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Coordinated Expression of Ig-Like Inhibitory MHC Class I Receptors and Acquisition of Cytotoxic Function in Human CD8+ T Cells

Nicolas Anfossi,* Jean-Marc Doisne,† Marie-Alix Peyrat,§ Sophie Ugolini,* Olivia Bonnau,** David Bossy,† Vincent Pitard,¶ Pierre Merville,¶ Jean-François Moreau,¶ Jean-François Delfraissy,‡ Julie Dechanet-Merville,¶ Marc Bonneville,2§ Alain Venet,2‡ and Eric Vivier2‡

MHC class I-specific inhibitory receptors are expressed by a subset of memory-phenotype CD8+ T cells. Similar to NK cells, MHC class I-specific inhibitory receptors might subserve on T cells an important negative control that participates to the prevention of autologous damage. We analyzed here human CD8+ T cells that express the Ig-like MHC class I-specific inhibitory receptors: killer cell Ig-like receptor (KIR) and CD85j. The cell surface expression of Ig-like inhibitory MHC class I receptors was found to correlate with an advanced stage of CD8+ T cell maturation as evidenced by the reduced proliferative potential of KIR+ and CD85j+ T cells associated with their high intracytoplasmic perforin content. This concomitant regulation might represent a safety mechanism to control potentially harmful cytolytic CD8+ T cells, by raising their activation threshold. Yet, KIR+ and CD85j+ T cells present distinct features. KIR+CD8+ T cells are poor IFN-γ producers upon TCR engagement. In addition, KIR are barely detectable at the surface of virus-specific T cells during the course of CMV or HIV-1 infection. By contrast, CD85j+CD8+ T cells produce IFN-γ upon TCR triggering, and represent a large fraction of virus-specific T cells. Thus, the cell surface expression of Ig-like inhibitory MHC class I receptors is associated with T cell engagement into various stages of the cytolytic differentiation pathway, and the cell surface expression of CD85j or KIR witnesses to the history of qualitatively and/or quantitatively distinct T cell activation events.


Abbreviations used in this paper: KIR, killer cell Ig-like receptor; ITIM, immunoreceptor tyrosine-based inhibition motif; ECD, phycoerythrin-Texas Red; BLCL, B lymphoblastoid cell line; AICD, activation-induced cell death.

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3 Abbreviations used in this paper: KIR, killer cell Ig-like receptor; ITIM, immunoreceptor tyrosine-based inhibition motif; ECD, phycoerythrin-Texas Red; BLCL, B lymphoblastoid cell line; AICD, activation-induced cell death.
far. Whereas most CD85j\(^{+}\) cells are KIR\(^{+}\), a minority coexpresses KIR (13). Although each inhibitory KIR interacts with specific determinants that characterize groups of HLA class I molecules, i.e., lys\(^{+}\) HLA-C, Asn\(^{+}\) HLA-C, HLA-B\(^{w4}\), HLA-A3 or HLA-A11, CD85j is an ITIM-bearing receptor for most (if not all) classical and nonclassical MHC class I molecules, via a direct interaction with their invariant α3 domain (14). CD85j is also a high affinity receptor for the UL-18 protein, a human MHC class I homologue encoded by human CMV (15). CD85j\(^{+}\) is expressed by more CD4\(^{+}\) T cells and more CD8\(^{+}\) T cells than are KIR\(^{+}\) (13). Within the CD8\(^{+}\) αβ TCR\(^{+}\) T cell compartment, CD85j is also expressed on CCR7\(^{+}\) memory phenotype T cells (7). We compared here the phenotypic and functional characteristics of KIR\(^{+}\)CD8\(^{+}\) T cells and CD85j\(^{+}\)CD8\(^{+}\) T cells, as well as their mode of induction.

Materials and Methods

Patients

Twenty-nine HIV-1-infected subjects who were not receiving therapy were studied. These individuals were diagnosed during acute HIV-primary infection, according to previously described criteria (16). After providing informed consent, they were included in the French PRIMO cohort, approved by institutional ethical review committee. Blood samples were then collected (day 0) and every 6 mo thereafter. After informed consent, kidney allograft male recipients diagnosed to develop CMV infection as described previously (17) were studied longitudinally.

Immunofluorescence

Immunostainings were performed using the following Abs purchased from Beckman Coulter (Fullerton, CA), BD Biosciences (San Diego, CA), or Caltag Laboratories (Burlingame, CA): unlabeled or PE-anti-CD158a/b (KIR2DL1/KIR2DS1), unlabelled or PE-anti-CD158b1/b2j (KIR2DL2/KIR2DS2), unlabelled or PE-anti-CD158e1 (KIR3DL1), unlabeled or PE-anti-CD85j, FITC- and PC5 anti-CD3, FITC-anti-TCRαβ, FITC-anti-perforin, biotin-anti-IFN-γ, PerCP-Cy5.5-anti-CD3, PerCP-anti-CD8α, phycoerythrin-Texas Red (ECD)-, PC5-, PC7- and allophycocyanin-anti-CD56, FITC- and PE-anti-CD27, FITC- and PE-anti-CD57, ECD-anti-CD580, FITC-anti-CD45RA and PE-anti-CD45RA. Unlabelled Abs were revealed using FITC- and PE-anti-mouse (polyclonal goat IgG) or biotin-mouse (polyclonal goat IgG). Biotinylated Abs were revealed using PerCP-streptavidin. In all the experiments, anti-KIR mAbs were used as a mixture of anti-CD158a/b (KIR2DL1/KIR2DS1), anti-CD158b1/b2j (KIR2DL2/KIR2DS2), and anti-CD158e1 (KIR3DL1). Samples were analyzed using a FACSCalibur flow cytometer and Cellequest software (BD Biosciences), an EpicsXL flow cytometer, or a FCS500 flow cytometer and RXP software (Beckman Coulter).

Multimer staining

HIV-1-specific CD8\(^{+}\) T cells were detected by PE-conjugated multimers purchased from ProImmune (Oxford, U.K.): gag 77–85, SLYNTVATL, HLA-A2; gag 20–28, RLPGPKKKL, HLA-A3; nef 90–97, FLKEKGLL, HLA-B8; gag 263–272, KRWILGLNK, HLA-B27. CMV-specific CD8\(^{+}\) T cells were detected by allophycocyanin-conjugated multimers purchased from Beckman Coulter: pp65, NLVPVMAVT, HLA-A2. Cells were incubated with pretitrated multimers (0.5 μg/ml) for 30 min at 4°C, followed by addition of a mAb panel and incubation for 15 min at room temperature. Cells were then washed and stored in 1% paraformaldehyde solution at 4°C until flow cytometry analysis was performed. Samples were acquired on a Beckman Coulter Epics XL and analyzed by the use of RXP software (Beckman Coulter).

Cell stimulation

For proliferation analysis, 2 × 10\(^{6}\) PBMC were stimulated for 4 days by different combinations of the following stimuli: anti-CD3 mAb (UCHT1) immobilized at 10 μg/ml, PMA (5 ng/ml; Sigma-Aldrich, St. Louis, MO) with ionomycin (0.5 μg/ml; Calbiochem, La Jolla, CA), IL-2 (1000 IU/ml, Proleukin; Chiron, Emoryville, CA), and recombinant human IL-15 (100 ng/ml; Prepeptech; London, U.K.). For cytokine production, 2 × 10\(^6\) cells were stimulated for 6 h in anti-CD3 mAb (UCHT1)-coated wells in the presence of pirebeldin A (10 μg/ml; Sigma-Aldrich). After surface staining, cells were permeabilized with PBS containing 1% saponin (Sigma-Aldrich) and 1% BSA (Invitrogen Life Technologies, Carlsbad, CA), and stained with biotin-anti-IFN-γ.

CFSE labeling

Cells were stained for 12 min at 37°C, in a 1 μM CFSE (Molecular Probes, Eugene, OR) containing medium (RPMI 1640) supplemented with 2% FCS.

Results

Comparison of KIR\(^{+}\) and KIR\(^{+}\) T cell clones sharing identical TCR

We previously reported the characterization of a panel of EBV-specific CD8\(^{+}\) T cell clones that share identical productive and nonproductive TCR transcripts but differ by the presence or absence of KIR cell surface expression (9). In all clones expressing an inhibitory KIR, engagement of KIR by cognate MHC class I ligand leads to the inhibition of Ag-induced T cell cytotoxicity (9). Further comparison of KIR\(^{+}\) and KIR\(^{+}\) T cell clones revealed that the Ag-induced proliferation of KIR\(^{+}\) T cell clones was drastically reduced when compared with that of KIR\(^{+}\) T cell counterparts (Fig. 1A). This proliferative hyporesponsiveness was observed within at least seven EBV-specific KIR\(^{+}\) T cell clones directed against two distinct MHC-peptide complexes (BMLF1/A*0201 and BZLF1/B*3501) (Fig. 1 and Table I). The inhibitory potential of KIR molecules prompted us to investigate whether the reduced proliferative capacity of KIR\(^{+}\) T cell clones was a consequence of KIR engagement by MHC class I ligands. A KIR2DL3\(^{+}\) T cell clone (BGL19) and its KIR\(^{+}\) counterpart (B2.5) characterized earlier (9) were thus compared for their HLA-B35-restricted proliferative response to the EBV Ag BZLF1 in the presence or absence of KIR2DL3 engagement. These experiments were performed using autologous HLA-B35, HLA-Cw3, HLA-Cw7 B lymphoblastoid cell lines (BLCL) or allogenic HLA-B35, HLA-Cw4, HLA-Cw4 BLCL as APCs. As shown in Fig. 1A, the proliferative capacity of the BGL19 KIR\(^{+}\) T cell clone was reduced in both situations, where KIR2DL3 can be engaged with its HLA-Cw7 and HLA-Cw3 cognate ligands (autologous BLCL) or not (allogenic BLCL). Addition of blocking anti-KIR2DL2/KIR2DL3 mAb (GL183) did not restore the Ag-dependent proliferation of BGL19 cells, making it unlikely that the endogenous expression of HLA-Cw7 and HLA-Cw3 on T cells can be involved in a putative KIR2DL3-mediated inhibition of BGL19 proliferation (Fig. 1B). Broad dose-response curves with anti-CD3 mAb as well as agonist and partial agonist antigenic peptides have been performed on KIR\(^{+}\) and KIR\(^{+}\) T cell clones, and confirmed the proliferative defect of KIR\(^{+}\) T cells as compared with the KIR\(^{+}\) T cell clones (data not shown).

![FIGURE 1. Reduced in vitro proliferative capacities of KIR\(^{+}\) T cell clones. Proliferation of KIR\(^{+}\) (B2.5) and KIR\(^{+}\) (BGL19) T cell clones was assessed by thymidine incorporation after 48 h in culture. A, T cell clones were cultured in the presence of autologous (HLA-Cw3/Cw7) or heterologous (HLA-Cw4/Cw4) Ag-loaded BLCL. B, T cell clones were cultured with IL-2, anti-KIR2DL2/KIR2DL3 (GL183, mouse IgG1), or both. These data are representative of six independent experiments with eight pairs of KIR\(^{+}\) and KIR\(^{+}\) T cell clones generated from two individuals.](http://www.jimmunol.org/content/173/5/7224/F1)
not shown). Of note, the pair of KIR\(^+\) and KIR\(^-\) T cell clones used in these experiments displayed similar TCR levels, were devoid of any other known inhibitory receptors (such as NKG2A/CD94, KIR2DL1, KIR3DL1, and KIR3DL2) (Table I), and did not show any difference in terms of costimulatory or adhesion receptors (data not shown). These experiments thus show that KIR\(^+\) T cell clones have a reduced proliferative capacity as compared with their KIR\(^-\) T cell counterparts, which is not the consequence of KIR engagement. They further suggest that the acquisition of KIR cell surface expression on T cells is associated with a differentiation program in which the proliferative potential is drastically weakened, as it is not restored by addition of cytokines, such as IL-2, IL-15, IL-6, alone or in combination (Fig. 1B and data not shown).

### Proliferative hyporesponsiveness of peripheral blood KIR\(^+\) and CD85\(^+\) T cells

Pairs of KIR\(^+\) and KIR\(^-\) T cell clones sharing identical TCR represent unique Ag-specific tools to dissect the biological relevance of KIR expression on T cells. Yet, we further compared KIR\(^+\) and KIR\(^-\) T cells freshly isolated from peripheral blood to rule out the possibility that the generation and propagation of the clones substantially influence their function. KIR\(^+\) and KIR\(^-\) T cells from PBMCs were thus assayed for their proliferation in response to anti-CD3 mAb stimulation by flow cytometry using CFSE dilution analysis, cells were stained with allophycocyanin-anti-CD8\(\alpha\), followed by biotinylated anti-mouse IgG mAb and mouse serum treatments. For monitoring surface expression, cells were stained with a mixture of FITC-anti-TCR\(\beta\), allophycocyanin-anti-CD8\(\alpha\), PE-anti-CD85\(\alpha\), and PerCP-streptavidin. For intracytoplasmic staining, cells were stained with a mixture of FITC-anti-TCR\(\beta\), allophycocyanin-anti-CD8\(\alpha\), and PerCP-streptavidin, fixed using 4% paraformaldehyde, permeabilized using saponin and then incubated either with PE-anti-CD85\(\alpha\) or isotype matched PE-IgG1 mAbs. Dot plots are gated on TCR\(\beta\)/CD8\(\alpha\) T cells.

![FIGURE 2. Reduced proliferative potential of CD8\(^+\)KIR\(^+\) and CD8\(^+\)CD85\(^+\) T cells. CFSE-labeled PBMCs derived from a healthy volunteer were stimulated for 4 days with indicated stimuli. Before cytometric analysis, cells were stained with allophycocyanin-anti-CD8\(\alpha\), with a mixture of PE-anti-KIR or with PE-anti-CD85\(\alpha\). Histograms are gated on the indicated CD8\(^+\) T cell subset. Indicated numbers represent percentages of T cells (CD8\(^{bright}\) cells) undergoing division. These data are representative from two independent experiments.](http://www.jimmunol.org/)

![FIGURE 3. Cell surface and intracytoplasmic expression of CD85\(\alpha\) in T cells. Freshly isolated PBMCs from three healthy donors were first incubated with a mixture of unlabeled anti-KIR mAbs, followed by biotinylated anti-mouse IgG mAb and mouse serum treatments. For monitoring surface expression, cells were stained with a mixture of FITC-anti-TCR\(\beta\), allophycocyanin-anti-CD8\(\alpha\), PE-anti-CD85\(\alpha\), and PerCP-streptavidin. For intracytoplasmic staining, cells were stained with a mixture of FITC-anti-TCR\(\beta\), allophycocyanin-anti-CD8\(\alpha\), and PerCP-streptavidin, fixed using 4% paraformaldehyde, permeabilized using saponin and then incubated either with PE-anti-CD85\(\alpha\) or isotype matched PE-IgG1 mAbs. Dot plots are gated on TCR\(\beta\)/CD8\(\alpha\) T cells.](http://www.jimmunol.org/)
FIGURE 4. Cell surface expression of KIR and CD85j upon anti-CD3 mAb-induced T cell activation. Proliferation of CD8+ T cells expressing KIR molecules (left panels) or CD85j (right panels) under anti-CD3 mAb triggering. CFSE-labeled PBMCs derived from healthy volunteers were stimulated for 4 days with indicated stimuli. Before cytometric analysis, cells were stained with allophycocyanin-anti-CD8α, with a mixture of PE-anti-KIR or with PE-anti-CD85j. Dot plots are gated on CD8α+ cells. Indicated numbers represent cell percentages in each quadrant. These data are representative from two independent experiments performed from distinct healthy donors.

blood T cells correlates with a reduction in their proliferative potential.

Effect functions of KIR+ and CD85j+ T cells

Functional analysis of pairs of KIR+ and KIR− clones expressing the same TCR indicated that while the former showed weaker proliferative responses after Ag exposure than the latter, KIR+ T cell clones tended to yield a stronger cytolytic potential against Ag-loaded allogeneic target cells (lacking KIR ligands), as well in lectin-induced killing assays. By contrast, cytokine production of the KIR+ BGL19 clone tended to be weaker than that of its KIR− counterpart (clone B2.5, data not shown). These preliminary indications prompted us to further analyze peripheral blood KIR+ vs KIR− and CD85j+ vs CD85j− T cells for their capacity to produce IFN-γ upon TCR triggering. As shown in Fig. 5, left panel, a severe reduction in anti-CD3 mAb-induced IFN-γ secretion was observed when KIR− T cells were compared with KIR+ T cells. In contrast, a substantial fraction of CD85j+ T cells produce IFN-γ, and most IFN-γ-secreting CD8+ T cells were CD85j+ (Fig. 5, right panel).

Peripheral blood KIR+ vs KIR− and CD85j+ vs CD85j− T cells were also analyzed for their engagement into a cytolytic differentiation program, as judged by the intracytoplasmic expression of perforin. Three populations of CD8+ T cells can be distinguished based on their perforin content: perforin+, perforindim, and perforinbright. Most (if not all) KIR+ and CD85j+ T cells express high intracytoplasmic levels of perforin (Fig. 6). Remarkably, the vast majority of perforinbright CD8+ T cells were CD85j+ (Fig. 6). Thus, KIR+ and CD85j+ T cells harbor multiple features of highly differentiated CD8+ T cells. KIR+ T cells represent a subset with a low proliferative potential, a reduced capacity to produce IFN-γ and a high intracytoplasmic level of perforin. CD85j+ T cells share with KIR+ T cells a low proliferative potential and perforinbright phenotype, but differ in their high capacity to produce IFN-γ.

Expression of KIR or CD85j on CD8+ T cells upon viral infection

Increased cell surface expression of KIR and CD85j on differentiated CD8+ T cells incited us to investigate whether these molecules are expressed on T cells during the course of viral infections. This analysis was first performed on PBMCs isolated from kidney transplanted HLA-A2 patients undergoing CMV infection upon immunosuppressive therapy. The immunodominant HLA-A2-restricted pp65 T cell response was monitored over time postinfection using pp65/A2 multimers. As seen in a representative individual, a pp65 response was detectable up to 9 mo after infection (Fig. 7). Only a minor fraction of CMV-specific T cells express KIR. Yet, a substantial fraction of CMV-specific T cells express CD85j (39 to 75%) several months postinfection (Fig. 7). This differential expression of KIR and CD85j during CMV infection prompted us to analyze the expression of these MHC class I-specific receptors on other virus-specific CD8+ T cells and particularly during HIV-1 infection, because HIV-1- and CMV-specific CD8+ T cells are known to harbor distinctive features of a CD8 differentiation program (22–24).

FIGURE 5. CD8+ KIR+ T cells and CD8+ CD85j+ T cells display opposite IFN-γ production under in vitro TCR stimulation. PBMCs derived from healthy volunteers were stimulated for 6 h with indicated stimuli in the presence of brefeldin A. After cell surface staining with FITC-anti-TCRαβ, allophycocyanin-anti-CD8α, a mixture of PE-anti-KIR (left panels) or with PE-anti-CD85j mAbs (right panels), cells were fixed, permeabilized and stained with biotin-anti-IFN-γ mAbs revealed by PerCP-streptavidin. Dot plots are gated on CD8α+ TCRαβ+ cells. Indicated numbers represent cell percentages in each quadrant. These data are representative from six independent experiments.

FIGURE 6. CD8+ KIR+ T cells and CD8+ CD85j+ T cells express high levels of perforin. PBMCs derived from three healthy volunteers were stained with FITC-anti-perforin, PerCP-Cy5.5-anti-CD3, allophycocyanin-anti-CD8α and a mix of unlabeled-anti-KIR (upper panels) or with unlabeled-anti-CD85j mAbs (lower panels). Unlabeled mAbs were revealed by biotin-anti-mouse followed by PE-streptavidin staining. Dot plots are gated on CD8+ CD3+ cells. Indicated numbers represent cell percentages in each quadrant.
The analysis was performed on PBMCs isolated from patients developing primary HIV-1 infection, where HIV-1-specific CD8+ T cells were monitored using a panel of HLA-class I multimers. Although KIR+ T cells were detected, the size of this subset was quite low (3.1 ± 2.6% of multimer cells, n = 17), regardless of the MHC-peptide complexes used, and even slightly lower than the percentages of KIR+ observed in the total CD8+ T cell population (6.2 ± 4.6, n = 29) (Fig. 8). No variation was observed according to the duration of HIV-1-infection, the viral load or CD4+ T cell counts (data not shown). Contrasting with the low expression of KIR, a significant proportion of HIV-1-specific CD8+ T cells expressed CD85j molecules (46 ± 12%, n = 7) (Fig. 8). Along this line, KIR+, CD85j+, and HIV-1+ CD8+ T cell subsets greatly differed by their maturation status. While KIR+ cells displayed phenotypic features of terminally differentiated T cells (CD27lowCD45RA+ perforinhigh), CD85j+ cells showed heterogeneous CD27 and CD45RA profiles, consistent with their less differentiated phenotype (Fig. 9). Moreover, despite the similar CD28+CCR7+ phenotype of both KIR+ and HIV-1-specific CD8+ T cells, the latter were CD27+CD57+CD45RO+ perforinlow, unlike the former (Fig. 9).

We then asked whether the expression of CD85j on HIV-1-specific CD8+ T cells was linked to the high level of activation observed in acute HIV-1 primary infection or to permanent Ag exposure. For this purpose, sequential samples from four patients were analyzed, from acute HIV-1 infection up to 24 mo of infection, for the expression of KIR and CD85j on four distinct subsets of HIV-1-specific CD8+ T cells (Fig. 10). We selected untreated patients with viral loads continuously positive over the 24 mo of follow-up and ranging from 3.4 to 5.0 log10 HIV-1 RNA copies/ml, suggesting continuous antigenic exposure throughout the follow-up period. Activation was maximal during acute infection (at the day of detection of HIV-1 infection: day 0) as illustrated by the very high expression of CD38 on HIV-1-specific CD8+ T cells (97 ± 2%), while CD85j was expressed on only 21 ± 13% specific T cells. At 24 mo of follow-up, activation dropped to 55 ± 22% of CD38+ cells (p < 0.05 compared with day 0). Meanwhile, the percentages of CD85j-expressing cells gradually increased in both patients for all specificities, reaching 46 ± 12% (p = 0.001 compared with day 0). No increase of KIR expression was observed on

**FIGURE 7.** A large fraction of CMV-specific CD8+ T cells express CD85j. Thawed PBMCs, harvested at indicated time points after renal transplant of a representative patient developing CMV infection, were stained with FITC-anti-CD3, a mix of PE-anti-KIR mAbs (left panels) or PE-anti-CD85j (right panels), PC7-anti-CD8α mAbs and allophycocyanin-p65/HLA-A2 multimers. Dot plots are gated on CD8+ CD3+ T cells. Indicated numbers represent cell percentages in each quadrants.

**FIGURE 8.** A large fraction of HIV-1-specific CD8+ T cells expresses CD85j but not KIR. PBMCs derived from three HIV-1-infected subjects were stained with indicated PE multimers and a mix of unlabeled-anti-KIR (upper panels) or with unlabeled-anti-CD85j mAbs (lower panels). Unlabeled mAbs were revealed by FITC-anti-mouse. Dot plots are gated on T cells (CD8bright cells). Indicated numbers represent cell percentages in each quadrant.

**FIGURE 9.** CD8+CD85j+ T cell phenotype differs from CD8+ KIR+ T cell phenotype. Phenotypic characterization of CD8+ KIR+ T cells (left panels), CD8+CD85j+ T cells (middle panels), and HIV-1-specific (gag 263–272) CD8+ T cells (right panels) using PE- or FITC-CD27, PE- or FITC-CD45RA, PE- or FITC-CD57 and FITC-perforin mAbs.
KIR cell clones sharing identical TCR indicate that KIR engagement is
NK-CTLs) have been shown to be HLA-E-restricted (32–34). NK-
express inhibitory MHC class I receptors (i.e., inhibitory KIR,
inhibitory MHC class I receptor is associated with various stages
of HIV-1 infection. PBMCs from four HIV-1
TCR engagement. Data obtained with KIR
induced cell death (AICD) of T cells upon antigenic stimulation
receptors can also contribute to the negative control of activation-
ies show that T cell activation can be down-regulated or even
class I inhibitory receptors has also been shown to contribute to the
self MHC class I molecules (25). On T cells, engagement of MHC
for cytotoxicity and cytokine production upon engagement with
NK cells by raising up the threshold of activating signals required
control these potentially harmful cells.
The differential IFN-γ response of KIR+CD8+ T cells and
CD85j+CD8+ T cells was the most distinctive feature between
these two subsets, as KIR+ cells were poor IFN-γ producers
whereas most IFN-γ-producing cells expressed CD85j. It has been
previously suggested that the T cell surface expression of CD85j
precedes that of KIR (13, 35). This sequential expression of Ig-like
inhibitory MHC class I receptors is in keeping with a linear dif-
ferentiation model of CD8+ T cells that would result from an
ordered expression of genes during peripheral maturation (36).
Among this line, it is possible to speculate that memory effector
CD8+ T cells gradually acquire their cytotoxic function at the cost
of their proliferative response first (CD85j+CD8+ T cells), fol-
lowed by a progressive loss of their cytokine potential
(KIR+CD8+ T cells). This model of the CD8+ T cell develop-
mental program is supported by previous data showing that per-
formin expression and production of IFN-γ in CD8+ T cells can be
largely segregated (37). Similar to KIR+CD8+ T cells, mouse
Ly49+ CD8+ T cells are poor IFN-γ producers upon TCR trigger-
and this defect in cytokine production appears independent of
the engagement of inhibitory Ly49 molecules on T cells (38). It is
noteworthy that freshly isolated KIR+CD56bright NK cells present a
higher cytolytic potential and are poor cytokine producers as com-
pared with KIR+CD56bright NK cells (39). The acquisition of some
cell surface inhibitory MHC class I receptors on mouse and human
CD8+ T cells as well as on human NK cells thus appears to corre-
late with a commitment into cytolytic cells with a reduced ability
to produce cytokines.
The similarities between KIR+CD8+ T cells and Ly49+CD8+ T
cells, as well as the differences between KIR+CD8+ T cells and
CD85j+CD8+ T cells are further emphasized when their mode of
induction is compared. Indeed, we provide here the first evidence
that CD85j is induced on a substantial fraction of virus-specific
cells upon HIV-1 or CMV infection. The latter result is consistent
with the increased CD85j expression on circulating T cells from
CMV-infected individuals that has led to propose CD85j as a
marker for the early identification of CMV disease ex vivo (40).
Yet, CD85j does not behave as a typical T cell activation marker.
Indeed, the fraction of CD85j+ virus-specific CD8+ T cells pro-
gressively increases over time, suggesting that the persistence of
the antagonistic stimulation favors the induction of CD85j on the T
cell surface. In contrast, we could not detect significant induction
of KIR on HIV-1-specific or CMV-specific CD8+ T cells. Simi-
larly, no detectable induction of Ly49+ on CD8+ T cells specific

FIGURE 10. Increase in CD8+KIR+ T cell percentage during the
course of HIV-1 infection. PBMCs from four HIV-1-infected
individuals were isolated at indicated time points postdetection of
HIV-1 infection. KIR (■) and CD85j (□) expression was assessed on four distinct HIV-1-specific
CD8+ T cell subsets using PE multimers. Analyses are gated on
CD8brightmultimer+ cells. iNKR, inhibitory NK receptors (CD85j and
KIR); nd, not determined.
for *Listeria monocytogenes* or lymphocytic choriomeningitis virus has been detected upon primary or persistent infection (41). Yet, we recently obtained evidence that persistent stimulation with self-Ag leads to induction of Ly49 on Ag-specific CD8+ T cells (38). To reconcile these findings, it is tempting to speculate that Ly49+ CD8+ T cells like KIR+ CD8+ T cells do not merely witness a persistent antigenic stimulation but rather the inflammatory/environmental context in which antigenic stimulation would take place. Thus, as in the mouse, these results suggest that the expression of inhibitory MHC class I receptors on T cells is part of an integrative program of CD8+ T cell differentiation that witnesses to a history of persistent challenges and contributes to the functional heterogeneity of the intracellular CD8+ T cell response.

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