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BRIEF COMMUNICATION

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Functional expression of the NKB1 killer cell inhibitory receptors on a γδ CTL clone

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The receptors for major histocompatibility complex (MHC) class I molecules on NK cells prevent NK cell-mediated cytolysis (Moretta et al. 1992; Gumperz and Parham 1995; Colonna 1996; Lanier and Phillips 1996). The known receptors for HLA-Bw4 and HLA-C molecules recognize polymorphic residues on residues 77–80 of MHC class I molecules (Colonna et al. 1993; Cella et al. 1994). Recent studies using monoclonal antibodies (mAb) specific for these receptors demonstrated that in addition to NK cells, a small percentage of peripheral T cells express these receptors (Aramburu et al. 1990; Ferrini 1994; Phillips et al. 1995). Moreover, it has been shown that superantigen-dependent cytotoxicity by αβ T cells is inhibited via the binding of the NKB1 receptor to HLA-Bw4 molecules (Phillips et al. 1995) and that NK-like activity of CTL is inhibited via the binding of p58 and CD94 molecules to HLA-Cw molecules (Mingari et al. 1995). Thus, since these receptors are expressed on both NK cells and CTL, it was recently proposed to call them killer-cell inhibitory receptor (KIR; Long et al. 1996). We recently showed that the recognition of some γδ T-cell clones is inhibited by the surface expression of HLA class I molecules on target cells (Nakajima et al. 1995). However, it still remains unknown whether these γδ CTL clones express known KIR for HLA class I molecules. In the present study, we attempted to demonstrate directly the surface expression and functions of these receptors on γδ CTL clones.

Our previous study suggested that six γδ CTL clones may carry heterogeneous KIR (Nakajima et al. 1995). To directly demonstrate KIR on γδ CTL, we examined the surface expression of known KIR on these γδ CTL clones using HP3E4 anti-p58, DX9 anti-NKB1, and HP3B1 anti-CD94 mAb. The results showed that these γδ CTL clones did not express these receptors (data not shown), suggesting that these γδ CTL clones may express KIRs for HLA-A or new KIRs that are not recognized by the available mAb. Therefore, in order to obtain γδ CTL clones expressing the KIRs that can be detected by these mAbs, we attempted to further establish γδ CTL clones from peripheral blood lymphocytes (PBL) of an HIV-1-infected individual as described previously (Nakajima et al. 1995). We selected killer cell clones which killed the NK-sensitive cell line C1R but did not kill the C1R transfectants expressing each HLA class I molecule. Of these killer cell clones, one clone (YY-D3-1) failed to effectively kill the C1R transfectants expressing HLA-B*5101(C1R-B*5101) and HLA-B*5201(C1R-B*5201) expressing the Bw4, while it effectively killed C1R transfectants expressing HLA-B*3501 (C1R-B*3501) expressing the Bw6 (Fig. 1). These results imply that this clone carries the NKB1 receptor. In fact, the flow cytometric analysis of this

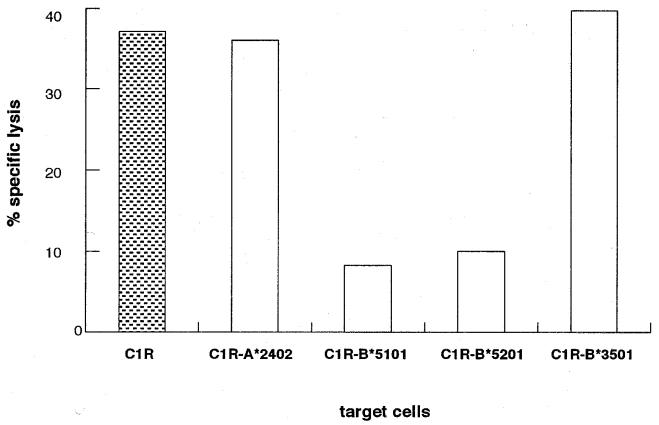

Fig. 1 The cytolysis of C1R cells expressing various HLA class I molecules by YY-D3-1 clone. Cytolysis of the YY-D3-1 clone for C1R and C1R transfectants was tested at an effector:target (E:T) ratio of 6:1. ^{51}Cr release in the supernatants of the mixture of ^{51}Cr-labeled target cells and the CTL clone was measured at 6 h after both cells were mixed. Percent-specific lysis was calculated as previously described (Nakajima et al. 1995). The surface expression of each HLA class I molecules on the transfectants was measured using flow cytometry with W6/32 mAb. The mean fluorescence intensity of the C1R transfectants was as follows: C1R;30.4, C1R-A*2402;389.5, C1R-B*3501;1437.2, C1R-B*5101;614.9, C1R-B*5201;1094.3

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Table 1 Flow cytometric analysis of surface molecules on the YY-D3-1 clone

	Monoclonal antibodies*										
CTL clone	TCR-αβ	TCR-γδ	CD3	CD4	CD8	CD16	CD56	p58	NKB1	CD94	–⁺
YY-D3-1	9.63‡	32.33	83.87	2.95	9.60	3.63	3.71	5.18	67.83	4.09	3.25

* mAb used for staining the CTL clone are as follows: WT31, 11F2, anti-Leu4, OKT4, anti-Leu2b, 3G8, B159, HP3E4, DX9, and HP3B1mAb for TCR-αβ, TCR-γδ, CD3, CD4, CD8, CD16, CD56, p58, NKB1, and CD94, respectively.

‡ Mean fluorescence intensities

⁺ The cells were stained with only FITC-labeled mouse-specific Ig antibodies

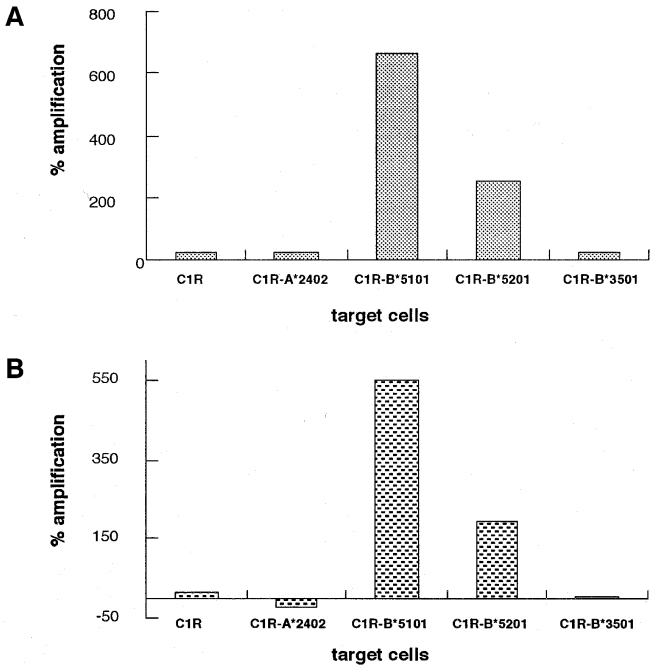

Fig. 2 A, B Restoration of cytotoxic activity of the YY-D3-1 clone for the C1R transfectants by blocking W6/32 anti-HLA class I mAb and DX9 anti-NKB1 mAb. **A** Cytotoxicity of the YY-D3-1 clone for C1R and the transfectants was examined at an E:T ratio of 4:1 after blocking of target cells with W6/32 mAb at a concentration of 10 μg/ml. Percent amplification of specific lysis was calculated as follows: % amplication = {(% specific lysis of target cells blocked with the mAb – % specific lysis of target cells without blocking of mAb)/% specific lysis of target cells without blocking of mAb}×100 **B** Cytotoxicity of the YY-D3-1 clone for C1R and the transfectants was examined at an E:T ratio of 4:1 after blocking of the CTL clone with DX9 mAb at a concentration of 10 μg/ml

clone revealed the surface expression of the NKB1 receptor (Table 1). This clone expressed CD3 and γδ TCR but neither CD4, CD8, CD16, nor CD56 (Table 1). Taken together, the results suggested that the γδ CTL clone carries functional NKB1 receptors.

In order to clarify the function of the NKB1 receptor on the YY-D3-1 CTL clone, we investigated the effect of W6/32 anti-HLA class I mAb and DX9 anti-NKB1 mAb on the cytolysis of C1R-transfectants. The cytolysis of C1R-B*5101 and C1R-B*5201 was effectively restored in the presence of W6/32 mAb, while this W6/32 mAb effect was not found in the cytolysis of C1R-B*3501 cells (Fig. 2). Moreover, the cytolysis of C1R-B*5101 and C1R-B*5201 cells by this clone was restored by the blocking of DX9 mAb (Fig. 2). These results indicate that the NKB1 receptors on YY-D3-1 CTL clone negatively regulate antigen recognition.

It is well known that NKB1 receptors contain three immunoglobulin superfamily domains in the extracellular domain (D'Andrea et al. 1995; Colonna et al. 1995) and recognize HLA-B molecules carrying the Bw4 epitope (Litwin et al. 1994). Residues 77–83 forming serological Bw4 epitope provide the binding site for these receptors (Gumperz et al. 1995). HLA-A*2402 also carries the Bw4 epitope that is detected by anti-Bw4 mAb (Müller et al. 1989), but a recent study showed that NKB1 receptors on NK cells do not negatively regulate the recognition of target cells expressing HLA-A*2402 by NK cells (Gumperz et al. 1995), suggesting that NKB1 receptors fail to recognize the Bw4 epitope on HLA-A*2402 molecules. In order to confirm the inability of NKB1 receptors to reorganize HLA-A*2402, we investigated recognition of the YY-D3-1 CTL clone for C1R-A*2402 cells. The cytolysis of C1R-A*2402 cells is almost identical to that of C1R cells (Fig. 1). Moreover, the blocking of W6/32 mAb and DX9 mAb failed to amplify cytolysis of the C1R-A*2402 cells (Fig. 2). These results indicate that NKB1 receptors on the γδ CTL clone do not recognize the Bw4 epitope on HLA-A*2402 molecules. HLA-A*2402 molecules carry the same sequences in the residues 77–82 with HLA-B*5101 and B*5201 (Sekimata et al. 1989). Our recent study showed that position 8 of nonamer peptides critically influence the binding of TÜ109 anti-Bw4 mAb to HLA-B*5101 molecules (Takamiya et al. 1996). Since this mAb can bind to HLA-B molecules carrying the Bw4 epitope but not to HLA-A molecules carrying the Bw4 epitope, it is speculated that NKB1 receptors also recognize the sequence of peptides bound to HLA class I molecules carrying the Bw4 epitope, and position 8 of peptides bound to HLA-A molecules carrying the Bw4 epitopes may affect the binding of NKB1 receptors.

NKB1 is preferentially expressed on T cells with a memory phenotype (Lanier and Phillips 1996). This molecule may control an immune reaction via T cells. Functional characterization of NKB1 molecules on T cells has been carried out only in the inhibition of superantigen-mediated cytolysis by $CD4^+$ and $CD8^+$ αβ T cells (Phillips et al. 1995). Here we directly demonstrated the functional expression of NKB1 receptors on the γδ T-cell clone. Thus, the present study provided additional evidence that NKB1 receptors negatively regulate recognition of T cells. Human γδ T cells have a crucial role in the elimination of tumor cells as well as virus- and bacteria-infected cells lacking

MHC class I molecules. Further studies concerning the role of KIR on γδ T cells are expected to clarify the molecular recognition via TCR and the inhibitory receptors.

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