The CD4 Molecule Is Not Always Required for the T Cell Response to Bacterial Enterotoxins

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Summary

T cells respond in a Vβ-restricted fashion to bacterial enterotoxins bound to major histocompatibility complex (MHC) class II molecules. The requirement for CD4 in MHC class II–restricted T cell responses is very well established. We have assessed the role of CD4 in the T cell response to the bacterial enterotoxins Staphylococcal enterotoxin A (SEA), SEB, and toxic shock syndrome toxin 1. Three $CD4^-$ murine T cell hybridomas were transfected with the human CD4 molecule and assayed for interleukin 2 production in the presence of accessory cells bearing human MHC class II molecules and of the appropriate enterotoxin. The results clearly indicate that $CD4^-$ cells responded even to suboptimal concentrations of enterotoxin(s) equally well as $CD4^+$ cells. Furthermore, expression of CD4 did not result in the acquisition of previously undetectable reactivity to enterotoxins. These results suggest that unlike the case with antigen-specific responses, formation of a T cell receptor–CD3/CD4 supramolecular complex is not always essential for T cell activation by bacterial enterotoxins.

T cells recognize bacterial toxins bound to MHC class II molecules in a Vβ-restricted fashion (1–6). A role for the coreceptor molecules CD4 and CD8 in antigen-specific T cell recognition and T cell activation has been well established (7, 8). These coreceptor molecules are thought to increase the affinity and avidity of the TCR/CD3 complex for the antigen plus MHC (9, 10). In addition, a signaling function for CD4 and CD8 molecules upon their engagement by their respective MHC molecule ligands (class II and class I) has been demonstrated (11, 12). The role of CD4 and CD8 molecules in the T cell response to bacterial enterotoxins has not been established. Proliferation of $CD8^+$ T cells to Staphylococcal enterotoxin A (SEA)[1] and SEB in the presence of monocytes (13, 14) suggests that CD4 MHC class II interaction may not be required for T cell recognition of toxins bound to MHC class II molecules. However, these experiments do not rule out an enhancing role for CD8-MHC class I interaction in this response. The reported inhibition of the T cell response to bacterial enterotoxins by mAbs to CD4 (15) is difficult to interpret in view of the known capacity of anti-CD4 mAbs to deliver negative signals to T cells. In this study, we have introduced the human CD4 molecule into three $CD4^-$ murine T cell hybridomas and examined the effect of CD4 expression on the T cell response to a panel of enterotoxins presented by cells expressing human MHC class II molecules. We find that in contrast to its well-known role in enhancing antigen-specific responses, CD4 does not always play a role in enterotoxin-mediated T cell activation.

[1] *Abbreviations used in this paper:* SE, Staphylococcal enterotoxin; TSST-1, toxic shock syndrome toxin 1.

Materials and Methods

Effector Cell Lines. 3DT52.5.8 is a murine T cell hybridoma specific for D^d (16), kindly donated by Drs. J. Kappler and P. Marrack (National Jewish Hospital, Denver, CO). I.1.B3 is a human $CD4^+$ subclone derived from 3T52.5.8 after infection with a retroviral vector containing a full-length cDNA encoding for the human CD4 molecule (17). 3DT tCD4 is a subclone of 3DT52.5.8 expressing, after infection, a truncated form of the human CD4 molecule lacking amino acids 31–38 within its cytoplasmic domain. BI is a beef insulin specific T cell hybridoma (18) obtained from Dr. A. Reske-Kunz (Mains, FRG). BI CD4 is a human $CD4^+$ subclone derived from BI as described above (17) for I.1.B3. By155.16 is a murine T cell hybridoma specific for HLA-DR4.6, and 16CD413.13 is a subclone of By155.16 expressing the human CD4 after retroviral infection as described earlier (19).

Accessory Cell Lines. The DAP-3 murine fibroblast line and the DAP 3 $DR1^+$ cells have been previously described (20). The human $DR5^+$, DRw5 lymphoblastoid cell line WT17 was obtained from the HIGHS human cell repository (Camden, NJ). L cells transfected with $E\alpha^d/E\beta^d$ (RT10 3H2) or TK only RT1.12) were a generous gift from Dr. Ronald N. Germain. Human monocytes obtained from a DR3,5-positive donor were prepared as described previously (5).

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Toxins. Staphylococcal toxins SEA, SEB, and toxic shock syndrome toxin 1 (TSST-1) were purchased from Toxin Technology Lab Inc. (Madison, Wisconsin).

Flow Cytometry. T cell hybridomas were stained with a panel of mAbs specific for different T cell surface markers. These mAbs included 145 2C11 directed to the CD3 ϵ chain, mAb 12-15 M specific for the murine CD2 molecule, mAb FD44 M specific for the murine LFA-1 molecule, and OKT4, which recognizes the human CD4 molecule. Cells were stained and analyzed by flow cytometry on a FACScan® (Becton Dickinson & Co., Mountain View, CA) as previously described (17). Briefly, cells were incubated for 30 min at 4°C with saturating amounts of the specific antibody. Cells were then washed and incubated for 30 min at 4°C with a commercially available FITC-labeled goat anti-mouse Ig (Becton Dickinson & Co.). 10^4 live cells gated by propidium iodide staining were analyzed for each sample. Results are expressed as mean fluorescence intensity on a four-decade logarithmic scale.

IL-2 Assay. T cell hybridoma (7.5×10^4 cells for 3DT52.5.8, I.1.B3, 3DT tCD4, BI, BI CD4, and BI tCD4; and 5×10^4 cells for By155.16 and 16CD4.13.13) were stimulated with 1 μg/ml of TSST-1, SEA, or SEB in the presence of 7.5×10^4 irradiated (5,000 rad) DAP3 cells or 2×10^5 irradiated WT17, RT10.3H2, or RT1.12 cells, or monocytes. Coculture of effector and target cells was performed in 1.0 ml RPMI 1640 supplemented with 10% FCS, 100 U/ml penicillin, 10 μg/ml streptomycin, and 2 mM glutamine. After 24 h at 37°C, the culture supernatants were assayed for IL-2 by the hexaminidase colorimetric assay (17). The absorbance at 405 m was measured in a Multiscan EIA autoreader (E310; Biotek Instruments, Inc., Burlington, VA). The proliferation of CTTL-2 in the presence of variable amounts of rIL-2 (Cetus Corp., Emeryville, CA) was performed in parallel as a standard curve.

Results and Discussion

To assess the role of CD4 in the T cell response to bacterial enterotoxins, we examined the response of three murine T cell hybridomas with or without transfection of a full-length cDNA encoding the human CD4 molecule. Table 1 depicts the characteristics of these hybridomas, namely their antigen specificity, Vβ expression, and capacity to respond to a panel of different enterotoxins (SEA, SEB, and TSST-1). Flow cytometry analysis reveals identical levels of TCR cell surface expression on the $CD4^-$ and $CD4^+$ T cell hybridomas. These T cell hybridomas also expressed comparable levels of LFA-1 (data not shown), while cell surface expression of CD2 was not detected.

In the current study, we used accessory cells that express human MHC class II molecules because it is not yet established if human CD4 can effectively interact with murine MHC class II molecules. Fig. 1 shows that the murine T cell hybridoma By155.6 responded equally well to SEB in the presence of human Ia^+ monocytes, EBV-transformed B cells (WT17), or murine L cells transfected with $I\text{-}E^d$ (RT10). Production of IL-2 was not detected with accessory cells in the absence of SEB, nor when control L cells transfected with the TK gene (RT1 cells) were added together with SEB. Similar results were obtained with the other two hybridomas used in this study after stimulation with SEB (data not shown). The equivalent capacity of murine and human MHC class II^+ accessory cells to support the activation of the T cell hybridomas by bacterial enterotoxins is surprising in view of

Table 1. *Characteristics of T Cell Hybridomas*

Hybridoma*	Vβ‡ expression	CD3/TCR§	CD4‖	ED_{50}¶	Antigen specificity	Enterotoxin response
3DT52.5.8	8.1 and 1	19.1	6.07	11.2	H2 D^d	SEA, SEB TSST-1
IIB3**	8.1 and 1	19.6	31.2	2.2	H2 D^d	SEB
3DT tCD4‡‡		19.7	33.8	18.5		
BI	8.2	36.3	6.3	4.8	Beef insulin	SEB
BI WT CD4**	8.2	40.6	26.3	6.9	Beef insulin	SEB
By155.6	8.1	345.0	339	5.2	DR4,6	SEA, SEB
16CD4.13.13**	8.1	367.3	346.5	10.0	DR4,6	SEA, SEB

* Pairs of $CD4^-$ and $CD4^+$ hybridomas were selected for equivalent cell surface expression of TCR, as assessed by flow cytometry with the anti-CD3ϵ-specific mAb 2C11.

‡ Vβ expression as assessed by flow cytometry and reactivity with Vβ-specific mAbs kindly provided by Dr. P. Marrack.

§ Cells were stained with a mAb specific for the CD3ϵ 145 2C11 and analyzed by flow cytometry as described in Materials and Methods. Cells stained with an isotype-matched FITC-labeled irrelevant mAb were used as a negative control. Mean fluorescence of negative controls averaged 6.1 on a four-decade logarithmic scale.

‖ Cells were stained with mAb OKT4 specific for the human CD4 molecule and analyzed for flow cytometry as described in Materials and Methods.

¶ ED_{50} is the concentration (μg/ml) of enterotoxin required for half-maximal stimulation of T cell hybridomas. This concentration was obtained by nonlinear regression analysis using the ALL-FIT statistical program.

** Cells were transduced with a retroviral vector containing a full-length cDNA encoding the CD4 molecule.

‡‡ Cells were transfected with a truncated form of CD4 lacking 31 out of 38 amino acids of the cytoplasmic domain.

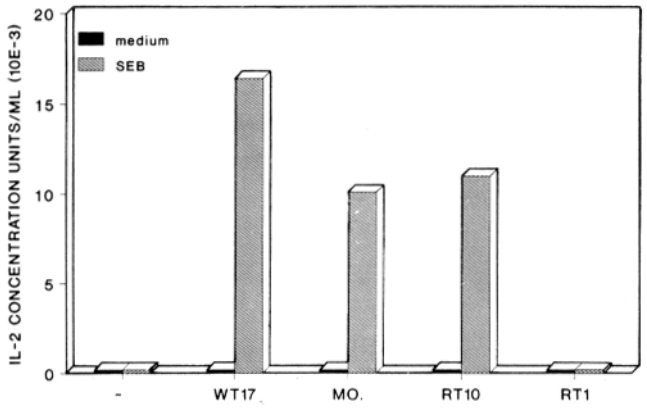

Figure 1. Response of the murine T cell hybridoma By155.6 to SEB in the presence of human or murine accessory cells. By155.6 recognizes human DR4, DR6 B cells. WT17 is a DR5, DRw5 homozygous EBV-carrying lymphoblastoid B cell line. DR3.5$^+$ monocytes were obtained by adherence on plastic plates at 37°C as described (18). RT10 is an L cell transfected with Eα^d + Eβ^d and selected by flow cytometry for elevated levels of the I-E^b molecules, whereas RT1 is a control L cell transfected with the TK gene (7, 16). After an overnight incubation of effector and MHC class II-positive cells in the presence or absence of 1 μg/ml of SEB, supernatants were harvested and assayed for IL-2 production using the IL-2-dependent line CTLL-2, and measured for hexaminidase release by colorimetry (19).

the fact that in antigen-specific T cell responses, murine TCRs interact inefficiently with human class II MHC molecules (7). These results validated the use of human class II MHC molecules to assess the role of human CD4 in the response of these hybridomas to bacterial enterotoxins. The use of L cell transfectants was further validated by the fact that interaction between LFA-1 and the intercellular adhesion molecules 1 and 2 is not required when L cells are used as APCs (21, 22).

Fig. 2 compares the response of CD4$^-$ and CD4$^+$ T cell hybridomas to all three toxins used at optimal concentrations (1–2.5 μg/ml) in the presence of DR1-transfected and control-untransfected DAP3 cells. Fig. 2 *A* demonstrates that the H2 D^d-specific hybridoma 3DT52.5.8, which expresses both Vβ8.1 and Vβ1, responded to all three enterotoxins tested. The CD4 transductant of this hybridoma, which expresses equivalent amounts of surface TCR/CD3 (Table 1) as the parent hybridoma, gave an equivalent response to all three toxins. Fig. 2 *B* shows that the beef insulin-specific hybridoma BI, which expresses the Vβ8.2 gene segment, responded to SEB but not to SEA or TSST-1. Expression of human CD4 in this hybridoma did not enhance its response to SEB. More importantly, human CD4 expression did not result in the acquisition of a response to SEA or TSST-1, although it was previously shown that expression of the human CD4 molecule in this hybridoma leads to enhanced response to beef insulin and in the acquisition of a new specificity to pork insulin. Fig. 2 *C* shows similarly that expression of human CD4 in the DR-specific hybridoma By155.6 did not enhance the response of this hybridoma to SEA or SEB and did not confer on it the ability to respond to TSST-1. These results indicated that the T cell response to optimal concentrations of bacterial enterotoxins was not dependent on the expression of CD4.

It was previously shown that CD4 plays a critical role in increasing the avidity of the TCR under limiting antigen concentrations (22). It was therefore important to examine the role of the CD4 molecule in T cell activation under limiting concentrations of enterotoxins. Results presented in Fig. 3 clearly indicate that CD4 expression did not enhance IL-2 production when the CD4$^+$ beef insulin-specific and the DR-specific T cell hybridomas were stimulated with suboptimal concentration of enterotoxins. Table 1 shows that the

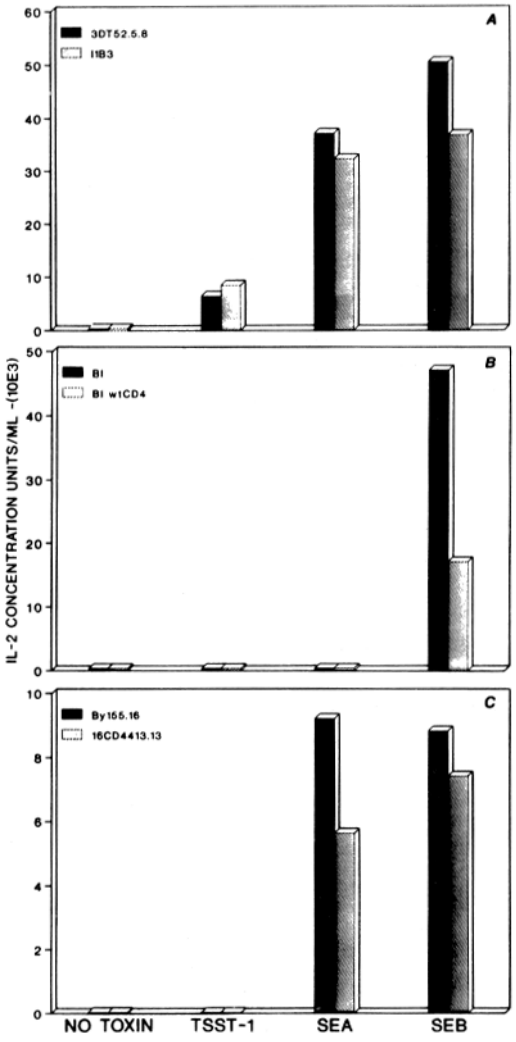

Figure 2. Response of T cell hybridomas to TSST-1, SEA, and SEB. The human CD4$^-$ and CD4$^+$ hybridomas described in Table 1 were stimulated with the bacterial enterotoxins SEA (1 μg/ml), SEB (1 μg/ml), and TSST-1 (2.5 μg/ml). (*A* and *B*) The fibroblastic DAP3 cells transfected with cDNAs encoding the DR1 molecule (16) were used as accessory cells. (*C*) Accessory cells consisted of the DR5, DRw5 lymphoblastoid cell line WT17. After overnight incubation of effector and MHC class II-positive cells in the presence of the above mentioned enterotoxins, supernatants were harvested and IL-2 was measured as described in Fig. 1.

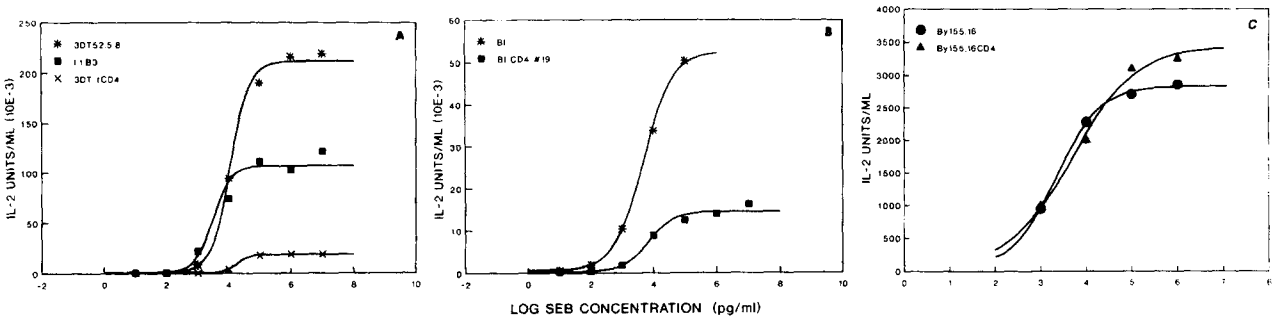

Figure 3. Enterotoxin dose-dependent IL-2 release by CD4$^-$ T cell hybridomas and CD4$^+$. T cell hybridomas were stimulated with decreasing concentrations of SEB covering a range of 4 to 6 logs in the presence of transfected cells expressing MHC class II molecules on their surface. IL-2 release was assayed as described for Fig. 1. Results of the experiments were analyzed using the ALL-FIT statistical program and a four-parameter logistic equation. Solid lines represent the computerized least square fit of the experimental data. Results are plotted on a semi-logarithmic scale with the log concentration of SEB on the *x*-axis and IL-2 units on the *y*-axis.

concentration of SEB required to obtain half-maximal stimulation (ED_{50}) of these hybridomas is not affected by the expression of CD4 or lack thereof.

A different picture emerged from the analysis of the H2 D^d-specific T cell hybridoma. This T cell hybridoma has the peculiar characteristic of expressing a TCR specific for a class I molecule; hence, TCR and CD4 can interact with two independent ligands unlike the above-mentioned systems, where CD4 and TCR probably interact with the same class II molecule on target cells. Half-maximal stimulation of the CD4$^+$ T cell hybridoma, as compared with the CD4$^-$ 3DT52.5.8 cells, occurred at 10-fold lower concentrations of SEB, clearly demonstrating an enhancing role for CD4 (Fig. 3 and Table 1). Moreover, the enhancement of the SEB response mediated by CD4 is dependent on the presence of the cytoplasmic domain of CD4. Indeed, Table 1 and Fig. 3 show that the ED_{50} for the 3DT52.5.8 cells transfected with a mutant CD4 molecule lacking the majority of its cytoplasmic domain (3DT tCD4) is comparable with that of the CD4$^-$ 3DT52.5.8 cells, suggesting that CD4 is not enhancing the response to SEB merely through an adhesion function. The results also suggest that the enhancing effect of CD4 in the hybridoma is not likely due to increased affinity of the TCR to the class II toxin complex but rather to positive signaling via CD4 upon engagement of CD4 by class II MHC molecules. It has been recently shown that mutant CD4 molecules lacking the last 32 amino acids of their cytoplasmic domain are not associated to the lymphocyte-specific tyrosine kinase $p56^{lck}$ (24, 25, and data not shown). The fact that the cytoplasmic domain of CD4 is required for the enhanced response to bacterial enterotoxins brings further evidence for the role of the cytoplasmic domain of CD4 and of the CD4-$p56^{lck}$ interaction in transducing positive signals.

In the present study, we have directly demonstrated that T cell triggering by bacterial enterotoxins occurs independently of the expression of CD4. Furthermore, CD4 does not appear to always play an accessory role in T cell triggering by bacterial enterotoxins. This is in direct contrast with the well-documented role of CD4 in enhancing T cell responses to antigenic peptides (23). Differences in avidity of the TCR could not account for the lack of a demonstrable role for CD4 in the T cell response to bacterial enterotoxins. Indeed, CD4 failed to enhance the T cell responses under conditions where the occupancy of the MHC class II molecule based on the ED_{50} of the toxin (26) was no more than 1%. This is in the proposed range of the occupancy of MHC class II molecules by antigenic peptides (27). The failure to detect a role for CD4 in T cell activation by enterotoxins may reflect an intrinsically higher affinity of the TCR for the toxin MHC class II complex compared with the antigenic peptide MHC class II complex. Alternatively, signal transduction after TCR/CD3 engagement by bacterial toxins may differ qualitatively or quantitatively from signal transduction after TCR/CD3 engagement by antigenic peptide. Consequently, effective signaling to T cells by toxins via the TCR may not require additional positive signaling via CD4. The availability of soluble TCR molecules to measure TCR affinity to class II toxin complexes combined with detailed signal transduction studies will help distinguish between these possibilities.

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