Superantigens and Their Role in Immune-Mediated Disease

Conrad Hauser* and Hans Acha Orbeat†

*Department of Dermatology, Hôpital Cantonal Universitaire, Geneva and †Ludwig Institute for Cancer Research, Lausanne Branch, Epalinges, Switzerland

The discovery of superantigens and their biologic effects has elicited much excitement in the scientific and medical communities. Because superantigens have the potential to interfere with recognition and elimination of conventional antigens and thus normal host defense or self-tolerance, intensive investigations on their physical properties and biologic effects have been undertaken and are currently being performed in many areas of fundamental and applied research including investigative dermatology. Here, we have therefore attempted to outline some basic properties of superantigens to see how the relationship between immune-mediated disease and superantigens could be investigated. It is not within the scope of this editorial/comment to review superantigens. The interested reader is referred to a recent series of excellent reviews [1].

Superantigens are gene products that are recognized by a large fraction of T cells. This recognition is mediated by the clonally distributed receptor for antigen, i.e., the T-cell receptor (TCR). The TCR of most T cells is composed of a heterodimeric αβ TCR that contains, in analogy to immunoglobulins, constant regions (MHC class II) and variable (V) regions. This allows the immune system to generate on the order of $10^{12}$ different clonally distributed TCR. Whereas the interaction of conventional antigens with the TCR requires multiple elements of both the α and β chains of the TCR, superantigens interact with a restricted set of TCR with distinct Vβ elements. This results in superantigens reacting with a large fraction of T cells, in some cases up to 20% of T cells. Some of these Vβ elements can be detected by monoclonal antibodies, whereas others must be identified by DNA sequence analysis. The genes for the TCR Vβ elements are located close together on the DNA and all share a significant sequence homology. Some of the identified superantigens have been determined to be microbial gene products. One class interacts with bacteria and has previously been characterized as toxins. For example, staphylococcal enterotoxin B (SEB) was identified in Staphylococcus aureus isolates associated with acute gastro-enteritis. The activity responsible for the gastrointestinal symptoms and the activity on T cells are not located on the same protein site but can be induced by chemical modification of SEB. Recombinant SEB reacts selectively with the murine TCR Vβ 7 and 8.1-8.3 elements and human TCR Vβ 3, 12, 14, 15, 17, and 20 elements. Other S. aureus-derived toxins well known to dermatologists are the toxic shock syndrome toxin-1 and the exfoliative toxin associated with the staphylococcal scalded skin syndrome. These toxins also possess superantigenic properties. Bacterial superantigens do not require processing by antigen-presenting cells to exert effects on T cells as do conventional antigens. They can directly bind to major histocompatibility complex (MHC) class II molecules on one hand and to the relevant Vβ chains on the other. This multimeric interaction results in T-cell response. Experiments with mutated MHC class II molecules gave evidence that bacterial superantigens bind to the outer side of the pocket formed by the MHC class II polypeptides. Conventional antigen is bound within the MHC pocket once it has been processed within the cell. Thus superantigens appear to require interaction with both MHC class II molecules and TCR Vβ elements, but do not display the typical MHC class II restriction seen with conventional antigens.

The investigation of another class of superantigens has recently resulted in a series of important findings. The minor lymphocyte stimulating (Mls) determinants of the mouse have puzzled immunologists for years. These determinants were discovered in 1970s by the mixed lymphocyte reaction because the frequency of responding T cells in a non-immunized population was on the order of 1:10 to 1:30. This was higher than the frequency of allo-MHC reactive T cells that were also investigated by the mixed lymphocyte reaction. The frequency of T cells for conventional antigens is on the order of 1:10^3 and thus can not be detected in a primary lymphocyte culture.

Although Mls reactivity required the presence of MHC class I+ cells, Mls determinants mapped outside the MHC. When it was discovered that Mls determinants mapped to the integration sites of the mammary tumor viruses (MTV), some light began to be shed on the Mls system. MTV are endogenous or exogenous retroviruses that integrate into the mouse genome and can cause mammary tumors. Today, there is good evidence that MTV are responsible for the superantigenic effects of all the known Mls determinants. Superantigenic effects could be induced in MTV+ sucklings fed with milk from mothers harboring a distinct MTV type. In addition, mice transgenic for MTV showed the relevant superantigenic effects. Recently, the ORF gene of MTV was transfected into B-cell lines and introduced into the germline to produce transgenic mice. The transfectants as well as the transgenic animals exhibited the expected superantigenic effects thus identifying the responsible MTV gene. MTV-dependent superantigenic activity was high in normal B cells but low or nondetectable in dendritic cells and macrophages. Only minute quantities of the ORF gene product could be identified at the surface of B cells. Comparing the sequence of the ORF gene of different MTV strains it has become clear that the extracellular carboxy terminal is polymorphic and correlates with Vβ selection. The precise molecular mechanisms of MTV-dependent superantigenic effects, including the requirement for processing of the ORF protein, remain to be elucidated. Cell-transfer experiments revealed that B cells as well as CD8+ T cells can induce superantigen effects in superantigen-negative recipients. The mechanism of the superantigen effect transferred by CD8+ T cells is for the moment not clear because these cells cannot directly stimulate T cells due to the lack MHC class II expression.

Reprint requests to: Dr. Conrad Hauser, Clinique de Dermatologie, Hôpital Cantonal Universitaire, CH1211, Geneva, Switzerland.

Abbreviations: Mls, minor lymphocyte stimulatory determinant; MTV, mammary tumor virus; SEB, staphylococcal enterotoxin B; V, variable.

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As T cells can recognize antigen only in physical association with major histocompatibility (MHC) gene products, investigators determined the MHC requirement for superantigen recognition. In general, it was found that MHC class II gene products were required for superantigen recognition, although recently activation of T cells in the presence of MHC class II* cell lines has been observed. In sharp contrast to the recognition of conventional antigen, no restriction to self-MHC was found even though preference for certain MHC class II isotypes and haplotypes was noted.

What are the effects of superantigen activation of T cells? In vivo experiments have shown that administration of superantigen or cells containing superantigens can have three principal effects that are selective for T cells with the relevant Vβ elements: 1) T cells can be deleted intrathymically or extrathymically; 2) T cells may expand in relative and absolute numbers; or 3) T cells may show altered responsiveness such as unresponsiveness to stimulation with conventional antigen also referred to as "anergy." The complexity of superantigen biology was further demonstrated when opposing effects on the relevant Vβ bearing T cells were observed depending on length of time since administration of superantigen. For example, the injection of SEB first leads to an expansion, subsequently to anergy, and finally to disappearance (deletion) of the relevant postthymic T cells. In vitro assays have been very extensively employed for the study of superantigenic effects. Depending on the conditions, activation and expansion, but also induction, of anergy have been observed in T cells. The response of T cells depends not only on their developmental and activational state but also on the accessory signals provided by the superantigen presenting cell, which may explain in part the variable responses of T cells to superantigens.

HOW CAN WE STUDY THE ROLE OF SUPERANTIGENS IN IMMUNE-MEDIATED DISEASE?

From the study of the biology of superantigens, a seductive hypothesis has emerged that superantigens originating from microorganisms may induce or contribute to T cell-mediated disease. One can imagine various ways in which superantigens may induce T-cell-dependent disease. Superantigens may lead to deletion or unresponsiveness of T cells that are crucial for host defense or tolerance. It is questionable whether such a mechanism exists because inbred mice carry multiple MTV with deletion of the relevant self-reactive T cells are activated by superantigens and thus induce or contribute to disease. Finally, it can also be hypothesized that normal T cells become functionally altered. Such altered T cells may not be capable of fulfilling their normal function in host defense or self-tolerance, thus causing disease.

Although there is no fully convincing report that animals exposed to superantigen develop T-cell-mediated disease, this should nevertheless be further tested. Today, it is not clear whether a superantigen encounter results in unresponsiveness or whether the function of superantigen-activated T cells can be rescued by presentation of conventional antigen thus resulting in disease such as autoimmunity. To address the potential roles of superantigens in autoimmune diseases, one should take advantage of the simple experimental models of autoimmune and the excellent transgenic mouse models available. Conditions with high susceptibility to autoimmune disease may be required for such experiments because administration of superantigen may only induce disease in animals with other factors or genes that favor the development of autoimmunity. For example, in chronic relapsing experimental allergic encephalomyelitis (EAE), a varying percentage of mice injected with the autoantigen myelin basic protein develop disease. Because the disease-causing T cells express Vβ8.2, SEB can be used to address the question of whether a superantigen can trigger autoimmunity in mice that are prone to autoimmune disease. In addition, in these mice, the question can be asked whether animals that have recovered from the first attack of EAE can be forced into relapse with superantigen. To cite one example of transgenic mice useful for such an analysis, the effect of superantigens can be tested on animals that are transgenic for a TCR that recognizes an autoantigen on the surfaces of the pancreas and crossed with SEB [6]. If in such models the autoimmune disease is dependent on the relevant T cells, further evidence for a role of superantigens in autoimmunity could be collected.

To date, however, there is only limited evidence that T cells activated by superantigen have the potential to mediate disease. T cells expanded in vitro with superantigens induce inflammation when injected into naive animals [3]. The possibility that T cells activated in vivo by superantigen may not have the same properties as T cells activated in vitro limits the value of this finding.

In clinical settings, cause-effect relations can often not be tested directly. Therefore, the question arises of what criteria should be fulfilled to postulate a cause-effect relation between superantigen derived from microorganisms and immune-mediated disease. We propose that the following correlations should be analyzed when a cause-effect relationship is postulated for superantigens and human disease. 1) A good correlation between the induction or exacerbation of a T-cell-mediated pathology and the identification of a superantigen-producing microorganism(s) has to be documented. 2) The suspected microorganism(s) should be isolated and the Vβ-selective effect of its (their) superantigen(s) determined. 3) The T-cell effects induced by the superantigen(s) from this (these) microorganism(s) have to be correlated with the disease-associated T-cell abnormalities. In the case of deletion or expansion of T cells with distinct Vβ subsets, this may be possible by determining the relative number of T cells carrying defined Vβ determinants using a panel of appropriate monoclonal antibodies and comparing them with expected numbers. In the case of nonresponsiveness of T cells, reduction of interleukin-2 secretion after monoclonal antibody crosslinking of the TCR could be measured. Determination of disease-associated T-cell numbers may not yield clear-cut results when multiple superantigens and multiple Vβ families are involved. In addition, simple determination of relative cell numbers does not detect superantigen-induced functional alterations within a distinct Vβ subset causing disease. Moreover, if the Vβ-selective effect is anatomically compartmentalized it may not be detected easily. 4) Exposure to superantigen derived from the suspected infectious agent should correlate with flare or induction of disease as well as with the expected alteration in T cells. Clearly, the difficulty lies in the establishment of the entire network of correlations. One has to bear in mind that a lack of correlation may not disprove the role of superantigen in T-cell-mediated disease. The crucial step that is waiting to be taken is the demonstration that superantigen-induced quantitative and/or qualitative alterations of T cells may induce or exacerbate disease.

Examples of diseases in which superantigenic effects have been suspected to be operative are rheumatoid arthritis [4,5], human immunodeficiency virus infection [6], and Kawasaki's disease [7]. Furthermore, administration of superantigen has been observed to induce arthritis flare in a model of streptococcal cell wall-induced arthritis [8]. Acute guttate psoriasis may be preceded by a streptococcal infection and the skin of patients with atopic dermatitis is heavily colonized by S. aureus. Because T cells are suspected of playing a crucial role in both disorders, superantigens may play a precipitating or aggravating role. However, evidence published to date for streptococcal or staphylococcal superantigenic effects in these disorders is scant [9-11]. As more monoclonal antibodies to various human Vβ families of the TCR are becoming available further studies on this topic are expected in the near future. It is possible that the symbiosis of superantigen-producing microorganism and host may not result in immediate and overt disease. Because this interaction has survived evolution, it may provide an advantage for selection. It will be the task of future investigations undertaken by basic and clinical scientists to further elucidate the relationship between superantigen and T-cell-mediated immunopathology.
REFERENCES