Review article
Pregledni znanstveni članek

SUPERANTIGENS

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A B S T R A C T

Superantigens are bacterial, viral, retroviral and some naturally occurring proteins that can specifically activate a large proportion of T and/or B cells. In contrast to classical peptide antigen recognition, superantigens do not require processing to small peptides. T-cell superantigens interact with the immune system by binding to major histocompatibility complex (MHC) class II proteins outside the classical antigen binding groove and activate T cells through the variable region of the T cell receptor beta-chain. B-cell superantigens target B cells, which have restricted usage of variable heavy and light chain and by binding to immunoglobulins outside the conventional antigen binding site, stimulate a high frequency of B cells. Studies of T-cell and B-cell superantigens are important since they are involved in many human diseases and represent a great tool in unravelling some of the basic mechanisms of immune response.

Key words: immunology / microbiology / superantigens / T cells / B cells

SUPERANTIGENI

I Z V L E Č E K

Superantigeni so bakterijske, virusne, retrovirusne beljakovine ter beljakovine naravnega izvora, ki specifično aktivirajo veliko število T in/ali B celic. V nasprotju s klasičnim prepoznavanjem peptidnih antigenov, se superantigeni ne procesirajo v manjše peptide. T-celični superantigeni reagirajo z imunskim sistemom tako, da se vežejo na beljakovine glavnega histokompatibilnostnega kompleksa razreda II izven klasičnega veznega mesta za antigene in aktivirajo T celice z vezavo na beta verigo variabilne regije T celičnega receptorja. B-celični superantigeni delujejo na omejene populacije B celic z izraženo določeno različico variabilne lahke in težke verige protiteles. Z vezavo na imunoglobuline izven klasičnega veznega mesta za antigene stimulirajo veliko število B celic. Proučevanje T-celičnih in B-celičnih superantigenov je pomembno predvsem zaradi njihove vpletenosti v potek mnogih bolezni pri človeku, predstavljajo pa tudi pomembno orodje za študij bazičnih mehanizmov pri imunskem odgovoru.

Ključne besede: imunologija / mikrobiologija / superantigeni / T celice / B celice

INTRODUCTION

Superantigens (SAgs) denote a group of proteins, made by infectious agents that have in common unusual, specific, an extremely potent stimulatory activity for T and/or B lymphocytes.

Unlike conventional antigens (Ag), T-cell SAgs bind to major histocompatibility complex (MHC) class II molecules outside the antigen-binding groove and are presented as unprocessed proteins to certain T cells expressing specific T-cell receptor (TCR) Vβ genes and as a consequence stimulate a high frequency of T cells. New data from the last few years suggest that there are also SAgs that activate B cells. These SAgs target B cells that have restricted usage of variable heavy or variable light chain family genes, bind to immunoglobulins outside the sites that bind conventional antigens and as a consequence stimulate a high frequency of B cells (Levinson *et al*., 1995).

The activity of superantigens was first noticed almost 20 years ago when Festenstein observed that T cells of some strain of mice responded strongly to spleen cells from other strain (Festenstein, 1973) and he knew, that this was not mixed lymphocyte reaction (MLR) because the responders and stimulators could be identical at the MHC. He called the target antigen Mls for minor lymphocyte stimulating antigen. In early 90's it became clear that this are actually retroviral proteins all encoded in the 3' long terminal repeat sequence of mouse mammary tumor viruses (MMTV) (MTVs, Woodland *et al*., 1989, 1991; Marrack *et al*., 1991; Frankel *et al*., 1991; Dyson *et al*., 1991; Choi *et al*., 1991; Archa Orbea *et al*., 1991a) which have integrated a number of times at a number of positions in the genomes of different mice. These SAgs represent a group of viral T-cell SAgs. Since early 80's more and more viral antigens have been observed to have the properties of Mls. An interesting discovery was that some bacterial toxins, which are responsible for the production of elevated amounts of cytokines resulting in toxic shock, food poisoning and scarlet fever in man and animals, are actually SAgs. *Staphylococcus aureus* enterotoxins (SE) groups A-H, toxic shock syndrome toxin-1 (TSST-1) and the streptococcal pyrogenic exotoxins (SPE) groups A-C are the most well studied T-cell SAgs to date (Papageorgiou and Acharya, 1997). In late 80 and early 90 another microorganism joined group of SAg producing microorganisms. *Mycoplasma arthritis*, an organism responsible for inducing chronic arthritis in rodents, produces a soluble protein (72 kDa, 213 residues) that acts as a SAg (Cole *et al*., 1989; Cole *et al*., 1990; Friedman *et al*., 1991). All these SAg now belong to the second broad group of bacterial T-cell SAgs. Among B-cell SAgs the best known are staphylococcal protein A (SPA), HIV gp 120, staphylococcal enterotoxins (SE) and red blood cell antigens i/I (Domiati-Saad and Lipsky, 1997). All microbial SAgs are listed in Table 1.

VIRAL SUPERANTIGENS

First and the most studied SAgs by now are endogenous viral SAg Mls produced by MMTV. MMTV is a B-type retrovirus that is maternally transmitted from mother to offspring via milk and is a major causative agent for mammary tumor development in mice (Acha-Orbea, 1993a; Salmons and Günberg, 1987). The wide variety of endogenous MMTV have been integrated into the germ line (Kozak *et al*., 1987) and number of copies are inherited like normal mouse genes following Mendelian rules. The number of copies varies greatly and the polymorphism of the integration sites is extensive (Callahan *et al*., 1982; Frankel *et al*., 1991; Jouvin-Marche *et al*., 1992). Most of these proviruses have lost the ability to produce infectious viral particles but have retained the capacity to produce functional MMTV proteins. A group of these endogenous proteins called Mls, has superantigenic properties and they all are encoded by an open reading frame (*orf*) which is found in the 3' long terminal repeat (LTR) of MMTV (Acha-Orbea *et al*., 1991a; Choi *et al*., 1991). Superantigenic proteins encoded by different MMTV integrands are predicted to be very closely related in amino acid sequence except for their C-terminal 30 residues, which seems to predict TCR Vβ specificity (Fasel *et al*., 1982; Donehower *et al*, 1983; Crouse and Pauley, 1989; Pullen *et al*., 1992). SAg Mls-1^a is the strongest endogenous superantigen known. It has TCR Vβ6, -7, -8.1 and possibly –9 specificities what is reflected in

its capacity to induce *in vivo* and *in vitro* a vigorous stimulation of T cells with these particular Vβ chain (Acha-Orbea, 1993a). In contrast to stimulation, clonal deletion of all Mls-1a reactive Vβ domains occurs in mice that express the relevant superantigen (Acha-Orbea, 1993b). Mls-1^a causes also a deletion of T cells with this particular Vβ chain when this SAg comes in contact with the immune system in the very first days of life, that is when mice are feeded on milk infected with MMTV, encoding Mls-1^a. There is at least 19 known different variants of MMTV Mls with defined Vβ usage (Acha-Orbea, 1993b). With the finding that Mls antigens are encoded by endogenous MMTV proviruses it became clear that infectious MMTV should also express these superantigens. This is a group of exogenous MMTV SAgs.

Several other viruses are now suspected of encoding viral SAgs. Epstein-Barr virus (EBV), the ethiologic agent of infectious mononucleosis is capable of inducing B-cell and T-cell proliferation *in vivo* and *in vitro* (Hanto *et al*., 1985). The nucleocapsid (N protein) of rabies viruses possess superantigenic properties (Lafon *et al*., 1992). Herpes virus saimiri (HVS) encodes a protein with strong sequence homology with the MMTV SAgs (Thomson and Nicholas, 1991). The protein $pr60^{gag}$ of murine acquired immunodeficiency syndrome (MAIDS) has also been associated with superantigenic activity (Hüginn *et al*., 1991; Kanagawa *et al*., 1992) as well as a protein, gp 120, encoded by HIV-1 (Imberti *et al*., 1991; Dalgleish *et al*., 1992; Laurence *et al*., 1992) (Table 1).

BACTERIAL SUPERANTIGENS

Like Mls the ability of certain bacterial toxins to stimulate T cells on unconventional way was first recognised in the early ’70 (Peavey *et al*., 1970; Smith and Johnson, 1975) and later on these toxins were classified as superantigens. They have been found in several different pathogenic bacteria: *Staphylococci, Mycoplasma, Streptococci, Yersinia* (Marrack and Kappler, 1990) (Table 1)

Bacterial superantigens are globular medium size proteins with 22-29 kDa and characterised by high resistance to proteases and to heat denaturation. Amino acid sequence comparison suggests that they can be classified into four groups consisting of (1) SEA, SED and SEE; (2) SEB, staphylococcal enterotoxins C1-C3, SPE-A and SPE-C; (3) TSST; (4) exfoliative toxins A and B and SPE-B. Members of each group tend to share specificity for Vβ chain. Differences between the SAgs are located mostly in high variable regions consisting of several surface loops. According to the effects the bacterial SAgs fall into two categories: (1) those which cause acute response, as for example toxic-shock syndrome or food poisoning; (2) those which cause chronic autoimmune related response. (Ulrich *et al*., 1995; Schlievert, 1997) (Table 2). The bacterial SAgs may also be subdivided into pyrogenic toxin superantigens and not pyrogenic toxins. The first ones (TSST-1, enterotoxins, group A streptococcal pyrogenic exotoxins) are distinguished from other SAgs in that they have in addition to superantigenicity numerous biological properties such as enhancement of endotoxic shock, type I hypersensitivity, cardiotoxicity, ability to bind endothelial cells and to endotoxin, and some others. Members of this group are associated with causation of life threatening toxic shock syndrome, whereas other SAgs have not (Schlievert, 1997).

Strains of *S. aureus* and group A streptococci that produce SAgs are appearing most frequently in clinical isolates, but the infection of domestic animals with TSST-1 for example or SE producing strains have also been observed. Most strains produce only one SAg although a few produce several. Most SAgs are encoded by mobile genetic elements as are antibiotic-resistance plasmids, where SED is encoded on phages (Ulrich, 1995).

Table 1. Known and possible T-cell and B-cell superantigens
Preglednica 1. Znani in možni T-celični in B-celični superantigeni

	T-cell SAg	B-cell SAg
Viral	Murine mammary tumor virus (MMTV)	
	Rabies virus (RV)	
	Epstein-Barr virus (EBV)	
	Herpesvirus saimiri (HVS)	
	Murine acquired immunodeficiency syndrome (MAIDS)	
	Simian immunodeficiency virus (SIV)	
	Human immunidificiency virus (HIV-1)	gp 120 (HIV-1 protein)
Bacterial	Staphylococcal toxic shock syndrom toxin-1 (TSST-1)	Staphylococcal protein A
	Staphylococcal enterotoxins (SE) A-H (not F)	
	Group A streptococcal pyrogenic exotoxins (SPE) A-C	
	Group A streptococcal superantigen	
	Group A streptococcal pyrogenic exotoxin F	
	Staphylococcal exfoliative toxins A and B	
	Mycoplasma arthritidis mitogen	
	Yersinia enterocolitica superantigen	
	Yersinia psedotuberculosis mitogen	
Possible bacterial	Coagulase negative staphylococci	
	β-hemolytic streptococci B,C, F, G	
	α- hemolytic streptococci (including *S. pneumoniae*)	
	Enterococci	
	Bacillus cereus	
Naturally occurring proteins		CD4
		Human liver protein pFv
		Protein L
		i/I carbohydrate Ag on RBC

Table 2. Known and possible disease association with superantigens
Preglednica 2. Znane in možne bolezni povezane s superantigeni

Acute diseases	Chronic diseases
Staphylococcal toxic shock syndrome	Arthritis
Streptococcal toxic shock syndrome	Atopic dermatitis
Scarlet fever	Guttate psoriasis
Staphylococcal scalded skin syndrome	Rheumatic fever
Kawasaki syndrome	Multiple sclerosis
Fevers of unknown origin	

MOLECULAR MECHANISM OF ACTION

Molecular mechanism of action of T-cell SAgs have been studied earlier and much more intensive and is consequently much more defined than mechanism of action of B-cell SAgs. The model of action is presented in Figure 1.

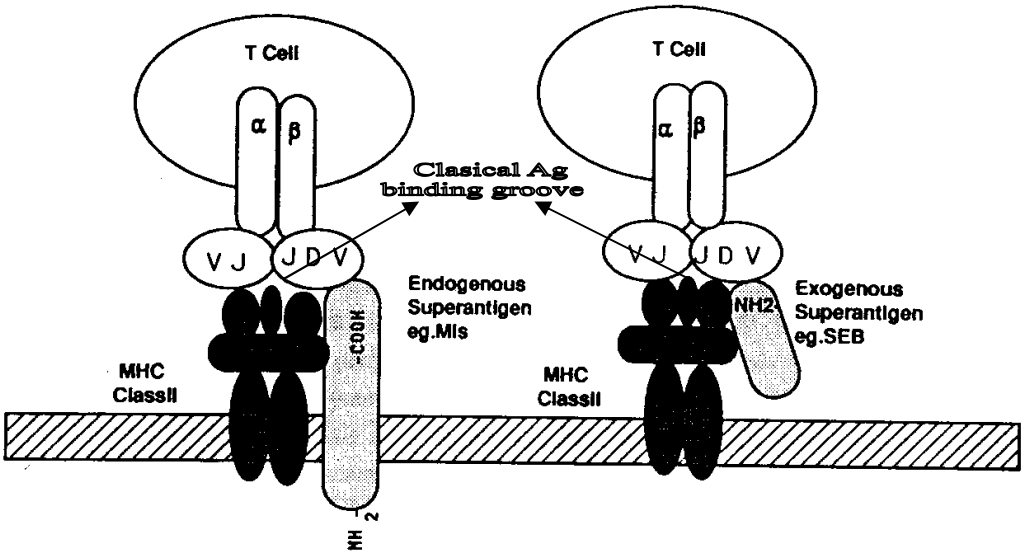

Figure 1. Model of endogenous and exogenous superantigen interaction with antigen- presenting cell and responding T-cell. SEB is represented as a model for exogenous SAg, Mls as a model for endogenous SAg, both shown interaction with variable (V) domain of β chain of TCR on T-cell and with MHC class II on Ag-presenting cell (Simpson *et al*., 1993).

Slika 1. Model interakcije endogenih in eksogenih superantigenov z antigen-predstavljajočo celico in ciljno celico T. SEB predstavlja model za eksogene SAg , Mls pa model za endogene SAg. Pri obeh je prikazana interakcija med β verigo variabilnega (V) dela TCR na ciljni celici T in MHC molekulo razreda II na Ag-predstavljajoči celici (Simpson in sod., 1993).

T-cell superantigens

Classical antigens are after processing bound to MHC II molecules and presented to TCR α/β on T cell as peptides in the well defined peptide-binding groove. Similarly to antibodies, the variable portion of the α/β TCR responsible for ligand recognition is put together from five variable elements, two on the α chain (Vα, Jα) and three on the β chain (Vβ, Dβ, Jβ). The α/β TCR must interact with amino acids both, on the peptide antigen in the groove and the MHC molecule. This complex interaction requires the right combination of all five of the TCR variable elements. The frequency of specific T cells (with one combination of these five elements) is very low (~10^{-5}).

Superantigens bind to MHC and TCR at the site distinct from the peptide binding groove at the lateral surface without any previous processing. It is thought that SAgs must bind MHC molecule before they can be recognised by TCR and at least for bacterial SAgs the evidence for this is quite straightforward (Mollick *et al*., 1989; Uchiyama *et al*., 1989; Fraser 1989; Russel *et*

al. 1990). A simple model has been described in which the SAg activates the T cell by directly crosslinking the MHC class II molecule on the Ag presenting cell and the TCR Vβ element on the T cell (Figure 1). There are some supporting data for this model of binding. Functional presentation of bacterial SAgs is not dependent on Ag processing while there is recent evidence that viral SAgs may undergo processing (Woodland and Blackman, 1993).

MHC-binding

SAgs appear to be able to interact with nearly any class II MHC molecule. Most, but not all, T cell-Sag interactions are independent of MHC polymorphism (Herman *et al*., 1990; Mollick *et al*., 1991; Yagi *et al*., 1991). Thus, a single superantigen can be recognised in the context of multiple class II alleles and isotypes, including xenogeneic class II molecules. However, individual class II molecules vary in the effectiveness at presenting SAgs to T cells (Woodland and Blackman, 1993) and some interaction preference is seen, in particular, interaction with HLA DR molecules is preferred. For bacterial SAgs was shown that the MHC binding site is on the outside of the molecule rather than in the peptide binding cleft (Dellabona *et al*., 1990; Herman *et al*., 1991, Karp and Long, 1992). There are some reports describing details about interactions between some particular SAgs and MHC molecule. Most information about the interactions of bacterial SAgs with MHC II molecule have been derived from the studies of structures of SEB and TSST-1 in complex with an MHC II molecule HLA-DR1. Residues 43-47 from the N-terminal hydrophobic domain of SEB and Phe44, Leu45 and Phe47 from a hydrophobic pocket in the DR 1 α chain are involved in the first contact (Kim *et al*., 1994). In TSST-1 the corresponding region is located around Leu30 while in the side of MHC molecule the same site as for SEB is involved. Despite the similarity and the apparent overlap of their respective MHC binding sites, SEB and TSST-1 do not compete with each other because, as it was proposed for both SAgs the cellular factors may affect their presentation to MHC molecule. (Wen *et al*., 1996).

For bacterial SAgs SEA, SEC2 and SED the role of zinc ion was demonstrated (Fraser *et al*., 1992; Fraser *et al*., 1993). It was suggested that the zinc ion could act as a bridge cross-linking the SAg with the MHC II molecule, where an appropriate fourth zinc ligand is provided for stabilisation of the complex. The detailed description of binding sites in the complex SEA-MHC II introduces the zinc binding site H187.H225.D227 in the SEA side and His81 in DRβ1 in the MHC side (Fraser *et al*., 1993).

Next to almost nothing is reported about binding of viral SAgs to the MHC molecule. Additionally to the antibody inhibition data (Peck *et al*., 1977) there are reports that the ability of cells to present viral SAgs depends upon the alleles of class II MHC molecule (Lynch *et al*., 1985) and that the expression of endogenous viral SAgs in mice almost always deletes $CD4^+$ cells better than $CD8^+$ T cells (Kappler *et al*., 1989).

TCR binding

Interaction of SAgs with TCR is also outside of the typical antigenic peptide groove and requires only the appropriate Vβ element which is predicted to lie on the outer, solvent exposed face of the molecule, far away from the putative binding site for conventional antigen-MHC (Pontzer *et al*, 1992; Mollick *et al*., 1993). Other three elements of TCR have very little influence on this interaction although there are some reports indicating the influence of non-Vβ regions as well as of polymorphic regions of MHC molecule. However, binding is determined mostly with Vβ region encoded by only a small number of Vβ genes (22 Vβ elements in mouse and 50 in humans), each expressed at high frequency among T cells. Each SAg can bind one or a few of the different Vβ regions. The consequence of this relatively low specificity of interaction only with one instead of five elements is powerful T cell activation with up to 20% of all T cells

while only 0.001 to 0.0001% of T cells are activated upon normal antigen presentation. This massive cell activation and release of immunological mediators such as TNF-α, IL1, IL2 and γ interferon (γ-INF) by CD4 T cells, which are predominant responding population, leads to systemic toxicity and suppression of the adaptive immune response and could cause shock and even death. SAgs cause serious diseases in humans such as food poisoning, scarlet fever, toxic shock syndrome, Kawasaki disease, Necrotising Fasciitis and have been implicated in rheumatic fever and AIDS (Table 2). There are some reports describing amino acid residues of some particular SAgs involved in binding to TCR. The binding site of SEB involves a shallow cavity between the two domains of the molecule created by the defined residues (Papageorgiou and Acharya, 1997; Schlievert, 1997). Analogous sites have been proposed for SEC2, SEC1 and SEA. The TCR biding site of TSST-1 is distinct from that of SEs and is probably located in the C-terminal domain (Papageorgiou and Acharya, 1997).

B-cell superantigens

B-cell SAgs interact with unconventional site of variable regions of soluble and membrane associated immunoglobulins. Several molecules have been postulated to be B-cell SAg: staphylococcal protein A (SPA) (Sasso *et al*., 1991), Staphylococcal enterotoxins A (SEA) and D (SED) (Domiati-Saad *et al*., 1996), the envelope protein gp 120 of HIV-1 (Berberian at al., 1991; 1993), CD4 (Lenert *et al*., 1990), protein L (Nilson *et al*., 1993), human liver sialoprotein, termed Protein Fv (pFv) (Silverman *et al*., 1995; Patella *et al*., 1993), certain reed blood cell proteins. To be classified as B-cell SAg proteins must simultaneously stimulate a large percentage of B cells, bind to antibodies (Ab) encoded by restricted VH or VL gene segments generating oligoclonal response and bind as intact proteins outside of the conventional antigen binding site. The variable region of Ig molecule is made of three elements of heavy chain (variable: VH, diversity: DH and joining: JH) and of two elements of light chain (variable: VL, joining: JL). Diversity is generated by the combinatorial assembly of the V genes. Complementarity determining regions (CDR) 1, 2 and 3 are hipervariable parts of the variable region and are separated by four framework regions (FR). In classical Ag recognition CDRs come in contact with Ag, actually they recognise three-dimensional structure of intact Ag molecule. B-cell SAgs induce B-cell response only through a VH or VL specific interaction. They don't interact with CDRs (at least not at the part where classical Ag does) but usually with the solvent exposed surfaces of the FR 1 or 3.

SPA is a prototypic B-cell SAg. It binds to human IgM, IgA and IgG containing VH3 gene segment (Inganas, 1981; Romagnani *et al*., 1992; Sasso *et al*., 1989). Detailed studies from many authors indicate that the binding site of SPA lies in the solvent exposed loops of Ig in FR1 or FR3 (where the highly conserved residues within this VH family are) and in the 3'end of the CDR2, outside the conventional paratope of the hypervariable loops. *In vitro* it induces polyclonal B-cell proliferation and differentiation into Ig secreting cells and consequently induces the production of VH3 specific Ig products (Vasquez Kristiansen *et al*., 1994).

A viral SAg, envelope glycoprotein of HIV-1, gp 120, also binds to Ig expressing VH3 gene segment independently of light chain utilisation (David *et al*., 1995). SEA and SED are T-cell dependent B-cell SAgs. SEA stimulates VH3 expressing B-cells while SED stimulates VH4 expressing B-cells. i/I carbohydrate antigens are molecules on the surface of red blood cells (RBC) which bind to the IgM cold agglutinins. This i/I Ag also interact with IgM in a SAg manner since it uses restrictly VH4-VH34 gene segments in the FR1 region (Pascual *et al*., 1991; 1992; Li *et al*., 1996). CD4, the 55 kDa human cell molecular marker and high affinity receptor for the HIV, also interacts with the Ig in a VH-dependent and possibly VH-restricted way. Many reports discuss the CD4 molecule as possible endogenous B-cell SAg because of restricted usage of VH domains and costimulation effects with some other molecules (Lenert *et al*., 1990; 1997).

But it may not entirely fit with stringent criteria of SAg since in soluble, monomeric form it did not stimulate B-cells.

INFLUENCE OF SUPERANTIGENS ON T-CELL END B-CELL REPERTOIRE

B and T cells derive from bone marrow steam cells and undergo gene rearrangements in a specialised microenvironment to produce a unique antigen receptor on each cell. B cells differentiate in the bone marrow while T cells migrate at a very early stage to the thymus, where their receptor rearrangement and maturation occur. It is essential that each individual's T cells are able to recognise foreign antigenic peptides when they are bound to his own MHC molecules so they should be self MHC restricted. It is equally essential that T cells are unable to recognise self peptides as self and not as Ag so they must be self tolerant. To reach all these qualities T cells undergo the two selective processes known as positive selection, in which they are screened for self MHC restriction and negative selection which eliminates those cells that are specific for self peptides bound to self MHC. Endogenous Mls SAg in mice mediate negative selection of T-cell receptors. Since MMTV's genes are inherited like normal mouse genes their proteins are already present at the time of selection and maturation of T cells. T cell receptors containing particular Vβ regions to which the Mls proteins bind are signalled during intrathymic maturation causing apoptosis and thus elimination of such T cells. Since integrated loci of MMTV are different in mice, the repertoire of selected T cells is also different. For example, one variant of the Mls SAg (Mls-1a) deletes all thymocites expressing the Vβ6 variable region in one mouse whereas such cells are not deleted in mice that lack Mls-1a gene locus (Janeway and Travers, 1994).

There are more and more data that also B-cell SAgs have the ability to influence the expression of the B-cell repertoire (Levinson *et al*., 1995). In last few years, maybe the most studied B-cell SAg is gp 120 since it may play a role in the pathogenesis of HIV-induced diseases. Early in HIV infection an increase in VH3 expressing B-cells activation associated with increased corresponding Ig production was observed (Domiati-Saad and Lipsky, 1997; Levy, 1994). Later in the infection several studies have reported depletion of some VH3 B-cell population (David *et al*., 1995).

PERSPECTIVES

There are several reasons why SAgs had such a tremendous interest in recent years. The knowledge about chemical interaction and biological, physiological and pathological consequences may be used to study some still not well understand interactions in immune system. By interacting with T cells trough the Vβ domain of TCR and with MHC of antigen presenting cells SAgs represent useful reagents to probe the MHC-TCR interaction as well as for studying of mechanisms of T and B cell gene rearrangement in the development of T and B cell repertoire. It is still puzzling to observe that the acute activation of SAg-reactive T cells *in vivo* is immediately followed by the induction of tolerance, so SAgs can further be used to study the mechanisms of T-cell tolerance versus T-cell inactivation (tolerance) (Miethke *et al*., 1995). Further the pathology of some human diseases, where SAgs are involved as for instance T-cell mediated septic shock, Kawasaki syndrome, rheumatoid arthritis and AIDS can be studied in new perspective. Maybe the most important is the possible pharmaceutical use of SAgs or their engineered derivatives in treatment of autoimmune diseases, infectious diseases and cancer therapy. SAgs have already been tested as superantigen vaccines in treatment of toxic shock in animal model (Ulrich *et al*., 1995). In animal model of autoimmunity, treatment with

staphylococcal superantigen has resulted in cure of disease or reduction of autoimmune symptoms (Abrahmsen, 1995). Very promising results were obtained with monoclonal antibody-superantigen fusion protein as tumor-specific agent for T-cell-based tumor therapy (Dohlstein *et al*., 1994; Hansson *et al*., 1997). By coupling a superantigen to a carcinoma-specific antibody, activated T-cells may be targeted to cancer cells and furthermore, T-cell activation/proliferation resulting from SAg presentation in the context of a MHC stimulates lymphokine production what results in activation of other less specific lymphocytes. This cascade mechanism of immunoconjugate containing SAg enabling a T-cell attack against tumor cells with minimal systemic activation (Hansson *et al*., 1997).

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