Brief Definitive Report

Bacterial Superantigens Mediate T Cell Deletions in the Mouse Severe Combined Immunodeficiency–Human Liver/Thymus Model

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Summary

The ability to analyze T cell receptor (TCR) thymic repertoire shaping in humans by self and foreign ligands is hampered by the lack of suitable models. We recently documented that the mouse severe combined immunodeficiency (SCID)–human fetal liver/thymus model recapitulates the TCR Vβ gene repertoire of human thymocytes. Here, we show that an exogenous superantigen, staphylococcal enterotoxin B, administered to such mice induces clonal deletions in both $CD4^+8^-$ and $CD8^+4^-$ cells involving the same human Vβ clones that are selected in vitro by this toxin. This model, therefore, may allow comprehensive studies into the effects of microbial and other agents on human T cell thymic selection processes in a biologically relevant setting.

Studies in the mouse, have shown that the functional TCR repertoire is primarily shaped intrathymically by positive and negative selection processes (for reviews see references 1–3). In particular, negative selection imposed by mouse mammary tumor provirus (Mtv)–encoded endogenous superantigens causes the deletion of T cell clones expressing specific Vβ genes (for reviews see references 4 and 5). Similarly, depletion of particular Vβ T cell clones can be observed in mice (6) and rats (7) exposed to bacterial superantigens during the maturation of the TCR repertoire (for reviews see references 8 and 9). Through the use of transgenic mice, deletion of TCR-expressing cells recognizing conventional self-antigens has also been documented (3, 10). In humans, analysis of Vβ transcript levels in immature and mature thymocyte (11) and peripheral blood T cell subsets (12–15) have suggested positive and negative selection, but definitive evidence for such processes is lacking. Moreover, although bacterial superantigens engage specific sets of human Vβ clones in vitro (8 and 9), their effects on in vivo thymic selection are unknown.

To address thymic selection in humans in a setting amenable to experimentation, we studied TCR Vβ expression in homozygous C.B-17 *scid/scid* mice reconstituted with human fetal liver and thymus (16–18). It has been documented that in this model, human progenitor cells of fetal liver origin emigrate into the coimplanted fetal thymus and differentiate into immature double-positive $CD4^+8^+$ and mature single-positive $CD4^+8^-$ and $CD8^+4^-$ T cell subsets (17, 18). Using a multiprobe RNase protection assay, we recently showed (19) that the TCR Vβ repertoire in the reconstituted thymus is virtually identical to the unselected repertoire in the thymus of the stem cell (liver) donor. Moreover, when identical stem cells developed in different thymic environments, significant differences in the Vβ transcript levels of the selected mature T cells were observed, indicating the influence of thymic genetic background–dependent polymorphic ligands in the selection process. Here, we document that injection of a bacterial superantigen (staphylococcal enterotoxin B [SEB]) in such SCID–human fetal liver/thymus mice induces deletion of T cells expressing the same Vβ engaged by this toxin in vitro, thereby establishing the use of this system in assessing the effects of microbial superantigens on the developing human immune system.

Materials and Methods

SCID–Human Fetal Liver/Thymus Mice. Human fetal livers and thymuses were obtained from donors between 15 and 21 wk gestational age. Parts of the thymuses were kept for RNA preparation and the remainder used in the transplantation experiments. C.B-17 *scid/scid* mice were transplanted under the kidney capsule with small pieces of thymus and syngeneic or allogeneic liver, as described (16–18). 6 mo later, the thymuses were removed for analysis.

Clonal Selection by SEB. For studying SEB in vivo effects on the developing human TCR repertoire, eight SCID mice transplanted 6 wk earlier with a syngeneic combination of human fetal liver and thymus were injected intraperitoneally with 12 μg SEB (Sigma Chemical Corp., St. Louis, MO) three times per week for 4 wk. Thymuses were then removed from such animals, pooled,

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and $CD4^+8^-$ and $CD8^+4^-$ single-positive cells were separated by sorting on a FACStar Plus® (Becton Dickinson & Co., Mountain View, CA) using FITC-conjugated anti-CD4 or PE-conjugated anti-CD8 antibodies (Becton Dickinson & Co.). Control $CD4^+8^-$ and $CD8^+4^-$ thymocytes were similarly prepared from human fetal liver/thymus transplanted mice not treated with SEB.

For assessing in vitro Vβ selection by SEB, PBLs (2.5×10^6 per ml) from a normal adult donor were cultured in RPMI 1640 medium containing 10% fetal calf serum, 5×10^{-2} mM 2-ME, 2 mM L-glutamine, and SEB at a final concentration of 0.25 μg/ml. After 4 d at 37°C in 6% CO_2, cultures were supplemented with 20 U/ml recombinant human IL-2 (Genzyme Corp., Boston, MA), incubated for an additional 48 h, and harvested.

RNase Protection Assay. RNA isolation from the above cell preparations, riboprobe design and labeling and RNase protection assays, using 22 human Vβ probes organized into three probe sets, were performed as we have recently described (11). Briefly, 2–3 μg of lyophilized total T cell RNA were hybridized with radiolabeled Cβ probe (10^5 cpm), and with each of the probe sets (2×10^3 cpm/uridine) in 5 μl hybridization buffer (80% formamide, 0.4 M NaCl, 1 mM EDTA, 40 mM Pipes, pH 6.7) at 56°C for 12–16 h. Unhybridized probes and target RNA were digested (1 h at 30°C) with RNase A (5 μg/ml) and T1 (10 U/ml) in 50 μl of digestion buffer (10 mM Tris, pH 7.5, 5 mM EDTA, 0.3 M NaCl). Purified "protected" probe/RNA duplexes were electrophoresed in a standard 6% polyacrylamide sequencing gel, and autoradiography (10–20 h at −70°C with intensifying screens) was performed on Kodak XRP film. Quantitation of Vβ transcript levels, was performed using a radioanalytic imaging apparatus (AMBIS; Ambis Systems, Inc., San Diego, CA). The net cpm at a given band corresponding to a specific protected Vβ probe was calculated by the formula: [(cpm of Vβ-specific band) − (cpm background around the band)]/(number of uridine residues in the specific Vβ probe). This value was then expressed as percent of total Cβ transcripts.

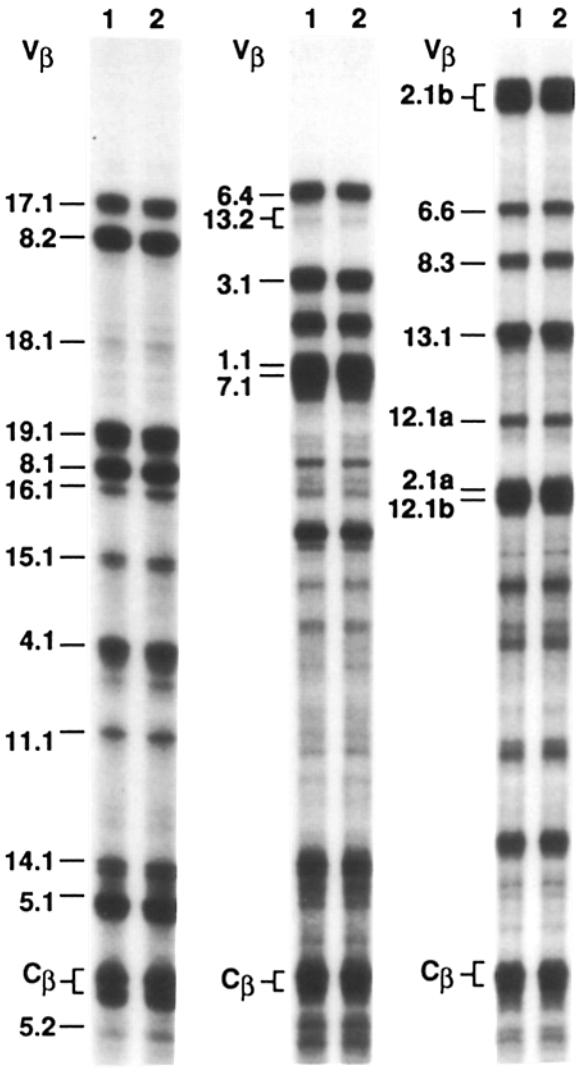

Figure 1. Vβ gene repertoire in SCID–human fetal liver/thymus mice. Autoradiographic profiles of Vβ gene expression in a human fetal thymus before (lanes *1*) and after (lanes *2*) coimplantation with the syngeneic fetal liver. Full-size bands are identified as specific Vβ. Secondary bands result from incompletely hybridized RNA cleaved into smaller fragments by RNase.

Results and Discussion

When thymus implants are engrafted alone into SCID mice, most (>90%) begin to atrophy (16), and within 3 mo, are almost totally devoid of cortical thymocytes, in agreement with the long-standing notion that the self-renewing capacity of thymic stem cells is limited (20). Continuous human T lymphopoiesis, however, occurs when a fragment of fetal liver, regardless of whether syngeneic or allogeneic, is coimplanted with the fetal thymus into the SCID mouse (16–19). To verify stability and completeness of thymic reconstitution in the set of mice used for the present study, total human thymocytes from control SCID mice implanted 6 mo earlier with human fetal tissues, were analyzed for Vβ gene expression. As depicted in Fig. 1, transcript levels for the 22 Vβ genes analyzed were similar in the transplanted human thymus and in the same thymus before implantation. These and our previous results with different sets of such mice (19) indicated that T cell reconstitution, encompassing precursors for the majority of clonotypes, reproducibly occurs in SCID–human fetal liver/thymus mice, thereby strengthening the use of this model in the study of human T cell biology.

In SCID mice reconstituted with allogeneic liver/thymus combinations, it has been shown, using mAbs against polymorphic HLA determinants, that the vast majority of thymocytes at ~3 mo after implantation are of fetal liver origin (16–18). We have further documented this by defining expression patterns for a set of allelic Vβ genes (11, 21) distinguishable by the RNase protection assay, wherein the allelic Vβs present in the reconstituted thymus were identical to those in the genome of the liver donor (19).

Having clearly established that the thymocytes and their Vβ repertoires in SCID–human fetal liver/thymus mice are similar to normal human fetal repertoires and are derived from liver progenitors, we then addressed whether confrontation with a bacterial superantigen during development of the repertoire leads to clonal deletions, as has been found in rodents (6, 7). For this purpose, SCID mice coimplanted with syngeneic human fetal thymus and liver were repeatedly injected intraperitoneally with staphylococcal enterotoxin B (SEB).

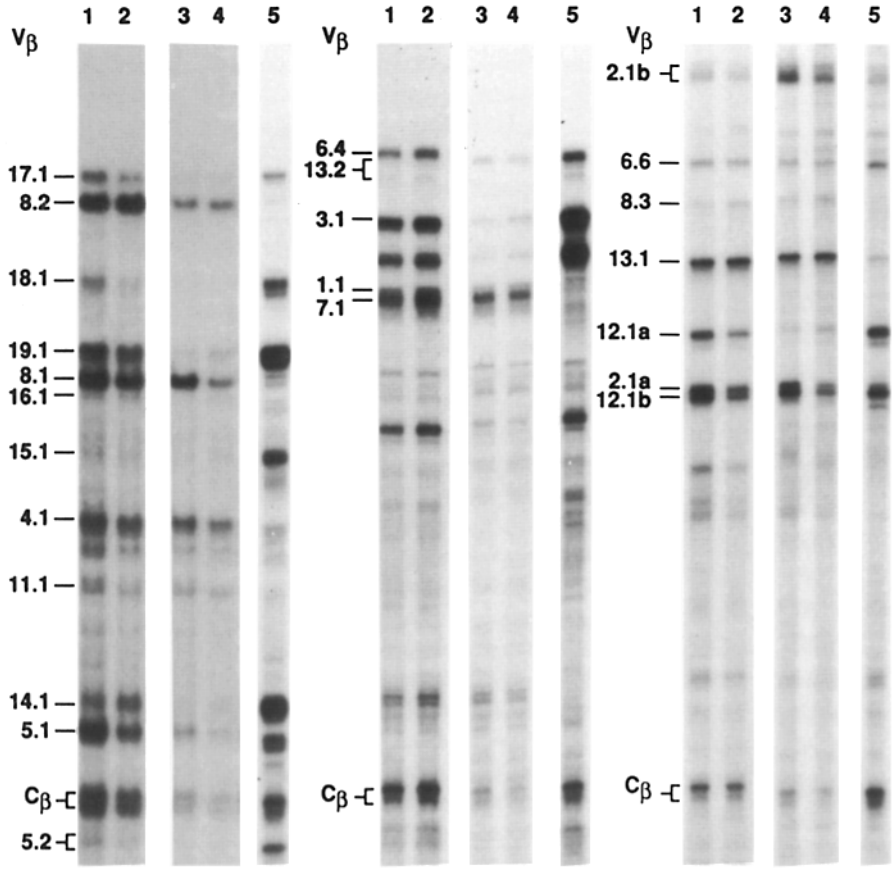

Figure 2. Documentation of Vβ clonal deletions by SEB. Autoradiographic profiles with three Vβ gene probe sets of thymocytes from control (lanes *1* and *2* for $CD4^+8^-$ and $CD8^+4^-$ cells, respectively) and SEB-injected (lanes *3* and *4* for $CD4^+8^-$ and $CD8^+4^-$ cells, respectively) SCID–human fetal liver/thymus mice. Lane *5* depicts human PBLs incubated in vitro with SEB.

Injections were initiated 6 wk after implantation, when the number of cells already selected in the fetal thymus transplant is very low, and continued thereafter three times per week for 4 wk during the emigration and intrathymic maturation of the liver progenitors. Total thymocytes were then isolated and sorted into $CD4^+8^-$ and $CD8^+4^-$ subsets, and RNA from these single-positive populations was analyzed for Vβ transcript levels by the RNase protection assay. Controls included thymocytes from SCID mice reconstituted with human fetal liver/thymus but not injected with SEB and PBLs from an unrelated adult normal human donor stimulated in vitro with SEB. The autoradiographic profiles are shown in Fig. 2, and the actual Vβ transcript values are listed in Table 1. In agreement with previous reports (5, 8, 9, 12), human PBLs cultured with SEB showed expansion of cells expressing Vβ3, 12, 14, 15, 18 (alternatively Vβ20) (12), and Vβ19 (alternatively Vβ17) (12), but engagement of Vβ5.1, 5.2, 6.4, 6.6, and 17 (alternatively Vβ18) (12) was also noted (Fig. 2, lane *5*). SCID–human fetal liver/thymus mice injected with SEB (Fig. 2, lanes *3* and *4* for $CD4^+8^-$ and $CD8^+4^-$ cells, respectively), compared with noninjected counterparts (Fig. 2, lanes *1* and *2* for $CD4^+8^-$ and $CD8^+4^-$ cells, respectively), remarkably showed a 50–90% decline in transcript levels for the same Vβs shown to be engaged in vitro by this toxin. Although the amount of TCR mRNA, as evidenced by Cβ intensity, is lower in lanes *3* and *4* compared with lanes *1* and *2*, the values given are calculated as percent Cβ in each lane and thus, are unbiased. Moreover, the SEB-induced deletions are selective, affect both low and highly expressed Vβs and, conversely, Vβs not engaged by SEB remain unaffected or compensatorily increased. Incomplete deletion for some SEB-selected clonotypes may be due to lower affinity and/or to the presence of residual, already selected, single-positive cells in the human thymus that are resistant to deletion. Although, in some instances, the decline was more pronounced in the $CD4^+8^-$ than the $CD8^+4^-$ single-positive subset, both subsets were affected. As documented in mice with both exogenous (6) and endogenous (22, 23) superantigens, as well as in mice expressing transgenic TCR specific for a self-peptide (3, 10), it is likely that the clonal deletions had occurred at the immature (double-positive) stage of differentiation. However, because class II MHC presented superantigens can bind Vβ without the participation of accessory coreceptors (for a review see reference 8), it is also possible that deletions affecting both subsets may be induced at, or continue after, transition to the single-positive stage. These findings, together with our previous observation of clonal deletions and/or functional inactivation of T cells reactive against alloantigens of fetal liver donors (18), clearly establish that the SCID–human fetal liver/thymus model is fully functional in that it displays negative selection processes against both conventional antigens and superantigens.

Table 1. *Concordance of SEB-induced Vβ Clonal Deletion in SCID-hu Fetal Liver/Thymus Mice with the In Vitro SEB-engaged Human Vβ Clones*

	SCID-human					
	Control		SEB injected		PBL	
Vβ	CD4	CD8	CD4	CD8	Control	SEB
1.1	0.89	1.54	0.27	0.62	0.92	1.01
2.1	0.84	0.96	3.85	3.05	2.34	0.07
3.1	1.63	1.70	0.20	0.83	2.27	11.83
4.1	2.80	3.08	7.02	6.10	2.33	0.63
5.1	6.49	3.90	2.59	1.66	4.38	6.01
5.2	0.36	0.54	0.14	0.16	0.47	3.57
6.4	1.10	1.80	0.46	0.57	1.85	2.47
6.6	2.23	2.54	0.62	0.69	0.69	1.07
7.1	0.94	2.35	3.03	4.18	1.14	0.27
8.1	2.76	1.75	5.75	3.27	1.85	0.12
8.2	1.20	1.11	1.61	2.44	1.09	0.06
8.3	0.43	0.40	0.60	0.70	0.23	0.02
11.1	1.74	1.75	1.31	1.60	0.37	0.30
12.1	2.41	2.93	0.52	0.71	1.30	1.80
13.1	3.53	3.43	4.74	5.27	1.53	0.36
13.2	0.31	0.63	0.14	0.25	0.32	0.35
14.1	1.83	2.94	0.53	0.45	2.43	10.19
15.1	0.97	0.67	0.16	0.28	0.76	3.39
16.1	0.23	0.16	0.61	0.37	0.24	0.05
17.1	0.92	0.45	0.11	0.13	0.83	1.34
18.1	1.77	0.98	0.15	0.14	1.02	4.05
19.1	1.98	1.75	0.25	0.85	1.70	10.55

Values represent Vβ transcript levels expressed as a percentage of total Cβ transcripts.
Vβs selected by SEB are underlined. Transcript levels for Vβs engaged by this superantigen in vitro are higher than in control nonstimulated peripheral lymphocytes, whereas transcript levels for the same Vβs are lower in SEB-injected than noninjected SCID-human fetal liver/thymus mice, apparently because of clonal deletions.

Clonal deletions in mice have also been shown to be conferred by molecules encoded by various mouse Mtv proviral integrants (4, 5) that might also affect the human Vβ repertoire in the SCID-human fetal liver/thymus model. Such endogenous superantigens of the normal BALB/c mouse (the background of the SCID mouse) delete T cells expressing Vβ3, 5, 11, and 12 (5, 8). Mouse Vβ3 is most homologous to human Vβ10 and 20, mouse Vβ5 is most homologous to human Vβ1 and 5, and mouse Vβ11 and 12 are most homologous to human Vβ8 (24). Although Vβ10 and 20 were not included in our human Vβ probe sets, the Vβ1-, 5-, and 8-expressing cells were not deleted in the single-positive subsets of the SEB nontreated SCID-human fetal liver/thymus mouse (Fig. 2, lanes *1* and *2*). The most likely explanation for the absence of Mtv-mediated effects on the human repertoire is the lack of Mtv-presenting B cells in the SCID mouse. Nonetheless, because SCID mice transplanted with syngeneic fetal thymus were shown to display some of the BALB/c-derived endogenous superantigen-mediated Vβ deletions (20), additional mechanisms might be involved, including Vβ structural differences between mouse and humans, and the inability of Mtv-encoded superantigens to be transferred to, and presented by, human APCs.

The present study documents that: (*a*) the human Vβ repertoire is fully displayed in SCID mice reconstituted with human fetal liver and thymus; (*b*) the human repertoire is unencumbered by endogenous superantigen of the SCID mouse; and (*c*) clonal deletions can be manifested in the maturing human T cell repertoire upon encounter of an exogenous superantigen. This is the first clear documentation of superantigen-induced clonal deletion in developing human T cells. In view of the mounting evidence that microbial superantigens may play a role in the pathogenesis of several human disorders, including AIDS (25, 26), autoimmune diseases (27, 28) and toxic shock syndrome (29), the use of the SCID-human liver/thymus model in assessing superantigenic and, in general, thymic repertoire-modifying effects of human pathogens in a biologically relevant setting is evident.

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Note added in proof: While this paper was under review, Waller et al. (*Blood.* 80:3144. 1992), using a limited set of anti-human Vβ antibodies, also reported that Staphylococcal enterotoxins can induce human T cell deletions in SCID-hu mice.

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