Vβ-Specific Stimulation of Human T Cells by Staphylococcal Toxins

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The staphylococcal toxins are responsible for a number of diseases in man and other animals. Many of them have also long been known to be powerful T cell stimulants. They do not, however, stimulate all T cells. On the contrary, each toxin reacts with human T cells bearing particular Vβ sequences as part of their receptors for major histocompatibility complex protein–associated antigen. The specificity of these toxins for Vβs puts them in the recently described class of superantigens and may account for the differential sensitivity of different individuals to the toxic effects of these proteins.

THE STAPHYLOCOCCAL TOXINS ARE A group of proteins secreted by *Staphylococcus aureus* that cause food poisoning or shock in man and experimental animals (*1*). Many of these proteins can also stimulate powerful T cell proliferative responses (*2*) in the presence of class II major histocompatibility (MHC)–bearing cells (*3–6*). Recently, we showed that one of these proteins is not a true mitogen for mouse T cells (*5*). The toxin does not stimulate all mouse T cells to respond; rather it stimulates murine T cells that bear particular Vβ sequences as part of their αβ T cell receptors (*5, 6*). In this paper we show that this is true for a large collection of staphylococcal toxins and applies when the toxins are used to stimulate human T cells. The specificity of the toxin superantigens for human T cell receptors with particular Vβs may account for the differential sensitivity of different individuals to these proteins.

In our previous experiments we used antibodies to study Vβ usage by mouse T cells responding to various toxins (anti-Vβs). Four similar antibodies against members of the human $V_\beta5$, $V_\beta6$, $V_\beta8$, and $V_\beta12$ families have been described (Fig. 1) (*8–11*). Although in every case the family of Vβs with which these antibodies react is known, it is not clear that the antibodies react in every case with all members of the family.

T cells were isolated from the peripheral blood of a single individual and stained with the anti–human Vβs. Samples of these T cells were also challenged with anti-CD3 or various staphylococcal toxins. The resulting blasts were stained with the anti-Vβs or anti-CD3 and analyzed by using a cytofluorograph. Results were calculated in each case as the percentage of cells stained with anti-CD3.

Each anti-Vβ stained a definable percentage of the peripheral resting T cells from this donor (Fig. 1). The percentage stained ranged from 5.2% with anti-$V_\beta6$ to 1.5% with anti-$V_\beta12$ (Fig. 1, A to D). Culture with anti-CD3 and interleukin-2 hardly changed the percentage stained with each anti-Vβ (Fig. 1, E to H), indicating that this combination of T cell stimuli affected T cells bearing different αβ receptors similarly. Culture with the toxins had variable effects on the percentage of T cells stained with each anti-Vβ (Fig. 1, I to L). Staphylococcal enterotoxin (SE) D, for example, greatly increased the percentage of T cells bearing $V_\beta5$ in the blast population and nearly excluded cells bearing $V_\beta6$. In contrast, T cell blasts stimulated with SEC2 were depleted of $V_\beta6$- and $V_\beta8$-bearing T cells and were greatly enriched in $V_\beta12$-bearing T cells. Finally, SEE stimulated $V_\beta8^+$ T cells, while excluding cells bearing $V_\beta12$. Reciprocal results for each of the toxins were found if the resting T cells contaminating the blast populations were analyzed for Vβ usage. After SEE stimulation, for example, the resting T cells were selectively depleted of $V_\beta8^+$ cells. This result indicates that the toxins are stimulating most of the T cells bearing the appropriate V_βs, not a minor population of these cells.

Five different donors were used in our experiments. These donors were HLA-typed by standard serological techniques, and their resting peripheral T cells were stained with anti-CD3 and the anti-Vβs. Each of the anti-Vβs reacted with a low but measurable percentage of peripheral blood T cells from each of the individuals (Table 1). For a particular individual these percentages were extremely reproducible from one day to another. The percentages of T cells that bore the different Vβs varied somewhat among individuals. As reported previously, this was particularly true for those cells bearing members of the $V_\beta6$ family, reactive with OT145 (*9*).

Cells from the different donors were stimulated with anti-CD3 or the staphylococcal toxins and analyzed for CD3 and Vβ expression (Fig. 2). For each individual, results were calculated as the percentage of T cell blasts bearing a particular Vβ after stimulation divided by the percentage of T cells bearing that Vβ before stimulation. This calculation was designed to correct for variations in Vβ expression from one person to another. As before, anti-CD3 stimulated T cells bearing the different Vβs uniformly; the ratio of T cells bearing a particular Vβ before and after CD3 stimulation was close

Table 1. Vβ expression on unstimulated human peripheral T cells.

Cell donor	HLA type					Percentage of T cells bearing			
	A	B	C	DR	DQ	$V_\beta5$	$V_\beta6$	$V_\beta8$	$V_\beta12$
BK	26 28	14 38		1,4 w53	w1 w3	3.9	3.3	3.2	1.3
CW	24 31	7 60	3 7	4,6 w52,w53	w3	2.7	2.0	4.0	1.5
LS	2	8 62	w7	3,6 w52	w1	2.6	5.2	3.6	1.5
RC	1 11	35 37	w4	1,7 w53	w1 w2	3.2	6.1	6.5	1.2
SL	1	8 63	w7	3,6 w52	w1 w2	3.1	4.4	3.7	1.8

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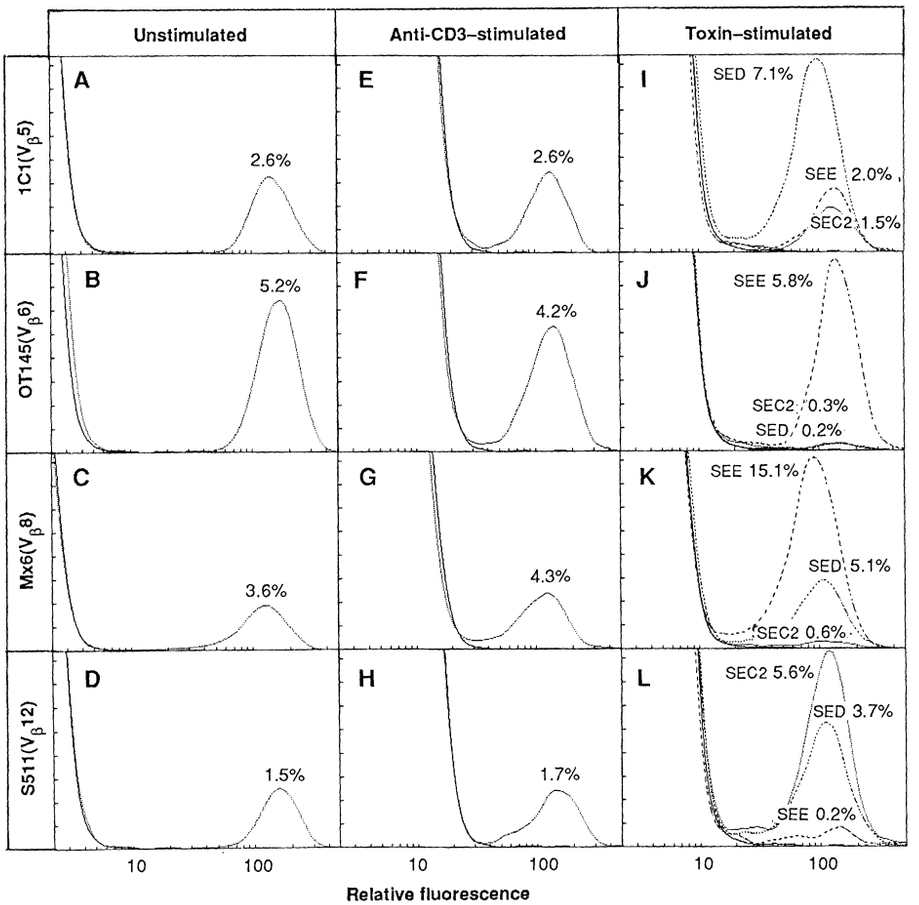

Fig. 1. Staphylococcal toxin stimulation of human T cells is Vβ-specific. Peripheral T cells were isolated from the blood of a single donor and analyzed before (**A–D**) or after (**E–H**) stimulation with anti-CD3 or with SEC2, SED, or SEE (1 μg/ml) (Toxin Technology, Madison, Wisconsin) (**I–L**). Purified anti-CD3 was rendered stimulatory by adherence to plastic bottles as follows. A solution of the protein (1 μg/ml) was incubated on the plastic surfaces for 8 hours at 4°C. Nonadherent antibody was removed by extensive washing. This plastic-adherent antibody or *S. aureus* toxins were used to stimulate the peripheral blood T cells in the presence of irradiated, autologous non–T cells as previously described (*7*). Three days later, live cells were collected and cultured for another 24 hours in recombinant human interleukin-2 (25 unit/ml) (Amgen) to allow regeneration of potentially modulated receptors. At this point about 10% of the surviving cells were true blasts. The surviving cells were incubated with purified antibodies to CD3 or with one of four monoclonal antibodies to human Vβ; 1C1, an antibody to members of the $V_\beta5$ family (*8*); OT145, an antibody to members of the $V_\beta6$ family (*9*); MX6, an antibody to members of the $V_\beta8$ family (*10*); and S511, an antibody to members of the $V_\beta12$ family (*11*). The cells were then stained with fluorescein-conjugated goat antibody to mouse immunoglobulin (Calbiochem, San Diego, California), by standard methods (*16*) and analyzed on an EPICS C. Forward angle and 90° light-scatter patterns were used to gate on the large blasted cells, which were easily distinguished from small lymphocytes and which constituted 50% or more of all the surviving cells in these cultures.

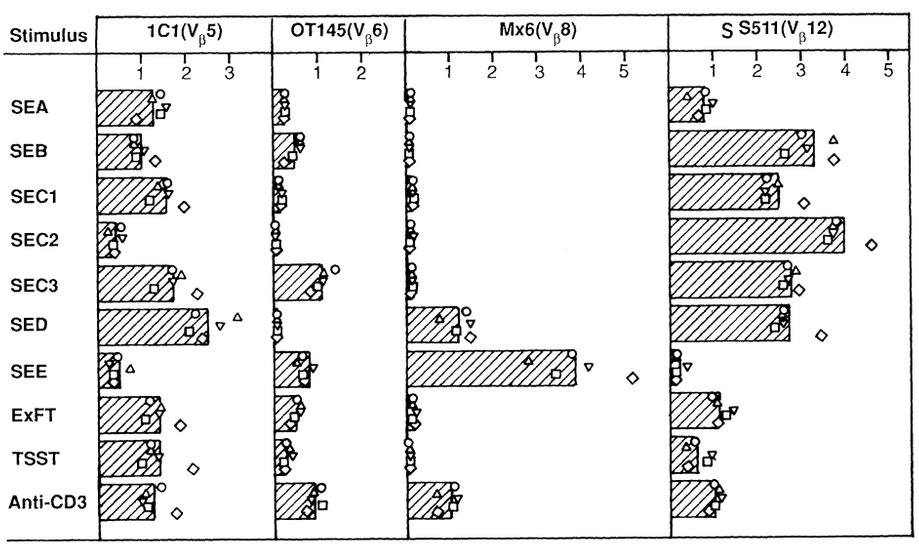

Fig. 2. Vβ-specific stimulation of T cells by toxins is donor-independent. Peripheral T cells from five different donors were stimulated with anti-CD3 or staphylococcal toxins (obtained from Toxin Technology, except SEB, which was obtained from Sigma). ExFT is exfoliating toxin, TSST is toxic shock stimulating toxin 1. Vβ expression was analzyed as described in Fig. 1. For each donor, results are expressed as the percentage of blasts that, after a particular stimulus, reacted with an anti-Vβ, divided by the percentage of T cells that reacted with the anti-Vβ before stimulation. Each symbol represents results from a single donor: ◇ BK, ○ CW, ▽ LS, △ RC, and □ SL.

to 1. In contrast, it was clear that the staphyloccocal toxins varied markedly in their ability to stimulate T cells bearing different Vβs. For example, T cells bearing $V_\beta5$ and $V_\beta12$ were quite rich in blasts produced by challenge with SEC3, whereas T cells bearing $V_\beta8$ were specifically excluded from the SEC3 blasts. One or more of the toxins was a stimulus for T cells positive for each of the Vβ families (albeit weakly for $V_\beta6$), indicating that a toxin superantigen had been identified for each of the Vβ families. Conversely, toxins could be identified which specifically failed to stimulate T cells bearing each of the Vβs.

In view of the relatively small number of different human Vβs we could analyze in this study, it is remarkable that a characteristic stimulation pattern could be identified for almost each toxin. SEC2, for example, stimulated T cells bearing $V_\beta12$ and excluded cells bearing Vβs from the other three families. This pattern was not seen with any of the other toxins. SED stimulated T cells bearing $V_\beta5$ and $V_\beta12$, had marginal effects on T cells bearing $V_\beta8$, and excluded cells bearing $V_\beta6$. Again, this pattern was unique to this toxin.

In some cases, stimulation with a given enterotoxin yielded blasts that were neither enriched nor depleted for expression of a given Vβ by comparison with the starting population. Starting and ending percentages of $V_\beta5$-bearing cells were similar, for example, in responses to toxic shock toxin (TSST). Such a result might indicate that only some $V_\beta5$-bearing T cells were stimulated by TSST. Perhaps the other variable components of the receptor, Vα, Jα, or Jβ, could quite often prevent interaction of this toxin with $V_\beta5$, a phenomenon we have noticed before for superantigen reaction

with mouse T cell receptor Vβs. Alternatively, TSST may react with only one member of the $V_\beta 5$ family. Thus, in responses to TSST, the increase in blasts bearing this member may be offset by a disappearance of T cells bearing other members of the family, but also reactive with 1C1. Discrimination by superantigens among different members of Vβ families has been seen in mice, where the self superantigen Mls-1^a stimulates T cells positive for $V_\beta 8.1$ but not those bearing $V_\beta 8.2$ or $V_\beta 8.3$ (*12*), and SEC1 stimulates T cells bearing $V_\beta 8.2$ but not those bearing $V_\beta 8.1$ or $V_\beta 8.3$ (*13*).

In some experiments, the percentages of T cells that stained with anti-CD4 or anti-CD8 were checked before and after stimulation. The starting percentages were virtually unchanged by toxin stimulation. T cells from one donor, for example, were initially 78% $CD4^+$ and 23% $CD8^+$. After stimulation with the nine different toxins the percentages in the blasts of $CD4^+$ cells ranged from 74% to 79%, and of $CD8^+$ cells from 20% to 26%, suggesting that all these stimuli affected CD4 and CD8 cells equally. It might have been expected that the toxins, which are dependent on class II MHC for presentation (*3–6*), would have preferentially stimulated $CD4^+$ cells, but such is not the case. This result is in line with our findings in analogous systems in mice (*5*).

One of the most striking features of the data in Fig. 2 is the consistency of the results from one individual to another. Thus, although the five people tested had different HLA types and different starting percentages of T cells bearing the various Vβs (Table 1), the proportional changes in Vβ expression in blasts stimulated by each toxin were almost the same from one individual to another. These properties are similar to those we and others have described for superantigen stimulation of T cells in mice (*5, 6, 14*). Although the superantigens require class II MHC for presentation, the allele of class II has much less impact on superantigen presentation than it does on recognition of conventional antigens plus MHC by T cells.

These results show that the staphylococcal toxins are not indiscriminate mitogens for human T cells, but are, in fact, Vβ-specific, as they are for murine T cells (*5, 6*). This result accounts for the previously noted clonal specificity for such toxins (*4*). Although each toxin is able to stimulate only a subpopulation of all T cells in man, they are still powerful T cell stimulants, active at low concentrations. Some or all of the toxic effects of these proteins in man may be mediated by their ability to stimulate large numbers of human T cells. For example, the ability of these toxins to induce secretion of large quantities of lymphokines (*15*) is probably secondary to their ability to stimulate, in a Vβ-specific way, a sizable percentage of T cells. It is also possible that the ability of these and other microbial-derived superantigens to stimulate populations of T cells bearing particular Vβs may be related to the differential resistance of different individuals to the effects of these toxins and also to the ability of microbial attack to induce immune consequences, such as autoimmunity, in certain individuals.

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Transmembrane Channels Based on Tartaric Acid–Gramicidin A Hybrids

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The gramicidin A transmembrane channel is believed to consist of two head-to-head β helices. Computer-generated models were used to formulate the structure of new single-chain channel molecules based on the gramicidin motif. The chemical synthesis of two tartaric acid–gramicidin A hybrids and single-channel analyses of their conducting properties are reported. These studies illustrate the rational design and synthesis of long-lived channels with tunable conductance properties and provide support for current molecular models of the natural (dimeric) gramicidin channel.

TRANSMEMBRANE CHANNELS SERVE as the conduits through which ions may traverse a cellular membrane. The naturally occurring peptide antibiotic gramicidin A [gA (*1*), OHCHN-L-Val-Gly-L-Ala-D-Leu-L-Ala-D-Val-L-Val-D-Val-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-D-Leu-L-Trp-$CONHCH_2CH_2OH$] conducts monovalent cations across lipid bilayers. Its study, which has stimulated a considerable body of research (*2*), has shown that gA dimers are the functional transmembrane elements (*3*). The solid-state structures of free (*4*) and cesium chloride–complexed (*5*) gA dimers