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Therapeutic Vaccines Against Cancer and Autoimmune Diseases

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In the spirit of the invitation to this Plenary Session, on the topic of 'The Scientific Legacy of the 20th Century', I shall try to mention here some of the highlights of my research in the last sixty years at the Weizmann Institute of Science.

Elucidation of antigenicity and immunogenicity

My laboratory pioneered the design of amino acid oligomers to define the minimal and precise chemical characteristics of antigens – molecules that could be bound by antibodies (1). Using these tools, we determined the functional size of the antibody binding pocket, and characterized the effects of charge, hydrophobicity, and side chain interactions in the antibody-antigen binding complex (1). These studies defined the chemistry of antigen binding by antibodies and laid the foundation for subsequent structural investigations based on x-ray analysis of crystallized preparations and NMR studies. In the course of these studies, I called attention to the essential difference between antigenicity (the capacity of a molecule to bind antibodies) and immunogenicity (a term coined to designate the capacity of a molecule to induce an active immune response). This distinction has become a guiding principle in immunology (1).

Discovery of a chemical basis for the action of immune response genes

In the light of my characterization of antigenicity, I went on to apply amino acid oligomer chemistry to the question of immune response genes. We synthesized amino acid oligomers with defined, minimal chemical differences and, together with Hugh McDevitt, discovered and analyzed the role of MHC genes in mediating genetic control of the immune response (2,3). Work with amino acid oligomers, which proceeded independently of that research, established a solid, synthetic chemical foundation for subsequent biologic studies. The chemical research was seminal in providing the mindset for subsequent biological studies and for the x-ray crystallography that definitely solved the structure of the antigen-binding site of the antibody.

Based on the high water-solubility of poly-DL-alanine, I could open all the disulfide bridges of an immunoglobulin without the product dropping out of solution. Upon reoxidation, all the immunological properties, whether as antigen (4) or as antibody (5), have returned, thus proving the correctness of the selection theory of antibody formation (6).

Vaccines are prophylactic in the sense that they are administered to healthy individuals to prevent a disease. Nevertheless, there is a growing trend to use vaccines to alleviate the suffering of those already with a disease. Great effort is being devoted to develop vaccines against tumors, AIDS, hepatitis, tuberculosis, and possibly against the bacteria that cause gastric ulcers. Copolymer 1 (Copaxone, glatiramer acetate) 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 the 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 and Creutzfeldt-Jacob disease and antibodies to a peptide derived from amyloid plaques could degrade plaques and be used as a therapeutic vaccine against Alzheimer's disease.

By its definition, a preventive vaccine is sufficiently similar in its chemistry to the etiological agent 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.

Development of an effective therapy for multiple sclerosis

Therapeutic vaccines become more and more important, especially as life expectancy increases. Efforts to develop vaccines against such diseases as cancer, AIDS, hepatitis, tuberculosis, Alzheimer's disease, and mad cow disease have not yet reached the stage where they can be successfully used on a daily basis. However, significant progress has been made in the realm of autoimmune diseases, resulting, (at least in one case) in an immunomodulatory vaccine against multiple sclerosis that was developed in my laboratory, and that is in daily use by more than 200,000 patients in 50 countries. The drug or therapeutic vaccine against exacerbating-remitting type of multiple sclerosis is a copolymer of four amino acid residues, denoted Copaxone, which is related to myelin basic protein (7-9).

The story began when we started synthesizing a series of amino acid copolymers composed of four amino acids to create an artificial immunogen

that would mimic myelin basic protein (MBP) and might induce the experimental autoimmune disease EAE, a model of MS. This bold step failed; none of the copolymers were encephalitogenic. But we countered this failed idea with an even bolder idea: the copolymer might not induce EAE, but it might, by mimicking MBP, induce the immune system to resist the disease. This turned out to be the case, and for the next two decades we realized the clinical application of Copaxone to human MS. Today, Copaxone is the most widely used treatment for MS. It is remarkably low in undesirable side effects, yet it significantly reduces the attack rates in relapsing-remitting MS and it prolongs considerably the ability of MS patients to maintain a relatively tolerable quality of life.

Speaking historically, 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 EAE. The Cop 1 primarily used, now called GA or Copaxone, is composed of a small amount of glutamic acid, a much larger amount of lysine, some tyrosine, and a major share of alanine. Thus, its overall charge is positive. There is significant immunologic cross-reaction (both at the antibody and cell levels) between Cop 1 and the MBP. 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 neither generally immuno-suppressive nor toxic. Actually, it is not helpful in any other autoimmune disease except MS and its animal model, experimental allergic encephalomyelitis (EAE). GA (glatiramer acetate, Copaxone) was demonstrated to suppress EAE induced by MBP in a variety of species: guinea, pigs, rabbits, mice and two species of monkeys (rhesus monkeys and baboons). In contrast to rodents, in which GA inhibits the onset of the disease, in primates it was used as treatment of the ongoing disease. After a couple of early clinical trials, it was clear that GA showed efficacy in treating patients with the relapsing-remitting disease. In three randomized double-blind trials, GA, at a dose of 20 mg once daily, administered s.c. in patients, was significantly more effective than placebo for the respective primary endpoint of each trial (proportion of relapse-free patients, relapse rate, and number of enhancing lesions on MRI scans) (10, 11).

Progression to sustained disability, as measured by the Kurtzke expanded disability status scale, was secondary endpoint in the two long-term trials. Patients with relapsing-remitting MS treated with GA in the pivotal US trial were significantly more likely to experience reduced disability, and placebo recipients were more likely to experience increased disability.

Three different clinical trials investigated humoral and cellular immune responses in MS patients treated with Copaxone 1 (12). All patients devel-

oped Cop 1-reactive antibodies, which peaked at 3 months after initiation of treatment, decreased at 6 months, and then remained low. The proliferative response of peripheral blood mononuclear cells to Cop 1 was high initially and gradually decreased during treatment. Several studies showed that MS patients mainly produce the Th2 type of GA-specific T cells after receiving GA (13,14). Cross-reactivity between GA and MBP is seen at several levels: antibodies, T cells, and cross-triggering of cytokines.

Disseminated demyelination is the primary morphological hallmark characterizing multiple sclerosis (MS) and its animal model, experimental autoimmune encephalomyelitis (EAE), leading to axonal loss and neurological impairments. It is, therefore, important to evaluate MS treatments for their neuroprotective capability to prevent demyelination and/or enhance remyelination. The interplay between pathological demyelination and the corresponding repair mechanism remyelination involves, on the one hand, the inflammatory immune cells that mediate the damage and on the other hand, the myelin-producing cells, the oligodendrocytes. The latter are terminally differentiated cells with a limited capacity to respond to injury that are destroyed in the actively demyelinating lesions. Accordingly, remyelination requires the recruitment of oligodendrocyte precursor cells (OPCs) by their proliferation and migration into the demyelinating area and their further differentiation into mature myelinating oligodendrocytes through distinct stages characterized by morphological transformation, and sequential expression of developmental markers.

The interplay between demyelination and remyelination is critical in the progress of MS and its animal model EAE. In a recent study (15), we explored the capacity of glatiramer acetate (GA, Copaxone) to affect the demyelination process and/or lead to remyelination in mice inflicted by chronic EAE, using both scanning electron microscopy and immunohistological methods. Spinal cords of untreated EAE mice revealed substantial demyelination accompanied by tissue destruction and axonal loss. In contrast, in spinal cords of GA-treated mice, in which treatment started concomitantly with disease induction (prevention), no pathology was observed. Moreover, when treatment was initiated after the appearance of clinical symptoms (suppression) or even in the chronic disease phase (delayed suppression) when substantial demyelination was already manifested, it resulted in a significant decrease in the pathological damage.

Presently, Copaxone (GA, Cop 1) is the most used drug against multiple sclerosis. It has already crossed one million years of use without significant side effects.

Reformation of the native structure of a protein.

It was a very early stage (1956) that I spent a most exciting period of my research in the laboratory at the NIH of my close friend and mentor, Christian Anfinsen, the deceased member of the Pontifical Academy of Sciences. By reducing the four disulfide bridges in bovine pancreatic ribonuclease and letting it stay overnight in solution, the enzymatic activity of ribonuclease was largely restored, and this essentially proved that there is no need for additional genetic information to tell the open polypeptidic chain how to refold into the unique protein architecture (16, 17) .

Synergistic effects in immunotherapy of cancer

After synthesizing a peptide corresponding to the amino-terminus of the carcinoembryonic antigen (CEA) we could show that antibodies to the peptide could recognize CEA in the blood of patients. Later on, we used to link by a weak covalent bond a small chemotherapeutic drug to an anticancer antibody (still polyclonal as monoclonal antibodies were not yet discovered). As a spacer between the drug and the antibody we used either dextran or polyglutamic acid. Despite interesting results, we concentrated later on the quality of the monoclonal antibody per se, and thus we found out an important synergistic effect between a small drug and the monoclonal antibody against ErbB1 (referred also as EGFR-epidermal growth factor receptor (18)). As a result of this discovery, the drug Erbitux is used only with a small chemotherapeutic drug, covered by our patent. Later on, we found a strong synergistic effect between two antibodies against the same receptor, provided they were against epitopes sufficiently removed (19, 20). In one case, it was against ErbB1 (19), in the other case against ErbB2 (20). Thus monoclonal antibodies prolong survival of cancer patients. However, the effectiveness of such therapeutic antibodies is low and patients evolve resistance. Thus, there is place for improvement. We found that pairs comprising an antibody reactive with the dimerization site of ErbB-2 and an antibody recognizing another distinct epitope better inhibit ErbB-2-overexpressing tumors than other pairs or the respective individual mAbs. Because the superiority of antibody combinations extends to tumor cell cultures, we assume that nonimmunological mechanisms contribute to mAb synergy. One potential mechanism, namely, the ability of mAb combinations to instigate ErbB-2 endocytosis, is demonstrated. Translation of these lessons to clinical applications may enhance patient response and delay acquisition of resistance.

Conclusion

The common denominator of the studies described is the use of a molecular approach to medical problems, starting with developing the tools of amino acid polymer chemistry, applying them to elucidate fundamental questions in immunology, culminating in a copolymer treatment for a tragic human disease, and in improving cancer treatment by synergy.

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