Summary

M proteins, the major virulence factor of group A streptococci, have been implicated in the pathogenesis of acute rheumatic fever (ARF) and other streptococcal related autoimmune diseases. A 22-kD fragment of M type 5 protein is a potent stimulant of human T cells and has recently been shown by our laboratory to belong to the newly designated family of superantigens. Using flow cytometry and the polymerase chain reaction, we demonstrate that this molecule reacts with subsets of human T cells expressing specific T cell receptor (TCR) Vβ elements, namely Vβ2, 4, and 8. We employed similar techniques to analyze the TCR Vα usage of pepM5-stimulated T cells. These studies revealed that the preferential usage of particular Vα elements is not specific for the superantigen; rather, it may reflect the repertoire of the individual being tested. The expansion of a large number of T cells bearing specific TCR Vβ sequences by M protein may account for its role in mediating the pathogenesis of post-streptococcal diseases. Furthermore, the preferential usage of TCR Vα elements in certain individuals may be an important factor that predisposes them to development of self-reactivity.

Materials and Methods

M protein was purified by limited pepsin digestion of type 5 group A streptococci as described (6). The mAb to Vβ 5.2-3, 8 and 12 were a gift from Dr. J.-C. Cerottini (Ludwig Institute Cancer Research, Epalinges, Switzerland). Anti-CD3 (OKT3) was from Coulter Immunology (Hialeah, FL).

Stimulation of T Cells. PBMC were purified into T and APC-enriched populations by 1 cycle of E-rosetting, and stimulated for 3-5 d with 1 µg/ml pep M5, 10 µl anti-CD3 Ab, or 1 µg/ml Staphylococcal enterotoxin B (SEB). Viable cells were isolated on a ficoll-gradient and cultured for an additional 24 h in medium containing 10 U/ml rIL-2 (Collaborative Research, Cambridge, MA) to regenerate potentially modulated receptors. Flow cytometric analysis of Vβ expression was done as described (6).
cDNA Amplification Using Polymerase Chain Reaction (PCR) and Quantitation of the Coamplified Products. RNA was extracted from stimulated cultures using RNAzol (Cinna/Biotecx, Friendswood, TX (20)). First-strand cDNA was prepared from 2 μg RNA using reverse transcriptase, superscript reverse transcriptase (Gibco-BRL, Gaithersburg, MD) and random hexanucleotides. To terminate the reaction, the samples were heated for 10 min at 95°C. The cDNA from each culture was aliquoted into 22 tubes each containing a 5' Vβ-specific primer, representing the 20 Vβ families along with a 3' Cα anti-sense primer amplifying products ranging from 170–220 bp. As an internal control, each tube contained 5' and 3' Cα primers which amplified a 600-bp product. All primers were used at 0.3 μM. The sequences of the primers used for Vβ and Cα amplification have been described by Choi et al. (10).

To determine the TCR Vα usage of cultures we used the above procedure with several modifications. The 5' Vα specific and 3' Cα primers (synthesized according to the published sequences (11) were used at 1.0 μM. As an internal control, we designed a set of Cβ primers: the 5' Cβ is TTT GAGCCATCA GAA GCA GAGATCT and the 3' reverse Cβ primer sequence is TCAGGGC TGC TGCTCAGGT A. The 5' Cβ and 3' Cβ primers were used at 0.1 μM. Amplification for both TCR Vβ and Vα usage was performed with 2.5 U of Taq Polymerase (Perkin Elmer Cetus, Norwalk, CT) using the following conditions: 95°C denaturing, 55°C annealing of the primers, and 72°C elongation, for 1 min each. All reactions were run for 25 cycles. Quantitation of the amplified products was achieved by incubation in the presence of 5' 32P-labeled Cα and Cβ primers (500,000 cpm of each/reaction). Radiolabeled products were separated on 2% agarose gels and exposed to x-ray film. The relative amounts of Cα, Vβ, Cβ, and Vα bands were determined by scanning the autoradiograms and integrating the areas of the relevant peaks. PCR values were normalized by dividing the area of Vβ or Vα by their respective internal control. The normalized values for pep M5- and SEB-stimulated cells were divided by those obtained for anti-CD3-stimulated cells to determine specific expansion. The PCR value = [area Vβ (Vα)/area Cα (Cβ)] pep M5 divided by [area Vβ (Vα)/area Cα (Cβ)] anti-CD3.

Results

Correlation of T Cell Receptor (TCR) Vβ Usage by Flow Cytometry and Polymerase Chain Reaction (PCR) Analysis. In previous studies we employed mAbs and flow cytometry to determine if pep M5 stimulated human T cells bearing specific Vβ elements. These studies revealed preferential expansion of cells expressing receptors of the TCR Vβ8 family (6). Analysis of the utilization of other Vβ families was not feasible due to the limited availability of mAb specific to these families. Thus, the PCR method described by Choi et al. (12) offered an opportunity to evaluate TCR gene usage of all 20 families of Vβ gene products.

T cells were stimulated with either anti-CD3, or the superantigen, pep M5, and SEB and then analyzed by flow cytometry, and PCR. The normalized PCR value for each TCR Vβ family from pep M5- or SEB-treated cells was divided by the normalized PCR value obtained for each TCR Vβ family from anti-CD3 stimulated cells. A PCR value of less than one would suggest 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. There was a strong correlation between our analysis of Vβ usage by flow cytometry and PCR (Fig. 1 A and B, and Table 1). Expansion of Vβ8 and Vβ12 bearing cells by pep M5 and SEB, respectively was observed using either procedure, thus confirming our previous reports (6) and others (8, 10).

Table 1. Flow Cytometry Analysis of T Cell Subsets Stimulated with Pep M5

| Stimulant | Vβ5.2-3 | Vβ8 | Vβ12 | CD3 |
|-----------|---------|-----|------|-----|
| anti-CD3  | 2.6     | 7.3 | 1.8  | 95.0|
| Pep M5    | 0.4     | 20.3| 0.6  | 93.6|
| SEB       | 3.0     | 3.5 | 4.1  | 97.8|

* See legend to Fig. 1.
Analysis of the Entire Panel of T Cell Receptor (TCR) Vβ families in T Cells from Pep M5-Stimulated Cultures. PCR analysis on cDNA from cells obtained from several individuals that were stimulated in vitro with pep M5 revealed a consistent expansion of cells expressing TCR Vβ2, Vβ4, and Vβ8 elements when compared to cells stimulated with anti-CD3 (Fig. 2). Occasionally, we observed expansion of cells bearing one or two additional Vβ elements by pep M5, but this varied among individuals.

Analysis of T Cell Receptor (TCR) Vβ and Vα Usage by Pep M5 and SEB Stimulated T Cells. Although superantigens have been shown to preferentially interact with the β chain of the TCR, a role for TCR Vα-chain elements has been suggested (12). To determine if there is preferential use of TCR Vα elements by pep M5- or SEB-stimulated T cells, we applied the PCR method described above and amplified cDNA from the same individual using Vβ and Vα-specific primers as well as their appropriate internal controls (Fig. 3). Once again, T cells expressing Vβ2, 4, and 8 elements were expanded by pep M5 (Fig. 3 A); while cells bearing Vβ12, 3, 14, 15, and 17 were expanded by SEB (Fig. 3 C). In contrast, when TCR Vα usage was analyzed no consistent pattern emerged (Fig. 3 B and D). In one individual, expansion of Vα 6, 10, 14, and 15 bearing cells was observed in both pep M5 and SEB stimulated cultures (Fig. 3 B and D). However, when another individual was tested, T cells bearing these Vα elements were not expanded and a different pattern emerged (data not shown). In contrast, the pattern of Vβ usage remained unchanged. These results suggested that there is no preferential utilization of TCR Vα elements by pep M5 or SEB-stimulated T cells. Rather, the preferential expansion of the Vα elements may reflect the individuals repertoire. In support of these findings, a longterm pep M5-specific cell line analyzed for TCR Vβ and Vα usage expressed predominantly Vβ2, 4, and 8, but virtually all TCR Vα elements were represented albeit to different levels (data not shown).

Discussion

Superantigens, including a number of staphyloccocal (8, 10) and streptococcal (13) toxins and a soluble product from Mycoplasma arthritidis (9) stimulate T cells expressing specific TCR Vβ gene products. We have recently demonstrated that the streptococcal M protein belongs to this family of bacterial superantigens (6, 7). In this study, we show that pep M5 preferentially stimulates cells bearing TCR Vβ 2, 4, and 8 elements irrespective of the TCR Vα element used.

Reports of epitopes within the M protein molecule that are shared by mammalian proteins and which can elicit autoreactive Abs have propounded the view that the post-streptococcal diseases are caused by the molecular mimicry
of M proteins to tissues of the heart, kidney, or brain (3, 4, 14). To date, there is no evidence that these autoantibodies are harmful to humans (4). In contrast, clinical and experimental evidence suggest an important role for T cells in the pathogenesis of ARF (2, 15). The major virulence factor of S. pyogenes is M protein, which in addition to its antiphagocytic activity, is a powerful stimulator of human T cell blastogenesis (2). It is therefore tempting to speculate that the activation of T cells by M protein is important in the development of these diseases. However, since T cells from all individuals respond vigorously to pep M molecules, other factors, such as the MHC genotype of the individual, may be involved and may explain why only 10% of the population is at risk for developing ARF (4).

The contribution of the Vα region of the TCR in the response to pep M5 remains to be elucidated. Pullen et al. (13) have proposed that the Vα region of the TCR may be important in responses where the TCR Vβ-superantigen interaction is weak. Our data suggest that the expansion of T cells bearing specific Vβ, but not Vα elements is driven by the superantigen. These studies do not rule out the possibility that Vα elements influence the response of T cells to superantigens since certain individuals show preferential pairing of specific TCR Vβs with Vαs and this may be another mechanism regulating the susceptibility of individuals to autoimmunity. A particular combination of Vβ and Vα may be the determining factor for the reactivity of the expanded T cells to self epitopes.

We wish to dedicate this paper to the memory of Dr. Edwin H. Beachey who has inspired this work. We thank Ms. Jeannine Hermann for flow cytometry analysis.

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