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Structural Components Responsible for Peptide Antigenicity

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Abstract

The conformation of a peptide defines its antigenic specificity. In most cases, a free peptide is in a random form, whereas the same peptide within a protein has a defined conformation. Immunological cross-reactions between the two are rare. Nevertheless, there are cases where an anticonformational antibody may transconform (change the conformation of) the free peptide, allowing the reaction to occur.

Based on such considerations, synthetic vaccines against infectious diseases may be constructed. The same is true for autoimmune diseases, where, at least in one case—that of the exacerbating-remitting type of multiple sclerosis—we have developed a polypeptidic drug-vaccine, copolymer 1 (denoted Copaxone), capable of suppressing the autoimmune phenomena in a specific way. This drug-vaccine has been approved for treatment by the US Food and Drug Administration (FDA).

Index Entries: Copolymer 1; multiple sclerosis; immunogenicity; conformation.

Antigenic Specificity of Peptides, Proteins, and Haptens

The term "antigenicity" has often been used to describe both the ability to elicit antibodies and the capacity to react with antibodies. For the sake of clarity, I distinguish between two separate concepts: "immunogenicity," which I define 1) as the capacity to provoke an immune response and which is independent of the specificity of the antibodies formed, and 2) "antigenic specificity," which is reflected in the nature of the antibody-combining site. Only rarely do peptides derived from a protein cross-react immunologically in an efficient way with the parent protein. This happens only if the peptide, which is random, is derived from a stretch of the protein that is in a completely random form, or if the peptide persists in keeping a unique conformation while in the free form, very similar to the conformation it possesses when within the protein. More often, antibodies to the free peptide will not cross-react with the protein, and antibodies

to the peptide epitope, which is conformationally constrained within the protein, will not react with the free peptide. As I will demonstrate, there is a third possibility—that of a peptide being able to transconform itself to fit the antibody-combining site. In cases where the antibody can help constrain the shape of the peptide, the actual antigenic cross-reaction tells us not what the conformation of a peptide is, but into what shape it is able to transconform.

Hapten–protein conjugates may be small haptens, less than the size of a complete epitope, or big haptens, e.g., sizable peptides that may be bigger than an epitope, but—more importantly—may react in various ways with portions of the protein to which they are covalently attached.

Antibodies produced in response to immunization with such a synthetic conjugate may be classified into four main categories in terms of specificity: 1) Antihapten antibodies, operationally defined as those which react with hapten alone or with the hapten conjugated to another carrier. Such antibodies may be further subdivided into: a) those that have combining sites just large enough to accommodate the hapten group and little else or b) those in which the hapten plays an immunodominant role although it is smaller than the total determinant group. 2) Antiprotein antibodies. Operationally, this is the population of antibodies that reacts with homologous protein to which no hapten is conjugated. 3) Antibodies directed against determinants "shared" by hapten and protein. In these antibodies the haptenic group no longer plays an immunodominant role. They can either a) react with protein alone or hapten alone, i.e., free hapten or hapten conjugated to a different carrier, or b) be incapable of doing either of the above, but react only with a determinant made up of part of the hapten and part of the carrier. 4) Antibodies against protein determinants formed as a result of the conjugation of hapten to protein. These antibodies are distinguished by the fact that they will not react at all with the protein, but will react very well with the hapten–protein conjugate. This reaction, however, is not inhibited by hapten. Such antibodies are most probably directed toward determinants produced by conformational changes in the protein molecule, which were induced by attachment of the hapten at some distant site.

The Role of Conformation in Peptide Specificity

Many years ago we made a clear distinction between sequential and conformational epitopes *(2)*. Sequential determinants are segments of a peptide, oligosaccharide, or oligonucleotide, whereas conformation-dependent epitopes are caused by a juxtaposition of atoms in space, which results from the higher-order structure of the macromolecule (e.g., protein).

When the tripeptide Tyr-Ala-Glu was attached to multichain poly-DL-alanine (multi-poly-DL-alanyl–poly-L-lysine), and the resulting macromolecule was used for immunization, antibodies were formed against the attached tripeptide (sequential epitopes). When the same tripeptide

was polymerized to yield poly(Tyr-Ala-Glu), this polymer possessed an α-helical shape in aqueous solution, and antibodies formed against it did not react with the tripeptide or even oligomers in which the tripeptide was repeated several times (conformational epitopes) *(3)*. Even higher oligomers were not yet helical *(4)*, but transformed into a helical shape when reacted with the antihelical antibody cavity *(5)*.

Vaccines Against Infectious Diseases

Following a clear distinction between sequential and conformation-dependent epitopes, we showed that the attachment of the synthetic "loop" peptide of lysozyme to a multichain poly-DL-alanine carrier results in a conjugate that elicits antibodies reacting with the intact lysozyme *(6)*. The reaction occurs in a unique region within the native protein (the "loop" region), and is conformation-dependent. The inevitable conclusion of these studies is that a new approach to vaccination is possible, for the simple reason that if this holds for one protein, it may hold for others, including viral coat proteins and bacterial toxins.

The concept of synthetic vaccines must include the ability to produce immunogenic molecules capable of provoking antibodies and specifically sensitized cells of the appropriate specificity to induce protection as well as several other "ingredients," crucial to any strategy for the development of new vaccines. These should include attempts toward built-in adjuvanticity, consideration of the genetic background of the species immunized, concern about the possibility of antigenic competition, and efforts to obtain prolonged immunity.

The synthesis of the epitopes desired for the vaccines may be done through chemical means or by genetic engineering. Most of the studies in our laboratory have been devoted to the chemical approach. In early studies we showed that it is possible to prepare synthetic antigens provoking antibodies capable of neutralizing a virus—namely, MS2 bacteriophage *(7)*—thus demonstrating for the first time the feasibility of the approach.

Actually, an antiviral response induced by a peptide was reported as early as 1963. Using a natural hexapeptide obtained from an enzymatic digest of tobacco-mosaic virus protein, conjugated to bovine serum albumin, Anderer succeeded in eliciting antibodies with a limited inhibitory capacity toward the infectivity of the virus. Ruth Arnon and colleagues have shown that such a response can be elicited by synthetic peptides and their conjugates, such as in the case of influenza (quoted in ref. *7a*).

In the last 15 years, synthetic antigens have been prepared that are capable of producing antibodies to neutralize bacterial toxins, such as diphtheria *(8)* and cholera *(9)*. Furthermore, using these systems as experimental models, we also showed that it is possible to prepare conjugates in which the appropriate synthetic epitope related to the biological system investigated, and a synthetic adjuvant, MDP (N-acetylmuramyl-D-alanyl-L-isoglutamine), are attached covalently to the same polymeric carrier, and

that the resulting conjugate, when administered in aqueous solution, leads to neutralizing antibodies *(10–12)*. These findings demonstrate that it is possible to design synthetic peptide vaccines with built-in adjuvanticity.

Vaccines Against Autoimmune Diseases

Vaccines against infectious diseases are known to be highly specific. We have extended this concept to autoimmune diseases: whenever it is possible to identify the putative cause of the diseases, it should be possible to find a close molecular analog that will combat the disease. Indeed, Cop 1—a synthetic amino-acid copolymer prepared by polymeric techniques—is related immunologically to the myelin basic protein (MBP), a substance in the myelin sheath of the brain that seems to be the main cause of the autoimmune phenomena in multiple sclerosis (MS). It all began as basic research into the mechanisms involved in the induction and suppression of experimental allergic encephalomyelitis (EAE), which is the primary animal model for MS. EAE is an acute neurological autoimmune disease mediated by CD4+ autoreactive T cells, which recognize the encephalitogenic antigen(s) in association with major histocompatibility-complex (MHC) class II molecules. These autoreactive cells migrate into the central nervous system (CNS) and mediate the pathogenic process.

Our approach to the study of EAE and its suppression was the synthetic one, using copolymers of amino acids whose composition resembled that of natural MBP to a certain extent, in order to stimulate its ability to induce or suppress EAE. None of the copolymers proved to be encephalitogenic, even after conjugation with brain lipids, but some—particularly Cop 1—showed high efficacy in suppressing EAE *(13)*.

Cop 1 is a synthetic amino-acid copolymer composed of L-alanine, L-lysine, L-glutamic acid, and L-tyrosine in a residue molar ratio of 4.2:3.4:1.4:1.0. It was shown to suppress EAE induced by MBP in a variety of animals, including guinea pigs, rabbits, mice, and two species of monkeys—rhesus monkeys and baboons *(14)*. The results clearly indicated that there was a remarkable degree of suppression of EAE by Cop 1 in all species studied, although different encephalitogenic determinants of MBP were involved in disease induction in the different species. Indeed, our studies have shown that the suppressive effect of Cop 1 in EAE is a general phenomenon and is not restricted to a particular species, disease type, or the encephalitogen used for EAE induction *(15,16)*.

Immunological Cross-Reactivity Between MBP and Cop 1

Since EAE is autoimmune in nature, and its pathogenicity involves T cells sensitized to MBP, the specific inhibition by Cop 1 may be explicable in terms of an immunological cross-reaction between Cop 1 and MBP. Studies have been performed to test this hypothesis at both the cellular and humoral levels of the immune response.

Using monoclonal antibodies raised against MBP, we could demonstrate clearly that several monoclonal anti-MBP antibodies reacted with Cop 1 and vice versa *(17)*. At the cellular level, a marked cross-reaction was observed both in vivo and in the delayed hypersensitivity skin test, and in vitro by measuring lymphocyte transformation *(18)*. Of particular interest is the very good correlation between the extent of immunological cross-reactivity and suppressive effect on EAE of various materials. Thus, D-Cop 1—a polymer resembling Cop 1 in all parameters except that it is composed of D-amino acids rather than L-amino acids—does not cross-react with MBP, and has no suppressing activity whatsoever *(19)*.

Induction of Antigen-Specific Suppressor Cells

It was demonstrated that mice pretreated with Cop 1 in incomplete adjuvant became resistant to further EAE induction. This state of unresponsiveness could be adoptively transferred to normal recipients by spleen cells from Cop 1-treated donors, and the cells responsible for the suppressive activity were identified as T lymphocytes. Furthermore, we have demonstrated the generation of suppressor T-cell hybridomas and lines from spleen cells of mice rendered unresponsive to EAE by Cop 1. Both cell types produce in vitro inhibition of MBP-specific effector lines and in vivo inhibition of clinical EAE *(20)*. Recent results revealed that these T-suppressor cells secrete Th2 cytokines after exposure to either Cop 1 or MBP *(21)*. These cytokines may mediate the therapeutic effect of Cop 1 in disease induced not only with MBP, but also with other encephalitogens by the mechanism of "bystander suppression."

Proposed Mode of Action of Cop 1 in EAE and MS

Cop 1 affects EAE—and therefore by extrapolation MS—at various levels of the immune response involved, which differ in their degree of specificity. Binding of Cop 1 to the MHC class II molecules, which is the least specific step, is a prerequisite for its effect by any mechanism. 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, which are activated by suppressive determinants shared between MBP and Cop 1. This mechanism is a specific one and results from the cross-reactivity between Cop 1 and MBP. 2) Cop 1 can compete for binding to MHC class II molecules with several myelin-associated antigens, resulting in inhibition of antigen-specific T-cell effector functions (i.e. proliferation, interleukin secretion, and cytotoxicity).

This mechanism may be less specific, as MHC blockade may lead to interference with other immune responses. However, this does not seem to be the case, as Cop 1 did not inhibit responses to ovalbumin or lysozyme. Furthermore, D-Cop 1—which bound to MHC class II molecules as efficiently as Cop 1 and competed with MBP for binding—did not inhibit

MBP-specific T-cell lines, and did not inhibit EAE when coinjected with the encephalitogenic emulsion. These findings may suggest that nonspecific MHC blocking is a necessary process, but one which requires an additional step involving antigen-specific mechanisms such as induction of cross-reactive T-cell tolerance, or T-cell receptor antagonism. We have shown recently *(22)* that competition also occurs at the level of the T-cell receptor between the complex of MBP-derived peptides with class II MHC antigen, and the complex of Cop 1 with class II antigen. Regardless of the mechanism involved, the ability of Cop 1 to suppress disease—which is induced not only by MBP but by other myelin-associated proteins as well—is very important, since these antigens might be potential autoantigens in MS.

Clinical Studies with Cop 1 in MS

In view of the putative resemblance between EAE and MS and the assumption that MBP may be involved in the pathogenesis of MS, preliminary clinical trials using Cop 1 were conducted on MS patients. These were initiated after toxicity studies in experimental animals showed that Cop 1 was nontoxic following both acute and subchronic administration to mice, rats, rabbits, and beagle dogs, and that there was no significant uptake by any of the animal organs.

The results of the phase II trial on ER patients demonstrated a remarkable decrease in the number of relapses and rate of progression in Cop 1-treated patients compared with the placebo control *(23)*. After a successful pivotal multicenter phase III clinical trial *(24,25)*, which was conducted in 11 medical centers in the US and involved 251 patients, the FDA decided to approve Cop 1 ("Copaxone") as a treatment for MS. Copaxone has since been approved in Israel, Canada, Argentina, and several countries in Europe.

Studies on Vaccination Against Myasthenia Gravis

We approached a vaccination against myasthenia gravis within the same concept of specificity, but this time we worked with defined peptides. Analogs in which one amino acid has been replaced have been prepared for two of the most myasthenogenic T-cell epitopes, derived from the acetylcholine receptor (p195–212 and p259–271 in α-subunit). These analogs inhibited proliferation of the T cells, and suppressed or prevented experimental myasthenia gravis induced by the myasthenogenic peptides. A dual analog—a peptide containing both the inhibitory analogs—was prepared and found to be very effective in suppressing the experimental disease, even when given by the oral route *(26–28)*.

Concluding Remarks

The structure of a peptide is of crucial importance to its antigenic specificity, whether it is within the sequence of a protein or free in solu-

tion. This largely determines the choice of antibodies for a peptide reacting with the parent protein. Based on this knowledge, it is possible to prepare synthetic antigens that may serve as vaccines against infectious diseases, or fight autoimmune diseases. In the latter case the role of the "vaccines" is to lower the immune response. This rather novel approach to specific molecules whose efficacy is based on the molecular resemblance to the "troublemaker" provoking the disease has allowed us to develop a drug–vaccine—copolymer 1 (known as Copaxone)—that has been approved as a treatment for the exacerbating-remitting type of MS. We hope that a similar approach will be successful for other autoimmune diseases, and we are currently studying another autoimmune neurological disease—namely, myasthenia gravis.

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Discussion

Unidentified: What are the side effects of copolymer I?

Sela: Rashes in few persons for one in 300,000 injections.

Koentgen: You apparently block presentation.

Sela: It is not enough to block presentation. You must realize this is the first time that the polymer has been accepted as a drug. You have a lot of biopolymers used in formulations of drugs. But here it is the active ingredient. There are no two identical molecules in any batch. The biopolymer contains molecules belonging to a series, the DR series: DR1, DR2, DR4, and so on. All of the molecules react. Again, I always say the immunologists love erotic expressions like "promiscuity." We have been collaborating with Jack Strominger to study the reaction of cells with recombinant biopolymers, and all of the different molecules are reactive. The great question is, can we fractionate the biopolymer preparation, and will we have different reactivities for different polymers? The answer, at least at the level of amino-acid composition, will depend on what has been released from the DR1 group, DR2 group, DR4 group, and so forth.

Marchalonis: It's a fascinating point now. Art Vandenbark and Steve Brostoff have independently and separately been using peptides to treat multiple sclerosis—a CDR2 peptide and a CDR3-J peptide. Both work. We've been doing an opposite experiment to relieve immunosuppression using a peptide. The immunosupression does not work without amplification and promiscuity. The worst part, of course, is getting the FDA to approve what we want to do. But you have that behind you.