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Regulation of T Cell Lymphokine Production by Killer Cell Inhibitory Receptor Recognition of Self HLA Class I Alleles

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Summary

The killer cell inhibitory receptors (KIRs) are surface glycoproteins expressed by natural killer (NK) and T cells that specifically recognize defined groups of polymorphic human histocompatibility leukocyte antigen (HLA) class I molecules. Interactions between KIRs on NK or T cells and major histocompatibility complex (MHC) class I molecules on potential target cells inhibit cell-mediated cytotoxicity, presumably by delivering a negative signal preventing lymphocyte activation. In this study we examined whether KIRs also regulate cytokine production induced in response to T cell receptor-dependent T cell activation. CD4+ and CD8+ T cell clones were stimulated by bacterial superantigens in the presence or absence of monoclonal antibodies (mAbs) against the KIR NKB1 or MHC class I molecules, and production of tumor necrosis factor α and interferon γ was evaluated. When bacterial superantigen was presented by an autologous antigen-presenting cell (APC) to a KIR+ T cell clone, cytokine production was always enhanced in the presence of anti-MHC class I mAb. Similarly, anti-KIR mAb also augmented cytokine production, provided that the APC expressed a HLA class I allele recognized by the KIR. These results suggest that recognition of autologous MHC class I molecules by KIR+ T cells provides a regulatory mechanism acting to modulate the potency of their responses to antigenic challenge.
Figure 1. KIR regulation of cytokine production by T cell clones. (A and B) A NKB1+ CD8+ T cell clone (TC867.9) (10^6 cells/well) was cultured with 721.221 cells or 721.221 cells transfected with HLA-B*5801 (10^6/well) in the presence of control IgG1 (anti-Leu 19, CD56), anti-NKB1 (DX9), or anti-HLA-A,B,C (DX17) (10 μg/ml). The 721.221 cells and transfecants were preincubated with or without 10 ng/ml of SEB (30 min at 37°C) and washed, before coculture with T cells. (C and D) The T cell clone was incubated (without 721.221 cells) in the presence or absence of 10 ng/ml of soluble SEB and anti-NKB1 or anti-HLA-A,B,-C (10 μg/ml), as indicated. After 20-h culture at 37°C, cell-free supernatants were collected and assayed for TNF-α (A and C) or IFN-γ (B and D) by ELISA (mean of triplicate cultures, SD <10%).

**A**

Unstimulated

| clg/ media | 4 hr stimulation | clg/ SEB |
|------------|------------------|---------|
| <1%        | <1%              | <1%     |

**B**

anti-NKB1 / media

| anti-NKB1 / SEB | 3.1% |
|-----------------|------|
| <1%             |      |

**C**

anti-MHC I / media

| anti-MHC I / SEB | 17.0% |
|------------------|-------|
| <1%              |       |

**D**

anti-MHC I / SEB

| anti-MHC I / SEB | 12.8% |
|------------------|-------|
| <1%              |       |
were collected from each well and IFN-γ and TNF-α were measured by ELISA, as previously described (17, 18).

**Intracellular Staining.** Intracellular cytokines were detected by immunofluorescent staining, as described previously (19). PE-labeled anti-TNF-α and FITC-labeled anti-IFN-γ mAb were generously provided by Drs. Ken Davis and Skip Maino (Becton Dickinson Immunocytometry Systems).

**Northern Blot Analysis.** Northern blot analysis was performed as previously described (20). RNA (10 µg/lane) was transferred to nylon membranes and hybridized with a 32P-labeled KIR-NKB1 cDNA probe (2). Radioactivity was detected using a PhosphorImager (Molecular Dynamics, Sunnyvale, CA).

**Results and Discussion**

Superantigens are viral and bacterial proteins that activate T lymphocytes by binding to MHC class II molecules on APCs and to the V region of TCR β chains on the responding T cells (21). We previously demonstrated that KIR + CTL kill superantigen-coated class I-negative B-LCL, but are inhibited when the potential target cells express a HLA class I allele reactive with the KIR. Whereas KIRs have been implicated in regulation of cytotoxicity in both NK and T cells, less is known about the role of these inhibitory receptors in the regulation of other antigen-induced T cell functions, in particular, cytokine synthesis. To this end, we generated KIR+, TCR-α/β+ T cell clones (both CD4+ and CD8+) and selected them for further study based on their ability to produce cytokines in response to stimulation with SEB presented either by the HLA class I-deficient B-LCL 721.221 cell line or 721.221 transfectants stably expressing certain HLA class I alleles. The results presented in Fig. 1 are from a representative CD8+ T

![Figure 2](image_url). **Fig. 2.** SEB-induced cytokine production by KIR+ and KIR− T cell clones. For detection of intracellular cytokines, NKB1+ (AD130.2) (A) and NKB1− (T81) (B) T cell clones were either untreated or stimulated with 10 ng/ml of soluble SEB (4 h) in the presence or absence of the control IgG1, anti-NKB1, or anti-HLA-A,-B,-C (10 µg/ml). Cells were harvested after 4 h, fixed, and stained with fluorochrome-conjugated control IgG, PE anti-TNF-α, or FITC anti-IFN-γ. Data are displayed as bivariate dot plots (x and y axes, 4-decade-log scales). In samples stained with FITC- and PE-conjugated IgG control mAb, >98% of cells were in the lower right quadrant (not shown). The total percentage of cells secreting TNF-α, IFN-γ, or both cytokines (sum of upper right, upper left, and lower right quadrants) is indicated in each panel. For detection of secreted cytokines, T cell clones were cultured in the presence of increasing concentrations of SEB for 20 h at 37°C with 10 µg/ml of control IgG1 mAb (○), anti-HLA-A,-B,-C (DX17) (◇), anti-NKB1 (DX9) (△), or medium (□). TNF-α and IFN-γ in the cell-free supernatants of the NKB1+ (A) or NKB1− (B) T cell clones were measured by ELISA (mean of triplicate cultures, SD <10%).
cell clone (Tc867.9) expressing the KIR, NKB1 which recognizes HLA-Bw4 ligands (7). In the absence of SEB, no cytokines were produced in response to coculture with 721.221 or a 721.221 transfectant expressing HLA-B*5801 (a HLA-Bw4 allele). Higher levels of TNF-α and INF-γ were detected by the T cell clone when SEB was presented by the class I–negative APC, compared with the HLA-B*5801+ transfectant (Fig. 1, A and B). Suppression of cytokine production by the HLA-B*5801+ APC apparently is regulated by the KIR present on the T cell clone because anti-NKB1 mAb or anti-MHC class I mAb enhanced cytokine production to a level equal to or higher than when the SEB is presented by the HLA class I–negative APC. In parallel with experiments measuring cytokine secretion, this T cell clone was assayed for its ability to kill SEB-coated 721.221 or HLA-B*5801+ 721.221 cells, and consistent with prior findings, the NKB1 KIR also inhibited superantigen-induced cytotoxicity against targets expressing HLA-B*5801 (not shown). These results demonstrate that binding of the KIR to its class I ligand is not only capable of modulating T cell–mediated cytotoxicity but also has inhibitory effects on T cell activation pathways leading to lymphokine production. These results are consistent with a prior study demonstrating effects of anti-KIR mAb on cytokine production (10).

Whereas studies with MHC-deficient EBV-transformed B-LCL cell lines transfected with a single class I allele are useful to analyze the specificity of KIRs, they do not address the role of these inhibitory receptors in immune responses initiated by normal, autologous APCs. Because activated human T cells express MHC class II, they are able to serve as APCs for superantigens. Therefore, this provides the opportunity to study KIR regulation of a T cell response in a completely autologous system with SEB presented on autologous class II molecules and KIRs interacting with self-class I ligands. Preliminary studies demonstrated that HLA-DR+ human T cell clones are capable of autore cognition of SEB and that the response is blocked by antihLA-DR, mAbs (data not shown). To examine the role of KIRs in the regulation of cytokine production induced by autologous APCs, we selected a NKB1+ T cell clone from a HLA-Bw4+ individual so that the KIR is capable of interacting with a self-MHC class I ligand. These KIR+ T cells were cultured with SEB in the presence or absence of anti-NKB1 or anti-HLA class I mAbs, and cytokines were measured (Fig. 1, C and D). When the T cell clone autore cognition of SEB, lymphokine production (TNF-α and INF-γ) was substantially increased if self-class I recognition by the KIR was disrupted by either anti-NKB1 or anti-class I mAb. These results demonstrate that interactions between KIRs and self–HLA class I molecules may regulate TCR–induced immune responses initiated by autologous APCs. It should be noted that we used the same NKB1+ T cell clone for the experiments shown in Fig. 1, A–D to directly compare SEB presentation by the 721.221 APC (Fig. 1, A and B) and the autologous T cell as APC (Fig. 1, C and D). Similar results were obtained in both situations.

Further experiments were performed to evaluate the kinetics and the effects of superantigen concentration on KIR regulation of cytokine production. As shown in Fig. 2 A, a NKB1+ CD4+ T cell clone (AD130.2) was stimulated with SEB in the presence or absence of anti-NKB1 or anti-HLA class I mAb. To examine early time points in the SEB-induced response, cytokines were detected by using immunofluorescence staining of intracytoplasmic lymphokines. Substantially more TNF-α and INF-γ were present in the cytoplasm of KIR+ T cells stimulated in the presence of anti-NKB1 or anti–HLA class I mAb even within 4 h after SEB activation. After overnight culture, the ability of KIRs to suppress cytokine secretion by SEB-activated T cells was evident at all concentrations of superantigen tested, although at higher antigen doses the inhibition was not absolute, implying a quantitative regulation of activation. In general, KIR regulation of TNF-α production was more profound than control of IFN-γ secretion. IL-2 was not detected in these cultures and superantigen stimulation did not induce substantial proliferation under the conditions studied.

Whereas KIRs are present on a subset of T cells, many T cell clones lack expression of these molecules. For comparison with the KIR+ T cell clones, we selected T cell clones that were unreactive with all existing anti-KIR mAbs (including EB6, GL183, HP-3E4, and DX9) and did not possess KIR transcripts as determined by Northern blot analysis (Fig. 3). These KIR+ T cells were evaluated for SEB responsiveness in the presence or absence of anti–HLA class I mAb. A representative KIR+ CD4+ T cell clone (T81) is shown in Fig. 2 B. In this case, anti–HLA class I mAb had no effect on cytokine production induced by SEB, consistent with the lack of inhibitory receptor for self–HLA class I.

Whereas the NKB1 KIRs recognize HLA-Bw4 ligands, other members of the KIR family react with HLA-C or HLA-A molecules (5, 22, 23). KIR reactive with HLA-C may also regulate SEB-induced cytokine production. For example, the CD8+ T cell clone TC867.4 expresses a GL183 KIR that presumably can interact with this individual’s HLA-Cw*0703 allele. Consistent with this possibility, anti–HLA class I mAb enhanced SEB-induced cytokine production by this T cell clone (Table 1). An individual NK cell clone can simultaneously express two or more KIRs on the cell surface (22, 24). Moreover, often KIRs are present on NK cells of individuals who apparently lack self–class I alleles able to bind the receptor (25). Similarly, we have observed T cell clones expressing more than one KIR and...
Table 1. Regulation of TNF-α and IFN-γ Production by KIR Recognition of Self-class I Alleles

| Clone     | Phenotype | HLA type            | KIR expression | IFN-γ Medium | αNKB1 | αHLA-I | TNF-α Medium | αNKB1 | αHLA-I |
|-----------|-----------|---------------------|----------------|--------------|-------|--------|--------------|-------|--------|
| TC867.9   | CD8⁺      | A*0101, A*2902, B*0801(Bw6), B*1302(Bw4) CW*0603, CW*0703 | NKB1          | 223          | 456   | 427    | 385          | 1,405 | 1,338  |
|           | TCR-α/β   |                     |                |              |       |        |              |       |        |
| AD130.2   | CD4⁺      | A3, A30, B49 (Bw4), B62 (Bw6), CW3, CW3 | NKB1          | 2,566*     | 8,315* | 7,202* | 944          | 6,548 | 5,880  |
|           | TCR-α/β   |                     |                |              |       |        |              |       |        |
| TC867.4   | CD8⁺      | A*0101, A*2902, B*0801(Bw6), B*1302(Bw4) CW*0603, CW*0703 | GL183         | 113          | 168   | 342    | 137          | 174   | 1,332  |
| AD169.5   | CD8⁺      | A2, A3, B35 (Bw6), B50 (Bw6), CW4, CW6 | NKB1, GL183   | 589          | 474   | 1,287  | 153          | 102   | 687    |
| T81       | CD4⁺      | A1, A29, B8 (Bw6), B13 (Bw4) CW6, CW7 | None          | 978          | 946   | 778    | 2,634        | 2,490 | 1,927  |
|           | TCR-α/β   |                     |                |              |       |        |              |       |        |

TC867.9 and TC867.4 are monotypic for CD8⁺ and CD4⁺, respectively; AD130.2 and AD169.5 are monotypic for CD4⁺ and CD8⁺, respectively; T81 is monotypic for CD4⁺.

IFN-γ and TNF-α were analyzed by ELISA after stimulation for 20 h with 10 ng/ml of SEB in the presence or absence of anti-NKB1 (DX9, 10 μg/ml) or anti-MHC class-I (DXIT, 10 μg/ml) mAbs. IgG1 control mAb had no effect on cytokine production (not shown).

*SEB concentration = 5 ng/ml.

Receptors apparently unable to interact with self-class I ligands. T cell clone AD169.5 represents a cell expressing two KIRs, NKB1 and GL183; however, this individual does not express a HLA-B or HLA-C allele known to interact with NKB1 or GL183 (Table 1). Nonetheless, in functional assays, anti-HLA class I mAb augmented cytokine production, suggesting that yet another KIR or inhibitory receptor for a self-class I allele may be expressed by this clone. Results from these studies indicate that the distribution and function of KIRs on T cells are quite similar to NK cells and reveal the complexity of analyzing T cell clones able to simultaneously express multiple KIRs, only some of which may interact with self-class I ligands.

In summary, our findings suggest that KIRs affect not only NK cell–mediated cytotoxicity, but also cytokine production by CD4⁺ and CD8⁺ T cells, implying a more general regulatory role in the immune system. The exact mechanisms governing KIR expression on NK and T cells are unclear at present and an understanding of how the KIR repertoire of an individual is shaped by self-HLA is limited. We have observed that KIRs are preferentially expressed on peripheral T cells with a “memory” phenotype and are rare in thymus and cord blood, suggesting that these inhibitory receptors may be expressed late during differentiation and serve to regulate responses against antigens, although this remains to be proven. It is tempting to speculate that in the T cell lineage, KIR may provide a novel mechanism to increase the threshold of activation for effector T cells, thereby prohibiting stimulation due to encounters with low affinity, self-peptide/MHC complexes. That these receptors, upon binding to their class I ligands, are able diminish the potency of T cell responses, suggests a mechanism for limiting T cell activation possibly operative in the maintenance of tolerance or prevention of autoimmunity.

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793 D'Andrea et al. Brief Definitive Report
References

1. Wagtmann, N., R. Biassoni, C. Cantoni, S. Verdiand, M.S. Maltam, M. Vitale, C. Bottino, L. Moretta, A. Moretta, and E.O. Long. 1995. Molecular clones of the p58 natural killer cell receptor reveal Ig-related molecules with diversity in both the extra- and intracellular domains. *Immunity.* 2:439–449.

2. D’Andrea, A., C. Chang, K. Franz–Bacon, T. McClanahan, J.H. Phillips, and L.L. Lanier. 1995. Molecular cloning of NKB1: a natural killer cell receptor for HLA-B allotypes. *J. Immunol.* 155:2306–2310.

3. Colonna, M., and J. Samaridis. 1995. Cloning of Ig-superfamily members associated with HLA-C and HLA-B recognition by human NK cells. *Science (Wash. DC).* 268:405–408.

4. Wagtmann, N., S. Rajagopalan, C.C. Winter, M. Peruzzi and E.O. Long. 1995. Killer cell inhibitory receptors specific for HLA-C and HLA-B identified by direct binding and by functional transfer. *Immunity.* 3:801–809.

5. Moretta, A., M. Vitale, C. Bottino, A.M. Oreno, L. Morelli, R. Augugliaro, M. Barbarets, E. Ciccone and L. Moretta. 1993. p58 molecules as putative receptors for major histocompatibility complex (MHC) class I molecules in human natural killer (NK) cells. Anti-p58 antibodies reconstitute lysis of MHC class I-protected cells in NK clones displaying different specificities. *J. Exp. Med.* 178:597–604.

6. Litwin, V., J. Gumperz, P. Parham, J.H. Phillips, and L.L. Lanier. 1994. NKBI: an NK cell receptor involved in the recognition of polymorphic HLA-B molecules. *J. Exp. Med.* 180:537–543.

7. Gumperz, J.E., V. Litwin, J.H. Phillips, L.L. Lanier, and P. Parham. 1995. The Bw4 public epitope of HLA-B molecules confers reactivity with NK cell clones that express NKBI, a putative HLA receptor. *J. Exp. Med.* 181:1133–1144.

8. Burshtyn, D.N., A.M. Scharenberg, N. Wagtmann, S. Rajagopalan, K. Berrada, T. Yi, J.-P. Kinet, and E.O. Long. 1996. Recruitment of tyrosine phosphatase HCP by the killer cell inhibitory receptor. *Immunity.* 4:77–85.

9. Karlofer, F.M., R.K. Ribau and W.M. Yokoyama. 1992. MHC class I alloantigen specificity of Ly-49+ IL-2–activated natural killer cells. *Nature (Lond.).* 358:66–70.

10. Mingari, M.C., C. Vitale, A. Cambiaggi, F. Schiavetti, G. Meloli, S. Ferrini, and A. Poggi. 1995. Cytotoxic T lymphocytes displaying natural killer (NK)–like activity: expression of NK-related functional receptors for HLA class I molecules (p58 and CD94) and inhibitory effect on the TCR–mediated target cell lysis of lymphokine production. *Int. Immunol.* 7:697–703.

11. Phillips, J.H., J.E. Gumperz, P. Parham and L.L. Lanier. 1995. Superantigen-dependent, cell-mediated cytotoxicity inhibited by MHC class I receptors on T lymphocytes. *Science (Wash. DC).* 268:403–405.

12. Ferrini, S., A. Cambiaggi, R. Meazza, S. Forzini, S. Marciano, M.C. Mingari, and L. Moretta. 1994. T cell clones expressing the natural killer cell–related p58 receptor molecule display heterogeneity in phenotypic properties and p58 functions. *Eur. J. Immunol.* 24:2294–2298.

13. Yssel, H., J.E. De Vries, M. Koken, W. van Blitterswijk, and H. Spits. 1984. Serum-free medium for the generation and the propagation of functional human cytotoxic and helper T cell clones. *J. Immunol. Methods.* 72:219–227.

14. Litwin, V., J. Gumperz, P. Parham, J.H. Phillips, and L.L. Lanier. 1993. Specificity of HLA class I antigen recognition by human NK clones: evidence for clonal heterogeneity, protection by self and non-self alleles, and influence of the target cell type. *J. Exp. Med.* 178:1321–1336.

15. Shimizu, Y., and R. DeMars. 1989. Production of human cells expressing individual transferred HLA-A, -B, -C genes using an HLA-A, -B, and -C null human cell line. *J. Immunol.* 142:3320–3328.

16. Lanier, L.L., and D.J. Recktenwald. 1991. Multicolor immunofluorescence and flow cytometry. *Methods (Orlando).* 2:192–199.

17. Yssel, H., K.E. Johnson, P.V. Schneider, J. Wideman, A. Terr, R. Kastelein and J.E. De Vries. 1992. T cell activation–inducing epitopes of the house dust mite allergen Der p 1. Proliferation and lymphokine production patterns by Der p 1–specific CD4+ T cell clones. *J. Immunol.* 148:738–745.

18. Favre, C., J. Wijdenes, H. Cabrillat, O. Djossou, J. Banchereau, and J.E. de Vries. 1989. Epitope mapping of recombinant human gamma interferon using monoclonal antibodies. *Mol. Immunol.* 26:17–25.

19. Openshaw, P., E.E. Murphy, N.A. Hosken, V. Maino, K. Davis, K. Murphy and A. O’Garra. 1995. Heterogeneity of intracellular cytokine synthesis at the single-cell level in polarized T helper 1 and T helper 2 populations. *J. Exp. Med.* 182:1357–1367.

20. Lanier, L.L., C. Chang, and J.H. Phillips. 1994. Human NKIR-P1A: A disulphide linked homodimer of the C-type lectin superfamily expressed by a subset of NK and T lymphocytes. *J. Immunol.* 153:2417–2428.

21. Scherer, M.T., L. Ignatowicz, G.M. Winslow, J.W. Kappler, and P. Marrack. 1993. Superantigens: bacterial and viral proteins that manipulate the immune system. *Annu. Rev. Cell Biol.* 9:101–128.

22. Lanier, L.L., J. Gumperz, P. Parham, I. Melero, M. Lopez-Botet, and J.H. Phillips. 1995. The NKBI and HP-3E4 NK cell receptors are structurally distinct glycoproteins and independently recognize polymorphic HLA-B and HLA-C molecules. *J. Immunol.* 154:3320–3327.

23. Dohring, C., D. Scheidegger, J. Samaridis, M. Cella, and M. Colonna. 1996. A human killer inhibitory receptor specific for HLA–A. *J. Immunol.* 156:3098–3101.

24. Moretta, A., C. Bottino, D. Pende, G. Trpodi, G. Tambussi, O. Viale, A. Oreno, M. Barbarets, A. Merli, E. Ciccone, and L. Moretta. 1990. Identification of four subsets of human CD3–CD16+ natural killer (NK) cells by expression of clonally distributed functional surface molecules: correlation between subset assignment of NK clones and ability to mediate specific alloantigen recognition. *J. Exp. Med.* 172:1589–1598.

25. Gumperz, J.E, N.M. Vallele, P. Parham, L.L. Lanier, and D. Tyan. 1996. Heterogeneous phenotypes of expression of the NKBI natural killer cell class I receptor among individuals of different HLA types appear genetically regulated, but not linked to MHC haplotype. *J. Exp. Med.* 183:1817–1827.