Superantigens produced by infectious pathogens: molecular mechanism of action and biological significance

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Received: 7 March 1994

Summary. "Superantigens" have in common an extremely potent stimulatory activity for CD4*, CD8*, and some γδ+ T lymphocytes. Superantigens use a unique mechanism: they crosslink variable parts of the T cell receptor with MHC class II molecules on accessory or target cells. The interaction site on the T cell receptor is the variable part of the β-chain (Vβ). There are several reasons why these molecules have aroused such tremendous interest in recent years. First, they have provided key information on tolerance mechanisms, both on the deletion of T cells in the thymus and on the induction of peripheral tolerance by anergy and apoptosis. Second, of all polyclonal T cell stimulators they are the ones that most closely mimic the recognition of specific antigen. Finally, they have been recognized as important factors in the pathogenicity of the producing pathogens, inducing shock and immunosuppression. Moreover, it has been postulated that superantigens could be involved in the pathogenesis of certain human diseases.

Key words: Superantigens – Infectious pathogens – Mechanism – Biological significance

Superantigens of Gram-positive cocci

The enterotoxins and the toxic shock syndrome toxin-1 (TSST-1) of Staphylococcus aureus and the streptococcal pyrogenic (erythrogenic) exotoxins (SPE) A and C of Streptococcus pyogenes form a family of genetically related exotoxins of 21–30 Kilodaltons (Kda) [1]. The SE have been characterized by reactivity with antisera and are divided into five different serotypes [2]. Most of the toxins do not show any serological crossreactivity. Some of the toxins have strong sequence homologies, e.g., SEA and SEE are more than 80% homologous in their amino acid sequence, whereas TSST-1 is hardly homologous to any other of the toxins [1]. Many of the genes encoding these proteins are present on mobile genetic elements, possibly the reason for their presence in two different Gram-positive bacteria. In fact, certain streptococcal toxins are more related to certain enterotoxins than enterotoxins among themselves. In addition to these long-known exotoxins described, there are a number of novel members of this family that have only recently been detected [3, 4]. The polymorphism of the SE and SPE is probably much higher than presently appreciated, as revealed by sequencing of naturally occurring SE variants [5]. They are soluble proteins of approximately 230 amino acids (AA) and have a central disulfide loop (TSST-1 has only 194 AA and does not possess cysteines). Only few AA are conserved between all these exotoxins, probably those required for conservation of tertiary structure [6, 7].

The Mycoplasma arthritidis superantigen

Mycoplasma arthritidis is a pathogen for rodents and induces an acute inflammatory infection in rats and mice. In mice this inflammation is followed by a chronic joint disease. Cell-free supernatants of M. arthritidis contain a potent T cell mitogen, acting on T cells of several species. Although this mitogenic property has been known for more than 13 years [8], it has so far not been possible to sequence or clone this protein. This is due to the small amounts of M. arthritidis superantigen (MAS) in the mycoplasma culture and to the lability and adhesive properties of MAS. The data available show that MAS has a molecular weight of 15 Kda as detected by gel filtration or 27–30 Kda by sodium dodecyl sulfate polyacrylamide gel electrophoresis [8]. It is a hydrophobic protein with an isoelectric point around pH 9. The protein is heat labile at 56°C and susceptible to serine proteases.

MAS has all the functional properties of superantigens [8, 9]. Its T cell stimulatory properties are strictly dependent on the presence of H2-IE molecules or HLA-DR molecules. MAS stimulates both CD4* and CD8* T lymphocytes, and responsive γδ+ T cells have been described [8, 9]. There is a preferential stimulation of mouse T cells expressing Vβ6, 8.1, 8.2, and 8.3, and of human
T lymphocytes expressing Vβ17.1 (also designated 19.1) [8]. In addition, there are other T cell receptors (TCR) that respond to MAS, but with lower affinity [10]. Injection of MAS into experimental animals induces a toxic shock syndrome and pronounced T cell suppression. This is only found in MAS-responsive IE+ mouse strains. The role of the superantigen in the induction of the arthritic disease is not clear.

Retroviral superantigens

Inbred and wild mice express superantigens encoded by different strains of endogenous mouse mammary tumor viruses (MMTV) [11, 12]. MMTV occurs as infectious virus or as integrated provirus. More than 40 different highly homologous MMTV proviruses are known, and a given mouse has two to eight proviruses, encoding “endogenous” superantigens that lead to deletion of T cells with the appropriate Vβ during ontogeny. The superantigen is the gene product of the open reading frame in the 3' long terminal repeat [11, 12]. The viral superantigens are highly homologous among themselves but completely unrelated to the bacterial superantigens or to MAS. They are synthesized as a 45-Kda transmembrane glycoprotein precursor, but may be proteolytically cleaved to yield an 18.5-Kda surface protein that probably is the functional form of the superantigen. The very polymorphic carboxy terminus appears to determine the Vβ specificity [11, 12].

Infectious MMTV is transmitted from mother to offspring via the milk. Expression of the viral superantigen on B lymphocytes in the gut leads to stimulation of T lymphocytes carrying the appropriate Vβ [13]. Infected B lymphocytes are stimulated by T lymphocytes and proliferate. This stimulation of B lymphocytes leads to the expansion of long-lived memory cells that keep the virus until the mammary gland has matured and is susceptible to MMTV infection. Thus, superantigen-dependent T cell/B cell interaction is required to transport the virus from the site of infection in the gut to the mammary gland. Expression of an endogenous MTV gene greatly reduces and eventually prevents the transmission of an infectious MMTV with the same Vβ specificity. Taken together, the advantage of producing a superantigen for MMTV is obvious: T lymphocyte stimulation is required for effective multiplication and transmission.

Molecular mechanism of T lymphocyte stimulation

MHC class II molecules are specific receptors for superantigens. This has been investigated extensively and in detail for the SE and the MAS. There are at least two different binding sites on HLA class II molecules, unrelated to the peptide binding site [14], and different toxins bind with different affinities to these sites, depending on the isotype and the allotype [15, 16]. The binding affinity of a given toxin does not necessarily determine its stimulatory activity. For example, SEE binds with 100-fold lower affinity to HLA-DR than SEA, but is equally efficient in stimulating T cells. Generally, the DR molecules display the highest affinity for the toxins among HLA class II molecules. The murine homologue of HLA-DR, the IE molecule, appears to be the dominant but not exclusive receptor for the toxins.

Recently, it has been reported that MHC class II binding of SEA, SEE, and SED is dependent on the presence of zinc ions (Zn²⁺) [17]. The toxins appear to bind Zn²⁺ directly, via histidine residues. Mutational analysis of MHC molecules has shown that the α-helix of DRβ1 is responsible for toxin binding [18–20]. The Zn-dependent toxins require a histidine at position 81, whereas residues 46 (methionine) and 39 (lysine) are essential for TSST-1 binding. A histidine to alanine substitution at position 81 did not prevent binding of TSST-1 [20].

The binding of a toxin to class II molecules does not only lead to a complex recognizable by T cells but also may have direct consequences for the presenting cell. This interaction can lead to the transduction of signals via class II molecules. TSST-1 and SEB have been reported to induce interleukin-1 (IL-1) and tumor necrosis factor-α (TNF-α) in human monocytes and in a monocyte line in the absence of T cells [21–23]. In contrast, other authors have found that the stimulation of monocytes by SEA to produced IL-1 and TNF-α depends strictly on the presence of T cells [24–26]. In human B lymphocytes, TSST-1 activates the adhesive function of lymphocyte function-associated antigen-1 (LFA-1) by an effect on protein kinase C [27]. This interaction also synergizes with anti-IgM to induce B cell proliferation in the absence of T cells. Thus, TSST-1 delivers a cotransgenic effect on B cells via HLA-DR molecules as signal transduction structures [28]. SEB did not have this effect.

The major interaction site on the αβ-TCR is the Vβ. Stimulation of murine or human T cells with a given toxin in vitro leads to selective expansion of T cells carrying certain Vβ. A similar preference was reported for the response of γδ T cells [29]. That the response is critically dependent on the Vβ part of the TCR was shown by Choi et al. [30]. The introduction of 8 AA of the human Vβ13.2 into the human Vβ13.1 was sufficient to confer the toxin reactivity of Vβ13.2 to a transfected T cell. These AA are located at a site of Vβ (CDR4) not involved in peptide antigen recognition. Different Vβs bind to different toxins with different affinities, therefore T cells with the highest affinity for a given toxin are preferentially expanded in bulk culture. This explains why T cells can respond to a toxin carrying certain Vβs that are not preferentially expanded in such bulk cultures [31].

In spite of the requirement for class II molecules in T cell stimulation, there is evidence that the toxins interact with the TCR directly, i.e., in the absence of class II molecules. Binding to class II molecules is not a prerequisite for T cell activation, because SE-mediated cytotoxicity has been found against several class II-negative target cells [32, 33]. In addition, although a physical binding of the toxin to the T cell cannot be detected, there are a number of observations that argue for a direct effect of the toxins on the TCR [34]. The interaction of the toxin with the TCR is apparently of low avidity and is usually insufficient to generate full responses.

The molecular mechanism of T cell stimulation is thus a multivalent crosslinking of the TCR with MHC class II