)

ii) dedifferentation of already differentiated cells which then re-differentiate (e.g. Urodeles)

iii) turn-over of the cells in differentiated adult tissues via stem cells whose differentiation capabilities are thought to be restricted to the cell phenotypes of the tissues to which they belong.

Plasticity of the Commitment of Adult Stem Cells

The dogma according to which developmental potential of adult stem cells is restricted was challenged during the past few years by several authors. A large amount of work has been done in order to determine if the SC from adults could be used in cellular therapy in mouse and subsequently in human. Despite early reports that stem cells from the adult CNS or skeletal muscle could reconstitute the hematopoietic system of a lethally irradiated mouse (5), these results have not been confirmed and may well have been due to contamination by blood cells within these tissues (6). On the other hand, reports about cells from the adult bone marrow, used in many transplantation experiments, seem to indicate that these cells are endowed with far more extensive differentiation capabilities than previously thought (e.g. 7-9).

Although some of the results that have been reported in the flurry of scientific literature (most often in high standard journals) during the past few years failed to be confirmed by other research groups, this phenomenon reflects the great significance and high expectations that this technology will aid human health.

A curious and unexpected phenomenon was reported to occur when normal cells and Embryonic Stem cells (ES cells) were co-cultured *in vitro*: cell fusion. However, strong selective pressure led to the fusion of ES cells or hematopoietic stem cells with normal cells, thus generating rare aneuploid hybrids. Given the prior work of Harris, Ephrussi and Davidson, this was not surprising (e.g. 10). However, that fusion could also occur *in vivo* to form stable binucleate heterokaryons after injection of bone marrow cells was not anticipated. This finding was demonstrated by one of today's speakers, Prof. Helen Blau who will also give us an overview of the state of the art. In 1983, she first showed that previously silent genes could be activated in non dividing cell fusion products. This reprogramming of nuclear gene expression has now been shown by her and other groups to occur in heterokaryons that form *in vivo* during life (11-16). These and other findings suggest that adult stem cells hold promise for cellular therapy in certain human tissues based on currently available reliable experimental data.

Embryonic Stem Cells

The discovery of the embryonic stem cell constitutes one of the greatest achievements of modern biotechnologies. These cells are obtained from mammalian embryos at an early stage of development, when certain cells are still multipotent. Their history dates back to the 1950s (see 17 for references) when it became possible to fertilize the mammalian ovocyte *in vitro* and to culture the embryo up to the blastocyst stage. Once the blastocyst has 'hatched', i.e. has exited from the membrane (*zona pellucida*) that surrounds the egg, it can only survive and develop further if it becomes implanted into the uterine wall. At this stage it is a ball surrounded by a cell sheet that will become the placenta and is occupied by a cavity – the blastocoele – in which an '*inner cell mass*', will further develop into the embryo.

The potential to culture the mammalian embryo *in vitro*, an embryo which normally develops in the mother's womb, was an important advance: for the first time, the mammalian embryo could be subjected to close observation and experimentation. As an example, I wish to mention the remarkable historical experiments carried out independently by Andrzej Tarkowski in England and Beatrice Mintz in the US in the 1960s which demonstrated the high level of plasticity displayed by mouse embryos at early stages of their development. Two embryos were closely juxtaposed when the egg began to divide (*morula* stage), one derived from a strain with

white and the other with black fur. The two aggregated morulas were implanted into the womb of a foster mother where they developed into a single mouse which had stripes of black and white fur showing that cells from both embryos participated in the skin of the chimera. The same observation was made for virtually all tissues of the body.

These findings showed that at this early stage, the fate of embryonic cells is not yet determined and that the early mammalian embryo exhibits a large degree of plasticity. The demonstration that, at the blastocyst stage, each embryonic cell is essentially as totipotent as the egg itself was shown by an experiment in which a few cells of the *inner cell mass* were removed from a black strain embryo and introduced into the blastocoel of a white strain embryo. The injected cells adhered to the host's inner cell mass and participated in the formation of an embryo in which virtually all tissues were chimeric. The same result was obtained when one single cell was implanted: the tissues and organs of the recipient embryo were chimeric, showing the pluripotentiality of every single cell of the embryo at that stage (18).

In normal development, soon after the inner cell mass is formed in the embryo, decisive morphogenetic events take place: the cells become arranged into germ layers (ectoderm, mesoderm, endoderm) by the process called 'gastrulation' and they lose their totipotency to become restricted to definite fates. These experiments proved the existence of embryonic stem cells (below).

In 1981, Gail Martin in the US and Martin Evans in the UK (19-20) published articles reporting that the embryonic stem cells of the inner cell mass of the preimplantation embryo could be grown in tissue culture *in vitro* under conditions that allowed them to proliferate without differentiating. Cell lines of pluripotent embryonic stem cells (ES cells) could then be maintained in culture indefinitely. These results were striking because the embryonic stem cells obtained due to the pioneering work of Gail Martin and Martin Evans corresponded to the *in vitro 'capture and maintenance'* of a stage which in normal development is only transitory.

The ES cell lines have provided biologists with a remarkable tool with which to study the molecular mechanisms underlying the differentiation process. They have allowed gene targeting techniques to be developed in the mouse. Through this technique, the activity of virtually any gene of the mammalian genome can be either abolished or modified thus providing an experimental means to explore the role of these genes and their mode of action.

The ES cells can be maintained in an undifferentiated and proliferative state only if they are subjected to appropriate culture conditions involving factors which prevent them from differentiating. Otherwise,

they will give rise to a multitude of diverse cell types. If they are cultured in the usual type of medium, ES cells differentiate as they would in the course of normal embryogenesis. However, the ES cells are not able to give rise to an embryo within the culture dish and merely yield anarchically distributed differentiated tissues.

In the recent years, culture conditions that rely on the use of various cytokines and growth factors, have made it possible to induce the differentiation of a high proportion of ES cells into selected cell types such as neurons, pancreatic islet cells producing insulin, cardiomyocytes, etc… This has nurtured the hope of using these cells for therapy in order to replace cells that are deficient or have died in human tissues.

For many years, biotechnology was able to produce ES cells from only mouse embryos (and even exclusively from certain strains of mice such as the strain 129). This technology attracted more attention when in 1998, Thomson and his colleagues (21) published that they had been able to derive ES cells lines from human embryos. Moreover, it was shown that germ cells isolated from the gonads of older human embryos can also give rise to permanent lines of embryonic stem cells endowed with properties that are very close to those of the embryonic stem cells derived from the inner cell mass at the blastocyst stage. The stem cells that result from germ cell proliferation are designated as EG cells in order to distinguish them from the now classical ES cells (22).

Chapters in this book constitute symposium presentations by eminent specialists of the ES cells and germ cells. They discuss the possibilities that may be expected from these biotechnological advances to cure diseases for which there is currently no effective treatments: Prof. Ron McKay from the NIH, Prof. Rudolf Jaenisch from the MIT and Prof. Azim Surani from the University of Cambridge (UK).

These spectacular advances in cellular biotechnologies generate hope that human beings may be relieved from pain and disease. They, however, also raise undeniable ethical problems. The aim of this symposium volume is to inform as much as possible the members of the Pontifical Academy of the progress of science, as it stands at this moment, with the hope that these reports will stimulate future interest in following the progress that will certainly occur in this very rapidly moving field.

We, scientists, are convinced that we have the duty to provide everyone with all of the available information resulting from the advances of science, since we believe that knowledge is critical for every reasonable human to make informed ethical decision regarding medical interventions.

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BIOETHIK UND MENSCHENRECHTE
REFLEXIONEN ÜBER DIE GEFÄHRDUNG UND DIE WÜRDE DES MENSCHLICHEN EMBRYO

KARL KARDINAL LEHMANN

Im Lauf der letzten Zeit haben sich außerordentlich viele Themen im Bereich der Bioethik in den Vordergrund geschoben, die auch eine große öffentliche Resonanz erhalten haben: das Human-Genomprojekt, die Pränatale Diagnostik, die Präimplantationsdiagnostik, die Stammzellforschung, das Klonen in verschiedenen Formen, Patente auf Leben und Gentherapie. Aber es gibt in der gesamten Thematik auch so etwas wir einen roten Faden oder eine durchlaufende Perspektive, die von Anfang an die Fragestellung beherrscht und leitet, wobei dies oft verborgen, indirekt oder implizit geschieht. Dazu gehört zuerst die Frage nach dem anthropologischen, ethischen oder auch moralischen Status des Menschen im Anfang seiner Existenz. Gerade die Frage nach der Schutzwürdigkeit ist entscheidend von der Antwort auf die Grundfrage geprägt: Wann beginnt menschliches Leben? Zugespitzt wird die Frage in diesem Beitrag durch den Bezug zu den Menschenrechten.[1]

[1] Ich benutze in diesem Beitrag viele Erkenntnisse und Einsichten, die ich in einem größeren Kontext mehrfach vorgetragen habe. Dort findet sich auch ausführlichere Literatur, vgl. K. Lehmann, Das Recht ein Mensch zu sein und zu bleiben = Der Vorsitzender der Deutschen Bischofskonferenz 22, Bonn 2004. Ergänzt wird dies durch: Vom Staunen vor dem Leben als bleibender Imperativ, in: Festschrift für Prof. Dr. R. Schröder, erscheint zum 60. Geburtstag 2004 – In diesem Beitrag habe ich die Fragen der verschiedenen Traditionen zur Beseelung des Menschen (Sukzessiv- und Simultanbeseelung) und ihrer Wirkungsgeschichte bis in die Neuzeit hinein übergangen. Ebenso wäre ein ausführlicheres Eingehen auf die extrakorporale Befruchtung mit allen ethischen Einzelproblemen in diesem Rahmen nicht mehr zu leisten, vgl. nur M. Rhonheimer, Etica della procreazione, Rom 2000 (Pont. Univ. Lateranense); M. Aramini, La procreazione assistita, Mailand 1999.

I.

Gelegentlich wird die These vertreten, die Frage nach dem Zeitpunkt für den Beginn des menschlichen Lebens habe an Bedeutung und Gewicht verloren. Durch die Entwicklung der Familienplanung und Geburtenkontrolle habe sich die Verantwortung überhaupt auf die Zeit vor der Zeugung verlagert. Das Recht zum Leben sei darum nicht mehr eine Naturgegebenheit, sondern eine Aufgabe der Eltern und der Gemeinschaft, die dieses Recht zu erteilen hätten.

Kinder müssen angenommen werden. Menschliches Leben ist angenommenes Leben. Der Akt dieser Annahme geht der bewussten Zeugung eines Kindes seitens der Eltern voraus und muss in den Verhältnissen seines Aufwachsens immer wiederholt werden. Dies offenbart bei aller Richtigkeit einiger Aspekte eine grundlegende Problematik: Denn hier wird gegenüber einem gewiss eindrucksvollen personalistisch orientierten Denken die Leiblichkeit des Menschen mit seinen biologisch-somatischen Grundlagen übergebührlich in den Hintergrund gedrängt.[2] Nicht wenige Gedankengänge in der heutigen Diskussion ziehen daraus die Konsequenz, ein entscheidender Einschnitt in der Entwicklung menschlichen Lebens bestehe eben darin, dass der wirkliche menschliche Embryo der Mutter bedarf, um sich zu einem Menschen entwickeln zu können. Dies erfülle sich eben erst in der vollen Annahme und Aufnahme einer befruchteten menschlichen Eizelle. Vom Menschen könne angemessen nur dialogisch geredet werden, nicht in der Aussageweise einer Substanz. Sein heißt zusammensein und zusammenleben. Eine solche Überzeugung findet es höchst problematisch, wenn das menschliche Leben vom Zeitpunkt der Befruchtung an als „embryonaler Mensch" betrachtet wird.

Wenn diese Meinung – wie man meint – voll entfaltet wird, würde dies bedeuten, dass alle extrakorporal gezeugten Embryonen, die nach der Entscheidung des Arztes und der Frau nicht zur Einnistung bestimmt sind, ungeschützt und für einen verschiedenen Gebrauch und Verbrauch verfügbar sind. Geltung und Reichweite des Lebensschutzes hingen so letztlich von der Willkür der Mutter ab. Das Lebensrecht wird jedoch nicht durch

[2] Diese mangelnde Beschäftigung von Anthropologie und Philosophie, aber auch der Theologie mit der konkreten Leiblichkeit des Menschen, besonders auch z.B. mit der Geburt, habe ich ausführlicher dargelegt in: Zusammenhalt und Gerechtigkeit, Solidarität und Verantwortung zwischen den Generationen = Der Vorsitzende der Deutschen Bischofskonferenz 24, Bonn 2004.

die Annahme seitens der Mutter begründet, sondern durch das Lebensrecht des Embryo.

Dies ist ein Argumentationsmuster, das in modifizierter Form immer wieder vorkommt und sich sogar noch weiter zu verbreiten scheint. Dies steht im Gegensatz zu einer grundlegenden Überzeugung, die noch heute in vielen Verfassungen und auch in den Erklärungen der Kirchen festgehalten wird, dass nämlich ein menschliches Lebewesen mit der Verschmelzung von Ei- und Samenzelle seinen Anfang nimmt. Damit beginnt eine neue biologische Realität. Dazu gehören ein eigenes Steuerungssystem, ein eigenes Lebensprinzip und ein genetisches Programm, aus dem sich dieses Lebewesen konsequent entwickelt. Mit diesem Anfang ist also ein vollständiges, in diesem Sinne auch individuelles menschliches Leben gesetzt. Darum haben Embryonen auch schon auf den frühesten Stufen ihrer Entwicklung am Schutz des menschlichen Lebens Anteil. Dass dieses Grundmodell eine differenzierte Betrachtung zulässt und fordert, sei nur nebenbei bemerkt.

Es gibt jedoch unterschiedliche Einschätzungen, die zu konkurrierenden Antworten auf die Frage führen, von wann an der Mensch ein Mensch ist Man muss diese anderen Betrachtungsweisen[3] kennen:

Eine heute sehr stark verbreitete Konzeption lässt das menschliche Lebewesen mit der Einnistung in der Gebärmutter (Nidation) um den fünften bis achten Tag beginnen. Manche vertreten nun die These, erst mit dieser ursprünglichen „Adoption“ durch die Mutter entstehe die Menschenwürde und damit auch die Schutzbedürftigkeit. Richtig daran ist, dass sich ein Embryo ohne eine Mutter – es braucht nicht die genetische Mutter zu sein – nicht zum Fötus entwickeln und geboren werden kann. Es geht hier gewiss um die einzigartige Zwei-Einheit zwischen der Frau und dem Kind. Die Verbindung mit dem mütterlichen Organismus ist für die Entwicklung des Embryos unersetzlich. Doch dies bedeutet nicht, dass die im Embryo angelegte genetische Information durch die Nidation eine Ergänzung erfährt oder in seinem ontologischen Status grundlegend verändert wird.

Die Nidation hat im Fall der In-vitro-Fertilisation eine andere Bedeutung als im Fall der natürlichen Zeugung. Im letzteren Fall wird die Entstehung des Embryos der Mutter erst im Nachhinein bewusst. Gerade bei der künstlichen Zeugung gibt es jedoch seitens der Mutter zu den

[3] Die Belege für die folgenden Leitsätze habe ich in dem in Anm. 1 angeführten Beitrag ausführlich dargelegt.

Embryonen in vitro eine lebendige Beziehung, denn die Mutter wartet ja oft in großer Erwartung und mit Bangen auf das neue Leben. Es geht dabei nicht nur um eine „physische Einnistung".

Es bleibt das Argument, dass ein großer Teil der im Mutterleib gezeugten Embryonen vor der Einnistung unerkannt abgeht. Man spricht von bis zu 70 % befruchteter Eizellen, deren Entwicklung auf diese Weise abgebrochen wird. Man überträgt dann diese „Verschwendung der Natur" gerne auf die nun vom Menschen her bewusst zu ergreifende Möglichkeit steuernden Handelns. Der Mensch entscheidet dann, was mit dem Embryo in der Petri-Schale geschieht. Er wird instrumentalisiert.[4]

Ein Argument wird bei dem Zeitpunkt gesucht, zu dem die Möglichkeit einer Mehrlingsbildung ausgeschlossen ist. Dies ist ungefähr am dreizehnten Tag der Embryonalentwicklung der Fall. Bis dahin könnte die Forschung an Embryonen bis zum vierzehnten Tag freigegeben werden, wie es in Großbritannien durchgesetzt worden ist. Man ist der Überzeugung, dass man vom individuellen Leben nur sprechen könne, wenn dieses Leben sich nicht mehr teilen könne. Man hat darauf hingewiesen, dass man von einem komplexeren Begriff von „Individuum" ausgehen muss, einer gegliederten, in enger Kooperation stehenden Funktionseinheit, die sich von innen her differenziert und so einen ganzen Embryo hervorbringt. Man muss also den Begriff des Individuums im Zusammenhang der Zellteilung nochmals neu durchdenken. Im übrigen darf man Individualität nicht mit Singularität verwechseln.

Oft wird auch die These vertreten, dass Selbstbewusstsein und Selbstbestimmungsfähigkeit den Menschen ausmachen. Damit schiede der nasciturus von vornherein als Träger des Lebensrechtes aus. Damit wird auch die Frage provoziert, ob der Lebensschutz während des Schlafes nicht suspendiert wäre, für den Geisteskranken, vor allem aber für den Komatösen gar nicht gegeben sei. Aber der Mensch ist in seiner aktuellen Entfaltung nicht einfach Vernunftwesen. „Das Leben aber ist keine Erscheinung der Freiheit, sondern deren vitale Basis. Die Freiheit setzt Leben voraus, nicht umgekehrt".[5]

Es gibt noch eine Reihe anderer Konzeptionen, die z.B. sich am Gedanken der selbstständigen Lebensfähigkeit des ungeborenen Kindes

[4] Dazu und zum Folgenden J. Isenssee, Der grundrechtliche Statuts der Embryos, in: O. Höffe u.a., Gentechnik und Menschenwürde, Köln 2002, 37-77, bes. 55ff. W. Huber, Der gemachte Mensch, Christlicher Glaube und Biotechnik, Berlin 2002, 38-47.

[5] J. Isensse, a.a.O., 57.

außerhalb des Mutterleibes orientiert und den Zeitpunkt mit der Lebensfähigkeit eines Fötus im Fall einer Frühgeburt identifizieren möchte. Dieser Zeitpunkt ist jedoch durch die medizinische Entwicklung und durch die Qualität der medizinischen Versorgung sehr relativ. Es gibt außerdem immer noch und immer wieder einige Leute, die das menschliche Leben mit der Geburt beginnen lassen möchten, womit das Lebensrecht ganz von der sozialen Anerkennung abhängen würde. Unter solchen Voraussetzungen würde die Forderung nach einer unantastbaren Menschenwürde von Grund auf verloren gehen.

Diese verschiedenen Theorien gehen davon aus, dass es bei der Menschwerdung um einen gestuften Prozess handelt, der weitgehend dann auch Stufen des Lebensschutzes mit sich bringt. In Wirklichkeit gibt es zwar Zäsuren und Einschnitte, aber sie entstammen einem einheitlichen, dynamischen und sehr konsequenten Prozess. Jede „Stufe" folgt kontinuierlich aus den vorangegangenen Prozessen. Einzelne Abschnitte werden vor allem auch durch unsere Beobachtungsgenauigkeit und unsere begrifflichen Abgrenzungen identifiziert. Manche wollen auch durch die Tatsache verschiedener Namen (Zygote, Morula, Blastozyste, Prä-Embryo usw.) unterschiedliche Phasen ablesen, die auch einen qualitativ differenten moralischen Status des menschlichen Lebens begründen. In Wirklichkeit sind dies eher „Parameter der Reifungsvorgänge (...), um eine Eindeutigkeit der Beschreibung zu erreichen".[6] Es wird aber nichts Wesentliches ergänzt. In diesem Sinne haben nach meinem Dafürhalten die Spezies-, Identitäts- und Potenzialitätsargumente zwar eine Differenzierung, aber zugleich auch im Prinzip eine Bestätigung und Bekräftigung erfahren.[7] Es gibt keinen Zeitpunkt in der Entwicklung, an dem man sagen könnte, hier werde der Embryo erst zum Menschen. Es

[6] G. Rager, in: Ärztliches Urteil und Handeln. Zur Grundlage einer medizinischen Ethik, hrsg., von L. Honnefelder und G. Rager, Frankfurt 1994, 86.

[7] Vgl. dazu ausführlich G. Damschen/D. Schönecker (Hg.), Der moralische Status menschlicher Embryonen, Berlin 2003; A. Holderecker u.a. (Hg.), Embryonenforschung, Freiburg/Schweiz 2003; R. Beckmann/G. Loer (Hg.), Der Status des Embryos, Würzburg 2003; F.S. Oduncu u.a. (Hg.), Stammzellenforschung und therapeutisches Klonen = Medizin – Ethik – Recht 1, Göttingen 2002; M. Düwell/K. Steigleder, Bioethik, Franfurt 2003, J.P. Beckmann, Fragen und Probleme einer medizinischen Ethik, Berlin 1996; A. Lienkamp/C. Söling (Hg.), Die Evolution verbessern, Paderborn 2002; I. Schmid-Tannwald/M. Overdick-Gulden (Hg.), Vorgeburtliche Medizin, München 2001; B. Nacke/St. Ernst (Hg.), Das Ungeteiltsein des Menschen, Mainz 2002; Th. Zoglauer, Konstruiertes Leben, Darmstadt 2002; J. Reich, „Es wird ein Mensch gemacht", Berlin 2003.

handelt sich in jedem Stadium um einen menschlichen Embryo. Der Mensch wird nicht zum Menschen, sondern er ist von Anfang an Mensch. Es scheint mir auch die Einsicht wichtig zu sein, dass sich die Steuerung der Entwicklung menschlichen Lebens differenziert. Die Entwicklung unterliegt am Anfang, bis zum Vierzellstadium, noch weitgehend der Steuerung durch das mütterliche Genom, während im weiteren Verlauf zunehmend das embryonale Genom aktiviert wird.

II.

Es soll gar nicht geleugnet werden, dass das eine oder andere Element und Moment einen wichtigen Gesichtspunkt in die Gesamtdebatte bringen kann. Es scheint mir aber wichtig zu sehen, dass diese manchmal etwas sperrigen Einsichten keine grundlegende Zäsur in der Entwicklung menschlichen Lebens für die Anerkennung von Würde und Lebensschutz bedeuten. Dies hat Konsequenzen für die Methode der ethischen Urteilsfindung und Abwägung. Die Konzeption sollte den Vorrang erhalten, die am besten mit der Gesamtheit der einzelnen Teilergebnisse in der Embryonalentwicklung zusammenstimmt und auch am besten willkürliche Entscheidungen vermeidet. Dies scheint mir nach wie vor und ohne jeden Zweifel die Verschmelzung von Ei- und Samenzelle zu sein, daraus unmittelbar ein menschliches Leben beginnt. Das daraus entstehende Gebilde enthält die Potenz zur vollständigen Entwicklung einer individuellen menschlichen Person. Diese Entwicklung verläuft auch so, dass die Kriterien der Identität und der Kontinuität eine Rolle spielen. Mit Recht kann man also von einer Kontinuität der Entwicklung sprechen.

Schließlich geht es in diesem Zusammenhang um ein – wenn es doch ein Menschenwesen ist oder wäre – mindestens hypothetisches Menschsein, das dann einen entsprechenden Schutz verlangt und verdient. Hier gelten das Axiom „Im Zweifelsfall für die Annahme zu schützenden Menschseins und der Menschenwürde“ und ein Tutiorismus, den es übrigens auch in zeitgenössischen ethischen Entwürfen gibt. Wer so denkt, wird auch darauf verzichten, eine bestimmte Stufe in der Entwicklung menschlichen Lebens so auszuzeichnen, dass erst jenseits dieses Einschnitts eine verbindliche Schutzwürdigkeit beginnt. Man darf auch aus einem Mangel an Schutzmöglichkeiten im Fall natürlich gezeugter Embryonen, die ohne unser Zutun abgehen können, nicht folgern, dass wir künstlich erzeugte Embryonen, die uns ganz anders zugänglich sind, beliebig für verbrauchende Forschung freigeben dürfen. Gewiss erhalten nicht

alle Embryonen die Möglichkeit, sich zu entwickeln, aber für diejenigen, die sich entwickeln können, beginnt ein bestimmtes und individuelles Leben mit der Verschmelzung von Ei- und Samenzelle. „Somit folgt aus der embryologischen Betrachtung der menschlichen Entwicklung, dass der Embryo von der Befruchtung an menschliches Leben darstellt und die Möglichkeit besitzt, dieses menschliche Leben voll zu entfalten, wenn ihm die dafür nötigen Umgebungsbedingungen geboten werden“.[8] Damit wird man auch sagen dürfen, dass beim Embryo mehr festliegt als die Zugehörigkeit zur menschlichen Gattung. Das individuelle, als solches einzigartige Genom, also die genetische Identität des Menschen, ist in hohem Maß festgelegt. Diesem Befund steht nicht entgegen, dass sich das Genom in einem oder in mehreren Lebewesen verkörpert.

Es gibt hier viele Versuche, mit Hilfe einer bestimmten Sprachregelung und einer Semantik doch Zäsuren und Unterschiede auszudrücken, die einen qualitativ verschiedenen, abgestuften Lebensschutz rechtfertigen. So möchte man gerne im Sinne qualitativer Unterschiede von der Zugehörigkeit zur Gattung Mensch und der Ausprägung einzelnen menschlichen Lebens, von menschlichem Leben und Menschen, von latentem menschlichen Leben und Menschen sprechen. Im Blick auf die Nidation muss dagegen mit aller Deutlichkeit erklärt werden, dass sie eine notwendige Bedingung für die weitere Entwicklung darstellt, aber keine qualitative Zäsur. Es ist auch notwendig zu erkennen, dass das individuelle Leben des Ungeborenen, so notwendig und prägend es auch mit der Mutter in Verbindung steht, nicht bloßer Bestandteil der Mutter ist und ihrem Willen allein unterworfen ist. Der Embryo wird um seiner selbst willen geschützt, nicht um der Mutter willen. Darum hat im Blick auf die Schutzwürdigkeit der Embryo auch eine eigene Bedeutung.

Die Menschenwürde ist weder an Alter noch an Vernunft gebunden. Darum besitzen auch Geisteskranke Menschenwürde. Dabei gibt es auch Vor- und Nachwirkungen des Schutzes der Menschenwürde. So gibt es einen Persönlichkeitsschutz nach dem Tod. Verfassungsrechtlich mag es formell umstritten sein, ob der Embryo Menschenwürde besitzt oder ob die Würde des geborenen Menschen nur auf ihn ausstrahlt. De facto wird dem Embryo jedoch Menschenwürde zugesprochen. In diesem Sinne kann der Verfassungsrechtler J. Isensee nach einer eingehenden Darlegung der Problematik erklären: „Letztlich haften an allen Versuchen, dem Lebensschutz auf einen Zeitpunkt nach der

[8] G. Rager, in: Ärztliches Urteil und Handeln, 82.

Kernverschmelzung zu verlegen, Momente von Willkür. Willkürfrei und folgerichtig ist die Anknüpfung an die Verschmelzung. Das Grundgesetz (der Bundesrepublik Deutschland) schützt das Leben von Anfang an. Aus seiner Sicht wächst und entfaltet sich das Leben seit der Vereinigung der weiblichen mit der männlichen Keimzelle ‚nicht erst zum Menschen, sondern als Mensch'. Das Schutzkonzept ist umfassend und folgerichtig. Die grundrechtliche Anerkennung erfolgt bedingungslos, ohne Ausnahme, ohne Vorbehalt. Sie erfasst das Leben vor und nach der Nidation, vor und nach der Geburt".[9] Das Leben im frühen Stadium ist nie einfach Biomasse oder ein „Zellhaufen", sondern ein bestimmter Mensch als Individuum. Dann kann es auch kein würdeloses Menschenleben geben. In diesem Sinne ist der Embryo ein „Grundrechtsträger", Subjekt individueller Rechte. Menschenwürde kann nicht auf den Kreis der Vernünftigen und Leistungstüchtigen reduziert werden. Darum ist der Embryo auch nicht einfach Schutzobjekt, sondern selber Subjekt eines Rechtsanspruchs. Diese Menschenwürde liegt allen Normen und Rechtswerten voraus. Sie ist der Grund aller Grundrechte. Sie verkörpert sich in ihnen. Um sie zentrieren sich die übrigen Rechtswerte. Damit strahlt die Menschenwürde auch in das Vorfeld aus, in dem Leben entsteht. Hier wird unter Umständen nicht die Würde eines einzelnen Menschen verletzt, sondern die der Menschheit überhaupt.

Es ist nicht leicht, hier mit dem Menschenrechtsbegriff umzugehen. Es gibt gerade seit einiger Zeit einen erweiterten Sprachgebrauch von Menschenrecht. Er bezeichnet grundlegende Rechte, die jedem Menschen als Menschen zustehen. Ihr Anspruch auf Gültigkeit ist universal. Dieses Recht ist lediglich an das Menschsein gebunden. Die konkreten inhaltlichen Ansprüche stellen Antworten auf kollektive Erfahrungen historischen Unrechts dar. Es kann deshalb keine erschöpfende und zeitlos gültige Liste von Menschenrechten geben. Dennoch habe sich bestimmte Bedrohungen durch staatliche und gesellschaftliche Mächte als so tief verletzend, als immer wiederkehrend oder als latent vorhanden gezeigt, dass sie als typisch zu regelrechten Katalogen zusammengefasst wurden. Dazu gehört auch das Recht auf Leben, wie auch z.B. auf Religions- und Gewissensfreiheit. Insofern Menschenrechte auf die Umsetzung sittlicher Normen in der politischen Gestaltung hindrängen und dadurch freilich auch positives Recht auf die ethische Idee der Gerechtigkeit hin kritisierbar machen, stellen sie eine Schnittstelle von Recht und Sittlichkeit dar. In diesem Sinne formuliere ich in diesem Zusammenhang gerne: Vom Recht, ein

[9] J. Isensee, a.a.O., 61; vgl. zur Nidation ebd., 59f.

Mensch zu sein und zu bleiben. Dies ist das elementarste und fundamentalste Menschenrecht.[10]

III.

Das kirchliche Lehramt erkennt den menschlichen Embryo grundsätzlich den gleichen ethischen und rechtlichen Status zu wie jedem geborenen Menschen. In der lehramtlichen Sichtweise beginnt mit der Befruchtung ein kontinuierlicher, koordinierter und abgestufter Prozess, der jede Einteilung in vor- oder nichtmenschliche Lebensphasen von vornherein ausschließt wie jede Klassifizierung mit einem abgestuften Lebensrecht. Das Leben des Embryos ist wie jedes menschliche Leben, unabhängig in welchem Entwicklungsstadium es sich befindet, ein absolut zu schützendes Gut. Entschieden wendet sich die Kirche gegen Rechtfertigungsversuche, die etwa im Hinblick auf gute Folgen experimenteller Forschung an Embryonen für nachfolgende Generationen von Nutzen sein könnten. Die Würde des Embryos ist unantastbar und darf nicht dem Kalkül folgenorientierter Handlungsbeurteilungen geopfert werden. Die Menschenwürde verbietet auch jegliche kommerzielle Verwendung lebender oder toter Embryonen. Weil das Leben des Embryos menschliches personales Leben im Vollsinn ist, verbieten die Gewährleistung seines Wohls und damit die Pflicht zur Wahrung seiner Integrität schließlich auch seine Kryokonservierung.[11]

[10] Dazu außer der schon genannten Literatur B. Hamm, Menschenrechte, Oblaten 2003; W. Odersky (Hg.), Die Menschenrechte, Düsseldorf 1994; St. Gosepath/G. Lohmann, Philosophie der Menschenrechte, Frankfurt 1998; M. Fleischhacker (Hg.), Der Schutz des Menschen vor sich selbst, Graz 2002; O. Höffe, Sittlich-politische Diskurse, Frankfurt 1981, 173-278; Ders., Moral als Preis der Moderne, Frankfurt 1993; E. Iliadou, Forschungsfreiheit und Embryonenschutz = Schriften zum Öffentlichen Recht, 799, Berlin 1999; R. Merkel, Forschungsobjekt Embryo. Verfassungsrechtliche und ethische Grundlagen der Forschung an menschlichen embryonalen Stammzellen, München 2002.

[11] Zu den kirchenamtlichen Quellen vgl. Chr. Götz, Medizinische Ethik und katholische Kirche = Studien der Moraltheologie 15, Münster 2000 (mit einer umfangreichen Sammlung kirchlicher Quellentexte: 363-620; vgl. auch die Textsammlung von G. Filibeck, I diritti dell´uomo nell´insegnamento della chiesa. Da Giovanni XXIII a Giovanni Paolo II, Vatikan 2001 (Texte 1958-1998). Texte zum Lebensrecht: 527-561; Dazu auch alle Veröffentlichungen der Pontificia Academia pro Vita, vor allem: Identità e statuto dell'embrione umano, Città del Vaticano 1998; Natura e dignità della persona umana a fondamento del diritto alla vita, Città del Vaticano 2003; Vgl. auch die Textsammlung von Papst Johannes Paul II zum Lebensschutzthema anlässlich des 25. Jubiläums seines Pontifikates: Difesa della vita e promozione della salute, Roma 2003.

Der jüngere Sprachgebrauch der kirchlichen Dokumente wird vielleicht am deutlichsten in der Instruktion der Kongregation für die Glaubenslehre über die Achtung vor dem beginnenden menschlichen Leben und die Würde der Fortpflanzung, die 1987 unter den Anfangsbuchstaben „Donum vitae" veröffentlicht worden ist.[12] Dort heißt es (I,1): „Jedes menschliche Wesen muss – als Person – vom ersten Augenblick seines Daseins an geachtet werden". In derselben Instruktion heißt es kommentierend: „Sicherlich kann kein experimentelles Ergebnis für sich genommen ausreichen, um eine Geistseele erkennen zu lassen; dennoch liefern die Ergebnisse der Embryologie einen wertvollen Hinweis, um mit der Vernunft eine personale Gegenwart schon vor diesem ersten Erscheinen eines menschlichen Wesens an wahrzunehmen. Wie sollte ein menschliches Individuum nicht eine menschliche Person sein? Das Lehramt hat sich nicht ausdrücklich auf Aussagen philosophischer Natur festgelegt, bekräftigt aber beständig die moralische Verurteilung einer jeden vorsätzlichen Abtreibung. Diese Lehre hat sich nicht geändert und ist unveränderlich". (I.l)

Das Zweite Vatikanische Konzil hatte schon deutlich zum Ausdruck gebracht, dass es aus diesem Grund auch das menschliche Leben von der Empfängnis an mit höchster Sorgfalt schützt (vgl. GS 51). Im Anschluss daran hat die „Charta der Familienrechte" von 1983 formuliert: „Menschliches Leben muss vom Augenblick der Empfängnis an absolut geachtet und geschützt werden". (Nr. 4)[13] In der „Erklärung zur vorsätzlichen Abtreibung" aus dem Jahr 1974[14] heißt es: „Von dem Augenblick an, indem die Eizelle befruchtet wird, beginnt ein neues Leben, welches weder das des Vaters noch das der Mutter ist, sondern das eines neuen menschlichen Wesens, das sich eigenständig entwickelt. Es würde niemals menschlich werden, wenn es das nicht schon von diesem Augenblick an gewesen wäre. Die neuere Genetik bestätigt diesen Sachverhalt, der immer eindeutig war (...), in eindrucksvoller Weise. Sie hat gezeigt, dass schon vom ersten Augenblick an eine feste Struktur dieses Lebewesens vorliegt: Eines Menschen nämlich, und zwar dieses konkreten menschlichen Individuums, das schon mit all seinen genau umschriebenen charakteristischen

[12] Vgl. den lateinischen und italienischen Text mit wichtigen Kommentaren: Congregazione per la Dottrina della Fede, Istruzione „Donum vitae" = Documenti e studi 12, Città del Vaticano 1990.

[13] Vgl. die deutsche Ausgabe als Nr. 52 der Verlautbarungen des Apostolischen Stuhls (1983).

[14] Vgl. den lateinischen Text in: AAS 66 (1974) 730-747, hier: 738.

Merkmalen ausgestattet ist. Mit der Befruchtung beginnt das Abenteuer des menschlichen Lebens, dessen einzelnen bedeutenden Anlagen Zeit brauchen, um richtig entfaltet und zum Handeln bereit zu werden". [15]

Dabei muss die sorgfältige Argumentation im Kreuzungsfeld von Embryologie, Philosophie und Theologie beachtet werden. Wir haben den eher kommentierenden Text aus „Donum vitae" (I,1) bereits angeführt, kommen aber nochmals auf ihn zurück. Man kann in ihm leicht erkennen, dass der Argumentationsgang behutsam vor sich geht. Die unterschiedlichen Methoden und Erkenntnisweisen der Humanwissenschaften und der Philosophie sowie der Theologie werden angesprochen. Jedoch enthalten die empirischen Forschungen auch wertvolle Hinweise, „um mit der Vernunft eine personale Gegenwart schon vor diesem ersten Erscheinen eines menschlichen Wesens an wahrzunehmen". Es wird klar zum Ausdruck gebracht, dass die empirischen Hinweise einer weiteren Reflexion bedürfen, auf diesem Weg aber auch zu einer gültigen Einsicht hinführen können. Dabei ist die Aussage, dass es sich beim Embryo um eine „Person" handelt, einerseits eindeutig (auch in den anderen zitierten Quellen!), anderseits wird aber auch gegenüber dem Begriff Person eine gewisse Nachdenklichkeit zur Sprache gebracht, vor allem durch die unerwartete *Frage*: „Wie sollte ein menschliches Individuum nicht eine menschliche Person sein?" Mit überraschender Deutlichkeit wird festgestellt, dass sich das Lehramt auch beim Gebrauch des Personenbegriffs „nicht ausdrücklich auf Aussagen philosophischer Natur festgelegt" hat. Außerdem geht man sehr stark auch von der ursprünglichen Intention dieser Aussagen aus, dass nämlich die Lehre der Kirche jede vorsätzliche Abtreibung beständig verworfen hat. Schließlich gilt die Anerkennung als Person vor allem auch dem Schutz des Embryos.

Diese differenzierte Beschreibung ist durch die Enzyklika „Evangelium vitae", die eine der großen Achsen der Lehrverkündigung von Papst Johannes Paul II ist, im Jahr 1995 wieder aufgenommen und bekräftigt worden, und zwar in einer lehramtlich nun noch stärker verbindlichen Form. In diesem Weltrundschreiben wird besonders auch die Begründung in der Offenbarung dargelegt. Aber im Ganzen herrscht trotz aller Verklammerung mit den Lehrtexten eher ein auf die Katechese und

[15] Zur Interpretation vgl. aus der oben Anmerkung 33 genannten Reihe der Glaubenskongregation „Documenti e studi" Nr. 3: Dichiarazione sull'aborto procurato, Città del Vaticano 1988 (dort auch lateinischer und italienischer Text).

Verkündigung abgestimmter Ton. Zusammengefasst ist diese jüngere Lehrentwicklung im „Katechismus der katholischen Kirche“: „Da der Embryo schon von der Empfängnis an wie eine Person behandelt werden muss, ist er wie jedes andere menschliche Wesen im Rahmen des Möglichen unversehrt zu erhalten, zu pflegen und zu heilen“.

Schon aus den lehramtlichen Texten geht eine gewisse Ambivalenz im Gebrauch des Wortes Person für den Embryo hervor.[16] Darum ist die Anwendung des Begriffs in einem ersten Schritt eher etwas zögernd. Man geht von der Individualität des Embryos, seiner Schutzwürdigkeit, seinen Rechten und der ihm zugeschriebenen Menschenwürde aus. Von diesen Intentionen her geht man auf den Begriff der Person zu. Aufschlussreich ist dafür die gewiss nicht nur rhetorische Frage in „Donum vitae“: „Wie sollte ein menschliches Individuum nicht eine menschliche Person sein?“ Die Zurückhaltung geht von dem verschiedenen Gebrauch des Personenbegriffs aus und möchte offenbar die Sache selbst nicht durch einen Streit um Begriffe gefährden. Der Mensch ist zunächst Person, weil er mit Vernunft und Gewissen begabt ist, d.h. moralisch verantwortbares Subjekt ist. Dass jemand der Schutz der Würde der Person zukommt, ist von nichts anderem abhängig als dem Umstand, Mensch zu sein. In der klassischen Philosophie und Theologie gibt Person eine Antwort auf die Frage, wer jemand ist und was jemand ist. Eine Person ist eine von allen anderen Gegebenheiten unterschiedene und nicht weiter zu vervielfältigende Einheit, die vor allem durch das Vermögen der Vernunft ausgezeichnet ist. In der römischen Tradition werden die Verantwortlichkeit für das eigene Handeln und die Menschenwürde betont. Dabei ist besonders für den klassischen Gebrauch des Personenbegriffs wichtig, dass sich der Personencharakter auch in der Unverletzlichkeit des menschlichen Leibes manifestiert. Dies bedeutet eine substanzielle Einheit von Person und Natur im individuellen Menschen. Deshalb bezeichnet z.B. Thomas von Aquin die vom Leib getrennte Seele für die Zeit dieser Trennung nicht als Person. Im Lichte des klassischen Verständnisses gibt es keine Trennung zwischen Person und Menschsein.[17]

[16] Vgl. dazu C. Breuer, Person von Anfang an? Der Mensch aus der Retorte und die Frage nach dem Beginn des menschlichen Lebens = Abhandlung zur Sozialethik, 36, Paderborn 1995 (mit sehr umfangreicher Bibliographie: 308-400); Chr. Götz, Medizinische Ethik und katholische Kirche, Kap. 3, bes. 120ff. Die Aussagen des päpstlichen Lehramtes zu Fragen der medizinischen Ethik seit dem Zweiten Vatikanum = Studien der Moraltheologie, 15, Münster 2000.

[17] Zur Geschichte des Personbegriffs vgl. die große Arbeit von Th. Kobusch, Die Entdeckung der Person. Metaphysik der Freiheit und modernes Menschenbild, Erste

Der neuzeitliche, moderne Personbegriff hat gewisse Gemeinsamkeiten, schlägt aber doch eine andere Richtung ein, indem die Person vor allem durch die Einheit des Bewusstseins konstituiert wird. In der klassischen Fassung des Personbegriffs sind alle Menschen Personen. Für weite Teile des neuzeitlichen Denkens ist die Person aber bewusstes, sittliches Subjekt. Offensichtlich gibt es aber menschliche Lebewesen, die nicht im aktuellen Zustand handelnde Subjekte sind, wie z.B. Ungeborene oder irreversibel Bewusstlose. Es spricht aber sehr viel dafür, dass man an der Einheit von Mensch und Personsein festhalten muss. L. Honnefelder hat dies überzeugend gerade durch das früher entwickelte Potenzialitäts-, Kontinuitäts- und Unverfügbarkeitsargument aufgezeigt.[18] Personalität wird nicht anerkannt, nicht zuerkannt oder von irgendjemand verliehen; sie ist das Fundament für jede Beziehung. Mit der entgegengesetzten Haltung würde man das Personsein von nachzuweisenden Eigenschaften abhängig machen und die Gleichheitsforderung einschränken.

Es ist ganz offenkundig, dass das moderne Denken aus den angegebenen Gründen sich scheut, den Personbegriff auf Embryonen und ungeborene Kinder anzuwenden. Mit einer konstanten Argumentation wird dabei auf das Fehlen des Bewusstseins, der reziproken Anerkennung und der Empfindungsfähigkeit verwiesen, wobei gerade das letzte Argument im Blick auf moderne Entdeckungen recht differenziert und vorsichtig gehandhabt werden muss.[19] Immerhin sieht I. Kant Personsein und menschliche Natur in einem unlöslichen Zusammenhang, was freilich die theoretische Vernunft nicht erkennen kann, die praktische Vernunft muss dies postulieren. Kant unterstellt den Zusammenhang, vermag ihn aber von seinem Ansatz her nicht auszuweisen.

Es ist gewiss eine Frage der Sprachregelung, ob man das ungeborene menschliche Leben – gerade im Licht des modernen Sprechens von Person

Auflage, Freiburg 1993, zweite durchgesehene und erweiterte Auflage, Darmstadt 1997; L. Honnefelder, Person und Menschenwürde, in: L. Honnefelder/G. Krieger (Hg.), Philosophische Propädeutik, Bd. 2: Ethik = UTB für Wissenschaft: Uni-Taschenbücher 1895, Paderborn 1996, 213-266; J. Reiter, Menschliche Würde und christliche Verantwortung, 103f. Zum Ganzen vgl. auch die wichtigen Ausführungen von G. Pöltner, Grundkurs Medizin-Ethik = UTB 2177, Wien 2002; D. Mieth, Was wollen wir können? Ethik im Zeitalter der Biotechnik, Freiburg i.Br. 2002; Zum weiteren Umfeld vgl. G. Maio, Ethik der Forschung am Menschen = Medizin und Philosophie 6, Stuttgart 2002.

[18] L. Honnefelder, a.a.O., 252-254.

[19] Vgl. dazu nur mit vielen Analysen, Beispielen und Bildern: Irene von Hardenberg, Erlebnisraum Mutterleib, in: GEO, Juli 2001, Heft Nr. 7, 18-42.

– wirklich mit personalen Kategorien beschreiben soll. Es ist jedenfalls schädlich gewesen und ist es noch, den Embryo vom Personsein und irgendwie auch vom Menschsein auszunehmen. Dafür ist vor allem Lockes Personbegriff verantwortlich. Dies hat aber nicht dazu führen können, dem Embryo auch in weiten Teilen der neuzeitlichen Philosophie abzusprechen, dass er ein „ens morale" ist.[20] Für den, der in der klassischen Philosophie geschult ist, lässt sich menschliches Denken, das nicht personales Denken ist, gar nicht konzipieren. „Denn was würde sonst das menschliche Leben nachträglich zu einem personalen Leben machen, etwa die Selbstbestimmung oder die Anerkennung durch andere... Personsein setzt doch gerade eine ursprüngliche Fähigkeit zur Selbstbestimmung voraus, kann also nicht durch diese erst konstituiert werden. Und würde Personsein durch Anerkennung durch andere konstituiert, würde die Person zum Produkt der menschlichen Gesellschaft, während sie dieser Gesellschaft doch als etwas zu Respektierendes vorgegeben ist".[21] Im Horizont des neuzeitlichen Denkens, das hier freilich auch schon zum Teil überwunden ist,[22] wird man vielleicht mit einer stark philosophisch pointierten Diktion etwas zurückhaltender sein. Auf jeden Fall muss man den Begriff erklären, was nicht ganz leicht ist, und die Intentionen aufzeigen, die diese Sprache erforderlich machen. In diesem Sinne ist die Rede von der Personalität oder von einem personalen Anfang des Embryos der Sache nach gerechtfertigt. Man sollte um der Klarheit willen diesen Begriff auch auf keinen Fall aufgeben.[23]

Der Personbegriff hat einen stark praktischen Einschlag. Als Theologe, der vor allem mit dem Personenverständnis in der Trinitätslehre und in der Christologie vertraut ist, kann man dies nicht auf Anhieb erkennen. Von der römischen Welt her, besonders von Cicero, ist das Abendland gewohnt, den Rang des Menschen in der Menschenwürde zu sehen. Sie verbindet sich schon bei Boethius und vor allem bei Thomas von Aquin mit dem Status, Person zu sein. Die Verantwortlichkeit für das eigene Handeln steht dabei in der Mitte. Inhaltlich wird dieser Personbegriff sehr stark von der Lehre

[20] Vgl. dazu Th. Kobusch, Die Entdeckung der Person, 102ff. 267ff.; L. Honnefelder, Person und Menschenwürde, 230ff.

[21] J. Reiter, Menschliche Würde und christliche Verantwortung, 104f.

[22] Vgl. Th. Kobusch, Die Entdeckung der Person, bes. das Nachwort der zweiten Auflage, 263-280.

[23] Für die hier anstehenden Fragen sind die beiden Bände „Personen" (Stuttgart 1996) und „Grenzen" (Stuttgart 2001) von R. Spaemann noch längst nicht in ihrer Bedeutung erkannt. Vgl. dazu auch B. Gillitzer, Personen, Menschen und ihre Identität = Münchener philosophische Studien 18, Stuttgart 2001.

der Gottebenbildlichkeit gefüllt, fällt aber nicht schlechthin einfach mit ihr zusammen. Deshalb wird der Begriff der Menschenwürde auch in der frühen Neuzeit in Denksystemen, die eine gelockerte Bindung an die christliche Glaubenslehre haben, aufgegriffen und dazu benutzt, das schöpferische Vermögen, aber auch die Gleichheit aller Menschen zum Ausdruck zu bringen.[24] Kant begreift die dem Menschen eigene Würde als Selbstzwecklichkeit und also von der Autonomie her. In den Texten „Charta der Vereinten Nationen" (1945), der „Allgemeinen Erklärung der Menschenrechte" (1948) und ähnlichen Texten kommt der Begriff zu einer hohen Anerkennung. Er bezeichnet den unverlierbaren und unantastbaren Eigenwert der Person im Unterschied zu ihrer Verzwecklichung und Vernutzung in totalitären Gesellschaften.[25]

Die Menschenwürde ist keineswegs nur eine Leerformel, wie immer wieder behauptet wird. Gewiss besteht ihre Grenze darin, dass sie vorwiegend eine formale Größe darstellt, aus der keine konkreten Normen positiver Art unmittelbar abgeleitet werden können. Eine heute manchmal inflationäre Berufung auf die Menschenwürde kann diesen großen Gedanken gewiss entwerten. Aber gerade von der Thematik des moralischen Status des Embryos her gewinnt der Begriff durchaus an Gehalt und ist besonders auch im Blick auf die Menschenrechte inhaltlich bestimmt und ethisch fordernd. In diesem Sinne ist es hilfreich, wenn sowohl der Begriff der Person als auch der Menschenwürde von ihrer praktischen Aufgabe her gesehen werden. In diesem Sinne verbinden beide Begriffe die Menschen untereinander, denn sie veranlassen ihn zur gegenseitigen Anerkennung in ihrer Würde. Damit wird auch der konkrete Menschenrechtsgedanke gestützt. „Zum Menschenrechtsgedanken gehört daher das Gebot der Unantastbarkeit

[24] Vgl. G.P. della Mirandola, Über die Würde des Menschen, Zürich 1988; E. Schockenhoff, Naturrecht und Menschenwürde, Mainz 1996; Ph. Balzer u.a., Menschenwürde vs. Würde der Kreatur, Freiburg i.Br. 1998; F.J. Welz, Die Würde des Menschen ist antastbar, Stuttgart 1988, bes. Kap. 7, 271 – 399.

[25] Vgl. dazu knapp und klar L. Honnefelder, a.a.O., 221ff.; zum weiten Hintergrund vgl. auch mit reicher Lit. J. Reiter, Über die Ethik der Menschenwürde, in: Weg und Weite. Festschrift für Karl Lehmann, hrsg. von A. Raffelt unter Mitwirkung von B. Nichtweiß, Freiburg 2001, 443-454. Vgl. auch zu den säkularen Quellentexten St. F. Winter u.a., Genmedizin und Recht, München 2001; H. Hasskarl (Hg.), Europäisches Gentechnikrecht, Aulendorf 2002; A. Eser (Hg.), Biomedizin und Menschenrechte. Die Menschenrechtskonvention des Europarates zur Biomedizin, Frankfurt 1999; Enquete-Kommission: Recht und Ethik der modernen Medizin. Stammzellforschung und die Debatte des Deutschen Bundestages zum Import von menschlichen embryonalen Stammzellen, Berlin 2002.

der Person und das Verbot, dies von etwas anderem abhängig zu machen als der Tatsache, Mensch zu sein.[26] R. Spaemann bringt seinerseits die Sache auf eine gute Formel, wenn er dies alles mit dem Eintritt eines Menschen in die Menschheitsfamilie zusammenbringt: „Es kann und darf nur ein einziges Argument für Personalität geben: die biologische Zugehörigkeit zum Menschengeschlecht".[27] Darum besteht aber das große Recht des Menschen, der ursprüngliche Schutz, darin, dass dem Embryo als Menschen nicht schon die Eintrittskarte in die Welt und die Menschheitsfamilie verwehrt wird. Dies wäre gerade bei der Ohnmacht des Ungeborenen, die seine Menschenwürde nicht aufhebt, sondern noch mehr zur Beachtung aufgibt, eine ganz und gar unerlaubte Verletzung der fundamentalen Menschenrechte.

Gewiss kann man darauf hinweisen, dass zwischen der Embryologie und einer philosophisch theologischen Auswertung der empirischen Befunde da und dort noch einige Fragen offen sind. Aber dies kann die Kraft des hier vorgetragenen Argumentes letztlich nicht schmälern. Man muss nämlich die Frage nach dem, was das menschliche Leben im Anfang bestimmt, immer wieder auch von der Endgestalt des Menschen auf den Anfang hin zurückverfolgen. Wenn man dann ohne Schwierigkeiten die Menschenwürde des Erwachsenen anerkennt und sieht, wie konsequent die Verwirklichung des genetischen Erbes des Menschen mehr oder weniger bruchlos und ohne erkennbare anthropologische Zäsuren erfolgt, dann muss man selbst im Zweifel vorsichtshalber und zur Sicherheit, also tutioristisch davon ausgehen, dass der Embryo bereits ein menschliches Wesen ist, dem Individualität und damit ein personaler Charakter zu Eigen ist. Im Sinne dieser Regel des Tutiorismus – in der modernen Ethik heißt es: Benefit of the doubt argument – ist man bei einem nicht behebbaren Zweifel in der moralischen Bewertung einer Handhabung verpflichtet, dem Prinzip zu folgen: idem est in moralibus facere et exponere se periculo faciendi (eine Tat zu begehen und sich der Gefahr auszusetzen, sie zu begehen, ist moralisch gleich zu bewerten). In diesem Fall ist man also verpflichtet, um der Wahrung der Menschenwürde und der Menschenrechte willen der jeweils strengeren Meinung zu folgen.[28] Vielleicht gelten diese Überlegungen in keinem ethischen Bereich so schwer und ernst wie auf

[26] L Honnefelder, a.a.O., 261.

[27] R. Spaemann, Personen, 264; Grenzen.

[28] Vgl. zum Tutiorismus in unserem Zusammenhang: Beginn, Personalität und Würde des Menschen, 238, 309f., 389, 396 (Zitat: 396)

dem Feld des vorgeburtlichen Lebens des Menschen. Denn das Leben ist zwar nicht das höchste, wohl aber das fundamentalste Gut des Menschen.

IV.

Im Zentrum aller dieser Überlegungen bedarf sicher der Begriff der Menschenwürde einer stärkeren Reflexion. Er ist gerade in jüngster Zeit immer wieder kritisiert worden, da er „leer" und kaum anwendungsfähig sei. Man würde ihn in seiner Reich- und Geltungsweite lieber begrenzen, um einen größeren Spielraum für die Zulassung verbrauchender Forschung zu erhalten.[29]

Deshalb ist es unbedingt notwendig, den Begriff der Menschenwürde grundlegend zu vertiefen. Dies gilt nicht nur für den Bereich der Ethik,[30] sondern auch für die Theologie.[31] Dabei muss die Philosophie sich fragen, wie weit sie – zunächst von Kant her – die Menschenwürde als absoluten Wert begründen kann und ob dies allein vom philosophischen Denken aus möglich ist. Dabei kommt es besonders darauf an, den Schutz des Lebens gerade bei den Schwächsten sicher zu stellen. Sein Gelingen ist ein Maßstab für den Stand des Lebensschutzes in einer Gesellschaft. Dabei bleibt es eine ernsthafte Frage, ob die Begründung eines wirklich absoluten Wertes der Menschenwürde ohne Rückgriff auf Religion und Bibel möglich ist. J. Habermas hat in seiner bekannten Rede bei der Verleihung des

[29] In diesem Zusammenhang kann ich nicht näher eingehen auf den Versuch von Frau Bundesministerin B. Zypries „Vom Zeugen zum Erzeugen? Verfassungsrechtliche und rechtspolitische Fragen der Bioethik" vom 29.10.2003 an der Humboldt-Universität in Berlin, zwar an der absoluten Geltung der Menschenwürde festzuhalten, aber in Frage zu stellen, ob dem Embryo in vitro dieser Schutz zukomme. Diese Überlegungen stehen gewiss auch in einem größeren Zusammenhang, den Gehalt und den Rang der verfassungsrechtlich garantierten Menschenwürde zu relativieren. Vgl. dazu E.-W. Böckenförde, Menschenwürde als normatives Prinzip. Die Grundrechte in der bioethischen Debatte, in: Juristen-Zeitung (JZ), 58 (2003), Nr. 17 vom 5.09.2003, 809-815.

[30] Vgl. dazu zahlreiche Arbeiten von O. Höffe, z.B. Prinzip Menschenwürde, Frankfurt 2002, 49-69. Ders., in: Medizin ohne Ethik?, Frankfurt 2002; Dazu auch S. König, Zu Begründung der Menschenrechte: Hobbes-Locke-Kant = Praktische Philosophie, Freiburg 1994.

[31] Dazu E. Schockenhoff, Ethik des Lebens, Mainz 1993, 168ff., K. Hilpert, Die Menschenrechte, Düsseldorf 1991, 94ff., 181ff., 185ff. u.ö.; Ph. Balzer u.a., Menschenwürde vs. Würde der Kreatur, Freiburg i.Br. 1998; W. Vögele, Menschenwürde zwischen Recht und Theologie = Öffentliche Theologie 14, Gütersloh 2000 (umfangreiche Bibliografie).

Friedenspreises des deutschen Buchhandels 2001 an die Unersetzlichkeit religiösen Denkens und Sprechens erinnert.[32] Es wird besonders notwendig sein, den Sinn der biblischen Rede, dass der Mensch Ebenbild Gottes ist (vgl. Gen 1,27), angesichts der heutigen Herausforderungen zu vertiefen.[33] Dabei muss zugleich gefragt werden, ob ein Ernstnehmen der absoluten Geltung der Menschenwürde über unser Thema hinaus[34] nicht auch Konsequenzen hat für den Umgang mit dem Leben in anderen Bereichen.[35] Dies gilt nicht zuletzt für alle Formen der Tötung, wie z.B. Notwehr, Krieg und Todesstrafe.[36] Auch wenn es Unterschiede in der Wahrnehmung des Lebensschutzes geben muss, so ist der Lebensschutz selbst letztlich unteilbar. Es spricht vieles dafür, dass die Theologie und die Sozialverkündigung der Kirche seit einigen Jahrzehnten sich in dieser Richtung bewegen. Dies gibt auch neue Unterstützung für die Wahrung der Menschenwürde und der Menschenrechte im Bereich der Bioethik.[37]

[32] Vgl. die Rede unter dem oben genannten Titel, Frankfurt 2001.

[33] Vgl. dazu W. Vögele, Menschenwürde zwischen Recht und Theologie, 467ff.; K. Koch, Imago Dei – Die Würde des Menschen im biblischen Text = Sitzungen der Joachim Jungius-Gesellschaft der Wissenschaften e.V. Hamburg, Jahrgang 18 (2000, Heft 4), Hamburg 2000 (Bibliografie: 67f.); vgl. J. Habermas, Friedenspreis 2001, 53f.; Ders., Die Zukunft der menschlichen Natur. Auf dem Weg zu einer liberalen Eugenik, Frankfurt 2001, 46ff., 56ff., 105ff.

[34] Dabei finden sich auch noch unerledigte ökumenische Probleme, vgl. R. Anselm/U.H. Körtner (Hg.), Streitfall Biomedizin. Urteilsfindung in christlicher Verantwortung, Göttingen 2003.

[35] Vgl. dazu auch H. J. Albrecht u.a., Wechselwirkungen. Beiträge zum 65. Geburtstag von A. Eser, Freiburg i.Br. 2001.

[36] Dazu die Studien zum Tötungsverbot von W. Wolbert, Du sollst nicht töten = Studien zur Theologischen Ethik 87, Freiburg/Schweiz 2000; Was wollen wir, wenn alles möglich ist? Fragen zur Bioethik, hrsg., von H. Zirden, München 2003.

[37] Zur Diskussion um die Menschenrechte vgl. auch W. Kerber (Hg.), Menschenrechte und kulturelle Identität, München 1991; G. Höver (Hg.), Religion und Menschenrechte = Schriften des Zentrum für Europäische Integrationsforschung 29, Baden-Baden 2001. Zu der Kraft von Glaubenstraditionen in unserem Thema vgl. auch H. Joas (Hg.), Was sind religiöse Überzeugungen? = Preisschriften des Forschungsinstituts für Philosophie, Hanren Welt, Bonn 2003 (Konrad-Adenauer-Stiftung e.V.).

BIOETHICS AND HUMAN RIGHTS
REFLECTIONS ON THE JEOPARDIZATION AND THE DIGNITY OF THE HUMAN EMBRYO

CARDINAL KARL LEHMANN

Many topics in the field of bioethics have come to the fore just recently, becoming the focus of much public attention. These include the Human Genome Project, pre-implantation diagnostics, stem cell research, various forms of cloning, life patents and genetic therapy. There is, however, a kind of common leitmotif or a perspective that runs through them all, which has dominated and directed these concerns from the very beginning, whereby this frequently occurs cryptly, indirectly or implicitly. First and foremost, this involves the question of the anthropological, ethical or indeed moral status of the human at the beginning of his or her existence. It is precisely this question of the need for protection that depends crucially on the answer to the fundamental question: When does human life begin? The question is even more acute in this address because of the reference to human rights.[1]

[1] In this address I am using many realizations and insights, which I have already spoken of on several occasions in a broader context. These also contain more details on the relevant literature, cf. K. Lehmann, Das Recht ein Mensch zu sein und zu bleiben = The President of the German Bishops' Conference 22, Bonn 2004. This is supplemented by: Vom Staunen vor dem Leben als bleibender Imperativ, in: Festschrift for Prof. R. Schröder, to be published on his 60th birthday in 2004 – In this article I have omitted the approach of various traditions to the question of when the soul/spirit develops in humans (successively and simultaneously) and the history of its influence into modern times. Equally, it would be impossible to go into the matter of extracorporeal fertilization with all its individual ethical problems in more detail in this context, cf., as examples, M. Rhonheimer, *Etica della procreazione*, Rome 2000 (Pont. Univ. Lateranense); M. Aramini, *La procreazione assistita*, Milan 1999.

I.

Occasionally, the thesis is put forward that the question of the time at which human life begins has lost meaning and importance and that owing to the development of family planning and birth control, responsibility has shifted entirely to the time prior to procreation. The right to life is therefore no longer something that is naturally given; it has become the responsibility of parents and the community to grant this right.

Children must be accepted. Human life is accepted life. This act of acceptance precedes the conscious procreation of a child by the parents and must always be repeated in the circumstances of its growing up. No matter how correct this may be, it reveals some aspects of a fundamental problem complex: for here, vis-à-vis a certainly most impressive, personalistically oriented way of thinking, the physical being of humans with its biological, somatic fundaments is pushed into the background.[2] A not inconsiderable number of trains of thought in the current discussion conclude that a crucial hiatus in the development of human life is the fact that a real human embryo requires the mother in order to develop into a human being. And this is only fulfilled by the full acceptance and reception of a fertilized human ovum. To speak appropriately of a human must occur in the form of a dialog; a substance cannot declare itself. Being means being and living together. With this kind of conviction it is extremely problematic if human life is regarded as an 'embryonic human' from the time of fertilization.

If this opinion is carried to the end – as some people believe – it would mean that all embryos engendered by extracorporeal means, which the physician and the woman involved decide are not to be nidated, have no protection and are available for another purpose and use. The effectiveness and the scope of the protection of life would therefore ultimately depend on the arbitrary will of the mother. However, the right to life is not founded on acceptance by the mother, but on the embryo's right to life.

This is one argumental pattern that occurs repeatedly in a modified form and that appears to be spreading even further. It stands in opposi-

[2] I have described in greater detail this lack of focus on the actual physical being of mankind and particularly, for example the subject of birth in anthropology and philosophy as well as in theology: Zusammenhalt und Gerechtigkeit, Solidarität und Verantwortung zwischen den Generationen = Der Vorsitzende der Deutschen Bischofskonferenz 24, Bonn 2004.

tion to a fundamental conviction, which is written into many constitutions and the declarations made by the Churches – namely, that a human begins life when the ovum and sperm fuse. This is the beginning of a new biological reality. It has its own control system, its own life principle and a genetic program from which this living being evolves consistently. This marks the beginning of a complete, in the sense of an individual, human life. This is why embryos share in the right to the protection of human life from the very earliest stage of their development. Incidentally, this basic model permits – and indeed demands – differentiated reflection.

There are, however, different assessments, which result in competing answers to the question as to the moment from which a human being is a human being. It is important to be familiar with these other ways of seeing it:[3]

One very wide-spread conception today defines human life as beginning with nidation in the womb, which is around the fifth to sixth day. Some advocate the theory that it is this original 'adoption' by the mother that gives rise to human dignity and thus also the need for protection. It is correct that an embryo without a mother – and this does not have to be the genetic mother – cannot develop into a fetus and be born. The point here is certainly the unique union of two between the woman and child. The link with the maternal organism is indispensable for the development of the embryo. But this does not mean that the genetic information inherent in the embryo is supplemented in any way through nidation or that it is fundamentally changed in its ontological status.

In the case of in-vitro fertilization nidation has a different meaning than in natural procreation. In case of the latter the mother does not become aware of the creation of the embryo until afterwards. Precisely in the case of artificial procreation, the mother has a live relationship to the embryos in vitro, because the mother often waits with great expectation and apprehension for this new life. It is not simply a matter of the 'physical nidation'.

There remains the argument that a large proportion of the embryos generated in the mother's body abort undetected before nidation takes place. Figures stated refer to up to 70% of fertilized ova the development of which is thus aborted. There is a tendency to transpose this 'extravagance of nature' to man's conscious potential for active intervention. A

[3] I have presented the evidence for the following guiding principles in detail in the article mentioned in footnote 1.

person then decides what is to happen to the embryo in the Petri dish. It thus becomes exploited.[4]

Some favor the moment when the formation of multiple embryos can be precluded. This is approximately on the thirteenth day of embryonic development. Up to that time, i.e. up to the fourteenth day, research on embryos could still be given clearance, as has been enforced in Great Britain. There is the conviction that one can only speak in terms of individual life if this life is no longer able to divide. It has been pointed out that one must assume a more complex concept of 'individual', namely a structured, closely cooperating functional unit which takes its differentiation from within, thus bringing forth a complete embryo. So, one must again rethink the concept of the individual in respect of cell division. What's more, individuality must not be confused with singularity.

Frequently the thesis is put forward that self-awareness and the capacity for self-determination is the essence of being human. This means that the nasciturus is discounted a priori from possessing the right to live. This, in turn, provokes the question as to whether the protection of life is not suspended during sleep, and whether it does not even exist for the insane or, in particular, for those in a coma.

But human beings in their current forms of evolvement are not merely rational beings. 'However, life is not a phenomenon of freedom, it is its vital basis. Life is prerequisite for freedom and not the other way round'.[5]

There is a whole series of other conceptions based on the notion of the capacity for independent life of the unborn child outside the mother's womb; this seeks to identify the relevant time as the moment from which a fetus is able to live if it is born prematurely. However, this moment is very relative owing to medical developments and the quality of medical care. Furthermore, there are still people – and they keep on cropping up – who would like to see human life beginning at birth, which would mean that the right to live depends on a social acceptance. Under such conditions, the demand that human dignity be sacrosanct would be lost completely.

All these various theories assume that incarnation proceeds in stages, thus implying that the protection of life also manifests itself in stages. In

[4] On this point and the following: J. Isenssee, Der grundrechtliche Statuts der Embryos, in: O. Höffe *et al.* Gentechnik und Menschenwürde, Köln 2002, 37-77, particularly 55ff. W. Huber, Der gemachte Mensch, Christlicher Glaube und Biotechnik, Berlin 2002, 38-47.

[5] J. Isensse, loc. cit. 57.

reality, although there are indeed ruptures and decisive moments, they are rooted in a unified, dynamic and extremely consistent process. Each 'stage' follows from the previous processes in a continuum. Individual sections are identified above all owing to our precision as observers and our conceptual classifications. Some people take the fact that there are different names (zygote, morula, blastocyte, pre-embryo etc.) to mean that there are different phases forming the basis for a qualitatively different moral status of human life. In reality, these are rather 'parameters of maturation processes (…), in order to arrive at an unambiguous description'.[6] Nothing significant is added. In this sense, in my opinion, the species, identity and potentiality arguments may have become more differentiated, yet at the same time they have, in principle been confirmed and reinforced.[7] There is no point in time in its development where one can say this is when the embryo first becomes a human being. At all stages, it is always a human embryo. A human being does not become a human being; he or she is a human being from the very outset. I also regard the insight that the control of the evolvement of human life is in itself differentiated as eminently important. At the beginning – up to the four-cell stage – it is largely subject to control by the maternal genome, whereas in its further course the embryonic genome becomes increasingly activated.

II.

There is no denying that there are elements and moments which can add a significant aspect to the overall debate. Nevertheless, I feel it is important to see that these sometimes rather clumsy insights do not signify a fundamental, decisive moment in the development of human life in

[6] G. Rager, in: Ärztliches Urteil und Handeln. Zur Grundlage einer medizinischen Ethik, Editors: L. Honnefelder and G. Rager, Frankfurt 1994, 86.

[7] Cf. in greater detail G. Damschen/D. Schönecker (Ed.), *Der moralische Status menschlicher Embryonen*, Berlin 2003; A. Holderecker *et al.* (Ed.), Embryonenforschung, Freiburg/Switzerland 2003; R. Beckmann/G. Loer (Ed.), Der Status des Embryos, Würzburg 2003; F.S. Oduncu *et al.* (Ed.), Stammzellenforschung und therapeutisches Klonen = Medizin – Ethik – Recht 1, Göttingen 2002; M. Düwell/K. Steigleder, Bioethik, Franfurt 2003, J.P. Beckmann, Fragen und Probleme einer medizinischen Ethik, Berlin 1996; A. Lienkamp/C. Söling (Ed.), Die Evolution verbessern, Paderborn 2002; I. Schmid-Tannwald/M. Overdick-Gulden (Hg.), Vorgeburtliche Medizin, München 2001; B. Nacke/St. Ernst (Ed.), Das Ungeteiltsein des Menschen, Mainz 2002; Th. Zoglauer, Konstruiertes Leben, Darmstadt 2002; J. Reich, „Es wird ein Mensch gemacht", Berlin 2003.

terms of the acknowledgement of dignity and the protection of life. This has consequences for the method whereby ethical judgments and deliberations take place. The conception should be given priority, which best harmonizes with the totality of the individual partial results in the course of embryonic development and which best avoids arbitrary decisions. I still believe – and I have no doubts about this – that with the fusion of the nuclei of the ovum and sperm, life begins immediately. The entity emerging from this contains the potential for fully developing into an individual human person. This development indeed takes place in such a way that the criteria of identity and continuity play a part in it. So, one is justified in referring to a development continuum.

After all, in this context we are concerned with an at least hypothetical human existence – if it is indeed, or would be, a human being – requiring and deserving the appropriate protection. The axiom that applies here is: In case of doubt one must decide in favor of the assumption of human existence and human dignity requiring the appropriate protection, as well as a form of tutiorism which, by the way, is also to be found in contemporary ethical concepts. Anyone who thinks along these lines will desist from featuring a specific stage in the evolvement of human life in such a way that official worthiness of protection does not begin until after this decisive point. Simply because there is little way to protect embryos engendered naturally, which abort without our interference, we may not infer that we may at will give clearance for embryos produced by artificial means, allowing us a quite different access, to be used in research. Clearly, not all embryos have the chance to develop, but for those that can develop, a specific and individual life begins with the fusion of the nuclei of the ovum and sperm. 'Consequently, the embryological view of human development leads to the conclusion that the embryo represents human life from the time of fertilization and possesses the potential to unfold this human life to the full if it is provided with the necessary environmental conditions'.[8] Hence one must be permitted to say that in the embryo there is more that is predefined than the fact of belonging to the human race. The individual – and as such unique – genome, in other words the genetic identity of the person is to a large degree predetermined. The fact that the genome incarnates in one or more organisms in no way contradicts these findings.

Many attempts are made by way of specific linguistic tricks and semantics to convey decisive points and differences to justify a qualita-

[8] G. Rager, in: Ärztliches Urteil und Handeln, 82.

tively varied, graded protection of life. Correspondingly, there is the desire to speak in terms of belonging to the human race and of the diverse characteristics of individual human life, of human life and human beings, of latent human life and human beings in the sense that these constitute qualitative differences. However, in respect of nidation it must be stated categorically, that it is a necessary condition of the continued development, but not a qualitative turning point in it. It is also imperative to realize that the individual life of the unborn child, no matter how necessary and formative its link with the mother may be, is not merely a part of the mother and subject to her will alone. The embryo is protected for its own sake, not for the sake of the mother. Hence, with regard to being worthy of protection, the embryo has a significance in its own right.

Human dignity is bound neither by age nor reason. This is why the insane also possess human dignity. And the protection of human dignity has influence and consequences. For example, there is protection of the personality after death. From the point of view of constitutional law, it may be formally controversial whether an embryo possesses human dignity or whether the dignity of the already born human being radiates onto it. However, de facto human dignity is attributed to the embryo. With this in mind, J. Isensee, expert on constitutional law, having described the problem complex in detail, is able to state: 'Ultimately, all attempts to adjourn the protection of life to a point in time after the fusion of the nuclei, involve elements of arbitrariness. The reference to fusion is without arbitrariness and it is logical. The Basic Law (constitution of the Federal Republic of Germany) protects life from the very beginning. Its point of view is that life grows and unfolds from the moment when the female and male nuclei unite, 'not to *become* a human being, but *as* a human being'. Recognition under constitutional law is unconditional, without exception and without reservations. It includes life before and after nidation, before and after birth'.[9] Life at this early stage is never simply biomass or a 'pile of cells', but a specific person, an individual. This means that there can be no such thing as human life without dignity. In this sense, the embryo is a 'bearer of fundamental rights', a subject with individual rights. Human dignity cannot be restricted to those who are sensible and capable of performing well. This is why the embryo is not merely a protected object; it is indeed the subject of a legal claim. Thus conceived, human dignity exists prior to any kind of

[9] J. Isensee, loc. cit., 61; cf. with Nidation *ibid*., 59f.

standards and legal values. It is the foundation for all basic rights. It is inherent in them. All other legal values are centered round it. Consequently, human dignity also radiates into the sphere in which life emerges. Maybe it is not so much the dignity of an individual human being that is violated but that of mankind as a whole.

It is not easy to handle the concept of human rights here. For some time now, the term human rights has been used with an extended meaning. It signifies fundamental rights to which each human being is entitled as a human being. It has a universal claim to validity. The only thing to which this right is attached is the state of being human. The practical claims arising from its content represent the answers to the collective experience of historic wrong. There can therefore be no exhaustive and eternally valid list of human rights. Nevertheless, certain threats by national and social powers have proved to be so profoundly injurious, constantly recurring or latently present that, being typical, they have been compiled into literal catalogs. These include the right to life and, for example the right to religious freedom and freedom of conscience. Inasmuch as human rights urge the implementation of moral standards in practical politics, thus indeed exposing the positive right to the ethical conception of justice to criticism, they represent an interface between law and morality. Bearing this in mind, in this context I like to put it this way: It is about the right to be and remain a human being. This is the most elementary and fundamental human right.[10]

III.

In principle official church teaching bestows the same ethical and legal status on the human embryo as it does for each human being born. In the view of official church teaching, a continuous, coordinated and graduated process begins at fertilization; this precludes any categoriza-

[10] Further references on this subject: B. Hamm, Menschenrechte, Oblaten 2003; W. Odersky (Hg.), Die Menschenrechte, Düsseldorf 1994; St. Gosepath/G. Lohmann, Philosophie der Menschenrechte, Frankfurt 1998; M. Fleischhacker (Ed.), Der Schutz des Menschen vor sich selbst, Graz 2002; O. Höffe, Sittlich-politische Diskurse, Frankfurt 1981, 173-278; Idem., Moral als Preis der Moderne, Frankfurt 1993; E. Iliadou, Forschungsfreiheit und Embryonenschutz = Schriften zum Öffentlichen Recht, 799, Berlin 1999; R. Merkel, Forschungsobjekt Embryo. Verfassungsrechtliche und ethische Grundlagen der Forschung an menschlichen embryonicen Stammzellen, Munich 2002.

tion in pre-human or non-human phases of life, as well as any kind of classification with a graduated right to live. The life of an embryo is like any human life, independent of its current stage of development; it is precious and must be given absolute protection. The Church emphatically contradicts attempts at justification that might be of use with regard to the positive consequences of experimental research on embryos for coming generations. The dignity of the embryo is sacrosanct and may not be sacrificed to the calculating approach of assessments of actions that take their orientation only from the possible consequences. Human dignity also forbids any kind of commercial use of living or dead embryos. Since the life of the embryo is a personalized human life in the fullest sense, the guarantee of its wellbeing and therefore the obligation to uphold its integrity ultimately also forbid its cryoconservation.[11]

The language used more recently in church documents is possibly clearest in the instruction to the congregation in the doctrine on respect for the very beginnings of a human life and the dignity of reproduction, which was published in 1987 under the title of 'Donum vitae'.[12] It states that (I,1) 'The human being must be respected – as a person from the very first instant of his/her existence'. The same instruction comments:

> Certainly no experimental datum can be in itself sufficient to bring us to the recognition of a spiritual soul; nevertheless, the conclusions of science regarding the human embryo provide a valuable indication for discerning by the use of reason a personal presence at the moment of the first appearance of a human life: how could a human individual not be a human person? The Magisterium has not expressly committed itself to an affirmation of a philosophical nature, but it constantly reaffirms the moral condemnation of any

[11] For official church teaching sources cf. Chr. Götz, Medizinische Ethik und katholische Kirche = Studien der Moraltheologie 15, Münster 2000 (with an extensive collection of ecclesiastical source texts: 363-620; cf. also the collection of texts by G. Filibeck, I diritti dell´uomo nell´insegnamento della chiesa. Da Giovanni XXIII a Giovanni Paolo II, Vatikan 2001 (Texte 1958-1998). Texte zum Lebensrecht: 527-561; See also the publications by the Pontificia Academia pro Vita, especially: Identità e statuto dell'embrione umano, Città del Vaticano 1998; Natura e dignità della persona umana a fondamento del diritto alla vita, Città del Vaticano 2003; cf. also the collection of texts of Pope John Paul II on the topic of protection of life on the occasion of the 25th anniversary of his pontificate: Difesa della vita e promozione della salute, Rome 2003.

[12] Cf. the Latin and Italian text with important comments: Congregazione per la dottrina della fede, Istruzione 'Donum vitae' = Documenti e studi 12, Città del Vaticano 1990.

> kind of procured abortion. This teaching has not been changed and is unchangeable (I.1).

The Second Vatican Council had clearly expressed that, for this reason, it protects human life with the utmost care from the moment of conception (cf. *GS* 51). This was continued by the 'Charta of Family Rights', dated 1983 which states: 'Human life must be respected and protected absolutely from the moment of conception' (n. 4).[13] The Declaration on Procured Abortion in 1974[14] explains that

> from the moment in which the ovum is fertilized, a new life begins that is neither that of the father nor of the mother, but a new human being that develops independently. It would never become human if it had not been human from this moment on. Recent genetics confirm this fact, which has always been unambiguous (…) in an impressive way. It has shown that from the very first moment this living being has a fixed structure of its own: namely that of a human being, indeed this particular human individual already containing all its precisely defined characteristic features. The adventure of human life begins at fertilization; its various significant inherent qualities take time to fully unfold and become ready to act.[15]

It is important to heed the painstaking argumentation at the interface of embryology, philosophy and theology. We have already cited text from 'Donum vitae' (I,1), which is more of a commentary, and we will return to it later. The circumspection in the course of its argumentation is perceptible. This refers to the various methods and cognition processes of the human sciences, philosophy and theology. However, empirical research also holds valuable findings for 'perceiving an individual human presence by means of reason even before this human person becomes manifest'. There is a clear statement that the empirical findings require further reflection, via which they may lead to a valid deeper understanding, whereby the statement that the embryo is a 'person' is unambiguous on the one hand (also in the other sources quoted!), while on the other hand a certain reflectiveness is expressed concerning the term 'person', and particularly the

[13] Cf. the German edition as n. 52 of the announcements of the Holy See (1983).

[14] Cf. the Latin text in: *AAS* 66 (1974) 730-747, here: 738

[15] For the purposes of interpretation cf. the series of the Sacred Congregation for the Doctrine of the Faith cited in note 33 'Documenti e studi' n. 3: Dichiarazione sull'aborto procurato, Città del Vaticano 1988 (also with Latin and Italian text).

unexpected *question*: 'How is it possible for a human individual to not be a human person?' It asserts with surprising perspicuity that official church teaching has not 'committed itself explicitly to statements of a philosophical nature' when using the term person. Moreover, the original intention of these statements, namely that the doctrine of the Church has constantly rejected procured abortion is taken up fervently. Ultimately, recognition as a person applies primarily also to the protection of the embryo.

In 1995 this differentiated description was included and reinforced in an even more binding form in terms of official church teachings by the Encyclical 'Evangelium vitae', one of the great axis among the doctrinal documents of Pope John Paul II. This circular letter to the world in particular explains how it is rooted in revelation. In spite of being closely bound to the doctrinal texts, overall the tone of it is more in harmony with the Catechism and preaching. Summarizing this more recent doctrinal development in the Catechism of the Catholic Church: 'Because the embryo must be treated as a person from the moment of conception, like any other human being it must, as far as possible, be kept intact, be cared for and healed'.

Even in the texts containing the official Church teachings there is a degree of ambivalence in using the word person for embryo.[16] This is why the use of the term is initially somewhat hesitant. One is proceeding on the assumption that the embryo is an individual, that it is worthy of protection, that it possesses rights and the human dignity ascribed to it. These intentions form the basis for approaching the term 'person'. The rhetorical question in 'Donum vitae', but certainly not only this, is instructive in this respect: 'How can a human individual not be human person?' The restraint stems from the various different ways the term person is used and evidently seeks to avoid endangering the matter by a dispute on terminology. In the first instance a human being is a person, because he or she is gifted with reason and conscience, i.e. is a subject capable of moral responsibility. The fact that someone is accorded protection of their dignity as a person depends on nothing other than the circumstance of being a person. In classical philosophy and theology 'person' is the answer to the question of who

[16] Cf. in this respect C. Breuer, Person von Anfang an? Der Mensch aus der Retorte und die Frage nach dem Beginn des menschlichen Lebens = Abhandlung zur Sozialethik, 36, Paderborn 1995 (with a very extensive bibliography: 308-400); Chr. Götz, Medizinische Ethik und katholische Kirche, Chapter 3, particularly 120ff. The statements in the official teachings of the Pope on questions of medical ethics since the second Vaticanum = Studien der Moraltheologie, 15, Münster 2000.

someone is and what someone is. A person is a unit that is distinguished from all other phenomena and that cannot be copied and is above all endowed with the capacity for reason. Roman tradition emphasizes responsibility for one's own action and human dignity. Particularly for the classic use of the term person it is important that the character of 'person' also manifests itself in the sanctity of the human body. This means that person and nature form a substantive unity. This is why, for example, Thomas Aquinas describes the soul separated from the body as not being a person for as long as they are separate. In the light of classical understanding there is no division between person and being human.[17]

The modern concept of person has certain common aspects, but it takes a different turn in that the person is constituted by the unity of consciousness. In the classical version of the concept of person, all human beings are persons. However, much of modern reasoning sees the person as a conscious, moral subject. But there are evidently human organisms which, in their current state, are not capable of acting, such as the unborn or those who have irreversibly lost consciousness. There are many indications that we should adhere to the unity of being human and being a person. L. Honnefelder has demonstrated this convincingly, precisely with the argument favoring potentiality, continuity and unavailability.[18] Personality is not acknowledged, accorded or awarded by somebody; it is fundamental to all relationships. With the reverse attitude one would make being a person dependent on characteristics which would have to be proved, limiting the demand for equality.

It is quite obvious that for the reasons already cited, modern thinking balks at applying the term person to embryos and unborn children. It is consistently argued that consciousness, mutual recognition and sensibil-

[17] On the history of the person concept, d.f. the great work by Th. Kobusch, Die Entdeckung der Person. Metaphysik der Freiheit und modernes Menschenbild, Erste Auflage, Freiburg 1993, second corrected and extended edition, Darmstadt 1997; L. Honnefelder, Person und Menschenwürde, in: L. Honnefelder/G. Krieger (Hg.), Philosophische Propädeutik, Vol. 2: Ethik = UTB für Wissenschaft: Uni-Taschenbücher 1895, Paderborn 1996, 213-266; J. Reiter, Menschliche Würde und christliche Verantwortung, 103f. On this whole topic, cf. also the significant writings of G. Pöltner, Grundkurs Medizin-Ethik = UTB 2177, Vienna 2002; D. Mieth, Was wollen wir können? Ethik im Zeitalter der Biotechnik, Freiburg i.Br. 2002; Zum weiteren Umfeld cf. G. Maio, Ethik der Forschung am Menschen = Medizin und Philosophie 6, Stuttgart 2002.

[18] L. Honnefelder, loc. cit., 252-254.

ity are lacking, whereby this last argument must be handled with a good deal of differentiation and caution in view of modern discoveries.[19] At least I. Kant sees being a person and human nature in an inseparable correlation, although this cannot be cognized by theoretical reason, and practical reason is forced to postulate: Kant imputes it but is unable to identify it with his basic approach.

Undoubtedly it is a matter of linguistic convention whether one should really describe unborn human life – particularly in the light of the modern way of speaking of persons – in terms of personal categories. At all events it was, and still is, harmful to exclude the embryo from being a person and in a way also from being a human being. It is primarily Locke's concept of person that is responsible for this. However, it cannot have caused the embryo to have been denied the status of 'ens morale' by a large part of modern philosophy.[20] For those schooled in classical philosophy, it is impossible to conceive of human thought, which is not personal thought.

> For what would transform human life into personal life at a later date? Could it, for example, possibly be self-determination or the acknowledgement of others… being a person indeed presupposes the original capacity for self-determination and cannot therefore be constituted by it in the first place. And even if being a person were constituted by acknowledgement by others, the person would become a product of human society, whereas it is indeed given as something, which this society must respect.[21]

In the perspective of modern thought, which has indeed been partially overcome by now,[22] there will perhaps be a greater restraint in using diction with a strong philosophical emphasis. At all events, the concept must be explained, which is not so easy, as well as pointing out the intentions that necessitate this language. In this sense, the matter at hand justifies one in speaking of the personality or of the personal beginning of the embryo. For the sake of clarity, this term should on no account be abandoned.[23]

[19] Cf. here, only with many analyses, examples and pictures: Irene von Hardenberg, Erlebnisraum Mutterleib, in: GEO, July 2001, issue n. 7, 18-42.

[20] Cf. on this subject: Th. Kobusch, Die Entdeckung der Person, 102ff. 267ff.; L. Honnefelder, Person und Menschenwürde, 230ff.

[21] J. Reiter, Menschliche Würde und christliche Verantwortung, 104f.

[22] Vgl. Th. Kobusch, Die Entdeckung der Person, particularly the epilogue in the second edition, 263-280.

[23] For the concerns addressed here the significance of the two volumes, "Personen" (Stuttgart 1996) and "Grenzen" (Stuttgart 2001) by R. Spaemann has by no means yet been

The term has strong practical connotations. As a theologian familiar, above all, with the concept of person in the Trinity Doctrine and in Christology, this is not immediately recognizable. From the Roman world, especially from Cicero, the Occident has been accustomed to considering the position of the human being as being found in human dignity. As early as Boethius and, particularly, Thomas Aquinas it was linked with the status of being a person. It centers on the responsibility for ones own actions. From the point of view of content, this concept of person is largely filled with the doctrine of man's likeness to god, but it does not simply coincide with it. This is why the early modern era adopted and used the concept of human dignity to express both the creative potential and the equality of all humans in thought systems that have a looser connection with the Christian doctrine.[24] Kant conceives the innate dignity of humans as being an end in itself and therefore on the basis of its autonomy. In the Charter of the United Nations (1948) and the Universal Declaration on Human Rights (1948) and similar texts the term attains high recognition. It stands for the inalienable and indefeasible innate value of the human person in contrast to the way in which in totalitarian societies the human person is valued according to his or her expediency and usefulness.[25]

Human dignity is by no means a meaningless concept as is often claimed. Its limitation is certainly the fact that for the most part it represents a formal dimension from which it is impossible to directly derive any concrete standards of a positive nature. The sometimes inflationary invocation of human dignity as it is practiced today can indeed invalidate this

recognized. Cf. also B. Gillitzer, Personen, Menschen und ihre Identität = Münchener philosophische Studien 18, Stuttgart 2001.

[24] Cf. G.P. della Mirandola, Über die Würde des Menschen, Zürich 1988; E. Schockenhoff, Naturrecht und Menschenwürde, Mainz 1996; Ph. Balzer *et al.*, Menschenwürde vs. Würde der Kreatur, Freiburg i.Br. 1998; F.J. Welz, Die Würde des Menschen ist antastbar, Stuttgart 1988, particularly chapter 7, 271-399.

[25] Cf. clearly and precisely on the topic: L. Honnefelder, loc.cit., 221ff.; cf. also concerning the broader background and with extensive bibl. J. Reiter, Über die Ethik der Menschenwürde, in: Weg und Weite. Festschrift for Karl Lehmann, edited by A. Raffelt in collaboration with B. Nichtweiß, Freiburg 2001, 443-454. cf. also on the secular source texts: St. F. Winter *et al.*, Genmedizin und Recht, München 2001; H. Hasskarl (Ed.), Europäisches Gentechnikrecht, Aulendorf 2002; A. Eser (Ed.), Biomedizin und Menschenrechte. The European Council Human Rights Convention on Biomedicine, Frankfurt 1999; Investigative Committee: Law and Ethics of Modern Medicine. Stem cell research and the debate in the Federal German Parliament on the import of human embryonic stem cells, Berlin 2002.

great idea. But it is precisely the subject matter of the moral status of the embryo, which gives the concept greater content and particularly in respect of human rights it is authoritative in terms of content as well as being ethically demanding. With this in mind, it is helpful if both the concepts of person and human dignity are seen from the point of view of their practical function. Seen from this standpoint, both concepts join people together, because they are prompted to acknowledge each other's dignity. This also supports the practical aspects of the concept of human rights. The concept of human rights therefore involves the precept of the inviolability of the person and the forbiddance of making this depend on anything other than the fact of being a human.[26] R. Spaemann has found a good formula for the matter by making all this coincide with the entry of a human to the whole human family: 'There can and indeed there may be only one single argument favoring personality: the fact of belonging biologically to the human race'.[27] This is also why the greatest right of humans, namely original protection, consists in the fact that as a human the embryo cannot be refused the entrance ticket to the world and the human family. Precisely because of the helplessness of the unborn child, which does not cancel its human dignity, but indeed calls even more for attention, this would be an utterly unlawful infringement of fundamental human rights.

Clearly, one may point out that here and there several questions are still open between embryology and the philosophical and theological analysis of the empirical findings. Ultimately, however, this cannot diminish the power of the argument put forward here. The question as to what determines life in the beginning must be traced back from the final gestalt of the human person. If one then has no problem in acknowledging human dignity and realizes how consistently the actualization of the genetic heritage of the human takes place, more or less without any breaks, and without any discernible anthropological caesurae, then – even if one has doubts – as a precaution and for safety's sake, i.e. from a tutioristic point of view – one must assume that the embryo is already a human being possessing individuality and therefore a personal character. In the spirit of this tutioristic principle – modern ethics calls it the benefit of the doubt argument – if there is a doubt that cannot be eliminated in the moral assessment of how to act, one is under obligation to follow

[26] L Honnefelder, loc. cit., 261
[27] R. Spaemann, Personen, 264; Grenzen.

the principle: idem est in moralibus facere et exponere se periculo faciendi (to commit an act and to expose oneself to the danger of committing it rates the same in terms of morals). In this case, therefore, in order to safeguard human dignity and human rights one is always bound by the duty to act in compliance with the more rigorous opinion.[28] It may be that these deliberations apply so strongly and earnestly to no other ethical sphere than that of prenatal human life. For, while life is not the supreme good of mankind, it is its most fundamental.

IV.

At the heart of all these deliberations, the concept of human dignity surely requires more intense reflection. Particularly just recently it has been criticized repeatedly because it is 'empty' and almost impossible to apply. There is a tendency to want to limit its range and scope in order to obtain greater latitude for the approval of research using stem cells.[29]

This is why it is absolutely imperative to give a deeper fundamental dimension to the concept of human dignity. This applies not only to the field of ethics,[30] but also to theology,[31] whereby philosophy must ask itself to what extent – initially owing to Kant – it is able to reason that human dignity is an absolute value and whether this is possible based only on

[28] Cf. on tutiorism in this context: Beginn, Personalität und Würde des Menschen, 238, 309f., 389, 396 (quotation: 396)

[29] In this context am unable to elaborate on the attempt by Minister B. Zypries 'Vom Zeugen zum Erzeugen? Verfassungsrechtliche und rechtspolitische Fragen der Bioethik' of 29.10.2003 at the Humboldt University in Berlin, to adhere to human dignity as an absolute axiom on the one hand, while questioning whether the embryo in vitro is entitled to this protection. These thoughts certainly have a larger context and they qualify the content and priority of human dignity, which is guaranteed under constitutional law. Cf. in this context E.-W. Böckenförde, Menschenwürde als normatives Prinzip. Die Grundrechte in der bioethischen Debatte, in: Juristen-Zeitung (JZ), 58 (2003), n. 17 dated 5.09.2003, 809-815.

[30] Cf. many of the works of O. Höffe, e.g. Prinzip Menschenwürde, Frankfurt 2002, 49-69. *Ibid.*, in: Medizin ohne Ethik?, Frankfurt 2002; on the same topic also S. König, Zu Begründung der Menschenrechte: Hobbes-Locke-Kant = Praktische Philosophie, Freiburg 1994.

[31] Cf. E. Schockenhoff, Ethik des Lebens, Mainz 1993, 168ff., K. Hilpert, Die Menschenrechte, Düsseldorf 1991, 94ff., 181ff., 185ff. *et al.*; Ph. Balzer *et al.* Menschenwürde vs. Würde der Kreatur, Freiburg i.Br. 1998; W. Vögele, Menschenwürde zwischen Recht und Theologie = Öffentliche Theologie 14, Gütersloh 2000 (extensive bibliography).

philosophical thought. In so doing the most important thing is to safeguard the life of the weakest. Success in this is a gauge for the status of the protection of life in a society. It still remains a serious question whether it is indeed possible to establish a genuinely absolute value of human dignity without taking recourse to religion and the bible. In his famous speech on the occasion of the awarding of the Peace Prize of the German book trade in 2001 J. Habermas reminds us of the indispensability of religious thought and speech.[32] It will be especially necessary to reinforce the meaning of the biblical discourse that man is the image of God (cf. *Genesis* 1, 27) in the face of today's challenges.[33] At the same time, it is also necessary to ask whether taking the absolute validity of human dignity seriously beyond our topic[34] does not also have consequences for how life is treated in other spheres.[35] This applies not least of all for all forms of killing, e.g. self-defense, war and the death penalty.[36] Even though there have to be differentiations in the cognition of the protection of life, the protection of life itself is ultimately indivisible. There are many indications that show that theology and the social doctrine of the Church have been moving in this direction for some decades. This also gives new support for the preservation of human dignity and human rights in the sphere of bioethics.[37]

[32] Cf. the speech with the aforementioned title, Frankfurt 2001.

[33] Cf. here W. Vögele, Menschenwürde zwischen Recht und Theologie, 467ff.; K. Koch, Imago Dei – Die Würde des Menschen im biblischen Text = meetings of the Joachim Jungius-Gesellschaft der Wissenschaften e.V. Hamburg, volume 18 (2000, Heft 4), Hamburg 2000 (Bibliografie: 67f.); cf. J. Habermas, Peace Prize 2001, 53f.; Ders., Die Zukunft der menschlichen Natur. Auf dem Weg zu einer liberalen Eugenik, Frankfurt 2001, 46ff., 56ff., 105ff.

[34] There are still ecumenical problems that have not been handled, cf. R. Anselm/U.H. Körtner (Ed.), Streitfall Biomedizin. Urteilsfindung in christlicher Verantwortung, Göttingen 2003.

[35] Cf. also H.J. Albrecht *et al.* Wechselwirkungen. Beiträge zum 65. Geburtstag von A. Eser, Freiburg i.Br. 2001.

[36] On this topic the studies by W.Wolbert on the prohibition to kill, Du sollst nicht töten = Studien zur Theologischen Ethik 87, Freiburg/Schweiz 2000; Was wollen wir, wenn alles möglich ist? Fragen zur Bioethik, Editor H. Zirden, Munich 2003.

[37] In respect of human rights cf. also W. Kerber (Ed.), Menschenrechte und kulturelle Identität, Munich 1991; G. Höver (Hg.), Religion und Menschenrechte = publications of the Center for European Integration Studies 29, Baden-Baden 2001. On the power of faith traditions with regard to our topic cf. also H. Joas (Ed.), Was sind religiöse Überzeugungen? = Preisschriften des Forschungsinstituts für Philosophie, Hannover 1, Götten 2003, particularly 9ff.; P. Nolte, Bürgergesellschaft und christliche Verantwortung in der postsäkularen Welt, Bonn 2003 (Konrad-Adenauer-Stiftung e.V.).

STEM CELLS: LESSONS FROM THE PAST, LESSONS FOR THE FUTURE

IRVING L. WEISSMAN

The beginning of the search for stem cells was the bombing of civilian populations in Hiroshima and Nagasaki, the first use of nuclear weapons in which humans were the target. In retrospect those people who did not die from the blast or the fire but died with the lowest dose of lethal radiation, and the longest time from radiation to death, were those that had destruction of sufficient amounts of their blood forming (hematopoietic) systems that they could not regenerate enough white cells to fend off otherwise nonpathogenic (disease causing) infections, or did not have enough platelets to clot their blood. Higher doses of irradiation killed not only the blood forming system but the stem and progenitor cells of the intestinal tract; because the time of regeneration of the entire intestinal tract is five to seven days, by five to seven days the lining between the body and the intestinal lumen in these higher dose irradiated humans was essentially gone. It was later demonstrated that mice could be given doses of whole body X-irradiation and the same radiation syndromes developed; at the minimal lethal dose, about 8.5 to 10.5 Gy, the mice died at about two weeks post radiation of hematopoietic failure (Ref 1). Shielding even a single bone or the spleen from radiation prevents this irradiation syndrome. Soon thereafter, using inbred strains of mice, whole body radiated mice could be recovered by injection of suspensions of cells from the blood forming organs, for example, the bone marrow (Ref 2). In 1956 three laboratories demonstrated that the injected bone marrow cells regenerated the blood forming system, rather than release radiation factors that caused endogenous cells to repair irradiation damage (Ref 3-5). Then, and now, the only treatment for hematopoietic failure following whole body radiation is transplantation of bone marrow cells, or in fact, the hematopoietic stem cells (HSC) that are responsible entirely for rapid and sustained regeneration of the blood forming system in these hosts (for reviews, see 6,7).

The hematopoietic system is not only destroyed by the minimal doses of lethal X- radiation or nuclear radiation, but also by chemotherapeutic agents which, like radiation, largely kill dividing cells. By the 1960s physicians pushing to treat cancer that had spread (metastasized) beyond the primary cancer site to lymphatics and draining lymph nodes, or via the bloodstream to the rest of the body were attempting to take advantage of the fact that cancer cells, are cells that have a high fraction of their cells undergoing cell division. They began using agents that kill dividing cells derived from nitrogen mustards and other empirically discovered agents, as well as broad fields of radiation, to attempt to kill back cancer cells from the approximately 10^{11} – $2x10^{12}$ cancer cells that exist at the time of diagnosis to no cancer cells at all. The field of radiation therapy of local and draining fields of cancer was advanced by the newly developed linear accelerators that deposited most of the radiation at the depth of the tumor rather than at the skin. But this required the development of a quantitative assessment of damage to cancer cells compared to damage to normal cells. To test whether the appearance of dose dependent killing of cancer cells was different in kind to the dose dependent killing of normal cells J. Till and E. McCulloch in Toronto began a quantitative assessment of the radiation sensitivity of a normal cell type as it exists in the body – bone marrow cells useful in transplantation. They began with lowering the dose of bone marrow cells to save just lethally irradiated mice; they found that at sub-radioprotective doses, mice dying at 10 to 15 days developed in their spleens colonies of myeloid and erythroid cells, and these colonies showed a direct variation in their number with the number of bone marrow cells injected (approximately one colony per 7,000 bone marrow cells injected) (Ref 8). To test whether these colonies of myeloerythroid cells derive from single clonogeneic precursors, Wu, Becker, Till, Siminovitch and McCulloch preirradiated donors with doses of irradiation that would induce unique chromosome breaks in most hematopoietic cells; surviving cells that had sustained radiation induced and repaired chromosomal breaks which marked for each clonogenic hematopoietic cell (Ref 9). They found that all of the dividing cells within a single mixed cell type spleen colony contained the same unique chromosomal marker, different from all the dividing cells in a second colony, all of which shared their own unique chromosomal marker (Ref 9). They could take the cells within a single spleen colony and reinject them to secondary hosts, often seeing donor derived spleen colonies that had been regenerated from the single cell that generated the first colony; and rarely these cells contained sufficient numbers of regenerative cells that they could radioprotect

secondary hosts, and in addition, give rise to lymphocytes as well as the myeloerthyroid cells within a colony, all bearing markers of the donor injected cells (Ref 10,11). These genetic marking experiments established the fact that there must exist in bone marrow single cells that can both self-renew and generate all of the populations of cells in the blood; these cells were called at that time pluripotent hematopoietic stem cells, a term which has later been modified to multipotent hematopoietic stem cells (HSCs) (Ref 12,13). But knowing that stem cells exist by retrospective analysis of randomly gene marked cells is not the same as having pure populations of HSC for study that can be prospectively isolated.

The Isolation of HSC in Mouse and Man

To search for those cells in bone marrow that contain the activity of HSCs we developed assays for the clonal precursors of T lymphocytes (Ref 14) and of B lymphocytes (Ref 15-16) in addition to the clonal precursors of myeloerythroid cells found in spleen colonies (Ref 8-11). At the point we began, it was already known that the spleen colony-forming cells that Till and McCulloch studied were in fact probably the outcome of oligopotent myeloerythroid progenitors rather than stem cells, and that they were different from colonies that arise at about 14 days – close to the time of death – which come from more primitive hematopoietic cells (Ref 17). For each of those assays a single colony could be derived from 10^3 to 10^4 cells, so we had a quantitative assessment for the enrichment of these clonal precursors of T, B and myeloerythroid cells. We then needed a way to fractionate prospectively cells from the bone marrow to put into these colony assays. We chose to produce large numbers of monoclonal antibodies (Ref 18) that detected subsets of cells found in bone marrow, and to use high speed fluorescence activated cells sorters (FACS) to purify these cells (Ref 19). In 1986 we reported a high degree of enrichment of multipotent HSC, and in 1988 their full isolation (Ref 13, 16). At that point mouse hematopoietic multipotent marrow cells represented 1 in 2000 cells in the young adult mouse marrow bones, and were 2000 fold enriched for the ability to radioprotect lethally irradiated hosts by donor derived reconstitution of all blood cell types for life (Ref 13). There were no other cells than these cells in bone marrow capable of long term multi-lineage reconstitution (Ref 20), and at the single cell level these were all multipotents, although some reconstituted for weeks, or a few months, and others reconstituted for life (Ref 21-23). We later showed that these mouse multipotent cells in fact could be subdi-

vided into three subsets – long term HSCs (LT-HSC), short term HSC (ST-HSCs), and multipotent progenitors (MPPs), and that these cells existed in a lineage (Ref 24-26). Of these only LT-HSC self-renewed in apparent perpetuity, while ST-HSCs had a programmed self renewal life span of 6 to 8 weeks, and multipotent progenitors, of less than two weeks (Ref 24-26). The search for human HSCs didn't take long. Using similar assays for clonogenic, lymphoid and myeloerythroid progenitors it was possible to isolate candidate human hematopoietic and multipotent stem and progenitor cells, with their own distinctive and similar cell surface markers (Ref 27-29). We demonstrated long term multilineage reconstitutive activity with these, but no other cells in human hematopoietic tissues by using them to reconstitute human fetal bone, human fetal thymus, human fetal liver, and human fetal lymphoid organs implanted into fully immunodeficient SCID-hu mice (Ref 27, 30, 31). Later, it was shown that these cells too could be divided into LT-HSC and shorter-lived multipotent stem and progenitors (Ref 28). All of this wok was carried out at a company of which I was the co-founder (Systemix, now, Cellerant Inc), and an account of the founding of the company and the academic issues involved can be found in a book by Donald Kennedy called *Academic Duty* (Ref 32).

Clinical Trials Using Purified Human HSC to Regenerate the Blood Forming System of Patients Receiving Otherwise Lethal Doses of Chemotherapy

Modern cancer chemotherapy, the attempt to kill cancer cells spread throughout the body in a predictable way, is derived from an understanding of spontaneous mutations in phage and bacteria. In the 1940s Luria and Delbruck (Ref 33) and Lederberg (Ref 34) found that spontaneous variants of genes that, for example, determine antibiotic sensitivity or resistance, exist in populations of microorganisms prior to their exposure to the selecting agent such as the antibiotic. These usually exist in an unselected population at a frequency of about 1 in 10^6 organisms. If one starts with a population of 10^{11} to 10^{12} organisms, it is a virtual certainty that there will be many variants resistant to any single antibiotic or chemotherapeutic agent. The probability that any single bacteria will be resistant to two agents acting on different molecular targets within the cell is the product of that probability, about 10^{-10} to 10^{-12}. The probability of spontaneous resistance to three independent agents is infinitesimal, unless the resistance is derived by multidrug resistant transporters that remove most or all hydrophobic agents from the cell (Ref 35). Modern cancer chemotherapy usually involves simul-

taneous administration of three or four independent chemotherapeutic agents, plus or minus local or whole body radiation. In the 1960s, this led to the first cure of childhood acute lymphocytic leukemia (Ref 36). For many cancers, however, the dose to kill all existing cancer cells in the body is, as described above, at a level which kills hematopoietic stem cells down to the level that regeneration is not possible before death. In theory, these patients could be cured by removing their bone marrow before these myeloablative therapies are instituted, freezing it, and rescuing them after the chemotherapy administration is finished (for review see 37). However, for many late stage cancers and leukemias the spread of metastatic cancer cells also contaminates blood and bone marrow. It makes little sense to go to the trouble to destroy all cancer cells within the body, if you follow that with re-seeding of the body with marrow transplants or mobilized blood transplants containing cancer cells. But we were able to demonstrate that high speed FACS isolation of HSC using two or more marker antibodies resulted in at least a 100,000 fold depletion of contaminating cancer cells (breast cancer cells, or non-Hodgkin's lymphoma cells, or multiple myeloma cells), whereas flow or magnetic particle or bead isolation for enrichment of HSC using a single monoclonal antibody (anti-CD34) was insufficient to remove all cancer cells (Ref 7, 38). Thus we could for the first time deliver back to the patient HSC with little or no contaminating cancer cells from their own bodies. Three phase 1/2 clinical trials were carried out with prospectively isolated human HSC: stage IV (widely metastatic) breast cancer (Ref 39), non Hodgkin's lymphoma (Ref 40), and multiple myeloma (Ref 41). In all three trials the purified HSCs were given at doses that enable very rapid return of white cells and platelets, comparable to the time of regeneration of these blood elements within fractionated bone marrow or immobilized blood (Ref 39-41). At about four years post transplantation about 35 to 40% of Stage IV breast cancer treatment patients receiving this treatment were alive without evidence of disease, and about 55% of the non Hodgkin's lymphoma were alive without evidence of disease (R. Negrin, personal communication and Ref 39). In different trials using unpurified mobilized peripheral blood, the four year Stage IV breast cancer survival without disease progression was about 6% (Ref 42). The data for patients with multiple myeloma are still being collected. Because these were not experiments where patients were prospectively randomized into pure stem cell versus unmanipulated mobilized blood transplant, it is not possible to tell whether these increased cancer free survival rates are significant and reproducible, so further trials are warranted.

Transplantation of HSC from Genetically Distinct Donors Enables Donor Specific Tolerance of Tissue Grafts, and Donor Specific Abrogation of Autoimmune Diabetes in Mice

Transplantation of HSC from marrow or mobilized blood between genetically distinct donors, even if they are matched at the major histocompatibility (H-2) in mice (and HLA in man) (Ref 43, 44), leads to a serious complication; T cells from the donor make an immune attack against all host tissues, called graft vs host disease (GVHD) (for review see 45). Purification of HSC in mouse and man completely eliminates contaminating T cells. We have carried out many HSC transplants in different mouse strain combinations, some matched at the MHC but different otherwise, and others where no match at MHC (H2), or other loci were expected. Higher doses of HSC were required in the allogeneic mismatched transplants than in syngeneic transplants to achieve rapid and sustained engraftment (Ref 46-48). We also showed that hosts whose immune and blood forming systems were generated from genetically distinct donors were permanently and specifically tolerable of donor and host tissue and organ transplants, whether the organ transplants were given the same day as HSC, or up to a year later (Ref 47, 49). We have now achieved submyeloablative regimens for such combined HSC and organ transplants, so that one can expect that the host would not be at risk for death by the regimen that conditions the host for HSC engraftment (Ref 46, 50). Translation to man should enable a switch from chronic immunosuppression for organ, tissue, or other tissue specific stem cell transplants to protocols wherein a single conditioning dose allows HSC and tissue, organ, or other tissue specific stem cell engraftment for life. This should eliminate both GVHD and chronic host transplant immunosuppression, which leads to many complications, including life threatening opportunistic infections and the development of tolerated malignant neoplasms.

These mouse preclinical experiments also allow one to assess whether various genetically defective hematolymphoid systems and hosts can be replaced by healthy blood forming systems. These include not only genetic defects such as aplastic anemia, thalasemia, severe combined immunodeficiency and sickle cell anemia, but also replacement of immune systems prone to attack self that create auto-immune diseases (Ref 6,50). These auto-immune diseases include Type 1 (juvenile) diabetes (autoimmune T cells destroy insulin producing pancreatic islets) (Ref 51-53), multiple sclerosis (autoimmune T cells destroy myelinating oligodendrocytes), rheuma-

toid arthritis (Ref 54), systemic lupus erythematosus, and ankylosing spondylitis, to name a few of the genetically-determined autoimmune disorders. We have shown that normal HSC can replace the autoimmune-prone hematolymphoid system of mice (NOD) with developing Type 1 diabetes at the stages in which polydypsia and polyuria precede complete islet destruction; these mice are cured of the development of this disease (Ref 50). Mice already having complete destruction of the islets can also have the autoimmune part of their disease abrogated with HSC transplants from donors lacking the genetic risk for diabetes, and in some cases frank diabetic animals treated with HSC and islet transplants from healthy donors are also cured of their need for insulin and the complications of their disease (Ref 50). Many organ systems are susceptible to these kinds of autoimmune attacks, and so we need to search for engrafting or stem cells specific for each of these tissues and organ systems, such as pancreatic islets, myelinating oligodendrocytes, cartilage producing chondrocytes, and liver cells, to name a few. The subject of other stem cell types will be addressed later in this manuscript.

Expansion of HSC

The number of HSC that one can isolate from mobilized blood, or from umbilical cord, or from bone marrow limits the full application of HSC transplantation in man, whether in response to accidental or intentional nuclear radiation exposure, or transplantation in the treatment of diseases as described above. Engraftment times of 50 days or more used to be standard when limiting numbers of bone marrow or umbilical cord bloods were used in a transplant setting, reflecting the low level of HSCs found in these native tissues. Attempts to expand HSC with the known cytokines stem cell factor/steel factor (SLF), thrombopoietin (TPO), interleukins 1,3,6,11, plus or minus the myeloerythroid cytokines GMCSF, GCSF, MCSF, and erythropoietin have never resulted in a significant expansion of HSC. Rather, they induce many, if not most HSCs into cell divisions which are accompanied always by cell differentiations (Ref 55). Yet there are many experiments wherein single or a few HSC were transplanted into animals, and in those settings animals expanded the number of HSCs at least 100,000 fold at the steady state while permitting the daughters of HSCs to regenerate full blood forming systems (Ref 21, 22, 23, 24). Thus we did not have in hand the factors that were present in the body to regenerate HSCs by self-renewing cell divisions. We have recently found the pathway that

enables at least mouse HSC to under go massive self-renewing cell divisions, with progeny that are functional in hematopoietic cell transplantations into lethally irradiated hosts (Ref 56,57). By investigating genes transcribed in purified mouse LT-HSC we have found that these cells contain expressed elements of the Wnt/fzd/beta-catenin signaling pathway (unpublished data of E. Ranheim, S. Prohaska, C. Forsberg, A. Wagers, K. Li, S. Cheshier, and I. Weissman). Wnt was first discovered as a mouse gene rescued by milk transferred (Ref 58) mammary tumor virus (Ref 59) upon its integration into breast cells, resulting in uncontrolled proliferation of breast cells resulting in breast cancer (Ref 59, 60). Transgenic mice having mammary cell specific enforced expression of that Wnt developed breast cancers (Ref 60). It was shown in Drosophila that Wnt was a secreted ligand for a frizzled (FZD) receptor, and specified developmental commitments (for review see Ref 61). The interaction between Wnt fzd and the complementary FZD receptor along with a coreceptor related to LDL receptors (LRP6) resulted in the release of cytoplasmic beta-catenin from a multiprotein complex that phosphorylated and destroyed beta-catenin (Ref 61). Released, unphosphorylated beta-catenin, translocated to the nucleus, where it bound to DNA binding proteins of the LEF/TCF family, converting them from repressors of transcription to activators of transcription (Ref 61). Proteins in the multiprotein destruction complex are negative regulators of beta-catenin activation and stimulation of LEF/TCF transcription, and it has been found empirically that destructive mutations of each of those negative regulatory elements can play a role in the development of at least colorectal carcinomas (Ref 62). Breast cancers and colorectal cancers are diseases wherein uncontrolled self renewal leads to expansion of malignant cell populations that we call cancer stem cells ([63] see below). We then tested the possibility that the Wnt/Fzd/beta-catenin pathway is a regulator of self-renewing divisions of at least mouse HSC, and demonstrated that addition of highly purified Wnt3A to HSC leads to their expansion, as does transfection of activated beta-catenin genes into HSC (Ref 56, 57). HSC cell lines requiring no serum could be established that expanded 100 to 1000 fold, and these expanded HSC were also transplantable (Ref 56); such tissue culture expansions of HSC in serum-free conditions could only be accomplished if these cells were blocked in the programmed cell death pathway by BCL-2 (Ref 55, 64, 65). Wnt activation of HSC leads to up-regulation of other genes implicated in HSC self renewal, including Notch 1 and HOX B4 (Ref 56, 66, 67). Therefore it is critical to discover whether the same pathways operate in the expansion of human HSC, and if we can take

advantage of these pathways to expand rare populations of HSC at will. In that way HSC transplants might be possible starting from small collections of HSC rather than massive mobilizations and apheresis (see below) and one might convert collections of HSC from volunteer donors or umbilical cords into storable expanded and aliquoted stem cell banks useful on demand for clinical transplantation and/or for protection against radiation accidents. In mice successful HSC transplants that regenerate fully normal immune and blood forming systems can be accomplished when there is only a partial H-2 (mouse MHC) match, and the establishment of useful human HSC banks might require as little as a 3 out of 6 match of subcomponents of the HLA gene haplotype. This might be accomplished with stem cell banks of as little as 4-10,000 independent samples.

HSCs Normally Traffic From Bone Marrow to Blood, and This Can Be Greatly Amplified by HSC Mobilization Protocols

Research in the late 1950s and early 1960s indicated that bone marrow in mice was the major source of hematopoietic reconstituting cells, the mouse spleen was about one-tenth as efficient and mouse blood about 1/100th as efficient (Ref 68). But we did not know whether the blood HSC had recently derived from marrow, or if they represented a stable recirculating population. The optimal mobilization regimen for HSC currently used in the clinic is to treat the marrow donor with a drug such as cytoxan which kills most dividing cells (cytoxan is a nitrogen mustard derivate). Normally, only about 8% of LT-HSC enter cell cycle per day (69), so these are hardly affected by a short treatment with cytoxan, but most of the downstream multipotent and oligopotent progenitors are mainly in cell cycle (70, 71) and their numbers are greatly depleted by this dose, creating a demand for hematopoiesis to regenerate a blood forming system. Empirically, cytokines such as GCSF and SLF can increase the number of HSC in the blood, especially if administered for several days following a cytoxan pulse. We have shown that the optimized protocol of cytoxan plus G-CSF results in every resident LT-HSC in mouse bone marrow undergoing several self-renewing cell divisions, expanding the number of HSC 12 to 15 fold in a matter of two to three days (Ref 72, 73). It appears that on the second or third day following mobilization, up to one-half of the daughter cells of self-renewing dividing LT-HSC leave the bone marrow, enter the blood, and within minutes engraft other bone marrow, spleen, or even liver hematopoietic sites (Ref 74). In normal mice transfused LT-HSC also rap-

idly emigrate from blood to hematopoietic tissues (Ref 74), and to maintain the steady state of 100 LT-HSC the bone marrow must produce and release between 10^4 and 10^5 LT-HSC per mouse per day into the blood stream (Ref 74). Resident cells in the blood undergoing this massive HSC flux can and do enter empty hematopoietic niches elsewhere in the bone marrow, and provide sustained hematopoietic stem cell self-renewal and hematopoiesis (Ref 74). We assume that this property of mobilization of HSC is highly conserved in evolution (it has been shown in mouse, dog and humans), and presumably results from contact with natural cytocidal agents in the wild, after which regeneration of hematopoiesis requires restoring empty HSC niches. This means that coursing through each and every tissue of the body in very large numbers everyday in normal individuals are functional, transplantable HSC. Concurrent with the discovery of this massive HSC flux through all tissues were the early *claims* that brain cells in developed animals contained stem cells that could turn into blood forming stem cells (Ref 75), that bone marrow blood forming cells could give rise to neurons in the brain (Ref 76, 77), skeletal myocytes in muscle (Ref 78-80), regenerating myocardium and blood vessels in the heart (Ref 81-83). These latter studies were not carried out in a way, initially, that would distinguish between tissue specific stem cells coexisting with itinerant HSCs in the tissues; but these were in fact experiments wherein it was claimed that a single stem cell population within the tissue could give rise to resident tissues (myocardium, skeletal muscle, brain) as well as blood formation (Ref 84). These experiments were the basis of claims that demonstrated the *plasticity* of adult stem cells, and were contrasted to the embryonic and fetal development of organs and tissues, wherein commitment appeared to be from more pluripotent to multipotent to oligopotent to unipotent cells, without transdifferentiation of one germ line tissue (for example mesoderm) to another (for example ectoderm or endoderm). We discuss below where these well publicized claims stand currently.

Can Blood Forming Stem Cells or Other Resident Bone Marrow Cells Transdifferentiate to Other Tissue Specific Stem and Progenitor Cells, or Can They Contribute to Regeneration of Nonhematopoietic Damaged Tissues?

We had called into question the claims that one adult tissue type stem cell could turn into another tissue type cell (Ref 22, 85, 86). We proposed that one could only claim one tissue could turn into another if one began with highly purified well known tissue committed stem cells, best were sin-

gle stem cells (for example, HSC), transferred to genetically distinct hosts, and demonstrated the progeny of that stem cell included both its normal tissue derivatives as well as other kinds of tissues (blood and other tissues in the HSC case). The existence of large numbers of circulating hematopoietic stem cells through each tissue requires this kind of single cell analysis, or purification to homogeneity of cells. The initial claim that skeletal muscle contained a common stem cell for blood and muscle (Ref 83), was later amended when this second criterion was applied, and it turned out that the cells giving rise to the blood and blood forming system were simply hematopoietic stem cells in muscle with hematopoietic stem cell markers, whereas the population giving rise to muscle were cells within muscle lacking hematopoietic lineage markers like CD45, and that could give rise to muscle only; it is not yet clear whether these are purified muscle stem cells (Ref 87). There was a second claim that neurospheres derived from a clonogenic brain cell could give rise in vitro to brain cells, but upon injection in vivo, and after a delay of about seven months, could replace the full hematopoietic system of sublethally irradiated allogeneic hosts (Ref 75). This failed replication. There was another claim that single surface model mouse brain cells with 80% neurosphere-initiating cell potential could be isolated, and these cells could give rise to at least muscle and brain (Ref 89). We cannot replicate that finding (Raveh, T., Pham, K. and Weissman, I.L., in preparation). There is another experiment wherein bone marrow cells depleted of mature and maturing lineage positive cells, labeled *in vitro* with a vital dye, could be transplanted into an irradiated host, and cells not undergoing cell division reisolated from bone marrow two days later; these cells at the single cell level were reported to reconstitute the blood forming system and many epithelial tissues in irradiated mice (Ref 84). None of these experiments have been repeated in the published literature.

In another experiment single, visually-observed lateral ventrical ependymal cells were isolated, cultured extensively to produce neurospheres, and the daughter cells placed in the blastocyst of the same species (mouse) or directly injected into a developing chick embryo; donor markers could be found in a wide variety of tissues, though oddly excluding hematopoietic tissue (Ref 90), different than tissue produced from another neurosphere experiment (Ref 75). One published attempt to reproduce this experiment failed (Ref 91). There are several experiments wherein bone marrow cells, often characterized as hematopoietic stem cells but without the attendant purification, injected into lethally irradiated animals give rise to donor derived myofibers in muscle and cortical neurons as well as cerebellar

Purkinje cells in the brain (Ref 76-82). We and others have attempted to reproduce the demonstration of production of cortical neurons from marrow or hematopoietic stem cells precursors, and have failed (Mei, H., Wagers, A. and Weissman, I.L., unpublished data). However, donor markers can appear in muscle, in Purkinje cells in the cerebellum, rarely in myocardial epithelium, and in liver cells (Ref 81, 92, 93 and see below). There are experiments wherein bone marrow cells and cells enriched but not highly purified for hematopoietic stem cells injected into the injured myocardium shortly after a left anterior descending coronary ligation has been carried out, and the donor cells are claimed not only to give rise to cardiac muscle, blood vessel, and perivascular smooth muscle cells containing donor markers, as well as providing a functional regeneration of the heart (Ref 81, 100). These experiments have led to extensive clinical trials in humans (Ref 95-99). We have carried out extensive experiments attempting to repeat these findings, and always find that either hematopoietic stem cells or bone marrow cells having donor markers can be found in the inflamed and damaged cardiac muscle, but none of those cells express the markers, the morphology, nor the function of cardiac muscle, or of cardiac blood vessels, or of cardiac smooth muscle cells (Ref 101).

Cell Fusion and Claims of Stem Cell Plasticity

The fusion of two cells to produce a heterokaryon, a cell with two different nuclei, is a rare event in nature and in pathology, but it has been recorded at several different levels. First, in many pathological states following infection with agents as widespread as Sendai virus or Myobacterium tuberculosis, multinucleated giant cells derived from the fusion of cells of the monocyte/macrophages series are a constant and often pathognomonic sign of the infection type (Ref 102). Second, cells of the monocyte/macrophage series normally fuse by the thousands every day in every bone to form osteoclasts, cells whose phagocytosis and digestion processes remodel the bones to make space for bone marrow (Ref 103). Third, the Sendai virus from Sendai virus pneumonia has been used experimentally to produce artificial heterokaryons since the mid 1960s (Ref 104-106), and these have been used to understand the role of nucleus and cytoplasm in determining gene expression profiles (Ref 106). Fourthly, Sendai virus and polyethelyne glycol (PEG) have been used experimentally since the mid 1970s to create heterokaryons between normal antibody forming cell precursors and malignant plasma cell line cul-

tures in order to make hybridomas that produce monoclonal antibodies (Ref 18, 107). Fifth, in the normal development of all skeletal muscles, myoblast progenitors fuse to each other or to existing myotubes to form multinucleated skeletal muscle cells (Ref 108), a physiological phenomenon. Sixth, true embryonic stem cells derived from mouse inner cell mouse blastocysts have been shown when mixed together *in vitro* to fuse as well (Ref 109, 110). It is unclear which of these cell fusions is physiological and which pathological, especially when cells of the monocyte macrophages lineage are involved, whose normal function is to adhere to dying and dangerous cells during the process of phagocytosis. Helen Blau reviews elsewhere in this volume her argument that cell fusions represents a physiological repair and rejuvenation process (111, 112). As described above, there are several well documented cases wherein stem cells or tissues containing stem cells, can upon in vivo injection give rise to the donor markers in other tissue types cells. In the first case, for example, purified hematopoietic stem cells could contribute donor markers as well as a functional enzyme to highly damaged liver cells in a host lacking FAH (Ref 92). In these experiments the donor marker positive cells underwent several rounds of drastic selections; FAH lacking cells die from toxic products such as fumary/acetol acetic acid, and only those cells that contained normal FAH are selected and can survive. In fact significant and functional liver regeneration in those situations occurs only with a few animals and only after months of repeated selection, as most hosts died during the selection. A systematic analysis of the chromosomes in these regenerated livers have demonstrated that in every case there was a fusion between host and donor hematopoietic cell progeny (Ref 93-94). Mice restored with highly purified hematopoietic stem cells, some with a single hematopoietic stem cell that gives rise to hematopoiesis, or reconstitute with whole bone marrow containing that number of stem cells used in the hematopoietic stem cell reconstitution were tested with and without cardiotoxin and crush damage to skeletal muscles in a variety of muscles to test for donor markers in skeletal muscle (Ref 113). For most muscles bone marrow give rise to donor marked myocytes in a cell fusion with an efficiency far better than HSC transplanted hosts, even though the bone marrow that was transplanted in these instances have the same number of HSCs as the host (Ref 113). In no cases did this exceed 0.1% of myofibers and all were cell fusions. Thus rare cell fusions in for the most part highly damaged muscles do come from blood borne circulating precursors (Ref 113). In addition to the two well described cases of liver

and muscle, donor derived nuclei have been found to reside in the same cell body as a host nucleus in bone marrow transplanted animals in the case of the rare Purkinje cells in the cerebellum (Ref 22, 94, 114), rare cells in cardiac muscles that have donor markers (Ref 94), and rare liver cells (Ref 93, 94). The brains of mice restored with purified hematopoietic stem cells and or bone marrow do contain a class of normal hematopoietic cell derived microglia, and these can increase in frequency over time; many of these microglia have long processes, but retain markers of the myelomonocyte lineage (Ref 115). There are several recent accounts that bone marrow derived cells can give rise to regenerating pancreatic islet beta cells producing insulin (116, 117). Finally, mesenchymal stem cells (MSC) isolated by a particular protocol have been reported to be fully pluripotent (119, 120). These claims have been presented to and published by the President's Bioethics Council.

The claims in the above paragraphs received broad public attention before the demonstrations that they might not be reproducible, usually even before the manuscripts were published in peer reviewed journals. These claims of transdifferentiation plasticity of adult tissue stem cells appeared to contradict extensive studies on the embryological origin of the three tissue germ lines, and the tissues derived from them (Ref 118) and were taken in public venues to mean that adult stem cells were also pluripotent, and obviated the need for the study of pluripotent stem cells derived from the blastocyst stage of development, whether this blastocyst derived from a sperm-egg fusion or nuclear transfer (see below). Several self-described stem cell experts and the organization Do No Harm, cited 228 publications claiming adult stem cell activities and transdifferentiation in a letter to Ruth Kirschstein, then Director of NIH. However, on close inspection zero out of the 228 papers fit the criteria described in Refs 85, 86 that a known stem cell of one type (perhaps at the single cell level), in an *in vivo* differentiation system demonstrated the functional regeneration of cells from another tissue (I. Weissman, personal observations). Some members of the US Senate, US House, and the President's Bioethics Counsel (121, 122) also cited such claims in developing their own positions against the use of human pluripotent stem cells derived from the blastocyst stage of development as a legitimate object of scientific and medical study in the United States. Before I consider the scientific explanations for those few experiments wherein donor markers were found in different types of host tissues I should reiterate what is generally accepted in the scientific community as a discovery and an established finding.

TABLE 1

The Transition from Discovery to Accepted Scientific Fact

1) The initial discovery must be published in fully peer-reviewed journals.
2) The experiment as published must be repeatable in many independent laboratories.
3) The phenomenon described should be so robust that other experimental methods must reveal it.

Without the triad of published accomplishments, shown in Table 1, it is inappropriate for any body or agency to take the initial claims as true enough to affect clinical care protocols or public funding and policy decisions.

Using the criteria in Table 1 for the acceptance of an experimental phenomenon as a biological fact, we do not believe there is sufficient evidence for any of the transplant claims of transdifferentiation. In fact, most if not all reports of donor markers in unexpected tissues are the result of cell fusions, and the rarity of cell fusions makes it questionable that such events are regenerative rather than reflect the functions of post-injury phagocytic cells.

It is conceivable that some of these very rare cell fusions could be part of a regenerative process, but there is no evidence today that such is the case. Such evidence would require that donor marked cells fusing with generating or regenerating host cells contributed to a robust regeneration process.

Do Other Tissues Have Tissue Specific Stem Cells Used in Their Generation and Regeneration Throughout Life?

In addition to hematopoietic stem cells, the following stem cells have been prospectively isolated to homogeneity: peripheral nervous system stem cells (Ref 123, 124) and central nervous system stem cells (Ref 125-127). In addition highly enriched populations that contain stem cells have been found for the skin (Ref 128), as well as mesenchymal stem cells (Ref 119, 129, 130, 131, 132). In the case of human CNS stem cells, extensive experiments using their transplantation into the lateral ventricles or into the brain or into the spinal cord of SCID mice have shown that they con-

tributed in a robust way to engraftment of the neurogenerative cells (the subventricular zone of the lateral ventricles and the subgranular zone of the dentate gyrus in the hippocampus) in adult animals, as well as daughter cells that appear to be undergoing site specific self-renewal, migration, and differentiation into oligodendrocytes, various neurons, and astrocytes in an apparent site-appropriate manner (Ref 127, 133). These cells can remyelinate mouse axons in the Shiverer myelin deficient mice, can participate in the formation of appropriately placed interneurons in the olfactory bulb and elsewhere, can participate to ameliorate the effects of fresh crush-type spinal cord injuries (Ref 134, 135), and can migrate selectively towards an ischemic area in the central nervous system following middle cerebral artery blockage in the immunodeficient mouse (Ref 127, 133-136). They can also provide PPTI enzymes to PPTI deficient mice with Batten's disease, ameliorating the build-up of lipoprotein lipofuscin deposits and the loss of neuronal cells and their axonal and dendritic processes (Ref 137). These robust regenerations stand in contrast to microglial contributions and the rare Purkinje cell fusion derived from bone marrow and hematopoietic stem cells (Ref 138-139).

We do not yet know that all organ systems are based on the stem → progenitor → progeny model, but the method that led to the isolation of hematopoietic stem cells, peripheral nervous system stem cells, and central nervous stem cells is general enough to look for such tissue and organ specific stem cells. It should be added that the same method was used for prospective isolation of most if not all hematopoietic progenitor cells downstream of hematopoietic stem cells in mouse and man, and these cells too are capable of more limited and more specific regeneration of various important blood cell types, although none of these cells self-renew, and therefore all such transplants are transient in nature (Ref 140-152).

Cancer Stem Cells

Elsewhere we and others have provided evidence that cancerous tissues, which are obviously derived from normal tissues following somatic genetic and epigenetic changes, contain within them cancer or leukemia stem cells, and for the most part these cancer and leukemia stem cells differ from normal tissue stem cells (153-163) as well as the nonmalignant progeny they produce in a failed attempt to make normal tissue. These can include progenitor cells within tissues that have acquired the capacity for self renewal, as well as multiple genetic and epigenetic events to avoid pro-

grammed cell death, immune mediated death and phagocytosis, and limitation of replication life spans by loss of telomere protective sequences (Ref 164-171). The subject of cancer and leukemia stem cells is better described elsewhere (Ref 159).

Nuclear Transplantation to Produce Reproductive Clones and Nuclear Transplantation to Pluripotent Stem Cell Lines

A central tenet of developmental biology is that following sperm-egg fusion the early preimplantation embryo develops two kinds of specified cells – the pluripotent cells within the inner cell mass, and the trophoblasts/placenta committed cells that surround the inner cell mass of the preimplantation blastocyst (Ref 172). Key experiments on transfer of cells just after implantation from the implanted mouse conceptus to genetically distinct preimplantation mouse blastocysts reveals that pluripotentiality is lost as the cells follow epigenetic programs to commit to maturing and different cell fates, first the formation of the three germ layers, and later the tissues derived from them (Ref 172, 173). Except in the case of mature lymphocytes, there is no evidence that the nuclei of highly differentiated cells have lost genetic material, and in the case of lymphocytes, it is merely the potential to rearrange subgenic elements into new antigen receptors and the high degree of variability they endow to an immune system that must have diversity to protecting against infections and neoplasms (reviewed in 174, 175). Therefore it was quite surprising when, in the 1950s and 1960s, Briggs and King; and Gurdon provided evidence that the frog has cells after the blastocyst stage which upon transfer into the enucleated egg can participate in early (and rarely later) developmental events, implying that the epigenetic events that change the differentiation commitments and functions of various cells might be, at least in part, reprogrammed to that of pluripotentiality (Ref 176, 177). However, this field lay fallow for many years thereafter, and reemerged in a popular and in a scientific sense when Ian Wilmut reported the birth of a cloned sheep (Ref 178). Dolly was derived by nuclear transfer of a cell found in the mammary gland into an enucleated sheep egg which was carried *in vitro* to the blastocyst stage before implantation in a prepared sheep uterus (Ref 178). It was later shown in mice that the same kind of nuclear transfer technology could give rise, rarely, to preimplantation blastocysts, and rarely, from these, pluripotent stem cell lines (179-180) could be derived that were similar in properties to the embry-

onic stem cell lines that had previously been isolated from the inner cell mass of mouse blastocysts (Ref 181, 182). At about the same time as the publication of the sheep reproductive clone called Dolly, Thomson *et al*. in Wisconsin reported adaptation of the technology first used to produce mouse embryonic cell lines from mouse blastocysts (181, 182) to the production of human embryonic stem cell lines from human preimplantation blastocysts (available from *in vitro* fertilization clinics) (Ref 183). Hogan and Donovan (Refs 184, 185) had shown that the primitive germ cells that exist in the mouse embryo between 9 and 11 days of gestation prior to their arrival in the genital ridges and commitment to gamete outcomes, could be used to produce mouse pluripotent stem cell lines. Gearhart *et al.* used culture conditions, adapted from the mouse studies, to produce human pluripotent stem cells lines from human primitive germ cells (Ref 186).

Briggs and King (176), Gurdon (177), and over 30 years later, Wilmut *et al.* (Ref 178) demonstrated that nuclear transfer in several subprimate species could result in sufficient reprogramming of the nucleus to allow early stages of development to be revealed, and rarely developed organisms to be born (by reproductive cloning). This opened the possibility that one might be able to produce human pluripotent stem cells lines not just from those blastocysts resident in various in vitro fertilization clinics but also from predefined genotype donors; such pluritpotent stem cells developed in mice by nuclear transfer (NT) lines have the genetic characteristics of the cell nucleus donors (179, 187).

The possibility of human reproductive cloning and the possibility of NT to produce human pluripotent stem cell lines led the Presidents of the National Academies to assemble a panel of scientists, physicians, and medical ethicists to examine in an unbiased manner the scientific, medical, and medical ethics issues surrounding nuclear transfer technologies both for reproductive cloning and to produce human pluripotent stem cell lines, and to report back to them with recommendations. I was chairman of that panel (Ref 188). The panel had at its disposal the ability to research all areas relevant to the subject, to hold a workshop (which was on August 7th, 2001) to make sure that the panel had current knowledge of all attempts in animals at reproductive cloning and production of pluripotent stem cell lines by nuclear transfer. This workshop was arranged as an open forum so that all would-be reproductive cloners could state the medical and scientific basis of their plans, hear the actual outcomes of animal research in the area, and question and be questioned

by experts in the field. We found that in over 17,500 attempts at reproductive cloning in at least five mammalian species, about 99.2% of those implanted blastocysts died in utero. Of those that were born, many died soon thereafter (Ref 188). In the case of many species a common syndrome was discovered to be dangerous to the life of the fetus and the mother that bore it – the large offspring syndrome; this syndrome is due to defective placentation (Ref 188). Given these and other examples, and given the lack of evidence that any would-be cloners or any animal reproductive cloners had developed technologies that in the future would advance or change these outcomes, and taking into account a long history of the medical ethics of human participants in medical research, starting from the Nuremburg Code (Ref 189), the panel voted unanimously to call for a legally enforceable ban on human reproductive cloning. The panel defined reproductive cloning as implantation of blastocyst stage nuclear transfer products into the uterus with the intent of reproductive cloning (Box 2 from Ref 188).

We also examined the issues surrounding NT to produce human pluripotent stem cells lines. There are at least four areas of research that could not be accommodated with already established human embryonic stem cell lines, all derived from *in vitro* fertilization clinics (Ref 190). The first was to diversify the genetic diversity of the human ES cell lines. Although it was claimed that 64 such cell lines existed for experiments funded by the US government in President Bush's August 9, 2001 executive order, in fact, very few of those cell lines proved to be available for wide use. More importantly, they only represented the ethnic and racial diversity of people who need assisted reproductive technologies to establish a pregnancy, and in the United States this includes a bias for people who are Caucasian, who are well to-do, and always includes people who are infertile, a frequent abnormality that may have a genetic component. A second reason is the possibility of 'therapeutic cloning' wherein the individual who donates the nucleus to produce a pluripotent stem cell line is recipient of cell products from that cell line. Because the nucleus of any body cell contains genes encoding most of the major and minor histocompatibility antigens (mHC and MHC respectively), it was hoped that stem and progeny cells from a donor derived pluripotent stem cell line would be histocompatible with that donor. This is true for the nucleus and chromosomal encoded genes, but there is extensive genetic polymorphism of mitochondrial genes and it was well established in studies of mice that the proteins produced by the mitochondrial genes can serve

as peptides for MHC presentation, which are mHC (Ref 191, 192). In nuclear transfer to produce pluripotent stem cells, the egg mitochondria usually are retained and therefore give rise to mitochondria in the pluripotent stem cell lines produced from them. Nevertheless such immune responses to mitochondrial specified mHC are relatively easily overcome with low doses of immunosuppressive drugs so this is not an ultimate barrier to the eventual practice of therapeutic cloning. In fact, there is an excellent mouse example of therapeutic cloning, wherein the somatic cell nucleus from a genetically immunodeficient mouse was used to create a pluripotent stem cell line, and the genetic defect that had led to the immunodeficiency was corrected in the cell line (Ref 187, 193). These gene-corrected ES lines were converted to transplantable hematopoietic cells *in vitro*, and those cells were transplanted into the genetically immunodeficient mice, partially curing their immunodeficiency (Ref 193).Therapeutic cloning represents the possibility that one can find stem and progenitor cells for tissues that have not yet yielded their adult phase stem cells for transplantation, as well as the possibility that organs or tissues produced this way can provide life saving transplants when the patients own organs or tissues are irreversibly damaged.

A third, and I believe the most important reason for doing nuclear transfer to produce human pluripotent stem cell lines rests with the finding in mice that genetic abnormalities contained in the donor somatic cells, in the case of the immunodeficient mouse, skin fibroblasts, give rise to cell lines that faithfully reproduce the genetic abnormality in the pluripotent stem cell line, and in mature tissues derived from the pluripotent cell line (Ref 187). An example of a genetic immunodeficiency was described in the previous paragraph. Other mouse pluripotent stem cell lines derived by nuclear transfer from T lymphocytes or B lymphocytes had the specified rearranged immune receptor genes of the nucleus donors (Ref 179). Thus, every time that a known genome from a donor cell was used to produce a pluripotent stem cell line, the genetic program of that genome was replicated in the cell line derived from it and in mice, the *in vivo* tissues and organs.

The human genome project has provided for us the tools to discover the many gene alterations that are inherited in families and that cluster in patients with particular diseases. It turns out that a very large number of human diseases are genetically determined or strongly genetically influenced, including diseases as common as type I and type II diabetes, early onset cardiovascular diseases, autoimmune diseases, most neurodegenerative diseases such as ALS, Alzheimer's, Huntington's, etc, many endocrino-

logical disorders, all lysosomal storage diseases, and most cancers, to name a short list of the large number that are present. To reiterate, the human genome project is changing our understanding of the inheritance of these diseases, from knowing which chromosomes carry the familial traits to identifying the genes that show correlated genetic differences with the diseases. But understanding which genes are involved in a particular disease does not allow one to understand the pathogenesis of these diseases. For virtually all of these genetically determined or genetically influenced diseases, the role that each of the particular mutant genes (that correlate with the disease) plays in the pathogenesis of each disease is simply unknown. For example, not everybody who has the high risk MHC gene for type I diabetes actually gets diabetes; several other unlinked gene loci are involved. The person with the disease had an unfortunate inheritance of the high risk genes to develop this disease. This is also true of amyotrophic lateral sclerosis, (Lou Gehrig's Disease), and in fact most of the diseases cited above. Understanding the pathogenesis of the disease requires being able to take its components apart in a reductionist manner, and the many steps in that reductionist exercise that are relevant cannot be done with living patients, or even tissue from patients taken post mortem. For these multigenic diseases that involve more than one tissue or organ system, it is a very important and daunting task to be able to put together how development or function goes awry in any particular disease. But if one had a pluripotent stem cell line, or several, from patients harboring known genomes and known genetically determined diseases, and could compare them to people in the same families who don't have the disease, one can begin to identify the pathogenic genes; and by isolating the adult-type stem cells that are involved in their development, as well as transplanting these maturing cells into the cognate organ of severe combined immunodeficient mice, one can follow for the life of the mouse the potential pathogenesis of the disease in each of the interacting organ systems (Ref 127, 194-196). One could therefore use such genetically determined disease-derived pluripotent stem cell lines to carry out reductionist experiments *in vitro* and *in vivo* to begin to determine which of the correlated gene abnormalities are more important in the causation of the disease than others, in which order, and in which tissues they occur; and by gene correction techniques, show whether the particular disease susceptibility was ameliorated, validating that gene and it's product as a target for therapeutic intervention.

The fourth kind of pluripotent stem cell research in humans that cannot be carried out with existing human embryonic stem cell lines is the use

of nuclear transfer technology of cells that have diverged from their inherited genes by a process of somatic mutations and/or alterations of gene expression. These include all cancers and leukemias, and some neurodegenerative diseases such as Huntington's disease. There is no reason to believe that the genetic changes that lead to the generation of cancer stem cells and leukemia stem cells alter the ability of the genome of those cells to undergo reprogramming following nuclear transfer to establish pluripotent stem cell lines. Therefore, in addition to inherited genetic diseases, NT pluripotent stem cell lines from all patients with cancers can fall under the same reductionist approaches. This would require the identification and isolation of cancer stem cells for each different type of cancer, production of human pluripotent stem cell lines from them by nuclear transfer, and identification by genome analysis of genes mutated or changed in their gene expression profiles in the cancer stem cells.

From the above it is obvious that we are talking about hundreds of diseases that afflict a very significant proportion of humans, and that knowledge of which genes are important for which particular diseases could lead to innovative approaches of these validated targets of experimental therapeutic inquiry, whether it be by pharmaceutical corporations, or for gene therapy, or even for cell therapy groups within and outside academia. For all of these constituencies it is urgent to be able to wrestle with the technological barriers that stand in the way of making nuclear transfer to produce human pluripotent stem cell lines from predefined disease donors for the broadest of our medical research communities. Not to do so, or to delay doing so, quite clearly will affect the lives of people who might have been helped when they had a small window of opportunity for relevant therapies derived from the knowledge carried by these cell lines. This changes the equation, from comparing the ethical and religious status of cells in preimplantation blastocysts produced by nuclear transfer to the alternative ethical consideration of the lives of millions of people who have these diseases now, people who are already born (Ref 190). This is a platform technology, much like 25 years ago recombinant DNA was a platform technology. Like recombinant DNA none of us can predict what kinds of discoveries will result if the best and brightest investigators can use these cells in biomedical research institutions, under strict regulatory guidelines. Recombinant DNA technology was regulated, not banned, and hundreds of thousands of patients per year are successfully treated with its products. *My personal opinion is that any group that has authority to ban this NT research is responsible for the fate*

of patients that could have been treated with relevant therapies created during their short window of opportunity.

These are just a few of the obvious avenues of biomedical research that would be blocked by a ban on research leading to the nuclear transfer to produce human pluripotent stem cell lines from predefined donor nuclei. None of these kinds of experiments can be done with current human pluripotent stem cell lines.

Some Lessons from History

Approximately 75 years ago geneticists were divided as to how natural selection works. One line of thought came from the teachings of Lamarck who held that changes in the selection environment would lead to adaptive changes in the genetics of all organisms, so that over time, one could rapidly develop resistant populations that are heritably altered. This Lamarckian view (in the 1920s) was championed by a Russian geneticist, Trofim Lysenko (reviewed in Ref 200). The alternative Darwinian view stated that rare variants pre-exist in any population prior to the exposure to the selecting environment, so that perhaps one in 100,000 to one in a million organisms already had altered the correct genes for resisting, for example, hostile environments. If one used Darwinian selection methods to isolate resistant organisms to obtain a resistant population, it would obviously take very many generations of selection. When Lysenko became an adviser to Stalin, Stalin chose only to support the Lamarkian approach, and the Darwinian approach was essentially banned (Ref 197-200). Several leading Russian geneticists ended up in jail, and at least one, Theodoseus Dobzhansky, emigrated to the United States (Ref 197-200). Dobzhansky joined with the eminent American geneticist Thomas Hunt Morgan at Cal Tech and together they laid the foundations for modern genetics research, modern biology, and modern medicine, including the recombinant DNA revolution described above. For at least the 50 years that followed this decision, Russia and the Soviet Union produced few or no eminent scientists in the genetics field, the genetic revolution did not occur in the Soviet Union, and the medical treatments that derive from it, as well as the biotechnology companies that develop them, did not happen in the Soviet Union. Thus when governments or societies or religious organizations ban research on the basis of anything but strict scientific and medical merit, medical ethics, and laws made in secular societies, they risk large scale changes for their entire society, and for several human generations.

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EMBRYONIC STEM CELLS – THEIR PLACE IN MEDICINE

RONALD McKAY

Some 'truths' are more central than others to the core concepts of all ideological systems. Current work on the earliest stages of mammalian development can be viewed as fundamental to our understanding of life or as a transient fashion. I will argue here that work on embryonic stem (ES) cells is part of the tradition of development biology established by 200 years of research. The argument will be extended to suggest that manipulating embryonic stages of cell development is central to progress in medicine and that ES cells uniquely provide access to these early events.

Galileo Galilei (1564-1642) mapped the movement of objects in the solar system, and by 1687 in his *Philosophiae naturalis principia mathematica* Isaac Newton (1643-1727) proposed that unseen forces accounted for their movements. Galileo and Newton made their own telescopes and the resulting insights became eternal truths. Two of Newton's contemporaries are important for our argument because they made lenses into microscopes. Anton von Leeuwenhoek (1632-1723) in Holland and Robert Hooke (1635-1703) in England used microscopes to suggest that organisms were composed of cellular subunits.

In Paris, the Boulevard Raspail named after Francois Vincent Raspail (1794-1878) leads away from the Blvd. St. Germain bisecting the 5th and 6th arrondissements. The plate naming the street describes Msr. Raspail as a chemist and a politician. In the history of science, Raspail played a political role in establishing public funding for Universities, a central feature of modern French culture. He is also known for his insight that all cells come from other cells (Omnis cellula e cellula – 1825). The subject that concerns us here is the relationship between cells. Msr. Raspail recognized the importance of this when he stated 'Give me an organic vesicle endowed with life and I will give you the whole of the organized world' (see *The Birth of the Cell*, Henry Harris, Yale University Press). This quote

shows that Raspail knew that understanding how one cell transforms into another is at the center of life.

The idea that all plants and animals are composed of cells became accepted between 1820 and 1840. The existence and functions of bacteria and yeast remained controversial until later in the 19th century when Louis Pasteur (1822-1895) is recognized the many contributions of these smaller cells. By 1900 the cellular history of many simple animals and plants was known. The marine biology laboratories at Naples and Woods Hole, Massachusetts were established in 1872 and 1888. The microscopic analysis of the early developmental stages in invertebrate marine animals was a major activity at these centers. T.H. Morgan (1866-1945), the founder of genetics, trained at Woods Hole. At the same time the cells of the brain were identified. The Spanish anatomist Ramon y Cajal (1852-1934) was amongst the most distinguished students of brain structure. These anatomists clearly understood they were reporting about the way the cells in living organisms behave in time and space.

Interest in the cellular development of animals continued in the decades from 1900-1950 with a growing focus on the more complex development of vertebrates. The name of Hans Spemann (1869-1941) may not be spoken in every home in Germany but his contributions to knowledge have been recognized in many ways. He received the Nobel prize with Hilda Mangold in 1935. The main street in the Max-Planck Instutute in Tubingen is named Spemanstrasse. Spemann and his colleagues showed that the basic body plan of vertebrates is established rapidly when the embryo contains only few cells suggesting that fundamental aspects of animal form are established at an early stage of development.

Spemann's work was focused on the embryos of birds and amphibia because they were easy to obtain and observe. The small numbers and inaccessibility of mammalian embryos makes them much more difficult to study. But by 1958, techniques were developed to place a mammalian embryo in a foster mother (McLaren A., Biggers, J.D., 'Successful development and birth of mice cultivated in vitro as early as early embryos', *Nature*, 182, pp. 877-8, 1958). In 1978, the first test-tube baby Louise Brown was born in England (Edwards, R.G., Steptoe, P.C., Purdy, J.M., 'Establishing full-term human pregnancies using cleaving embryos grown in vitro', *Br. J. Obstet. Gynaecol.*, 87, pp. 737-56, 1980). This process is named in vitro fertilization, because mature eggs are recovered from the genetic mother and fertilized with sperm in a dish. The fertilized egg is then matured for a few days and implanted in the mother who will carry the developing baby.

Scientists call the earliest stages where there are few cells an embryo and the word fetus is used to describe later stages of vertebrate development (http://www.wordiq.com/definition/Embryo). There are many stages to development, when the embryo implants into the uterus it is at a stage called the blastula. The blastula is a very simple structure containing few cells that will form the body surrounded by supporting cells that will form tissues like the placenta. So in the procedure of in vitro fertilization (IVF) only the very first steps of development occur outside the body.

Immediately after the blastula stage the mammalian embryo generates the basic body plan. This process is called gastrulation and is of great interest but it occurs hidden from analysis. Researchers interested in tumors of the ovaries and testes showed that these tumors contain many cell types that are all derived from a single cell type. A cell that has the potential to generate all the cells of the body was then obtained from the normal blastula of the mouse and it was shown that these cells could be grown in large numbers in the laboratory. Remarkably these cells can be introduced again into a blastula, the blastula implanted into a foster mother mouse and normal offspring are born containing cells derived from the cells grown in the lab (Beddington, R.S., Robertson, E.J., 'An assessment of the developmental potential of embryonic stem cells in the midgestation mouse embryo', *Development*, 105, pp. 733-7, 1989). These lab grown cells can generate complete mice and this has become part of a technique that generates mice carrying modified genes. The cultured cells are called embryonic stem cells and their use in the genetic manipulation of mice has become one of the most powerful techniques in modern medicine.

Although it was known that embryonic stem cells could give rise to all the cells of the body, at first few people directly studied the development of ES cells into other cell types. Scientists interested in the somatic stem cells that make specific tissues in the body were the first to show that these intermediate cells could be obtained from mouse embryonic stem cells in the laboratory. They developed ways to show that these lab generated cells showed appropriate functions expected in the blood, the brain and other tissues. Many brain diseases are caused by cell loss or degeneration. Parkinson's disease is in a large part caused by loss of midbrain neurons that use the neurotransmitter dopamine. In animals, grafted cells that make dopamine can restore functions of the lost neurons (Kim, J.H. *et al.*, 'Dopamine neurons derived from embryonic stem cells function in an animal model of Parkinson's disease', *Nature*, 418, pp. 50-6, 2002). Cells derived from either the developing brain or from ES cells can restore function but the ES cells

have the distinct advantage that they can easily grow in the laboratory and still produce large numbers of the right type of neurons. At present, it is not possible to obtain large numbers of these neurons from any other source. When in the 1990s primate embryonic stem cells were obtained, it was easy to imagine using these cells as a source of specific human cells (Thomson, J.A. *et al.*, 'Embryonic stem cell lines derived from human blastocysts', *Science*, 282, pp. 1145-1147, 1998). Increasing evidence suggests that it will be possible to isolate large numbers of cells with specific functions of clinical interest from human ES cells.

There are several reasons for believing that interest in ES cells must continue to grow. First the point discussed above, that many cells of the body may have evolved to have limited growth and ES cells may be the only way to generate large numbers of these cells. Second, it will likely be clinically important to study the events of early human development that are replayed every time ES cells differentiate. Third, the long-term growth of ES cells will allow a new understanding of human genetics. The interest in human ES cells is clearly in a tradition of investigation of vertebrate development that has developed over two hundred years. Seen in historical context, the current interest in differentiating human ES cells is not a transient fad but a fundamental transition that brings together developmental biology and medicine. Just as the insights of Galileo and Newton still inform us, the continued use of human ES cells will likely be central to medical research for many decades. The perspective first stated by Raspail is now a reality.

GERM CELLS: THE ETERNAL LINK BETWEEN GENERATIONS

M. AZIM SURANI

Germ cells are potentially immortal since they provide the link between all generations. The fusion between eggs and sperm gives rise not only to a new individual, but also, theoretically at least, to an endless series of generations. Since germ cells are totipotent, they also exhibit unique properties (Surani, 2001). Thus, analysis of the germ cell lineage is highly relevant for the elucidation of the uniqueness of pluripotent stem cells.

An egg or oocyte is probably the most complex and extraordinary cell because this single cell can potentially develop into a whole organism. William Harvey was the first to recognise this in 1651 when he claimed that 'all things come from an egg', although it was nearly 200 years later that Bischoff and Van Beneden first observed fertilisation. Harvey thought that development of the embryos and fetus from an egg occurred gradually. Thus, Harvey thought of development in terms of epigenesis, which is a gradual emergence of the embryo and fetus from an egg, rather then endorsing the concept of preformation. Waddington later depicted this concept in his famous 'epigenetic landscape' in a symbolic representation of the developmental potential of an egg. Epigenesis is thus the formation of entirely new structures during development of the embryo. A relatively recent concept of epigenetics refers to the study of mitotically or meiotically heritable changes in gene function that cannot be explained by changes in DNA sequences. Indeed, the derivation of all differentiated cell types from pluripotent stem cells occurs without changes in DNA sequences, thus emphasising the importance of epigenetics in these processes.

Mammalian Germ Line Cycle and Parthenogenesis

The germ cell cycle is repeated in every generation (Figure 1, see p. 138), which also shows the relationship between early development and the deriva-

tion of pluripotent embryonic stem cells (ES) and pluripotent embryonic germ cells (EG), from blastocysts and primordial germ cells, respectively. Fully mature gametes or sperm and egg may be considered as the end products of the germ cell cycle. The oocyte (Figure 2, see p. 139) contains critical information necessary for development from this single cell into an adult (Surani, 2001). The oocyte contributes genetic information in the form of approximately 30,000 genes, which constitutes the genetic blueprint for generating the organism. There are also maternally inherited factors that are synthesised and stored during the maturation of the oocyte, which play an essential role in regulating early development. Finally, there is epigenetic information, which defines the maternal origin of the oocyte genome in mammals, which is distinct from the paternal epigenetic information contributed by the sperm.

Since the oocyte seems to contain the necessary information for development, there have been many studies to determine the development potential of this cell in the absence of fertilisation. Such development is referred to as parthenogenesis. Richard Owen first used this term in 1849 to describe 'procreation without the immediate influence of a male'. His work on frogs showed that artificially activated eggs in the absence of any paternal contribution could develop into tadpoles and adults although at a low frequency. Mouse eggs can also be similarly activated artificially, for example, by simply incubating them in medium containing alcohol. Such parthenogenetic mouse eggs can develop at a high frequency to the preimplantation blastocyst stage consisting of about 40-60 cells, a stage from which pluripotent ES cells are normally derived. If transplanted into recipient mothers, parthenogenetic embryos can develop to an advanced 25-somite stage but they never reach adulthood (Kaufman *et al*., 1977). However when parthenogenetic embryos are combined with normal embryos to make chimeras, parthenogenetic cells can contribute to many tissues in adult animals (Surani *et al.*, 1977). It was intriguing why parthenogenetic embryos failed to develop to term. Further investigations into this question led to the discovery of the phenomenon of genomic imprinting, suggesting that parental genomes are functionally non-equivalent and therefore both are required for development to term (McGrath and Solter, 1984; Surani and Barton, 1983; Surani *et al*., 1984).

Genomic Imprinting

Using micromanipulation techniques, it is possible to make zygotes consisting of two maternal genomes called gynogenones (which are similar

to parthenogenones), and zygotes, which contain two paternal genomes called androgenones. Neither of these two types of zygotes can develop to term. More importantly, at the most advanced stage of their respective postimplantation development at midgestation, they exhibit distinct and opposite characteristics. The former has better embryos and the latter, better development of placental tissues. This observation suggested that the parental genomes are functionally non-equivalent despite the fact that they have equivalent genetic information (Saitou *et al*., 2002; Surani *et al*., 1984). The term genomic imprinting in this context is used to describe the functional differences between parental genomes during mammalian development, and both are crucial for development to term. Much work has been carried out to look at the mechanism of genomic imprinting. From these studies it emerges that there are a number of genes whose expression is strictly dependent on their parental origin. Some are expressed only when inherited from the father and others are expressed only when inherited from the mother (Saitou *et al*., 2002). A major growth factor, insulin like growth factor 2 (*Igf2*), for example, is only expressed when inherited from the father and therefore this gene is repressed in parthenogenetic embryos. Currently approximately 50-100 imprinted genes have been identified in the mouse and human genomes. The activity of these genes explains the necessity for both sets of parental genomes for full development in mice and probably in all mammals.

Since both parthenogenones (or gynogenones) and androgenones have the potential to develop to the blastocyst stage, it has been possible to derive pluripotent Es cells from them. While they have many of the attributes of normal pluripotent ES cells, they show differences in their potential to differentiate into a variety of somatic cell types. Parthenogenetic ES cells have a greater potential for differentiation into neuronal cells while androgenetic ES cells can differentiate more readily into mesodermal tissues such as skeletal muscle.

The Origin of the Germ Cell Lineage

Eggs and sperm are the products of the germ cell lineage. It is important to consider the origin of this lineage during development. Although the establishment of germ cell lineage is one of the fundamental necessities in all-living organisms, it is striking that the mechanism by which this is achieved is not conserved amongst different model organisms. Broadly speaking there are two major ways used to establish the germ cell lineage. The first mecha-

nism is referred to as preformistic, where only those cells that eventually form the founder germ cells inherit the germ cell determinants that are already present in the egg. The second mechanism involves a stem cell model where development first leads to the establishment of pluripotent stem cells. These cells then respond to signalling molecules to acquire germ cell competence, and eventually germ cell fate. In both cases however, the germ cell lineage is amongst the first to be established during development. A key requirement for germ cell specification is to have a mechanism that prevents germ cells from acquiring somatic cell fate. Thus repression of the somatic cell fate is a key event during germ cell specification, which involves epigenetic mechanism for repression of genes that confer somatic cell fate on neighbouring cells (Saitou *et al.*, 2002; Saitou *et al.*, 2003).

In mice, germ cell competence is induced in pluripotent proximal epiblast cells of the early postimplantation embryos (Lawson and Hage, 1994; McLaren, 2003). Signalling molecules, such as BMP4, are critical for conferring germ cell competence on pluripotent epiblast cells. Germ cells are specified from amongst these competent cells by mechanisms that remain to be fully elucidated. The neighbouring cells that share common ancestry acquire somatic cell fate. Germ cells continue to express some of the key genes associated with pluripotency, particularly *Oct4*. However, germ cells do not behave as pluripotent ES cells. Unlike ES cells, which can differentiate into all the different cell types when introduced into blastocysts, germ cells fail to respond in any way at all, and they eventually die without undergoing differentiation. It would be informative to know why germ cells are refractory to differentiation under these conditions.

Programming the Germ Cell Lineage

Following establishment of the germ cell lineage, there follows an extensive reprogramming of the genome (Hajkova *et al.*, 2002; Surani, 2001). This process involves a cycle erasure and re-initiation of epigenetic information, which may be essential for restoring totipotency to this lineage. Some aspects of the mechanism involved at this time may be important for understanding many aspects of how epigenetic information can be removed and re-introduced, which affects the properties of cells. Such a mechanism presumably also operates during differentiation of diverse cell types from pluripotent stem cells. Germ cells also subsequently inherit epigenetic information concerning their parental origin. Finally, during oocyte development, there is inheritance of maternal factors that are synthesised

and stored in a fully mature oocyte. These factors play a critical role during early development. Amongst them must also be molecules that have the potential to restore totipotency to a somatic nucleus when transplanted into the oocyte. Since fusion between somatic cells and stem cells also results in the restoration of pluripotency in the somatic nucleus of hybrid cells, it follows that stem cells must also contain key factors that are capable of genomic reprogramming (Surani, 2001).

Germ Cells and Pluripotent Stem Cells

There are two aspects of particular interest when considering the relationship between germ cells and pluripotent stem cells. The first is the observation that germ cells under specific conditions in culture can change and acquire properties similar to pluripotent ES cells. These cells generated from primordial germ cells are referred to as embryonic germ cells (EG). Pluripotent ES and EG cells are very similar but they are not identical (Surani, 2001). Nevertheless, EG cells can differentiate into all the different cell types as seen with ES cells. The mechanism, which leads to the development of pluripotent stem cells from the highly specialised germ cells, the precursors of sperm and eggs, is unknown but an understanding of this process would add to knowledge concerning the pluripotent state.

It is also known that pluripotent stem cells express certain key genes to maintain this state. Apart from *Oct4,* there is *Nanog,* which is essential for the retention of pluripotency. It is interesting to note that the gene *Nanog* is closely linked in both mouse and humans genomes to a gene called *stella* that is expressed specifically in founder germ cells in mice (Payer *et al*., 2003). It will be informative to explore the reasons for this close linkage between these genes that show expression in pluripotent stem cells and germ cells.

From Pluripotent Stem Cells to Primordial Germ Cells, Sperm and Oocytes

Recent work has shown that it is possible to derive primordial germ cells as well as sperm and eggs from pluripotent mouse ES cells (Geijsen *et al*., 2004; Hubner *et al*., 2003; Payer *et al*., 2003; Surani, 2004; Toyooka *et al*., 2003). Sperm cells generated from ES cells undergo meiosis (Geijsen *et al.*, 2004). When injected into the oocyte, these spermatogenic cells derived from ES cells apparently participated in development at least up to the blastocyst stage. In another set of experiments, it was found that

it is also possible to generate oocytes in culture from pluripotent ES cells. These oocytes were able to activate and develop spontaneously into blastocysts (Hubner *et al*., 2003). This is the first demonstration that ES cells can develop into early embryos via development of eggs.

While the generation of germ cells from stem cells is still a relatively unrefined process, further work may improve the efficiency of this process. Such system would be of great benefit if human pluripotent ES cells can be used to generate human germ cells. This would allow detailed studies of the human germ cell lineage, which is currently difficult if not impossible, and consequently, we know relatively little about the human germ cell lineage. If it becomes possible to generate human oocytes from the existing human ES cells, this would also allow detailed investigations on the properties of human eggs. In addition, such oocytes generated from ES cultures could be used to study genomic reprogramming following transplantation of somatic nuclei. Recent studies have shown that it is possible to generate pluripotent stem cells from somatic cells following transplantation of somatic nuclei into human oocytes. This procedure may be possible to carry out using human oocytes generated in culture from human ES cells. If so, it would allow generation of additional lines of human ES cells, thus providing a larger repertoire of these cells then currently available. Furthermore, human ES cells may also be generated by this procedure using somatic cells from patients. Following their differentiation into, for example, cardiac or neural cells, they can be used for transplantation to alleviate diseases. More importantly, it may also become possible to generate ES cells from various patients with complex diseases. These cells could provide a means to study how various disease states arise, which may serve to develop therapeutic agents to alleviate many human diseases (Surani, 2004).

Conclusion

An investigation of the germ cell lineage is important for variety of reasons. Firstly, these studies are informative concerning the pluripotent state and therefore they will help to increase our knowledge of pluripotent stem cells. Germ cell lineage has many unique properties including extensive epigenetic reprogramming of the genome. A greater understanding of the underlying mechanism may allow development of methods to restore pluripotency to somatic cells without the use of oocytes. Alternatively, it may become possible to generate human germ cells from the existing human ES cells. This will have a significant impact on our understanding

of the human germ cell lineage. It may also pave the way towards development of human oocytes from human ES cells. Recent studies showed that it is possible to derive human ES cells from somatic cells following somatic nuclear transplantation into human oocytes (Hwang *et al.*, 2004). If oocytes generated from ES cells could be used for this purpose, it would obviate the need for donor oocytes from patients. More importantly, such oocytes would facilitate generation of a larger repertoire of human pluripotent ES cells through somatic nuclear transplantation. These cells would have the potential for use in cell therapy to alleviate various diseases. And they could also be used to study the mechanisms underlying complex human diseases, and for the development of therapeutic agents to combat such diseases. The latter could turn out be the most significant use of advances in stem cell research in human medicine (Surani, 2004).

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ADULT STEM CELLS: PROSPECTS FOR REPROGRAMMING BONE MARROW DERIVATIVES IN STABLE HETEROKARYONS

HELEN M. BLAU

Introduction

Stem cells have stimulated tremendous excitement because they provide hope that cellular therapy can aid in the treatment of diseases that have been refractory to other treatments. There are a range of stem cell types (for review see Chapter by Prof. Le Douarin in this volume or Blau, Brazelton *et al.*, 2001). The ultimate goal for each type is the same: to induce the nucleus of the cell to perform functions needed to maintain, replace, or rescue a particular tissue. Thus, the essence of stem cell-mediated therapy is nuclear reprogramming, which entails the induction of gene expression patterns unique to cell types in diverse tissues and organs. Consequently all stem cell types should be studied in parallel, as one may be better suited to the treatment of a given disease than another and all will yield findings fundamental to nuclear programming, critical to using any stem cell to replace or rescue damaged tissues.

Research in my laboratory and in that of others working on adult stem cells has focused on nuclear reprogramming in relation to adult bone-marrow-derived cells (BMDC), and in particular, the well characterized hematopoietic stem cells (HSC) and their derivatives. There is now ample evidence that these cells contribute naturally to adult tissues during life and that damage (traumatic or genetic) enhances that contribution. It therefore behooves us to better understand the mechanisms by which this contribution is achieved in the hope that it can be amplified and enlisted therapeutically. Adult cells (HSC and their derivatives) are advantageous in that they are not subjected to growth in tissue culture and they derive from the patient, thereby overcoming potential immunological obstacles. Ultimately

the goal of adult stem cell research is to enlist the hematopoietic stem cell derivatives of an individual to treat his or her own disease by delivering the appropriate chemical factors.

The finding that BMDC, and even HSC derivatives, can contribute to non-hematopoietic tissues that suffer from injury or are genetically defective has drawn worldwide attention, as it may have significant therapeutic applications for tissue repair due to trauma or disease. However, major challenges must be overcome before these cells can be considered as a potential therapy for non-hematopoietic tissues such as brain, skeletal muscle, and liver. The efficiency with which they contribute to tissues must be substantially augmented and their efficacy in treating disease must be clearly demonstrated in several animal models of human disease. To achieve these goals, the relevant HSC derivative must be identified and the cellular and molecular mechanisms leading to the contribution of adult bone marrow derived cells to specific tissues *in vivo* must be elucidated.

Two mechanisms have been reported. The bone marrow derived cells may fuse and then be reprogrammed in response to *intra*cellular cytoplasmic factors, e.g., as stable binucleate heterokaryons. Alternatively, reprogramming may occur in response to *extra*cellular signals in the microenvironment. Finally, the two mechanisms may act in combination in a given tissue. Regardless of which of these two mechanisms is used, reprogramming of the BMDC nucleus is of central importance. It has only recently been recognized that the contribution of BMDC constitutes a natural repair mechanism in adult muscle. Enhancement of such an existing mechanism may have a major impact on regenerative medicine.

In this article, I speculate that the different mechanisms for reprogramming nuclei described above depend on their inherent mitotic activity and tissue context. For example, a tissue such as epidermis, intestine or skeletal muscle that has a relatively simple morphology and is undergoing constant renewal through cell division (Janes, Lowell *et al.* 2002; Alonso and Fuchs 2003) might be expected to be replenished by the provision of cells with the capacity to proliferate and augment cell number. In response to environmental signals, tissues such as intestine and skeletal muscle are known to generate new cells throughout life and one source could be BMDC. By contrast, a highly elaborate cell such as a Purkinje neuron, which has more than one million synaptic connections to other cells and is not known to divide or be made anew in adulthood, would be hard to replace. In this case, 'rescue' by cell fusion and subsequent reprogramming of an incorporated nucleus may constitute a more plausible scenario than *de novo* cell production.

The growing excitement regarding the potential of BMDC and hematopoietic stem cells (HSC) to contribute to adult tissues has also stimulated growing controversy and attention and questions regarding its potential impact on normal life. As described in this article, although the quality of recent published reports varies, there is sufficient evidence to support the existence of bone marrow derived cells that can assume new functions in adulthood. Gene expression patterns can change, differentiation can be manipulated, and cells can incorporate into tissues from which they did not originate. The current state of the field is described. That BMDC contribute to tissues in adults now seems indisputable. Whether this phenomenon can be readily augmented will be determined by future research. The ultimate goal is to employ endogenous cellular components (directly or after brief *ex vivo* manipulation by genetic engineering) to repair the body using a combination of cell and gene therapy.

Finally, I would like to propose that the definition of a stem cell may benefit from revision and expansion based on the recent unexpected findings described in this report. The most well described stem cell, the hematopoietic stem cell (HSC), is a cell source of the donor nucleus that is reprogrammed to participate in foreign tissues, irrespective of mechanism. Whether an HSC derivative changes its gene expression program in response to the external microenvironment or in response to the internal milieu following cell fusion, the outcome is the same: nuclear reprogramming. If the nucleus derived from an HSC derivative is capable of self-renewal and has the potential to alter its genetic program in accordance with the diverse differentiated states typical of the tissue in which it resides, it has the attributes of a multipotential cell. These are the criteria for defining a cell as a stem cell as described in the introduction of this Symposium by Prof. Le Douarin. According to current definitions, whether the nuclear gene expression program changes as a result of exposure to different signals (extracellular vs. intracellular) does not dictate whether the cell harboring that nucleus is a stem cell. Since specific fusion of hematopoietic stem cell derivatives to other cells was never previously envisioned as a potential property of these cells, this novel feature presumably should not prohibit a cell from being designated as a stem cell. Specifically, if an HSC, the quintessential 'tissue specific stem cell' can give rise to progeny that naturally become reprogrammed to participate in the function of tissues other than blood such as skeletal muscle and Purkinje cells, then the definition of a stem cell may need to be changed. Taken together, these considerations suggest that stem cells may be better defined not as an entity but rather based on function (Blau, Brazelton *et al.* 2001).

Here, I report examples from my laboratory and that of others showing that BMDC and HSC contribute progeny to diverse tissues in adulthood. A historical perspective on mammalian cell fusion provides a context for these new findings. The goal of this report is to stimulate further investigation of adult stem cells, the mechanisms responsible for nuclear reprogramming and their potential therapeutic application.

Mammalian Cells Fused in Heterokaryons in Vitro

In the 1980s it was still considered highly unlikely that specialized mammalian cells could be altered. Despite the ground-breaking experiments on nuclear transplantation into oocytes (cloning) in amphibians (Briggs 1952; Briggs 1959; Gurdon 1962; Gurdon and Uehlinger 1966; Di Berardino and King 1967), it was generally thought that once a cell had assumed a 'terminally differentiated' state in liver or skin, for example, that state was irreversible. To test whether this theory held true, experiments were performed in culture dishes in which cells were fused to one another with polyethylene glycol. In the first studies using synkaryons (cell hybrids that exhibit cell division and nuclear fusion), gene repression was the norm and only transient gene activation was observed. Interpretation of results was complicated by the multiple rounds of cell division that occurred, resulting in chromosome reduction and rearrangement (Ephrussi, Davidson *et al.* 1969; Harris, Wiener *et al.* 1971; Davidson 1974). However, some results of major importance were obtained with synkaryons: the transformed state was found to be recessive to normal and activation of genes by trans-acting factors was first shown. Nonetheless, such unstable fusion products made it difficult to determine whether the gene of interest or its regulator was lost or to derive information regarding the underlying mechanisms.

By contrast, in cell fusion products known as heterokaryons, each nucleus remained distinct and intact, as there was no cell division or accompanying chromosome loss (Fig. 1, see p. 140). Gene expression could therefore be monitored over time. The demonstration that specific genes were progressively activated or repressed following fusion of diverse cell types in heterokaryons provided evidence that differentiation in adult human cells is not irreversible, but instead, dynamic and continuously regulated by the stoichiometry of proteins present in the cells at any given time (Blau 1989; Blau and Baltimore 1991).

Again, a historical perspective is useful. In early work with heterokaryons, Ringertz and coworkers showed that fusion of rat myoblasts

and chick erythrocytes caused the nuclei of the red blood cells to swell and chromatin to become diffuse, a finding that was in agreement with the initial steps known to be involved in nuclear reprogramming (Ringertz 1976). Later, my laboratory showed that nuclear reprogramming could occur in heterokaryons. A means of inhibiting cell division to prevent aneuploidy and the use of cells of two different species that were not transformed were critical to the success of these experiments. When mouse muscle cells were fused in tissue culture with human primary diploid cells derived from all three embryonic lineages, endoderm (hepatocytes), ectoderm (keratinocytes) and mesoderm (fibroblasts), strikingly, nuclear reprogramming was observed in each of these cell types. In each case, human muscle gene expression was detected, providing evidence that the differentiated state could be altered in highly diverse specialized cells derived from human adults. More than ten previously silent muscle genes were activated; organelles, such as the Golgi, were redistributed; and certain cell surface proteins were shown to be localized in nuclear domains for the first time (Blau, Chiu *et al.* 1983; Chiu and Blau 1984; Blau, Chiu *et al.* 1985; Chiu and Blau 1985). In the ensuing years, these results were confirmed by others for muscle, hepatocyte and globin gene activation following fusion with other cell types in stable heterokaryons. As a result, the plasticity of the differentiated state was generally accepted as a property of normal, non-transformed specialized mammalian cell types (Wright 1984; Wright 1984; Baron and Maniatis 1986; Spear and Tilghman 1990). These *in vitro* heterokaryon studies showed that activation of previously silent genes could be achieved, resulted from the balance of cytoplasmic factors present in somatic cells at any given time, and did not require passage through the oocyte or embryogenesis.

BMDC and HSC Contribution to Non-Hematopoietic Tissues

In the past few years a number of investigators have focused on BMDC in adults as a source of cells with potential for tissue regeneration, because they have access to all tissues of the body via the circulation. When the first remarkable report appeared demonstrating that bone marrow cells could contribute to muscle in mice (Ferrari, Cusella-De Angelis *et al.* 1998), it remained to be determined whether this was a rare and sporadic event or of fundamental physiologic significance. In these experiments, following transplantation of lethally irradiated mice with bone marrow from a transgenic mouse that expressed a myosin light chain

enhancer driving beta galactosidase, a few muscle fibers that were blue due to reporter gene expression were detected. There followed a number of reports of similar findings, using marrow cells genetically marked with b-galactosidase, Y-chromosome or green fluorescent protein (GFP). Such cells were reported to be present in the brain, the liver, heart, skeletal muscle, and epithelia of the kidney, lung and skin (Gussoni, Soneoka *et al.* 1999; Jackson, Mi *et al.* 1999; Petersen, Bowen *et al.* 1999; Brazelton, Rossi *et al.* 2000; Lagasse, Connors *et al.* 2000; Mezey, Chandross *et al.* 2000; Krause, Theise *et al.* 2001; Priller, Persons *et al.* 2001; LaBarge and Blau 2002; Wang, Montini *et al.* 2002; Camargo, Green *et al.* 2003; Corbel, Lee *et al.* 2003; Ianus, Holz *et al.* 2003; Kale, Karihaloo *et al.* 2003; Vassilopoulos, Wang *et al.* 2003; Weimann, Charlton *et al.* 2003) (Fig. 2, see p. 141). Although the incidence was generally low (0.01 to 0.1% of total cells) and the results were not always definitive due to differences in the experimental techniques employed and lack of replication, these studies provided the impetus for further investigation.

Of particular interest was the finding that the 'rare spontaneous' contribution of BMDC to non-hematopoietic tissues could be significantly increased in response to tissue stress or damage. Injection of toxins, exercise on a running wheel, or the use of an otherwise underutilized muscle caused a significantly increased contribution of BMDC to tissues by at least 20 fold, or a 4-5% of the total fiber within the tibialis exterior muscle. (LaBarge and Blau 2002; Camargo, Green *et al.* 2003; Corbel, Lee *et al.* 2003).

BMDC Contribution to Skeletal Muscle and Purkinje Neurons in the Brain

Experiments regarding the basis for BMDC incorporation into non-hematopoietic tissues are now well underway. There are two major questions of interest: (1) Which cell type in bone marrow is responsible? (2) By what mechanism does this cell type contribute to diverse tissues? Although clearly other excellent examples exist (see above), here I focus on two that have been extensively studied by my laboratory and are illustrative: skeletal muscle and Purkinje neurons in brain.

To understand and enhance the above findings, it is critical to identify the cell type with the capacity for contributing to these specialized cell types. Single cell transplants have now shown definitively that one very well characterized cell type within the bone marrow can be implicated. This cell is the hematopoietic stem cell (HSC), with characteristic mark-

ers ckit+, sca-1+, lin- (Spangrude, Heimfeld *et al.* 1988; Smith, Gasparetto *et al.* 1991; Uchida and Weissman 1992; Morrison and Weissman 1994; Osawa, Hanada *et al.* 1996). This cell, the HSC, is the quintessential tissue-specific stem cell capable of reconstituting all lineages of the blood in lethally irradiated mice. Definitive evidence that this cell or its derivatives could contribute to other tissues was provided when we and others showed that a *single* HSC yielded GFP-expressing cells that not only replenished the blood, but also were incorporated into mouse muscle fibers (Camargo, Green *et al.* 2003; Corbel, Lee *et al.* 2003). (Fig. 3, see p. 142). Key for eliciting contribution of such bone marrow-derived cells to muscle was injury, either caused by local toxin injection (Camargo, Green *et al.* 2003; Corbel, Lee *et al.* 2003) or caused by exercise (LaBarge and Blau 2002) (Figs. 4-6, see pp. 143-145). It had been postulated that the bone marrow contained determined precursors to cells such as neurons of the brain and skeletal muscle cells (Anderson, Gage *et al.* 2001; Korbling and Estrov 2003). Although this may still be true, these findings suggested that HSCs could give rise not only to all of the cells of the blood, but also cross lineages to contribute to skeletal muscle, and neurons in the brain.

Bone marrow contribution to non-hematopoietic tissues also occurs in the cerebellum of the brain (Priller, Persons *et al.* 2001; Wagers, Sherwood *et al.* 2002; Alvarez-Dolado, Pardal *et al.* 2003; Weimann, Charlton *et al.* 2003; Weimann, Johansson *et al.* 2003) (Fig. 7, see p. 146). In both humans and mice, BMT-derived nuclei are present in Purkinje neurons. In mice, a low frequency is observed that increases over time after BMT. Essentially all GFP-expressing Purkinje neurons exist as binucleate heterokaryons (Alvarez-Dolado, Pardal *et al.* 2003; Weimann, Johansson *et al.* 2003) (Fig. 8, see p. 147). The use of transgenic mice harboring the Purkinje specific promoter, L7, first showed that BMDC nuclei that are present in heterokaryons are reprogrammed: their chromatin is dispersed and L7 promoter expression is induced (Weimann, Johansson *et al.* 2003) (Fig. 9, see p. 148). After transplantation of male bone marrow, Y chromosomes were detected in the female Purkinje cells of both mouse and human brains (Fig. 10, see p. 149). To our knowledge, these examples constitute the first evidence of reprogramming of gene expression in stable binucleate *heterokaryons* existing *in vivo* in mammals. Based on these results, we hypothesize that the tissue culture phenomenon (changes in gene expression commensurate with nuclear reprogramming in heterokaryons) which we observed decades ago (1983), constitutes a mechanism by which complex, post-mitotic cells can receive aid from endogenous cells throughout life.

Reprogramming In Vivo

Two different mechanisms have been proposed whereby BMDC contribute to non-hematopoietic tissues. (1) Reprogramming via extracelllar signaling and (2) reprogramming via fusion and cytoplasmic mixing. Either mechanism ultimately involves nuclear reprogramming. Initial reports of 'plasticity' in adult stem cells suggested the first mechanism, i.e., that a developmentally immature BMDC could alter its typical course of differentiation to that of non-hematopoietic tissues (Anderson, Gage *et al.* 2001; Blau, Brazelton *et al.* 2001; Raff 2003). Therefore, BMDC could function as stem cells for other tissues. This first mechanism implies a response to extracellular signals that are detected by the BMDC and result in an alteration of gene expression and differentiation along an alternative developmental pathway. Examples that have been reported include bone marrow derived kidney epithelium (Krause, Theise *et al.* 2001), pulmonary epithelium (Krause, Theise *et al.* 2001), pancreatic islet cells (Ianus, Holz *et al.* 2003) and muscle satellite cells (LaBarge and Blau 2002) (Fig. 4, see p. 143).

As evidence for a second mechanism, fusion was detected in humans and mice leading to reprogramming by the cytoplasm, as previously seen with *in vitro* heterokaryon formation. Initial reports of contribution of BMDC to non-hematopoietic tissues by fusion described the phenomenon as 'merely fusion' (Blau 2002) or 'random' (Camargo, Green *et al.* 2003). However, time has granted a new respect for fusion. Indeed, fusion of BMDC with other tissues appears to be a specific process limited to particular donor and recipient cells, such as BDMC and Purkinje cells of the cerebellum (BMDC do not fuse with other cells within the cerebellum) and only binucleate, not mononucleate or trinucleate cells are detected. I propose that the observed fusion of BMDC with other cells may represent a way for cells from different parts of the body with diverse histories to contribute to one another. For example, one could envision a situation where a postmitotic cell in a complex tissue environment suffers a deleterious loss of function due to mutation or trauma and is rescued by expression of a reprogrammed, wild-type copy of the genome present in a donated nucleus.

Conclusion

Beginning with amphibian research, in the past five decades we have been endowed with a large fund of information and extraordinary technical achievements. We have learned that: (1) genetic material is not lost

during development and differentiation, (2) the cytoplasm of the oocyte as well as somatic cells have the ability to reprogram gene expression, (3) most genes can be reactivated, even in terminally differentiated cells, (4) a single somatic cell nucleus has the replicative capacity to yield sufficient progeny to produce the tissues necessary for a whole new organism, and (5) in life cells change their phenotypes, and in some cases, nuclear gene expression is reprogrammed across lineages. These findings indicate that theoretical roadblocks based on long held dogma that might have precluded the use of cells from one source to repair another have already been overcome.

Why should there be two mechanisms by which BMDC or HSC derivatives can contribute to adult tissues? In the case of tissues in which proliferation is ongoing, BDMC may contribute, like tissue specific stem cells (e.g. HSC or satellite muscle cells), via mitosis and subsequent specialization in response to extracellular signals. On the other hand, cells in tissues without proliferative capacity may not have the option for achieving tissue renewal. Thus, the concept of rescuing a defective cell, especially one that cannot and does not need to divide, may serve as an alternative means of tissue repair. Consequently, nuclear donation and subsequent reprogramming may constitute an elegant solution used by nature, and one that could be capitalized upon therapeutically.

The goal of controlling nuclear reprogramming *in vivo* will likely be made using numerous approaches. Investigation of reprogramming in nuclear transplantation is lending insight into the epigenetic changes that affect reprogramming (Jaenisch and Bird 2003). Indeed, chromatin remodeling enzymes and embryonic transcription factors are emerging as key players. Further understanding of the proteins responsible for regeneration in tissues in simpler organisms such as newts and zebrafish may provide insights. Characterization of fusion molecules or secreted factors that recruit and induce BMDCs may allow the efficiency of this form of tissue repair to be increased. Investigation of reprogramming in both BMDCs *in vivo* and *in vitro* in cultured heterokaryons using novel technologies will shed light on these important control mechanisms.

If the existence of technological hurdles and questions of degree or 'low frequency' had stopped researchers, then many significant discoveries would not have materialized. The study of *in vivo* reprogramming of BMDC warrants vigor, rigor and persistence. The future use of adult stem cells in regenerative medicine will rely on thorough investigation by numerous talented scientists.

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NUCLEAR CLONING, EMBRYONIC STEM CELLS AND THE PROMISE FOR TRANSPLANTATION THERAPY

RUDOLF JAENISCH

Summary

An emerging consensus is that somatic cell nuclear transfer (SCNT) for the purpose of creating a child (also called 'reproductive cloning') is not acceptable for both moral and scientific reasons. In contrast, SCNT with the goal of generating an embryonic stem cell line ('therapeutic cloning') remains a controversial issue. Although therapeutic cloning holds the promise of yielding new ways of treating a number of degenerative diseases, it is not acceptable to many because the derivation of an embryonic stem cell line from the cloned embryo (an essential step in this process) necessarily involves the loss of an embryo and hence the destruction of potential human life.

In this article, I will develop two main arguments that are based on the available scientific evidence. 1) In contrast to an embryo derived by *in vitro* fertilization (IVF), a cloned embryo has little if any potential to ever develop into a normal human being. This is because, by circumventing the normal processes of gametogenesis and fertilization, nuclear cloning prevents the proper reprogramming of the clone's genome, which is a prerequisite for development of an embryo to a normal individual. It is unlikely that these biological barriers to normal development can be solved in the foreseeable future. Therefore, from a biologist's point of view, the cloned human embryo, used for the derivation of an embryonic stem cell and the subsequent therapy of a needy patient, has *little if any potential* to create a normal human life. 2) Embryonic stem cells developed from a cloned embryo are functionally indistinguishable from those that have been generated from embryos derived by in vitro fertilization (IVF). Both types of embryonic stem cells have an *identical potential* to serve as a source for therapeutically useful cells.

It is crucial that the ongoing debate on the possible therapeutic application of SNCT is based on biological facts. The goal of this article is to provide such a basis and to contribute to a more rational discussion that is founded on scientific evidence rather than on misconceptions or misrepresentations of the available scientific data.

It is important to distinguish between 'reproductive cloning' and 'nuclear transplantation therapy' (also referred to as 'SCNT' or 'therapeutic cloning'). In reproductive cloning a cloned embryo is generated by transfer of a somatic nucleus into an enucleated egg with the goal to create a cloned individual. In contrast, the purpose of nuclear transplantation therapy is to generate an embryonic stem cell line (referred to as 'ntES cells') that is 'tailored' to the needs of a patient who served as the nuclear donor. The ntES cells could be used as a source of functional cells that would be suitable for treating an underlying disease by transplantation.

There is now experience from cloning of seven different mammalian species that is relevant for three main questions of public interest: 1) Would cloned human embryo be 'normal'? 2) Could the problems currently seen with cloning be solved in the foreseeable future? 3) Would ES cells derived from a cloned human embryo be 'normal' and useful for cell therapy? The arguments summarized in this article are based upon a review prepared for the President's Bioethics Committee. Here I will only summarize the key arguments and the reader is referred for details and literature to (Jaenisch, 2003)

Most Cloned Animals Die or Are Born With Abnormalities

The majority of cloned mammals derived by nuclear transfer (NT) die during gestation, and those that survive to birth frequently display 'Large Offspring Syndrome', a neonatal phenotype characterized by respiratory and metabolic abnormalities and enlarged and dysfunctional placentas. In order for a donor nucleus to support development into a clone, it must be reprogrammed to a state compatible with embryonic development. The transferred nucleus must properly activate genes important for early embryonic development and also suppress differentiation-associated genes that had been transcribed in the original donor cell. Inadequate 'reprogramming' of the donor nucleus is most likely the principal reason for developmental failure of clones. Since few clones survive to birth, the question remains whether survivors are fully normal or merely the least affected animals carrying through to adulthood despite harboring subtle abnormalities that originate in faulty reprogramming but that are not severe enough to interfere with survival to birth or beyond.

Adult Cloned Animals: How Normal Are They?

The observation that apparently healthy adult cloned animals have been produced in seven mammalian species (albeit at low efficiency) is being used by some as a justification for attempting to clone humans. In fact, even those that survive to adulthood, such as Dolly, may succumb relatively early in adulthood because of numerous health problems. The available evidence indicates that most clones die soon after implantation. But even those that survive to birth an beyond are not 'normal'. A direct comparison of gene expression profiles of over 10,000 genes (of the 30,000 or so in the mammalian genome) showed that approximately 4% of the expressed genes in their placentas differed dramatically in expression levels from those in controls, and that the majority of abnormally expressed genes were common to both types of clones. When imprinted genes, a class of genes that express only one allele (either from maternal or paternal origin), were analyzed, between 30 and 50% were not correctly activated. These observations represent strong molecular evidence that cloned animals, even those that survive to birth, suffer from serious gene expression abnormalities.

Nevertheless, despite these widespread gene expression abnormalities, a small fraction of clones developed to seemingly normal adults. However, when cloned mice were aged, serious problems, not apparent at younger ages, became manifest leading to serious pathological alterations in multiple organs and premature death. Thus, severe abnormalities in cloned animals may often become manifest only when the animals age.

It is a key question in the public debate whether it is ever possible to produce a normal individual by nuclear cloning, even if only with low efficiency. The available evidence suggests that it may be difficult if not impossible to produce normal clones for the following reasons: 1) All analyzed clones at birth showed dysregulation of hundreds of genes. The development of clones to birth and beyond despite widespread epigenetic abnormalities suggests that mammalian development can tolerate dysregulation of many genes. 2) Some clones survive to adulthood by compensating for gene dysregulation. Though this 'compensation' assures *survival*, it may not prevent maladies to become manifest at later ages. Therefore, most if not all clones are expected to have at least subtle abnormalities that may not be so severe as to result in an obvious phenotype at birth but will cause serious problems later as seen in aged mice.

Is it Possible to Overcome the Problems Inherent in Reproductive Cloning?

It is often argued that the 'technical' problems in producing normal cloned mammals will be solved by scientific progress that will be made in the foreseeable future. The following considerations argue that this may not be so.

A principal biological barrier that prevents clones from being normal is the 'epigenetic' difference between the chromosomes inherited from mother and from father, i.e. the difference between the 'maternal' and the 'paternal' genome of an individual. Such methylation of specific DNA sequences is known to be responsible for shutting down the expression of nearby genes. Parent-specific methylation marks are responsible for the expression of imprinted genes and cause only one copy of an imprinted gene, derived either from sperm or egg, to be active while the other allele is inactive. For cloning to be made safe, the two parental genomes of a somatic donor cell would need to be physically separated and separately treated in an 'oocyte-appropriate' and a 'sperm-appropriate' way, respectively. At present, it seems that this is the only rational approach to guarantee the creation of the epigenetic differences that are normally established during gametogenesis. Such an approach is beyond our present abilities. These considerations imply that *serious biological barriers* exist that interfere with faithful reprogramming after nuclear transfer. It is a safe conclusion that these biological barriers represent a major stumbling block to efforts aimed at making nuclear cloning a safe reproductive procedure for the foreseeable future.

Therapeutic Applications of SCNT

In spite of the biological and ethical barriers associated with reproductive cloning, nuclear transfer technology has significant therapeutic potential that is within our grasp. There is an enormous distinction between the goals and the end product of these two technologies. The purpose of reproductive cloning is to generate a cloned embryo that is then implanted in the uterus of a female to give rise to a cloned individual. In contrast, the purpose of nuclear transplantation therapy is to generate an embryonic stem cell line that is derived from a patient (referred to as 'ntES cells') and can be used subsequently for tissue replacement.

In a 'proof of principle' experiment, nuclear cloning in combination with gene and cell therapy has been used to treat a mouse genetic disorder that has a human counterpart. To do so, the well-characterized *Rag2*

mutant mouse was used as 'patient'. This mutation causes *severe combined immune deficiency (SCID),* because the enzyme that catalyzes immune receptor rearrangements in lymphocytes is non-functional. Consequently, these mice are devoid of mature B and T cells, a disease resembling human *Omenn syndrome*. The successful treatment of the mutant mice indicates that, unlike the situation with reproductive cloning, no *biological* barriers exist that in principle prevent the use of SCNT to treat human diseases. The *technical* issues in using SCNT and human stem cells for therapeutic purposes need, however, to be solved, but there are no indications at present that these represent formidable problems that will resist relatively rapid solution.

SCNT for Cell Therapy: Destruction of Potential Human Life?

A key concern raised against the application of the nuclear transplantation technology for tissue therapy in humans is the argument that the procedure involves the destruction of potential human life. From a biological point of view, life begins with fertilization when the two gametes are combined to generate a new embryo that has a unique combination of genes and has a high potential to develop into a normal baby when implanted into the womb. A critical question for the public debate on SCNT is this one: is the cloned embryo equivalent to the fertilized embryo?

In cloning, the genetic contribution is derived from one individual and not from two. Obviously, the cloned embryo is the product of laboratory-assisted technology, not the product of a natural event. From a biological point of view, nuclear cloning does not constitute the creation of new life, rather the propagation of existing life because no meiosis, genetic exchange and conception are involved. Perhaps more important is, however, the overwhelming evidence obtained from the cloning of seven different mammalian species. As summarized above, the small fraction of cloned animals that survive beyond birth, even if they appear 'normal' upon superficial inspection, are likely not so. The important conclusion is that a cloned human embryo would have little if any potential to develop into a normal human being. With other words, the cloned human embryo lacks essential attributes that characterize the beginning of *normal* human life.

Taking into account the potency of fertilized and cloned embryos, the following scenarios regarding their possible fates can be envisaged. Fertilized embryos that are 'left over' from IVF have three potential fates: disposal, generation of normal embryonic stem cells or generation of a nor-

mal baby when implanted into the womb. Similarly, the cloned embryo has three potential fates: it can be destroyed or could be used to generate a normal ntES cell line that has the same potential for therapy as an ES cell derived from a fertilized embryo. In contrast to the fertilized embryo, the cloned embryo has little if any potential to ever generate a normal baby. An embryonic stem cell line derived by nuclear transfer may, however, help sustain existing life when used as a source for cell therapy that is 'tailored' to the need of the patient who served as its nuclear donor.

If SCNT were accepted as a valid therapeutic option, a major concern of its implementation as medical procedure would be the problem of how to obtain sufficient numbers of human eggs that could be used as recipients. Commercial interests may pressure women into an unwanted role as egg donors. The recent demonstration that embryonic stem cells can be coaxed into a differentiation pathway that yields oocyte-like cells may offer a solution to this dilemma. If indeed functional oocytes could be generated from a generic human ES cell line, sufficient eggs could be generated in culture and serve as recipients for nuclear transfer without the need of a human egg donor. It seems that technical issues, not fundamental biological barriers, need to be overcome so that transplantation therapy can be carried out without the use of human oocytes.

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VACCINATION OF CANCER PATIENTS WITH TUMOR ANTIGENS RECOGNIZED BY T LYMPHOCYTES

THIERRY BOON, BERNARD LETHÉ, CHRISTOPHE LURQUIN, PIERRE VAN DER BRUGGEN, FRANCIS BRASSEUR, PIERRE COULIE

It is now generally recognized that human tumors bear antigens that can be recognized by T lymphocytes of cancer patients. The approach that led to the identification of these antigens was based on in vitro stimulation of T lymphocytes of cancer patients with irradiated cells of the autologous tumor. This provided cytolytic T lymphocytes (CTL) clones that were capable of lysing with great specificity the autologous tumor cell line. Gene transfection experiments combined with recognition of the transfected cells by these CTL clones then led to the identification of the genes coding for the antigens.

When these approaches were applied to human melanoma, four classes of antigens were identified, according to the genetic or epigenetic processes that produced them (Figure 1). First, there were antigens encoded by cancer-germline genes. These genes are not expressed on normal adult cells except on male germline cells. They are expressed in a fraction of tumors of many histological types. As major histocompatibility complex molecules are not expressed on male germline cells, this implies that the antigens recognized by T lymphocytes are present only on tumor cells. These antigens are therefore common to many tumors and strictly tumor-specific. A second class of antigens was found to be encoded by genes that are expressed in normal cells but are overexpressed in a number of tumors. A third class results from point mutations found in a wide array of genes. Interestingly many of these mutations have clear oncogenic potential, such as one found in cyclin-dependent kinase 4 and another found in caspase 8. A fourth class was unexpected: many T lymphocytes of melanoma patients recognized melanocyte differentiation antigens, such as tyrosinase. One would have expected that natural tolerance would have eliminated these T

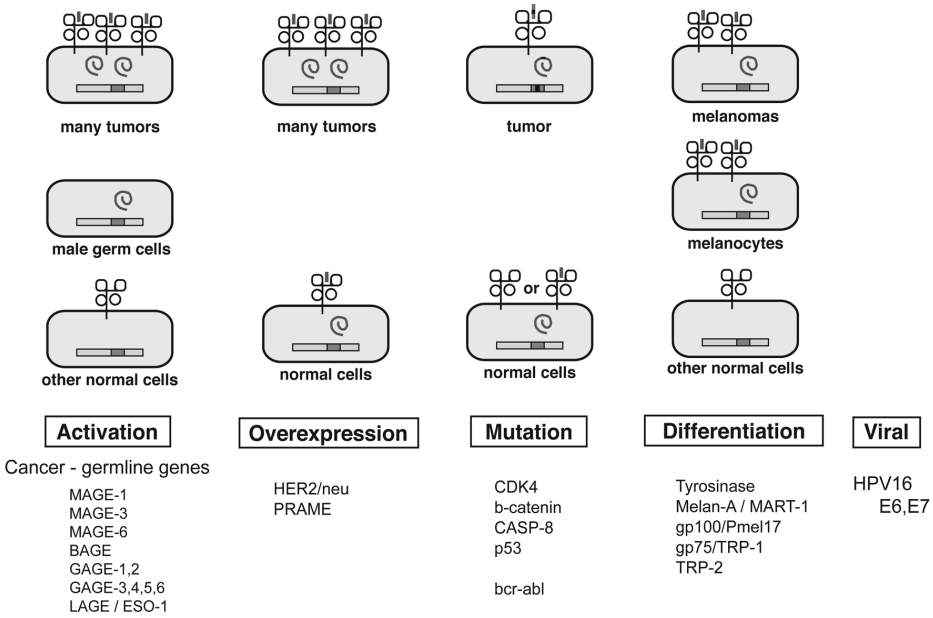

Figure 1. Main categories of tumor antigens.

lymphocytes. We believe that these four classes of antigens will also be found with other tumors. A fifth class should be mentioned: oncoviral antigens. For instance, uterine cervix cancer is caused by human papilloma virus (HPV16) and viral genes E6 and E7 code for antigens that are recognized by T cells.

The antigens encoded by cancer-germline genes ought to be good candidates for the therapeutic vaccination of cancer patients as they are strictly tumor-specific and present on many tumors, unlike the mutational antigens which are highly specific for each individual tumor. The first cancer-germline genes that were identified belong to the MAGE-A family, which comprises 12 genes. By now, about 10 families of cancer-germline genes have been identified. Most of these genes are located on the X chromosome. Some of these genes are expressed in many tumors (Table 1). For instance MAGE-3 is expressed in 74% of metastatic melanomas and in 47% of non-small cell lung cancer. These genes are activated in tumors as a result of the demethylation of their promoter.

PERCENTAGE OF TUMORS EXPRESSING GENES

	MAGE-1	MAGE-3
Melanoma	46	74
Esophagus	53	63
NSCLC	46	47
Head & Neck	31	51
Bladder	32	57
Advanced myeloma	32	32

Table 1.

Much of our effort on therapeutic vaccination of cancer patients has focused on gene MAGE-3. A large number of MAGE-3 antigens presented by class I and class II major histocompatibility complex molecules have been identified. A MAGE-3 encoded peptide presented by HLA-A1 (MAGE-3.A1 antigen) has been used in several clinical trials. A first trial involved three subcutaneous and intradermal injections of this peptide, in the absence of adjuvant. The patients were metastatic melanoma patients with detectable disease. Tumor regressions were observed in 7 out of the 26 patients who completed the trial. We then examined whether more frequent injections or the addition of adjuvant improved the results. No improvement was observed. Immunization with MAGE-3 protein mixed with an adjuvant did not produce a higher rate of tumor regression. Neither did vaccination with recombinant poxvirus ALVAC harboring a minigene coding for MAGE-3.A1.

After vaccinating a total of about 200 metastatic melanoma patients with detectable disease, our results can be summarized as follows. No significant toxicity has been observed, implying that this form of therapy

ought to be applicable to earlier stages of the disease and to patients with better prognosis. Tumor regressions are observed in about 20% of the patients, but only in 10% of the patients are these tumor regressions medically significant (complete or partial responses). When tumor regressions occur, they proceed slowly and in the absence of noticeable inflammation.

Originally, our notion was that high-level anti-vaccine responses, i.e. anti-MAGE-3.A1 CTL, would be necessary, but perhaps not sufficient, in order to cause tumor regressions. We were therefore very surprised to find little or no CTL responses in the blood of most patients who showed tumor regressions. Much of our present effort is aimed at the analysis of low-level CTL responses in vaccinated patients. For MAGE-3.A1, the level of CTL precursors found in the blood of non-cancerous individuals is 4.10^{-7} of CD8 T cells. The diversity of this anti-MAGE-3.A1 T cell repertoire is high: our observations lead to an estimate of at least 100 different T cell receptors (clonotypes). As humans have a total of 4.10^{10} CD8 T cells, this implies that each of the 100 clonotypes is present at a frequency of 4.10^{-9} and comprises about 160 T cells.

For the evaluation of the frequency of anti-MAGE-3.A1 CTL, we restimulate in vitro blood T lymphocytes with peptide tetramers for two weeks, in limiting dilution conditions. The microcultures are then assessed with HLA-peptide for the presence of positive cells. The positive cultures are cloned and the specific lytic ability of the clones on tumor cells expressing the MAGE-3.A1 antigen is evaluated. This approach enables us to detect responses with frequencies as low as 10^{-6} of CD8 T cells. This approach has been applied to a number of patients vaccinated with the MAGE-3.A1 peptide, with recombinant ALVAC virus carrying the MAGE-3.A1 minigenes. The anti-vaccine CTL responses that are identified are usually low: mostly between 10^{-6} and 10^{-5}, even though one response as high as 10^{-3} has been observed (an example is shown in Figure 2). Strikingly, these responses are monoclonal. This enables us to evaluate the responses directly in the blood with clonotypic PCR, with results that have been in good agreement with those obtained after restimulation in vitro. For the ALVAC trial, there is clearly a correlation between anti-MAGE-3.A1 CTL responses and tumor regressions. Out of 5 patients who showed tumor regressions, 4 showed a CTL response. Out of 14 patients who did not, only 2 showed anti-MAGE-3.A1 CTL and one of them already had elevated CTL before vaccination. This correlation suggests, but does not rigorously prove, that the occasional tumor regressions that are observed following vaccination, are caused by the vaccination.

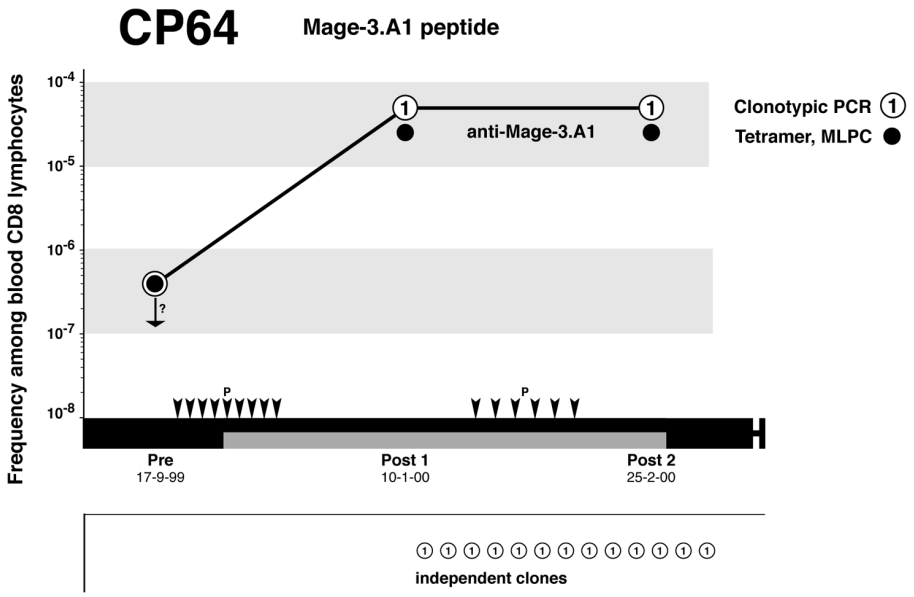

Figure 2. EXAMPLE OF ASSESSMENT OF FREQUENCY OF ANTI-VACCINE CTL CLONES.
The patient was vaccinated with the Mage-3.A1 peptide. The blood frequency of anti-Mage-3.A1 CTL is shown with a black dot. The response comprised a single CTL clonotype labelled 1. The frequency obtained by clonotypic PCR is shown by the symbol '1'. Peptide injections are indicated with symbols labelled 'P'. The patient showed a mixed tumor reponse.

A similar analysis has been carried out on patients vaccinated with autologous dendritic cells pulsed with peptide MAGE-3.A1. In contrast with the results obtained with peptide clone or with ALVAC, polyclonal responses were observed.

In most of the patients who show evidence of tumor regression following vaccination, the anti-vaccine cytolytic T cell (CTL) response is either undetectable or present at a low level, which might be deemed insufficient to produce tumor rejection. We therefore set out to examine whether T cells recognizing other tumor antigens might participate in the tumor regression process. As a first step, we estimated in 6 patients the blood frequencies of anti-tumor CTL, namely lytic effectors that recognized the autologous

melanoma cells but not autologous B cells nor NK target K562. After vaccination, frequencies of anti-tumor CTL in the blood ranged from 10^{-4} to 3×10^{-3} of the CD8 T cells, i.e. 10 to 10,000 times more than the anti-vaccine CTL in the same patient. Similar anti-tumor CTL frequencies were already present in the blood prior to vaccination. From a patient who had shown nearly complete tumor regression following vaccination, we derived 15 anti-tumor CTL clones. Ten CTL clones recognized antigens encoded by cancer-germline gene *MAGE-C2*, and 3 recognized antigens encoded by melanocyte differentiation gene *gp100*. These antigens were also recognized by CTL present at high frequency in the blood of this patient before vaccination. These results suggest that melanoma patients carry very high frequencies of anti-tumor CTL, which are directed against the main categories of tumor antigens defined previously.

As a second step in assessing the respective contribution of anti-vaccine and anti-tumor CTL to tumor regression, we investigated the presence of anti-tumor and anti-vaccine CTL inside metastases in the same patient who showed tumor regression after vaccination against antigen MAGE-3.A1. The frequency of anti-MAGE-3.A1 CTL was 2.5×10^{-6} of CD8 T cells in the blood and it was 6-fold higher in an invaded lymph node. An anti-tumor CTL recognizing an antigen encoded by MAGE-C2 showed a considerably higher enrichment. Whereas in the blood the frequency of this CTL was 9×10^{-5}, in the invaded lymph node it was about 400 times higher. Several other anti-tumor T cell clonotypes had frequencies of above 1% inside metastases. These results suggest that the anti-vaccine CTL may not be the principal effectors that kill the bulk of the tumor cells. They may exert their effect mainly by an interaction with the tumor, which creates conditions that enable the stimulation of large numbers of other anti-tumor CTL, which then proceed to destroy the tumor cells. Consistent with this model, we observed that new anti-tumor CTL clonotypes appeared following vaccination and were present in the tumor at a very high frequency.

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HEMATOPOIETIC *STEM CELL* GENE THERAPY

ALAIN FISCHER

To date the best evidence for the efficacy of stem cell therapy has been provided by the success of allogeneic hematopoietic stem cell transplantation to cure a number of diseases. The potential ability of hematopoietic stem cell (HSC) to give rise, life long to all blood cell lineages [1] is a rationale for the treatment of a number of genetically determined blood cell diseases, by relevant gene transfer into HSC. There are however a number of technical limitations, the major one being related to the resting stage of HSC. Indeed, retrovectors so far used in clinical practice to induce gene transfer into host genome, can only permit gene integration into dividing cells in which the nuclear membrane is temporally dissolved. Thus clinical applications will require to develop vectors such as lentiviral based vectors enabling gene transfer into non dividing cells and/or mean to induce HSC to cycle without loosing their self renewal capacity.

Gene Therapy of Severe Combined Immunodeficiencies (SCID)

At present, however, there is a favorable setting for which gene therapy has demonstrated its efficacy [2]. It is the treatment of severe combined immunodeficiencies (SCID). SCID are rare inherited conditions characterized by a complete block in T lymphocyte differentiation. Genetic defects leading to SCID conditions have been almost all identified, opening the possibility to perform relevant gene transfer in autologous progenitors. SCID conditions are lethal within the first year of life in the absence of treatment. Allogeneic HSCT can cure these conditions although, when the donor is not fully matched, risk of failures is significant and long-term decline in immune functions makes this therapeutic approach partially unsatisfactory. Within the SCID condition, SCID-X1 characterized by a block in both T and NK lymphocyte development is the most frequent. It is

caused by mutations of the gene encoding γc, a cytokine receptor subunit expressed by all hematopoietic-derived cells. γc role in T lymphocyte development is known, it enables IL-7 to provide survival and proliferation signals to T cell precursors. IL-15 is similarly acting on NK cell precursors. It was thus thought that γc expression triggered by γc gene transfer into a few deficient lymphocyte progenitors could give rise to a tremendous proliferation of T cell precursors giving rise to long-lived mature T cells. This is the concept of selective advantage conferred by the transgene expression on which gene therapy of SCID is based. Experimental data as well as consequences of spontaneous reverse mutation correcting the γc gene mutation provided further rationale for this approach [2].

Principle of gene therapy consists in ex vivo inserting the γc gene into bone marrow precursors cells by using a retroviral vector made unable to replicate. The γc gene is placed under the transcriptional control of the viral Long Terminal Repeat (LTR). Insertion of the provirus occurs in active regions of the genome. This method has been tested in vitro showing efficacy, including long lasting expression of γc in cell lines as well as in vivo in a murine model of the γc deficiency. The immunodeficiency has been corrected, efficacy is sustained and no adverse effects have been observed. Based on these preclinical data, gathered in a 6-year period of time, a clinical trial has been initiated in 1999. Between 1999 and 2002, 10 patients with SCID were treated likewise in Paris. In 9 out of 10, a sustained correction of the immunodeficiency has been achieved [3, 4]. T cell became detectable in the blood within 10 to 12 weeks, reaching normal cell counts, now being sustained for almost 5 years for the first treated patients. The γc transgene is expressed in all T and NK cells, as well as by a minority (1‰ to 1%) of B lymphocytes and myeloid cells. All characteristics of transduced T cells indicate a full correction of the immunodeficiency, including T cell receptor diversity, ongoing production of T cells in the thymus, in vitro and in vivo reactivities to antigens.

By analyzing the transgene integration sites, which represent a 'signature' of the transduced precursors, it was estimated that a few hundred clones gave rise to the whole T cell pool, emphasizing the selective advantage conferred to these transduced precursors. Each cell contains on average one copy of the provirus. Production of immunoglobulins has been also restored to an extent, which is strong enough to avoid immunoglobulin supplementation in 7 out of 9 patients. Similar findings have since been observed in 4 additional patients with SCID-X1 similarly treated by A. Thrasher's group in UK [5]. The analysis of the provirus integrations sites

in transduced myeloid cells as well as in marrow precursors cells provided evidence that progenitors able to differentiate in both lymphoid and myeloid cells were transduced. These data together with the sustained detection of both transduced lymphocytes and myeloid cells over time strongly suggest that very immature progenitors, possibly stem cells were targeted, rising hope for very long term efficacy. Thus, this therapy is bringing a clear benefit to the patients, enabling them to live a normal life and coping with infections. It seems, that albeit the low number of treated patients and the maximum 5 years-follow up, gene therapy is more efficient that HSCT, the alternative treatment. Efficacy of gene therapy has now be demonstrated for another form of SCID (Adenosine deaminase deficiency) in 4 patients [6]. It is now logical to try to extend this strategy to the treatment of other forms of T cell immunodeficiencies for which the selective advantage concept will apply. This altogether should include about 10 diseases including SCID and the Wiskott-Aldrich syndrome. Extension to other genetic defects of hematopoiesis will require the usage of vectors enabling to transduce a much higher number of HSC possibly combined with chemotherapy to reduce the competition with non-transduced HSC.

Safety Issue

The occurrence of a gene therapy related serious adverse event (SAE) in 2 patients is raising an important safety issue to be thoroughly considered in the perspective of gene therapy development. A T cell clonal proliferation occurred approximately 3 years after treatment [7]. These T cells have a mature phenotype and do express the γc protein, without overexpression. Cells became blastic in appearance at a time of clinical manifestations while secondary genomic alterations became detectable, (a t 6;13 chromosomal translocation in one, a trisomy 10 and a sil-tal rearrangement in the second). Both patients were successfully treated by chemotherapy. One then required allogeneic HSCT and is still under treatment. In both cases, uncontrolled clonal proliferation was primarily caused by an insertional mutagenesis event, i.e. integration of the provirus in the LMO-2 locus. Aberrant expression of LMO-2 has previously been described in association with a rare form of T-cell acute lymphoblaste leukemia while forced expression of LMO-2 in mice is also causing leukemogenesis, albeit after a long delay (close to one year) [8]. Insertions occurred in one in the first intron of the LMO-2 locus, in reverse orientation while in the second, insertion is placed close to the hematopoietic promotor of LMO-2. In both cases, the viral LTR likely exerts

an enhancer activity on LMO-2 giving rise to aberrant continuous expression of LMO-2. That in both instances the LMO-2 locus was hit cannot be regarded as a random event. Either the LMO-2 locus is a physical hot spot for provirus integration, an hypothesis which is unlikely, given the preliminary results of the identification of the different provirus integration sites found in the patients, or among many integration sites, those occurring in the LMO-2 are selected because of their functional consequences. Since these SAE have not been observed in other clinical settings, nor in experimental conditions with a single exception [9], it is possible that functional additive or synergistic effects with the γc transgene expression plays an important role as also suggested in a murine model of leukemia [10]. This is difficult to assert, as the precise molecular function of LMO-2 is still unknown today. Another puzzling observation consists in the fact that the SAE occurred in the 2 youngest treated patients, and not in those aged 4 months or older. The likelihood that this occurs randomly is only 1.2%. It is thus very likely that age is a significant contributing factor possibly because characteristics of hematopoiesis at birth differ with a higher rate of proliferative cells and distinct pattern of gene expression [11]. These circumstances might favor integration into and selection of LMO-2-expressing clones. This hypothesis is being tested in an animal model.

These considerations are important because, if correct, they mean that the serious safety issue is limited to gene therapy of patients with SCID-X1, for the minority of patients under the age of 4 months. This would permit to continue to use this therapeutic strategy for patients above that age. In addition, in the future, vectors may be designed to increase safety, but not at the expense of efficacy. Three tracks are being tested: to use self inactivated LTR, as it is the case with designed HIV-derived lentiviral vectors, to add insulators with the hope to limit the enhancer activity to the provirus and avoid its effect on surrounding genes, and finally to add a suicide gene to be activated by a drug if a clone is escaping control.

Altogether, a careful appraisal of the benefit/risk ratio comparing the chance of alternative therapy, HSCT versus gene therapy has to be performed individually for each patient.

The Future

Further extension of stem cell gene therapy, beyond this present state, i.e. demonstration that it can work, will require further technological advances. As mentioned above, lentiviral vectors derived from HIV can pro-

vide an efficient tool to transduce HSC. Experimental results achieved in the correction of hemoglobin disorders in mice are encouraging [12, 13]. In order to reduce the risk of insertional mutagenesis, targeted integration will be a possible solution.

One strategy, which is presently tested, consists of using a bacterial integrase (Ψ C31) able to induce integration at rare positions in the mammalian genome. Some efficacy has been shown in targeting liver cells [14]. Another line of development consists of inducing homologous recombination to repair mutated genes. In order to increase the frequency of such events, a strategy based on the creation of DNA double strand break at mutation sites by a tailored integrase is being tested [15]. But a long way to go is needed to achieve high enough specific breaks without non-specific integration.

In conclusion, stem-cell gene therapy has been shown to be potentially effective. Its development requires both a careful pathophysiological analysis of diseases to be treated as well as development of technologies to improve gene transfer efficacy, and this in a safe way. HSC are very good target cells to treat a number of diseases. On the long term, newly generated stem cells following somatic nuclear cell transfer might be provide an optimal tool for disease correction [16, 17].

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TABLES

Figure 1. *Miracle of Saint Côme and Saint Damien*. Fernando del Rincon (1450-1517), Museum of Prado, Madrid.

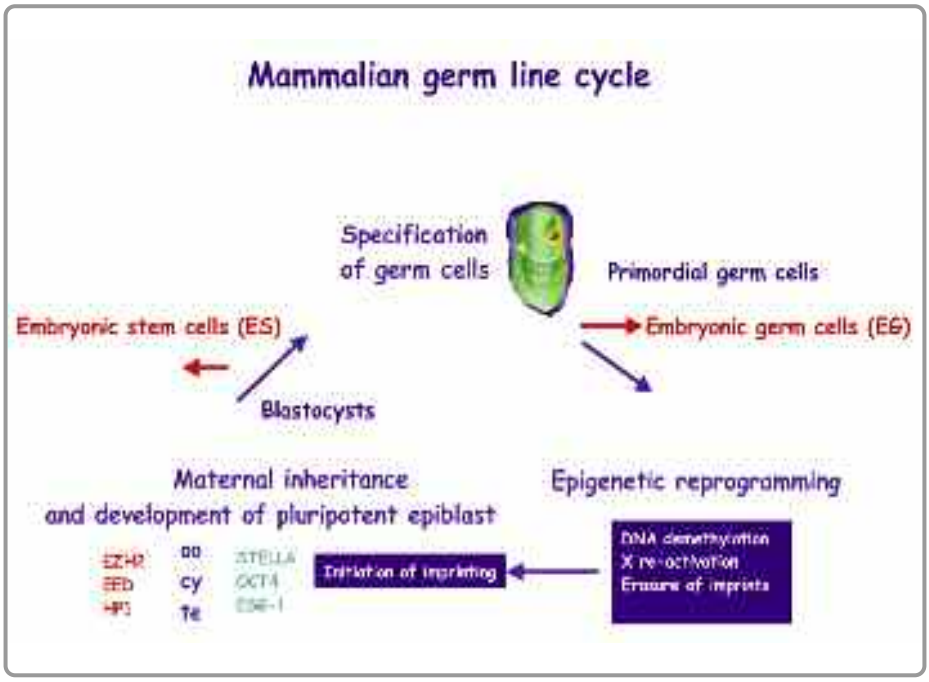
Mammalian germ line cycle
Specification of germ cells
Primordial germ cells
Embryonic stem cells (ES)
Embryonic germ cells (EG)
Blastocysts
Maternal inheritance and development of pluripotent epiblast
Epigenetic reprogramming
EZH2
EED
HP1
oo
cy
te
STELLA
OCT4
Initiation of imprinting
DNA demethylation
X re-activation
Erasure of imprints

Figure 1.

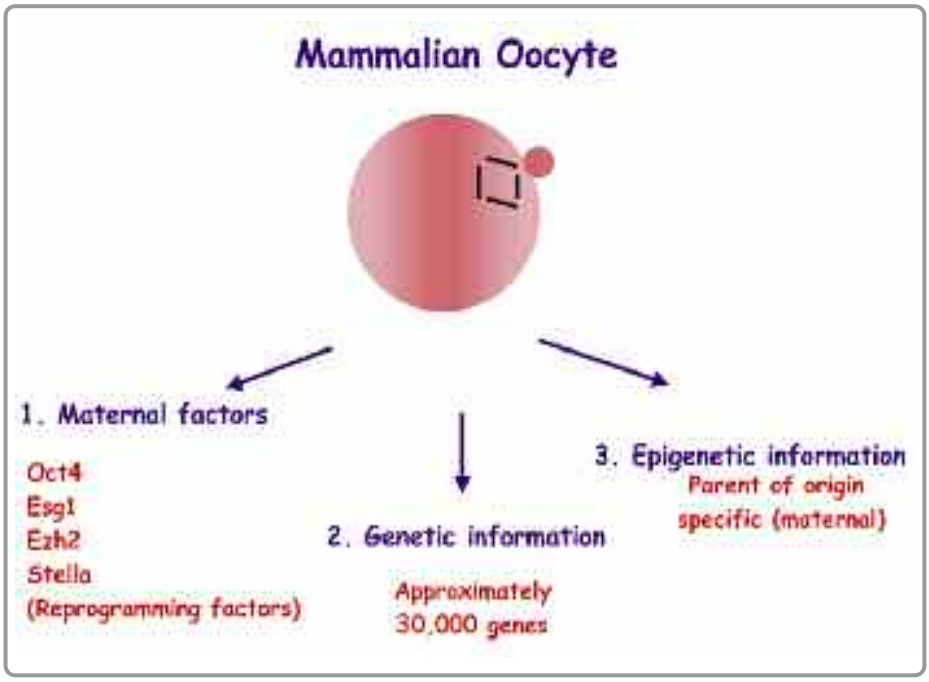
Mammalian Oocyte
1. Maternal factors
Oct4
Esg1
Ezh2
Stella
(Reprogramming factors)
2. Genetic information
Approximately
30,000 genes
3. Epigenetic information
Parent of origin
specific (maternal)

Figure 2.

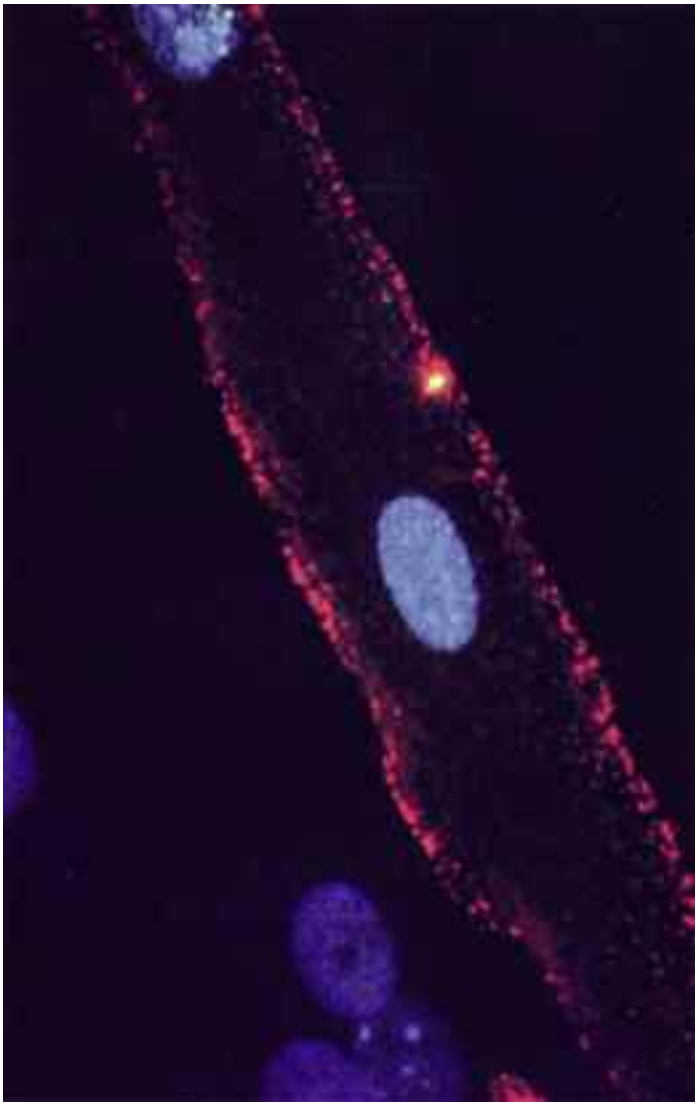

Figure 1. *Expression of a human muscle gene by a human hepatocyte*. nucleus in a heterokaryon formed in tissue culture. Mouse muscle cells (blue punctate nuclei) and human nonmuscle cells (blue uniformly stained nuclei) are fused to form heterokaryons. Upon exposure to muscle cytoplasm, cells specialized for different tissues can be induced to express gene products characteristic of muscle (red immunofluorescence). From Blau *et al.*, *Science*, 230, pp. 758-766 (1985).

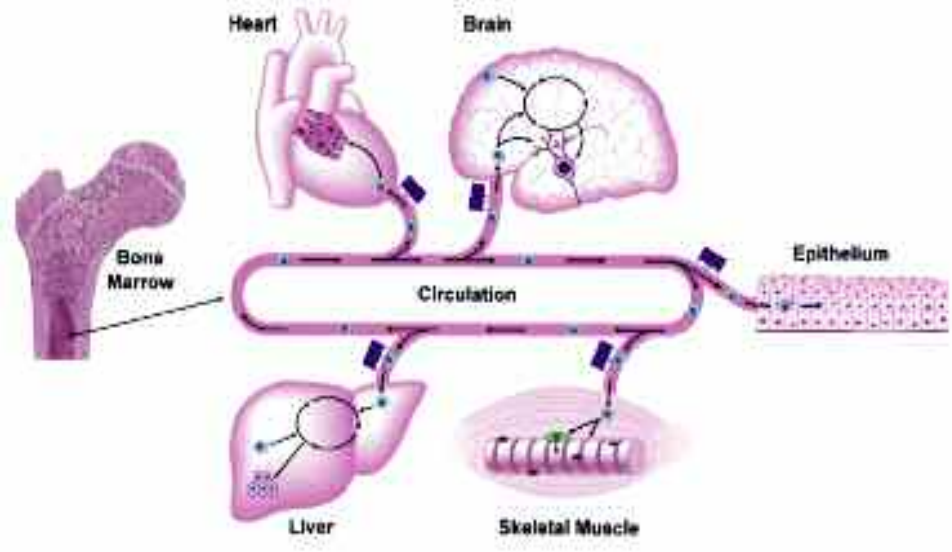

Figure 2. *Evolving concepts of stem cell plasticity*. Reported transitions in stem cell identity and differentiation are illustrated. In addition to localized tissue-specific stem cells, some stem cells may travel throughout the body via the circulation. The scheme also suggests that cell fate decisions may not be irreversible. Flexibility is the hallmark of this depiction allowing for regeneration and changes in cell fate in response to need.

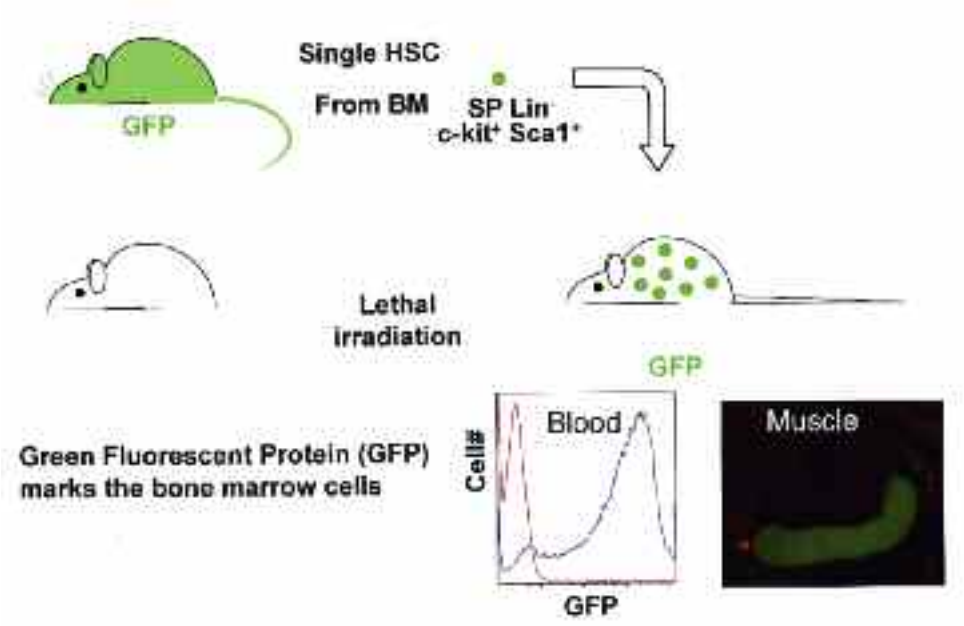

Figure 3. *General strategy for identifying cell fate transitions using bone-marrow derived cells.* Bone marrow cells from a genetically marked adult mouse are delivered intravascularly into isogeneic, lethally irradiated, normal adult hosts. The bone marrow can derive either from transgenic donor mice that constitutively express green fluorescent protein (GFP) or b-galactosidase in all of their cells. Alternatively, cells from a male mouse can be used which, following transplantation into female mice, can be detected based on their Y-chromosome. Following irradiation at high doses, mice will die unless bone marrow is administered leading to reconstitution of all the lineages of the blood. The success of a bone marrow transplant can be ascertained by survival of the animal and the degree of chimerism in the blood, i.e., the proportion of the cells in the circulation of the recipient that express the genetic marker of the donor, determined either by microscopy or FACS. Four to eight weeks are usually required to reconstitute the blood in adult mice (8-10 weeks of age) and detection in the tissue of interest requires another 2-4 weeks.

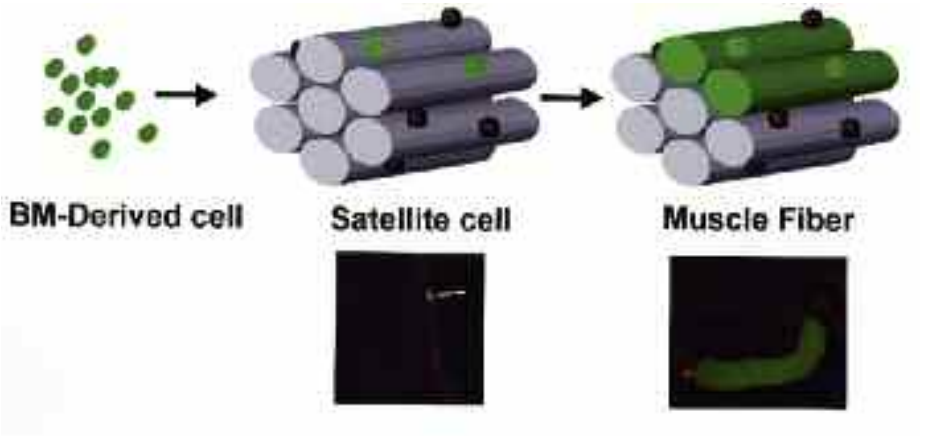

Figure 4. *Cells from the bone marrow give rise to muscle through a tissue-specific stem cells enroute to becoming mature muscle fibers*. Cells from the bone marrow, potentially hematopoietic stem cells or their progeny, contribute nuclei to multinucleate muscle fibers in adult mice that receive a bone marrow transplant. This schematic represents our hypothesis that the dominant mechanism for this contribution follows a series of differentiation steps from blood into muscle. First, a cell from the bone marrow differentiates into a muscle satellite cell. Muscle satellite cells are the entities in skeletal muscle that proliferate and fuse with muscle fibers during muscle regeneration. They are a biochemically and functionally diverse cohort of cells in adults and they may have multiple origins. Second, having adopted a muscle stem cell status, the bone marrow derived satellite cells are then activated by local cues to regenerate muscle, ultimately resulting in their fusion with existing and nascent muscle fibers in a manner typical of endogenous satellite cells.

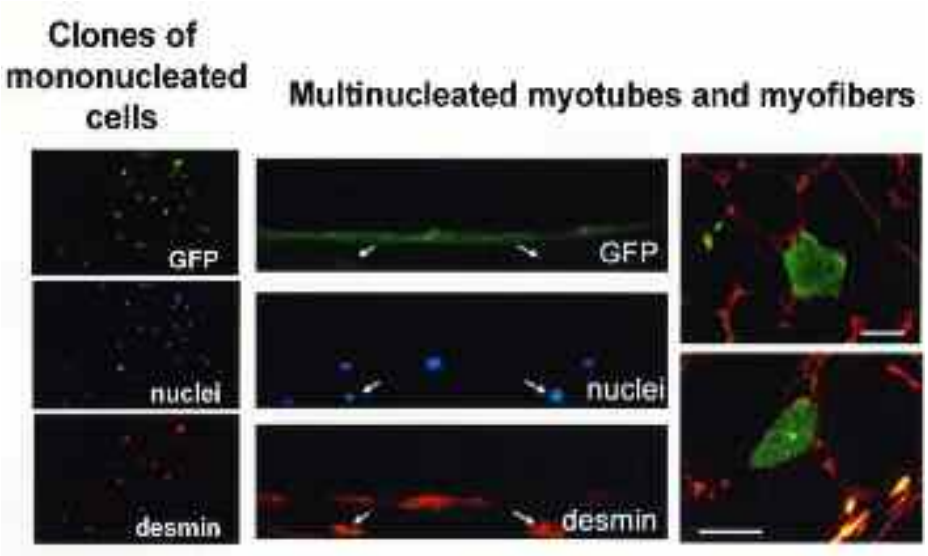

Figure 5. *The satellite cell niche (extracellular environment) causes reprogramming of nuclei of bone marrow derived cells and activationto of muscle-specific genes*. A heritable myogenic phenotype is characteristic of bone marrow-derived satellite cells. The descendents of satellite cells, myoblasts, were isolated from the skeletal muscle of recipients of transplanted green fluorescent protein (GFP(+)) bone marrow in three independent experiments. Clones of mononucleate cells: clones that originate from single donor-derived myoblasts express *GFP*, exhibit *nuclei* stained with Hoechst 3342 and express the intermediate filament protein *desmin* (magnification x200). Multinucleated myotubes: when induced to differentiate, clones formed multinucleate myotubes in culture that expressed *GFP*, exhibited *nuclei* stained with Hoechst 3342 and express *desmin* (magnification x200). Arrows show nuclei of myoblasts outside the myotubes. Multinucleated myofibers: Bulk FACS-sorted donor-derived myoblasts were injected into the TA muscles of SCID mice where they fused with existing skeletal muscle fibers. Transverse sections of TA are shown with antibody staining GFP (green) and laminim (red) that are representative of two sections taken at 200 mm intervals showing that GFP(+) fibers span up to 200 mm. Scale bar represents 20 mm.

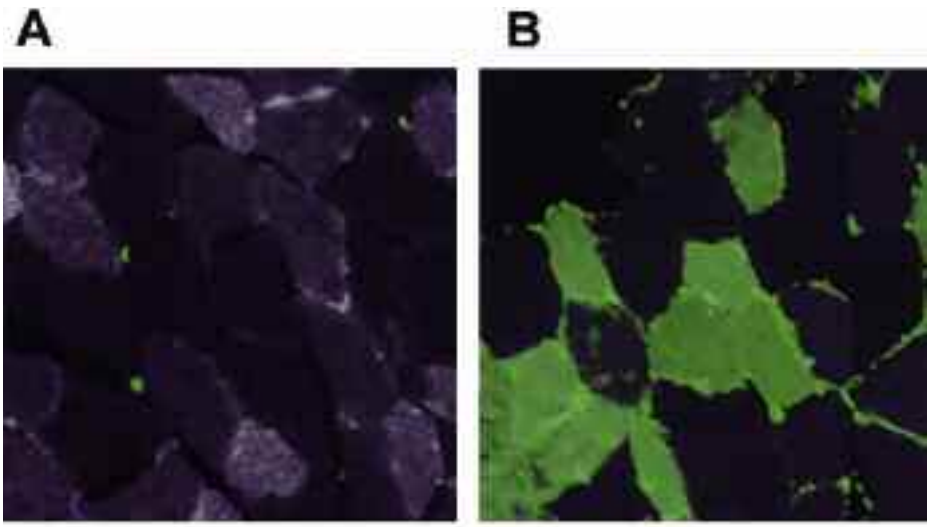

Figure 6. *Exercise causes damage which enhances the contribution of GFP(+) bone marrow-derived satellite cells to regenerating muscle*. GFP(+) fibers in the Tibialis Anterior muscle are shown in unexercised (A) and exercised (B) mice. The background GFP(-) myofibers are shown in dark gray. GFP(+) muscle fibers were in clusters in the exercised mouse (B) suggesting that regeneration could have resulted from single GFP(+) satellite cell clones in these regions.

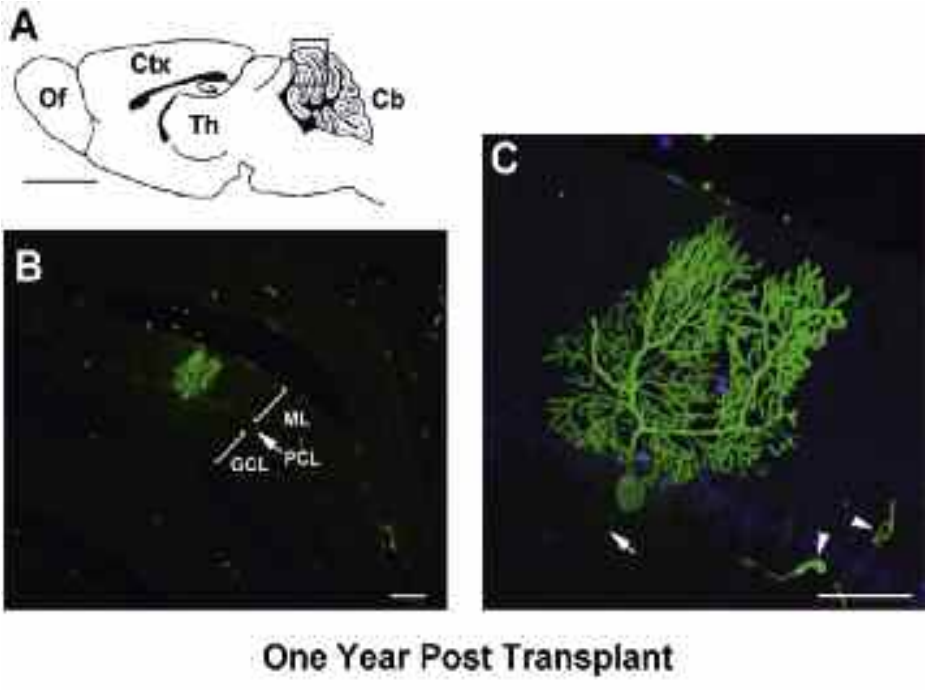

Figure 7. *Fusion with Purkinje cells causes bone marrow derived cells results in GFP+ Purkinje cells which and reprogramming of nuclei by intracellular signals.* (A). Schematic of a mouse brain showing the anterior olfactory bulb (OF), cerebral cortex (Ctx), thalamus (Th), and the caudally located cerebellum (Cb). (B). In thick sections (45μm) cut from the cerebellum of a mouse post-bone marrow transplant, individual donor-derived GFP^+ Purkinje neurons are evident in the Purkinje cell layer (PCL). The dendrites from these cells extend into the cell sparse molecular layer (ML), while their axon projects through the granular cell layer (GCL) and is the only output connection from the cerebellum to the rest of the brain. Three lobes of the cerebellum in the box in (A) can be seen in (B). Note the many bone marrow derived (GFP^+) cells in the parenchyma. (C). High power laser scanning confocal image of this cell show its many synaptic spines and single output axon (arrow). The 2 GFP^+ BMDC cells are probably microglia or macrophages in PCL and ML (arrowheads). Scale bars, (A): 2mm, (B): 100mm, (C): 50mm.

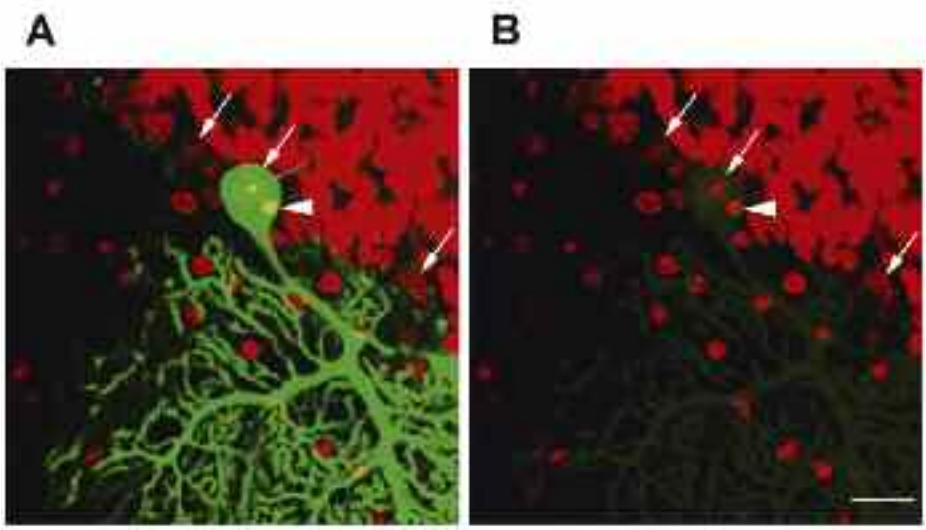

Figure 8. *100% of GFP+ Purkinje cells are stably binucleate heterokaryons due to fusion*. Mice were bone marrow transplanted at 2 months of age and cerebella were collected and analyzed at various time points. (A and B) All of the GFP^+ Purkinje cells observed had 2 nuclei. (A) This Purkinje cell has a distinctive dendritic tree with many synaptic spines, and an axon exiting the soma at the left. One of the 2 nuclei in the cell is compact (arrowhead) and is the putative BMD nucleus. (B) The other nucleus has dispersed chromatin similar to other Purkinje neurons (arrows). In 752 control Purkinje neurons from transplanted and normal mice, no binucleated cells were observed. Scale bar: 20mm.

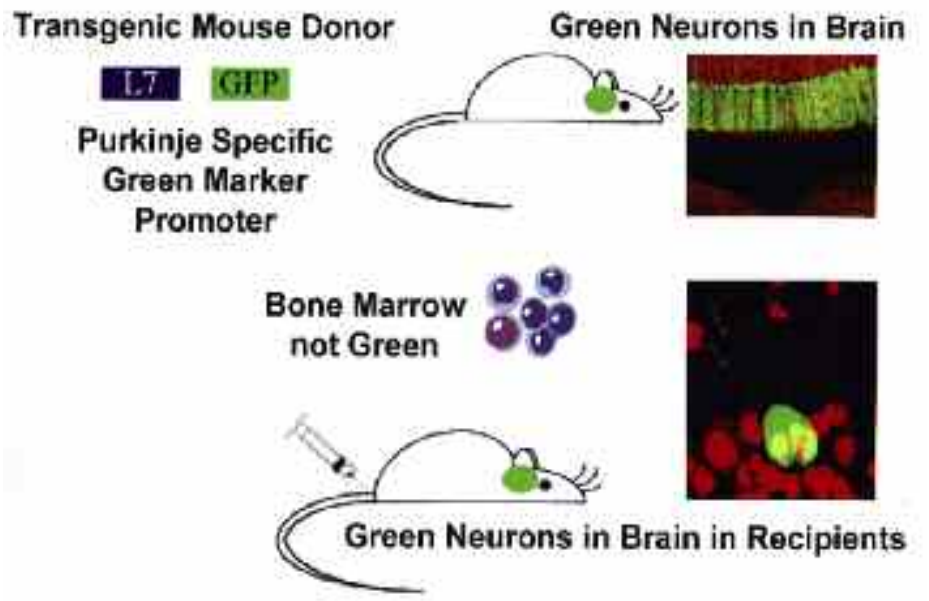

Figure 9. *Schematic drawing of the experimental design demonstrating reprogramming of bone marrow derived cells (BMDCs) after transplantation into wild-type recipients.* The upper panel illustrates, the Purkinje neuron-specific transgene, L7-EGFP-pcp-2 construct, left and the micrograph, the expression pattern of L7-EGFP promoter in a 5mm optical coronal section of the cerebellum at the level of the 4th ventricle to the right. All Purkinje cells express the Purkinje specific promoter L7-EGFP (green), the section is counterstained with a nuclei dye, To-Pro3 (red).

The lower panel illustrates the injection of bone marrow, harvested from L7-EGFP tg-mice, into the tail vein of a wild-type isogenic recipient. The bone marrow does not express the Purkinje cell-specific transgene and hence is not green. The micrograph is a 1mm optical section showing the cell body of a Purkinje cell that express the L7-EGFP transgene. This reveals that a BMDC has fused with a Purkinje cell, forming a stable reprogrammed heterokaryon. Note the two nuclei in the Purkinje cell.

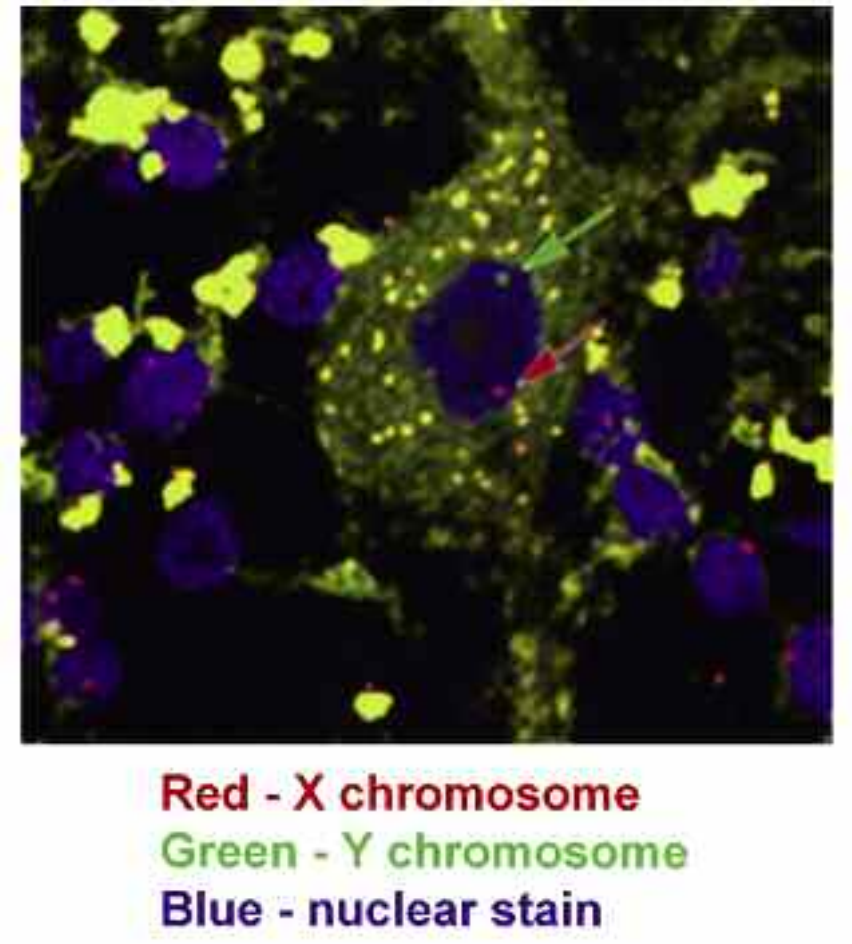

Figure 10. Evidence of *male* Y-chromosome in Purkinje neurons of *female* brains. The female-specific chromosomes (X) and the male-specific chromosomes (Y) in the cerebella were processed with X (red) and Y (green) probes, the nucleus counterstained with To-Pro-3 (blue) and imaged using a scanning confocal microscope at 1mm optical sections. The Purkinje neuron is clearly defined with a large nucleus surrounded by a large cytoplasmic region. The male bone marrow-derived nuclei (green arrow) can clearly be seen in the female (X-chromosome indicated by red arrow) Purkinje neuron.

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