Major Histocompatibility Complex Independent Clonal T Cell Anergy by Direct Interaction of *Staphylococcus aureus* Enterotoxin B with the T Cell Antigen Receptor

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Summary

The Staphylococcal enterotoxin superantigens stimulate vigorous responses in T cells bearing certain T cell antigen receptor (TCR) Vβ regions. In addition to activation, these superantigens also impart negative signals to T cells resulting in a profound state of unresponsiveness or anergy. The *Staphylococcus aureus* enterotoxins (SE) B and C_2 bind to a closely related site on major histocompatibility complex (MHC) human leukocyte antigen (HLA)-DR1 molecules. Only SEB, however, interacts with the TCR Vβ3 region of HA1.7, a human HLA-DR1 restricted T cell clone specific for influenza haemagglutinin. In competition experiments, we demonstrated that the induction of anergy in HA1.7 by SEB is unaffected by the presence of SEC_2. These results suggest that SEB-induced anergy is MHC independent and involves a direct interaction between the TCR and SEB. To resolve definitively whether SEB binds directly to T cells in the absence of MHC class II molecules, the cDNAs encoding the HA1.7 TCR were transfected into an MHC class II–negative human T cell line. The addition of SEB to these transfectants resulted in the downregulation of cell surface TCR expression, an increase in the concentration of intracellular calcium ions, the production of lymphokines, and reduced responsiveness to a subsequent challenge with SEB. We conclude that SEB interacts directly with the TCR in the absence of cointeraction with MHC class II molecules, and that this interaction may induce anergy in HA1.7.

A group of exotoxins produced by certain strains of *Staphylococcus aureus* induce vigorous responses in T cells expressing particular TCR Vβ elements (1–4). Such exotoxins have thus been termed superantigens. In common with endogenous murine retroviral superantigens, bacterial superantigens also have the capacity to shape the TCR repertoire by clonal deletion (2, 5) or the induction of anergy (6–8). Since thymic deletion alone fails to explain self-tolerance to antigens expressed exclusively by adults or in sites remote from the neonatal thymus, anergy, which can be induced in mature T cells, is proposed to account for T cell unresponsiveness to these antigens both in in vivo (6–9) and in vitro models (10–12).

Incubation of human T cell clones with supraoptimal concentrations of nominal peptide antigen renders the cells anergic to a subsequent immunogenic challenge (10, 11, 13). In common with antigen-specific T cell activation, antigen-induced anergy is initiated by MHC class II–restricted antigen presentation (14).

More recently, it has been demonstrated that *Staphylococcus aureus* enterotoxin (SE)[1] superantigens, are also able to induce T cell anergy to their native ligand (12, 15). Unlike peptide antigen–induced anergy, however, enterotoxin-induced anergy was not inhibited by anti-MHC class II mAbs (R. E. O'Hehir and J. R. Lamb, unpublished observations).

The aim of the present study was, therefore, to determine whether a Staphylococcal enterotoxin superantigen was able to interact directly with the TCR and induce clonal anergy in the absence of MHC-dependent antigen presentation.

Materials and Methods

Reagents and Cell Lines. HA1.7 is a human $CD4^+$ T cell clone specific for influenza haemagglutinin (HA) peptide 307–319 in the

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[1] *Abbreviations used in this paper:* AnPCR, anchored polymerase chain reaction; HA, influenza haemagglutinin; SE, *Staphylococcus aureus* enterotoxin.

context of HLA-DR1. LNAT is an autologous EBV-transformed B cell line used as an APC (16). JRT3T3.5, a CD3-TCR negative, MHC class II-negative mutant of Jurkat (17), was a gift from Dr. A. Weiss (University of California, San Francisco). CTLL-M, a murine IL-2-dependent T cell line used for the bioassay of Jurkat-derived supernatants (18), was obtained from the European Collection of Animal Cell Cultures (EACC) (Porton Down, UK). Oligonucleotides were synthesized by Dr. Ian Goldsmith (Imperial Cancer Research Fund). The HA peptide 307–319 was synthesized by Dr. J. Rothbard (ImmuLogic Pharmaceutical Corp.). FITC-conjugated goat antibodies specific for mouse Igs and anti-CD3 were obtained from Becton Dickinson & Co. (Mountain View, CA). MX6, an anti-TCR Vβ8 mAb was obtained from Dr. S. Carrel (Ludwig Institute, Epalinges, Switzerland). SEB, SEC$_1$, and SEC$_2$ were obtained from Sigma Chemical Co., (Poole Dorset, UK) or Toxin Technology, Inc. (Madison, WI).

Cloning of TCR α and β chain cDNA. HA1.7 was purified free of APC and stimulated for 6 h with 1 μg/ml phytohaemagglutinin-P (Sigma Chemical Co.) and 10 ng/ml PMA (Sigma Chemical Co.) to increase TCR α and β chain mRNA expression (19). RNA was purified from 5×10^6 cells by the guanidine isothiocyanate-acid phenol method (20). cDNA was synthesized by a standard oligo-dT primed method (21). cDNAs encoding the HA1.7 TCR α and β chains were then amplified using the anchored PCR (AnPCR) (22) as modified by Dr. P. Marche and Dr. O. Acuto (Institut Pasteur, Paris, France).

For amplification of TCR α chain cDNA, an antisense primer was designed to include part of the C region (**bold type**), and EcoRI, and BglII sites: 5' GCGAATTCAGATCTTAGGCAGA-**CAGACTTGTCACTGG.** 3'; the sense primer for the TCR α chain contained a dC anchor, XhoI, NotI, and SalI sites: 5' CACTC-GAGCGGCCGCGTCGACCCCCCCCCC 3'. The antisense primer used to amplify the TCR β chain contained part of the C region (**bold type**), KpnI, SalI, and ClaI sites: 5' GGTACC-**GTCGACATCGATCCACCAGCTCAGCTCCACGTGGTCG** 3'. The sense primer for the TCR β chain included a dC anchor, SphI, NotI, and SacII sites: 5' GCATGCGCGCGGCCGCGGA-GGCCCCCCCCCCCCCCC 3'. 25 cycles of AnPCR were performed, each cycle consisting of 1 min at 94°C, 2 min at 55°C, 3 min at 72°C, and a single final extension of 6 min at 72°C. AnPCR products were isolated, cloned, and sequenced by standard methods. Full α and β coding sequences were constructed using constant region cDNAs derived from the Jurkat TCR α and β chains.

Functional Expression of TCR. The HA1.7 TCR α and β constructs (UBα14/4 and CUBβ1.5) were cloned into the eucaryotic expression vector PJ6Ω, in which cDNA is transcribed under the control of the rat actin promoter (23). pJ6Ω expression vectors containing the hygromycin B (pJ6Ωhygro) or puromycin (pJ6Ωpuro) resistance genes, were made available by Dr. H. Land (Imperial Cancer Research Fund).

The HA1.7 TCR β expression construct (50 μg/ml) was cotransfected with pJ6Ω hygro (25 μg/ml) into 2×10^7 JRT3T3.5 by electroporation (450 V at 125 μF). Cells were immediately placed on ice and resuspended in RPMI 1640 containing 10% FCS. After 3 d of culture, the medium was supplemented with 400 μg/ml hygromycin-B (Sigma Chemical Co.).

After 3 wk, the α chain expression construct was cotransfected with pJ6Ω puro into the β chain transfected line by electroporation. Cells were selected in medium containing 400 μg/ml of hygromycin-B, and 4 μg/ml of puromycin (Sigma Chemical Co.), and the resulting cell line assessed for CD3 expression by flow cytometer analysis. Cells were sorted for high CD3 expression three times

and then cloned by limiting dilution. Clones were screened for responses to peptide 307–319 and SEB in the presence of LNAT APC. One particularly efficient clone CH7C17 was used for the majority of these studies.

A mock transfected subline designated OGSL1 was also generated. This line underwent all the procedures outlined above, but was electroporated in the absence of plasmid DNA.

Functional Assays. Anergy was induced by incubation of T cells (10^6/ml) with HA peptide 307–319 (up to 100 μg/ml) or SEB (up to 100 μg/ml) for 16 h at 37°C. Cells were thoroughly washed and then challenged with an immunogenic concentration of HA peptide 307–319 or SEB in the presence of irradiated (6,000 rad) LNAT APC. Experiments with HA1.7 were pulsed with 1 μCi of [³H]thymidine (Amersham International, Amersham, Bucks, UK) for the last 16 h of a 72-h culture. For experiments with CH7C17, culture supernatants were taken at 72 h and used to stimulate CTLL-M cells for 24 h. Proliferation was then measured by [³H]thymidine incorporation for the last 8 h of culture.

Assay of Intracellular Calcium. Cells were washed three times with serum-free RPMI, then incubated with 1 μg/ml Fura-2 in RPMI at 37°C for 15 min. Cells were washed twice with HBSS containing 20 mM Hepes at pH 7.3, and resuspended at 10^6/ml. Two ml of cells were placed in a cuvette and warmed to 37°C with stirring in a fluorimeter (The Perkin-Elmer Corp., Norwalk, CT) set to excite at 325 nm and measure fluorescence at 510 nm. The stimulus was added and the fluorescence recorded for 5 min.

Immunofluorescence. Cells were stained for sorting or cell surface phenotype analysis by standard methods using mAb directly conjugated to FITC, or a primary mAb followed by a second layer of FITC-conjugated goat antibodies specific for mouse Ig.

SEB and SEC$_2$ Competition Binding Assay. SEB was biotinylated by incubating a twofold molar excess of N-hydroxysuccinimide active ester of long chain biotin with SEB (1 mg/ml) overnight at 4°C. Unbound biotin was removed by Sephadex G-25 gel filtration. Biotinylated SEB (50 μg/ml) was incubated with 1BW4, an HLA-DR1-Dw1 EBV-transformed B cell line, for 2 h at 4°C in the presence of various concentrations of unlabeled SEB or SEC$_2$. Cells were washed with PBSA containing 0.1% BSA and incubated at 4°C with FITC-avidin D (Vector Labs., Inc., Burlingame, CA) at 10 μg/ml for 30 min. Cells were washed and resuspended in PBSA containing 0.1% BSA for flow cytometer analysis.

Results

SEC$_2$ Fails to Compete with SEB during the Induction of Anergy. The Staphylococcal enterotoxins SEC$_2$ and SEB are known to bind to the same site on MHC class II antigens (24). Unlike SEC$_2$, however, SEB stimulates T cells expressing TCR Vβ3.1 elements (12). To confirm the identity of the SEB and SEC$_2$ binding sites on HLA-DR1, biotinylated SEB was incubated with HLA-DR1$^+$ B cells in competition with unlabeled SEB and SEC$_2$. Consistent with the report that the two enterotoxins bind to a closely related site on the HLA-DR1 molecule, the binding of SEB to HLA-DR1$^+$ B cells was specifically competed with both by unlabeled SEB and SEC$_2$ (Fig. 1 *A*).

If SEB-induced anergy occurs by a mechanism independent of MHC class II binding, then it should be possible to anergize HA1.7 with SEB after pretreatment with an excess of SEC$_2$. A 100-fold molar excess of SEC$_2$ failed to inhibit the induction of anergy in HA1.7 by either SEB or the HA
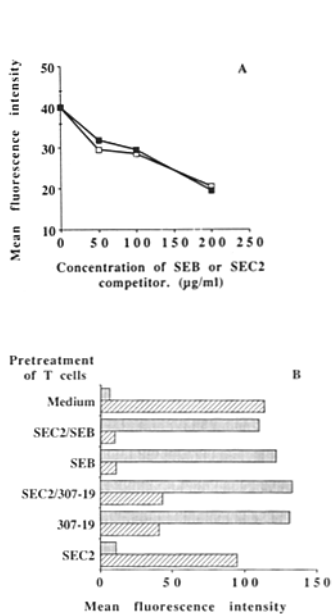
Figure 1. SEC$_2$ interacts with the SEB binding site on HLA-DR1, but fails to inhibit anergy induction by SEB or HA peptide 307–319 in HA1.7. (*A*). Biotinylated SEB at 50 μg/ml was incubated with IBW4, HLA –DR1-Dw1 EBV-transformed B cells for 2 h at 4°C in the presence of various concentrations of unlabeled SEB (*filled squares*) or SEC$_2$ (*open squares*). Biotinylated SEB bound to the cell surface was detected using FITC-conjugated avidin and flow cytometer analysis. (*B*) HA1.7 was treated with 50 μg/ml of SEC$_2$ for 1 h at 37°C then incubated with an anergizing concentration of SEB (0.5 μg/ml) or peptide 307–319 (50 μg/ml) for 16 h at 37°C. Cells were washed and stained with mAb specific for CD3 (*hatched bars*) or CD25 (*shaded bars*).

peptide 307–319 (Fig. 1 *B*). The fall in CD3 expression, and rise in CD25 expression, both characteristics of anergy (12), combined with the failure of HA1.7 to incorporate thymidine after a subsequent immunogenic challenge with HA peptide 307–319 and APC (data not shown), suggested that anergy induction by SEB may not require MHC class II molecules.

Generation of MHC Class II Antigen-negative T Cells Expressing the HA1.7 TCR. The TCR α and β chain cDNAs encoding the HA1.7 TCR were cloned from HA1.7 RNA using AnPCR. 20 clones containing a PCR fragment of the correct size for TCR α were sequenced. All 20 clones consisted of an identical sequence with no evidence for a second TCR α transcript. The HA1.7 Vα region differed from the Vα1.2 subfamily sequence by a single, conservative base change
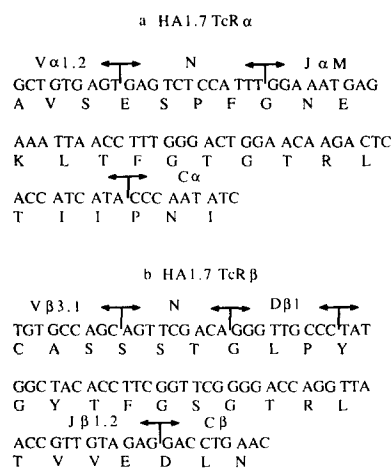
(25). The N region consisted of twelve nucleotides, and was unique to HA1.7 (Fig. 2 *A*). The core sequence of the HA1.7 Jα region was identical to that used by clone AA13 (26), and was most similar, but not identical, to a member of the M Jα family (27).

20 clones of the single TCR β chain PCR fragment were also sequenced. All 20 clones consisted of a Vβ3.1 region identical to that used by the clone PL4.4 (28), a unique 9-bp N region, and a Dβ region with the conserved core sequence Gly, Leu, Pro (Fig. 2 *B*). The J region was identical to the Jβ1.2 region used by the cell line JM (29).

Full-length coding sequences of the TCR α and β chains were constructed by ligation with the TCR α and β constant regions of Jurkat. Constructs were sequenced to ensure the correct joining of the constant regions. Each TCR cDNA was recloned into expression vectors and transfected into the CD3-TCR negative, MHC class II-negative mutant T cell line JRT3T3.5. The transfected line was cloned by limiting dilution, and the clone CH7C17 was characterized further. A mock transfected line OGSL1 was also characterized in parallel.

The mock transfected subline OGSL1 and the HA1.7 transfectant CH7C17 were examined for surface expression of CD3, TCR-α/β, and TCR Vβ8 by immunofluorescence. OGSL1 was completely CD3, TCR-α/β, and Vβ8 negative, and CH7C17 expressed high levels of CD3 and the TCR-α/β, but no TCR Vβ8, demonstrating that the TCR expressed by CH7C17 was not derived from the native TCR Vβ8 positive Jurkat cell line (data not shown). CH7C17 was also assayed for the expression of MHC antigens. Immunofluorescence analysis using mAbs, specific for monomorphic epitopes of HLA-DR antigens, HLA-DP antigens, all MHC class II antigens (including HLA-DQ), and all HLA class I antigens showed that both the native Jurkat cell line, OGSL1, and CH7C17 expressed no detectable MHC class II antigens (Fig. 3). All cell lines however, were strongly positive for MHC class I antigens (data not shown; Fig. 4 *A*). This confirms results from independent laboratories which demonstrate the
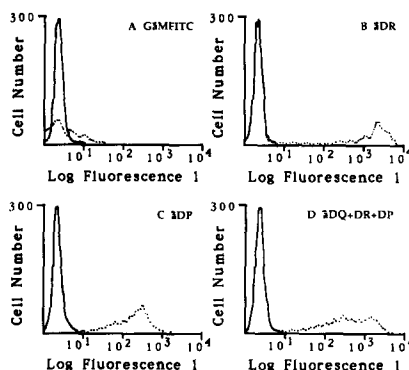

a HA1.7 TcR α

Vα1.2 ◄─┬─► N ◄─┬─► Jα M
GCT GTG AGT GAG TCT CCA TTT GGA AAT GAG
A   V   S   E   S   P   F   G   N   E

AAA TTA ACC TTT GGG ACT GGA ACA AGA CTC
K   L   T   F   G   T   G   T   R   L
◄─┬─► Cα
ACC ATC ATA CCC AAT ATC
T   I   I   P   N   I

b HA1.7 TcR β

Vβ3.1 ◄─┬─► N ◄─┬─► Dβ1 ◄─┬─►
TGT GCC AGC AGT TCG ACA GGG TTG CCC TAT
C   A   S   S   S   T   G   L   P   Y

GGC TAC ACC TTC GGT TCG GGG ACC AGG TTA
G   Y   T   F   G   S   G   T   R   L
Jβ1.2 ◄─┬─► Cβ
ACC GTT GTA GAG GAC CTG AAC
T   V   V   E   D   L   N

Figure 2. Sequence of HA1.7 TCR α and β chain V-N-(D)-J-C junctional regions. (*a*) TCR α chain. (*b*) TCR β chain. These sequence data are available from EMBL/Genbank/DDBJ under accession numbers X63455 (HA1.7 TCR alpha chain) and X63456 (HA1.7 TCR beta chain).

Figure 3. Jurkat transfectants fail to express surface MHC class II antigens. CH7C17 (*solid line*) and LNAT (*dotted line*) were stained with (*A*) goat anti–mouse Igs conjugated with FITC (GaMFITC); (*B*) FITC-conjugated anti-HLA-DR (Becton Dickinson & Co., Mountain View, CA); (*C*) anti-HLA-DP (B7/21/2) and GaMFITC and (*D*) anti-HLA-DQ + DR + DP (CA2.11) + GaMFITC, then analyzed by flow cytometry.
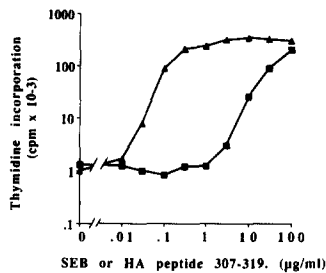
Figure 4. Reconstitution of HA1.7 TCR antigen-MHC specificity. JRT3T3.5 was transfected by electroporation with cDNAs encoding the TCR α and β chain of HA1.7. Transfected cells were cloned (CH7C17) and stimulated with LNAT APC and peptide 307–319 (*filled squares*) or SEB (*filled triangles*). T cell activation was assessed by lymphokine production.

Figure 5. SEB induces downregulation of CD3, and stimulates lymphokine production by CH7-C17 in the absence of MHC class II antigens (A) CH7C17 was incubated for 16 h with various concentrations of SEB in the absence of APC, cells were washed and stained with anti-CD3 (*filled squares*, left hand scale) or anti-MHC class I (*filled triangles*, right hand scale) and analyzed by flow cytometry. CH7C17 was stimulated for 16 h with various concentrations of SEB in the absence of APC (*filled squares*); supernatants were taken and assayed for lymphokines. Various concentrations of SEB (*filled triangles*) were incubated for 16 h in the absence of cells. Supernatants were then tested for the direct activation of CTLL-M cells.

complete absence of both cell surface MHC class II antigens on various sublines of Jurkat, (S. Marsh and J. Bodmer, personal communication), and mRNA for HLA-DP, DQ, and DR (45).

Unequivocal evidence that the TCR expressed by CH7C17 was identical to that expressed by HA1.7, was obtained by functional analysis. CH7C17 and OGSL1 were challenged with various concentrations of HA peptide 307–319 and SEB in the presence of LNAT APC. After 24 h of culture, supernatants were assayed for lymphokine content in a CTLL-M bioassay.

As shown in Fig. 4, CH7C17 responded to both HA peptide 307–319 and SEB with a concentration-dependent response, demonstrating that the specificity of the HA1.7 TCR had been reconstituted in CH7C17. The mock transfected cell line, OGSL1, was completely unresponsive to HA peptide 307–319 and SEB presented by LNAT APC. Neither of the cell lines responded to SEC_1 (data not shown).

MHC Class II Independent SEB-mediated Signaling in CH7C17. CH7C17 was incubated in the complete absence of MHC class II-positive APC with SEB at concentrations up to 100 μg/ml. After overnight incubation, cells were washed and assessed for the level of CD3 expression. The culture supernatants were taken for lymphokine assay.

The level of cell surface CD3 on CH7C17 cells decreased in an SEB concentration-dependent manner, such that higher concentrations of SEB resulted in lower cell surface expression of CD3 (Fig. 5 A). In contrast to CD3, the level of MHC class I antigen expression was increased with the concentration of SEB (Fig. 5 A). MHC class II expression, however, was not induced after incubation with SEB. Using CA2.11, a mAb specific for HLA-DR + DP + DQ antigens, no change was observed in the mean fluorescence intensity of CH7C17 after incubation in medium alone, or in the presence of SEB. A mean fluorescence intensity of 3.4 was observed with CA2.11 and secondary antibody in both the presence and absence of 20 μg/ml SEB. Downregulation of CD3 was specifically due to SEB as SEA, SEC_1, SEC_2, and SEE failed to induce any change in CD3 expression (data not shown). Lymphokine production by CH7C17 cells also increased with the concentration of SEB. This increase was not due to the direct interaction of SEB with the CTLL-M cells (Fig. 5 B).

CH7C17 Preincubated with SEB in the Absence of MHC Class II Antigens Fails to Respond to a Subsequent Immunogenic Challenge with SEB CH7C17 was incubated without MHC class
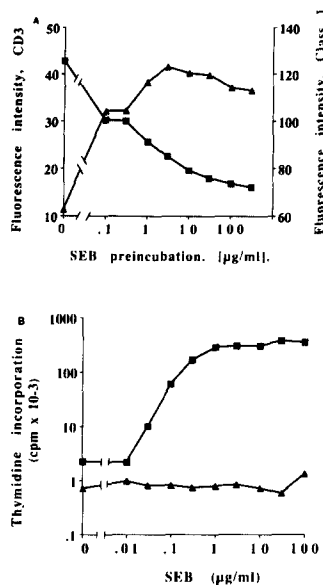
II-positive APC with SEB at various concentrations up to 100 μg/ml. After overnight incubation, cells were washed and loaded with Fura-2 for assay of changes in intracellular calcium ion concentration. Fura-2-loaded cells were then stimulated with SEB (15 μg/ml) in the absence of APC in stirred cultures. Changes in intracellular calcium ion concentration upon stimulation were measured using a fluorimeter.

Within 10 s of restimulation with SEB, the concentration of intracellular calcium increased, peaking at a level dependent upon the concentration of SEB used in the overnight incubation, such that preincubation with higher concentrations of SEB rendered CH7C17 less responsive to the subsequent exposure (Fig. 6). This effect was specific to SEB, as cells incubated overnight with various concentrations of SEC_1 (which does not activate HA1.7) had no effect on the increase in intracellular calcium ions stimulated by 15 μg/ml SEB. Neither SEB nor SEC_1 was able to increase intracellular calcium above basal levels in the TCR-negative cell line OGSL1 (data not shown).
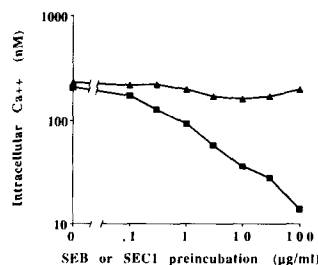

Figure 6. SEB induces unresponsiveness in CH7C17 cells. CH7C17 was incubated for 16 h with various concentrations of SEB (*filled squares*) or SEC_1 (*filled triangles*). Cells were washed and loaded with Fura-2, then stimulated in the absence of APC with 15 μg/ml of SEB. Changes in fluorescence induced by an increase in intracellular calcium ion concentration were measured, and the concentration of intracellular calcium calculated.

Discussion

This study demonstrates that a TCR of defined antigen specificity is able to interact with SEB in the absence of MHC class II antigens. Furthermore, the direct interaction of SEB with the TCR was able to stimulate a program of events leading to clonal anergy in the T cell clone HA1.7.

Characteristically, superantigens are able to bind to all TCRs using particular $V\beta$ regions irrespective of the antigen-MHC specificity of the TCR (3). This implies that the superantigen binding region shared by certain TCR $V\beta$ regions is remote from the CDRs involved in MHC-restricted peptide-antigen recognition. In support of this, two recent studies have analyzed the reactivity of TCR mutants and close $V\beta$ family members with different reactivities, and predicted that superantigen binding sites lie on the side of the TCR molecule, away from the peptide antigen-MHC binding site (30, 31). Similar studies on the MHC requirement of superantigen recognition have shown that many MHC class II antigen isoforms are able to present a particular superantigen to TCR. This suggests that superantigens bind to common sites on MHC class II antigens remote from the peptide antigen binding groove.

The present results demonstrating anergy induction by peptide 307–319 in the presence of SEC_2 (Fig. 1 B) support this conclusion. Others have characterized this binding site using cell lines that express MHC molecules with mutations in the peptide antigen binding groove (32), or by demonstrating the binding of labeled superantigens to structurally similar MHC class II molecules (33).

In a recent study of clonal T cell anergy in HA1.7, we demonstrated that human T cells of defined antigen-MHC specificity, when exposed to high concentration of SEB, become anergic to a subsequent immunogenic stimulus with their natural peptide ligand and APC (12). The present report demonstrates that, in contrast to peptide-mediated anergy, SEB-induced anergy in HA1.7 is not MHC dependent. Thus, pretreatment of HA1.7 with anti-MHC class II mAb or SEC_2, which binds to an identical site on HLA-DR1, but fails to interact with the HA1.7 TCR, is unable to inhibit SEB-induced anergy.

To demonstrate directly that SEB interacts with the TCR in the absence of MHC antigens, cDNAs encoding the HA1.7 TCR were introduced into a T cell tumor line which expressed no cell surface TCR and MHC class II. Incubation of these cells with SEB in the absence of APC resulted in SEB concentration-dependent T cell activation, as measured by a rapid increase in the concentration of intracellular calcium ions and lymphokine production. In common with HA1.7, incubation of CH7C17 with SEB in the absence of APC reduced cell surface CD3 expression in a dose-dependent manner. This downregulation was of functional significance, as the cells mounted a reduced Ca^{2+} response to a subse-

quent exposure to an optimal concentration of SEB. Studies in vivo (34) support the conclusion that the level of TCR-CD3 expression is of fundamental importance in the mechanism of both T cell activation and anergy.

The absence of cell surface MHC class II expression by the transfected mutant cell line derived from Jurkat is of central importance to this study. In addition to immunofluorescence results reported here, (Fig. 3), and those by independent groups (S. Marsh and J. Bodmer, personal communication) analysis of mRNA specific for HLA-DP, DQ, and DR has determined that the parental cell line of CH7C17, Jurkat, does not produce mRNA for HLA-DP, DQ, or DR (45). Furthermore, J. D. Fraser (35), reports that SEA and SEB are unable to bind to the surface of Jurkat cells, confirming that these cells are MHC class II negative. The complete MHC class II negativity of Jurkat was also shown functionally by demonstrating the inability of Jurkat cells to present enterotoxins to unprimed human PBLs (35).

There is a single report that small amounts of MHC class I H chain can be immunoprecipitated from MHC class II–positive cells pulsed with enterotoxin. This study concludes that a small amount of class I H chain might interact with preexisting enterotoxin-MHC class II complexes (35). There is no evidence, however, that MHC class I antigens are able to bind directly to, or present enterotoxins to T cells (36). Indeed, even CD8 positive, class I-restricted CTL TCRs interact with SE only when bound to MHC class II (37). We therefore conclude that SEB-induced downregulation of CD3 in CH7C17 and anergy in HA1.7 is MHC independent and involves a direct interaction between the TCR and SEB.

Other groups have also obtained circumstantial evidence that superantigens might bind directly to the TCR (37–40). Immobilized or cross-linked Streptococcal M toxin has been shown to stimulate native Jurkat cells to produce IL-2 (41). T cells may also be stimulated in the absence of APC by conjugation of a T cell-surface antigen-specific mAb to the same solid support as an enterotoxin (42). There is also a report that enterotoxins may behave in a similar manner to soluble anti-CD3 mAb in their ability to induce early activation events in T cells (38).

The ability to anergize T cells in the absence of MHC class II antigens using enterotoxins has clinical implications in, for example, autoimmune and allergic diseases, where there may be restricted TCR $V\beta$ usage in the harmful T cell responses, but where the antigen is either not determined or extremely complex, and involvement of MHC antigens is ill-defined. In these cases, enterotoxins modified to separate the enterotoxic (emetic) from TCR binding activity (43), and possibly the TCR from MHC binding activity (44), could be used to anergize T cells expressing particular $V\beta$ regions implicated in disease processes.

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