Localization of a T-Cell Epitope of Superantigen Toxic Shock Syndrome Toxin 1 to Residues 125 to 158

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Tissue shock syndrome toxin 1 (TSST-1) is a member of the staphylococcal enterotoxin superantigen family. So far, little is known about T-cell epitopes on superantigens. In this study, we developed an improved method for localizing T-cell epitopes on superantigens that involved synthetic peptides plus costimulation by CD28 or phorbol myristate acetate. Using this method, we localized a T-cell epitope to a 34-residue region, TSST-1 (residues 125 to 158), which possessed only two of four TSST-1-targeted β-chain variable element (VB) specificities of T-cell receptors in humans and mice, human VB2 and murine VB15.

Materials and Methods

Main reagents. The monoclonal antibody 9.3 (anti-human CD28) was donated by J. A. Ledbetter (Bristol-Meyers Squibb Pharmaceutical Research Institute, Seattle, Wash.). MPB2/D5 (anti-human TCR VB2) was a generous gift from F. C. Lancaster (University of Leeds, Leeds, England). Goat anti-mouse immunoglobulin (Ig) was purchased from Sino-America Biotechnology Co. (Shanghai, People’s Republic of China). PMA was obtained from Sigma Chemical Co. (St. Louis, Mo.). [3H]thymidine was obtained from Chinese Atomic Energy Institute (Beijing, People’s Republic of China). RPMI 1640 was obtained from GIBCO Laboratories (Grand Island, N.Y.). A set of reagents for peptide synthesis was provided by Perkin-Elmer Co. (Foster City, Calif.)

Murine VB-bearing T-cell hybrids. Murine T-cell hybridomas K25-49.16 (Vβ3), KOK-49.5 (Vβ15), 2023-34.7.9 (Vβ17), and KH-10.1 (Vβ13) were generously provided by P. Marrack (Howard Hughes Medical Institute, Denver, Colo.).

Peptide synthesis. Thirty-four-mer TSST-1 (residues 125 to 158) and 58-mer TSST-1 (residues 101 to 158) peptides, designated T34 and T58, respectively, were synthesized with solid-phase 9-fluorenylmethoxycarbonyl (Fmoc) chemistry (13) on an ABI model 431A automated peptide synthesizer. Peptides were cleaved from the resins with trifluoroacetic acid-ethanedithiol-thioanisole-anisole at a ratio of 90:3:5:2. The cleaved peptides were then extracted in ethyl acetate and subsequently dissolved in water and lyophilized. Each peptide was highly pure, showing a single peak in reversed-phase high-performance liquid chromatography with a C8 column (5 mm; Merck and Co., Inc., Rahway, N.J.). 0.1% trifluoroacetic acid, and a gradient of 0 to 50% acetonitrile. Actual amino acid compositions of the peptides corresponded closely to theoretical compositions. The sequence of control 10-mer peptide, designated C10 and prepared by Fmoc chemistry, mentioned above, was determined by computer at random. It had no homology with TSST-1.

Cell separation. Blood samples were obtained from healthy adults. Human peripheral blood mononuclear cells (PBMC) were isolated by the Ficoll-Paque gradient method (2). Murine single splenocytes were prepared by mincing freshly dissected spleens from B6Lc3 mice into small pieces and then teasing the pieces through stainless mesh. Erythrocytes were removed by hypotonic shock treatment. The T cells of human PBMC or murine splenocytes were purified with a nylon wool column (15). To ensure that the purified T cells used in our experiments were depleted of APC, cultures of purified T cells stimulated with 5 μg of phytohemagglutinin per ml or 10 ng of PMA per ml were included in the experiments. The lack of a proliferative response in those cultures was indicative of the absence of APC. Human VB2-depleted purified T cells were prepared by a panning technique with anti-human TCR VB2 as previously described (19).

Proliferation assay. Cell suspensions (6 × 10^5/well) were cultured in 96-well plates (Costar, Cambridge, Mass.) in 200 μl of complete RPMI 1640 containing 10% fetal bovine serum, 25 μM HEPES, 200 mM l-glutamine, 100 μM of penicillin per ml, and 100 μg of streptomycin per ml at 37°C in 5% CO2 and 95% humidity. Human PBMC or murine splenocytes were stimulated by various concentrations of synthetic peptides alone; human purified T cells or human VB2-depleted purified T cells were stimulated by various concentrations of

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synthetic peptides with 10 μg of anti-human CD28 per ml and 30 μg of goat-anti-mouse Ig per ml for cross-linking anti-human CD28; and murine purified T cells were stimulated by various concentrations of synthetic peptides alone. (E) Murine purified T cells were stimulated by various concentrations of synthetic peptides with PMA. Mitogenic response was measured by [3H]thymidine incorporation. All cultures were performed in triplicate, and data are shown as means ± standard deviations from six independent representative experiments.

IL-2 production assay. Supernatants of the murine Vβ-bearing T-cell hybridomas stimulated by various concentrations of synthetic peptides with PMA for 24 h at 37°C and 5% CO2 were tested for the production of interleukin-2 (IL-2). Therefore, the supernatants were added to the cultures of IL-2-dependent CTLL cells (10^5/well), with a final concentration of 25%. After the CTLL cells in the supernatants of the murine hybridoma cells were cultured for 18 h at 37°C in 5% CO2, they were pulsed with [3H]thymidine (0.5 μCi/well) for 6 h. Samples were harvested and counted as described above. All cultures were performed in triplicate. The results are representative of six independent experiments.

RESULTS

Failure of T34 and T58 to activate human PBMC and murine splenocytes. Human PBMC and murine splenocytes are T cells mixed with accessory cells. As shown in Fig. 1A and D, cultures of human PBMC or murine splenocytes were stimulated by T34 and T58 alone at various concentrations from 0.1
Induction of human or murine purified T-cell proliferation by T34 and T58 in the presence of costimulation by CD28 or PMA. It is evident that accessory cells are essential for the induction of T-cell proliferation by superantigens (23). However, PMA and anti-CD28 can substitute for accessory cells in the induction of T-cell proliferation by superantigens (6, 28, 29). To determine whether T34 and T58 cannot bind to accessory cells, and thus fail to activate T cells, cross-linked anti-human CD28 was added to the cultures of human purified T cells stimulated by T34 and T58, while PMA was added to murine purified T cells. With costimulation by CD28 or PMA, T34 and T58 could trigger the proliferative response of human or murine purified T cells in a dose-dependent manner (P < 0.05 or 0.01). Furthermore, the ability of T34 to activate human and murine purified T cells was stronger than that of T58 (P < 0.05) (Fig. 1B and E).

TCR Vβ specificity of T34 and T58. TSST-1 can activate T cells bearing human Vβ2 or murine Vβ3, -15, and -17 (23). Vβ specificity of T-cell proliferation by T34 and T58 was assessed in order to determine if the epitopes of T34 and T58 are TSST-1 specific. As shown in Fig. 1C, with costimulation by CD28, human Vβ2-depleted purified T cells could not be activated by T34 and T58 but could be activated by staphylococcal enterotoxin B, whose human TCR Vβ specificity excludes Vβ2 (data not shown). On the other hand, in the presence of PMA, T34 and T58 could activate murine Vβ15-bearing T-cell hybridomas but not murine Vβ3-, -17-, and -13 (control)-bearing T-cell hybridoma cells, as evaluated by IL-2 production and [3H]thymidine incorporation (Fig. 2). That is, T34 and T58 possessed only two of four TSST-1-targeted TCR Vβ specificities in humans and mice, human Vβ2 and murine Vβ15.

DISCUSSION

Historically, empirical epitope localization of protein antigens has relied upon either enzymatic digestion or cyanogen bromide cleavage into successively smaller fragments retaining epitopic specificity (12). Later, advances in peptide synthesis technology paved the way for the generation of small fragments with overlapping sequences and the construction of synthetic peptides corresponding to different areas of the protein, which could be probed for reactivity with T or B cells. At present, the peptide synthesis approach is widely applied for localizing T-cell epitopes on ordinary antigens (18).

Since recognition of antigens by T cells requires assistance from APC, antigen-MHC molecule complex formation is a prerequisite for subsequent TCR binding and mitogenic activity of antigens (31). Therefore, synthetic peptides must possess MHC-binding sites when peptide synthesis is used to localize T-cell epitopes on antigens. Otherwise, T-cell epitopes within the synthetic peptides cannot be found.

Before binding to TCR, ordinary antigens need to be processed into short peptide fragments of about 10 to 20 residues in length which bind to a cleft on the surface of the MHC molecules (33, 35). Thus, T-cell epitopes and MHC-binding
sites of ordinary antigens are within sequences of 10 to 20 residues. They are intertwined or overlapping. In contrast, superantigens do not require processing to small peptides but bind as intact proteins to MHC class II molecules of APC (14). T-cell epitopes and MHC-binding sites of superantigens may be located separately from each other. It is thus difficult for the shortened peptides to catch both T-cell epitopes and MHC-binding sites. This may be one of the main reasons why the use of peptide synthesis has had limited success in localizing T-cell epitopes on superantigens.

Some studies have shown that superantigens can induce the proliferation of purified resting T cells in the presence of APC-negative costimulatory signals such as anti-CD28 (28, 29) and PMA (6). Furthermore, we and others have previously demonstrated that the manner in which the superantigen activates purified T cells costimulated by CD28 or PMA is identical to that with APC (19, 22), suggesting that the main role of APC in superantigen-mediated T-cell activation may be to provide T cells with CD28 costimulation. Based on this knowledge, we have developed an improved method—with the use of synthetic peptides plus cross-linked anti-human CD28 or PMA—in order to solve the problem of MHC-binding sites in localizing T-cell epitopes on superantigens.

In this study, we found that T34 and T58 could not activate human PBMC or murine splenocytes but could activate human or murine-purified T cells with costimulation by CD28 or PMA; i.e., T34 and T58 do not encompass MHC-binding sites but do contain T-cell epitopes. Since the control peptide was 10 residues, while TSST-1 peptides were 34 and 58 residues, there may be something abnormal about the specificities of T34 and T58 to activate T cells with costimulation by CD28 or PMA. In fact, we verified that bovine serum albumin or a 36-mer peptide from superantigens can serve as classical antigens to activate T cells (32), we wanted to determine whether T34- and T58-induced T-cell proliferation is related to the superantigenic nature of the epitopes. In addition, the sequence of T58 included that of T34, and the epitope of T58 may be identical to that of T34, located within the common sequence of T34 and T58, TSST-1 (residues 152 to 158). As for the superantigenicity of T58 being less than that of T34, it is possible that the conformity of T58 makes the epitope in T58 less accessible.

Although it was previously reported that the synthetic peptides from superantigens can serve as classical antigens to activate T cells (32), we wanted to determine whether T34- and T58-induced T-cell proliferation is related to the superantigenicity of TSST-1. Therefore, we examined the Vβ specificity of T cells activated by T34 and T58 and found that T34 and T58 possess two of four TSST-1-targeted Vβ specificities in humans and mice, human Vβ2 and murine Vβ15. It is clear that the epitopes contained by T34 and T58 are TSST-1 superantigen specific. It is known that superantigens have several kinds of TCR Vβ specificities in humans and mice (23), but it is not known whether one epitope corresponds to all Vβ specificities or whether each epitope is associated with one Vβ specificity in a superantigen. Comparison of human and murine TCR Vβ protein sequences by Clark et al. revealed that human Vβ2 is the homolog of murine V15, whereas murine Vβ3 appears to be homologous to murine Vβ17. Human Vβ2 and murine Vβ15 show little similarity to murine Vβ3 and -17, respectively (7). Taken together, these results imply that TSST-1 may contain two T-cell epitopes, one responsible for human Vβ2 and murine Vβ15 and the other responsible for murine Vβ3 and Vβ17. Therefore, we suggest that TSST-1 (residues 125 to 158) should contain a T-cell epitope with specificity for human Vβ2 and murine Vβ15.

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