Regulation of Inhibitory and Activating Killer-Cell Ig-Like Receptor Expression Occurs in T Cells After Termination of TCR Rearrangements

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A small fraction of T cells expresses killer-cell Ig-like receptors (KIR), a family of MHC class I-specific receptors that can modulate TCR-dependent activation of effector functions. Although KIR+ cells are enriched within Ag-experienced T cell subsets, the precise relationships between KIR+ and KIR− T cells and the stage of KIR induction on these lymphocytes remain unclear. In this study, we compared KIR− and KIR+ αβ T cell clones, sorted by means of the CD158b (KIR2DL2/KIR2DL3/KIR2DS2) specific mAb GL183. We isolated several pairs of CD158b+ and CD158b− αβ T cell clones sharing identical productive and nonproductive TCR transcripts. We showed that expression of functional KIR on T cells is regulated after termination of TCR rearrangements. Transcriptional regulation of KIR genes was documented in multiple T cell clones generated from the same donor, and the presence of KIR transcripts was also detected in KIR− T cells. These results document a complex regulation of KIR expression in T cells at both pre and posttranscriptional levels, under the control of yet undefined signals provided in vivo. The Journal of Immunology, 2001, 166: 2487–2494.

N atural killer and T cells play central and complementary functions in immunity against transformed or virus-infected cells. Although T cell-mediated elimination of target cells is achieved through recognition of Ags bound to MHC molecules, NK cell activation is in most cases negatively affected by the presence of MHC class I products on target cells (1–3). The molecular basis of these opposite behaviors of T and NK cells was elucidated with the identification of several NKR able to deliver inhibitory signals to effector cells upon recognition of MHC class Ia or Ib molecules (4–7). In humans, these inhibitory MHC receptors fall into two major groups showing homology to either C-type lectins or Ig. Among members of the first group were identified several HLA-E-reactive heterodimeric receptors comprising an invariant CD94 subunit paired to NKG2 polypeptides (8–9). Although KIR2DL1 and KIR2DL2/2DL3 receptors recognize products of particular HLA-Cw alleles (e.g., Cw2/4/5/6/15/1602/1701 for the former, and Cw1/3/7/8/12/13/14/1601/1603 for the latter), KIR3DL1 receptors interact with multiple HLA-B molecules (Bw*04), KIR3DL2 receptors may recognize particular HLA-A (e.g., A*03 and A*011) alleles and KIR2DL4 receptor recognizes the nonclassical HLA-G molecule (11–17). A novel family of broadly distributed leukocyte Ig-like receptors (LIRs)/Ig-like transcripts (ILTs) has been more recently discovered (18–20). On lymphocytes, ILT-2 (LIR-1) has been shown to interact with a broad spectrum of HLA-A and HLA-B alleles as well as with HLA-G (18, 21).

Inhibitory NKR (KIR-long (KIR-L) molecules, LIR-1/ILT-2, NKG2A) harbor an intracytoplasmic immunoreceptor tyrosine-based inhibition motif, which is responsible for the inhibitory function of the receptor (5, 7). Activating NKR have also been described. Activating NKR (KIR-short (KIR-S) molecules, LIR-7/ILT-1) have no intracytoplasmic immunoreceptor tyrosine-based inhibition motif, but are associated with immunoreceptor tyrosine-based activation molecule-bearing transduction polypeptides such as killer cell-activating receptor-associated protein/DAP12 (KIR-S molecules, NKG2C) or FcγR (LIR-7/ILT-1) (7).

NKRs are not specific NK cell markers. Their distribution includes subsets of αβ and γδ T lymphocytes (14, 22–25). NKR+ αβ T cells are preferentially found within Ag-experienced subsets (26), thus suggesting selective NKR induction or expansion of NKR+ T cells during in vivo T cell responses. In this regard, cytokines such as IL-15 and TGF-β have been implicated in the

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4 Abbreviations used in this paper: KIR, killer-cell Ig-like receptor; BLC, B lymphoblastoid cell; RT, reverse transcription; LIB, leukocyte Ig-like receptor; ILT, Ig-like transcript; KIR-L, KIR-long; KIR-S, KIR-short.
induction of CD94/NKG2A dimers observed on some CD8+ T cells following their in vitro activation (27, 28). Whether a similar mechanism applies for KIR is yet unclear; in particular, direct evidence for in vitro or in vivo induction of KIR on mature lymphoid cells is still lacking.

Here, we have studied the Ag specificity and TCR features of KIR+ and KIR− αβ T lymphocytes derived from cells involved in a chronic in vivo response against EBV. We characterized several pairs of KIR+ and KIR− T cell clones expressing identical TCR transcripts, indicating that regulation of KIR expression occurs after termination of TCR rearrangements.

**Materials and Methods**

**Abs and reagents**

The following mAbs have been described earlier (4) and were used for flow cytometry analyses, immunomagnetic sorting, as well as for functional assays: ER6 (CD158a, anti-KIR2DL1 (p58.1) and KIR2DS1 (p50.1)); GL183 (CD158b, anti-KIR2DL2/3DL2/3DL2; GL183 (p50.1)); Z776 (anti-KIR3DL1 (p70)); Q66 (anti-KIR3DL2 (p41.0)); and FS172 (anti-KIR2DS4 (p50.3)). Synthetic peptides corresponding to previously defined EBV epitopes (BMLF1/HLA-A2, GLCTLVAML; BZLF1/HLA-B402, SENDRLL; and BZFL1/HLA-B35, PCVLWPVLPELPQGLTAY HVS) (29) were obtained from Chiron Mimotopes (Victoria, Australia).

**Cells**

B lymphoblastoid cells (BLC) were grown in RPMI 1640 medium with 2 mM l-glutamine and 8% pooled human serum (culture medium). PBMC and BLCS (25, 31).

**Analysis of TCR transcripts**

For TCR transcripts, RNA from 5 × 10^6 T cells was extracted using TRIzol reagent (Life Technologies) according to the manufacturer’s instructions. Total RNA (5 μg) was used for cDNA conversion in a total volume of 30 μl with a first-strand cDNA synthesis kit (Life Technologies). Determination of the KIR repertoire was performed by PCR using BCR for each amplification. For intracellular staining, cells were permeabilized in saponin buffer (PBS plus 0.1% BSA plus 0.1% saponin (Dyonal, Oslo, Norway). After eight washes, bead-anchored cells were either seeded on 0.3 cells/well (for direct cloning) or expanded in bulk cultures in culture medium supplemented with IL-2 (150 IU/ml; Chiron Mimotopes) and restimulated at 3-wk intervals with purified lectin (leucokinagglutinin, 0.5 μg/ml; Sigma, Les Ulis, France) in the presence of irradiated allogeneic PBMC and BLCS (25, 31).

**Flow cytometry analysis**

Cells were phenotyped by indirect immunofluorescence as described (25, 31). In brief, cells were incubated first with unconjugated mAb for 30 min at room temperature, washed, and incubated with FITC-conjugated rabbit anti-mouse Ig antisera (BioAtlantic, Nantes, France) for 30 min on ice. After washing, cells were analyzed by flow cytometry on a FACSscan apparatus (Becton Dickinson, Mountain View, CA) using the LYSYS II software package on a FACScan apparatus (Becton Dickinson, Mountain View, CA) using the LYSYS II software package on a FACScan apparatus. For intracellular staining, cells were permeabilized in saponin buffer (PBS plus 0.1% BSA plus 0.1% saponin (Sigma) previous staining and flow cytometry was performed as described above using reagents reconstituted in saponin buffer. All washes were performed in saponin buffer.

**Analysis of TCR transcripts**

For TCR transcripts, RNA from 5 × 10^6 T cells was extracted using TRIzol reagent (Life Technologies, Rockville, MD) according to the supplier’s instructions, and was dissolved in 40 μl water. Reverse transcription (RT) was performed on 2.5 μl of the RNA solution in a final volume of 12.5 μl for 30 min at 45°C in a mix containing 50 mM Tris-HCl (pH 8.3), 75 mM KCl, 3 mM MgCl2, 10 mM DTT, 10 U RNAsin (Promega, Madison, WI), 1 μM of each dNTP, 100 U Moloney murine leukemia virus reverse transcriptase (Life Technologies) and 25 μM of the Ca-specific primer 5′-TGAAGTCCATAGACCTCATGTC. For each clone, five PCR products were performed using sets of degenerate Vb primers described elsewhere (32). Each PCR was performed 50 μl with a 1× mix of Taq DNA polymerase (Pharmacia, Piscataway, NJ). 1.25 U of Taq DNA polymerase, 25 μl of each Vb primers. Amplification was performed as described (31, 33). Amplified products were directly sequenced (without previous plasmid cloning) as described (31–33) using Sequenase (Amersham, Arlington Heights, IL) and the Co sequencing primer, 5′-CTTTTGCGACACATTTTGGTAGG.

For TCR6 transcripts, RT was performed using a Cβ reverse primer primers 5′-GCACAGAGCACCCTTGTGCTG (see above) as described. Amplification was performed using the following degenerate Vβ primers: 5′-CAYN-RDVDYRTBTMYTGYTGA and 5′-CMYRMHMMPMTKTWYTGTTA (Y = C or T; R = A or G; N = A or C or G or T; A = A or C or G or T; B = G or T; H = A or T; K = G or T; W = A or T). A second PCR was then performed using a nested Cβ primer (5′-GGTCTCAGCGCCACCACTGGTG) as described (31–33). Bands of interest were purified and sequenced as described above.

**Functional assays**

COS cell transfection assay was performed by the DEAE-dextran chloroquine method (34). In brief, 1.5 × 10^6 COS cells were co-transfected with 100 ng of an expression vector coding for an EBV protein and 100 ng of an expression vector coding for an HLA allele. Transfected COS cells were tested 48 h after transfection for their ability to trigger TNF secretion by 10^6 responding T cells after 6-h incubation at 37°C. TNF released in culture supernatants was quantitated by a biosay using WEHI 164 cells (35).

For the peptide-induced “fratricide” assay, CTL were incubated for 2 h with 10 μM peptide and washed once, and cell lysis was estimated by flow cytometry on the basis of forward/side scatter patterns and propidium iodide exclusion (29).

KIR repertoire analysis

DNA and total RNA were prepared from 5 × 10^6 T cells using the TRIzol reagent (Life Technologies) according to the manufacturer’s instructions. Total RNA (5 μg) was used for cDNA conversion in a total volume of 30 μl with a first-strand cDNA synthesis kit (Life Technologies). Determination of the KIR repertoire was performed by PCR using for each amplification either 200 ng of genomic DNA for genomic typing, or 2 μl of cDNA synthesis reaction for transcripts detection. Specific oligonucleotides for genomic and cDNA sequences, and PCR conditions were described previously (36, 37). To verify the specificity of the primers designed to detect KIR sequences, vectors containing a single KIR cDNA sequence (KIR2DS1, KIR2DL1, KIR2DL3, or KIR2DS2) or a bacterial artificial chromosome (BAC) containing KIR2DL1, KIR2DL1, KIR2DL3, and KIR2DS4 genes (kindly provided by Dr. N. Wagtman), were used as matrices in the PCR-based assay. At a concentration of 0.1 pg of plasmid or 200 ng of BAC, only specific amplifications of the KIR cDNA or genomic sequences by appropriate primer pairs were detected.

**Semi-quantitative PCR analysis and sequencing**

To compare the amount of KIR2DS1 or KIR2DS2 transcripts in T cell clones, 5 μl of diluted (10^-1 to 10^-7) or not (10^0) cDNA were used in a PCR assay to co-amplify human β-actin and KIR2DL3 or KIR2DS2. The matrix concentration of the various T cell clones that led to a similar human β-actin amplification was determined and the amplification levels of KIR2DL3 or KIR2DS2 at these concentrations were compared.

Full-length cDNAs of KIR2DL3 were obtained from T cell clones RNA by RT-PCR using oligo(dT) RT and PCR amplification using 5′-KIR2DL3/2DS2 primer (TCCGTCAATGCTGCGACATGTTG) and 3′-KIR2DL3/2DS2 primer (AGGCTCTACGATCCTGGATCT). Amplified products were cloned in pGEM-T-easy vector and sequenced using M13 reverse and T7 primers (ABI Prism 3; Perkin-Elmer, Norwalk, CT).

**Results**

Isolation of CD158+ and CD158− αβ T cells sharing identical in-frame and out-of-frame TCR transcripts

Several indirect observations, such as the preferential KIR expression on T cells with memory features (26), suggest that surface evidence for in vitro or in vivo induction of KIR on mature lymphoid cells is still lacking.
KIR are induced at late stages of T cell differentiation, after termination of TCR rearrangements. However, this hypothesis remains to be formally proven. Indeed despite numerous attempts, it has not been possible to induce or modulate surface KIRs on established T cell clones in vitro, even in the presence of cytokines (e.g., IL-15, IL-4, IL-12, IFN-γ, and TGFβ) known to affect surface expression of lectin-like NKR s, such as CD94/NKG2 heterodimers (27, 38). A way to demonstrate this would be to isolate pairs of clones differing by their KIR phenotype but expressing identical TCRs. To this end, we attempted to isolate KIR+ and KIR− αβ T cell clones showing the same antigenic specificity. CD158b+ (GL183+) KIR+ T cell lines were generated from two synovial T cell lines derived from rheumatoid arthritis patients, which were previously shown to be selected in vivo by a restricted set of well-defined EBV-derived Ags (29, 39). The specificity of unsorted (predominantly CD158b+) and CD158b+ T cells derived from these patients was then determined by testing their reactivity to COS cells cotransfected with cDNAs coding for various EBV/HLA combinations (29, 34). The CD158b+ cell lines derived from both patients reacted to few dominant EBV/HLA combinations also recognized by unsorted autologous T cell lines (data not shown). T cell clones were then generated from CD158b+ and unsorted T cell lines, checked for CD158b expression, and screened for their reactivity against the aforementioned dominant viral Ag in a peptide-induced fratricide assay. Several CD158b+ and CD158b− clones recognizing the same EBV epitope (BMLF1/A2) were isolated from one patient (A). Similarly, we obtained from the other patient (B), CD158b+ and CD158b− T cell clones reacting against another EBV Ag (BZLF1/HLA-B35 epitope). Representative CD158b+ (A4.5, B2.5) and CD158b− (AGL10, BGL19) T cell clones derived from patients A (A4.5, AGL10) and B (B2.5, BGL19) are shown in Fig. 1. TCRα and β transcripts derived from these clones were then amplified by RT-PCR and the corresponding V(D)J functional regions sequenced. TCRα- and β-chains with identical junctional sequences were identified in the two pairs of KIR+ and KIR− T cell clones reacting against the above Ags (Table I). Of note, KIR+ and KIR− T cell clones shared not only in-frame but also out-of-frame TCR transcripts (Table I), thus proving that they were the progeny of the same mature T cell clone. Taken together, these data formally demonstrated that the regulation of KIR cell surface expression occurs after termination of TCR gene rearrangements.

### Functional analysis of KIR+ and KIR− αβ T cell clones

The functionality of KIRs on the aforementioned T cell clones was studied by analyzing the effect of KIR engagement on T cell clone cytolytic activity. Cross-linking of CD158b using mAb GL183 induced partial inhibition of anti-CD3 redirected lysis of murine FeR P815 cells by the CD158b+ clone BGL19, when compared with its CD158b− counterpart (clone B2.5) (data not shown). Similar results were obtained when KIR were cross-linked by their physiologic ligands (i.e., using HLA-Cw3+ or Cw7+ targets) and the T cell clone activated by its nominal peptidic Ag. Indeed, EBV peptide-loaded BLC lacking CD158b ligands (e.g., HLA-Cw4+ targets) were more efficiently lysed by the CD158b+ clone BGL19 than those expressing CD158b ligands (e.g., HLA-Cw3/7+ targets) (Fig. 2A, right). By contrast, Cw4+ and Cw3/7+ BLC loaded with the HLA-B35-restricted BZLF1 peptide Ag were lysed to a similar extent by the KIR− clone B2.5 (Fig. 2A, left). This difference was exclusively accounted for by KIR engagement because efficient cytolsis of Cw3/7+ target cells by clone BGL19 was fully restored when masking KIR during the CTL assay (Fig. 2B). Taken together, these results indicated that the CD158b molecules on clone BGL19 were functional as inhibitory receptors for HLA-Cw3/Cw7.

### Transcriptional and posttranscriptional control of KIR expression in αβ T cells

A PCR-based analysis of the KIR repertoire was performed on KIR+ and KIR− T cell clone DNA and RNA. Genotype of T cell clones derived from donor B (B2.5, BGL19) is 2DL1+, 2DL2, 2DL3+, 3DL1+, 3DL2+, 2DS1+, 2DS2, 2DS3, 2DS4+, 2DS5+, and 3DS1 (Table II and Fig. 3). Genotype of cells from donor A origin (AGL10 and A4.5) is 2DL1+, 2DL2+, 2DL3+, 3DL1+, 3DL2+, 2DS1+, 2DS2+, 2DS3, 2DS4+, 2DS5+, and 3DS1 (Table II and Fig. 3). Evidence for a transcriptional regulation of KIR genes was obtained by comparing the transcripts and the genes detected in a given cell (Table II and Fig. 3). In AGL10 cells, neither 2DS1 nor 2DS4 transcripts were detected, while 2DS1 and 2DS4 genes were present. Similarly, in A4.5 cells, no transcripts for 2DL1, 2DL2, 3DL1, 3DL2, 2DS1, or 2DS4 were detected despite the presence of the corresponding genes.

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**FIGURE 1.** Identification of pairs of KIR+ and KIR− T cell clones reacting against identical MHC/EBV peptide complexes. A. Flow cytometry analysis of T cell clones derived from patients A and B using KIR-specific mAb. Shown are the fluorescence histograms (in log scale) obtained by staining T cell clones with GL183 mAb (open histogram) or an irrelevant isotype-matched mAb (filled histogram). Background staining was obtained with all the other NKR-specific mAb tested (i.e., EB6, ZIN276, DEC66, and FSTR172). B. Identification of EBV Ags recognized by KIR+ and KIR− T cell clones. T cell clone specificity was evaluated against EBV peptides corresponding to the major EBV epitopes identified at the polyclonal level (see text). Because EBV Ags were recognized in the context of autologous HLA alleles, T cell specificity could be determined by an effector fratricide assay after a 2-h incubation with peptide as described (29). □, BMLF1/A2 peptide (upper panel), BZFL1/B35 peptide (lower panel); ■, BZLF1/B40 peptide (upper and lower panels).
Table I. 

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* A4.5 and AG110 clones were derived from donor A; B2.5 and BGL19 clones were derived from donor B. RF, Reading frame (+, in frame; –, out of frame). TCR genes were designated according to the last World Health Organization–International Union of Immunological Societies nomenclature (54).

The comparison of KIR transcription patterns in KIR+ vs KIR− T cell clones isolated from the same individuals prompted us to investigate whether an additional regulation pathway of KIR expression occurred in T cells, at posttranscriptional levels. B2.5 and BGL19 cells transcribed all their KIR genes, although the former did not express any surface KIR and the latter expressed exclusively KIR2DL3 protein at the cell surface (Table II and data not shown). A4.5 T cells transcribed two KIRs, 2DL3 and 2DS2, but did not express the corresponding protein at the cell surface. Similarly, out of the seven transcripts detected in AG110 T cell clone, only CD158b is expressed at the surface (KIR2DL2 and/or KIR2DS2) (Fig. 3). The “missing” KIR proteins were also absent in intact or permeabilized cells, as indicated by flow cytometry analysis using a large panel of KIR-specific mAb (see Table III). Similar complex control of KIR gene expression was documented in 14 additional T cell clones derived from donor A and 2 additional clones from donor B (Table III). Several explanations, such as low levels of KIR gene transcription and/or lack of reactivity of mAb to the corresponding KIR protein (e.g., due to KIR polymorphism), could account for the absence of KIR transcripts in the absence of the corresponding protein. To exclude that polymorphism of KIR2DL3 was responsible for the absence of staining using CD158b mAb, we cloned and sequenced the full-length KIR2DL3 cDNA from A4.5 and AG110 T cell clones. Both sequences were totally identical with KIR2DL3 clone 6 sequence (GenBank accession number U24074; data not shown). We then attempted to amplify full-length KIR2DL3 cDNA from A18.1 and AG116 T cell clones using 5′-KIR2DL3 primer and 3′-KIR2DL3 primer. In these experiments, only KIR2DS2 clone 49 (GenBank accession number U24079) was cloned and sequenced (data not shown). Therefore, B2.5 T cells and A18.1 T cells express bona fide KIR2DL3 and KIR2DS2 transcripts, although no cell surface expression of the corresponding protein could be detected. We then performed a semiquantitative PCR analysis of KIR2DS2 and KIR2DL3 transcripts in AG116 and A18.1, as well as in BGL18 and B2.5 T cell clones, respectively. A lower KIR2DL3 transcription level was observed in B2.5, as compared with BGL18 T cells (Fig. 4A). Similarly, a lower KIR2DS2 transcription level was observed in A18.1 as compared with AG116 T cells (Fig. 4B). These data thus indicate that quantitative differences in KIR transcripts are likely responsible for the distinct KIR surface phenotypes observed in A18.1 and AG116, as well as in B2.5 and BGL18 T cell clones.

Further analysis of the pathways that regulate KIR expression was performed in clonal NK cell lines. NK92, NK3.3, and NKL human NK cell lines were cell surface phenotyped using CD158b mAb. As described earlier, NK3.3 is CD158b+ whereas both NK92 and NKL are CD158b− (data not shown). Despite the lack of detectable KIR molecules at the cell surface, KIR2DL3 transcripts were detected in NK92 cells. The specificity of KIR2DL3 RT-PCR detection was assessed by the lack of KIR2DL3 transcripts in NKL (Fig. 4C, upper panel). Consistent with the results obtained in T cells, the level of KIR2DL3 transcripts is much higher in the CD158b+ NK3.3 cells, than in the CD158b− NK92 cells (Fig. 4C, lower panel).

**FIGURE 2.** KIR-mediated inhibition of clone BGL19 cytotoxic activity. A, CTL activity of B2.5 and BGL19 clones was estimated at a 10/1 E:T ratio against BZLF1/B35 peptide-loaded HLA-B35+ BLC carrying or not p58.2 KIR ligands (○, B BLC (Cw3/7); ●, BM9 BLC (Cw4/4)). B, Restoration of CTL activity of BGL19 T cell clone against KIR-L+ targets following masking of KIRs on effector cells. CTL activity of clones B2.5 and BGL19 was estimated at a 10/1 E:T ratio against B BLC (Cw3/7) or BM9 BLC (Cw4/4) loaded with 10 μM of BZLF1/B35 peptide, in the absence (□) or presence (■) of GL183 mAb (1/4 final hybridoma supernatant).
Discussion

Two groups of cell surface receptors have been identified in NK and T cell subsets, which define a repertoire of MHC class I recognition. Ig-like molecules include KIR and LIR/ILT molecules, whereas C-type lectins include CD94/NKG2 heterodimers in humans as well as CD94/NKG2 heterodimers and Ly-49 homodimers in the mouse (5, 6). The shaping of this MHC class I repertoire appears to differ between lectin-like and Ig-like molecules. Indeed the cell surface expression of murine Ly-49 is influenced by the level of expression of cognate MHC class I molecules (40, 41), whereas KIR cell surface expression does not seem to be directly affected by the nature or expression levels of HLA class I molecules (14, 42). Besides, activation of CD94/NKG2 gene transcription and/or cell surface export of intracytoplasmic CD94/NKG2 heterodimers can be induced on developing and mature lymphoid cells after short-term in vitro culture in the presence of IL-15 (27). By contrast, all attempts to induce KIR in vitro on mature T or NK cells have failed so far. These results, which confirm that expression of KIRs and NKR homologous to C-type lectins is regulated through distinct pathways (43), have left open the issues as to when and how KIR are induced. The absence of KIR transcripts (data not shown) and proteins (44) in thymocytes, and by contrast their selective expression on fully differentiated NK cells (45) and Ag-experienced T cells (26), suggests late KIR induction during lymphoid cell development. Along this line, the existence of KIR + and KIR − T cell clones sharing identical productive and nonproductive TCR transcripts formally proves that induction/modulation of KIR expression occurs after termination of TCR rearrangements. In line with previous studies (25, 27, 28), the KIR phenotype of our T cell clones was highly stable (i.e., they remained KIR + or KIR − irrespective of their activation status (data not shown)), thus strongly suggesting that KIR induction occurred in vivo and not during the process of synovial T cell line generation. Cytokines are probably involved in KIR cell surface expression, as suggested by the in vitro induction of KIR on developing CD34 + hematopoietic progenitor cells upon treatment with Flt-3 ligand and IL-15 (46). However, whether the same factors will be required to induce KIR on precursor and mature lymphoid cells remains open. In this regard, preliminary experiments failed to demonstrate any effect on KIR expression by T cell clones of exogenously added IL-15, either alone or in combination with IL-2 (M.-A.P. and M.B., unpublished observations).

KIR expression on T cells has been mainly described on CD8 + memory T cells (26). KIR engagement by autologous MHC class I molecules can regulate in vitro CD3/TCR-mediated cytototoxicity and cytokine production suggesting a role of KIR in the control of T cell activation (22, 23, 25, 27, 47–49). In a previous study, activation of KIR − CTL clones following recognition of tumor Ags was shown to be abrogated by KIR (50). Interestingly, in our study, the inhibitory effect of KIR on TCR-mediated activation of αβ T cell clones was partial. The efficiency of KIR-mediated inhibition is likely affected by numerous parameters such as the

<table>
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<tr>
<th>KIR</th>
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* Analysis of the KIR repertoire on T cell clone-derived genomic DNA or cDNA was performed using previously described primers (36). The KIR nomenclature is from Ref. 36. Presence of the corresponding KIR protein (Prot) either on the cell surface or intracellularly was studied by flow cytometry on, respectively, intact or saponin-permeabilized cells using a panel of KIR-specific mAbs: EB6 (CD158a, KIR2DL1/KIR2DS1), GL183 (CD158b, KIR2DL2-2DL3/KIR2DS2), ZIN276 (anti-KIR3DL1), DEC66 (anti-KIR3DL2), and FSTR172 (anti-KIR2DS4). While KIR recognized by GL183 mAb on clone BGL19 was unambiguously assigned to KIR2DL3, GL183 mAb on clone A4.5 recognized at least two different KIR molecules, AGL10 and FSTR172, and the antibody was not able to distinguish between them. For clone AGL10, FSTR172 was not useful to distinguish between KIR2DL3 and 2DS4. For clone B2.5, GL183 recognized at least two different KIR molecules, AGL10 and FSTR172, and the antibody was not able to distinguish between them.

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**Table II. KIR genotype and phenotype of CD158b + and CD158b − αβ T cell clones**

**FIGURE 3.** KIR gene transcription patterns of T cell clones. The detection of transcripts and genes for each KIR was performed by RT-PCR and genomic PCR as described previously (36). The same panels of KIR amplification were obtained in three independent experiments.
avoiding TCR/ligand interactions and more generally the global avidity of the interactions between effector and target cells. Such factors may easily explain the differences observed between the two situations and are in line with recent data revealing unexpected complexity in the biological function of KIR on T cells (51, 52).

KIR belong to a multigenic and multiallelic family, and the polymorphism of KIR genes is still incompletely documented (37, 53). KIR genes are currently subdivided into inhibitory KIR (KIR2DL1, KIR2DL2, KIR2DL3, KIR2DL4, KIR3DL1, and KIR3DL2) and activating isoforms (KIR2DS1, KIR2DS2, KIR2DS3, KIR2DS4, KIR2DS5, and KIR3DS). Eighteen distinct KIR genotypes have been previously documented (36). T cell clones derived from donor B shared the most common genotype corresponding to group 1 individuals described earlier (36), while cells from donor A harbored a newly described genotype (Table II). Given the limited number of individuals analyzed thus far (36), these numbers are certainly underestimated and, therefore, it is not surprising to detect here new KIR genotypes such as the one displayed by cells from donor A.

A variable KIR transcription pattern from one NK clone to another, but a good matching between the presence of a given KIR transcript and its corresponding protein within a given clone, has been previously reported (36). These data suggested that the regulation of KIR expression only operated at a transcriptional, but not at a posttranscriptional, level. We confirm here the transcriptional regulation of the genes encoding for KIR-L and KIR-S proteins in both T and NK cells. But, in addition, we found that KIR-L and KIR-S transcripts were frequently detected in T cell clones and in one NK cell line in the absence of the corresponding protein, revealing that RT-PCR data that document KIR transcription pattern should be interpreted with caution. This discrepancy between KIR-L/KIR-S gene transcription and protein expression could be explained by transient KIR modulation (e.g., following receptor cross-linking). However, we failed to detect any intracellular KIR within permeabilized cells, in addition to those already detected on the surface (Table III). Furthermore, kinetic analysis of KIR sur-

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Patient A Genotype

| CD158b<sup>a</sup> | BGL19   | Transcripts | + | + | + | ND | + | + | - | - | - | - | - |
| KIR<sup>b</sup>     | B2.5    | Transcripts | + | + | + | + | + | + | - | - | - | - | - |

Patient B Genotype

| CD158b<sup>a</sup> | AGL16   | Transcripts | + | + | + | + | + | + | - | - | - | - | - |
| KIR<sup>b</sup>     | A18.1   | Transcripts | + | + | + | + | + | + | - | - | - | - | - |
|                       | AZ4     | Transcripts | + | + | + | + | + | + | - | - | - | - | - |
|                       | A14.11  | Transcripts | + | + | + | + | + | + | - | - | - | - | - |
|                       | A5.23   | Transcripts | + | + | + | + | + | + | - | - | - | - | - |

FIGURE 4. Semiquantitative PCR analysis of KIR transcripts in CD158b<sup>a</sup> and CD158b<sup>−</sup> T cell clones and NK cell lines. Coamplification of either KIR2DL3 (A and C) or KIR2DS2 (B) and actin transcripts were performed using 5 µl of undiluted (10<sup>−1</sup> and C, upper panel) or diluted cDNAs (10<sup>−1</sup>, 10<sup>−2</sup>, and 10<sup>−3</sup>). A, Comparison of KIR2DL3 transcripts from CD158b<sup>+</sup> (BGL18) or CD158b<sup>+</sup> (B2.5) T cell clones. B, Comparison of KIR2DS2 transcripts from CD158b<sup>−</sup> (AGL16) or CD158b<sup>−</sup> (A18.1) T cell clones. C (upper panel), Detection of KIR2DL3 transcripts from CD158b<sup>+</sup> (NK3.3) or CD158b<sup>−</sup> (NK92 and NKL) NK cell lines. C (lower panel), Comparison of KIR2DL3 transcripts from CD158b<sup>+</sup> (NK3.3) or CD158b<sup>−</sup> (NK92) NK cell lines.
face phenotype and transcripts in T cell clones at various time points after antigenic activation demonstrated stable transcription and surface expression of KIR, irrespective of the T cell clone activation status (data not shown). In addition, we have shown that the level of KIR-L and KIR-S transcripts are positively correlated to the cell surface expression of the corresponding KIR protein.

Thus, although the factors that induce KIR gene transcription are still unknown, our data show that the regulation of KIR expression occurs in T cells after termination of TCR rearrangements, that a threshold of KIR-L and KIR-S transcripts must be reached to achieve efficient T and NK cell surface expression of the KIR proteins at the cell surface, and that the transcriptional and post-transcriptional pathways that regulates KIR genes expression in T and NK cells are at least in part shared between KIR-L and KIR-S.

Acknowledgments

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