An Analysis of the *in vivo* SEB response in a T Cell Receptor *scid* Transgenic mouse

by

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A thesis submitted in conformity with the requirements for the degree of Master of Science

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Abstract

The superantigen SEB was used to analyze the immune response of a C.B-17 *scid* mouse carrying a transgenic T cell receptor expressing Vα 13 and Vβ 8.2. There are no B cells present in this mouse and the only T cells present express the T cell receptor transgene. Therefore the response of a clonotypic population of T cells can be studied in the absence of all other T or B cells. The splenic T cells from these mice mounted a response to SEB *in vivo*, illustrating that a clonotypic population of T cells is able to mount an SEB induced immune response in the absence of other T cells or B cells. IL-2 Rα staining suggested that a majority of the T cells had been activated in response to SEB. In addition upon SEB injection a population of CD4- TCR+ cells was detected in the spleens of SEB injected transgenic *scid* mice. A similar population was seen in the thymus but not in the spleen of control mice.
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Abbreviations

- β2m: beta 2 microglobulin
- CM: complete media
- EAE: experimental allergic encephalomyelitis
- FITC: fluorescein isothiocyanate
- GVHD: graft versus host disease
- Ig: immunoglobulin
- IL: interleukin
- IL-2 R: interleukin 2 receptor
- IFN: interferon
- mAb: monoclonal antibody
- MBP: myelin basic protein
- MEM: minimum essential medium
- MHC: major histocompatibility complex
- MIP: macrophage inflammatory peptide
- MLs: minor lymphocyte stimulating antigens
- MMTV: mouse mammary tumor virus
- OVA: D0-11.10 T cell receptor transgenic mouse
- PLP: proteolipid protein
- PBS: phosphate buffered saline
- PE: phycoerythrin
- RAG -/-: recombinase activating gene deficient
- scid: severe combined immunodeficiency
- SE: Staphylococcal Enterotoxin
- SDC: SEB dependent cytotoxicity
- TCR: T cell receptor
- TNF: tumor necrosis factor
- Th1: T helper 1
- Th2: T helper 2
- 7AAD: 7 amino actinomycin D
Introduction

The immune response to foreign antigen involves the effector cells of the immune system. Lymphocytes are the key effector cells of the immune system. These cells are responsible for recognizing, mounting a response to and clearing the antigen. In addition to the response against antigen these cells may also serve to regulate the immune response. T cells bearing the αβ T cell receptor (TCR) are amongst the lymphocytes which are involved in regulating the immune response (1) (2) (3). Generally αβ T cells can regulate an immune response either by T cell mediated cytotoxicity or by the secretion of cytokines (4) (5). In order for an αβ T cell to exert its immunoregulatory activity it usually needs to be activated (5).

αβ T cells recognize antigens in the context of cell surface proteins called major histocompatibility complex (MHC) molecules (6) (7). There are two general classes of αβ T cells, each of which have two major subclasses. The two major subclasses of αβ T cells include helper T cells (CD4) and cytotoxic T cells (CD8). CD4 T cells recognize antigen in the context of MHC class II molecules, while CD8 T cells recognize antigen in the context of class I molecules. In addition to recognizing antigen plus MHC, T cells require costimulatory molecules to be activated. One of the key costimulatory molecules involved in T cell activation is CD28 (8). CD28 is a homodimeric molecule expressed on the surface of αβ T cells. Its interaction with members of the B7 family of molecules expressed on antigen presenting cells results in the activation of an αβ T cell.
T cells may regulate various phases of the immune response. For example, mice which lack the TCR α chain and thus lack αβ T cells had an increased number of B cells (1). These B cells secreted increased amounts of autoreactive antibodies. The authors suggest that this may point to a role for αβ T cells in regulating B cells. The presence of a TCR (T cell receptor) transgene specific for myelin basic protein (MBP) in a RAG 1 deficient (RAG -/- ) mouse resulted in spontaneous experimental allergic encephalomyelitis (EAE) in 100% of the mice (9). Only 14% of TCR transgenic mice that were not RAG 1 deficient spontaneously developed EAE. Since RAG 1 -/- mice are unable to initiate rearrangement of their antigen receptors and thus are immunodeficient and EAE involves primarily T cells (10) (11), the authors implicate the onset of spontaneous EAE in all MBP transgenic RAG -/- mice to be the result of a lack of regulatory T cells that are absent in these mice but present in the RAG 1 proficient TCR transgenic mice. However the onset of spontaneous EAE may also be due to other factors such as the EAE susceptible haplotype of the mice used.

T suppressor cells are proposed as a class of T cells which can actively downregulate an immune response. These cells can exert their suppressive activity through anti-idiotypic suppression. The suppressor T cell can recognize the antigen receptor of the activated T cells and thus suppress these cells. The suppressive activity of these cells could be adoptively transferred to a secondary recipient (12). Both helper T cells (CD4) and cytotoxic T cells (CD8) can influence the immune response. CD4 T cells exert their immunoregulatory capacity mainly by the secretion of cytokines (4) (5). CD8 T cells exert their immunoregulatory capacity by their cytotoxic activity (13) and the secretion of cytokines (14) (15).
CD4 cells may be important in regulating the immune response. Mice deficient in CD4 cells showed decreased antibody production illustrating that CD4 cells can help B cells make antibodies (16). However since these mice can still generate antibodies in the absence of CD4 cells, other cells may contribute to antibody production. In addition to helping B cells, CD4 cells can influence the immune response by the secretion of cytokines (17). Upon T cell activation, CD4 T cells can differentiate into two distinct subsets characterized by their pattern of cytokine secretion (17). These subsets, termed T helper 1 (Th1) and T helper 2 (Th2) were initially characterized in murine T helper cell clones that were divided into two distinct groups based on their cytokine profile (18). Th1 and Th2 cells secreted distinct arrays of cytokines which serve to regulate the other subset (4) as well as other immune responses such as antibody responses and cell mediated immune responses (17). Th1 cells produce IL-2, IFN-γ, TNF-β, TNF-α (17) (18) (19) and are involved in cell mediated inflammation and in delayed type hypersensitivity reactions (20). Th2 cells produce IL-4, IL-5, IL-10, TNF-α (17) (18) (19). Some of the Th2 cell produced cytokines encourage antibody production and are found in association with allergic responses (17). In addition the cytokine products of Th1 and Th2 cells serve to regulate each other (4). Thus Th1 produced IFN-γ selectively inhibits the proliferation of Th2 cells. Th2 produced IL-10 selectively inhibits cytokine synthesis by Th1 cells. This cross regulation can cause a bias in Th1 or Th2 responses in certain infections. In addition other cytokines can also serve to drive Th1 or Th2 development. For example, IL-12, a cytokine secreted by monocytes, can drive the development of IFN-γ producing Th1 cells (21).
Development of the appropriate Th subset during infection can determine the outcome of infection. For example, a Th1 type response in C57BL/6 mice correlated with the resolution of the protozoan parasite infection *Leishmania major* while a Th2 response in BALB/c mice correlated with dissemination of disease and ultimate death of the infected mice (5). Th1 and Th2 responses have been seen in autoimmune disease models. In EAE (experimental allergic encephalomyelitis), a mouse model of multiple sclerosis (22), there is evidence that Th1 cells are important for the initiation of the disease (23) (24). EAE was first induced in mice by Olitsky and Yager in 1949 (22). They treated albino mice of the Swiss strain with intramuscular and subcutaneous injections of normal mouse brain mixed in Freund's adjuvant. The mice developed symptoms such as wheezing and histological lesions similar to monkeys, rabbits and guinea pigs with EAE. In most current models, myelin basic protein (MBP), a dominant protein of the central nervous system myelin, can be used to initiate EAE (23) (24).

To determine the role of Th1 versus Th2 cells in EAE, two myelin basic protein peptide specific cell lines were used (24). Adoptive transfers of these clones into mice showed that only the Th1 clones were able to induce disease as measured by paralysis and immunohistology. However the ability to induce EAE amongst the Th1 clones was dependent on the presence of α4 integrin (VLA-4). Furthermore lymphocytes that infiltrate the brain and spinal cord of a mouse with EAE stained positively for IFN-γ and IL-2. IL-4 and IL-5 infiltrating lymphocytes were seen later on in the disease (23). Th2 cells can protect against EAE in some systems (25) but not in others (26). Mice orally administered myelin basic protein (MBP) developed mucosally derived Th2 like cells (25). These cells produced TGF-β, IL-4 and IL-10. Cloned Th2 cells from these mice inhibited the development of EAE in mice.
immunized with MBP. However, TGF β, which is not classically a Th2 cytokine, may play a critical role in mediating protection in this model. Results from another group that induced EAE in SJL mice by proteolipid protein (PLP), a major component of the central nervous system myelin membrane, showed that adoptive transfers of Th1 but not Th2 cells induced the disease (26). However, adoptive transfers of Th1 and Th2 cells still induced EAE. This suggests that although Th1 cells seem to be responsible for the initiation of EAE (23) (24) (26) it is not clear whether Th2 cells protect against EAE (26).

A differential role for Th1 and Th2 cells has also been implicated in graft versus host disease (GVHD) (27) (28). Chronic GVHD and acute GVHD are two forms of GVHD that have been well characterized in mouse models. While acute GVHD is induced by the transfer of C57BL/6 splenocytes into unirradiated F1 recipients (C57BL6 X DBA/2), chronic GVHD is induced by the transfer of DBA/2 splenocytes into these F1 recipients (27). Acute GVHD is characterized by the involvement of cytokines such as IL-1, IFN-γ and TNF-α while chronic GVHD is characterized by the involvement of cytokines such as IL-4 and IL-10. (28). Allen et al. found that in the chronic model of GVHD (DBA/2 into C57BL/6 X DBA/2), splenic mRNA showed more IL-4 signal than in control mice or mice with acute GVHD (C57BL/6 into C57BL6 X DBA/2) (27). In contrast, mice with acute GVHD showed more IL-10, IFN-γ and MIP-1α (a chemokine) signal and less TNF-β (lymphotoxin) signal. Since the mice with acute GVHD had increased IL-10 and decreased TNF-β, the Th1/Th2 distinction in acute versus chronic GVHD may not be clear-cut. Recently, Krenger et al. showed that polarized type 2 CD4 and CD8 T cells failed to induce acute GVHD as measured by reduced IFN-γ and TNF-α production as well as lethality though LPS challenge (28). LPS is used in models of
GVHD to induce TNF-α production and the extent of TNF α production is related to the severity of GVHD. They generated polarized alloreactive CD4 or CD8 T cells *in vitro* by adding rIL-2 plus or minus rIL-4 to a mixed lymphocyte reaction directed against class I differences (bm1 into C57BL/6) or class II (bm12 into C57BL/6) differences. Type 1 cells were generated in the cultures that lacked IL-4, while type 2 cells were generated in the cultures that had IL-4. Transplantion of polarized type 2 CD4 or CD8 plus donor bone marrow cells failed to induce GVHD as measured by reduced IFN-γ production, LPS induced lethality and reduced TNF-α production. Polarized type 1 CD4 and CD8 cells induced increased IFN-γ in response to Con A after transplantation and virtually no IL-4. Polarized type 2 CD4 cells produced IL-4 and reduced IFN-γ, while type 2 CD8 cells produced very low levels of IFN-γ. Mixing experiments of polarized Th2 CD4 cells with naive CD4 cells and donor bone marrow showed that these polarized type 2 cells could inhibit acute GVHD as measured by IFN-γ production in a secondary mixed lymphocyte reaction. Thus CD4 cells may play a role in regulating the immune response.

**The role of CD8 T cells in the immune response**

CD8 cells can play a role in the regulation of an immune response. These cells can mediate suppression of an immune response by directly killing activated T cells (13), by secreting cytokines (15) (28), or by controlling the activity of CD4 cells (2) (3). CD8 T cells can also suppress the immune response by a functional elimination of the reactive T cell. For example, veto cells are specialized antigen presenting cells that delete activated T cells that recognize
them (29). Veto cells generally carry the molecule CD8 on them and inhibit class I mediated immune responses.

Although classically CD8 cells have not been implicated in cytokine production, more recently alloreactive CD8 T cells clones have been shown to secrete a Th1 pattern of cytokines (14). CD8 T cell clones were stimulated with a panel of antigens and the supernatants were assayed for the production of cytokines by ELISAs and bioassays. These clones produced IFN-γ, TNF-β, and TNF-α and low amounts of IL-2. CD8 T cells can also be polarized in vitro to become type 1 and type 2 CD8 cells depending on their cytokine profiles (15). CD8 cells from H-Y transgenic mice (30) stimulated with B6 antigen presenting cells and cultured in the presence of IL-2 and IL-12 made high amounts of IFN-γ and IL-2 and no IL-4 or IL-5 upon restimulation with anti-CD3 antibody. CD8 cells generated with IL-2 and IL-4 made IL-4 and IL-5 but very little IL-2 and IFN-γ. The role that type 1 and type 2 CD8 cells may play in influencing the immune response in vivo was discussed earlier in a murine model of acute GVHD (28). These findings have important implications for type 1 and type 2 CD8 T cells in the regulation of the immune response.

CD8 T cells may also influence the fate of CD4 cells (2) (3) (31). LCMV virus infected β2m -/- mice (lack class I MHC) had exaggerated CD4 T cell proliferation in vivo (31). These CD4 cells also developed cytotoxic function. Although the authors implicate a role for CD8 cells in controlling CD4 T cell proliferation and cytotoxicity, a direct role was not demonstrated. Since CD8 T cells lyse virally infected cells (32), the exaggerated CD4 T cell responses could be due to an increased viral load. A role for CD8 cells has also been suggested in EAE (2) (3). Although, EAE is primarily mediated by CD4 cells (10) (11), in mice with
EAE a lack of CD8 cells prevented recovery from the disease (2). Mice lacking CD8 cells were bred with EAE susceptible PL/J H-2b mice for four generations. Although the disease onset and susceptibility in CD8 -/- mice was the same as in wild type mice, the mutant mice had milder acute EAE (less deaths) and suffered from a chronic (relapsing) form of EAE. This suggested that CD8 cells may play a role in the pathogenesis or regulate recovery from EAE. The role that CD8 cells have in EAE was also shown by Jiang et al (3). They depleted B10.PL (H-2b) mice of CD8 cells and showed that mice depleted of CD8 cells were no longer resistant to a secondary challenge with MBP. The authors suggest that CD8 cells may participate in the resistance to a secondary induction of EAE.

**Superantigens**

Superantigens stimulate particular populations of T cells (33) (34). In contrast to peptide antigens which activate T cells carrying the specificity for the peptide MHC complex, superantigens activate T cells that bear particular Vβ chains (33) (35). Since superantigens involve the activation of T cells with particular Vβ chains they are useful in following antigen specific responses in vivo.

The T cell superantigen family consists of bacterial superantigens produced by *Staphylococcus aureus* and *Streptococcus pyogenes* as well as virally encoded superantigens such as the minor lymphocyte stimulating (Mls) antigens (36). Mls antigens are encoded by open reading frames in the 3' long terminal repeat of mouse mammary tumor viruses (MMTV) (37). *Staphylococcus aureus* produces eight similar enterotoxins; SEA, SEB, SEC1, SEC2, SEC3, SED, SEE, and TSST (toxic shock syndrome toxin) which are superantigens (38). The superantigens SEA-E are involved in food poisoning while TSST is involved in toxic shock.
syndrome. *Streptococcus pyogenes* produces three superantigens SPA, SPB and SPC which have been associated with scarlet fever and toxic shock. Both the staphylococcal and streptococcal toxins are 27-28 kDA polypeptides. TSST is a 22 kDA polypeptide. The staphylococcal and streptococcal toxins share similarities in their primary amino acid sequence suggesting common origins. The crystal structure of SEB shows that it consists of a main chain with two domains (39). The first domain consists of residues 1-120 and has five \( \beta \)-strands and three \( \alpha \)-helices. The second domain consists of residues 127-239 and has seven \( \beta \)-strands and two \( \alpha \)-helices. A shallow cavity formed by these two domains is postulated as the TCR binding site. The MHC class II molecule binds to an adjacent site.

T cell superantigens stimulate mature T cells bearing particular T cell receptor V\( \beta \) chains (33) (35). Exposure of mature T cells to Mls\(^3\) antigens stimulates a strong T cell proliferative response *in vivo* (40) and *in vitro* (36). Staphylococcal enterotoxin B (SEB) potently stimulates murine T cells that bear the following T cell receptor \( \beta \) chains: V\( \beta \) 3, 7, 8.1, 8.2, 8.3, 11, 17 (33) (34) (38) (41). In general superantigens bind outside the peptide binding groove of the MHC molecule and to the TCR V\( \beta \) thus crosslinking the two molecules (42). Heterogeneity in the interaction of particular SEB reactive V\( \beta \) chains with superantigens suggests other contributing elements to superantigen recognition. For example the \( \alpha \) chain has also been implicated in influencing the response to SEB (43) (44) as well as Mls (45) (46) (47). Another factor that influences T cell recognition of SEB (34) or Mls is the MHC molecule (48). Furthermore, in human T cells, mutations in the peptide binding groove of class II MHC can effect the binding of toxic shock syndrome toxin-1 and SEB with the class II MHC molecule (49).
Superantigen response *in vivo*

The *in vivo* response of mature T cells exposed to superantigen is characterized by an initial phase of proliferation followed by a deletion (after day 4) of CD4 and CD8 cells bearing the appropriate Vβ chain (50) (51) (52). Results from Kawabe et al indicate that the deletion seen is partially due to cell death via apoptosis (53). Since deletion is defined as the loss of Vβ8.1 T cells in the spleen, lymph node or blood, after day 4 of SEB injection (50) (51) (52), it is possible that this loss may be due to other factors such as migration of the T cells to other anatomical sites or downregulation of the both the TCR and the CD4 molecule. For the purposes of this thesis deletion is defined as the loss of CD4 Vβ8 T cells from the spleen, lymph node or blood of mice after day 4 of SEB injection. This loss could be due to migration, apoptosis or a combination of both. The superantigen SEB can activate both CD4 and CD8 cells (54), however CD4 cells undergo more deletion than CD8 cells (50) (51) (53). Additionally only CD4 cells are impaired in their ability to respond to SEB upon *in vitro* restimulation and are termed anergic (50). These anergic T cells exhibit impaired tyrosine phosphorylation in response to TCR mediated stimulation (55). It is important to note that the anergy exhibited by these T cells is transient (56). Furthermore, competent antigen presenting cells and high concentrations of SEB can overcome anergy *in vitro* (57) (58). A second exposure to SEB *in vivo* results in the production of similar amount of IL-2 and tumor necrosis factor by CD4 Vβ8 cells as is present in mice given a single challenge of SEB. However this secondary challenge only results in expansion of CD8 Vβ8 cells (59). Therefore the CD4 Vβ8 cells are still unable to proliferate *in vivo* upon subsequent challenge with SEB and may be
anergic or may lack the ability to respond to SEB. Clonal anergy to superantigens has also been shown to exist with human lymphocytes (60).

An effector function of CD8 cells upon SEB injection is SEB dependent cytotoxicity (SDC) (54). Upon SEB injection CD8 cells are able to directly lyse class II bearing target cells incubated with SEB ex vivo. This effector function of CD8 cell has been documented in vitro and is termed SEB dependent cytotoxicity (SDC). SDC peaks after 2-3 days of SEB injection. The role of SDC in vivo has not been addressed.

Factors which influence the superantigen response in vivo
The response to superantigens in vivo is determined by a number of factors. These factors include age of the host (33) (61), dosage of the superantigen (62) (63), haplotype of the host and the particular VB studied (48). In neonatal mice, encounter with superantigen leads to a deletion of the reactive T cell without the induction of a proliferative response, while in mature mice encounter with superantigen leads to proliferation of the mature reactive T cells followed by a deletion of some of the superantigen reactive cells (33) (35) (64). In aged mice, encounter with superantigen leads to less expansion and less deletion (61). If the superantigen is injected in a very high dose in adult mice along with the hepatotoxin D-galactosamine, then there is an expansion of T cells and the mice die of toxic shock (63). If the superantigen is repeatedly injected into adult mice, there is a profound deletion of the SEB reactive T cells (62) (65).

The proliferative response to superantigens in vivo is dependent on the presence of many cell surface molecules (66) (67) (68) (69). CD28 is a homodimeric molecule expressed on T cells (8). Its binding to B7-1 or B7-2 molecules, which are expressed on the surface of antigen presenting cells, facilitates the activation of a T cell. While CD28 allows for the
activation of T cells, its homolog CTLA-4, which also binds members of the B7 family, inhibits activation (70). In contrast to CD28, which is constitutively expressed on T cells, CTLA-4 is transiently expressed on activated T cells. Antibodies that block the interaction of CD28 and B7 are able to block SEB induced proliferation in vivo (66) and in vitro (71). Evidence from CD28 deficient mice showed that though the initial proliferation is dependent on CD28, deletion is not (67). In contrast to anti CD28 antibodies, Fab fragments of anti CTLA-4 antibodies in vitro and in vivo enhance T cell responses to SEB (68). This shows that CTLA-4 and CD28 have opposing effects in T cell activation in response to SEB. Additionally the adhesion molecule, ICAM-1, also seems to play a role SEB induced T cell proliferation in vivo (69). Mice that lack ICAM-1 have impaired T cell proliferative responses to SEB but are able to undergo SEB induced deletion.

Factors which influence superantigen induced deletion in vivo
There are many factors which can influence superantigen induced deletion in vivo. These factors include the cell surface molecule Fas (72), other T or B cells (13) (73) (74), the initial proliferative response to the superantigen (75), as well as the cytokine IL-2 (65) (76). Fas plays a partial role in the apoptosis of T cell activated in response to SEB (72). Fas is an integral membrane protein which is a member of the tumor necrosis factor receptor family. T cells from both gld mice, which lack functional Fas ligand, and young MRL/lpr mice, which lack functional Fas, are able to proliferate in response to SEB (72). However only a fraction of the T cells undergo superantigen induced deletion. Thus Fas may play a partial role in the deletion of SEB activated T cells.
While the role that T and B lymphocytes play in the *in vivo* response to Mls responses is defined, the role that T and B lymphocytes play in the *in vivo* response to SEB response is less clearly defined. For the Mls response, B cells seem to be the most efficient presenters although other lymphocytes can present Mls (73). Furthermore both B cells and CD8 cells but not CD4 cells can induce peripheral deletion of Mls reactive T cells (73). In neonates CD8 cells seem to be more efficient than B cells in inducing deletion (52). The role of B cells in the SEB response has been recently addressed in B cell knockout mice (58) (77). B cells do not seem to be required in the initial proliferative response to SEB *in vivo* (77). However T cells from B cell knockout mice are not anergic *in vitro* (58). The role of B cells in *in vivo* anergy or deletion was not addressed. The role of other CD8 or γδ cells in the *in vivo* response to SEB response is not clearly defined. There are two reports that discuss the role of CD8 cells in the SEB induced deletion of CD4 Vβ8 T cells (13) (74). One report using CD8 depleted mice suggests that the role for CD8 cells is minimal if any, since CD8 depleted mice (depleted by anti-CD8 monoclonal antibody treatment) have CD4 Vβ8 T cells deleted (74). This study found no qualitative difference in the responses of untreated and CD8 T cell depleted mice (depleted by anti-CD8 monoclonal antibody treatment) to SEB. However they did find a small quantitative difference in deletion. Another report using both CD8 depleted and β2m knockout mice (which lack class I MHC) suggested that CD8 cells are required in SEB induced deletion of CD4 Vβ8 T cells (13). Both CD8 depleted and β2m knockout mice had a partial deletion of CD4 Vβ8 T cells. Additionally it was shown that CD8 cells lyse CD4 cells that have previously been activated in response to SEB *in vitro*. 
In contrast to the previous groups, Renno et al. have shown that T cells which undergo apoptosis \textit{in vivo} are ones that have proliferated in response to SEB (75). All T cells that underwent apoptosis had proliferated in response to SEB. Renno suggests that the V\(\beta\)8 cells that are weakly SEB reactive do not receive a signal that is strong enough to allow proliferation and then death. However other groups have shown that deletion of CD4 V\(\beta\)8 T cells can occur in the absence of significant proliferation (67) (69).

The role of IL-2 in SEB mediated deletion has recently been addressed. Mice that lack the IL-2 gene (IL-2 \(-/-\)) have normal \textit{in vivo} expansion of CD4 and CD8 cells in response to SEB (76). However fewer CD4 cells are subsequently deleted. Evidence from another group also points to a role for IL-2 in SEB mediated deletion. Lenardo has shown that injection of an antibody against the IL-2 receptor \(\alpha\) chain was able to prevent the reduction of lymph node V\(\beta\)8 cells in response to repeated injections of SEB (65).

**Mouse Models : The advantages of \textit{scid} and transgenic mice**

Since the immune response to antigen involves the activity of a specific lymphocyte, it is advantageous to follow that lymphocyte during the course of an immune response. Recent molecular genetic technology has allowed for the production of TCR transgenic mice engineered to express a high frequency of T cells specific for an antigen (30). Since the T cells in TCR transgenic mice express clonotypic TCRs, they can be followed \textit{in vivo} and \textit{in vitro} with antibodies that specifically bind to the antigen receptor. Thus the fate of a specific population of T cells during the course of an immune response can be studied. It is important to note that although a TCR transgenic mouse expresses a high percentage of antigen specific T cells it also carries other T cells since the lack of allelic exclusion at the \(\alpha\) locus results in T
cells that carry the transgenic β chain with an endogenous α chain (30). This can result in a loss of specificity for the antigen. To overcome this problem scid TCR transgenic mice can be used (78). Scid mice are naturally occurring mutant mice that have lost the ability to successfully rearrange their antigen receptors by VDJ recombination (79) (80). This inability is due to a defect in DNA repair that impairs the cells from rejoining the rearranged antigen receptor DNA. Therefore scid mice are generally devoid of T and B lymphocytes (80). Mice that bear the TCR transgene and harbor the scid mutation bear the transgenic T cell in absence of other T or B cells. These mice are a good in vivo system for studying an immune response of a clonotypic population of T cells in the absence of other T or B cells.

**Thesis**
The immune response to superantigens is controlled by a complex interaction of T cells, B cells and cell surface molecules. According to one model, the extent of deletion is dependent the initial proliferative response to SEB (75). Thus TCRs with low affinities for SEB do not proliferate or get deleted, whereas TCRs with high affinities for SEB proliferate and then are deleted. However evidence from other groups suggest that deletion can occur in the absence of significant proliferation (67) (69). Furthermore other groups implicate CD8 T cells as important in controlling the deletion of CD4 cells in response to SEB (13) (74). These reports are conflicting. Some groups have shown that in the absence of CD8 cells in β2m -/- mice or in mice treated with an antibody against CD8, the CD4 Vβ8 T cells are partially deleted (13). Other groups using an antibody against the CD8 molecule indicate that CD8 cells are minimally required in SEB induced deletion (74). The work presented in this thesis focused on the investigation of the SEB response in a mouse with a clonotypic population of T cells.
All the T cells in this mouse should have the same affinity for SEB. Therefore if they proliferate in response to SEB they should be deleted. Since the role of CD8 cells in SEB induced deletion of CD4 Vβ8 T cells is controversial, we wanted to investigate whether the absence of CD8 cells in this system would affect the extent of deletion of CD4 Vβ8 T cells.

**Model system**
The superantigen SEB was used to analyze the immune response in C.B-17 scid mice (80) carrying a transgenic αβ T cell receptor reactive to an ovalbumin peptide plus I-A<sup>d</sup> (81), a T cell receptor expressing Vα 13 and Vβ 8.2 and reactive with SEB (33) (82). These mice are hereafter referred to as OVA scid mice. Since OVA scid mice bear the scid mutation, these mice have no B cells and only have T cells that bear the αβ TCR transgene. Therefore the response of a clonotypic population of SEB reactive T cells can be studied in the absence of all other T or B cells. By adoptively transferring different T lymphocyte subsets into this mouse model, the role that T cells play in the SEB response can be examined. The role of CD8 T cells in SEB induced CD4 Vβ8 T cell deletion was investigated by adoptively transferring different T lymphocytes subsets into these mice. To overcome the problem of the high percentage of superantigen reactive T cells present in the OVA scid mouse, adoptive transfers of OVA scid splenocytes along with CD4 or CD8 cells were done into a C.B-17 scid mouse. The results indicate that a clonotypic population of T cells in the absence of any other T or B cells is able to mount a response to SEB in the spleen by increasing in transgenic T cell number. These transgenic T cells are able to undergo superantigen induced deletion. The deletion seen may be due to many factors including the migration of T cells from the spleen to lymph nodes or liver. The deletion seen may also be due to death of T cells by apoptosis or a combination of
migration and apoptosis. IL-2 Rα staining suggests that the T cells in the OVA scid were activated in response to SEB. Upon SEB injection a population of CD4+ TCR+ cells was detected in the spleens of SEB injected OVA scid mice. A similar population was seen in the thymus but not in the spleen of control mice.
Materials and Methods

Mice:
BALB/c mice were obtained from the Jackson Laboratories (Bar Harbor, ME). DO-11.10 TCR transgenic mice, hereafter referred to as OVA mice (83) were kindly provided by Dr. Dennis Y. Loh (Washington University School of Medicine, St. Louis, Mo.). These mice express a transgenic αβ T cell receptor reactive to an ovalbumin peptide (ova 326-336) plus I-A<sup>d</sup> (81) (83). The mice were produced by Loh et al as follows. A genomic library of the DO.11.10 hybridoma (84) was made. Probes specific for the rearranged DO.11.10 α and β genes were used to isolate the productively rearranged α and β genes. A 13 kb fragment containing the correct Vα rearrangement and a 12 kb fragment containing 5 kb of DNA upstream of the rearranged Vβ8.2 including Vβ 5.1 and the complete Cβ1 gene were obtained and verified through sequencing and restriction mapping. The 13 kb fragment with the correct Vα rearrangement was ligated into the cosmid CaBS2. This cosmid contained the constant region of the α chain. The 12 kb β chain fragment was ligated to a DNA fragment containing the entire Cβ2 gene, the Cβ enhancer and Vβ14 gene and the resulting construct was ligated into a PCK-X vector. The final functional α and β constructs used for injection were cut out of their respective vectors and were 47 kb and 38 kb respectively. Fertilized embryos were obtained from 3-4 week old superovulated (C3H/HeJ X C57BL/6) F1 females that were mated to BALB/c males. The rearranged α and β gene constructs were injected into the embryo pronuclei. The first transgenic progeny contained 4 copies of the β chain and two copies of the α chain and was of the H-2<sup>bd</sup> haplotype. This transgenic male was mated to BALB/c H-2<sup>d</sup> females to produce H-2<sup>bd</sup> transgenic mice used for breeding. The OVA mice express the Vα13
and Vβ 8.2 chains of the TCR. The Vα13 Vβ8.2 TCR is SEB reactive (33) (82). In the Miller lab, the OVA mice were backcrossed to BALB/c mice for nine generations. Mice were screened for the presence of the transgene by flow cytometry using a FITC conjugated MR 5-2 (anti Vβ 8.1, 8.2) antibody (Pharmingen, San Diego CA). C.B-17 mice are congenic mice originally made by mating BALB/c mice with B6 mice for 17 generations and selecting for the IgH locus of B6 mice on a H-2d background (85). C.B-17 scid mice (80) are C.B.17 mice which have a mutation in the scid gene (86). These mice lack T or B cells due to the scid defect and have the H-2d haplotype. The OVA scid mice were made in the Miller lab by mating OVA mice to C.B-17 scid mice for 6 generations. Mice were screened for the presence of the transgene and absence of any other T or B cells by flow cytometry using a PE conjugated 53-2.1 (Thy 1.2) antibody (Pharmingen, San Diego CA) and FITC conjugated MR 5-2 (anti Vβ 8.1, 8.2) antibody (Pharmingen, San Diego CA). Thereafter the mice were maintained by brother sister mating. All mice were bred and maintained in the defined flora animal colony at the OCI (Ontario Cancer Institute).

**Antibodies and Reagents:**

Staphylococcal enterotoxin B (SEB) was purchased from Sigma Immunochemicals (St. Louis, Mo). Monoclonal antibody KJ1 26.1 (mouse IgG2a) is specific for the transgenic TCR expressed by the OVA.scid mouse (87). KJ1 26.1 culture supernatant was collected from the KJ1 26.1 hybridoma (kindly provided by Kappler and Marrack). The antibody was purified on Protein A sepharose by affinity chromatography (88) and FITC labeled according to the Current Protocols in Immunology (The Gold Book Bulletin: Supplement 1 June 1991). PE conjugated H129.19 (anti-CD4) (89) and PE conjugated 53-6.7 (anti CD8) (90), PE
conjugated RA3-6B2 (anti B220) (91), FITC conjugated 29B (anti CD3) (92), Steptavidin Quantum Red and 7AAD (a DNA intercalating dye) (93) were purchased from Sigma Immunochemicals (St. Louis, Mo.). FITC conjugated MR 10-2 (anti Vβ9) (94) was a generous gift from Dr Danska (University of Toronto, Toronto, ON). Biotin conjugated 7D4 (anti IL-2 receptor α) (95) purchased from Pharmingen (San Diego, CA) was a generous gift from Dr Ohashi (University of Toronto, Toronto, ON). FITC conjugated MR 5-2 (anti Vβ 8.1, 8.2) (96), FITC conjugated RR4-7 (anti-Vβ6) (97) and PE conjugated 53-2.1 (Thy 1.2) (90) were purchased from Pharmingen (San Diego, CA). Anti-FCR γ II supernatant was produced from the hybridoma 2.4G2 (98) purchased from the American Type Culture Collection (Rockville, MD). Mouse serum was purchased from Jackson Immuno Research laboratories (BayHarbor, ME). Anti-CD4 ascites was produced from the hybridoma GK 1.5 (99), anti-CD8 ascites was produced from the hybridoma 53-6.72 (90). Both hybridomas were purchased from the American Type Culture Collection (Rockville, MD) and raised in C.B-17 scid mice. Magnetic beads coated with sheep anti-rat IgG were purchased from Dynal (Oslo, Norway). Magnetic beads coated with goat anti-mouse IgG beads were purchased from BIOMAG (Cedarlane, Hornby, ON).

**Culture Medium:**
α MEM purchased from Gibco (Missaugua, ON) was supplemented with 20μM Hepes pH 7.3, 50μM β2-mercaptoethanol, penicillin, streptomycin plus 10% heat-inactivated fetal calf serum purchased from Biowhittaker (Walkersville, MD). This was the media used for all the splenocyte suspensions and is hereafter referred to as complete media (CM).
**Flow cytometry:**
All monoclonal antibodies used have been titrated. The following monoclonal antibodies were used for staining: 1µg FITC conjugated KJ1 26.1 (anti OVA TCR), 0.5µg PE conjugated H129.19 (anti-CD4), 0.5µg PE conjugated 33-6.7 (anti CD8), 0.5µg PE conjugated RA3-6B2 (anti B220), 0.25µg FITC conjugated 29B (anti CD3), 0.5 µg Steptavidin Quantum Red and 1 µg 7AAD (a DNA intercalating dye) 0.5µg biotin conjugated 7D4 (anti IL-2 receptor α), 1µg FITC conjugated MR 5-2 (anti Vβ 8.1, 8.2), 1µg FITC conjugated RR4-7(anti Vβ 6) and 0.125µg FITC conjugated MR 10-2 (anti Vβ9). In some cases the splenocytes were harvested using Lympholyte separation medium (Cederlane Laboratories, Hornsby, ON) to remove dead cells. The cells were then washed three times with CM. 5 x 10^5 cells were washed with 1% BSA in PBS (+ Ca^{2+} and Mg^{3+}) and then incubated with 40 µl anti FCR γ II supernatant (not added in figure 3 and table 1 data), 5 µl mouse serum, titrated Biotin, FITC and PE conjugated antibodies in a final volume of 150-200 µl for 20 minutes. Stained cells were washed with 1% BSA in PBS. The secondary antibody steptavidin quantum red was added (0.5µg) and the cells were incubated for 20 minutes. Stained cells were then washed with 1% BSA PBS. For cells that were stained with FITC and PE only, one wash with 1% BSA PBS was done followed by an incubation of 5-20 minutes with 1 µg 7AAD as an indicator of dead cells. In cases where the splenocytes were passaged over Lympholyte M to eliminate dead cells no 7AAD was added (figure 3 and table 1). Cells were analyzed using a FACScan flow cytometer (Becton Dickinson & Co., Mountain View, CA). Fluorescence data were collected using logarithmic amplification; 10,000 events were collected.
**Determination of cell numbers:**

Spleen suspensions were stained as described above. Forward scatter was plotted on the X axis and side scatter was plotted of the Y axis of a dot plot. Red blood cells (RBC) were gated out of the analysis based on their forward scatter and side scatter characteristics. To exclude dead cells forward scatter was plotted on the Y axis of a dot plot and 7-AAD (FL-3) was plotted on the X axis. 7-AAD positive cells were gated out of the analysis. For two color analysis FL-2 was plotted on the Y axis and FL-1 was plotted on the X axis of a dot plot. The percentage of cells in the appropriate subset was determined. This percentage was multiplied by the total number of eosin excluding splenocytes counted on a haemocytometer.

**Injections:**

50 µg of SEB in PBS was injected intravenously in the tail vein of the mice. Fractionated or unfractionated splenocytes were resuspended in PBS and injected intravenously in the tail vein of the mice.

**Statistics:**

Independent T tests were used for the statistical analysis. All calculations were performed using the computer program Microcal Origin version 3.5 (Microcal Software, Inc., Northampton, MA).

**Preparation of unfractionated splenocytes and fractionation of B depleted splenocytes**

Mice were sacrificed by cervical dislocation and single spleen suspensions made by standard techniques (100). This was the source of unfractionated cells. For the B depleted T cells used in Table 1 the splenocytes were harvested using Lympholyte separation medium.
(Cederlane Laboratories, Hornsby, ON) and washed three times in CM. For the subsequent
fractionations no lympholyte was used. The suspension then was passaged over a nylon wool
column (101). The percentage of cells recovered from the nylon wool purification was about
30-40%. Splenocytes were collected and resuspended at $10^7$ cells/ml in CM. This suspension
was then treated with magnetic beads coated with goat anti-mouse IgG at the ratio of 20:1
beads/cell. The mixture was continuously mixed at 4°C for 45-60 minutes. B cells were then
removed magnetically. The purity of the populations was monitored by flow cytometry. Cells
and was 66-93% CD3+ and 0.4%-1.2% B220+.

**Preparation of T depleted splenocytes**

To obtain T depleted spleen, splenocytes were harvested using Lympholyte separation medium
(Cederlane Laboratories, Hornsby, ON) and treated with 1/300 dilution of rat ascites against
CD4 (GK 1.5) and CD8 (53-6.72) at 4°C for a 30 minutes. The suspension was washed and
then magnetic beads coated with goat anti-rat IgG were added (ratio of 2:1 beads/cell). The
mixture was continuously mixed at 4°C for 30-45 minutes. CD4 and CD8 cells were then
removed magnetically. The purity of the populations was monitored by flow cytometry. The
purity of the population was 90% B220+, 4.3% CD3+, 5.7% CD3- B220-.

**Preparation of fractionated CD4 or CD8 cells:**

For the fractionation of CD4 and CD8 cells, cells from the nylon wool column separation were
treated with 1/300 dilution of rat ascites against CD4 (GK1.5) or CD8 (53-6.72) at 4°C for a
30 minutes. The suspension was washed and then treated with magnetic beads coated with
goat anti-mouse IgG at the ratio of 20:1 beads/cell and magnetic beads coated with sheep anti-
rat IgG at the ratio of 2:1 beads/cell. The mixture was continuously mixed at 4°C for 45-60
minutes. B cells and CD4 or CD8 cells were then removed magnetically. The percentage of cells recovered from the magnetic bead sort was about 18-34% for CD4 cells and 12-20% for CD8 cells. The purity of the populations was monitored by flow cytometry. The purity of the CD4 cells was 90% CD4+, 2.0% CD8, 2.0% B220, 6.0% CD3- B220-. The purity of the CD8 cells was 79% CD8, 2.0% CD4, 2.0% B220, 17% CD3- B220-.
Results

**A characterization of OVA scid versus BALB/c thymocytes**

In order to compare TCR transgenic scid thymocytes with non transgenic thymocytes, thymocytes from OVA scid and BALB/c mice were stained with FITC conjugated anti-CD8, anti-Vβ8 or anti OVA TCR and PE conjugated anti-CD4 antibodies (figure 1). The OVA scid thymus (A) had a higher percentage of CD4+, CD8+ and CD4- CD8- thymocytes and a lower percentage of CD4+ CD8+ cells than a BALB/c thymus (B). All the OVA scid thymocytes expressed Vβ8 (C). Only a portion of BALB/c thymocytes expressed Vβ8 (D). All the OVA scid thymocytes also expressed the transgenic TCR (E). Only a portion of the Vβ8+ (C) and KJ1 26.1+ (E) thymocytes expressed CD4 and thus there was a population of CD4- KJ1 26.1+ and a population of CD4- Vβ8+ cells present in the OVA scid thymus.

**A characterization of OVA scid versus BALB/c splenocytes**

In order to compare TCR transgenic scid splenocytes with non transgenic splenocytes, splenocytes from OVA scid and BALB/c mice were stained with FITC conjugated anti-CD8, anti-Vβ8 or anti OVA TCR and PE conjugated anti-CD4 antibodies (figure 1). The OVA scid spleen (F) had a higher percentage of CD4+ cells than a BALB/c spleen (G). The OVA scid spleen had no CD8+ (F) or B220+ cells (J). The CD4+ cells in the OVA scid spleen expressed Vβ8 (H) and KJ1 26.1 (L). BALB/c spleen had CD8+ (G) cells and B220+ (K) and barely detectable levels of CD4+ KJ1 26.1+ (M) cells. Only a portion of the CD4 cells in the BALB/c spleen expressed Vβ8 (I).
Figure 1: OVA scid and BALB/c thymus and spleen

Splenocytes (F, G, H, I, J, K, L and M) and thymocytes (A, B, C, D and E) from OVA scid and BALB/c mice were stained with FITC conjugated and PE conjugated antibodies. A, B, F and G: PE conjugated anti CD4 and FITC conjugated anti CD8. C, D, H and I: PE conjugated anti CD4 and FITC conjugated anti Vβ 8.1,8.2. E and L: PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR). J and K: PE conjugated anti B220 and FITC conjugated anti CD3. Lymphocytes were gated on based on forward scatter, side scatter parameters. Dead cells were gated out using the DNA dye 7AAD. Both the BALB/c and OVA scid mice were 12 weeks old. Splenocytes from a BALB/c mouse were stained with anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR) (M) in an independent experiment. The percentage of splenocytes or thymocytes of the respective populations is shown in the upper right quadrant.
OVA scid thymus

KJ1 26.1 (3) vs CD4 (4)

E

CD4

KJ1 26.1

76%

23%
**OVA scid spleen**

**BALB/c spleen**

KJ1 26.1

29
**CD4 Vβ8 T cells respond to SEB**
In order to ascertain the antigen specificity of the SEB response, BALB/c mice were injected with SEB and assayed 4 or 11 days after injection (4 and 11); control (C) mice were given PBS and assayed 11 days after injection (figure 2). After 4 days or 11 days, mice were sacrificed and the number of CD4 Vβ8, CD4 Vβ6 or CD4 Vβ9 cells in the spleen was enumerated by flow cytometry. There was a significant increase in the splenic number of CD4 Vβ8 but not CD4 Vβ6 or CD4 Vβ9 cells 4 days following SEB injection. On day 11 there was a decrease in the splenic number of CD4 Vβ8 but not CD4 Vβ6 or CD4 Vβ9 cells as compared to day 4. Therefore SEB allowed for a specific increase and decrease in splenic CD4 Vβ8 T cell numbers.

**SEB response in OVA scid versus BALB/c mice**
According to some groups the extent of proliferation and deletion in response to SEB is dependent on the initial proliferative response to SEB (75). According to other groups CD8 T cells may control the reduction in the number of CD4 Vβ8 T cells in response to SEB (13) (74). Since OVA scid mice had only a clonotypic population of T cells in the absence of other T or B cells, a comparison was made of the SEB response in OVA scid mice versus BALB/c mice. Mice were injected with SEB and assayed 4 or 10 days after injection (4 and 10); control (C) mice were given PBS and assayed 10 days after injection. After 4 days or 10 days, mice were sacrificed and the number of CD4 Vβ8 (BALB/c) or the number of CD4 KJ1 26.1, KJ1 26.1 (OVA scid) cells in the spleen was enumerated by flow cytometry (figure 3). Both OVA scid mice and BALB/c mice were capable of responding to the SEB injection since there was
Figure 2: CD4 Vβ8 T cells respond to SEB
Splenocytes from SEB injected (4, 11) or PBS injected (C) BALB/c were assayed on day 4 or day 11 after injection. BALB/c splenocytes were stained with PE conjugated anti CD4 and FITC conjugated anti Vβ8.1, 8.2, PE conjugated anti CD4 and FITC conjugated anti Vβ6 or PE conjugated anti CD4 and FITC conjugated anti Vβ 9 at day 4 or 11 after injection. The number of cells per spleen in the appropriate subset was enumerated. BALB/c mice were 6 wks at time of injection. Dead cells were gated out using the DNA dye 7AAD. Control mice (C) were given PBS. Three mice were used per group. The standard error of the mean is shown.
Figure 3: SEB response in OVA scid versus BALB/c mice

Splenocytes from SEB injected (DAY 4, DAY 10) or PBS injected (C) BALB/c and OVA scid mice were harvested using lympholyte separation medium. BALB/c splenocytes were stained with PE conjugated anti CD4 and FITC conjugated anti Vβ8.1, 8.2 at day 4 or 10 after injection. The number of CD4 Vβ8 cells per spleen was enumerated. OVA scid splenocytes were stained with PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR) at day 4 or 10 after injection. The number of CD4 Vβ8 and CD4 KJ1 26.1 cells per spleen was enumerated. BALB/c mice were 12 wks at time of injection and OVA scid mice were 10-16 weeks old at the time of injection. The standard error of the mean is shown. Two (C), four (DAY 4) and four (DAY 10) mice were used.
an increase in the splenic number of CD4 VB8 (BALB/c) and CD4 KJ1 26.1 (OVA scid) in both mice 4 days following SEB injection. The response of OVA scid and BALB/c mice was compared on day 10. While the number of CD4 VB8 cells recovered from the BALB/c on day 10 was less than that of PBS injected (C) mice, the number of CD4 KJ1 26.1 or KJ1 26.1 cells recovered from OVA scid mice was not significantly less than that of PBS injected (C) mice. An independent T test comparing the number of CD4 VB8 T cells recovered from control and day 10 mice suggested that the difference seen in BALB/c mice were statistically significant (p<0.01). The differences seen in the number of CD4 KJ1 26.1 cells recovered from control versus day 10 OVA scid mice was not statistically significant (p>0.01).

Adoptive transfer of BALB/c splenocytes into OVA scid mice
Since the OVA scid mice lack any T or B cells and CD8 T cells have been implicated as being important in the reduction of CD4 VB8 cells T to below levels found in control mice (13), we wanted to investigate whether the addition of fractionated or unfractionated BALB/c splenocytes could affect the number of CD4 KJ1 26.1 T cells recovered from SEB injected OVA scid mice. To explore this question a set of adoptive transfer experiments was done (table 1). To examine the role of other T cells in the SEB response, fractionated B cell depleted (exp 4), T cell depleted (exp 5) or unfractionated BALB/c splenocytes (exp 1-4) were coinjected with SEB into OVA scid mice. The response of the OVA scid T cells (CD4 KJ1 26.1) was followed. Control mice were given SEB (SEB) or PBS (C) only. Splenocytes from the mice were stained with CD4 KJ1 26.1 antibodies at day 8, 9 and 10 in five independent experiments (exp 1-5). The number of CD4 KJ1 26.1 cells per spleen was enumerated. The addition of unfractionated BALB/c spleen (exp 1-4) was unable to reduce the number of CD4
TABLE 1: Adoptive transfer of BALB/c splenocytes into OVA scid mice

Adoptive transfers of varying numbers of unfractionated (uc) or fractionated (fc) BALB/c splenocytes plus SEB were done into an OVA scid. Control mice were given PBS (C) or SEB (SEB). The age of the recipient mice for each experiment is shown. At the appropriate day of assay (day) splenocytes from the mice were harvested using lymphocyte separation medium. Splenocytes were stained with PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR). In experiment 5 (exp) the splenocytes were also stained with PE conjugated anti B220. The number of CD4 KJ1 26.1 cells per spleen was enumerated.

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Exp (experiment number), age (age of mouse in weeks), day (day of assay), cells (type of cells injected), # cells (number of fractionated or unfractionated BALB/c splenocytes injected), group (group), c/s (number of cells per spleen), % inj cells (percentage of CD4+ KJ1 26.1- injected cells recovered)

1. % CD4 KJ1 26.1 splenocytes recovered
2. % CD4+ KJ1 26.1-
3. fractionated cells: 92% CD3+, 1.2% B220+, 6.8% CD3- B220-
   unfractionated cells: 43% CD3+, 52% B220+, 5% CD3- B220-
4. 90% B220+, 4.3% CD3+, 5.7% CD3- B220-
5. B220+
KJ1 26.1 cells recovered to less than that of control (C) mice. In experiments 1 (SEB 1), 4 and 5 the numbers of CD4 KJ1 26.1 recovered from SEB treated mice was less than the numbers recovered from control. However in experiments 1 (SEB 2), 2 and 3 the numbers of CD4 KJ1 26.1 recovered from SEB treated mice was more than the numbers recovered from control. The percentage of CD4 KJ1 26.1 cells in control mice varied from 69% (exp 5) to 74% (exp 1). The percentage of CD4 KJ1 26.1 cells in SEB injected mice varied from 36% (exp 5) to 69% (expt 2). Therefore the addition of unfractionated BALB/c splenocytes was unable to reduce the numbers of OVA scid T cells recovered from SEB injected mice to less than that recovered from PBS injected mice.

We postulated that since the percentage of CD4 Vβ8 T cells is high in the transgenic system the adoptively transferred cells may be unable to further reduce the numbers of CD4 Vβ8 T cells recovered. We tried adoptive transfer experiments using fractionated splenocytes from BALB/c mice, congenic to C.B-17 differing only at the IgH locus, to answer the above question. Adoptive transfer of OVA scid splenocytes plus BALB/c T cells into a C.B-17 scid allowed us to get over the problem of having a high percentage of CD4 KJ1 26.1/CD4 Vβ8 T cells. It also allowed us to use titration experiments to enhance the conditions required for the reduction CD4 Vβ8 T cell number.

**OVA scid T cells are detectable in C.B-17 scid mice upon adoptive transfer**

In order to determine whether OVA scid T cells could be transferred and detected in a C.B-17 scid mouse an adoptive transfer titration experiment was done. Adoptive transfers of OVA scid splenocytes plus SEB were performed in a C.B-17 scid. Varying numbers of OVA scid splenocytes were injected into C.B-17 scid mice. Control mice (0.00E+00) were given PBS
Adoptive transfers of OVA scid splenocytes was done into C.B-17 scid mice (12 weeks old). Varying numbers of OVA scid splenocytes (65% CD4+ KJ1 26.1+) were injected into C.B-17 scid mice. Control mice (0.00E+00) were given PBS. Splenocytes from the C.B-17 scid recipients were stained with PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR) four days after injection. The number of CD4 KJ1 26.1 cells per spleen was enumerated. Dead cells were gated out using the DNA dye 7AAD. One mouse was used per group.

<table>
<thead>
<tr>
<th># OVA scid cells injected</th>
<th>cells/spleen</th>
<th>% CD4+ KJ1 26.1+</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3.3E+06</td>
<td>0</td>
</tr>
<tr>
<td>3.00E+06</td>
<td>3.4E+06</td>
<td>0.47%</td>
</tr>
<tr>
<td>6.00E+06</td>
<td>6.7E+06</td>
<td>0.96%</td>
</tr>
<tr>
<td>1.00E+07</td>
<td>4.4E+06</td>
<td>2.15%</td>
</tr>
</tbody>
</table>
(figure 4). Splenocytes from the mice were stained with CD4 KJ1 26.1 antibodies 4 days after injection. The number of CD4 KJ1 26.1 cells per spleen was enumerated. OVA scid T cells were detectable after four days of transfer in the C.B-17 scid in a dose dependent manner.

**OVA scid T cells are capable of mounting a response to SEB upon adoptive transfer**

To determine whether the adoptively transferred cells could respond to SEB in the C.B-17 scid, OVA scid splenocytes plus SEB were transferred into a C.B-17 scid (table 2). Varying numbers of OVA scid splenocytes were injected with SEB into C.B-17 scid mice in three independent experiments (A-C). Control mice were given OVA scid splenocytes only $10^7$ (expt A), $5 \times 10^6$ (exp B) or $3 \times 10^6$ (expt C), and assayed on day 4 after injection (table 2). Splenocytes from the mice were stained with CD4 KJ1 26.1 antibodies at 4 (expt A-C), 7 (expt A), and 11 (expt B and C) days after injection. The number of CD4 KJ1 26.1 cells per spleen was enumerated. The adoptively transferred OVA scid T cells (CD4 KJ1 26.1) recovered from mice given SEB plus OVA scid splenocytes were higher than C.B-17 scid mice given OVA scid splenocytes alone (C versus day 4 experiments A-C). C.B-17 scid mice given OVA scid splenocytes plus SEB and assayed on day 7 (exp A) or day 11 (expt B and C) had a higher number of OVA scid T cells (CD4 KJ1 26.1) recovered than control (C) mice given OVA scid splenocytes (C versus day 7 expt A, C versus day 11 expt B and C) of OVA scid T cells (CD4 KJ1 26.1). However, independent experiments (data not shown) in which OVA scid cells were injected into C.B-17 scid mice without SEB and assayed on day 12 or 13 showed that in the absence of SEB the CD4 KJ1 26.1 T cell were barely detectable (0.03%-0.06%). Since the OVA scid T cells was were barely detectable in the absence of SEB, the
TABLE 2: OVA scid T cells are capable of mounting an immune response upon adoptive transfer

Adoptive transfers of OVA scid splenocytes plus SEB were done into C.B-17 scid mice in 3 independent experiments (A-C). $1 \times 10^7$ (68% CD4+ KJ1 26.1+) A, $5 \times 10^6$ (16% CD4 KJ1 26.1+) (B) or $3 \times 10^6$ (28% CD4+ KJ1 26.1+) C, OVA scid splenocytes were injected with SEB into C.B-17 scid mice. Control mice (C) were given OVA scid splenocytes only and assayed on day 4. Splenocytes from the C.B-17 scid mice were stained with PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR). The number of CD4 KJ1 26.1 cells per spleen was enumerated. Dead cells were gated out using the DNA dye 7AAD. One mouse was used per group.

**Experiment A**

<table>
<thead>
<tr>
<th>group</th>
<th>cells/spleen</th>
<th>%CD4 KJ1 26.1</th>
<th># CD4 KJ1 26.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>1.1E+07</td>
<td>2.4%</td>
<td>2.6E+05</td>
</tr>
<tr>
<td>day 4</td>
<td>7.2E+07</td>
<td>1.9%</td>
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<tr>
<td>day 7</td>
<td>6.5E+07</td>
<td>1.5%</td>
<td>9.8E+05</td>
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</tbody>
</table>

**Experiment B**

<table>
<thead>
<tr>
<th>group</th>
<th>cells/spleen</th>
<th>% CD4 KJ1 26.1</th>
<th># CD4 KJ1 126.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>8.8E+06</td>
<td>0.13%</td>
<td>1.1E+04</td>
</tr>
<tr>
<td>day 4</td>
<td>4.8E+07</td>
<td>4.6%</td>
<td>2.2E+06</td>
</tr>
<tr>
<td>day 11</td>
<td>3.7E+07</td>
<td>1.1%</td>
<td>4.1E+05</td>
</tr>
</tbody>
</table>

**Experiment C**

<table>
<thead>
<tr>
<th>group</th>
<th>cells/spleen</th>
<th>% CD4 KJ1 26.1</th>
<th># CD4 KJ1 26.1</th>
</tr>
</thead>
<tbody>
<tr>
<td>C</td>
<td>2.6E+07</td>
<td>0.19%</td>
<td>4.9E+04</td>
</tr>
<tr>
<td>day 4</td>
<td>1.0E+08</td>
<td>3.9%</td>
<td>3.9E+06</td>
</tr>
<tr>
<td>day 11</td>
<td>7.8E+07</td>
<td>1.2%</td>
<td>9.4E+05</td>
</tr>
</tbody>
</table>
reduction in splenic cell number of CD4 Vβ8 T cells in response to SEB cannot be compared to mice given OVA scid T cells alone.

**CD4 and CD8 T cells from BALB/c reduce the number of OVA scid T cells recovered**

Since CD8 T cells can influence the fate of CD4 T cells during an immune response (2) (3) (31), we wanted to examine whether the addition of CD4 or CD8 cells could influence the numbers of CD4 KJ1 26.1 cells recovered from a C.B-17 scid mouse injected with OVA scid splenocytes plus SEB. Since OVA scid T cells (CD4 KJ1 26.1) were not detectable upon adoptive transfer into C.B-17 scid mice in the absence of SEB on day 12 or 13, a comparison was made between mice given OVA scid splenocytes plus SEB versus OVA scid splenocytes plus SEB and CD4 or CD8 T cells. The role of the added CD4 or CD8 cells in influencing the number of CD4 KJ1 26.1 recovered was investigated. It is important to note that since there was no control (without SEB) in this experiment the issue of reduction in CD4 Vβ8 T cell number in comparison to mice not given SEB was not addressed. CD4 and CD8 cells from BALB/c spleen were transferred with OVA scid splenocytes plus SEB into a C.B-17 scid (figure 5). Control mice were given 5 x 10^6 OVA scid splenocytes plus SEB only. 1 x 10^7 fractionated BALB/c and 5 x 10^6 OVA scid splenocytes were injected with SEB into C.B-17 scid mice. Splenocytes from the mice were stained with CD4 KJ1 26.1, CD4 Vβ8 or CD3 B220 antibodies on day 11 after injection. The numbers of CD4 KJ1 26.1, CD4 Vβ8 and CD3 cells per spleen were enumerated. The number of CD4 Vβ8 T cells recovered from mice given BALB/c CD4 T cells (CD4) was more than the number recovered from SEB (SEB) or
Figure 5: CD4 and CD8 T cells from BALB/c reduce the number of OVA scid T cells recovered

Adoptive transfers of 1 X 10^7 CD4 (CD4) or CD8 (CD8) cells from BALB/c spleen and 5 X 10^6 (54% CD4 + KJ1 26.1 +) OVA scid splenocytes plus SEB was done into C.B-17 scid (12 weeks old). Control mice were given 5 x 10^6 OVA scid splenocytes and SEB (SEB).

Splenocytes from the C.B-17 scid mice were stained with PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR), PE conjugated anti CD4 and FITC conjugated anti Vβ8.1 8.2 or PE conjugated anti B220 and FITC conjugated anti CD3 at day 11 following the injections. The number of CD4+ KJ1 26.1+ cells, CD4+ Vβ8+, CD3+ B220- cells per spleen was enumerated. Dead cells were gated out using the DNA dye 7AAD. Three mice were used per group. The standard error of the mean is shown.

<table>
<thead>
<tr>
<th>Group</th>
<th>Cells/spleen</th>
<th>#CD4 KJ1 26.1</th>
<th>#CD4 Vβ8</th>
<th>#CD3</th>
<th>%CD4 KJ1 26.1</th>
<th>%CD4 Vβ8</th>
<th>%CD3</th>
</tr>
</thead>
<tbody>
<tr>
<td>SEB</td>
<td>8.8E+07</td>
<td>1.9E+05</td>
<td>2.2E+05</td>
<td>1.9E+05</td>
<td>2.2</td>
<td>2.5</td>
<td>2.2</td>
</tr>
<tr>
<td>CD4</td>
<td>1.6E+07</td>
<td>4.5E+04</td>
<td>7.7E+05</td>
<td>1.6E+06</td>
<td>0.28</td>
<td>4.8</td>
<td>10</td>
</tr>
<tr>
<td>CD8</td>
<td>9.1E+06</td>
<td>3.5E+04</td>
<td>9.00E+04</td>
<td>5.6E+05</td>
<td>0.39</td>
<td>0.99</td>
<td>6.2</td>
</tr>
</tbody>
</table>
BALB/c CD8 T cell (CD8) injected mice. This difference probably reflects BALB/c derived CD4 Vβ8 T cells that respond to SEB. It is interesting to note that as compared to the mouse given OVA scid splenocytes and SEB the addition of either CD4 or CD8 cells reduced the number of CD4 KJ1 26.1 cells recovered. However, in the three groups it seemed that a different number of T cells (CD3+) were detected in the spleen. Mice given BALB/c CD4 cells had more T cells (CD3+) in the spleen than mice given BALB/c CD8 cells. Therefore it is possible that the BALB/c CD4 or CD8 cells occupied the space in the spleen that otherwise be occupied by CD4 KJ1 26.1 cells. Although it seems that either CD4 or CD8 T cells were able to reduce the number of CD4 KJ1 26.1 cells recovered; it is not clear whether this result points to a direct role for CD4 or CD8 cells. This reduction in CD4 KJ1 26.1 cell number could be non specific since both populations were able to reduce the numbers of CD4 KJ1 26.1 cells recovered.

Adoptive transfer of BALB/c splenocytes into OVA scid mice
Since the experiments in table 1 had variable numbers of splenocytes recovered from control OVA scid spleens (1.50x10^6 - 4.70x10^6) it was possible that the lympholyte separation medium was causing non specific loss of splenocytes in the OVA scid spleen. Therefore we decided to repeat the observations seen in table 1 without the lympholyte separation medium. Adoptive transfers of fractionated (B depleted) or unfractionated (unfrac) BALB/c splenocytes were coinjected with SEB into OVA scid mice (figure 6). The response of the OVA scid T cells (CD4 KJ1 26.1) was followed. Control mice were given SEB only (SEB). Splenocytes from the mice were stained with CD4 KJ1 26.1 antibodies 11 days after injection. The number of CD4 KJ1 26.1 cells per spleen was enumerated. The addition of B depleted or
Adoptive transfers of B depleted BALB/c spleen plus SEB were done into an OVA scid. 1 x 10^7 B depleted splenocytes (B depleted) or 1 x 10^7 unfractionated (unfrac) BALB/c splenocytes were injected with SEB into OVA scid mice. Splenocytes from the mice were stained with PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR), PE conjugated anti CD4 and FITC conjugated anti Vβ8.1 8.2 or PE conjugated anti B220 and FITC conjugated anti CD3 at day 11 following the injections. The number of CD4+ KJ1 26.1+ cells, CD4+ Vβ8+, CD3+ B220- and B220+ cells per spleen was enumerated. Dead cells were gated out using the DNA dye 7AAD. Three mice were used per group. The standard error of the mean is shown. Control mice were given SEB alone (SEB). OVA scid mice were 8 weeks old at the time of injection.

1. 66% CD3+, 0.4% B220+, 34% CD3- B220-
2. 43% CD3+, 35% B220+, 23% CD3- B220-
unfractionated (unfrac) BALB/c spleen was unable to significantly reduce the number of CD4 KJ1 26.1 cells recovered from an SEB (SEB) injected OVA scid mouse.

**SEB response in OVA scid versus BALB/c mice**

Since figure 3 was done with a low number of mice two (control) four (day 4) and four (day 10), and experiments in table 1 had variable numbers of splenocytes recovered from control OVA scid spleens (1.50x10^6 - 4.70x10^6) it was possible that the lympholyte separation medium was causing non specific loss of splenocytes in the OVA scid spleen. Therefore the experiment shown in figure 3 was repeated with a larger number of mice and without lympholyte (figure 7). BALB/c or OVA scid mice were injected with SEB (4 and 11); control mice (C) were given PBS. After 4 days or 11 days, mice were sacrificed and the number of CD4 Vβ8 (BALB/c and OVA scid) and CD4 KJ1 26.1 (OVA scid) in the spleen was enumerated by flow cytometry (figure 7). Both OVA scid mice and BALB/c mice seemed to be capable of responding to the SEB injection since there was an increase in the number of CD4 Vβ8 in both mice at 4 days following SEB injection. The response of OVA scid and BALB/c mice on day 11 was compared. The number of CD4 Vβ8 T cells recovered from both the BALB/c and OVA scid mice on day 11 was less than that of PBS injected mice. An independent T test comparing the number of CD4 Vβ8 T cells recovered from control and day 11 mice suggested that the differences between BALB/c control and day 11 SEB injected were significant (p<0.05), and the differences seen between OVA scid control versus day 11 were not significantly different (p>0.05). However at the the 2% significance level neither difference was statistically significant. Therefore any differences seen between the BALB/c mice versus the OVA scid mice were minimal.
**Figure 7: SEB response in OVA scid versus BALB/c mice**

Splenocytes from SEB injected (DAY 4, DAY 11) or PBS injected (C) BALB/c and OVA scid were assayed on day 4 or day 11 after injection. BALB/c splenocytes were stained with PE conjugated anti CD4 and FITC conjugated anti Vβ8.1, 8.2 at day 4 or 11 after injection. The number of CD4 Vβ8 cells per spleen was enumerated. OVA scid splenocytes were stained with PE conjugated anti CD4 and FITC conjugated anti Vβ8.1, 8.2 or PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR) at day 4 or 11 after injection. The number of CD4 Vβ8 and CD4 KJ1 26.1 cells per spleen was enumerated. BALB/c mice were 12 wks at time of injection and OVA scid mice were 14 weeks old at the time of injection. Dead cells were gated out using the DNA dye 7AAD. Control mice (C) were given PBS. Four mice were used per group. The standard error of the mean is shown.

![Graphs showing SEB response in OVA scid versus BALB/c mice](image-url)
IL-2 receptor α expression on OVA scid versus BALB/c CD4 Vβ8 T cells

Since IL-2 has been implicated in SEB mediated deletion (76), (65), the expression of the IL-2 R on OVA scid T cells upon SEB injection was investigated. The levels of IL-2 receptor α were assayed in OVA scid and BALB/c mice 20 hours after SEB injection (B and D). Control mice were given PBS (A and C). Both BALB/c and OVA scid mice had elevated levels of IL-2 receptor α 20 hours following SEB injection (figure 8). Since OVA scid splenocytes expressed detectable levels of IL-2 receptor α upon SEB injection, they seemed to be activated in response to SEB.

CD4- TCR+ cells are seen upon SEB injection

Upon SEB injection a population of cells that do not express CD4 but do express TCR were seen. Splenocytes from SEB injected (B and D) or PBS injected (A and C) OVA scid mice were assayed on day 11 after injection (figure 9). The SEB injected mice had a splenic population of CD4- VB8+ (B) and CD4- KJ1 26.1 (D) cells. This population was barely detectable in PBS injected mice (panel A, C). In terms of number of cells per spleen the SEB injected mice had the following numbers: CD4- VB8+ (1.8 x 10^6 ± 1.6 x 10^5) and CD4- KJ1 26.1 (2.0 x 10^6 ± 1.8 x 10^5). The PBS injected mice had CD4- VB8+ (9.9 x 10^4 ± 2.8 x 10^4) and CD4- KJ1 26.1 (5.9 x 10^4 ± 2.1 x 10^4).
Figure 8: IL-2 receptor α expression on OVA scid versus BALB/c CD4 Vβ8 T cells

Splenocytes from OVA scid and BALB/c mice injected with SEB (B, D) or PBS (A, C) were stained with PE conjugated anti CD4, FITC conjugated anti Vβ8.1, 8.2 and biotin conjugated anti IL-2 Rα at 20 hours after injection. CD4 Vβ8 cells were gated for their IL-2 Rα. Data shown is representative of 3 SEB injected mice and 2 control mice. Mice were 10 weeks at the time of injection. The MFI is shown.

OVA scid

A

B

BALB/c

C

D

OVA scid
**Figure 9: CD4+ TCR+ cells are seen upon SEB injection**

Splenocytes from SEB injected (B and D) or PBS injected (A and C) OVA scid mice were assayed on day 11 after injection. OVA scid splenocytes were stained with PE conjugated anti CD4 and FITC conjugated anti VB8.1, 8.2 or PE conjugated anti CD4 and FITC conjugated KJ1 26.1 (anti OVA TCR) at 11 after injection. OVA scid mice were 14 weeks old at the time of injection. Dead cells were gated out using the DNA dye 7AAD. Lymphocytes were gated on based on forward scatter and side scatter parameters. The percentage of splenocytes of the respective populations is shown in the upper right quadrant.
Discussion

The CD4 Vβ8 response to superantigens \textit{in vivo} is characterized by a burst of proliferation that peaks on day 3-4 and a subsequent drop in cell number (day 8-11) that results in the deletion of the responding cells to below the levels found in PBS injected control mice (50) (54).

According to some groups the extent of deletion seen in response to SEB is dependent on the extent of proliferation (75). According to other groups CD8 cells may play a role in reducing the number of CD4 Vβ8 T cells to below levels found in PBS injected controls (13). The superantigen SEB was used to analyze the immune response in a C.B-17 scid mouse expressing a transgenic αβ T cell receptor reactive to a class II restricted I-A^d ovalbumin peptide. This TCR receptor expresses V\(\alpha\) 13 and Vβ 8.2 and is reactive with SEB. Since C.B-17 mice bear the scid mutation, OVA scid mice have no B cells and the only population of T cells that they will have will bear the transgenic TCR. Therefore using this mouse the response of a single clonotypic population of SEB reactive T cells can be studied in the absence of all other T or B cells. The monoclonal antibody KJ1 26.1 specific for the transgenic TCR expressed by the OVA mouse (87) was used to follow the course of these cells during the immune response.

In order to examine the SEB response in the OVA scid mouse, the response of OVA scid and BALB/c mice at 4 and 11 days after SEB injection was compared. Both these mice have the H-2^d haplotype and have TCRs bearing Vβ8 chains in varying proportions. Both mice seemed to be capable of responding to the SEB injection since there was an increase in the number of CD4 Vβ8 in mice 4 days following SEB injection. Since the OVA scid mouse mounted a comparable response to SEB \textit{in vivo}, a clonotypic T cell is able to mount an SEB
induced immune response in the absence or presence of other T cells or B cells. In order to further study the response to SEB in these mice, the response of OVA scid and BALB/c mice was compared on day 11. The number of CD4 Vβ8 cells recovered from both the BALB/c and the OVA scid mouse on day 11 was less than that of PBS injected mice. Although the OVA scid mouse has only the clonotypic population of SEB reactive T cells in the absence of other T cells, it is still capable of responding to SEB in the absence of other lymphocytes. Therefore a clonotypic population of T cells is able to mount an SEB response in the spleen from proliferation to deletion. It is important to note that deletion is defined as the loss of CD4 KJ1 26.1 cells from the spleens of mice after day 4 of SEB injection.

In order to examine the role for other T cells particularly CD4 and CD8 T cells in the response to SEB, we decided to investigate whether the addition of other CD4 and CD8 T cells could influence the number of CD4 KJ1 26.1 T cells recovered from SEB injected OVA scid mice. The role of other CD4 cells or CD8 cells in the SEB response is not clearly defined (13 (74). There are two reports that discuss the role of CD8 T cells in reducing the number of CD4 Vβ8 T cells recovered from SEB injected mice. One report using CD8 depleted mice suggests that the role for CD8 cells is minimal if any, since CD8 depleted mice seem to have less CD4 Vβ8 T cells recovered from SEB injected mice than from PBS injected mice (74). Another report using both CD8 depleted and β2m knockout mice (lack class I MHC) suggests that CD8 cells are needed to reduce the numbers of CD4 Vβ8 T cells recovered to less than that in PBS injected mice (13). They suggest that CD8 cells lyse CD4 cells that have previously been activated in response to SEB. It is important to note that the latter report defined CD8 cytotoxic function entirely *in vitro* and presented no evidence that this function
exists in vivo. The role for γδ T cells and CD4 cells other than the ones that carry the SEB reactive TCR in the SEB response is unknown.

In order to test the role that T cells, specifically CD8 cells, have in reducing the numbers of CD4 Vβ8 T cells in SEB injected mice, a set of adoptive transfer experiments was done. Fractionated (B depleted) or unfractionated BALB/c splenocytes were coinjected with SEB and the immune response was followed. The addition of any one of these populations was unable to affect the numbers of OVA scid T cells recovered. Therefore there was no further reduction in OVA scid T cell numbers seen by the addition of unfractionated or fractionated BALB/c splenocytes. It was postulated that perhaps the percentage of CD4 Vβ8 cells was so high in the transgenic system that the adoptively transferred cells were unable to effectively reduce them. An alternative adoptive transfer system was investigated. Adoptive transfers of OVA scid cells plus BALB/c cells, congenic to C.B-17 and differing only at the IgH locus, into a CB.17 scid mouse was investigated. This allowed one to get over the problem of having a large number of antigen reactive cells. This approach also allowed for titration experiments to optimize the conditions needed for the reduction in cell number. However, in the absence of SEB, the transferred OVA scid T cells were barely detectable. It is possible that the presence of the transgene or the presence of the scid mutation in these T cells results in a defect in long term recirculation (Dr Ohashi personal communication). Therefore the question of the reduction in number of CD4 Vβ8 T cells as compared to mice not given SEB cannot be addressed in this system.

Since CD8 T cells can influence the fate of CD4 T cells during an immune response (2) (3) (31), we wanted to determine whether the addition of CD8 cells could influence the
numbers of OVA scid T cells recovered in C.B-17 scid mice injected with OVA scid T cells plus SEB. Adoptive transfers of CD4 or CD8 T cells and OVA scid splenocytes plus SEB was done into a C.B-17 scid (figure 5). It is interesting to note that as compared to the mouse given OVA scid splenocytes and SEB the addition of either CD4 or CD8 cells seemed to reduce the number of CD4 KJ1 26.1 cells recovered. Since either CD4 or CD8 cells were able to reduce the number of OVA scid T cells recovered, the reduction in OVA scid T cell number may not be specific to CD4 or CD8 cells. The CD3 data in figure 5 showed that a different number of T cells were recovered from the spleen in the three situations. It is possible that in the presence of CD4 cells more T cells homed to the spleen than in the presence of CD8 cells. Therefore the CD4 T cells that homed to the spleen may occupy the space of the TCR transgenic cells.

Our results are consistent with Williams et al's results that showed that CD8 cells alone do not play an active role in mediating deletion of SEB reactive T cells (74). This result is in contrast to Jiang et al's work (13) that suggested that CD8 cells play an active role in SEB induced deletion of CD4 V$\beta$8 T cells. However since their work primarily defined the cytotoxic role of CD8 cells in vitro this phenomenon may not exist in vivo. Our results show that a clonotypic population of T cells is able to mediate all phases of the SEB induced immune response in the spleen from proliferation to deletion. IL-2 Rα staining suggests that the T cells in the OVA scid have been activated in response to SEB. Adoptive transfers of B depleted BALB/c spleen or unfractionated BALB/c spleen was unable to influence the extent of SEB induced deletion of CD4 KJ1 26.1 cells. Therefore a minimal role for CD8 T cells in SEB induced deletion was found in our model system. Adoptive transfers of CD4 or CD8 cells plus
OVA scid T cells into a C.B-17 scid was able to reduce the numbers of OVA scid T cells recovered from a single dose of SEB. However this reduction in the number of OVA scid T cell recovered is not specific to CD4 or CD8 cells, and does not relate to SEB induced CD4 Vβ8 T cell deletion.

Recent evidence from several groups has shown that there is heterogeneity in the interaction of particular SEB reactive Vβ chains with superantigens. This suggests that there are other contributing elements to superantigen recognition. For example the α chain has been implicated in influencing the response to SEB (43) (44), as well as Mls (45) (46) (47). Since there are many factors that influence the SEB response other than the presence of a Vβ8 chain, our results do not rule out the possibility of a suboptimal SEB response in these mice. The evidence from the SEB, MHC, TCR interactions suggest there is a gradient in reactivity towards SEB (34) (43) (44) (49). This gradient includes Vβ8 cells with differing reactivity towards SEB from highly reactive to non reactive. Therefore it is formally possible that the Vα13 Vβ8.2 TCR present in the OVA scid mouse is of a moderate to low or moderate to high affinity towards SEB. It would be interesting to study whether the Vα13 Vβ8.2 TCR present in the OVA scid mouse has a high or a low affinity for SEB as compared to Vβ8.2 TCRs with other α chains. This could be studied in the OVA transgenic mouse. All the T cells in this mouse express Vβ8 and only 50% express the αβ transgene combination (82). Therefore the influence of different α chains in the in vivo response to SEB could be studied using this mouse. Interestingly both BALB/c and OVA scid mice had elevated levels of IL-2 α receptor
20 hours following SEB injection (figure 8). This suggests that the transgenic TCR has a sufficient affinity for SEB to allow for T cell activation in response to SEB.

The adoptive transfer of OVA scid splenocytes into the C.B-17 scid without SEB suggested that TCR transgenic scid T cells are unable to recirculate for a long time in the absence of stimulation. This is because the TCR transgenic scid T cells were detected earlier (day 4) in the absence of SEB but were virtually undetectable at day 12 or day 13. Therefore one can study whether the scid defect or the presence of a TCR transgene results in a defect in recirculation by following the fate of adoptively transferred TCR transgenic scid T cells versus T cells from a transgenic mouse that does not harbour the scid mutation. The fate of these T cells could be compared to the fate of adoptively transferred T cells from a normal mouse.

Upon SEB injection a novel population of CD4- TCR+ cells was seen in the spleen of SEB injected OVA scid mice. A population of CD4- TCR+ (DN T cells) cells was also seen in the thymus of uninjected OVA scid mice. This population does not express the CD8 molecule. CD4-CD8- TCR+ (DN T cells) cells have been seen in various systems (102) including in MRL/lpr mice (103), CD4 knockout mice (16) (104), human peripheral blood (105), normal mouse liver (103) and TCR transgenic mice (30) (78) (82) (106). In some cases this population is thymically derived (30) (78) (102). These DN T cells can secrete cytokines (104) (106). In some cases these cells are cytotoxic towards class II bearing antigen presenting cells (106). It would be interesting to investigate the role of these cells in the OVA scid system. It is possible that in the absence of CD8 cells these cells are responsible for cytotoxicity (106).
References


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