Superantigen-Dependent, Cell-Mediated Cytotoxicity Inhibited by MHC Class I Receptors on T Lymphocytes

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Bacterial superantigens bind with high affinity to major histocompatibility complex (MHC) class II antigens on antigen-presenting cells and with T cell antigen receptor (TCR) β chains on T lymphocytes, which results in the T cell activation responsible for toxic shock syndrome and food poisoning. Many cytotoxic T lymphocyte (CTL) clones were shown to have receptors for human leukocyte antigen (HLA) class I molecules that inhibited superantigen-induced cytotoxicity against appropriate class I–bearing target cells. One type of inhibitory receptor, NKB1, was present on $CD4^+$ and $CD8^+TCR\alpha\beta^+$ CTL clones and blocked the killing of staphylococcal enterotoxin B (SEB)–coated targets bearing certain polymorphic HLA-B molecules. Expression of HLA-A, -B, and -C molecules on the SEB-coated targets also protected against cytolysis mediated by many NKB1-negative T cell clones, suggesting the presence of additional inhibitory MHC class I receptors. These HLA class I receptors may limit tissue destruction and possibly autoimmunity caused by activated T lymphocytes.

Superantigens are a group of unprocessed bacterial and viral proteins that activate T cells in vivo and in vitro (*1*) by directly binding to the lateral exposed surfaces of MHC class II molecules on antigen-presenting cells (APCs) and to the variable region of the TCR β chain (V_β) on the responding T cells (*2–7*). Superantigens can thus interact with a large fraction of T cells, resulting in cellular activation, proliferation, anergy, or deletion of specific T cell subsets (*8–13*). Bacterial superantigens include staphylococcal enterotoxins A (SEA), B (SEB), C (SEC), D (SED), and E (SEE) and the toxic shock syndrome toxin–1 (TSST-1), which are the causative agents for several human diseases such as food poisoning and toxic shock syndrome (*1*). Although structural analysis of superantigens has defined how they bind to MHC class II and TCR molecules (*2–5, 7*), little is known about the immune regulation of superantigen-induced responses, particularly the inhibitory mechanisms required to limit superantigen-induced tissue pathology.

Natural killer (NK) cells express receptors that bind polymorphic MHC class I molecules on potential target cells and presumably transmit inhibitory signals that prevent NK cell–mediated cytotoxicity (*14, 15*). A subset of human NK cells express an MHC class I receptor (NKB1) that inhibits NK cell–mediated cytotoxicity against Epstein-Barr virus–transformed B lymphoblastoid target cells that express HLA-B molecules of the Bw4 serotype (*16, 17*). Additional inhibitory NK cell receptors may exist that recognize polymorphic HLA-A and -C molecules (*18–21*). We have investigated the expression of the NKB1 receptor on peripheral blood T cells and now present evidence which suggests that these inhibitory MHC class I receptors may regulate superantigen-induced T cell responses.

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NKB1 is expressed on a small percentage of freshly isolated peripheral blood T lymphocytes (Fig. 1). Examination of lymphocytes from 47 healthy, adult blood donors revealed NKB1 expression on 0.2 to 15% (mean, 1.6%) of $CD3^+$ T cells. Within the $CD3^+$,$NKB1^+$ T cell population, we detected T cells that expressed either CD4 or CD8 and the presence of $TCR\alpha\beta^+$ and $TCR\gamma\delta^+$ T cells (Fig. 1). The NKB1 antigen present on the cell surface of T lymphocytes is an ~70-kD glycoprotein (*22*), indistinguishable from the NKB1 glycoprotein immunoprecipitated from NK cells (*16*). To determine whether the NKB1 receptor expressed on T lymphocytes regulates effector cell function, we established $NKB1^+$,$CD3^+$,$TCR\alpha\beta^+$ T cell clones from the peripheral blood of healthy adults by single-cell sorting. These T cell clones stably maintain expression of the NKB1 receptor, whereas T cell clones derived from NKB1-negative T cells do not acquire NKB1 during in vitro culture (*22*).

The $NKB1^+$ T cell clones (including both $CD4^+$ and $CD8^+CD3^+$,$TCR\alpha\beta^+$ T cell clones) were assayed for the ability to kill the HLA class I–negative (class II–positive) B lymphoblastoid cell line 721.221 (*18*) in the presence (but not in the absence) of SEB (Fig. 2). To determine whether expression of HLA class I on the target cells affects their susceptibility to cytolysis, we tested these T cell clones for the ability to kill SEB-coated 721.221 target cells that were transfected with HLA-A, -B, or -C

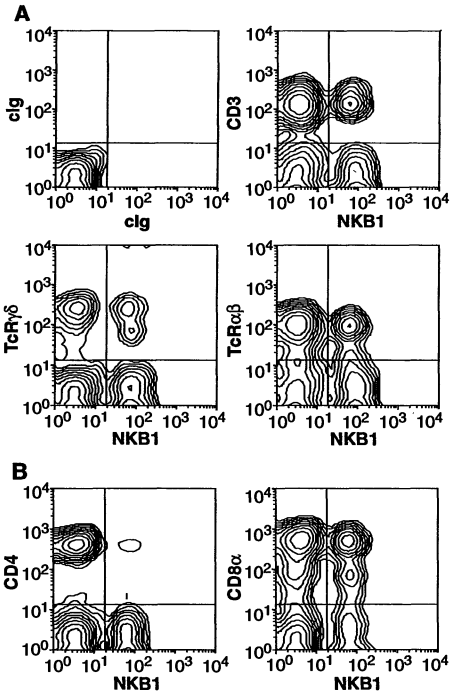

Fig. 1. Expression of NKB1 on peripheral blood T lymphocytes. (**A**) Peripheral blood mononuclear cells isolated from a healthy, adult donor were stained with fluorochrome-conjugated, isotype-matched control mAbs (cIg); fluoroscein isothiocyanate (FITC)–conjugated mAb to NKB1 (DX9 hybridoma) (*16*) and phycoerythrin (PE)-conjugated mAb to CD3 (mAb Leu 4); FITC-conjugated NKB1 and PE-conjugated mAb to TCRγδ (mAb 11F2); or FITC-conjugated NKB1 and PE-conjugated mAb to TCRαβ (mAb WT31), as indicated, by the techniques described previously (*16, 25*). (**B**) Peripheral blood mononuclear cells were stained with CyChrome-conjugated mAb to CD3 (Pharmingen, San Diego, CA), FITC-conjugated mAb to NKB1, and PE-conjugated mAb to CD4 (mAb Leu 3a) or CyChrome-conjugated mAb to CD3, FITC-conjugated mAb to NKB1, and PE-conjugated mAb to CD8 (mAb Leu 2a). Samples were analyzed by flow cytometry (*25*). An electronic gate was used to identify $CD3^+$ T lymphocytes, and the FITC and PE fluorescence of T cells are shown. Data are displayed as contour plots of emitted fluorescence (4 decade log scales). In the control sample, >98% of the lymphocytes are in the lower left quadrant of the contour plot. Results are shown for an individual with a relatively high percentage (~10%) of $NKB1^+$ T cells. In a study of 47 healthy adult donors, 1.6% (mean, range <0.1 to 15%) of $CD3^+$ T cells expressed the NKB1 receptor. The relative proportion of $TCR\alpha\beta^+$, $TCR\gamma\delta^+$, $CD4^+$, and $CD8^+$ T cells within the $NKB1^+$ T cell population varied among donors, but all subsets were represented in most individuals.

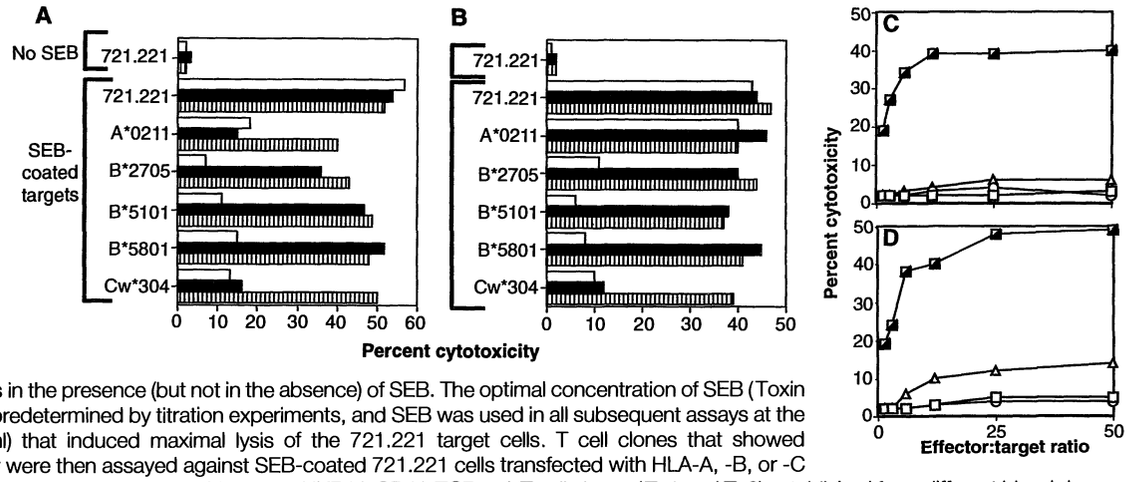

Fig. 2. Polymorphic HLA class I molecules protect 721.221 target cells from lysis by SEB-activated NKB1$^+$ T cell clones. NKB1$^+$,TCR$\alpha\beta^+$ T cell clones were established from four unrelated, healthy blood donors by single-cell sorting by means of flow cytometry and were cultured with conditions as described by Yssel *et al.* (*26*). These T cell clones were tested in a 4-hour radioisotope-release assay (*27*) for the ability to kill ^{51}Cr-labeled 721.221 target cells in the presence (but not in the absence) of SEB. The optimal concentration of SEB (Toxin Technology, Sarasota, FL) was predetermined by titration experiments, and SEB was used in all subsequent assays at the lowest concentration (3.5 ng/ml) that induced maximal lysis of the 721.221 target cells. T cell clones that showed SEB-dependent cytolytic activity were then assayed against SEB-coated 721.221 cells transfected with HLA-A, -B, or -C genes (*16, 17, 21*). Representative results are presented from two NKB1$^+$,CD4$^+$,TCR$\alpha\beta^+$ T cell clones (Tc1 and Tc2) established from different blood donors. NKB1$^+$ T cell clones Tc1 (**A**) and Tc2 (**B**) were assayed for lysis of ^{51}Cr-labeled 721.221 target cells or 721.221 transfectants that expressed the indicated HLA-A, -B, or -C alleles in the presence of SEB at an effector to target ratio of 12:1. No cytotoxicity (<10%) was observed against 721.221 (A to D) or the HLA class I transfectants in the absence of SEB. Cytolytic activity was determined in the presence of isotype-matched control mAb (□), F(ab′)$_2$ fragments of mAb to NKB1 (■), or F(ab′)$_2$ fragments of a mAb to monomorphic HLA class I [DX17 hybridoma (*17*)] (▥). Cytotoxicity at various ratios of effector to target cells for NKB1$^+$ T cell clones Tc1 (**C**) and Tc2 (**D**) against 721.221 target cells in the absence (□) or presence (◪) of SEB and the HLA-B*5801 transfectant of 721.221 in the absence (○) or presence (△) of SEB is shown. The concentration of mAb was 2.5 μg/ml in all assays.

genes. NKB1$^+$ T cell clones were unable to lyse SEB-coated 721.221 cells that expressed HLA-B*5801, -B*5101, and -B*2705 molecules; however, cytolytic activity against these SEB-coated class I transfectants was restored in the presence of F(ab′)$_2$ fragments of monoclonal antibody (mAb) to either NKB1 or to HLA class I (Fig. 2). Results from two NKB1$^+$,CD4$^+$,TCR$\alpha\beta^+$ T cell clones (Fig. 2) are representative of experiments with 35 NKB1$^+$ T cell clones established from four unrelated blood donors. These findings suggest that interactions between NKB1 on the CTL and the HLA-Bw4 (B*2705, B*5101, and B*5801) molecules on the 721.221 transfectants are responsible for the inhibition of SEB-induced cytotoxicity. These NKB1$^+$ T cell clones were also unable to kill SEB-coated 721.221 cells transfected with other HLA-A, -B, and -C genes; however, mAb to NKB1 did not affect target cell protection conferred by these other polymorphic HLA-A, -B, or -C class I molecules, showing the specificity of the NKB1 receptor and suggesting that other inhibitory receptors for HLA class I exist on these CTL clones (Fig. 2). For example, the target cell protection conferred by the HLA-A*0211 molecule was prevented by mAb to MHC class I, but not mAb to NKB1 (Fig. 2A), indicating the presence of another MHC class I receptor on this T cell clone specific for HLA-A*0211.

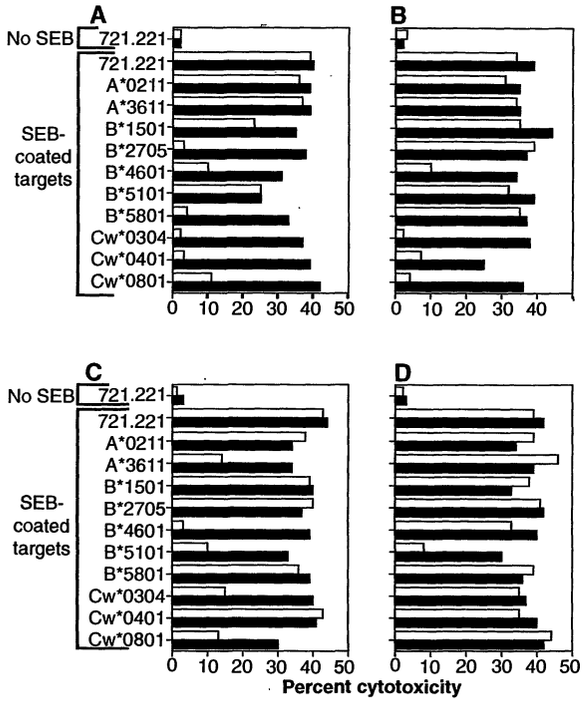

Fig. 3. Inhibition of SEB-induced cytotoxicity by polymorphic HLA class I molecules. CD3$^+$,TCR$\alpha\beta^+$ T cell clones were generated from the peripheral blood of healthy, adult donors with culture conditions as described (*26*). T cell clones were selected for the ability to kill the HLA class I–negative B lymphoblastoid cell line, 721.221, in the presence (but not in the absence) of SEB. These T cell clones were then tested in a 4-hour radioisotope-release assay for cytotoxicity against ^{51}Cr-labeled 721.221 cells or 721.221 cells stably transfected with the indicated HLA-A, -B, or -C alleles in the presence of SEB (3.5 ng/ml) at an effector to target ratio of 12:1. SEB-induced cytotoxicity of four representative T cell clones from the same blood donor are shown (A to D). T cell clones in (**A**) and (**B**) are CD4$^+$,TCR$\alpha\beta^+$ and T cell clones in (**C**) and (**D**) are CD8$^+$,TCR$\alpha\beta^+$. These T cell clones did not kill 721.221 (A to D) or the HLA class I transfectants in the absence of SEB. F(ab′)$_2$ fragments of a mAb to monomorphic HLA class I (DX17 hybridoma, 2.5 μg/ml; ■), but not an isotype-matched control mAb (□), reversed the protection conferred by the HLA class I molecules, demonstrating that expression of HLA class I on the target cells is responsible for target cell protection. The NKB1$^-$ T cell clone shown in (A) recognizes HLA-B*2705, -B*5101, or B*5801 molecules, suggesting that additional receptors with this specificity exist that are not recognized by the mAb to NKB1 (as we have observed previously with certain NKB1$^-$ NK cell clones) (*16*).

Although we have used NKB1$^+$ T cells to show that inhibitory MHC class I receptors exist, the ability of MHC class I on the target cell to prevent superantigen-dependent cytolysis is not restricted to T cells expressing NKB1. To show that these inhibitory MHC class I receptors are widely expressed on peripheral blood T lymphocytes, we generated NKB1$^-$,CD3$^+$,TCR$\alpha\beta^+$ T cell clones from the peripheral blood of healthy, adult donors. T cell clones were then assayed for the ability to kill the 721.221 target cells in the presence (but not the absence) of SEB. T cell clones (including both CD4$^+$ or CD8$^+$CD3$^+$,TCR$\alpha\beta^+$ T cell clones) that killed SEB-coated 721.221 targets were then tested for the ability to lyse SEB-coated 721.221 transfectants that expressed HLA-A, -B, or -C molecules. T cell clones from a given blood donor differed in their ability to kill the SEB-coated HLA class I transfectants, and target cell protection could be conferred by transfection with several MHC class I

genes. Results from four representative CTL clones (two $CD4^+TCR\alpha\beta^+$ and two $CD8^+TCR\alpha\beta^+$ T cell clones) are shown in Fig. 3. The protection from lysis is conferred by expression of HLA class I on the target cells because addition to the cytotoxicity assay of $F(ab')_2$ fragments of mAb to HLA class I restored killing of the SEB-coated transfectants (Fig. 3). Superantigen-induced cytolysis of 721.221 target cells was inhibited by mAb to CD3, showing involvement of the TCR-CD3 complex (*22*). Thus, CTL clones have, in addition to NKB1, a number of functionally similar receptors for HLA class I molecules that inhibit the cell-mediated cytotoxicity induced by the interaction between TCR on the effector T cells and SEB bound to MHC class II on the target APC. Although we used SEB in our experiments, similar results were obtained with other superantigens such as SEA and TSST-1 (*22*).

Our results indicate that many T cell clones may express inhibitory receptors that recognize polymorphic HLA class I molecules. Engagement of these receptors by their HLA class I ligands on potential target cells substantially limits the cytotoxicity induced by bacterial superantigens. These MHC class I receptors may also play a more general role in the regulation of T cell responses against alloantigens and conventional peptide antigens. In particular, inhibitory signals generated through these HLA class I receptors may prevent destructive autoimmunity against normal tissues in circumstances where T cells are activated during an inflammatory immune response (*23*, *24*).

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Cloning of Immunoglobulin-Superfamily Members Associated with HLA-C and HLA-B Recognition by Human Natural Killer Cells

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Cytotoxicity by natural killer (NK) cells is inhibited by major histocompatibility complex (MHC) class I molecules on target cells. This inhibition may be mediated by NK receptors with different MHC specificities. A family of four NK-specific complementary DNAs (cDNAs), designated NKATs (NK-associated transcripts), was identified that encoded related transmembrane proteins, characterized by an extracellular region with two or three immunoglobulin-superfamily domains and by a cytoplasmic domain with an unusual antigen receptor activation motif (ARAM). The distribution of these cDNAs was clonotypic and correlated with NK cell inhibition by particular class I alleles. Thus, NKAT cDNAs may encode receptors for class I molecules on NK cells.

Cytotoxicity by NK cells is controlled by activating and inhibitory receptors (*1*). Receptors that activate NK cells, such as the Fc γ receptor III (FcγRIII) and NKR-P1, belong to the immunoglobulin (FcγRIII) or to the C-type lectin (NKR-P1) superfamilies and trigger NK cytotoxicity when bound to an immunoglobulin Fc region or to a carbohydrate ligand on target cells, respectively (*2*). Receptors that inhibit NK cells turn off NK cytotoxicity when engaged with an MHC class I molecule on target cell (*3*). The only NK inhibitory receptor characterized, Ly-49, belongs to the C-type lectin superfamily (*4*). In humans, NK cell clones derived from the same individual are heterogeneous in their recognition of class I molecules, suggesting that there may be multiple NK inhibitory receptors with different MHC specificities that are clonotypically distributed (*5*). NK clones exist that are inhibited by Asn^{77}-Lys^{80} human leukocyte antigen–C (HLA-C) alleles (NK1-specific clones), by Ser^{77}-Asn^{80} HLA-C alleles (NK2-specific clones), and by Ile^{80} HLA-B alleles (NK3-specific clones) (*6*). Monoclonal antibodies for human NK inhibitory receptors have been reported, but the genes have not yet been identified (*7*–*9*).

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To clone these receptors, we amplified human NK cell mRNAs by reverse transcription–polymerase chain reaction (RT-PCR) using several sets of degenerate oligonucleotides derived from highly conserved regions shared by known molecules of the immunoglobulin-superfamily (Ig-SF) and C-type lectin superfamily. We searched for amplified cDNAs preferentially displayed by NK cells as compared to B, T, and myeloid cells (*10*).

Among several amplified fragments, we selected a ~240–base pair (bp) cDNA amplified from the NK cell clone 3D2 with one set of Ig-SF–specific degenerate primers (*11*). This fragment hybridized in Northern (RNA) blot analysis to a ~1.9-kb transcript and to a less abundant transcript of ~1.7 kb, which were detected in peripheral blood lymphocytes (PBLs), in the NK cell line NK3.3 (*12*), and in the tumor cell line SKW3 (*13*) (Fig. 1). No hybridization was observed with other tissues or with the B, T, and myeloid cell lines tested, suggesting that this cDNA is selectively expressed on NK cells (*14*).

A full-length cDNA sequence containing the 240-bp fragment was obtained by amplification of cDNA 5′ and 3′ ends (*15*). The resulting 1605-bp cDNA sequence, termed NKAT1 (NK-associated transcript 1), contains a single open reading frame that is predicted to encode a transmembrane protein of 348 amino acids with a