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Distinct T-Cell Receptor Vβ Gene Usage by Human T Lymphocytes Stimulated with the Streptococcal Pyrogenic Exotoxins and pep M5 Protein

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A number of streptococcal products, including the streptococcal pyrogenic exotoxin (SPE) types A, B, and C as well as a 22-kDa fragment of M type 5 protein (pep M5), are potent stimulants of human T-lymphocyte blastogenesis and belong to the newly designated family of superantigens. The Vβ usage of human T cells stimulated with these toxins was investigated by using the polymerase chain reaction. We demonstrate that SPE A, B, and C as well as pep M5 stimulate the proliferation of T cells in a dose-dependent manner. pep M5 stimulates cells bearing Vβ 2, 4, and 8 elements of the T-cell receptor (TCR), whereas SPE A stimulates TCR Vβ 2-, 12-, 14-, and 15-bearing cells. SPE B stimulated only cells expressing TCR Vβ 8 elements, while SPE C stimulated cells expressing Vβ 1, 2, 5.1, and 10. These studies reveal that the preferential usage of particular Vβ elements is distinct for these different superantigens, which may be important in the pathogenesis of various streptococcal diseases.

The streptococcal pyrogenic exotoxin (SPE) types A, B, and C belong to a family of closely related molecules including the staphylococcal enterotoxins and toxic shock syndrome toxin 1 (4, 29, 34). Structurally, these toxins show a wide range of sequence similarity at both the DNA and protein levels (4, 24, 27, 29, 34). Despite this variation, all are capable of inducing shock syndromes such as toxic shock syndrome, toxic shock-like syndrome, or scarlet fever (3, 4, 7, 8, 16, 17, 22, 25, 28–30, 34). One common feature among these molecules is that they all are powerful inducers of T-cell proliferation (10, 13, 20) and activate a variety of cells to produce lymphokines such as interleukin-1 (IL-1), gamma interferon, and tumor necrosis factor (9, 14, 15, 23). In addition, these toxins induce a nonspecific T-cell-dependent immunosuppression of antibody production as well as enhance susceptibility to lethal endotoxin shock.

T-cell stimulation by SPE and staphylococcal enterotoxins has been shown to require the presence of class II-bearing accessory cells which are necessary for the binding and presentation of these molecules to T lymphocytes (5, 21). However, the response of T cells to these toxins is not restricted by major histocompatibility complex elements as is the case with conventional antigens. Furthermore, the SPE are mitogenic for specific subpopulations of T cells that express certain Vβ elements of the T-cell receptor (TCR). On the basis of these observations, SPE have been proposed to be members of the superantigen family (20).

Recently, SPE A was found to stimulate murine T cells bearing Vβ elements, in particular, Vβ 8.2, whereas SPE B and SPE C did not stimulate TCR Vβ 3-, 5-, 6-, 8-, 9-, or 11-bearing cells (12, 18). Fleischer et al. (11) demonstrated that SPE A was much more effective in stimulating human T cells in comparison with mouse cells and actually stimulated human cells bearing Vβ 8 elements. The limited number of monoclonal anti-human Vβ antibodies has hindered the analysis of the human T-cell Vβ specificity of the various superantigens. A recent study by Abe et al. (1) utilizing polymerase chain reaction (PCR) methodology showed that SPE A- and SPE B-stimulated cells utilize different Vβs. No analysis of SPE C-stimulated cells was performed.

We have recently used a PCR method that was originally described by Choi et al. (6) and Tomai et al. (32) to analyze the TCR V-gene usage of T cells stimulated with another streptococcal superantigen, pepsin-extracted type 5 M protein (pep M5). We undertook this study (i) to determine the Vβ usage by SPE C-stimulated cells and (ii) to investigate the possibility that the expansion of particular Vβs by pep M5 is due to a minor contamination by one of the pyrogenic toxins. Here we report for the first time the Vβ usage by SPE C-stimulated human T cells, and we demonstrate that SPE A, SPE B, SPE C, and pep M5 show distinct patterns of TCR Vβ stimulation.

To prepare the toxins, large batch cultures of *Streptococcus pyogenes* 594 (SPE A), 86-858 (SPE B), or 86-104 (SPE C) were grown in dialyzed beef heart medium at 37°C with slow stirring. The toxins were then purified by preparative isoelectric focusing (26, 28). pep M was isolated from type 5 group A streptococci (Manfredo strain) by treating with pepsin at suboptimal pH as described elsewhere (2). pep M5 was purified from 60% ammonium sulfate over a DEAE–high-pressure liquid chromatography column (31). Homogeneity of toxins and pep M5 was verified by sodium dodecyl sulfate-polyacrylamide gel electrophoresis Monoclonal Ab to CD3 (OKT3) was obtained from Coulter Immunology, Hialeah, Fla.

T cells were purified from peripheral blood mononuclear cells by one cycle of E rosetting as described previously (31) and activated in the presence of autologous irradiated antigen-presenting cells with various concentrations of the stimuli for 3 days at 37°C. Proliferation was determined by [^{3}H]thymidine uptake (31). For PCR analysis, live cells were isolated on a Ficoll gradient (Sigma Chemical Co., St. Louis, Mo.), washed, and incubated for 24 h in medium containing

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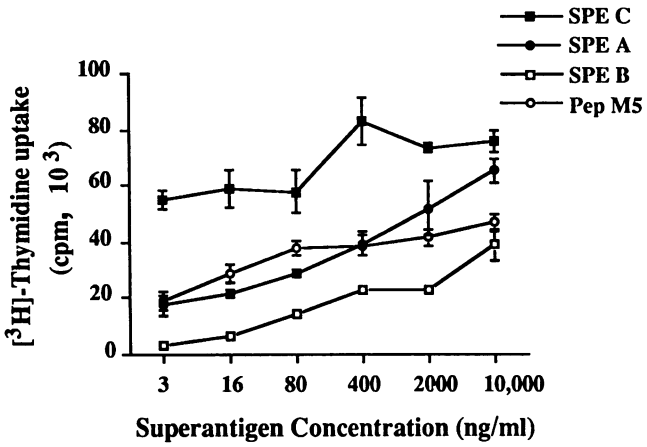

FIG. 1. Stimulation of T-cell proliferation by streptococcal products. Purified T cells (10^5) were incubated with 10^5 irradiated autologous antigen-presenting cells in the presence or absence of anti-CD3 antibody, 5 μg of phytohemagglutinin per ml, or various concentrations of pep M5, SPE A, SPE B, or SPE C. Proliferation was assessed after 3 days by [^{3}H]thymidine uptake, and data are presented as the mean cpm ± standard error of the mean. The background cpm for this study was 3×10^3 cpm.

recombinant IL-2 (10 U/ml; Collaborative Research, Cambridge, Mass.). Total RNA was extracted from viable cells with RNAzol as described elsewhere (32). First-strand cDNA was synthesized from RNA with random hexanucleotide primers and reverse transcriptase (BRL, Gaithersburg, Md.). The PCR was performed as previously described (32). Quantification was achieved by adding 5′-^{32}P-labeled primers (10^6 cpm for the Vβ or Cα product). Radiolabeled products were separated on 2% agarose gels and exposed to X-ray film for 1 to 4 h at −70°C. The relative amounts of Cα and Vβ bands were determined by scanning the autoradiograms and integrating the relevant peaks. The PCR value was as follows: (area of Vβ*n*/area of Cα)$_{\text{pep M5}}$ divided by (area of Vβ*n*/area of Cα)$_{\text{anti-CD3}}$. A PCR value of less than one would suggest a lack of stimulation of that particular Vβ family by the superantigen, whereas a value greater than one would indicate preferential expansion and utilization of that TCR Vβ family by the superantigen.

Purified T cells were stimulated in the presence of irradiated antigen-presenting cells with various concentrations of pep M5, SPE A, SPE B, or SPE C for 3 days. There was a dose-dependent response observed for all stimuli compared with the unstimulated control (Fig. 1). Anti-CD3 antibody and phytohemagglutinin were the most potent stimuli, augmenting proliferation approximately 25-fold (data not shown), whereas pep M5 augmented proliferation approximately 10-fold. SPE C at all doses tested was the most effective toxin in inducing proliferation, with peak activity at around 0.5 μg/ml. Although SPE A was less effective than SPE C, it induced a powerful response at 10 μg/ml which was equal to that observed for SPE C at the same concentration. SPE B was the least mitogenic of the toxins; however, at 10 μg/ml the response was comparable to that obtained with pep M5. Although on a molar basis SPE C is the most potent mitogen of the three toxins, SPE A is made in much greater quantities by the streptococci. One would therefore expect the SPE A-producing strains to cause more severe disease. Indeed, it has been reported that the recent toxic shock-like syndrome cases are SPE A associated, whereas milder scarlet fever and scarlatina are SPE B and/or SPE C associated (7, 16, 27, 30).

To test whether human T cells expressing specific TCR Vβ elements are preferentially activated, cells were stimulated with pep M5, SPE A, SPE B, or SPE C and analyzed for TCR Vβ gene usage by PCR (Fig. 2). As previously reported, pep M5 stimulated T cells expressing Vβ 2, 4, and 8 elements of the TCR (31, 32). The patterns of TCR Vβ usage from cells stimulated with SPE A, SPE B, or SPE C were quite different from that observed for cells stimulated with pep M5. SPE A consistently stimulated cells expressing Vβ 2, 12, and 14. The stimulation of these Vβs was seen in 11 different individuals tested. However, in three individuals we also observed expansion of Vβ 15-bearing cells. The data presented in this paper show results from one exceptional individual who in addition to the above Vβs also showed a low level of stimulation of Vβ 8- and 4-bearing cells. SPE C preferentially stimulated cells expressing Vβ 1, 2, 5.1, and 10, and in one case cells expressing Vβ 15 were also amplified. SPE B, however, stimulated only cells bearing Vβ 8 elements.

Group A streptococci produce a large number of products that may contribute to the pathogenicity of the organism. SPE A, SPE B, and SPE C are known to cause scarlet fever, and recently SPE A has been implicated in a number of toxic shock-like syndrome outbreaks (7, 16, 30). The mechanism by which these streptococcal toxins, as well as their staphylococcal counterparts toxic shock syndrome toxin and staphylococcal enterotoxins, cause these diseases is not fully understood. Recently, a number of reports have suggested that the massive proliferation of T cells may be responsible for the toxicity of these proteins (12, 19). This response is likely to cause overproduction of cytokines such as IL-1, IL-2, gamma interferon, and tumor necrosis factor alpha, which are known to induce toxic effects in both humans and experimental animals (15, 33). If this is the case, then individuals expressing high levels of toxin-responsive T cells would be expected to be more susceptible to diseases caused by organisms producing these toxins (19). Recent studies by Lee et al. (16a) suggested that the toxic effects of these agents are mediated by toxin-induced capillary leakage, since fluid replacement in animals treated with the toxins greatly enhanced their survival. This capillary leakage is likely in part due to macrophage production of IL-1 and tumor necrosis factor, which are known to be produced by peripheral blood mononuclear cells stimulated with these toxins, as well as direct toxin interaction with endothelial cells. Taken together, the results from various laboratories can be explained if one considers that the role of T cells is in exacerbating the toxic effects by producing cytokines such as gamma interferon which are known to augment the production of tumor necrosis factor alpha by macrophages. Despite the uncertainty in the mechanism of lethality induced by these toxins, T-cell mitogenicity is likely to cause several symptoms.

In this study we showed that each of the toxins stimulates distinct subsets of T cells and that each SPE has a signature Vβ stimulation. Since the severity of the streptococcus-associated diseases can vary among individuals, it is possible that these individuals have various baseline levels of Vβ expression and thus respond to the toxins to different degrees.

In addition to the consistent pattern of Vβ usage which is unique for each superantigen, expansion of additional Vβs has been occasionally observed. A recent report by Fleischer et al. (11) showed that SPE A induces a fourfold

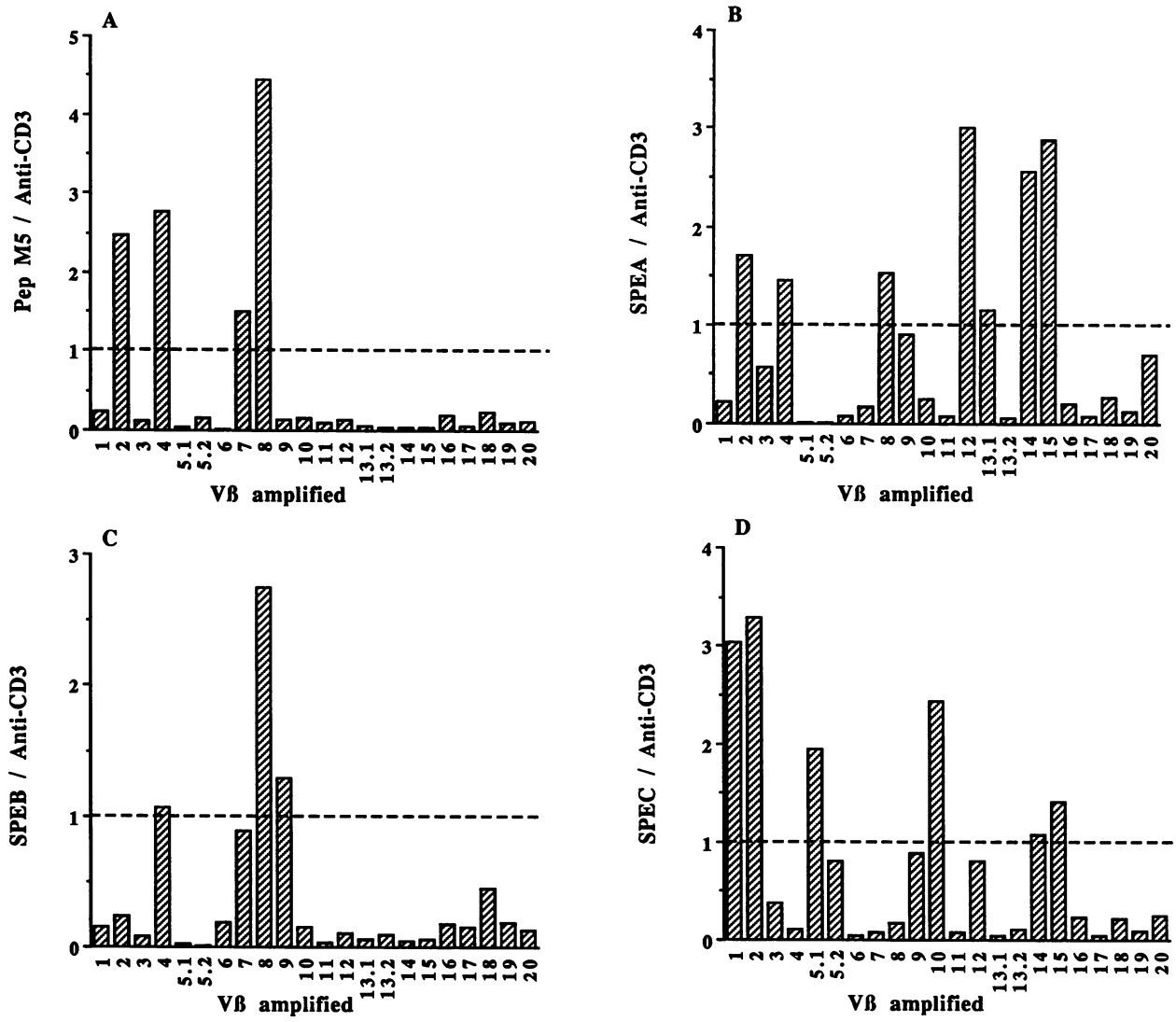

FIG. 2. PCR analysis of the TCR Vβ usage of pep M5-, SPE A-, SPE B-, and SPE C-stimulated T cells. Purified T cells from a single individual were stimulated for 3 days in the presence of irradiated antigen-presenting cells with 1 μg of the following stimuli per ml: pep M5 (A), SPE A (B), SPE B (C), and SPE C (D). All results were compared with the PCR values of cells stimulated by 10 μl of anti-CD3 (OKT3). RNA was extracted from the cells, and cDNA was prepared and analyzed by the PCR. The PCR value was as follows: (area of Vβ*n*/area of $C\alpha)_{pep\ M5}$ divided by (area of Vβ*n*/area of $C\alpha)_{anti\text{-}CD3}$.

enrichment in Vβ 8-bearing cells. Since these authors did not analyze the entire panel of Vβ usage, it was difficult to determine whether other Vβs were also stimulated. However, after we completed our study, Abe et al. (1) utilized PCR to analyze the Vβ usage for only SPE A- and SPE B-stimulated cells and found that SPE A selectively stimulates cells bearing Vβ 8, 12, and 14, while SPE B stimulates cells bearing Vβ 2 and 8. In contrast to their studies, we have tested a number of individuals for response to SPE A and saw a modest increase in Vβ 8 in only one individual, which is presented in this paper. The nature of this discrepancy is not clear but could be attributed to the HLA haplotype of the individual which may allow for the expansion of additional Vβ elements. The facts that we observed expansion of Vβ 15-bearing cells in some individuals and that only 1 of 11 individuals tested showed expansion of Vβ 8-bearing cells by SPE A may be in line with this possibility. An alternative explanation is that the minor structural variations in SPE A isolated from different M serotypes of *S. pyogenes* are reflected in their ability to interact with different Vβ elements. In fact, recent studies by Nelson et al. (21a) demonstrated that SPE A isolated from type M-1 as well as M-3 streptococci differ from other SPE A isolated from other M serotypes by a single amino acid in positions 110 and 106, respectively. The above two possibilities are not mutually exclusive and may prove important in studies of disease susceptibility.

When examining various streptococcal products for their effects on T cells, one is always concerned that minor contaminants are responsible for the mitogenic effects seen. Since pep M5 and the different SPEs have their own signature Vβ stimulation, it is unlikely that these products are cross-contaminated. Using the Vβ signature for superantigens may prove to be a powerful tool in studies of the role of

streptococcal products in disease. There have been speculations in the literature regarding the possible role of superantigens in autoimmune diseases (20). It should be noted that high levels of stimulation of T cells by SPE A and its other toxic effects result in acute-onset toxic shock-like syndrome with a high fatality rate, whereas M protein, which is not a toxin and which has been implicated in the pathogenesis of the post-streptococcal infection autoimmune diseases, is a milder superantigen in comparison with the toxins (31, 32). It is conceivable that milder superantigens that do not cause massive toxicity are more likely candidates for the induction of autoimmunity.

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