PATH OF DISCOVERY OF A THERAPEUTIC VACCINE

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Before discussing my specific case, I would like to approach the subject of 'paths of discovery' in a more general manner. Karl Popper in his many writings was of the opinion that the best way to contribute to building science is to progress by making conjectures and trying to refute them. A self-consistent model, even when it is wrong, is always useful if it is 'falsifiable' [1]. Disproving an accepted theory is the only way to advance knowledge. As Baruch Blumberg mentions in his discussion of the scientific process: 'not only should scientific experiments be planned in their technical aspects prior to starting, but their design should be part of a long range strategic plan' – all this because of the influence of Karl Popper's recommendations [2].

In my opinion, theories are very good as working hypotheses and as inducement to do some experiments, but they become terribly dangerous when they become dogmatic, and then they are also counterproductive. I mean theories are good as long as you do not take them too seriously. I must qualify this statement by one thing, because in the lab I want to do those experiments that excite and intrigue me: I do not want to have to spend a large part of the time just to disprove other people's theories or hypotheses. Sometimes you have a difficult situation because you cannot just say to somebody: 'No, I do not believe it, I am sure it is wrong'. Somebody has to test it and prove it, otherwise the scientific method falls apart.

I actually believe that one needs three things for research: optimism, perseverance, and serendipity, which is when luck meets the prepared mind. I also believe that some of the best ideas come totally unexpectedly during a time of relaxation, which could be either some wonderful bona fide relaxation, or some boring committee meetings.

I think it is very important to have good schooling, but it is desirable to have also a little bit of ignorance. I am a compulsive reader, or more precisely, a compulsive scanner of literature. But I think that if I had first

decided to study immunology in depth for a couple of years as it was practiced at that time, and then had started asking questions, I do not know whether I would even have dared to start conducting experiments. My approach was that if I came to a complex biological reality, I should try to figure out whether I could define precisely one question which could be answered in a clear-cut way, and then we could continue and move to another question.

I would like to make another remark: I know there are individuals who really prefer to be alone all the time, and maybe through daydreaming, they reach all their working hypotheses, but I am a great believer in the interaction and fertilization of ideas. I feel there is a tremendous need and great payoff for this kind of interaction, and I have been collaborating around the world with many colleagues, and was always keen on having many visiting scientists from various parts of the world spending extended periods of time in my laboratory.

As an example from my own research, I would like to describe here the path of discovery of the drug, which I prefer to call the therapeutic vaccine, for the exacerbating-remitting form of multiple sclerosis [3, 4]. We called it 'copolymer 1'. It was renamed 'glatiramer acetate' and industry named it Copaxone. It all started with our interest in the immunology of lipids.

COPOLYMER 1 AND MULTIPLE SCLEROSIS

In our early studies with Ruth Arnon, 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 [5], or sphingomyelin [6]. Furthermore, we demonstrated that both the sugar and lipid components of such molecules contributed to their immunological 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 (experimental allergic encephalomyelitis) caused by

MBP (myelin basic protein) 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 to see 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 EAE [7, 8]. Later we were able to suppress the actual disease in rhesus monkeys and baboons [9. 10]. The copolymer 1 we primarily used, denoted Cop 1, 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 [11, 12] and at the T cell level [13, 14], between Cop 1 and the 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 [15]. Cop 1 is not generally immunosuppressive, nor is it 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 phase II trials, one involving exacerbating-remitting patients [16] and another one on chronic progressive patients [17]. 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 ll medical centers in the United States [18], 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.

We were obviously very interested in the mode of action of Cop 1. We knew that the effect was specific for the disease, and we assumed that it had to do with the immunological cross-reaction between the 'villain' (myelin basic protein) and the drug (Cop 1). What we learned later is that Cop 1

binds almost immediately and strongly to the groove of major histocompatibility complex (MHC) class II antigens of most genetic backgrounds, and it displaces efficiently from the groove any peptides derived from the myelin protein [19]. This promiscuity is probably because of its polymeric character, permitting microheterogeneity in the amino acid sequence. The extensive and promiscuous binding to class II MHC molecules, without prior processing, leads to clustering of these molecules on the antigen-presenting cells, which may explain their high affinity binding [20].

This is the first necessary but not sufficient step in its mechanism of action. The binding, which is the least specific step, is a prerequisite for its later effects. Following this interaction, two mechanisms were clearly shown to be effective. 1) Cop 1 binding to the relevant MHC leads to the activation of T suppressor cells because of suppressive determinants shared between myelin basic protein and Cop 1. 2) Successful competition between the complex of Cop 1-MHC class II antigen with the complex of myelin basic protein-MHC class II antigen for the myelin basic protein-specific T cell receptor (a phenomenon called by immunologists the 'T receptor antagonism') is shown [21].

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 [22]. This was corroborated by clinical studies in multiple sclerosis patients [23]. 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 [24]. 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 summarized recently the story of specific vaccines against autoimmune diseases [25], as well as the successful use of Cop 1 (glatiramer

acetate, Copaxone) in the treatment of multiple sclerosis for exacerbating-remitting patients [26]. The majority of the patients in the large clinical trial have been 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 tens of thousands of MS patients feel better because of a drug/vaccine that we have 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.

THERAPEUTIC VACCINES

Copolymer 1 is just one example of a therapeutic vaccine in the field of autoimmunity. Great effort is being devoted to develop vaccines against tumors, AIDS, hepatitis, tuberculosis, and possibly against the bacteria that cause gastric ulcers. Copolymer 1, used today as a vaccine against multiple sclerosis (MS), is a good example of a beneficial treatment for this autoimmune disease, based on its similarity to the myelin basic protein (MBP), one of the putative causes of MS. This finding could lead to therapeutic vaccines against other autoimmune diseases such as myasthenia gravis, systemic lupus erythematosus (SLE) and rheumatoid arthritis. Furthermore, antibodies prepared against prions raise hopes for a vaccine against bovine spongiform encephalitis (BSE) and Creutzfeldt-Jakob disease (CJD) and antibodies to a peptide derived from b-Amyloid plaques could degrade plaques and be used as a therapeutic vaccine against Alzheimer's disease (AD) [27].

By its definition, a preventive vaccine is sufficiently similar in its chemistry to the molecule that provokes the disease so that the immune response directed against it can act against the causative agent. This situation is analogous in the case of therapeutic vaccines.

A colloquium on 'Therapeutic Vaccines' took place recently in the USA National Academy of Sciences in Washington, trying to put under one roof the manifold efforts in various areas in need of such a vaccine [28].

CONCLUSIONS

In conclusion, I feel that there must be some strategic planning of research; in my case this would be the molecular basis of antigenicity, synthetic vaccines, autoimmune diseases or cancer vaccines, but at the tactical level there must be space for spontaneity and serendipity, which I have already defined as a situation where luck meets the prepared mind.

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