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Historical review

Historic recollections: From polymers and proteins to autoimmunity and cancer

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1. Getting involved with polymers

My first work and research was a practical problem in efforts to dye cotton as if it were wool. For this purpose we introduced unsaturated links into cotton. Several months later I was already accepted by Ephraim Katchalski-Katzir to become a Ph.D student, working on polymers of amino acids as models for proteins.

As my Ph.D thesis was concerned with polytyrosine (a polymeric chain of phenols) and poly-p-aminophenylalanine (a polymeric chain of anilines), it was necessary to produce polypeptidic azo dyes from them. I reasoned that these might serve as synthetic models for azoproteins, of which one rare example, provided by Landsteiner, was the attachment of haptens including peptides, via an azo bond, to proteins. Reading Landsteiner's book *The Specificity of Serological Reactions*, I came across the statement that gelatin is probably not antigenic because it contains no tyrosine. This led me to study the possibility of increasing the antigenicity of gelatin by attachment of tyrosine peptides. To do these studies, the amino groups of the protein were used to initiate the polymerization of the tyrosine monomer. The continuation of this study was the Ph.D thesis of Ruth Arnon, and we showed that limited tyrosylation enhanced immunogenicity without significantly changing specificity, whereas more extensive tyrosylation converted gelatin into potent immunogen provoking antibodies mainly to tyrosyl peptides. It was at this time that we first clearly defined the notion of immunogen and immunogenicity, and distinguished it from antigenic specificity.

2. Polytyrosine

Poly-L-tyrosine was part of the subject of my Ph.D thesis. My thesis research included also the synthesis of poly-3,5diiodotyrosine and poly-*p*-amino-L-phenylalanine. Later on I was directly involved with the synthesis of polytryptophan and polycyclohexylalanine. Of special interest was the spectrophotometric titration of polytyrosine and of copolymers of tyrosine with positively or negatively charged amino acids; this showed the influence of the vicinal electrostatic field on the ease of ionization of the phenolic hydroxyl groups. We were very touched when John Edsall included figures from this study in his book with Jeffreys Wyman on *Biophysical Chemistry*.

For the preparation of poly-L-tyrosine, I remember that I tried 40 times to synthesize the monomer. N-carboxy-*O*-benzyloxycarbonyl-tyrosine anhydride, until I was successful. This taught me the need for perseverance and optimism in research. Of course serendipity – defined as luck meeting the prepared mind – also helps. I mentioned this because much of the story that follows actually depended on the availability of this monomer.

My Ph.D thesis was on the azo derivatives of some aromatic poly-*a*-amino acids. One could even diazotize poly-*p*-aminophenylalanine and couple it to polymers containing tyrosine, resulting in colored water-insoluble compounds.

We could use as initiators of polymerization of N-carboxyamino acid anhydrides (the monomers from which polyamino acids were built), not only monofunctional small molecules, but also macromolecules possessing several amino groups. If proteins were used as such polyvalent initiators, we ended up with polypeptidyl proteins, whereas, when polylysine or polyornithine were used, we had for the first time multichain polyamino acids.

3. Ribonuclease

After five years of working with protein models, I felt ready to go abroad for a postdoctoral period to work with proteins. Thus, I arrived in 1956 in the laboratory of Chris Anfinsen at the NIH in Bethesda. The friendship between us resulted in prolonged stays in Bethesda in 1956–1957, 1960–1961, and 1973–1974. On his part, Chris came to Rehovot on sabbaticals on several occasions and was an active, extremely valuable member of the Board of Governors of

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the Weizmann Institute of Science, serving for many years as the Chairman of the Scientific Advisory Committee. His death in May 1995 was for me personally a deeply felt loss. Chris was a great friend, helpful to all those who surrounded him, full of charm and modesty, and actually a great romantic.

From the National Institutes of Health we sent out our first joint paper, which Chris was in a hurry to prepare for a Festschrift honoring Linderstrom-Lang. We finished it before I managed to get my USA driver's license or rent an apartment. The topic was the selective splitting of protein chains by trypsin at arginine residues, after lysine residues were reversibly blocked by benzyloxycarbonyl groups.

Oxidative opening of the four disulfide bridges of bovine pancreatic ribonuclease, to permit its sequencing, was possible only because tryptophan was absent in this protein. A more general method, which could be used also for a protein containing tryptophan, such as lysozyme, was reductive cleavage followed by blocking of the sulfhydryl groups with iodoacetic acid. I was fortunate to participate with Chris in these studies and left part of the reduced ribonuclease without blocking its sulfhydryl groups to see if it could reoxidize properly, and whether the enzymatic activity would come back. The results of these experiments showed a total recovery of the activity, even though statistical considerations pointed to 105 various ways in which the four disulfide bridges could reform. Thus, we demonstrated that no additional information was needed for the correct, unique architecture of a protein molecule and that it is the genetic code dictating the amino acid sequence that is responsible for the conversion of the randomly coiled structure.

During my visit in Bethesda, I tried in two ways to combine my previous experience with polyamino acids and research on enzymes. One was the use of copolymers of glutamic acid with aromatic amino acids to efficiently inhibit ribonuclease, lysozyme, or trypsin. The other series of studies had to do with poly-DL-alanylation of proteins. In contrast to poly-L-alanine and poly-D-alanine, poly-DL-alanine, which is a random copolymer of L-alanine and D-alanine, is well soluble in water and may serve as a solubilizer. Poly-DL-alanyl ribonuclease could be reduced and reoxidized properly. Poly-DL-alanylation renders gliadin water soluble and converts myosin into a derivative soluble in distilled water and with all its ATPase activity preserved.

4. Immunology

Coming back to peptidyl proteins, it was easy to take gelatin (considered to be not antigenic at the time) and attach peptides of tyrosine by polymeric techniques. The resulting polytyrosyl gelatin became a very good antigen. This is when I first used the expressions “immunogen” and “immunogenicity.” I said that we have to distinguish between two properties: immunogenicity and antigenic specificity. Attaching just 2% tyrosine converted gelatin into a strong antigen, and the antibodies made were against gelatin. Attaching 10% tyrosine converted it into a very good antigen, but all the antibodies made were against tyrosine peptides.

As a result of the studies on tyrosylated gelatin, we assumed that gelatin was not necessary for immunogenicity. We, therefore, replaced the gelatin with multichain poly-DL-alanine as the carrier for peptides of tyrosine and glutamic acid and showed that the resulting copolymer, denoted (T,G)-A-L, led to specific, precipitable antibodies in experimental animals. We synthesized numerous linear and multichain polyamino acids and tested them for immunogenicity. Our preliminary communication on a “synthetic antigen” was rejected by *Nature* under the pretext that the journal does not publish papers that are part of a series, so we published it elsewhere. The final paper became a Citation Classic.

(*Current Contents* 1986). The availability of synthetic antigens permitted a systematic elucidation of the molecular basis of antigenicity. 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 immuno-genic only when they were administered in minute amounts and they led to no secondary response. Their immunogenicity was thymus-independent, as was that of several other polymers such as linear (Pro-Gly-Pro)*n* and multichain polymers of L-proline locked in with terminal polymeric side chains of.

D-Phe and D-Glu. The common denominator of all these “thymus-independent” antigens was that they not only possessed repeating antigenic determinants but they also were metabolized slowly, if at all.

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 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 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 @-helical structure. In addition we could demonstrate for the first time, by circular dichroism, how antibodies to the a-helical polymer could help transconform into a helical shape a small polymer that was not yet helical.

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. 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. In this connection, it should be remembered that partial degradation products of proteins may still be immunogenic. Furthermore, the sera we were investigating might have contained a myriad of antibodies against degradation products derived from the original immunogen.

5. Thyroxine

In 1955 I was back in Europe as a young scientist presenting the work on multichain polyamino acids at the International Congress of Chemistry in Zurich, and a week later, the work on spectrophotometric titration of polymers and copolymers of amino acids at the International Congress of Biochemistry in Bruxelles. This was a wonderful occasion to meet scientists whom until then I had known only by reputation and through their papers. Among the many, I want to mention two, Hans Neurath and Bill Harrington,

who later became close friends of mine. During the same trip, I went to see Sir Charles Harington in London. Harington was the man who elucidated the structure of thyroxine and predicted that it is formed from two diiodotyrosine residues by oxidation, rather than simple dismutation. I wanted to tell him that the availability of polytyrosine had permitted us to find out that, following alkaline incubation of iodinated polytyrosine, the hydrolysate contained 2% thyroxine and 2% serine. We had thus proved the validity of his prediction.

Actually, for a time there was a kind of "touch-and-go" as to whether I would go into immunology or into the world of thyroid, which I never continued.

6. Genetic code

As a result of studying the mechanism of polymerization of *N*-carboxyamino acid anhydrides with Arieh Berger, I was involved in an interesting story that I want to relate here while discussing polyamino acids. On my second visit to the National Institutes of Health (NIH) in 1960, Marshall Nirenberg came one day and asked me whether I had some poly-L-phenylalanine and whether I knew its solubility properties. I did not have the polymer in Bethesda, but I did ask Nirenberg why he was interested. Through these conversations I became one of the first to know about the breaking of the genetic code, UUU encoding Phe. Although I was somewhat skeptical of the story, I immediately looked for and found hidden somewhere in an experimental section of a paper in the *Journal of the American Chemical Society*, that poly-L-phenylalanine was insoluble in all the solvents we had tested with the exception of a saturated solution of anhydrous hydrogen bromide in glacial acetic acid. Because on that very day I was preparing just such a solution (used to remove carbobenzoxy groups) in the laboratory, I gladly gave the reagent to Nirenberg and was touched and surprised when he acknowledged this in the classical paper that resulted in his receiving the Nobel Prize. The real point of the story lies elsewhere. Why did we try to use such a peculiar solvent? The truth of the matter is that years earlier, together with the late Arieh Berger in Rehovot, we were investigating the mechanism of polymerization leading to linear and multichain polyamino acids. One day I had two test tubes, one with polyphenylalanine and one with poly-carbobenzoxylysine, stuck in a beaker on my desk. Arieh came to decarbobenzoxylate the lysine polymer, a reaction with hydrogen bromide in glacial acetic acid during which carbon dioxide is released. He took the wrong test tube away with him and returned, puzzled because the material had dissolved and he could not see any evolution of carbon dioxide. At once we realized the mistake and I noted in my laboratory book that, at long last, we had found a solvent for poly-L-phenylalanine.

7. 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.

In our studies 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 multichain synthetic polypeptide we investigated, possessed small amounts of tyrosine, histidine, or phenylalanine at the tips of their polymeric side chains. 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 strains investigated.

Some time later, using these multichain polypeptides, Hugh McDevitt 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. Not surprisingly, a very large proportion of studies using synthetic polypeptides in immunology has been devoted to this field of research.

8. Viroimmunoassay

I would also like to mention here work with Joseph Haimovich, on a viro-immunoassay, using chemically modified bacteriophage to detect minute amounts of antigen and antibodies to T4 bacteriophage haptens, such as dinitrophenyl and arsanil, small molecules such as penicillin, prostaglandin, angiotensin and nucleosides, and various peptides, as well as macromolecules, such as proteins and nucleic acids, were covalently attached. Some of the bacteriophages thus modified were covalently attached. Some of the bacteriophages thus modified were still viable and could be neutralized by antibodies to the molecules attached. The technique was extremely sensitive for the detection of antibodies. Moreover, antibody inactivation of the bacteriophage could be inhibited by free hapten molecules and thus could serve as a most sensitive technique for their detection and quantitation.

9. Antigen transformation

A detailed study of the antibody combining sites to a series of peptide determinants of increasing size and defined structure, led us to the conclusion that the size of the combining sites was in all cases such as to accommodate four amino acid residues, and that the most exposed portion of the epitope plays an immunodominant role. In two interesting studies,,we could show how the combining site of the antibody can transconform the structure of the antigen.

We showed how antibodies to the *a*-helical polymer could help transform into a helical shape a small polymer that was not yet helical. We could also demonstrate that the Fab of an antibody to *p*-azobenzenearsonate hapten may "suck out" the *p*-azobenzenearsonate moiety from its unavailable conformation within an ordered copolymer and convert it into another conformation, recognized by Fab.

10. Support of selection theory of antibody formation

Poly-L-alanine and poly-D-alanine are utterly insoluble in water alpha-helices. On the other hand, poly-DL-alanine, a random copolymer of L-alanine and D-alanine is not only well soluble in water, but also a great solubilizer. Thus, in a poly-DL-alanylated antibody, one can open all the disulfide bridges, and it does not drop off solution, as does unmodified gamma-globulin. Upon reoxidation, we found most of the activity recovered, both as an antigen (immunoglobulin) and as an antibody, thus giving strong support to the selective theory of antibody formation.

11. Synthetic vaccines

I mentioned earlier, while discussing the role of steric conformation in defining antigenic specificity, that we had prepared a totally synthetic antigen capable of provoking antibodies reacting with native lysozyme. These studies led to the inevitable conclusion that a new approach to vaccination was possible. We reasoned that synthetic vaccines might by a reality in the future, for the simple reason that if these conclusions held for one protein, they might hold for others, including viral coat proteins and bacterial toxins. Of course, it is not sufficient to have just a synthetic epitope that will provoke antibodies to the protein. I shall not repeat here the arguments I have made before as to why there is a place for improvement of vaccines today, but for a synthetic vaccine to be successful, it should contain at least five ingredients: (a) specificity; (b) built-in adjuvanticity; (c) the correct genetic background; (d) the capacity to cope with antigenic competition; and (e) the correct "texture", i.e. a form that will give persistent and long lasting immune protection. Much of the experimental work was done in collaboration with Ruth Arnon and various other colleagues.

We first synthesized a peptide from the amino terminus of the carcinoembryonic antigen of the colon; this showed a weak cross-reaction with the intact antigen. The first study related to viruses was the synthesis of a peptide from the envelope of the MS2 bacteriophage.The synthetic peptide inhibited phage neutralization by antiphage antibodies, and the same peptide elicited antibodies capable of neutralizing the virus after attachment to multichain poly-DL-alanine. Similarly, Ruth Arnon succeeded in preparing a conjugate of a synthetic peptide derived from influenza hemaglutinin,and it provoked antibodies and protected mice against influenza challenge. With Chaim Jacob, we showed that tetanus toxoid coupled with synthetic peptides of the B subunit of holera toxin led to the formation of antibodies capable of neutralizing the toxic activity of the native cholera toxin. Actually, some antibodies inhibited the entire spectrum of activities of the intact cholera toxin, including adenylate cyclase induction and intestinal fluid secretion. Attachment of a peptide composed of residues 50–64 within the sequence of B subunit of cholera toxin to our multichain polymer (T,G)-A-L produced a totally synthetic vaccine, which elicited in rabbits antibodies with neutralizing capacity.

12. The Odyssey of copaxone (copolymer 1 – glatiramer acetate) against multiple sclerosis

Of special interest was the immune response to lipid components which, due to solubility problems, was not easy to either elicit or investigate. However, conjugates in which synthetic lipid compounds were attached onto synthetic copolymers of amino acids elicited specific response to lipids such as cytolipin H, which is a tumor-associated glycolipid, or sphingomyelin. Furthermore, we demonstrated that both the sugar and lipid components of such molecules contributed to their immunological specificity. The resultant antilipid antibodies were capable of detecting the corresponding lipids both in water-soluble systems and in their physiological milieu. This was fascinating, since 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.

I would now like to come to a story started in 1967 which ultimately led to a drug against MS that was approved by the Food and Drug Administration in the USA in December 1996.

13. Beginnings of copolymer 1 in experimental allergic encephalomyelitis (EAE)

After extending our studies with Ruth Arnon on synthetic polyeptide antigens to synthetic antigens with lipid specificity, it was not surprising that I thought that EAE, 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., phospholipid). My conclusion was to prepare several positively charged copolymers of amino acids, and see whether we could induce EAE when the copolymers were administered into experimental animals (guinea pigs and rabbits), in complete Freund's adjuvant, similar to the successful administration of MBP. Ruth Arnon was a partner from the beginning, and we took Dvora Teitelbaum as a Ph.D student, and she continued with us until her early demise. This was the original trio in the long path to the development of copolymer 1 (Cop 1), as the drug against the exacerbating-remitting type of MS, which was approved by the Food and Drug Administration 29 years after the original concept. Only after we failed to induce the experimental disease did we try to see whether our copolymer could prevent or suppress the disease.

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. Later on, we could suppress the actual disease in rhesus monkeys and baboons. The copolymer 1 we 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 and at the T-cell level – 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 not generally immunosuppressive and 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.

Once the results of the clinical trials carried out in the United States by Murray Bornstein became available, and it was clear that Cop 1 was a candidate drug for MS, I called at some time in 1985, my friend, Ely Hurwitz, for many years the CEO of the Teva Pharmaceutical Industries in Israel, and suggested that I would come to their home for a small dinner, and that he should prepare a slide projector. I went with my wife Sara and we were received by Ely and his wife Dalia. We had a good look at the slides I had brought with me, and the rest is history. Ely took the decision to develop Cop 1 as a drug/vaccine against MS at a time when Teva was miniscule in comparison with what it is today. He had the vision and the courage. On the other hand, Teva had no experience in developing an innovative drug. This meant that it took much longer than expected, but luckily, Teva has increased its activities enormously and has become an international drug company of significant dimensions and has a very good reputation, and is a world leader in generic drugs.

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

14. Mode of action

We were obviously very interested in the mode of action of Cop 1. We know that the effect was specific for the disease and we assumed that it has to do with the immunological cross-reaction between the "villain" (MBP) and the drug (Cop 1). What we have learned later is that the Cop 1 binds almost immediately and strongly to the groove of MHC class II antigens of most genetic backgrounds, and it displaces efficiently from the groove any peptides derived from the MBP. This promiscuity is probably due to 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.

This is the first, necessary but not sufficient, step in the 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, due to suppressive determinants shared between MBP and Cop 1; and (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 MBP-specific TcR (a phenomenon called by immunologists the "T receptor antagonism").

We are all used to specificity when we think about vaccines against infectious diseases, but Cop 1 is the first successful specific drug (or should we call it a vaccine?) against an autoimmune disease. In both sets of diseases there is close chemical relatedness between the factor causing the disease and the one preventing it or suppressing it. In principle, for every autoimmune disease for which there is a candidate molecule that provokes it, it should be possible to find an analog that will prevent the exacerbation of the disease.

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 MBP and suppress experimental autoimmune encephalomyelitis. This was corroborated by clinical studies in MS patients. It was of interest to observe that Th2 suppressor lines and clones induced by Cop 1 cross-reacted at the level of Th2 cytokine secretion with MBP, but not with other myelin antigens. This bystander suppression may explain the therapeutic effect of Cop 1 in EAE and MS.

Both Cop 2 and MBP bind in a promiscuous way to different types of antigen-presenting cells of various H-2 and HLA haplotypes. Cop 1 exhibited the most extensive and rapid binding to antigen-presenting cells, and it was capable of inhibiting the binding of either intact MBP or its encephalitogenic epitope sequence, 84–102. Furthermore, Cop 1 was capable of displacing MBP from the cells, whereas once bound it could not be displaced. These results demonstrate that Cop 1 indeed competes with MBP for MHC binding and thereby inhibits the presentation of the latter to the TcR and consequently prevents T-cell responses to MBP.

It is evident that Cop 1 exerts its activity by an immunomodulating effect. It is clear that Cop 1 affects EAE and therefore, by extrapolation, MS, at various levels of the immune responses involved, which differ in their degree of specificity. The binding of Cop1 to the MHC class II molecules, which is a prerequisite step for any further involvement, is the least specific event. 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-THR ("the immunological synapse"), namely, as interference with the presentation of the encephalitogenic antigen to the TcR, which is a specific interaction.

Recent work by Rina Aharoni and her colleagues has shown, in the model disease EAE, that copaxone delays demyelination and even causes remyelination. The fact that at the present time, close to 300,000 patients in 50 countries receive the drug and stopped having the atttacks almost completely, is a reason for profound satisfaction.

15. Myasthenia gravis

Multiple sclerosis is mainly a T cell-mediated disease, whereas, in myasthenia gravis the attack of specific antibodies on the acetylcholine receptor (AChR) is the accepted cause of the disease. Nevertheless, assuming that most antibody responses need helper T cells, we have synthesized two immunodominant myasthenogenic T cell epitopes (p195-212 and p259-271), derived from an *a*–subunit of the nicotinic AChR. Ideally, the goal of therapy for MG should be the elimination of autoimmune responses to the AChR specifically, without interfering with immune responses to other antigens. To this end, the dual analog composed of the tandemly, reciprocally arranged two single analogs of p195-212 and p259-271, namely Lys-262-Ala-207, was prepared and shown to efficiently inhibit the proliferation of T cell lines specific to the myasthenic peptides and of lymph node cells primed *in vivo* to either of these peptides. The dual analog specifically inhibited *in vivo* T cell stimulation to either myasthenogenic peptide in >90% of the responding MG patients. The dual analog interferes with specific autoimmune responses, and when administered orally, the dual analog could treat experimental allergic myasthenia gravis induced in mice by immunization with the multideterminant native *Torpedo* AChR. Moreover, it had beneficial effects on the clinical manifestations characterizing EAMG. Thus, the dual analog is an efficient immunomodulator of EAMG in mice and could be of specific therapeutic potential for MG.

Regrettably, this study did not reach the clinic, because of the opinion of marketing people in the industry.

16. Cancer Research

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*. The Fab dimers were almost as effective as intact antibodies. Daunomycin linked to anti-tumor antibodies penetrated the cell membrane at a higher rate than daunomycin linked to dextran or to normal immunoglobulin. Together with Japanese colleagues, we could show a chemotherapeutic effect against hepatoma in rats.

With Meir Wilchek and Bilha Schechter we showed indirect immunotargeting of cis-platinum to a human epidermoid carcinoma, using the avidin-biotin system. The biotinylaated antibody was attached to the cancer cell, and this was followed by cis-platinum attached to avidin, 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. A strong synergistic effect was observed when the anti-ErbB1 antibodies were administered together with cis-platin.

This became of practical importance as the patent for the synergistic effect of anti-ErbB1 and small chemotherapeutic drugs is used by industry.

For the last 25 years I have been collaborating with Yossi Yarden, who has been working nn the family of ErbB receptors and their ligands. We produced and investigated antibodies against these interesting protooncogene products. ErbB2 (also known as HER-2/Neu) is a tyrosine kinase, and its dense appearance is correlated with a poor prognosis in breast cancer. The antibodies formed either inhibited or accelerated the tumorigenic growth of ErbB2-transfected fibroblasts in athymic mice. Suppression and promotion of tumor growth by monoclonal antibodies to ErbB2 differentially correlated with cellular uptake. ErbB2 has no known efficient ligand but has tyrosine kinase activity. On the other hand, ErbB3 has a ligand, heregulin, but has no tyrosine kinase activity. When they heterodimerize, the dimer is an efficient and active receptor. ErbB2 heterodimerizes also with ErbB1 (epidermal growth factor receptor) and with ErbB4.

We found a synergistic effect of anti-cancer effect when we used combinations of two anti-EGFR antibodies, provided they are not against the same epitope.

In recent years, the realization of a synergistic effect of two anticancer drugs, an antibody and a small chemotherapeutic drug, or two antibodies to the same receptor, led to our studies on pancreatic carcinoma.

Due to intrinsic aggressiveness and lack of effective therapies, prognosis of pancreatic cancer remains dismal. We assumed (with Yosi Yarden) that agents targeting EGFR and/or HER2 would effectively retard pancreatic ductal adenocarcinoma. Accordingly, two immunological strategies were tested in animal models: Firstly, two antibodies able to engage distinct epitopes of either EGFR or HER2 were separately combined, and secondly, we tested pairs of one antibody to EGFR and another to HER2. Unlike the respective single monoclonal antibodies, which induced weak effects, both types of antibody combinations synergized in animals in terms of tumor inhibition.

Aptamers, oligonucleotides able to avidly bind cellular targets, are emerging as promising therapeutic agents, analogous to monoclonal antibodies. We selected from a DNA-library an aptamer specifically recognizing ErbB-2/HER2, a receptor tyrosine kinase, which is overexpressed in a variety of human cancers, including breast and gastric tumors. Treatment of human gastric cancer cells with a trimeric version (42 nucleotides) of the selected aptamer (14 nucleotides) resulted in reduced cell growth *in vitro*, but a monomeric version was ineffective. Likewise, when treated with the trimeric aptamer, animals bearing tumor xenografts of human gastric origin, reflected reduced rates of tumor growth. The antitumor effect of the aptamer was nearly twofold stronger than that of a monoclonal anti-ErbB2/HER2 antibody (WithYossiYarden).

Could aptamers one day replace antibodies in fighting diseases?

17. Concluding Remarks

I have always been driven by curiosity and search. I know that there are individuals who prefer to be alone all the time, and may be through daydreaming they reach all their working hypotheses, but I am a great believer in the interaction and in the fertilization of ideas. I have been collaborating with many colleagues around the world, and I have always been keen on having many visiting scientists spending extended periods of time in my laboratory. As for theories, they are very good as working hypotheses, as long as you do not take them too seriously, but they are dangerous when they become dogmatic. Thus, what drove me all my life was curiosity, optimism, perseverance and the search for truth. Serendipity, when luck meets the prepared mind is also of help.

Close friendship with my mentor, Ephraim Katzir, until his demise, as well as similar close friendship and collaboration with Ruth Arnon, who was my first Ph.D. student, were characteristic of my whole scientific career.

18. Research articles

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