Thymic Selection of the Human T Cell Receptor Vβ Repertoire in SCID-hu Mice

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Summary

Implantation of pieces of human fetal liver and thymus into SCID mice results in the development of a human thymus-like organ, in which sustained lymphopoiesis is reproducibly observed. In this model, T cell development can be experimentally manipulated. To study the influence of thymic selection on the development of the human T cell repertoire, the T cell receptor (TCR) Vβ gene repertoire of double-positive ($CD4^+CD8^+$) and single-positive ($CD4^+CD8^-$ and $CD4^-CD8^+$) T cells was analyzed in the SCID-hu thymus using a multiprobe ribonuclease protection assay. TCR diversity in double-positive SCID-hu thymocytes was found to be comparable with that present in the thymus of the fetal liver donor, did not change with time, and was independent of the origin of the thymus donor. Thymic selection in SCID-hu thymus induces changes in Vβ usage by the single-positive $CD4^+$ or $CD8^+$ T cells comparable with those previously reported for single-positive cells present in a normal human thymus. Finally, significant differences were observed in the Vβ usage by CD4 or CD8 single-positive T cells that matured from genetically identical stem cells in different thymic environments. Collectively, these data suggest: first, that the generation of TCR diversity at the double-positive stage is determined by the genotype of the stem cells; and second, that polymorphic determinants expressed by thymic epithelium measurably influence the Vβ repertoire of mature single-positive T cells.

The TCR expressed by the majority of T cells is a heterodimer composed of an α and β chain. TCR diversity is generated by random rearrangement of multiple germline-encoded variable (Vα, Vβ), diversity (Dβ), and joining (Jα, Jβ) gene segments, nongermline-encoded N region insertions, and α and β chain pairing (for review see reference 1). These TCR dimers are expressed on the cell membrane in association with the CD3 complex, which appears in ontogeny at the double-positive stage. The TCR in association with CD4 or CD8 accessory molecules is at that stage available for thymic selection. Studies performed in transgenic mice (2, 3) showed that TCR-α/β^+ precursors capable of recognizing peptides in the context of self-MHC are rescued from apoptosis and continue to differentiate into single-positive T cells. This process is known as positive selection. In addition, strongly self-reactive T cell precursors are deleted from the repertoire (negative selection). Because of these two selection processes, the TCR repertoire of the CD4 or CD8 single-positive T cells is different from the unselected repertoire expressed by the double-positive cells (4).

We have previously used the SCID-hu model to study the influence of thymic selection on human T cell responsiveness (5). SCID mice were transplanted under the kidney capsule with pieces of human fetal liver and thymus as originally described by McCune et al. (6, 7). Stem cells migrate from the fetal liver to the thymic implant where they differentiate into double-positive and finally single-positive T cells that express high levels of TCR-α/β dimers. In this model, we have shown that polymorphic HLA determinants expressed by thymic epithelial cells, profoundly influence human T cell responses against these determinants, as assessed in cellular assays such as MLR and limiting dilution analyses. Whether these changes in T cell reactivity induced by thymic epithelium were accompanied by changes in the T cell repertoire because of positive or negative selection could not be determined with these assays.

In the present paper, we studied the Vβ gene usage in the human thymus of SCID-hu mice. We demonstrate, using RNase protection assay, that the human TCR repertoire in the SCID-hu mice displays similar characteristics to those in the human thymus of the fetal liver donor. We further document that selection in the SCID environment proceeds simi-

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larly to selection in the human fetus and that polymorphic factors in the thymic environment exert considerable influence on the Vβ usage by the CD4 or CD8 single-positive T cells.

Materials and Methods

Mice. C.B-17 *scid/scid* mice were transplanted with small pieces of fresh fetal liver and thymus under the kidney capsule as described (7). Each mouse was transplanted with fetal liver of one donor and its autologous thymus (A/A animals) or an allogeneic thymus (A/B animals). Fetal donors between 15 and 21 wk gestational age were used. No attempts were made to deplete the thymus grafts of endogenous thymocytes. However, in most animals, endogenous thymocytes are completely replaced by fetal liver-derived cells by 3 mo after transplantation (5, 7). Mice with thymocytes exclusively of fetal liver donor origin between 6 and 12 mo after transplantation were studied.

Cell Preparation. Up to five thymuses from SCID-hu mice transplanted with identical material were harvested, dissected free of renal tissue, and squeezed through a steel mesh. This cell suspension was washed once, counted, and incubated with 10 μg purified OKT6 mAb (anti-CD1; American Type Culture Collection, Rockville, MD) per 10^8 cells for 30 min. After two washes, the cells were incubated with one goat anti-mouse–coated magnetic bead (Dynal, Inc., Great Neck, NY) per cell for 30 min. The $CD1^-$ cells were subsequently labeled with CD4-FITC and CD8-PE and sorted on a FACScan® (Becton Dickinson & Co., San Jose, CA). RNA was isolated from the sorted $CD4^+CD8^-$, sorted $CD4^-CD8^+$ (both >98% pure), and the $CD1^+$ cells attached to the beads.

The Multiprobe RNase Protection Assay. Sequence and transcription of 22 Vβ probes was described earlier (4). Hybridization of 1–2 μg of RNA with radiolabeled Cβ probe and each of the probe sets was performed at 56°C for 12–16 h. Unhybridized probes and target RNA were digested with RNase A and RNase T1. Purified protected probe-target mRNA duplexes were electrophoresed in standard sequencing gels and autoradiographed on XRP film (Eastman Kodak Co., Rochester, NY). Quantitation of Vβ transcript levels was performed with a radioanalytic imaging apparatus (AMBIS Systems, Inc., San Diego, CA). The net cpm at a given Vβ band was calculated as described (4). The mean and SD of duplicate determinations is shown. Differences in Vβ expression levels of A/A and A/B (or A/C) mice were statistically analyzed using Student's *t* test.

Results and Discussion

Relative Vβ mRNA Levels of Unselected ($CD1^+$) Fetal and SCID-hu Thymocytes. SCID mice were grafted under the kidney capsule with liver and thymus from one fetal donor. The grafted thymuses were harvested at various times up to 10 mo after transplantation, and the Vβ repertoire of $CD1^+$, $CD4^+$ $CD8^+$ unselected thymocytes was analyzed. In Fig. 1, it is shown that relative expression levels for 22 Vβ genes in a thymus harvested at 10 mo after transplantation are comparable with those present in the fetal thymus of the donor before transplantation. Analyses at earlier points in time gave similar results (data not shown). These data support the notion that not only differentiation of the human T cell lineage, but also generation of T cell diversity proceeds similarly in the thymus of SCID-hu mice and the human fetus.

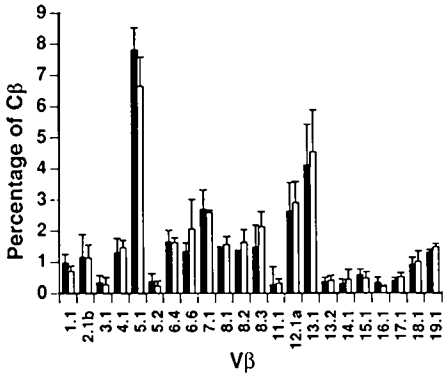

Figure 1. Relative TCR Vβ usage in unselected total thymocytes from a fetal donor (*filled bars*) and a SCID-hu mouse constructed with fetal liver and thymus of this donor (*unfilled bars*).

For the purpose of our study, it was important to determine whether differences in expression levels of certain Vβ genes observed in double-positive thymocytes of different donors (4), are caused by genomic properties of the hematopoietic stem cells or by exogenous factors. To address this point, SCID-hu mice were constructed with fetal liver from one donor and fetal thymus from another. In these animals, all endogenous thymocytes were replaced by fetal liver-derived cells by 6 mo after transplantation as could be demonstrated with mAb against polymorphic HLA determinants (5; and data not shown). This could be confirmed at the molecular level studying expression levels of allelic Vβ genes in such combinations: the Vβ2.1b allele is absent in a fresh thymus homozygous for the Vβ2.1a allele, but is expressed 6 mo after transplantation of this thymus with an allogeneic fetal liver, which was apparently heterozygous for this gene (Fig. 2). The consistency of the results is documented by the fact that the profile is identical in two recipient mice. The levels of 22 Vβ variable gene segments generated in these mice were then compared with the unselected repertoire present in the untransplanted thymus of the fetal liver and fetal thymus donors. In Fig. 3, an informative combination is shown, in which there are significant differences in the percentage of Vβ2, 3, 7, and 12 mRNA levels between both donors. For each of these four Vβ, the levels in the SCID-hu chimeric thymus and those in the thymus of the fetal liver donor were superimposable, indicating that the Vβ repertoire at the double-positive stage is defined by the hematopoietic stem cells of the fetal liver donor.

At this point it has to be noted that we cannot rule out an effect of age of the fetal thymus donor or fetal liver donor on the development of the TCR repertoire (8), since most of the livers and thymuses used in the experiments described above were 18–20-wk gestational age. However, in comparing the relative Vβ levels reported here for the fetal and SCID-hu thymus, with those previously reported for 17 postnatal thymuses (aged 5 d–10-yr), differences were noted in the relative levels of certain Vβ: Vβ5.1 and Vβ7.1 were expressed

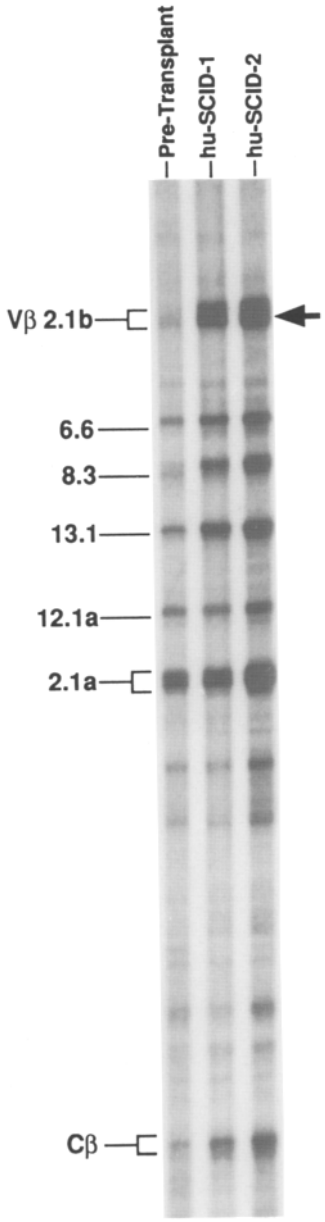

Figure 2. Vβ2.1 allele expression in a fetal thymus before transplantation (lane *1*) and in thymus of two SCID-hu animals 6 mo after transplantation of this thymus with allogeneic fetal liver (lanes *2* and *3*). In lane *1*, Vβ2.1b represents <0.1% of Cβ, whereas in lanes *2* and *3* the Vβ2.1b transcripts represent 3.2% of Cβ.

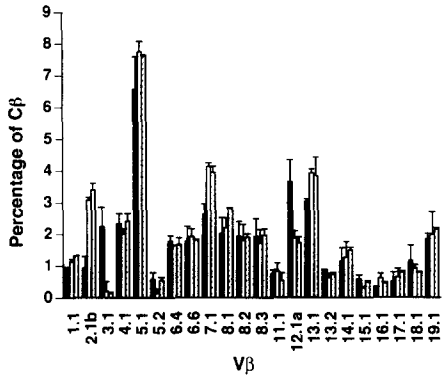

Figure 3. Relative TCR Vβ usage of fetal donor B (*filled bars*) and fetal donor A (*hatched bars*). Vβ usage of an A/B SCID-hu mouse, constructed with fetal liver of A and fetal thymus of B (*unfilled bars*).

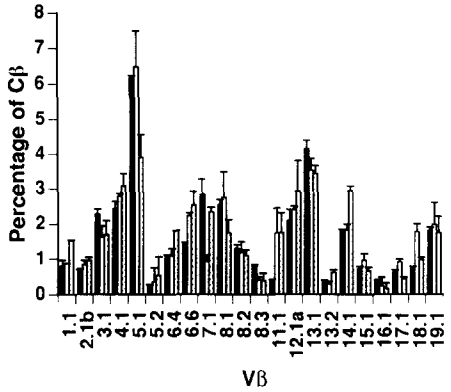

Figure 4. Relative TCR Vβ transcript level in SCID-hu CD1$^+$ thymocytes (*filled bars*), CD4 (*unfilled bars*), and CD8 (*hatched bars*) single-positive T cells.

at higher percentages in SCID-hu (Vβ5.1 = 6.6 ± 1.4% and Vβ7.1 = 2.9 ± 1.1%, eight animals) and fetal thymus (Vβ5.1 = 9.7 ± 4.4% and Vβ7.1 = 3.1 ± 0.7, four cases) than in postnatal thymus (Vβ5.1 = 3.7 ± 0.6% and Vβ7.1 = 1.3 ± 0.4%). On the other hand, Vβ5.2 levels were lower in the fetal and SCID-hu thymus, 0.4 ± 0.4% and 0.8 ± 0.5%, compared with 2.1 ± 0.2% in the postnatal thymus. Since the repertoire in SCID-hu mice remains similar to the fetal repertoire, it is unlikely that the differences between the postnatal and fetal repertoire are related to pregnancy, or to the relatively antigen-free uterine environment. The differences between fetal and postnatal repertoire might be age related, as has been described for the B cell repertoire (9, 10) and the γ/δ repertoire (11, 12). We are at the moment collecting thymuses of various gestational ages to further address this issue.

Thymic Selection Induced Changes in Vβ mRNA Levels. Although complete deletion of T cell populations expressing a particular Vβ gene are not normally observed in humans, shifts in Vβ usage are usually induced by thymic selection (4). We investigated changes induced by selection of the human TCR repertoire in the SCID-hu mouse. A representative example is shown in Fig. 4, in which the unselected repertoire at the CD1$^+$ double-positive stage in a particular animal is compared with the repertoire of the CD4$^+$ and CD8$^+$ single-positive populations: Vβ1.1, Vβ6.4, Vβ6.6, Vβ11.1, Vβ13.2, and Vβ14.1 increase; Vβ3.1, Vβ5.1, Vβ8.1, Vβ8.3, Vβ16.1, and Vβ17.1 decrease in the CD8$^+$ population relative to the unselected CD1$^+$ population. In the CD4$^+$ population, Vβ6.6, Vβ11.1, and Vβ18.1 increase, whereas Vβ3.1, Vβ7.1, Vβ8.3, and Vβ16.1 tend to decrease. Generally, the majority of the Vβ gene mRNAs that increase or decrease in the CD4$^+$ or CD8$^+$ population in the postnatal thymus (4), tend to do similarly in that population in the SCID-hu thymus (see also Fig. 5). Two conclusions can be drawn from these data. First, as in postnatal thymus, the Vβ usage is affected significantly by positive and or negative selection. Second, endogenous superantigens present in the BALB/c background genes seem to not noticeably affect the human repertoire, unlike staphylococcal superantigens, which

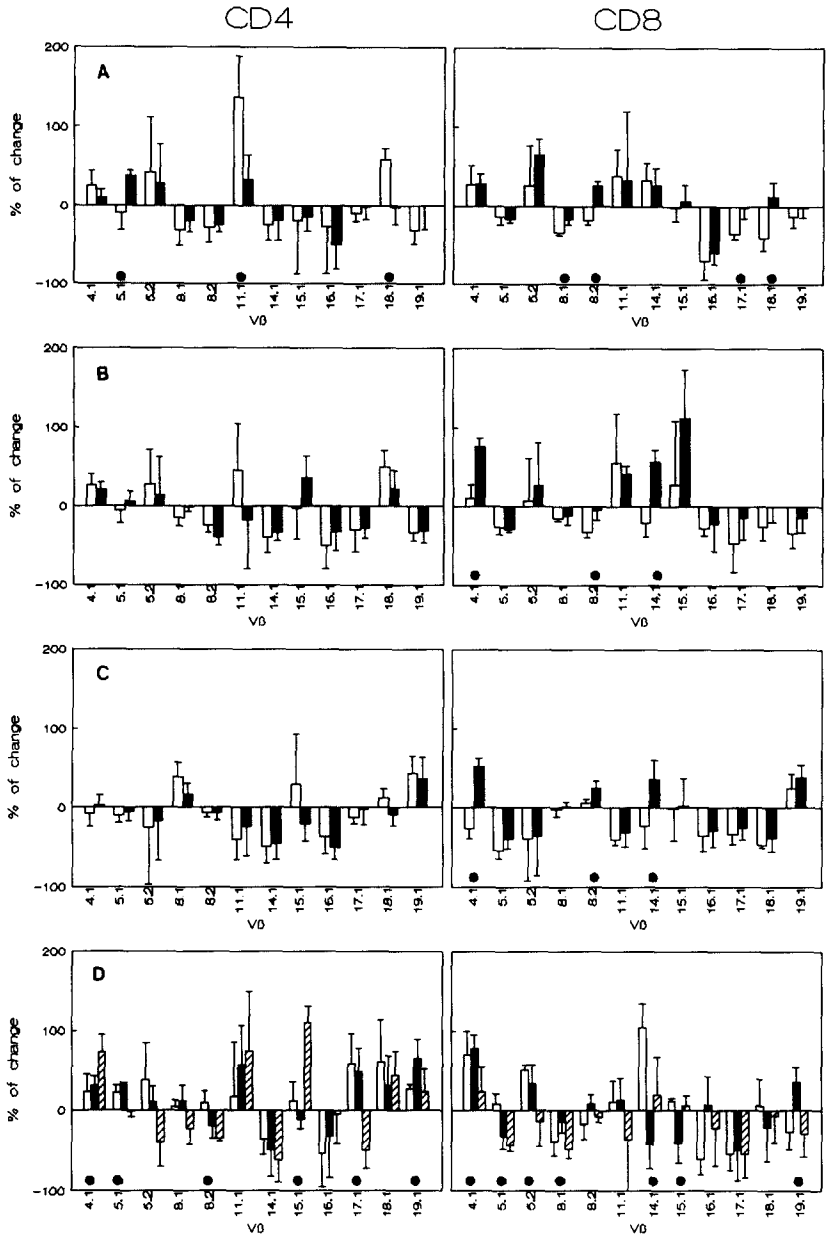

Figure 5. Comparison of the TCR Vβ repertoire of single-positive CD4+ and CD8+ thymocytes, isolated from pools of A/A and A/B SCID-hu thymus. For each Vβ gene, the percentage of change in levels relative to the levels in the double-positive thymocytes of the A/A mice, is shown. Results of three different A/A–A/B combinations (*A–C*) and one A/A–A/B–A/C combination (*D*), which were transplanted with fetal liver of a donor A and fetal thymus of either a donor A, a donor B or a donor C. (*Unfilled bars*) A/A combination. (*Filled bars*) A/B combination. (*Hatched bars*) A/C combination. (●) Significant differences (Student's *t* test, $p < 0.05$) in Vβ expression levels between A/A and A/B (or A/C) mice.

can cause deletion of human T cells expressing certain Vβ genes in the SCID-hu model (Baccala, R., A. E. Vandekerckhove, D. Jones, D. H. Kono, M.-G. Roncarolo, and A. N. Theofilopoulos, manuscript submitted for publication). This is not surprising, since endogenous superantigens are primarily presented by B cells (13) that are absent in the SCID mouse.

Influence of Thymic Epithelium on the Vβ T Cell Repertoire. To investigate the influence on the repertoire of HLA and other polymorphic determinants expressed by the thymic environment, SCID-hu mice were constructed with fetal liver and its autologous thymus (A/A animals) or an allogeneic thymus (A/B animals). To ensure complete repopulation of the thymus by fetal liver donor A-derived stem cells in both groups, the T cell repertoire was analyzed after at least 6 mo. In Fig. 5, the expression levels of 12 Vβ genes are depicted for four such A/A–A/B mouse combinations. As expected on the basis of the same fetal liver stem cell origin, the relative Vβ levels expressed by the CD1+ double-positive thymocytes was never significantly different between the A/A and A/B animals (data not shown). At the single-positive level, most of the changes induced by selection occurred concordantly in both the A/A and A/B animals, suggesting that they are induced by nonpolymorphic determinants on the HLA molecules, or by clonal changes induced by antigens expressed on the fetal liver-derived hematopoietic cells present in the thymus. However, superimposed on this common pattern are individual-specific alterations: Vβ5.1, Vβ11.1, and Vβ18.1 expression is significantly different in the CD4+ subset; and Vβ8.1, Vβ8.2, Vβ17.1, and Vβ18.1 in the CD8+ subset of the two animal group (Fig. 5 *A*). Variations

in expression levels of several Vβ are seen in the various combinations studied. Significant differences in the expression levels for a given Vβ are usually confined to one subset, either $CD4^+$ or $CD8^+$, and no or opposite changes are seen in the other subset. The discordant Vβ expression profiles in mice transplanted with three different thymuses (Fig. 5 *D*) further strengthens the conclusion that these variations are thymus dependent.

In this SCID-hu model, we have previously shown that the T cell reactivity of genetically identical T cells is influenced by polymorphic antigens expressed on the thymic epithelium (5). T cells generated in A/B mice, unlike T cells of A/A mice, were unresponsive to antigens expressed on the thymic epithelium of donor B. This nonresponsiveness could in part be attributed to functional changes in the thymus-specific T cells. However, whether only functional changes had occurred, or whether deletions of thymus-reactive T cells and therefore changes in the T cell repertoire had occurred, could not be determined. In addition, the influence of the thymic epithelium on the T cell repertoire because of positive selection was not studied. We demonstrate here significant changes in the repertoire of genetically identical T cells maturing in A/A and A/B SCID-hu mice. Although these experiments do not address the relative contribution of positive and negative selection, they clearly show that polymorphic determinants expressed in the thymic environment affect the T cell Vβ repertoire.

Selection-induced changes in Vβ usage in humans are more subtle compared with those in mice, since no complete deletion of T cells expressing certain Vβ determinants was observed. In the outbred human population, various genetic or environmental factors might be at play in thymic selection and counteract one another, thereby hampering the analysis of individual variables. Despite these difficulties, HLA phenotype was found to segregate with levels of T cells expressing Vβ5, Vβ6, Vβ8, Vβ12, or Vα2 in family studies (14), suggesting that polymorphic HLA determinants can influence the T cell Vβ repertoire. In this study (Fig. 5), we demonstrate that polymorphic determinants expressed by thymic epithelium, likely HLA determinants, indeed exert considerable influence on the Vβ T cell repertoire.

In summary, the results demonstrate that: (*a*) immature $CD1^+$ thymocytes express a Vβ repertoire determined by the genotype of the stem cells; and (*b*) polymorphic determinants expressed by the thymic epithelium influence the levels of Vβ expressed at the single-positive stage. These differences in the T cell repertoire may determine differences in immune responsiveness to antigens or vaccines, and susceptibility or resistance to diseases.

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