Predictability in Science: Accuracy and Limitations
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ON THE UNPREDICTABILITY OF INDIVIDUAL RESEARCH

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I would like to distinguish between predictability by science and predictability of science. In the first case science is used to predict, e.g. climate, tsunamis or earthquakes.

In the second case, we wonder what direction science will take.

Undoubtedly, the great discoveries of science lead, predictably, to research trends resulting from them. Thus, the discovery of the double helix led to thousands of studies, ultimately leading to the breaking of the genetic code, and – in turn – after close to fifty years, to the elucidation of the human genome. Similarly, in physics, after the discovery of the theory of relativity or the theory of atoms, it was to be expected that – usually only after several years – a stream of studies resulting from these theories – would appear in a predictable fashion. The same is true of exciting new techniques. In life sciences discoveries like the cell-sorter (FACS) or the polymerase chain reaction (PCR), to give just two examples, have revolutionized many areas of experimental research. I would like to generalize these observations by stating that – at a 'macro' level – it is safe to assume that science is, to a large extent, predictable.

My contention is that this is not true at the 'micro', the individual research level. As most scientists are expected to write grant proposals – in which they describe their plans for research and the results they expect to reach – it is of interest to ascertain to what extent their predictions resemble the actual results. It would be depressingly boring if there would be too much resemblance between the plans and the subsequent reality. In all fairness, it must be stated that 'predictable' is not necessarily 'predicted', and if the results are actually opposite to what was predicted, in many cases this leads to breakthroughs of uncommon interest. We must be continuously watchful because very often the 'unpredictable' is lost because of lack of attention. The discovery of Fleming in 1928 of penicillin is due to his having paid attention to a Petrie dish with transparent areas in which the bac-

teria disappeared. This stresses the importance of serendipity which I define as 'luck meeting the prepared mind'.

I would like to give a few examples from my own research experience, and I refer to the discovery of the first synthetic polypeptide antigens, to the discovery of determinant-specific genetic control of immune response, to the discovery of a synthetic copolymer of amino acids that became an efficient drug against the exacerbating-remitting stage of multiple sclerosis, and to the discovery of a synergistic effect of a specific monoclonal antibody and of a chemotherapeutic drug in fighting cancer.

Synthetic Polypeptide Antigens

The purpose of the study was to make a protein a better antigen. It was then that we promoted the notion of immunogen and immunogenicity. We wanted to increase the immunogenicity of a protein, and we chose gelatin, a very poor immunogen, to which we attached chains of polytyrosine [1]. A limited polytyrosylation converted gelatin into a potent immunogen which provoked in experimental animals the formation of gelatin-specific antibodies. A more intensive polytyrosylation led to an immunogen which led solely to anti-tyrosine peptide antibodies [1,2]. The inevitable conclusion was that gelatin could be replaced with a synthetic branched polyamino acid and when we attached peptides including tyrosine to such a polymer, we obtained a synthetic branched macromolecule which was a potent and specific immunogen in several animal species [3]. So, in this case we wanted to improve the antigenicity of proteins, and we ended up with a whole array of synthetic antigens, which permitted us to elucidate many molecular aspects of antigenicity [2,4,5].

We could learn a lot about the role of size, composition, and shape, as well as about the accessibility of those parts of the molecule crucial for immunogenicity. As a matter of fact, we learned that it was possible (provided one was prepared to invest the necessary effort) to prepare synthetic immunogens leading to antibodies of essentially any specificity.

Although in most cases a good immunogen had a molecular mass of at least several thousand Daltons, dinitrophenyl-hexalysine and arsanil-trityrosine were by themselves capable of triggering an efficient immune response. The minimal size for a molecule to be immunogenic depends, therefore, largely on its chemical nature.

Although electrical charge may be important in defining the antigenic specificity of an epitope, charge is not a minimum necessary cause for

immunogenicity; we could prepare water-soluble amino acid copolymers devoid of charge that were immunogenic. Polymers of D-amino acids were immunogenic only when they were administered in minute amounts and they led to no secondary response.

In the early days there was a wonderful feeling working on synthetic antigens because practically nobody else was working on the subject, but later on it was as pleasant and satisfying to know that so many laboratories had become interested in the synthetic approach to immunological phenomena. One of the most fascinating aspects of our studies with synthetic antigens had to do with the steric conformation of the immunogen and of its epitopes. We distinguished between conformational (conformation-dependent) and sequential determinants [6] and showed how the same peptide (Tyr-Ala-Glu) may lead to antibodies recognizing the sequence (when attached to multichain poly-DL-alanine) or recognizing an epitope defined by conformation (when the tripeptide was polymerized to give an α-helical structure). In addition, we could demonstrate for the first time, by circular dichroism, how antibodies to α helical polymer could help transconform into a helical shape a small polymer that was not yet helical [7]. These studies led us directly to study proteins and to synthesize a macromolecule in which a synthetic 'loop' peptide derived from hen egg white lysozyme was attached to branched polyalanine [8]. The resulting antibodies reacted with intact lysozyme through the 'loop' region, but the reaction was totally abolished when the disulfide bond within the 'loop' was opened, and thus the three-dimensional structure was collapsed.

Genetic Control of Immune Response

Even though some hints could be found in earlier literature, the actual establishment of the genetic control of the immune response became possible only through the study of synthetic antigens, simple chemically, in inbred strains of mice and guinea pigs, simple genetically.

I would now like to tell the story how it all started. In the summer of 1961, when I returned to the Weizmann Institute in Israel, from a year spent at the National Institutes of Health in Bethesda, Maryland, I stopped in London to discuss with John Humphrey and Brigitte Askonas a collaborative effort to follow the fate of strongly radioactive synthetic polypeptide antigens and to find out whether antigen molecules must be present in antibody-producing cells. Ultimately, this project was brought to a successful fruition [9], but in its initial stages, Hugh McDevitt, who joined Humphrey

from Boston, injected cold poly(Tyr,Glu)-poly(DL-Ala)-polyLys, (T,G)-A--L, into rabbits to study their immune response. Several weeks later, Humphrey informed me at a WHO meeting in Geneva that the sandylop rabbits they used did not produce antibodies, and we considered the genetic makeup of the animal as one possibility to explain this result. Within a short time, it was clear that New Zealand rabbits produced as many antibodies as did our rabbits in Rehovot, and Himalayan rabbits were almost an order of magnitude better. At this moment, it was natural for McDevitt to switch to inbred strains of mice.

In our studies [10, 11] we first showed determinant-specific (antigen-specific) genetic control of immune responses by making use of multichain polyamino acids as antigens and inbred mice as experimental animals. (The first paper became a Citation Classic, Curr Cont. 1987). The multichain synthetic polypeptides we investigated, possessed at the tips of their polymeric side chains, small amounts of tyrosine, histidine, or phenylalanine. These antigens were denoted (T,G)-A--L, (H,G)-A--L, and (Phe,G)A--L. We noted that when histidine was substituted for tyrosine, genetic control was completely reversed, whereas replacement with phenylalanine led to a material strongly immunogenic in both the strains investigated.

Some time later, Hugh McDevitt, using these multichain polypeptides, was able to show for the first time the link between the immune response and the major histocompatibility locus of the mouse, which in turn led to our present-day understanding of immune response genes and their products. Of all the contributions of synthetic polypeptides toward our present-day understanding of immunology, none has been more important than the discovery and the definition of the genetic control of the immune response, which in turn was a crucial trigger toward a better understanding of the cellular basis of immunological responsiveness.

So, the initial project was to find out whether a cell producing antibodies has some antigen in it, and we ended up with discovering the genetically defined differences in the immune response.

Drug Against Multiple Sclerosis

We tried to build synthetic amino acid copolymers that would resemble myelin basic protein (MBP) and would induce, similarly to this protein, experimental allergic encephalomyelitis in animals (EAE), and only after we failed, we realized that they cannot initiate the disease, but they can suppress it.

In our early studies, of special interest was the immune response to lipid components, which was not easy to either elicit or investigate because of solubility problems. However, conjugates in which synthetic lipid compounds were attached onto synthetic copolymers of amino acids elicited a specific response to lipids such as cytolipin H, which is a tumor-associated glycolipid [12], or sphingomyelin. Furthermore, we demonstrated that both the sugar and lipid components of such molecules contributed to their specificity. The resultant anti-lipid antibodies were capable of detecting the corresponding lipids both in water-soluble systems and in their physiological milieu. This was fascinating because it gave us a glimpse into some disorders involving lipid-containing tissue and consequently led to our interest in demyelinating diseases, namely, disorders in which the myelin sheath, which constitutes the lipid-rich coating of all axons, is damaged, resulting in various neurological dysfunctions. We thus thought that EAE, caused by MBP might actually be induced by a demyelinating lipid and that the positively charged MBP might serve only as a schlepper (carrier) for an acidic lipid (e.g. phospholipids). We prepared several positively charged copolymers of amino acids and tested whether we could induce EAE when the copolymers were administered into experimental animals (guinea pigs and rabbits) in complete Freund's adjuvant, similarly to the successful administration of MBP, but we failed. On the other hand, the injection of several positively charged amino acid copolymers in aqueous solution into mice, rabbits, and guinea pigs resulted in efficient suppression of the onset of the disease, experimental allergic encephalomyelitis [13,14]. Later on, we could suppress the actual disease in rhesus monkeys and baboons. The copolymer 1 that was primarily used, denoted Cop 1, now called glatiramer acetate, and by industry 'Copaxone', is composed of a small amount of glutamic acid, a much larger amount of lysine, some tyrosine, and a major share of alanine. To our pleasant surprise, there is a significant immunological cross-reaction (both at the antibody level [15] and at the T cell level [16]) between Cop 1 and myelin basic protein. Interestingly, when an analog of Cop 1 made from D-amino acids was tested, it had no suppressing capacity nor did it cross-react immunologically with the basic protein. Cop 1 is not generally immunosuppressive; it is not toxic; actually it is not helpful in any other autoimmune disease except in multiple sclerosis and its animal model, experimental allergic encephalomyelitis.

The clinical trials with Cop 1 have included two preliminary open trials and two double-blind II trials, one involving exacerbating-remitting patients

[17] and another one in chronic progressive patients [18]. The results of the phase II trial in exacerbating-remitting patients demonstrated a remarkable decrease in the number of relapses and in the rate of progression in Cop 1-treated patients compared with the placebo control. Cop 1 is a promising low risk multiple sclerosis-specific drug for treatment of the relapsing disease. As an antigen-specific intervention, Cop 1 has the advantage of reduced probability of long term damage to the immune system.

After a successful, pivotal multicenter phase III clinical trial conducted in 11 medical centers in the United States [19], Cop 1 was approved by the United States Food and Drug Administration as a drug for multiple sclerosis. This was a moment of gratification and deep emotion for my colleagues and myself, as well as for our industrial partners, Teva Pharmaceutical Industries.

An important step in our understanding of the mode of action of Cop 1 was the observation that copolymer 1 induces T cells of the T helper type 2 that cross-react with myelin basic protein and suppress experimental autoimmune encephalomyelitis [20]. This was corroborated by clinical studies in multiple sclerosis patients [21]. It was of interest to observe that Th2 suppressor lines and clones induced by Copolymer 1 cross-reacted at the level of Th2 cytokine secretion with myelin basic protein but not with other myelin antigens [22]. This bystander suppression may explain the therapeutic effect of Cop 1 in EAE and multiple sclerosis (MS).

Cop 1 binds promiscuously to many different cells regardless of their DR restriction. It binds avidly and fast and can also displace already bound antigens, and this holds for all the myelin antigens that may be involved in MS; and yet, Cop 1 exerts its activity in an antigen-specific manner (it is not a general immunosuppressive agent and does not affect other experimental autoimmune diseases). Its specificity must, therefore, be envisaged in the context of the trimolecular complex MHC-Ag-T-cell receptor ('the immunological synapse'), namely, as interference with the presentation of the encephalitogenic antigen to the T-cell receptor, which is a specific interaction.

I recently summarized the story of specific vaccines against autoimmune diseases [23], as well as the successful use of Cop 1 (glatiramer acetate, Copaxone) in the treatment of multiple sclerosis for exacerbating-remitting patients [24]. The majority of the patients in the great clinical trial continue to be followed in an organized fashion for more than 7 years. Their risk of an MS relapse was over 1.5 per year at onset and is now less than 1 every 6 years. On an average, these patients have experienced no increase in neurological disability, whereas natural history profiles would

have predicted substantial worsening. The accumulated experience with glatiramer acetate (Cop 1) indicates that its efficiency is apparently increased as a function of usage time, while the favorable side effect profile is sustained.

Personally, the whole odyssey of Cop 1 and its use in MS has been a source of great satisfaction and emotion. The awareness that over one hundred thousand MS patients feel better because of a drug/vaccine that we conceived and developed, moves me deeply. Twenty-eight years have passed from the moment of the idea to the approval of Cop 1 by the Food and Drug Administration. I have a feeling that discoveries resulting from basic research take a longer time to fruition, but on the other hand, they are probably more original in terms of concept.

Synergy Between a Monoclonal Antibody and Chemotherapeutic Drugs

In this case we covalently bound for long period chemotherapeutic drugs to anti-tumor antibodies, using the latter mainly as missiles to target the drug. Over the years we found out that some monoclonal antibodies are very efficient as anti-cancer drugs, but the greatest effect was obtained when we used the combination of the antibody and the chemotherapeutic drug [25].

The idea of binding anti-cancer therapeutic drugs covalently to antibodies reacting with cancerous cells has appealed to me from an early time. Instead of having the drugs given systemically, spread throughout the whole body, immunotargeting would focus the supply of the drug exclusively to the cancer area. However, we did not get to immunotargeting until many years later, when we bound daunomycin and adriamycin via a dextran bridge to antibodies against antigens of leukemia, lymphoma, and plasmacytoma cells. We showed that these are effective as ‘guided missiles’ both in vitro and in vivo [26].

Later on we moved to monoclonal antibody against the extracellular domain of the epidermal growth factor receptor, denoted today ErbB1, and found that its conjugate with daunomycin was quite efficient but so was the antibody by itself [25]. A strong synergistic effect was observed when the anti-ErbB1 antibodies were administered together with cis-platin. This observation became of great interest because of its therapeutic potential (e.g. in the review article by Mendelsohn and Baselga [27]). Over the years, I became more and more *convinced* that what matters most is the nature of monoclonal antibodies.

Conclusions

It is of crucial importance to have well defined plans in research, but it is at least as important to be flexible and open minded, and to conduct research in a way that leads to optimal results. These often lead to unexpected discoveries, as I hope I have showed in the four examples I have illustrated here.

REFERENCES

1. Sela, M., and Arnon, R., *Biochem. J.*, 75, 91 (1960).
2. Sela, M., *J. Biol. Chem.,* 278, 48507 (2003).
3. Sela, M., Fuchs, S., and Arnon, R., *Biochem. J.,* 85, 223 (1962).
4. Sela, M., *Adv. Immunol.*, 5, 29 (1966).
5. Sela, M., *Science,* 166, 1365 (1969).
6. Sela, M., Schechter, B., Schechter, I., and Borek, F., *Cold Spring Harbor Symp.* Quant. Biol., 32, 537 (1967).
7. Schechter, B., Conway-Jacobs, A., and Sela, M., *Eur. J. Biochem.*, 20, 321 (1971).
8. Arnon, R., Maron, E., Sela, M., and Anfinsen, C.B., *Proc. Natl. Acad. Sci. USA,* 68, 1450 (1971).
9. McDevitt, H.O., Askonas, B.E., Humphrey, J.H., Schechter, I., and Sela, M., *Immunology*, 11, 337 (1966).
10. McDevitt, H.O., and Sela, M., *J. Exp. Med.,* 122, 517 (1965).
11. McDevitt, H.O., and Sela, M., *J. Exp. Med.*, 126, 969 (1967).
12. Arnon, R., Sela, M., Rachaman, E.S., and Shapiro, D., *Eur. J. Biochem.,* 2, 79 (1967).
13. Sela, M., Arnon, R., and Teitelbaum, D., *Bull. Inst. Pasteur*, 88, 303 (1990).
14. Teitelbaum, D., Meshorer, A., Hirshfeld, T., Arnon, R., and Sela, M., *Eur. J. Immunol.,* 1, 242 (1971).
15. Teitelbaum, D., Aharoni, R., Arnon, R., and Sela, M., *Proc. Natl. Acad. Sci. USA*, 85, 9724 (1988).
16. Teitelbaum, D., Milo, R., Arnon, R., and Sela, M., *Proc. Natl. Acad. Sci. USA,* 89, 137 (1992).
17. Bornstein, M.B., Miller, A., Slagle, S., Weitzmann, M., Crystal, H., Dexler, E., Keilson, M., Merriam, A., Wassertheil-Smoller, S., Spada, V., Wein, W., Arnon, R., Jacobsohn, I., Teitelbaum, D., and Sela, M., *N. Engl. J. Med*. 37,408 (1987).

18. Bornstein, M. B., Miller, A., Slagle, S., Weitzmann, M., Drexler, E., Keilson, M., Spada, V., Wein, W., Appel, S., Rolak, L., Harati, Y., Brown, S., Arnon, R., Jacobsohn, I., Teitelbaum, D., and Sela, M. *Neurology,* 41, 533 (1991).
19. Johnson, K.P., Brooks, B.R., Cohen, J.A., Ford, C.C., Goldstein, J., Lisak, R.P., Myers, L.W., Panitch, H.S., Rose, J.W., Schiffer, R.B., Vollner, T., Weiner L.P., Wolinky, J.S., and the Copolymer 1 MS Study Group, *Neurology,* 45, 1268 (1995).
20. Aharoni, R., Teitelbaum, D., Sela, M., and Arnon R., *Proc. Natl. Acad. Sci. USA.,* 94, 10821 (1997).
21. Neuhaus, O., Farina, C., Yassouridis, A., Wiendl, H., Bergh, F.T., Dose, T., Wekerle, H., and Hohlfeld, R., *Proc. Natl. Acad. Sci. USA,* 97, 7452 (2000).
22. Aharoni, R., Teitelbaum, D., Sela, M., and Arnon, R., *J. Neuroimmunol.*, 91, 135 (1998).
23. Sela, M., *C.R. Acad. Sci. Paris Life Sci.* 322, 933 (1999).
24. Sela, M., and Teitelbaum, D., *Expert Opin. Pharmacother.,* 2, 1149 (2001).
25. Aboud-Pirak, E., Hurwitz, E., Pirak, M.E., Bellot, F., Schlessinger, J., and Sela, M., *J. Natl. Cancer Inst.,* 80, 1605 (1988).
26. Levy, R., Hurwitz, E., Maron, R., Arnon, R. and Sela M. and Sela, M., *Cancer Res.* 35, 1182 (12975).
27. Mendelsohn, J., and Baselga, J., *Oncogene,* 19, 6550 (2000).