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T CELL REPERTORE SELECTION BY SUPERANTIGENS

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SUMMARY

Superantigens are substances of bacterial or viral origin that interact specifically with the β-chain of the T cell receptor. During development, immature T cells encountering superantigens are physically eliminated in the thymus by a mechanism of programmed cell death (apoptosis). In contrast, mature T lymphocytes in the periphery respond to superantigens by proliferation and acquisition of effector functions. This initial response is often followed by inactivation and/or death of the superantigen-reactive T cells. Thus superantigens provide a useful model system to investigate the fate of T cells encountering neo-self antigens at different stages of development.

INTRODUCTION

The recognition of foreign antigens by T lymphocytes is mediated by the T cell receptor (TCR), which is a heterodimeric molecule composed of α and β chains (*Davis* et al., 1988; *Marrack* and *Kappler*, 1987). Like the immunoglobulins, TCR α and β chains contain variable (V) junctional (J) and constant (C) domains that are encoded by distinct genetic elements that recombine during ontogeny. In the mouse there are 20 Vβ elements and 12 Jβ elements whereas the number of Vα and Jα elements is estimated at ~100. This large genetic pool allows a considerable permutational variability in the specificity of the TCR, which is necessary to cope with the large number of foreign antigens and pathogens that can be encountered by a given individual.

In contrast to immunoglobulins, TCR recognize a complex ligand formed by the binding of a small (processed) peptide to molecules encoded by the major histocompatibility complex (MHC) (*Bjorkman* et al., 1987). Two types of mature T cells can be distinguished on the basis of their ligand specificity: $CD4^+$ cells recognize peptides bound to MHC class II molecules whereas $CD8^+$ T cells recognize peptide: MHC class I complexes (*Swain*, 1983).

During development in the thymus, T cells are positively selected by interaction with self MHC molecules (*von Boehmer*, 1986). In this way mature $CD4^+$ and $CD8^+$ T cells are subsequently able to recognize foreign antigens (processed peptides) only in association with appropriate self MHC class II or I molecules, respectively. (This process is frequently referred to as MHC restriction). At the same time, developing T cells are subjected to negative selection events to ensure that cells with autoreactive TCR are functionally eliminated from the repertoire (*Marrack* and *Kappler*, 1987; *von Boehmer*, 1990). This latter process (also referred to as immunological tolerance) will be discussed in detail in this review.

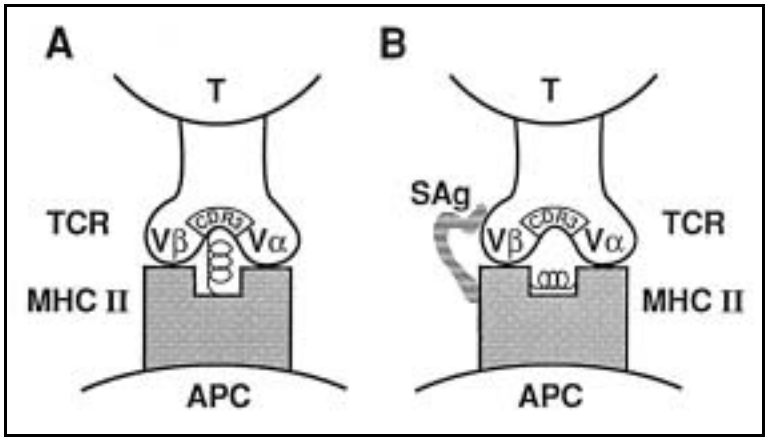

Figure 1: T cell receptor interaction with conventional (peptide) antigens or superantigens.

A. Processed peptides (▮) bound to MHC class II molecules on antigen-presenting cells (APC) interact specifically with the hypervariable CDR3 region of the TCR.

B. Superantigen (SAg) binds (either simultaneously or sequentially) to both MHC class II and a site on the outer surface of TCR Vβ. This binding does not require a specific peptide: CDR3 interaction as in A.

SUPERANTIGENS

As mentioned above, T cell recognition of conventional (peptide) antigens associated with MHC molecules is mediated by the TCR. In general the specificity of this type of recognition is dependent upon the contribution of most (or all) elements that confer TCR variability (Vα, Jα, Vβ, Jβ). An apparent exception to this generalization is found with a recently characterized group of substances referred to collectively as "superantigens". The recognition of superantigens by the TCR seems to depend only upon the Vβ domain, with no apparent contribution from any of the other variable TCR elements. However, like conventional antigens, superantigens require presentation by MHC molecules. To date only MHC class II molecules have been shown to bind and present superantigens to T cells. A schematic comparison of TCR recognition of conventional peptide antigens and superantigens is shown in Figure 1.

Two classes of superantigens are well characterized. The first group is exemplified by the exotoxins of *Staphylococcus aureus*. These substances, which have been known for some time as potent stimulators of T cells, have more recently been shown to act as superantigens in that they bind MHC class II and interact with particular TCR Vβ domains (*White* et al., 1989). The second group of "superantigens" was originally identified as genetic elements that encode a strong T cell stimulating capacity in the mouse (*Festenstein*, 1974). These so-called minor lymphocyte stimulatory (Mls) determinants were subsequently shown to behave as Vβ-specific superantigens (*Kappler* et al., 1988; *MacDonald* et al., 1988). Very recently it has further been shown that most (if not all) Mls-like determinants are encoded by endogenous copies of mouse mammary tumor virus (MMTV). The MMTV gene product responsible for this Mls activity is located in an open reading frame (orf) in the 3' long terminal repeat (LTR) of the virus (*Acha-Orbea* and *Palmer*, 1991).

Table 1: Summary of best characterised murine Vβ-specific superantigens

Superantigen	TCR Vβ
SEA	1, 3,10,1 1, 17
SEB	7, 8.1, 8.2, 8.3,17
SEC1	8.2, 8.3,11,17
SEC2	8.2,10, 17
SEC3	7, 8.1, 8.2
SED	7,8.1,8.2,8.3,11, 17
SEE	11,15, 17
TSST-1	3,15, 17
Mls-1 (Mtv-7)	6, 7, 8.1, 9
Mls-2 (Mtv-13)	3
Mls-3 (Mtv-6)	3
Mls-4 (Mtv- 1)	3
Etc-1 (Mtv-9)	5.1, 5.2, 11

For further information see: *Abe* and *Hodes* (1989), *Herrmann* and *MacDonald* (1991) and *Janeway* (1991)

A summary of known superantigens and their corresponding TCR Vβ specificities is given in Table 1.

Details of the presumed trimolecular interaction between superantigens, MHC class II molecules and TCR Vβ domains remain to be defined. For bacterial enterotoxins (which are readily available in pure form) it is clear that high affinity binding to MHC class II molecules occurs (*Marrack* and *Kappler*, 1990); however no direct enterotoxin; TCR Vβ binding has yet been demonstrated. The situation with Mls determinants is much less clear, since the MMTV orf protein has only recently been shown to encode the functional superantigen. Nevertheless site-directed mutagenesis experiments have provided indirect evidence that residues in TCR Vβ outside of the presumed peptide: MHC binding domain are involved in contacting both bacterial enterotoxins and Mls (MMTV) gene products (*Choi* et al., 1990; *Dellabona* et al., 1990; *Pullen* et al., 1990).

T cell responses to superantigens differ from conventional antigen responses in several aspects (Table 2). First, because of their Vβ specificity, superanti-

Table 2: Comparison of T cell responses to antigens and "superantigens"

Property	Antigens (peptides)	Superantigens	
		Mls	Enterotoxin
Frequency	low ($< 1/10^4$)	high (1/10)	high
T cell receptor	unique (α/β)	Vβ restricted	Vβ restricted
MHC requirement	class I or class II	class II (E>A)	class II (not species specific)
Phenotype	$CD4^+$ (class II) $CD8^+$ (class I)	$CD4^+$ and $CD8^+$	$CD4^+$ and $CD8^+$

gens elicit a response from a high proportion (up to 20%) of T cells in a primary response. In contrast, the frequency of T cells responding to peptide: MHC complexes are usually undetectable without prior immunization. Second, the presentation of superantigens by MHC molecules is distinct from conventional antigen presentation. All superantigens characterized to date are presented by MHC class II molecules, whereas both class I and II MHC molecules can bind and present antigenic peptides. Third, both mature T cell subsets ($CD4^+$ and $CD8^+$) respond to superantigens, despite the fact that only $CD4^+$ cells respond to peptide antigens presented in association with MHC class II.

Because of their specificity for TCR Vβ domains, superantigens are powerful tools to study the selection of the T cell repertoire *in vivo*. Since a given Vβ domain is expressed by a significant proportion of T cells (on average 5% in the mouse), it is feasible (with the aid of Vβ-specific monoclonal antibodies) to follow the fate of potentially autoreactive T cells in an unambiguous way. The following sections describe in more detail how this property of superantigens has been exploited experimentally.

CLONAL DELETION DURING INTRATHYMIC DEVELOPMENT

During development, the thymus is colonized by stem cells originating in the fetal liver (or adult bone marrow). These early stem cells of the T lineage have an immature $CD4^-$ $CD8^-$ phenotype (although they appear to transiently express low levels of CD4, at least in the mouse) and have not rearranged their TCR genes. Once in the thymus, $CD4^-$ $CD8^-$ cells sequentially rearrange and express their TCRβ and TCRα genes. In parallel they acquire surface expression of both CD4 and CD8. The majority population in the thymus, located primarily in the cortex, belong to this $CD4^+$ $CD8^+$ TCR^+ subset. At this stage of development, $CD4^+$ $CD8^+$ cells become committed to the CD4 or CD8 lineage, and those cells with appropriate TCR specificity for self MHC are positively selected for survival. This complex (and still poorly understood) series of events results in the generation of mature $CD4^+$ and $CD8^+$ T cells restricted to MHC class II or I, respectively. These mature cells are located primarily in the thymic medulla and are destined to emigrate to the periphery. A summary of these events is given in Figure 2.

The fate of autoreactive T cells during thymic development was first directly investigated by exploiting the correlation between TCR Vβ specificity and the presence of endogenous (MMTV-encoded) superantigens. These studies showed unequivocally that most autoreactive T cells are clonally deleted as they mature in the thymus (Kappler et al., 1987). Similar results were obtained by injecting exogenous superantigens (Staphylococcal enterotoxins) in neonatal rnice, resulting in clonal deletion of the appropriate Vβ subset (*White* et al., 1989). These conclusions were subsequently confirmed by independent experiments involving transgenic mice bearing TCR α and β chains of defined MHC antigen specificity. Again clonal deletion in the thymus was observed when developing T cells encountered self antigens (*von Boehmer*, 1990).

By employing superantigen: Vβ correlations as well as transgenic TCR models, the process of clonal deletion in the thymus has been intensively investigated over the last several years. From such studies, it is clear that clonal deletion can occur at several different stages

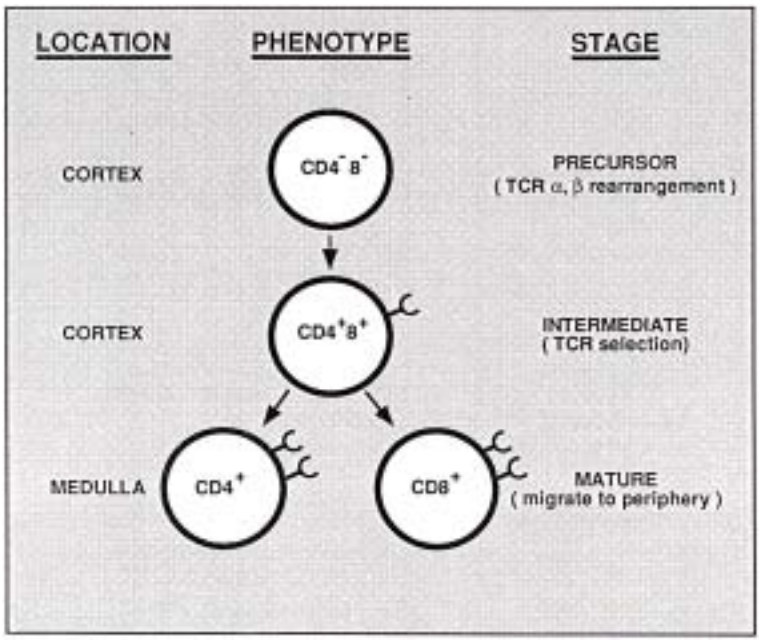

Figure 2: Stages of intrathymic development.
$CD4^-8^-$ precursor cells give rise to mature $CD4^+$ and $CD8^+$ T cells via a $CD4^+8^+$ intermediate stage. Following TCR α and β gene re-arrangement, the TCR (⊥) is first expressed at low levels on the cell surface at the $CD4^+8^+$ stage. Positive and negative selection then takes place, leading to the survival of mature $CD4^+$ and $CD8^+$ cells expressing high TCR levels. For further details see: *Fowlkes* and *Pardoll* (1989).

of intrathymic development. Depending on the self antigen encountered, clonal deletion may occur either very early ($CD4^+$ $CD8^+$ stage) or relatively late ($CD4^+$ or $CD8^+$ stage). The factors responsible for this variability in stage of deletion remain to be determined. One obvious explanation would be that clonal deletion occurs whenever there is sufficient TCR avidity for the self ligand. Since TCR avidity increases during development with increasing TCR surface density, it would follow that the threshold required for clonal deletion could be reached at different developmental stages for different self ligands. Alternatively it is possible that interaction with specialized thymic stromal cells (or production of specific co-factors) contribute in some way to the deletion event.

It is now widely accepted that the mechanism of death of autoreactive thymocytes is closely related to the programmed cell death (also referred to as apoptosis) that occurs in many developmentally regulated systems (*Cohen*, 1991; *Golstein* et al., 1991). Apoptosis involves rapid morphological changes (including conspicuous condensation of nuclear chromatin) followed by degradation of internucleosomal DNA (*Wyllie* et al., 1980). This type of death can be prevented (or delayed) by inhibitors of de novo RNA or protein synthesis. The nuclease responsible for DNA cleavage has not been identified, nor has it been determined whether DNA degradation per se is a causative (or secondary) event in apoptosis.

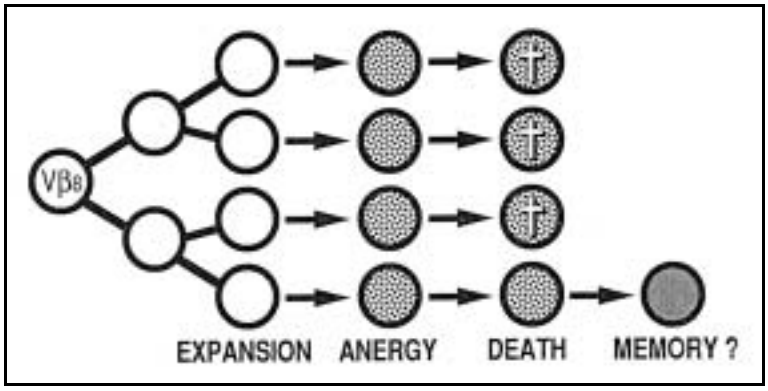

Figure 3: Sequence of events following *in vivo* injection of superantigens.
Administration of SEB (specific for $V\beta8^+$ T cells) results in an initial clonal expansion of $V\beta8^+$ cells in peripheral lymphoid tissues followed by cell death and anergy of the surviving $V\beta8^+$ cells. The relationship of this process to the generation of memory cells is unknown. A similar sequence of events occurs when other superantigens are injected, except in this case other TCR Vβ are involved (see Table 1).

PERIPHERAL TOLERANCE

Despite the potential efficiency of clonal deletion of autoreactive T cells, it is evident that not all self antigens can be expected to be expressed in the microenvironment of the developing thymus. For example, antigens expressed in specialized tissues (as well as antigens expressed late in ontogeny) would be expected to encounter mature T cells after they have left the thymus. For these reasons it would be anticipated that tolerance mechanisms distinct from clonal deletion should exist.

In the context of the superantigen models discussed here, peripheral tolerance can be investigated by injecting adult mice with superantigens and monitoring the subsequent behavior of reactive T cells expressing appropriate TCR Vβ domains. Such experiments have recently been carried out both for bacterial enterotoxins (*Kawabe* and *Ochi*, 1991; *MacDonald* et al., 1991; *Rellahan* et al., 1990) and Mls antigens (*Dannecker* et al., 1991; *Rammensee* et al., 1989; *Webb* et al., 1990). In both cases, injection of superantigens leads to an initial clonal expansion of T cells with reactive Vβ domains in lymph node and spleen. This expansion involves both $CD4^+$ and $CD8^+$ subsets, as would be predicted from the *in vitro* behavior of superantigens. Following this expansion period there is a rapid decline in the number of T cells expressing these Vβ domains. *In vitro* studies indicate that this decrease is due (at least in part) to selective death of reactive T cells; however it has not been excluded that other phenomena (such as altered migration patterns *in vivo*) may also contribute to this decrease. The mechanism of superantigen-induced peripheral T cell death is controversial; evidence both for (*Kawabe* and *Ochi*, 1991) and against (*MacDonald* et al., 1991) an apoptotic mechanism has been reported.

Despite extensive cell death, many T cells with superantigen-reactive Vβ domains survive *in vivo*. These remaining cells exhibit a tolerant (or anergic) phenotype inasmuch as they specifically fail to proliferate when re-challenged with the relevant superantigen *in vitro*. This

failure to proliferate correlates with a lack of interleukin-2 (IL-2) production by the tolerant cells. The molecular basis of this anergic state remains to be elucidated; however it is clear that IL-2 mRNA accumulation (as assessed by Northern blot) is greatly reduced in anergic T cells stimulated by superantigens. Further studies should reveal whether this represents decreased rates of IL-2 transcription and, if so, whether this in turn can be related to a deficiency in any of the known transcription factors acting on the IL-2 promoter. A summary of events occurring upon stimulation of peripheral T cells with superantigens is shown in Figure 3.

Few comparable studies on the mechanism of peripheral tolerance have been carried out in other model systems. Nevertheless recent data with TCR transgenic mice suggest that the same sequence of events (i.e. clonal proliferation, death and anergy) occur upon exposure of peripheral T cells to conventional antigens as well (*Rocha* and von *Boehmer*, 1991).

CONCLUDING REMARKS

Superantigens have proven to be a very useful paradigm for elucidating the fate of T cells confronting self (or foreign) antigens at different stages of development. The unique TCR Vβ specificity of these reagents allow (in combination with the relevant monoclonal antibodies) unambiguous conclusions with regard to clonal expansion, death or anergy of reactive cells in various experimental systems. These data complement studies with TCR transgenic mice, where similar conclusions have been reached in many instances. However in contradistinction to the transgenic models, superantigen: Vβ correlations present certain experimental advantages, not the least of which are the possibility to study unmanipulated animals and to use unreactive Vβ subsets as appropriate negative controls.

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