INFECTION AND IMMUNITY, Mar. 1996, p. 987–994 Vol. 64, No. 3
0019-9567/96/$04.00+0

Reactivity of Mouse T-Cell Hybridomas Expressing Human Vβ Gene Segments with Staphylococcal and Streptococcal Superantigens

BERNHARD FLEISCHER,[1]* ANTJE NECKER,[2] CORINNE LEGET,[2] BERNARD MALISSEN,[3] AND FRANÇOIS ROMAGNÉ[2]*

Bernhard Nocht Institute for Tropical Medicine, D-20359 Hamburg, Germany,[1] Immunotech S.A., 13276 Marseille,[2] and Centre d'Immunologie de Marseille Luminy, 13009 Marseille,[3] France

Received 19 May 1995/Returned for modification 10 July 1995/Accepted 18 December 1995

A panel of 15 mouse T-cell hybridomas, each expressing a different human Vβ gene segment (hVβ) in an otherwise mouse T-cell receptor (i.e., mouse α chain and CD3 complex), was constructed by transfection of hVβ/mouse Cβ chimeric T-cell receptor (TCR)-β genes into a mouse T-cell hybridoma recipient lacking the endogenous TCR-β chain. Several qualities that are conferred by the hVβ chain of the TCR are retained in the chimeric human-mouse TCR complex: a large panel of hVβ-specific antibodies specifically stained the hVβ expressed by the mouse T-cell hybridomas. Moreover, hVβ-transfected mouse cells could readily produce interleukin 2 when stimulated by superantigens presented by antigen-presenting cells. These characteristics made it possible to refine the reactivity of 17 superantigen preparations with the available transfected Vβs. Each superantigen gave a characteristic pattern of reactivity on the transfectants. Positive reactivities with some of these transfectants, which differ only by the expressed hVβ, demonstrate unambiguously the superantigenic character of a protein or fraction and its potential to react with the corresponding Vβs. Therefore, these hVβ-transfected cells constituted a valuable tool for determining "specificity fingerprints" of known or putative superantigens. First, commonly used, commercially available superantigens such as staphylococcal enterotoxin B and toxic shock syndrome toxin-1 (TSST-1) showed additional Vβ reactivities, compared with those of their recombinant counterparts. This stresses the importance of using defined preparations of superantigens for the definition of Vβ specificities. Second, the stimulatory pattern of a strain of *Streptococcus pyogenes* demonstrated that this strain, unlike others, produces a potent Vβ8-specific superantigen that is as yet undefined at the molecular level.

"Superantigen" is the collective term used to denote a heterogeneous set of molecules produced by infectious pathogens and endowed with an extremely potent mitogenic effect on T lymphocytes (11, 18, 24). Toxic shock syndrome toxin-1 (TSST-1) and the staphylococcal enterotoxins (SEs) constitute prototypic superantigens, the molecular mechanism of action of which has been investigated extensively. According to a currently accepted model, superantigens are bivalent molecules which, by binding simultaneously to major histocompatibility complex (MHC) class II molecules and to variable segments (Vβ or Vγ) of the T-cell antigen receptor (TCR) molecules, subsequently bring about T-cell activation and the expansion of T-cell populations carrying certain Vβs (13, 18, 24). The Vβ reactivity profile of the different toxins identified to date is based on stimulation of either bulk or cloned T cells (6, 19, 23). In bulk culture, expansion of T-cell populations carrying Vβs can be monitored by quantitative PCRs (6) or by the use of monoclonal antibodies (MAbs) specific for Vβ chains (19). The former approach requires cumbersome calibration steps to obtain reliable, quantitative results, whereas the latter is still hampered by the few anti-human Vβ (anti-hVβ) antibodies presently available. Moreover, when PCR is used, reactivity for a given superantigen may vary even within closely related family members that are not differentiated reliably even via carefully chosen oligonucleotides. Studies of individual T-cell lines with defined Vβs showed that reactivity to a given toxin is also found with T-cell populations that are not expanded preferentially in bulk culture, presumably because of a differential affinity of superantigens for Vβs (12, 16). Such studies are generally performed with human T-cell clones which have certain experimental limitations; e.g., maintenance of a large panel of untransformed T-cell clones is difficult, the activation and functional states of the clones are difficult to control, and, finally, superantigens, in addition to their capacity to induce T-cell activation restricted by the TCR-β chain variable region, can deliver activation signals to human T-cell clones via their MHC class II molecules (31).

To design a more flexible experimental model, we have constructed a panel of 15 mouse T-cell hybridoma transfectants, each of which expresses a different hVβ. These cells were tested for their reactivity with either highly purified or recombinant toxins. This extensive study allowed us to refine the patterns of reactivity of 17 toxin preparations on the 15 available transfected Vβs.

Moreover, by using such a panel of murine T-cell lines expressing hVβs, we could show that many of the commercially available staphylococcal enterotoxin preparations seem to be contaminated by other superantigens. Finally, we showed that this system is valuable for the detection of new superantigens, such as the ones produced by different strains of *Streptococcus pyogenes.*

* Corresponding author. Mailing address for François Romagné: Immunotech S.A., 130 Av. De Lattre de Tassigny, 13276 Marseille Cedex 9, France. Mailing address for Bernhard Fleischer: Bernhard Nocht Institute for Tropical Medicine, Bernhard Nocht Strasse 74, D-20359 Hamburg, Germany.

MATERIALS AND METHODS

Nomenclature. It is important to note that the system for numbering of subfamilies and subfamily members used in this paper is that of Wei et al. (37). The

TABLE 1. Origins of the cDNAs of the hVβs used for the transfection experiments

Specificity[a]	Sequence (5′ to 3′) of sense primer for cDNA derived by PCR from RNA and source of other sequences[b]	cDNA with published matching sequence
Vβ1.1	TAGAATTCATGGGCTTCAGGCTCCTC	HBVT73
Vβ2.1a	TAGAATTCATGCTGCTGCTTCTGCTG	TCRBV2S1*2 (9)
Vβ3.1	TAGAATTCATGGGAATCAGGCTCCTC	PL4.4
Vβ5.1	TAGAATTCATGGGCTCCAGGCTGCTCTGTT	HBVP51
Vβ6.1	Cloned cDNA (15)	HBVP50
Vβ8.1	From Jurkat mRNA: TAGAATTCATGGACTCCTGGACCTTC	YT35
Vβ9.1	TAGAATTCATGGGCTGCAGGCTCCTCTGCTGTG	PL2.6
Vβ12.2	TAGAATTCGAAATGGGCACAAGGTTGTTCTTC	Vβ12.2 (28)
Vβ13.1	GGGAATTCAAGATGGCCATCGGCCTCCTGTGCTGTGC	HBVP34
Vβ13.3	TAGAATTCGCACCTGCCATGAGCATCGGGCTCCTG	IGRb14
Vβ14.1	GCGAATTCCACCATGCTGAGTCCTCTGCTCCTTC	PH21
Vβ16.1	GCGAATTCCACCATGGTTTCCAGGCTTCTCAGTT	HT370
Vβ17.1	TAGAATTCTTGAGCAACCAGGTGCTC	HBVT02
Vβ21.3	Cloned cDNA (15)	IGRb01
Vβ22.1	Cloned cDNA (15)	IGRb03

[a] Nomenclature for Vβ is as defined in reference 37, where references for the HBVT, PL, PH, HBVP, YT35, HT370, and IgR sequences can be found.
[b] Unless otherwise stated, the cDNAs were obtained by PCR from RNA of peripheral blood lymphocytes by the sense primers indicated, each one annealing to the leader sequence of the cognate Vβ, and a common antisense primer hybridizing at the beginning of hCβ. The sequences of the cloned PCR products and other cDNAs matched the indicated published sequence exactly.

more common Vβi.j. designation is kept instead of the official World Health Organization denomination TCRBViSj.

Constitution of a panel of mouse T-cell hybridoma transfectants expressing distinct hVβs. The full-length hVβ genes were obtained either from available cloned cDNA (e.g., Vβ6.1, Vβ21.3, and Vβ22 described in reference 15) or from PCR-amplified products obtained from RNA of peripheral blood lymphocytes or T-cell lines (Table 1). The hVβs were subsequently linked to a mouse Cβ2 (mCβ2) gene and transfected into the DOIS19 cell line, a β chain-loss variant of the mouse DO.11.10 T-cell hybridoma (22).

PCR experiments and chimeric hVβ/mCβ gene construction. Total RNA was prepared from phytohemagglutinin-stimulated blasts or T-cell lines by the guanidium isothiocyanate method (27). cDNA was prepared from 1 μg of RNA with reverse transcriptase (Life Technologies Inc., Gaithersburg, Md.) according to the manufacturer's instructions and subjected to PCR amplification as described earlier (26). A set of sense primers specific for Vβ gene leader sequences (Table 1) was used in combination with the single antisense primer 5′GGGAGAT CTCTGCTTCTGATGG3′, specific for both hCβ1 and hCβ2 genes, to amplify the different hVβ genes as well as a short portion of Cβ (codons 1 to 22). Each sense primer contained an *Eco*RI site for cloning and the ATG initiating codon, whereas the single antisense primer straddles the unique *Bgl*II site present in both the hCβ1 and hCβ2 genes. After digestion with *Eco*RI and *Bgl*II, each amplification product was cloned in front of codons 23 to 173 of mCβ2 by ligation into the *Eco*RI- and *Bgl*II-digested pBSCβm15 plasmid (26) to reconstitute a complete chimeric TCR-β transcription unit. Several of the resulting clones were sequenced with the U.S. Biochemical Corp. (Cleveland, Ohio) Sequenase kit. Clones with Vβ gene sequence exactly matching previously published sequences were selected (Table 1). A sequence with in-frame Dβ, Jβ was picked randomly from each pool of hVβ-containing clones for the subsequent cloning steps. Vβ8.1 was amplified from mRNA from Jurkat cells, and the sequence of the Dβ, Jβ region was identical to the YT35 sequence, published previously for the β chain gene of this cell line.

Transfection of the hVβ/mCβ chimeric genes. The strategy used for the expression of the hVβ/mCβ genes was essentially the same as that described earlier with the pHβPr-1neo plasmid as an expression vector (26), except that we used here a new expression vector, NT, which was substantially more efficient for driving the expression of the chimeric genes. This vector was constructed for the stable expression of genes under the SRα promoter (32). Briefly, the *Sal*I fragment, containing the SRα promoter of pcDL-SRα296 (32), was cloned into the *Sal*I-linearized PSV2-neo plasmid (30). The plasmid NT was chosen for the following manipulations, in which the neomycin resistance gene and the cloned gene under the SRα promoter had the same direction of transcription. The different chimeric β chain genes were subcloned into the NT expression vector and electroporated into the DOIS19 cell line. To enhance surface TCR expression, the cells were cotransfected with the mCD3-ζ chain gene under the control of the β-actin promoter (36). This led to a 5 to 10 times higher level of expression of the TCR on the cell surface compared with transfection with the TCR-β gene only. Cells were selected and maintained in Dulbecco's modified Eagle's medium containing 10% fetal calf serum in the presence of 2 mg of G418 per ml. Surface expression of the TCR was monitored by MAbs specific for the TCR complex (see below).

Fluorescence-activated cell sorter analysis and MAbs. 145-2C11 (2C11) is an anti-mCD3 ε (21). Several anti-hVβ-specific antibodies were generated with the following transfected cells as immunogens: Vβ2 (E22E7.2), Vβ5.1 (Immu157), Vβ6.1 (CRI304.3), Vβ9.1 (Fin9), Vβ12.2 (Ver2.32), Vβ14 (Cas1.1.3), Vβ16 (Tamaya1.2), Vβ17 (E175F3), Vβ21.3 (Ig125), and Vβ22 (Immu546). These antibodies are available from Immunotech (Marseilles, France). Other antibodies raised against human T-cell clones or lines and specific for Vβ2 (MPB2/D5) (7), Vβ3 (JOVI3) (35), Vβ12 (S511) (2), Vβ17 (C1) (14), Vβ8 (56C5; Immunotech), and Vβ13 (JU74; Immunotech) were also used in this study. JU74 was originally described as an anti-Vβ13.6-specific antibody (10), but we showed during this study that it also recognized the closely related subfamily member Vβ13.3. For staining, 10^5 cells were incubated for 30 min at 4°C with primary anti-TCR MAb, and after being washed, the cells were then incubated for 30 min at 4°C with polyclonal goat anti-mouse $F(ab')_2$ fragments coupled to phycoerythrin (GAMIG-PE; Immunotech). Cells were analyzed with a FACScan flow cytometer (Becton Dickinson, Mountain View, Calif.).

Cell lines. The Burkitt's lymphoma line BJAB (HLA-DR2 and DR5), the Raji line (HLA-DR3 and -DR6) and its selectively HLA class II-deficient mutant RJ.225, and the mouse B-cell lymphoma A20/J and its HLA-DR3-transfected derivative A20D45 (the gift of U. Haemmerling, Heidelberg, Germany) were used as antigen-presenting cells (APCs). Jurkat cells, an $hV\beta8^+$ interleukin 2 (IL-2)-producing T-cell line, were obtained from S. Carrel, Lausanne, Switzerland. The Jurkat line was 99% positive by fluorescence-activated cell sorter analysis with the anti-Vβ8 antibody 56C5. The murine T-cell hybridoma 3D054.8, specific for ovalbumin and IA^d, was obtained from R. McDonald, Lausanne, Switzerland.

Superantigens. The SEs SEA, SEC2, SEC3, SED, SEE, and TSST-1 were purchased from Toxin Technology Inc. (Madison, Wis.). SEB was produced as a recombinant molecule (recSEB) in *Staphylococcus aureus* (12). SEE and the streptococcal pyrogenic exotoxins A and C (SPEA and SPEC, respectively) were produced as recombinant molecules in *Escherichia coli* (4). recSEA produced in *E. coli* was the gift of Michael Dohlsten, Kabi-Pharmacia Inc., Lund, Sweden. recTSST-1 produced in *S. aureus* (3) was the kind gift of P. Bonventre, Department of Microbiology, University of Cincinnati, Cincinnati, Ohio. Three *S. pyogenes* strains, which were isolated from patients with scarlet fever and which did not contain the genes for SPEA and SPEC, were obtained from J. J. Ferretti, University of Oklahoma, Norman, Okla. The type-specific antigens of strains 86, 116, and 391 were M−, T4, M12, T12, and M22,T− respectively. Supernatants of *Mycoplasma arthritidis* were used as a source of the *M. arthritidis* mitogen (MAM). They were dialyzed against cell culture medium and used at dilutions of 1:100 to 1:400.

T-cell stimulation assay. Stimulation of the T-cell hybridoma transfectants was done in triplicate 200-μl microcultures containing 5×10^4 T cells and 2.5×10^4 accessory cells, as described previously (4). The amount of IL-2 produced by the responding T-cell hybridomas was determined in an IL-2 bioassay with CTLL-2 cells and measured by [^{3}H]thymidine uptake after 24 h for at least 6 h. Data are given as the means of triplicate experiments. Standard errors of the mean were always below 10% (except for low values).

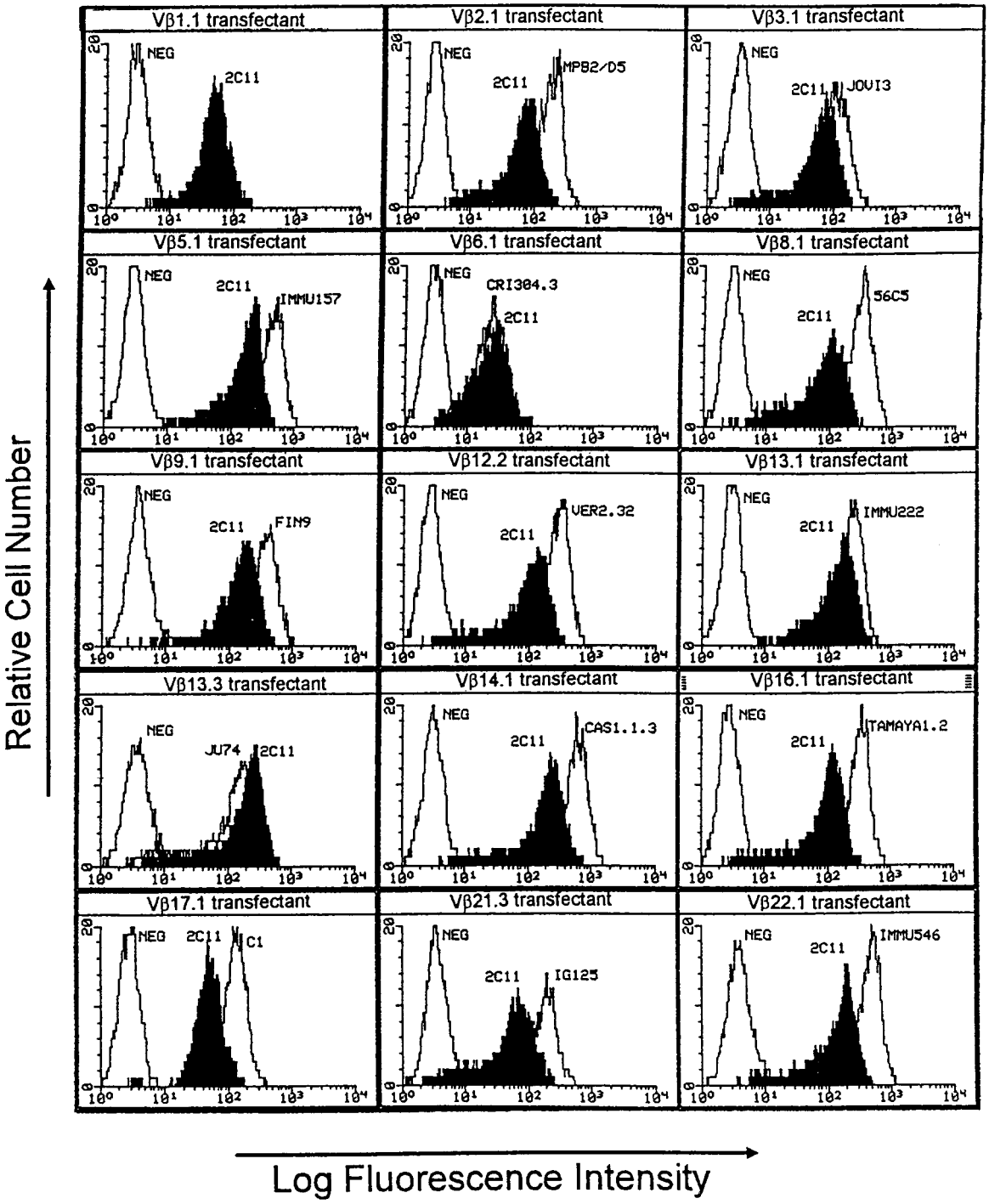

FIG. 1. Staining pattern of the mouse cell lines transfected with different hVβs. The anti-CD3 ε MAb 2C11 (black histograms) demonstrates the reconstitution of TCR-CD3 complexes at the surface of the mouse TCR-β⁻ T-cell hybridoma DOIS19, used as recipient cells, after transfer of the indicated chimeric hVβ/mCβ2 cDNAs. A panel of anti-Vβ MAbs, each specific for one of the transfected hVβs, except for hVβ1, was also used to stain the various transfectants. As expected, each antibody (name of each antibody is given by its histogram) specifically stained its corresponding Vβ-transfected cells and gave a background signal with all of the other transfectants (not shown). The histograms are compared with that of a negative control (NEG) obtained after staining of each of the cell lines with an irrelevant anti-human CD25 MAb (Immunotech). The signal given by a particular anti-Vβ on its specific transfectant was generally brighter than the one obtained with 2C11. This is likely to result from the higher affinity of the GAMIG-PE secondary reagent for the mouse antibodies (which was the case with all anti-hVβ-specific MAbs) than that for the hamster antibody (which was the case with 2C11).

RESULTS

Expression of 15 distinct hVβ gene products in mouse DOIS19 T cells. Fifteen hVβ/mCβ chimeric cDNAs were transfected individually into the mouse TCR-β-negative T-cell hybridoma DOIS19. As shown in Fig. 1, the DOIS19 cells stably transfected with the chimeric genes containing hVβ1.1, hVβ2.1, hVβ3.1, hVβ5.1, hVβ6.1, hVβ8.1, hVβ9.1, hVβ12.2, hVβ13.1, hVβ13.3, hVβ14.1, hVβ16.1, hVβ17.1, hVβ21.3, and

hVβ22.1 (nomenclature according to reference 37) were clearly stained by the anti-mCD3 ε MAb 2C11. As expected, no signal above background was found for DOIS19 nontransfected cells. The levels of TCR expression of the transfectants can be compared by using the signal given by the 2C11 antibody. The level of expression was a function of the transfected β-chain and varied by a factor of 10 between the weakest, Vβ6.1 (mean fluorescence of 25), and the strongest, Vβ13.3 (mean fluorescence of 250), TCR expressors.

Staining of the transfected cells with Vβ-specific reagents. A panel of MAbs specific for hVβs, was also used to specifically monitor TCR expression in the various transfectants. The pattern of staining with these specific anti-hVβ MAbs is shown in Fig. 1, except for that of hVβ1, for which no antibody is presently available. All of these anti-Vβ MAbs stained only the cells transfected with the corresponding Vβ and gave only background staining with all of the other transfected cells. We showed previously that antibodies raised against Vβ2 and Vβ17 transfectants specifically recognized human T cells carrying the corresponding Vβ (26). This original observation has been expanded to nine other new MAbs (see Materials and Methods). Conversely, all of the antibodies raised against human T-cell clones or lines that we tested, Vβ2 (MPB2/D5), Vβ3 (JOVI3), Vβ8 (56C5), Vβ12.2 (S511 [data not shown]), Vβ13.3 and Vβ13.6 (JU74), and Vβ17 (C1), stained their corresponding Vβ-transfected cell lines. Taken together, these results strongly suggest that the conformation of the hVβs expressed at the surface of the mouse cell in the otherwise mouse TCR-CD3 complex is similar to that in the fully human TCR-CD3 complex.

Expression of the hVβ8.1 is sufficient to confer specificity for superantigenic toxins. Vβ8.1$^+$ human cell line Jurkat cells and Vβ8.1-transfected mouse cells were compared for their responses to different toxins presented by Raji cells (Fig. 2). Both cell lines responded to SEA, SED, and SEE but not to TSST-1, SEB, SPEA, or SPEC. There was no response to any superantigen when the class II-negative RJ.225 cells were used as APCs (data not shown). SEE was the most potent toxin to elicit a response in the two cell lines, followed by SED and SEA in that order. It is evident from Fig. 2, that the relative patterns of responses to the different toxins were identical in the two cell lines. However, Jurkat cells responded to all toxins at approximately 30-fold lower concentrations than the hVβ8.1-transfected mouse cell line. Thus, the specificity of the response was determined by Vβ, whereas the sensitivity of the response was determined, for a given APC, by the responder cell. We therefore tested the responses of the two cell lines with different APCs. As shown in Fig. 3, Jurkat cells did not respond to SEE when presented by the murine B-cell line A20/J and showed only a marginal response to SEE presented by the HLA-DR3-transfected A20D45 cells. In contrast, the hVβ8.1-transfected mouse cells showed a good response to A20D45 cells with SEE but only a low-level response to nontransfected A20/J cells. This indicates that the human class II molecules (HLA-DR, -DQ, and -DP) are much more efficient in presenting SEE than the H-2^d class II molecules (IE and IA) expressed by the A20 cell line. Moreover, it suggests that additional species-specific interactions may contribute in an important way to the triggering induced by SEE when presented by A20D45 cells.

Specificity of Vβ transfectants for microbial superantigens. Finally, we tested the Vβ-transfected cells for their reactivity with a panel of staphylococcal and streptococcal superantigens. We used commercially available toxin preparations as well as a number of toxins produced as recombinant proteins in *E. coli* and *S. aureus*. A first screening was done with concentrations

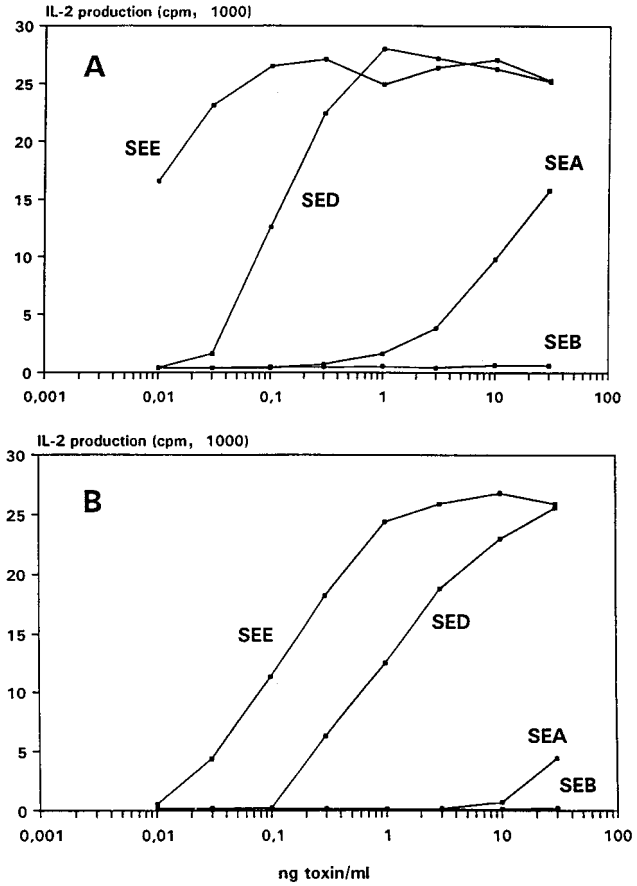

FIG. 2. Expression of the hVβ8.1 segment confers specific reactivity to bacterial toxins in both a mouse and a human TCR-CD3 complex. The IL-2 produced by Jurkat cells (A), expressing hVβ8.1 in the fully human TCR-CD3 complex, and the hVβ8.1 DOIS19-transfected cells (B), expressing hVβ8.1 in the chimeric hVβ/otherwise mouse TCR-CD3 complex, in response to recSEA, recSEB, SED, and recSEE presented by Raji cells was measured by incorporation of [^{3}H]thymidine in CTLL-2 cells. Maximal proliferation of CTLL-2 cells in the presence of saturating concentrations of IL-2 gave an incorporation of 54,000 cpm. The figure shows typical results from three independent experiments.

of 100 ng of toxins per ml or a 1/200 dilution of supernatants (see, for example, Table 3). A dose-response curve was then determined for each toxin preparation which showed positivity with a given transfectant, as illustrated in Fig. 2 and 3. The results of this screening are summarized in Table 2. These data are the synthesis of results from many different experiments (at least three independent experiments) in which each T-cell line was tested against the different toxins. The results have been collected by grouping the responses into the categories strong, intermediate, weak, and negative. Several conclusions can be drawn from this series of experiments. Each transfectant has its own specific pattern of response with different toxins. For Vβ6.1, Vβ13.1, and Vβ13.3 transfectants, no reactivity with any of the toxins used could be detected, despite their ability to produce IL-2 upon stimulation with phytohemagglutinin or 2C11 antibody (data not shown). The patterns of reactivity observed with different Vβs for a given toxin are consistent with what had been described previously with superantigen stimulation of bulk T cells. For example, SEB has been reported to elicit the proliferation of Vβ3, Vβ12, Vβ15, Vβ17, and Vβ20 populations (6). The results obtained with the panel of Vβ-transfected cells confirm this pattern of reactivity for the

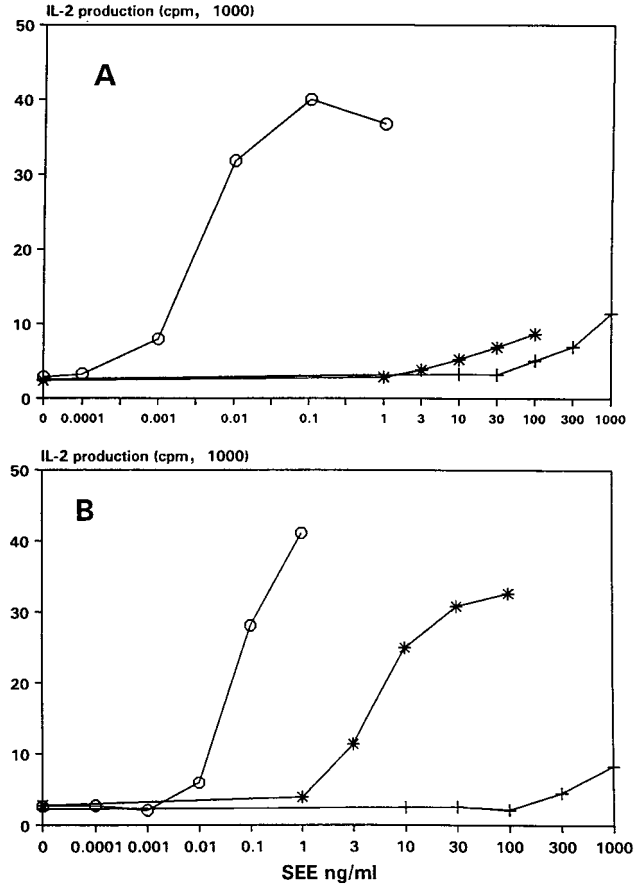

FIG. 3. IL-2 production by Jurkat cells (A) and the hVβ8.1 DOIS19 transfectant (B) in response to recSEE presented by different MHC class II-positive cells: Raji cells (○), nontransfected A20/J cells (+), and A20D45 cells (*), a DR3-transfected A20/J cell line. Typical results from three independent experiments are shown.

available hVβ-transfected hybridomas. The reactivity of TSST-1 (Vβ2) was also confirmed. For SEE, a reactivity was found with Vβ21.3 in addition to the one with Vβ5.1 and Vβ8.1 described previously (6). The results obtained with SEC2 were conflicting, because only reactivity with the Vβ14 transfectant was found and no reactivity with Vβ12 and Vβ13.1 was found, as originally reported (6). It should be noted that a similar mouse T-cell hybridoma transfected with Vβ13.1 also failed to respond to this toxin, whereas a transfected Vβ13.2 cell line did respond (5). Recently, Abe et al. investigated the response of human T cells to SPEA. They found a proliferation of Vβ8, Vβ12, and Vβ14 populations (1). In contrast, Tomai et al. (33) reported that SPEA stimulates Vβ2, Vβ12, Vβ14, and Vβ15. Using recSPEA on the panel of transfectants, we confirmed only the Vβ12 and Vβ14 stimulation and found an additional slight reactivity with Vβ9. The latter publication also reported the pattern of stimulation triggered by SPEC, in which Tomai et al. (33) found a reactivity of Vβ1, Vβ2, Vβ5.1, and Vβ10 T-cell populations. Vβ10 is very likely to be a nonfunctional pseudogene (HUMTCRB GenBank sequence, accession number L36092), which would stress the limits of the PCR-based data. Using recSPEC on the transfectants, we only confirmed the reactivity of SPEC with Vβ2. MAM has been reported to induce the proliferation of at least Vβ17- and Vβ3-carrying cells in bulk culture (14). MAM was the only defined superantigen which failed to trigger a response in any of the transfectants used here (see Discussion). We used the murine hybridoma 3DO54.8 as a positive control, showing that our MAM preparation in fact contained active superantigen. As shown in Table 3, 1:200 dilution of the supernatant was sufficient to obtain maximal response from this hybridoma. Our MAM preparation was also highly mitogenic for BALB/c spleen cells (not shown).

Comparison between toxin preparation from *S. aureus* and recombinant toxins. The patterns of reactivity differed between certain commercial toxins prepared from *S. aureus* strains (SE) and "the same" toxins produced as recombinant proteins (recSE). A previous study suggested that SEB may not have the same Vβ profile as that of recSEB (17). We refined and extended this observation. The SEB preparation was a

TABLE 2. Reactivity of Vβ transfectants with different pyrogenic exotoxin superantigens

Toxin	Reactivity with[a]:														
	Vβ1	Vβ2	Vβ3	Vβ5.1	Vβ6.1	Vβ8.1	Vβ9	Vβ12.2	Vβ13.1	Vβ13.3	Vβ14	Vβ16	Vβ17.1	Vβ21.3	Vβ22
SEA	++	−	−	+	−	+	+++	−	−	−	−	+++	−	−	++
recSEA	++	−	−	+	−	+	+++	−	−	−	−	+++	−	−	++
SEB	+++	−	+++	−	−	−	+++	++	−	−	+++	−	+++	−	−
recSEB	−	−	+++	−	−	−	−	++	−	−	+++	−	+++	−	−
SEC2	−	−	−	−	−	−	−	−	−	−	+	−	−	−	−
SEC3	−	−	−	+	−	−	−	−	−	−	++	−	−	++	−
SED	+++	−	−	−	−	+++	++	−	−	−	−	−	−	++	−
SEE	−	−	−	+++	−	+++	−	−	−	−	−	−	−	+++	−
recSEE	−	−	−	+++	−	+++	−	−	−	−	−	−	−	++	−
TSST-1	−	+++	−	+++	−	−	−	−	−	−	−	−	−	+	−
recTSST-1	−	+++	−	−	−	−	−	−	−	−	−	−	−	−	−
recSPEA	−	−	−	−	−	−	+	++	−	−	+++	−	−	−	−
recSPEC	−	+++	−	−	−	−	−	−	−	−	−	−	−	−	−
86	++	−	−	−	−	−	−	−	−	−	−	−	−	−	−
116	−	−	−	−	−	+++	−	−	−	−	−	−	−	−	−
391	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−
MAS	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−

[a] Transfected hybridomas were tested for their proliferative response against several concentrations of superantigens in the presence of Raji cells as presenting cells. Each toxin was tested in concentrations of 1, 10, 100, and 1,000 ng/ml. −, no response at the highest concentrations; +, 2- to 10-fold stimulation at 100 ng/ml; ++, 10- to 50-fold stimulation at 100 ng/ml; +++, >50-fold stimulation at 100 ng/ml. Also tested were supernatants of three *S. pyogenes* strains (86, 116, and 391) and supernatants from *M. arthritidis* cultures (MAS) at a 1:200 dilution.

TABLE 3. Reactivity of Vβ transfectants with MAM[a]

Superantigen	Reactivity (cpm) with MAM[b]						
	Vβ1	Vβ2	Vβ8.1	Vβ9	Vβ12.2	Vβ22	3DO54.8
Medium	240	230	314	248	288	215	223
MAM	268	206	316	240	208	320	25,000
recSEA	23,000	nt	nt	23,200	nt	20,700	21,100
recSEE	nt	nt	27,200	nt	nt	nt	nt
recSPEA	nt	nt	nt	nt	18,600	nt	21,600
recSPEC	298	27,000	nt	nt	nt	206	nt

[a] Transfected hybridomas and the ovalbumin-IA^d-specific murine T-cell hybridoma 3DO54.8 as a positive control were tested for their proliferative responses against MAM and the toxins indicated in the presence of Raji cells as presenting cells. Each toxin was tested at a concentration of 100 ng/ml; MAM was used as a 1:200 dilution of supernatant from *M. arthritidis* cultures. This preparation of MAM was highly mitogenic for mouse spleen cells and gave a small but significant response on human peripheral blood mononuclear cells, as described previously (12).

[b] Results are given as means of triplicate experiments in counts per minute incorporated by CTLL-2 cells. nt, not tested.

potent stimulant for Vβ1 and Vβ9 transfectants. This was not the case for recSEB. Similarly, TSST-1 stimulated Vβ5.1 and Vβ2 transfectants, whereas recTSST-1 stimulated only the Vβ2 transfectant.

Patterns of reactivity of different strains of *S. pyogenes*. A panel of *S. pyogenes* strains was tested for the production of superantigens with the transfectants. The patterns of reactivity of three strains are included in Table 2. These bacteria did not contain the genes for SPEA and SPEC but secreted a mitogen or mitogens that stimulate peripheral blood mononuclear cells (data not shown). As can be seen from Table 2, one of the strains, 86, contained a superantigen stimulating Vβ1$^+$ cells, whereas supernatants of strain 116 selectively stimulated Vβ8$^+$ cells. There was no other known streptococcal superantigen that had an identical pattern of reactivity.

DISCUSSION

S. aureus toxins are responsible for a significant percentage of all food poisonings and are also associated with severe shock. The pyrogenic, erythrogenic toxins are major determinants of the pathogenicity of group A streptococci. Moreover, superantigens have been suspected to play a role in important human diseases such as rheumatoid arthritis (25), diabetes (8), rabies (20), or even human immunodeficiency virus infection, although most of these results await confirmation. The stimulation of a sizeable fraction of T cells bearing a particular Vβ or Vβs is thought to be the most important mechanism of pathogenicity, and determination of the Vβ specificities of the toxins is essential to understand and counteract their actions. We present here a flexible experimental system with which to study the reactivity of a protein or fraction suspected to be superantigenic. The system relies on the stimulation of a panel of mouse T-cell hybridomas transfected with hVβs. Upon presentation by APCs of superantigens specific for a transfected Vβ, the cell lines readily produce measurable amounts of IL-2. Choi et al. (5) and we (4, 26) have illustrated the utility of this system by using a very limited panel of such transfected cells expressing hVβ2, hVβ13.1, and hVβ13.2. As documented in this paper, we have extended this approach to 13 additional hVβs. Moreover, during the course of this study, we have constructed five additional transfectants expressing hVβ4, hVβ6.5, the functional allele of hVβ20, hVβ23, and hVβ24. Therefore, the TCR-β chain-loss variant DOIS19 constitutes a very permissive recipient for the expression of chimeric hVβ/mCβ polypeptides, which, to date, has been only refractory to the expression of hVβ15. The cell lines described here offer all of the advantages of mouse T-cell hybridomas in that they are easy to grow in large quantities and display levels of TCR expression which are stable for several weeks of continuous culture. Moreover, the sensitivity reached by such cell lines in detecting superantigenic activity is several orders of magnitude greater than that of any biochemical or serological test. These advantages allowed us to test in a parallel manner most of the superantigens described to date. Our findings are on the whole consistent and in some cases extend the described specificities of these toxins for Vβs.

Some important discrepancies were also detected. For example, MAM has been shown clearly to promote the expansion of at least Vβ17- and Vβ3-bearing T cells in bulk culture, but MAM failed to trigger our Vβ17 transfectant. This was not due to the inability of this transfectant to respond, because it produced IL-2 upon stimulation with SEB, nor was it due to our preparation of MAM, which stimulated a mouse T-cell hybridoma when presented by Raji cells, was highly mitogenic for mouse spleen cells, and gave the usual small, but significant, response on human peripheral blood mononuclear cells (12). No definitive conclusions can be drawn from such negative results in this experimental system. A negative response may be due intrinsically to the fact that the toxin does not bind the expressed Vβ, but we cannot rule out a negative influence of the "mouse context," as discussed below. Comparison of the mouse Vβ8 transfectant with the human Vβ8$^+$ Jurkat cell line for the reactivity to toxins presented by human Raji cells showed that the patterns of reactivity were qualitatively the same but quantitatively different, giving a 30-fold lower sensitivity of the mouse transfectant than that of the human cell line. Our study of the reactivity of these two cell lines to SEE presented by either the mouse A20/J cell line and its HLA-DR3 counterpart or human Raji cells implies that apart from TCR engagement, additional interactions, presumably through adhesion molecules, play an important role in the triggering of the effector cells. This study also suggested that the interactions of the molecules involved are species specific and are retained to a greater degree when the effector cell is of human origin and the APC is of mouse origin, compared with the reverse combination. Besides this impaired sensitivity, the lack of reactivity for a superantigen of a mouse cell line transfected with an hVβ, which potentially reacts with that superantigen, can be explained by additional factors which are not specific to our experimental system. The human Dβ, Jβ region or mouse Vα can play a dominant negative role. Such a transdominant effect has been demonstrated for Vα in mice for the Mls1a superantigen (29, 34). Some superantigens may require MHC molecules not present on our APCs, and certain Vβs may also require special MHC molecules not present on Raji cells (12). All of our experiments were performed with Raji cells as APCs. In experiments not shown here, with cells from another Burkitt's lymphoma line, BJAB, as APCs, the patterns of reactivity of the transfectants did not change. Instead, only the dose-response curve for some toxins changed, indicating that the class II allotype was not a decisive variable in our system.

However, given the fact that all of the cell lines differ only by the transfected β-chain and that the chimeric TCR is very unlikely to react with peptide antigens or in an allogeneic manner, positive signals for given transfected cell lines strongly suggest the superantigenic character of a protein or fraction and its potential to react with the corresponding Vβ(s). Therefore, our Vβ-transfected cells are valuable for determining

"specificity fingerprints" when a superantigen is suspected in infectious agents or diseases and for orienting the research in polyclonal Vβ T-cell expansion. This is exemplified by the stimulatory pattern of *S. pyogenes* 116, which produces a strong Vβ8-stimulating mitogen despite being negative for the genes encoding SPEA and SPEC. None of the known streptococcal superantigens matches this reactivity. Vβ8 stimulation has been reported for SPEB; however, this molecule has been shown not to be a superantigen (4). recSPEB produced in *E. coli* did not stimulate either the Vβ8.1 transfectant or Jurkat cells, nor did it stimulate human peripheral blood mononuclear cells (data not shown).

Moreover, using our panel of cells, we have demonstrated that the patterns of reactivity of two commonly used toxins (SEB and TSST-1) differed significantly from those of their cognate toxins produced as recombinant proteins. These differences may be explained by at least the following two factors. First, the recombinant toxin was a molecular variant carrying a mutation or mutations in a region that determined the Vβ interaction, and second, the preparation derived from *S. aureus* was not pure but contained another superantigen. The attempts to map the reactivity of the different toxins for hVβs must take into account their potency in stimulating T cells at femtomolar to nanomolar concentrations. Even highly purified preparations of toxins, apparently pure by all biochemical criteria, may still contain trace amounts of superantigens sufficient to trigger a functional response (4). The present data stress the importance of using a defined preparation of "standard" superantigens with known nucleotide sequences for the definition of Vβ specificities.

ACKNOWLEDGMENTS

This work was supported in part by Eureka grant EU286 and by BMFT grant 01KI9201.

The expert technical assistance of Lydia Besnardeau and Sabine Herzog is gratefully acknowledged. We thank C. Rocher from Roussel Uclaf, Romainville, France, for providing the Vβ14 and Vβ16 transfectants and H. Rickenberg for critical reading of the manuscript.

REFERENCES

1. **Abe, J., J. Forrester, T. Nakahara, J. A. Lafferty, B. L. Kotzin, and D. Y. M. Leung.** 1991. Selective stimulation of human T cells with streptococcal erythrogenic toxins A and B. J. Immunol. **146:**3747–3750.
2. **Bigler, R. D., D. E. Fisher, C. Y. Wang, E. A. Rinnooy Kan, and H. G. Kunkel.** 1983. Idiotype-like molecules on cells of a human T cell leukemia. J. Exp. Med. **158:**1000–1005.
3. **Bonventre, P. F., H. Heeg, C. Cullen, and C.-J. Lian.** 1993. Toxicity of recombinant toxic shock syndrome toxin 1 and mutant toxins produced by *Staphylococcus aureus* in a rabbit infection model of toxic shock syndrome. Infect. Immun. **61:**793–799.
4. **Braun, M. A., D. Gerlach, U. F. Hartwig, J. H. Ozegowski, F. Romagné, S. Carrel, W. Kohler, and B. Fleischer.** 1993. Stimulation of human T-cells by streptococcal superantigen erythrogenic toxins (scarlet fever toxins). J. Immunol. **150:**2457–2466.
5. **Choi, Y., A. Herman, D. Digiusto, T. Wade, P. Marrack, and J. Kappler.** 1990. Residues of the T cell receptor that interact with *S. aureus* toxin superantigens. Nature (London) **346:**471–473.
6. **Choi, Y., B. Kotzin, L. Herron, J. Callahan, P. Marrack, and J. Kappler.** 1989. Interaction of *Staphylococcus aureus* toxin "superantigens" with human T cells. Proc. Natl. Acad. Sci. USA **86:**8941–8945.
7. **Clarke, G. R., H. Reyburn, F. C. Lancaster, and A. W. Boylston.** 1994. Bimodal distribution of V beta 2+ CD4+ T cells in human peripheral blood. Eur. J. Immunol. **24:**837–842.
8. **Conrad, B., E. Weidmann, G. Trucco, W. A. Rudert, R. Behboo, C. Ricordi, H. Rodriquez-Rilo, D. Finegold, and M. Trucco.** 1994. Evidence for a superantigen involvement in insulin-dependent diabetes mellitus aetiology. Nature (London) **371:**351–355.
9. **Cornelis, F., K. Pile, J. Loveridge, P. Moss, R. Harding, C. Julier, and J. Bell.** 1993. Systematic study of human alpha beta T cell receptor V segments shows allelic variations resulting in a large number of distinct T cell receptors. Eur. J. Immunol. **23:**1277–1283.
10. **Diu, A., F. Romagné, C. Genevée, C. Rocher, J. M. Bruneau, A. David, F. Praz, and T. Hercend.** 1993. Fine specificity of monoclonal antibodies directed at human T cell receptor variable regions: comparison with oligonucleotide driven amplification for evaluation of V beta expression. Eur. J. Immunol. **23:**1422–1429.
11. **Fleischer, B.** 1992. Biological significance of superantigens. Chem. Immunol. **55:**36–64.
12. **Fleischer, B., R. Gerardy-Schahn, B. Metzroth, S. Carrel, D. Gerlach, and W. Kohler.** 1991. A conserved mechanism of T cell stimulation by microbial toxins: evidence for different affinities of T cell receptor toxin interaction. J. Immunol. **146:**11–17.
13. **Fleischer, B., and H. Schrezenmeier.** 1988. T cell stimulation by staphylococcal enterotoxins: clonally variable response and requirement for MHC class II molecules on accessory or target cells. J. Exp. Med. **167:**1697–1708.
14. **Friedman, S. M., M. K. Crow, J. R. Tumang, M. Tumang, Y. Xu, A. S. Hodtsev, B. C. Cole, and D. N. Posnett.** 1991. Characterization of human T cells reactive with the *Mycoplasma arthritidis* derived superantigen (MAM): generation of a monoclonal antibody against V beta 17, the T cell receptor gene product expressed by a large fraction of MAM-reactive human T cells. J. Exp. Med. **174:**891–900.
15. **Giegerich, G., M. Pette, E. Meinl, J. T. Epplen, H. Wekerle, and A. Hinkkanen.** 1992. Diversity of T cell receptor alpha and beta chain genes expressed by human T cells specific for similar myelin basic protein peptide/MHC complexes. Eur. J. Immunol. **22:**753–758.
16. **Goronzy, J. J., U. Oppitz, and C. M. Weyand.** 1992. Clonal heterogeneity of superantigen reactivity in human V beta 6+ T cell clones: limited contribution of V beta sequence polymorphisms. J. Immunol. **148:**604–611.
17. **Herman, A. J., J. W. Kappler, P. Marrack, and A. M. Pullen.** 1991. Superantigens: mechanism of T cell stimulation and role in immune responses. Annu. Rev. Immunol. **9:**745–772.
18. **Janeway, C. A.** 1991. Selective elements for the V beta region of the T cell receptor: Mls and the bacterial toxic mitogens. Adv. Immunol. **50:**1–53.
19. **Kappler, J., B. Kotzin, L. Herron, E. W. Gelfand, R. D. Bigler, A. Boylston, S. Carrel, D. N. Posnett, Y. Choi, and P. Marrack.** 1989. V beta stimulation of human T cells by staphylococcal toxins. Science **244:**811–813.
20. **Lafon, M., M. Lafage, A. Martinez-Arends, R. Ramirez, F. Vuillier, D. Charron, V. Lotteau, and D. Scott-Algara.** 1992. Evidence for a superantigen in humans. Nature (London) **358:**507–510.
21. **Leo, O., M. Foo, D. H. Sachs, L. E. Samelson, and J. A. Bluestone.** 1987. Identification of a monoclonal antibody specific for a murine T3 polypeptide. Proc. Natl. Acad. Sci. USA **84:**1374–1378.
22. **Letourneur, F., and B. Malissen.** 1989. Derivation of a T cell hybridoma variant deprived of functional T cell receptor alpha and beta chain transcripts reveals a nonfunctional alpha mRNA of BW5147 origin. Eur. J. Immunol. **19:**2269–2274.
23. **Lundin, K. E. A., J. E. Brinchmann, and T. Hansen.** 1994. Interactions between staphylococcal superantigens and human T cell clones are predominantly but not exclusively governed by their T cell receptor V beta usage. Scand. J. Immunol. **39:**387–394.
24. **Marrack, P., G. M. Winslow, Y. Choi, M. Scherer, A. Pullen, J. White, and J. W. Kappler.** 1993. The bacterial and mouse mammary tumor virus superantigens; two different families of proteins with the same functions. Immunol. Rev. **131:**79–92.
25. **Paliard, X., S. G. West, J. A. Lafferty, J. R. Clements, J. W. Kappler, P. Marrack, and B. L. Kotzin.** 1991. Evidence for a superantigen in rheumatoid arthritis. Science **253:**325–329.
26. **Romagné, F., L. Besnardeau, and B. Malissen.** 1992. A versatile method to produce antibodies to human T cell receptor V beta segments: frequency determination of human V beta 2+ cells that react with toxic-shock syndrome toxin-1. Eur. J. Immunol. **22:**2749–2752.
27. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
28. **Slightom, J. L., D. R. Siemieniak, L. C. Sieu, B. F. Koop, and L. Hood.** 1994. Nucleotide sequence analysis of 77.7 kb of the human V beta T-cell receptor gene locus: direct primer-walking using cosmid template DNAs. Genomics **20:**149–168.
29. **Smith, H. P., P. Le, D. L. Woodland, and M. A. Blackman.** 1992. T cell receptor alpha chain influences reactivity to Mls1 in V beta 8.1 transgenic mice. J. Immunol. **149:**887–896.
30. **Southern, P. J., and P. Berg.** 1982. Transformation of mammalian cells to antibiotic resistance with a bacterial gene under control of the SV40 early region promoter. J. Mol. Appl. Genet. **1:**327–341.
31. **Spertini, F., H. Spits, and R. S. Geha.** 1991. Staphylococcal enterotoxins deliver activation signals to human T cell clones via major histocompatibility complex class II molecules. Proc. Natl. Acad. Sci. USA **88:**7533–7538.
32. **Takebe, Y., M. Seiki, J.-I. Fujisiwa, P. Hoy, K. Yokota, K.-I. Arai, M. Yokota, and N. Arai.** 1988. SRα promoter: an efficient and versatile mammalian cDNA expression system composed of the simian virus 40 early promoter and the RU-5 segment of the human T-cell leukemia virus 1 long terminal repeat. Mol. Cell. Biol. **8:**466–472.
33. **Tomai, M. A., P. M. Schlievert, and M. Kotb.** 1992. Distinct T-cell receptor

Vβ gene usage by human T lymphocytes stimulated with the streptococcal pyrogenic exotoxins and pep M5 protein. Infect. Immun. **60:**701–705.
34. **Vacchio, M. S., O. Kanagawa, K. Tomonari, and R. J. Hodes.** 1992. Influence of T cell receptor alpha expression on Mlsa superantigen specific T cell responses. J. Exp. Med. **175:**4893–4902.
35. **Viney, L. J., H. M. Prosser, C. R. A. Hewitt, J. R. Lamb, and M. J. Owen.** 1992. Generation of monoclonal antibodies against a human T cell receptor beta chain expressed in transgenic mice. Hybridoma **11:**701–713.
36. **Wegener, A.-M. K., F. Letourneur, A. Hoeveler, T. Brocker, F. Luton, and B. Malissen.** 1992. The T cell receptor CD3 complex is composed of at least two autonomous transduction modules. Cell **68:**83–95.
37. **Wei, S., P. Charmley, M. A. Robinson, and P. Concannon.** 1994. The extent of the human germline T-cell receptor V beta gene segment repertoire. Immunogenetics **40:**27–36.

Editor: J. R. McGhee