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Immunology

Selective manipulation of the human T-cell receptor repertoire expressed by thymocytes in organ culture

(T-cell development/clonal deletion/receptor modulation/staphylococcal enterotoxins)

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ABSTRACT **A recently described organ culture system for human thymocytes is shown to support the generation of a diverse T-cell receptor repertoire *in vitro*: thymocytes of the αβ lineage, including representatives of the Vβ families 5.2/5.3, 6.7, and 8, accounted for the majority of T-cell receptor-positive cells throughout a 3-week culture period. Thymocytes bearing γδ receptors were also identified, particularly among the CD4 CD8 double-negative subset. The T-cell receptor repertoire expressed in organ culture responded to experimental manipulation with staphylococcal enterotoxins. Staphylococcal enterotoxin D (a powerful activator of human peripheral T cells expressing Vβ 5.2/5.3 receptors) caused a marked reduction of Vβ 5.2/5.3 expression, as determined with the Vβ-specific antibody 42/1C1. Evidence is presented that this loss of Vβ 5.2/5.3 expression resulted from the selective deletion of activated thymocytes by apoptosis, in concert with T-cell receptor modulation. These effects of staphylococcal enterotoxin D were specific (since staphylococcal enterotoxin E did not influence Vβ 5.2/5.3 expression) and Vβ-selective (since expression of Vβ 6.7 remained unaffected by staphylococcal enterotoxin D). On the basis of these observations, we suggest that thymic organ culture provides a powerful approach to study the generation of the human T-cell repertoire.**

In the thymus, T-cell precursors first express the clonotypic T-cell receptor (TCR) and accessory molecules such as CD4 and CD8. Interactions of these structures with major histocompatibility complex (MHC) glycoproteins and MHC-associated ligands expressed by thymic stromal cells determine the fate of developing thymocytes. As a result of this process (called thymic education), T cells preferentially recognize foreign antigens in the context of self-MHC and are normally tolerant to many self components (reviewed in refs. 1–3). Self-tolerance is established in part by clonal deletion, and the elimination of thymocytes reactive to a class of Vβ-specific ligands (called superantigens) can be demonstrated by TCR Vβ-specific monoclonal antibodies (mAbs) (4, 5). In the mouse, endogenous superantigens stimulate mature T lymphocytes and cause the deletion of responsive thymocytes (reviewed in ref. 6). Both effects can be mimicked experimentally by exogenous superantigens such as staphylococcal enterotoxin (SE) (6–8). In humans, endogenous superantigens have not been described. In the absence of experimental approaches to study the human thymocyte repertoire, the analysis of SE effects remains limited to peripheral T cells (9–11).

Most of what we know about T-cell repertoire formation is based on animal studies, but, given the clinical importance of autoimmune diseases and immunodeficiencies, most of what we want to know concerns the human system. Autoreactive T cells are characterized by preferential TCR Vβ usage in some animal models for autoimmunity (reviewed in ref. 12), and limited evidence indicates a similar bias may exist in human diseases such as multiple sclerosis (13, 14) or rheumatoid arthritis (15). *In vitro* model systems for thymic selection might aid the understanding (and ultimately the resolution) of repertoire-associated pathology. We previously reported that alymphoid (2′-deoxyguanosine-treated) murine embryonic thymic rudiments support (in the presence of human stromal elements) the development of human thymocytes from fetal and postnatal sources (16, 17, 38). Without the addition of mitogens, lymphokines, or other exogenous factors that might override thymic selection events, this system allows for an approximately 30-fold increase of human thymocyte numbers over a 3-week period. Expansion is sustained by relatively immature precursor cells, and the thymocyte subpopulations generated *in vitro* are phenotypically similar to those seen *ex vivo*, including a major CD4 CD8 double-positive (DP) subset with the marker constellation $CD3^{dim}$, $CD5^{dim}$, and $CD1a^{bright}$ (16, 38). The present study probes the TCR repertoire of human thymocytes developing in these organ cultures by immunofluorescence staining and multiparameter flow cytometry, and it describes TCR Vβ-specific changes resulting from experimental exposure to exogenous superantigens.

MATERIALS AND METHODS

Thymic Organ Culture. Human thymocytes were obtained from pediatric patients undergoing cardiac surgery, and fetal tissue (14 to 19 weeks of gestation) was obtained from routine surgical procedures. Single-cell suspensions were prepared mechanically, and aliquots were stored frozen in liquid nitrogen until use. Murine thymic lobes from d14 (C57/BL6 × SJL)F_2 embryos were treated with 1.35 mM 2′-deoxyguanosine for 5–6 days (18), washed extensively, and cultured in hanging drops with 2–5 × 10^5 postnatal or 1–2 × 10^5 fetal thymocytes. Unattached cells were removed 48 hr later, and thymi were placed on Nuclepore filters (0.8 μm, Costar) floating on Iscove's modified Dulbecco's medium supplemented with 10% heat-inactivated fetal calf serum. Where indicated, SE (SED, SEE, or SEC-2; Toxin Technology, Madison, WI) was added (1 μg/ml) 1 week after initiation of cultures. At the end of the culture period, individual lobes were teased apart with cataract knives, and viable cells were counted by phase-contrast microscopy.

Immunofluorescence Staining and Fluorescence Activated Cell Sorter (FACS) Analysis. Staining was carried out at 4°C in phosphate-buffered saline A, 0.2% bovine serum albumin, and 0.1% sodium azide, and the cells were washed twice in

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Abbreviations: DN, double-negative (for CD4 and CD8); DP, double-positive; mAb, monoclonal antibody; MHC, major histocompatibility complex; PE, phycoerythrin; SE, staphylococcal enterotoxin (SED, SEE, SEC-2); SP, single-positive; TCR, T-cell receptor; TR, Texas red.

this buffer after each step. Cells were incubated for 45 min with saturating concentrations of the mouse mAbs BMA031 (anti-TCR αβ, IgG2b; ref. 19), 42/1C1 (anti-Vβ 5.2 and 5.3, IgG1; ref. 20), MX6 (anti-Vβ 8, IgG2a; ref. 21), OT145 (anti-Vβ 6.7; ref. 22), or TCR δ1 (anti-TCR γδ, IgG1; ref. 23), followed by fluorescein-conjugated goat antibody to mouse immunoglobulin for 15 min. After blocking with 5% mouse serum, phycoerythrin (PE)-coupled Leu3a (anti-CD4, Becton Dickinson) and biotinylated Leu2a (anti-CD8, Becton Dickinson) were added for 45 min. The latter was revealed by streptavidin conjugated to PE/Texas red (PE/TR; streptavidin tandem, Southern Biotechnology Associates) for 15 min. Between 15,000 and 50,000 events were collected per sample (depending on the availability of cells) on a FACScan (Becton Dickinson). For analysis, a live gate was set on forward and side scatter. Human origin and T-cell lineage were routinely verified by staining for human CD45 and CD2 (data not shown).

Gel Electrophoresis of DNA. Genomic DNA was prepared as described (24) from 2×10^6 thymocytes recovered from organ cultures treated for 24–72 hr with SE (1 μg/ml) or the anti-CD3 mAb UCHT1 [IgG1 (25), used as supernatant at a final concentration of 20%]. Samples were loaded onto 2% agarose gels containing ethidium bromide at 0.1 μg/ml and subjected to electrophoresis at 30 V for 3–4 hr prior to photography under UV light.

Isolation and Culture of Human Peripheral Blood Mononuclear Cells (PBMC). PBMC were prepared by centrifugation of heparinized blood over Ficoll–Hypaque (Pharmacia). For proliferation assays, 5×10^4 PBMC were cultured in 200 μl of RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum in round-bottom 96-well plates for 72 hr in the presence of the indicated stimuli. After pulsing with [^{3}H]thymidine (1 μCi per well; 1 Ci = 37 GBq) for 12 hr, the cultures were processed for liquid scintillation counting. To determine effects of SE on the TCR repertoire expressed by CD4 T cells, PBMC (5×10^5 per ml in 12-well Costar plates) were exposed to SE (1 μg/ml) for 72 hr, washed, and cultured either in human recombinant interleukin 2 (25 units/ml, Boehringer Mannheim) or on feeder layers of murine L cells (5×10^5 PBMC and 1×10^5 L cells pretreated for 30 min with mitomycin-C at 50 μg/ml); human and murine fibroblastoid cells can improve survival (26) and responsiveness (ref. 27 and unpublished data) of human T lymphocytes in the absence of exogenous growth factors. Immunofluorescence staining of PBMC was performed as described for thymocytes, and Vβ expression by CD4 T cells (>99% CD3-positive; see Fig. 1*a*) was determined by gating on the basis of CD4 reactivity and light-scatter parameters.

RESULTS

TCR Expression by Human T-Lymphoid Cells *ex Vivo* and *in Vitro*. Peripheral T lymphocytes express uniformly high levels of TCR, and populations of Vβ 5.2/5.3, Vβ 8, and Vβ 6.7 cells were readily identified by the panel of mAbs used in this study (Fig. 1*a*), providing a reference point for the heterogeneous TCR expression by human thymocytes *ex vivo* (Fig. 1*b*). Subsequently, these reagents were used in combination with mAbs to CD4 and CD8 to follow TCR expression by human thymocyte subsets, and Fig. 2 shows a characteristic phenotypic analysis after 3 weeks in organ culture. Thymocyte subpopulations are defined by CD4 and CD8 (Fig. 2*a*), and the three-color immunofluorescence approach allows TCR expression by each population to be resolved (Fig. 2*b*). We have previously shown that thymic organ cultures support the expansion of a phenotypically immature CD4 CD8 DP population (CD3^{dim}, CD5^{dim}, and CD1a^{bright}; ref. 38). These cells were αβ TCR$^{negative/dim}$, whereas αβ TCR^{bright} cells were found among the SP thymocyte populations (Fig. 2*b*). Between 85% and 95% of CD8 SP cells expressed high levels of αβ TCR, in agreement with their phenotypic maturity (CD3^{bright}, CD5^{bright}, and CD1a$^{negative/dim}$; ref. 38). In time course experiments (Fig. 3), most CD4 SP thymocytes initially displayed a similar phenotype, including bright staining for αβ TCR. Immature CD4 SP cells [present at very low numbers in human thymus *ex vivo* (28)] gradually accumulated in culture (Figs. 2*b* and 3*a*; ref. 38). Consequently, the percentages of CD4 SP thymocytes reactive with anti-TCR mAbs were lower than among the CD8 SP subset (Figs. 2 and 3). The TCR diversity expressed by αβ TCR-positive thymocytes in organ cultures was analyzed in more detail with mAbs to the Vβ families 5.2/5.3, 8, and 6.7 (20–22). The SP and DP thymocyte subsets contained representatives of each of these TCR Vβ families, and the profiles of TCR Vβ expression were similar to thymocytes *ex vivo* (compare Fig. 1*b* to Fig. 3 *c–e*). Few CD4 CD8 DN cells were αβ TCR-positive (Figs. 2*b* and 3*a*), precluding a reliable quantitation of Vβ expression among this subset. However, a sizeable fraction of γδ TCR-positive DN thymocytes was present throughout the culture period (Fig. 3*b*).

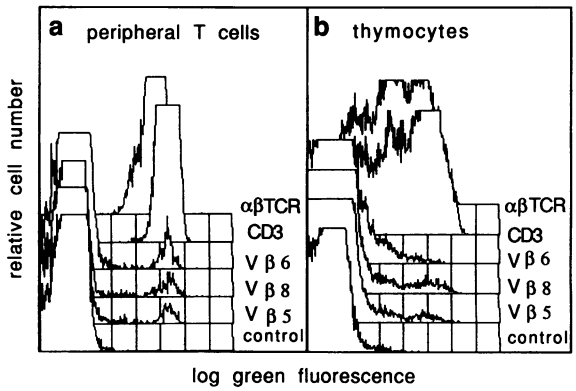

FIG. 1. Reactivity of human T-lymphoid cells with TCR-related mAbs. (*a*) PBMC were obtained by Ficoll–Hypaque centrifugation of heparinized blood, and the following percentages of stained cells were determined with a gate set on CD4-positive cells: αβ TCR, 98.9%; CD3, 99.8%; Vβ 5.2/5.3, 3.2%; Vβ 8, 4.6%; Vβ 6.7, 5.4%; negative control, 0.1% (fluorescein-conjugated goat antibody to mouse immunoglobulin). (*b*) Analysis of postnatal thymocytes gave the following percentages: αβ TCR, 70.0%; CD3, 71.9%; Vβ 5.2/5.3, 3.0%; Vβ 8, 4.0%; Vβ 6.7, 6.2%; negative control, 0.5% (fluorescein-conjugated goat antibody to mouse immunoglobulin).

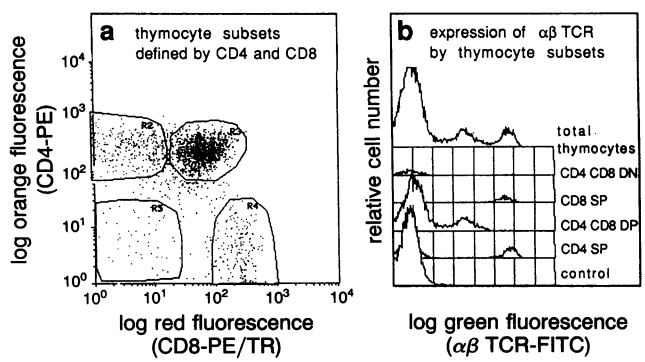

FIG. 2. αβ TCR expression by human thymocyte subpopulations after 3 weeks in organ culture. (*a*) CD4 and CD8 expression by cultured thymocytes and the gates used to define subpopulations. (*b*) Histogram overlay showing the distribution of fluorescein-conjugated αβ TCR among the indicated populations (DN, double-negative; SP, single-positive). Control staining was with fluorescein-conjugated antibody to mouse immunoglobulin.

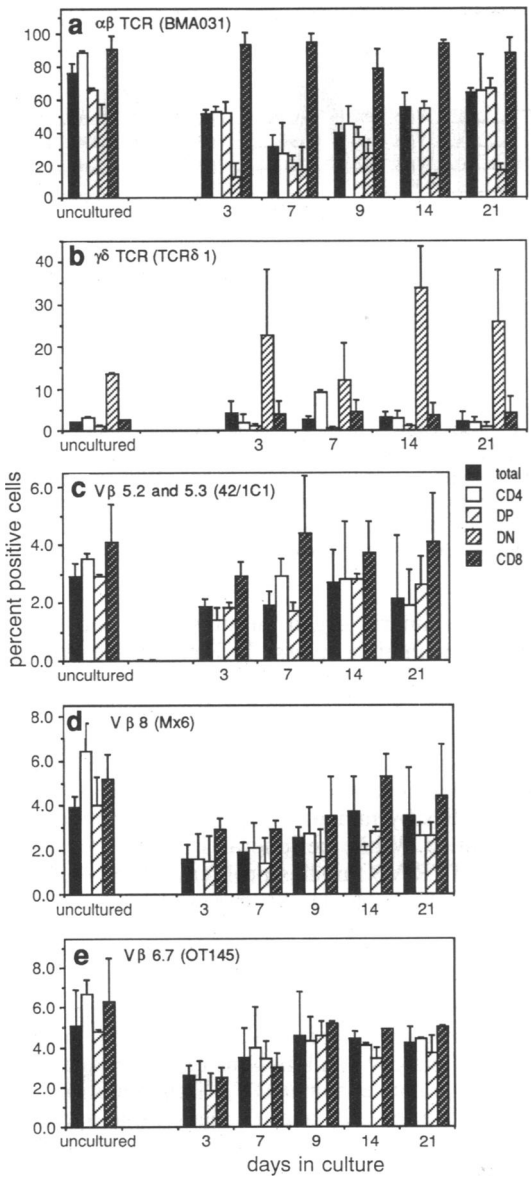

FIG. 3. Kinetic analysis of TCR expression in organ culture. Thymocyte subpopulations were defined by gating according to CD4 and CD8 expression (see Fig. 2). Shown are the percentages of cells positive for αβ TCR (*a*), γδ TCR (*b*), Vβ 5.2/5.3 (*c*), Vβ 8 (*d*), and Vβ 6.7 (*e*) among total thymocytes and the indicated subpopulations. Bars represent the means +SD of 3–9 determinations at each time point analyzed.

This analysis suggested that thymocyte populations generated in organ culture express a broad and relatively unbiased TCR repertoire. To explore the potential of this system for investigating human T-cell repertoire formation, we monitored the effects of exogenous Vβ-specific ligands.

Experimental Manipulation of the Human TCR Repertoire. To determine the effects of SE on peripheral T cells, human PBMC were exposed to SED or SEE (1 μg/ml) for 72 hr, washed, and maintained in interleukin 2 for 24 hr (9). As described by Kappler *et al.* (9), peripheral T cells responded by strong proliferation, and the analysis of Vβ expression in the blast population revealed the expansion of specific TCR Vβ families. As summarized in Table 1, SED stimulated T cells, expressing Vβ 5.2/5.3 and Vβ 8, but not Vβ 6.7, whereas SEE stimulated T cells expressing Vβ 8, but not Vβ 5.2/5.3 or Vβ 6.7. These data agree with previous reports (9, 10), with the exception that in our hands Vβ 8 T cells responded to the commercial SED preparations used. This result was confirmed with several Vβ 8 reagents (kind gifts of S. Carrel, Ludwig Institute for Cancer Research, Lausanne, Switzerland), including the standard Vβ 8 mAb, MX6 (21), and is supported by the independent observation that Vβ-8-expressing Jurkat cells respond to SED (29). No additional Vβ reactivities were revealed by providing a source of murine antigen-presenting cells (the BALB/c-derived B-cell lymphoma M12; data not shown).

Table 1. Vβ-specific expansion of peripheral T cells in responses to SED and SEE

Addition to medium	% positively staining CD4+ T cells			[3H]Thymidine incorporation, cpm × 10^{-3}
	Vβ 5.2/5.3	Vβ 8	Vβ 6.7	
None	2.9 ± 0.4	4.4 ± 0.2	4.5 ± 0.9	4.1 ± 2.2
PHA	3.8 ± 0.1	3.5 ± 0.9	4.3 ± 1.3	193.4 ± 7.9
SED	5.9 ± 1.2	8.3 ± 3.3	1.1 ± 0.1	169.2 ± 20.1
SEE	1.3 ± 1.1	10.1 ± 5.8	2.7 ± 1.2	142.5 ± 11.5

Values represent percentages (mean ± SD of three experiments) of positively staining cells detected among CD4-positive T-cell blasts. PHA, phytohemagglutinin.

In striking contrast to the response of peripheral T cells (refs. 9 and 10; Table 1), addition of SE to thymic organ cultures resulted in a decrease of viable cell numbers: compared with control cultures, recoveries after SE exposure were reduced by 23–33% at 24 hr and 55–63% at 72 hr (data from six experiments with SED, SEE, and SEC-2). Gel electrophoresis of thymocyte DNA revealed a moderate degree of oligonucleosomal fragmentation (Fig. 4). The smallest fragments corresponded to the size of individual nucleosomes (approximately 180 base pairs). This pattern, considered a hallmark of the cellular suicide mechanism apoptosis (30), was most pronounced 72 hr after exposure to SED (lane 3 of Fig. 4; SEE and SEC-2 gave indistinguishable results). Similar bands were seen after 24 hr (unpublished observations). The positive control (lane 2) shows DNA from cultures treated with the CD3 mAb UCHT1 (25), while little DNA fragmentation occurs in unmanipulated cultures (lane 1). The overall reduction in the numbers of viable cells and the occurrence of oligonucleosomal DNA fragmentation indicate that SE caused the deletion of thymocytes in organ culture.

Immunofluorescence staining suggested that those thymocytes expressing toxin-responsive TCR Vβ families (see Table 1) were selectively affected. Fig. 5 illustrates the marked loss of Vβ 5.2/5.3 determinants in response to SED (note that Vβ 5.2/5.3 expression was unchanged by SEE). Both SED and SEE reduced Vβ 8 staining, while neither toxin affected the frequency of Vβ 6.7 cells. Table 2 summarizes results for both total and DP thymocyte populations (the less numerous SP populations were not evaluated separately). SED diminished Vβ 5.2/5.3 expression in each experiment ($P < 0.0005$ by paired Student's *t* test), while SEE did not affect Vβ 5.2/5.3. Toxin effects on Vβ 8 expression were less clear-cut, but a comparison of control and SE-treated cultures within each experiment suggests a significant

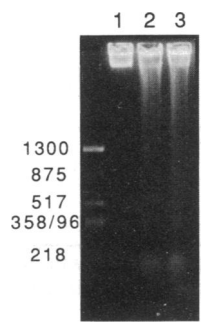

FIG. 4. SED causes the degradation of human thymocyte DNA into oligonucleosomal fragments. DNA was prepared from 2 × 10^6 thymocytes 24 (not shown) or 72 hr after exposure of organ cultures to SED (lane 3, 1 μg/ml) or the anti-CD3 reagent UCHT1 (lane 2, final concentration 20% supernatant; ref. 25). DNA from untreated control cultures is shown in lane 1. The size (in base pairs) of marker DNA (plasmid SP65 cut with *Hin*fI and *Pst* I) is indicated on the left (358/96 marks an unresolved triplet of 358, 369, and 396 base pairs).

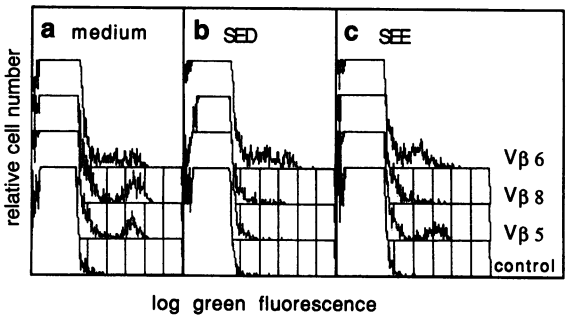

FIG. 5. SED and SEE selectively affect Vβ expression by human thymocytes in organ culture. Cultures were exposed to SED (*b*) or SEE (*c*) at 1 μg/ml. Cell suspensions were prepared after 48 hr, and the analysis of Vβ expression included small as well as large thymocytes. The numerical values obtained in this and additional experiments are summarized in Table 2.

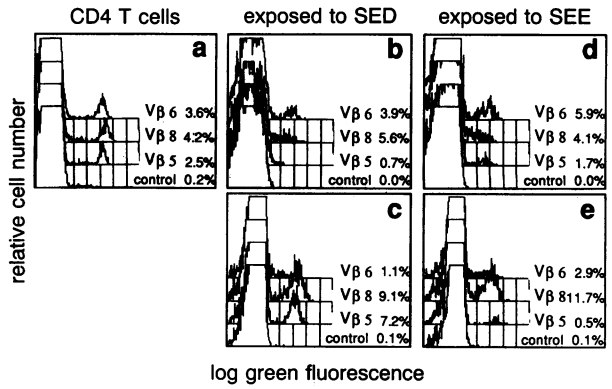

FIG. 6. Loss and recovery of TCR Vβ determinant expression by CD4 T cells exposed to SED or SEE. After exposure to SED (*b* and *c*) or SEE (*d* and *e*) at 1 μg/ml for 72 hr, PBMC were washed and cultured in fresh medium without exogenous lymphokines on a feeder layer of murine L cells for 12 hr (*b* and *d*) or 72 hr (*c* and *e*). Analysis of Vβ expression included small lymphocytes as well as blast cells.

impact of both SED and SEE ($P < 0.05$ by paired Student's *t* test). Residual cells often displayed reduced levels of Vβ 8 (data not shown). In agreement with results on PBMC (not shown), SEC-2 affected none of the Vβ families analyzed.

Loss of viability and DNA fragmentation occurred more slowly (see above) than the changes in Vβ expression induced by SE [essentially complete by 20 hr (unpublished observations)]. We therefore considered the possibility that additional mechanisms (such as receptor modulation) might affect the kinetics of TCR Vβ expression, and analyzed early SE effects on peripheral T cells. Vβ 5.2/5.3 and Vβ 8 (but not Vβ 6.7) determinants were drastically reduced after an 8-hr exposure to SED at 37°C (but not at 4°C), while SEE affected Vβ 8 but not Vβ 5.2/5.3 (not shown). Remarkably, Vβ expression was not restored when toxin-pulsed PBMC were washed and cultured (without exogenous lymphokines) for 12 hr in fresh medium (Fig. 6 *b* and *d*). Samples taken from the same cultures 60 hr later (72 hr after removal of toxins) revealed a completely different picture (Fig. 6 *c* and *e*): while undetected by Vβ-specific mAbs, Vβ 5.2/5.3 and Vβ 8 peripheral T cells had actually expanded in response to SED (diluting out Vβ 6.7), and Vβ 8 T cells were specifically enriched by SEE stimulation (compare Table 1, which shows similar results in interleukin-2-supplemented cultures). In parallel experiments with thymocytes, chimeric organs were exposed to SE for 72 hr, washed, cultured in the absence of toxins, and analyzed for Vβ expression at various times. As shown in Fig. 7, even 72 hr after removal of toxins (i.e., 144 hr after the initial exposure), both SED- and SEE-treated populations still contained reduced numbers of Vβ 8 cells, and the percentage of Vβ-5.2/5.3-expressing thymocytes after SED treatment was lower than in control cultures (or after exposure to SEE).

DISCUSSION

The present study uses antibody staining and multiparameter flow cytometry to probe the human TCR repertoire expressed by chimeric organ cultures. We find that human thymocytes maintain a diverse TCR repertoire over a 3-week culture period and that thymocyte subpopulations generated *in vitro* show TCR expression patterns similar to those seen *ex vivo* (with the exception of an expanded subset of phenotypically immature CD4 SP thymocytes). Using SEs as Vβ-specific ligands, we show that the expression of individual Vβ families by human thymocytes can indeed be selectively modified. While peripheral T cells expressing toxin-responsive TCR Vβ families proliferated in response to SE (refs. 9 and 10; Table 1), SE-exposed human thymocytes showed decreased viability and selective loss of corresponding TCR Vβ families, more pronounced for Vβ 5.2/5.3 than for Vβ 8. These changes were accompanied by oligonucleosomal DNA fragmentation, characteristic of apoptosis (30). This result is in agreement with earlier studies, in which CD3 mAbs or stimulatory combinations of CD2 reagents induced death and oligonucleosomal DNA fragmentation in human thymocyte cultures (17), and it suggests that apoptosis contributes to the Vβ-selective deletion of human thymocytes by SE.

In the murine system, similar experiments have established apoptosis as an important mediator of negative selection, demonstrating the susceptibility of murine thymocytes (*in vivo* or in organ culture) to deletion by CD3 reagents (24), stimulatory peptides (31), and SEs (7, 8). Death of human thymocytes appears as a relatively protracted process and may occur as late as 72 hr after exposure to mitogenic mAbs (17) or SEs (this study). SE-induced changes in TCR Vβ expression were rapid by comparison (complete after 20 hr). TCR modulation may at least in part account for this discrepancy, since both SED and SEE caused (within <8 hr) the reversible loss of specific Vβ determinants from peripheral T cells. On removal of SEs, peripheral T cells reexpressed

Table 2. Effects of SEs on the TCR Vβ repertoire expressed by human thymocytes in organ culture

Addition to medium	% positively staining cells							
	αβ TCR		Vβ 5.2/5.3		Vβ 8		Vβ 6.7	
	Total	DP	Total	DP	Total	DP	Total	DP
None	38.7 ± 15.6	30.8 ± 14.8	1.6 ± 0.6	1.6 ± 0.7	3.9 ± 1.2	3.3 ± 2.2	3.1 ± 1.6	2.7 ± 1.7
SED	36.0 ± 17.5	31.0 ± 16.8	0.5 ± 0.4*	0.4 ± 0.5*	2.6 ± 1.2	2.6 ± 1.4	3.7 ± 1.2	4.3 ± 1.1
SEE	35.3 ± 17.6	36.8 ± 16.6	1.5 ± 0.8	2.0 ± 1.0	2.2 ± 1.1	2.9 ± 1.5	3.0 ± 2.1	2.9 ± 1.4
SEC-2	35.2 ± 17.4	39.2 ± 14.3	1.8 ± 0.6	2.2 ± 0.7	4.1 ± 1.6	5.6 ± 0.3	3.5 ± 1.5	3.9 ± 0.9

Values represent percentages of positively staining cells with the listed reagents among total or DP thymocyte populations, including small thymocytes as well as blasts (mean ± SD of 13 experiments with SED and SEE and 7 experiments with SEC-2). *, $P < 0.0005$ by paired Student's *t* test.

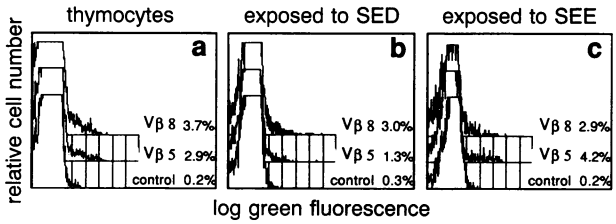

FIG. 7. TCR Vβ expression by thymocytes 6 days after exposure to SED or SEE. Chimeric organ cultures were exposed for 72 hr to SED (*b*) or SEE (*c*) at 1 μg/ml, washed, and cultured in fresh medium without toxin. Cell suspensions were prepared 72 hr later, and the analysis of Vβ expression included small as well as large thymocytes.

(with a lag period of >12 hr) toxin-responsive Vβ determinants at increased frequencies: SE-reactive peripheral T cells had expanded while undetectable by Vβ-specific mAbs. Whereas the early SE effects on TCR Vβ expression were similar for peripheral T cells and thymocytes, the frequencies of thymocytes expressing SE-reactive Vβ determinants remained below control levels even as late as 72 hr after the removal of toxins (144 hr after exposure). Residual Vβ 5.2/5.3 thymocytes 6 days after SED exposure were mainly found among the SP subsets (unpublished observation). This might be due to the selective survival, and possibly the limited expansion, of functionally mature thymocytes, consistent with the reported resistance of SP populations to clonal deletion in some murine systems (24, 31). However, since we do not know the developmental stage at which these cells first encountered SED, our observations allow no firm conclusions regarding the relative sensitivity of human thymocyte subpopulations to clonal deletion. The SE-induced modulation of Vβ determinant expression by peripheral T cells (described above) complicates estimates of the initial thymocyte deletion caused by SE, and it provides a cautionary note regarding experimental systems in which TCR Vβ determinants serve both as a target for experimental manipulation and as a means to identify cells.

The levels of TCR/CD3 expression by thymocytes are normally indicative of maturation stages, reflecting cellular signaling (32) and repertoire selection in the thymus (33). Occupancy of the CD3/TCR complex or other structures that mediate thymocyte/stromal cell interactions can disrupt thymocyte development, causing deletion or developmental arrest (24, 31, 34, 35). Experimental TCR modulation profoundly affects T-cell function, as it accompanies the induction of specific unresponsiveness (anergy) by antigen (36) and SE (11) in human T-cell clones, and it may mediate T-cell tolerance *in vivo* in mice transgenic for extrathymic MHC molecules (37).

While the relative contribution of human and murine MHC products to TCR repertoire selection in chimeric organ culture remains to be established, we have shown that human thymocyte populations developing in organ culture express a broad TCR repertoire that selectively responds to Vβ-specific stimuli. Thymic organ culture may provide a powerful experimental approach to define *in vitro* the forces capable of shaping the human T-cell repertoire.

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