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Phenotypic and Functional Characterization of CD4 T Cells Expressing Killer Ig-Like Receptors

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Killer Ig-like receptors (KIR) are commonly found on human NK cells, γδ T cells, and CD8 T cells. Although KIR+ CD4 T cells are found in certain patients, their prevalence in healthy donors is controversial. We now provide definitive proof that such cells are present in most individuals, and report on their frequency, surface phenotype, cytokine profile, and Ag specificity. The number of KIR+ CD4 T cells detected in peripheral blood increased with age. In contrast with regular KIR− CD4 T cells, the majority of KIR+ CD4 T cells lacked surface expression of CD27, CD28, CCR4, and CCR7, but did express CD57 and CD24. In addition, KIR were detected on approximately one-tenth of CD28− and CD57− memory CD4 T cells. In line with the absence of the Th2 marker CCR4, the KIR+ CD4 cells produced mainly IFN-γ and little IL-4, IL-10, or IL-17 upon TCR triggering. Furthermore, the KIR+ population contained cells that responded to recall Ags in an HLA class II-restricted fashion. Together, our data indicate that KIR-expressing CD4 T cells are predominantly HLA class II-restricted effector memory Th1 cells, and that a significant, previously unrecognized fraction of effector memory Th1 cells expresses KIR.


The killer Ig-like receptors (KIR) constitute the largest family of human NK receptors, with multiple inhibitory and activating members (1). The inhibitory receptors bind classical HLA class I molecules and mediate “missing self” recognition. The physiological ligands for the activating KIR are unknown. In addition to NK cells, T cells also frequently express KIR. However, the role these NK receptors play in T cell responses remains largely enigmatic (2).

The KIR family displays a high degree of polymorphism, which is associated with disease. The KIR locus is polymorphic with respect to both gene content and sequence (3–5). KIR haplotypes contain between 5 and 12 highly homologous KIR genes (4), and up to 9 alleles for individual KIR genes have been described (5). KIR3DS1 and its proposed ligand HLA-Bw4 confer resistance to the development of full-onset AIDS in HIV-infected individuals (6). Also, the KIR2DS2 gene is associated with various autoimmune conditions, such as psoriatic arthritis (7), diabetes (8), and vascular complications in rheumatoid arthritis (9).

KIR expression by TCR-γδ+ T cells and a subset of CD8+ TCR-αβ+ cells is firmly established (10–12), but information on KIR expression by CD4 T cells is scarce. However, in patients with certain diseases KIR are frequently detected on an expanded CD28− subset of CD4 T cells (13, 14). In rheumatoid arthritis, CD28− CD4 T cells are cytolytic and they proliferate in response to autologous PBMC (15, 16). Moreover, their number correlates with disease severity (17). When compared with CD28+ CD4 T cells from healthy control subjects, the cells preferentially express the disease-associated activating KIR2DS2 molecule (9), which costimulates TCR-mediated IFN-γ production and proliferation (13). In acute coronary syndromes, CD28− CD4 T cells can be activated by KIR cross-linking directly, without the need for TCR engagement (14). The Ag specificity of the above-described KIR+ CD4 cells is unknown.

There are conflicting reports on KIR expression on CD4 T cells in healthy individuals. According to Anfossi et al. (12), on average 7 ± 3% (±SEM) of KIR+ T cells carry CD4, indicating that the large majority of healthy donors has KIR+ CD4 T cells. Indeed, a number of KIR+ CD4 T cell clones have been isolated from healthy donors (18, 19). In contrast, Warrington et al. (20) detected KIR expression selectively on CD28− CD4 T cells, an extremely rare subset in healthy individuals. By consequence, they detected KIR+ CD4 T cells only in a small minority of individuals, containing increased frequencies of CD28− CD4 cells (9, 14). We now provide definitive proof that KIR+ CD4 T cells are present in most individuals, and report on their frequency, surface phenotype, cytokine production, and Ag specificity.

Materials and Methods

Subjects

After informed consent was obtained, peripheral blood cells were obtained from healthy adult and 90-year-old donors. Healthy adults were random blood bank donors ranging in age from 18 to 70 years (n = 30). Healthy 90-year-old donors (n = 10) were enrolled in the cohort of the Leiden 85-plus study (21, 22), a prospective population-based study of inhabitants of Leiden, The Netherlands. Health was defined as no acute illness, no death within 3 mo after blood sampling, no severe cognitive impairment and a plasma C-reactive protein level below 10 mg/L. Umbilical cord blood (UCB) cells were derived from the umbilical vein after normal full-term deliveries (n = 7). Heparinized blood was collected and PBMC or cord blood mononuclear cells were isolated by density gradient centrifugation.
over Ficoll-Hypaque, and subsequently stored at −80°C in 10% DMSO/20% FCS until use.

**Antibodies**

Directly conjugated Abs to CD3, CD8, CD25, CD27, CD5RA, CD45RO, CD56, CD57, CCR4, CCR7, TCR-αβ, KIR3DL1 (CD158e, clone DX9), and isotype controls were obtained from BD Biosciences (San Jose, CA) and to CD4 from DAKO (Cytometry, Glostrup, Denmark). The PE-conjugated EB6 (CD158a, CD158h, anti-KIR2DL1, KIR2DS1), GL183 (CD158b, CD158j), KIR2DL2, KIR2DL3, KIR2DS2), FES172 (CD158i, KIR2DS4a), and CD244 (2B4) Abs were from Beckman Coulter (Mijdrecht, The Netherlands).

Unconjugated Abs to NKG2D (BAT221), NKp30 (A76), NKp46 (BAB281), NKp44 (Z231), and Ig-like transcript (ILT)-F (F278) were a kind gift from Dr. D. Pende (Instituto Nazionale per la Ricerca sul Cancro, Genova, Italy), anti-KIR2D Ab (NKVSF1) from Dr. A. Poggi (Instituto Nazionale per la Ricerca sul Cancro, Italy), and anti-KIR2DL4 Ab (cl-33; Ref. 23) from Dr. E. O. Long (National Institutes of Health, Bethesda, MD). Binding of unconjugated Abs was detected using goat anti-mouse IgG F(ab)2-RPE (DAKO).

**Flow cytometry**

Cells were stained with CD3-PerCP, CD4-allophycocyanin, KIR-PE (EB6, GL183, DX9, FES172 mixed or separately), combined with an Ab to an additional surface marker conjugated to FITC. PE-conjugated Abs to a limited number of surface markers (CD94, CD244, CCR4, CCR7, CXCR4) were combined with the CH-L FITC Ab (directed to KIR2DL2, KIR2DL3, KIR2DS2). Staining was performed at 4°C for 1 h, followed by three washes with 0.1% BSA in PBS. The cells were then fixed in 0.5% paraformaldehyde in PBS and stored at 4°C until acquisition of at least 10⁶ cells per sample on a BD FACS_Calibur cyometer and analyzed using the CellQuest (BD Biosciences) analysis software.

To exclude possible staining artifacts, we performed a number of control experiments. First, >95% of the KIR⁺CD4 T cell population signal dissipated when CD3, CD4, or KIR Abs were replaced with isotype-matched control Abs. Second, when the small population of KIR⁺CD4 cells was isolated by FACS (1, 10, or 1000 cells/well), expanded and subsequently stained with KIR or control Abs, >99% of cells were indeed KIR⁺, whereas control KIR⁻CD4 T cells treated in the same manner always remained negative for KIR. Thus, low numbers of KIR⁺CD4 T cells could be detected reliably.

**T cell cloning**

PBMC were stained with CD3-PerCP, CD4-allophycocyanin and KIR-PE (a mix of EB6, GL183, DX9, FES172) Abs as previously described. KIR⁺ and KIR⁻CD4 cells were subsequently plated at 1 cell/well by a BD Biosciences (Becton Dickinson) FACS Vantage flow cytomter using CellQuest software. T cells were cultured in IMDM (Invitrogen Life Technologies, Gaithersburg, MD) supplemented with 20 U/ml recombinant human IL-2 (Cetus, Emeryville, CA) and to CD4 from DAKO (Cytometry, Glostrup, Denmark). CD4 T cells were subsequently plated at 1 cell/well by a BD Biosciences CellQuest (BD Biosciences) analysis software.

For the detection of recall responses, 100,000 irradiated (3000 rad) PBMC were incubated with one of the following recall Ags: tetanus toxoid (1 Lf/ml; Rijksinstituut voor Volksgezondheid en Milieu (RIVM), Bilthoven, The Netherlands); Mycobacterium tuberculosis protein purified derivative (100x diluted) from RIVM; the HLA-DR3-restricted Mycobacterium tuberculosis HSP65 3-13 peptide (1 μg/ml, Ref. 27); CMV lysate (100x diluted) from Microbix Biosystems (Toronto, Ontario, Canada); CMV pp65 protein (1 μg/ml; Austral Biologicals, San Ramon, CA); Canida albicans lysate (100x diluted); or staphylococcal enterotoxin A (0.1 μg/ml; Serva, Heidelberg, NY) in IMDM/10% human serum for 4 h in a 96-well V-bottom plate. Blocking Abs to HLA class I (W6/32) or class II (PdV5.2), or CD4 (RV4) were added during the final 30 min of this incubation, at a final concentration of 10 μg/ml. Subsequently, 50,000 autologous responder KIR⁺CD4 T cells per well were added. T cell proliferation was determined by measuring the incorporation of [3H]thymidine during the final 16 h of a 96-h culture period.

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**Results**

KIR⁺ CD4 T cells are present in most healthy individuals, and accumulate with age

We determined whether KIR⁺CD4 T cells could be detected in the peripheral blood of healthy individuals. To this end, PBMC were stained with directly conjugated Abs to CD3, CD4, and KIR. In healthy adult donors age 18–70, on average 0.2% of CD4 T cells stained with KIR Abs (Fig. 1). We also determined the frequencies of KIR⁺CD4 T cells in UCB and in PBMC from healthy 90-year-old individuals (Fig. 1). Although KIR⁺CD4 T cells were virtually undetectable in UCB, KIR were detected on 1.0% of CD4 T cells in PBMC from the average healthy nonagenarian. Thus, KIR⁺CD4 T cell numbers appeared to increase with donor age.

**KIR⁺CD4 T cells have an effector memory surface phenotype**

To compare the surface phenotype of KIR⁺ to that of KIR⁻ CD4 T cells, four-color flow cytometry was performed on PBMC from healthy adults (Fig. 2). As before, KIR⁺CD4 T cells were distinguished from other cells using directly conjugated Abs to KIR, CD3, and CD4.

Because KIR are characteristic of NK cells rather than T cells, T cell and NK cell markers were examined. KIR⁺CD4 T cells all carried an αβ TCR (Fig. 2A), and were largely negative for CD25,
which is present on most regulatory T cells (28), and also negative for TCR-α chain V24, a marker of CD1d-restricted NKT cells (29). Thus, they were regular TCRαβ T cells, and not CD4+CD25+ regulatory cells or NKT cells. CD244 (2B4), a co-stimulatory molecule that binds CD48 and is expressed on all NK and some T cells (30), was frequently expressed by KIR+ CD4 T cells, but not by KIR+ CD4 T cells (Fig. 2B). The large majority of KIR+ CD4 T cells did not express NK markers CD16, CD56, or CD94.

As the observed age-dependent increase in the frequency of KIR+ CD4 T cells was consistent with a memory phenotype, PBMC were stained with Abs to various memory T cell markers (Fig. 2C). Like memory T cells, KIR+ CD4 T cells were mostly CD45RO+CD45RA−, but this was not consistent in all donors (data not shown). The most striking differences between KIR+ and KIR- CD4 T cells were observed for CD27, CD28, and CD57. Although KIR- cells were almost always CD27+CD28+, CD57+, the majority of KIR+ CD4 T cells displayed the opposite phenotype, characteristic of effector memory cells.

Functionally distinct CD4 subsets are characterized by their chemokine receptor expression patterns (31). In contrast to KIR- CD4 T cells, KIR+ CD4 T cells did not express CCR7, confirming the effector memory phenotype of most KIR+ CD4 T cells indicated by the absence of CD27 and CD28 (Fig. 2D). Expression of the Th1 markers CCR5, CXCR3, and CXCR6 (31, 32) did not differ consistently between KIR+ and KIR- CD4s (data not shown). KIR+ CD4 T cells did not express the Th2 marker CCR4, whereas a large subset of KIR- CD4 T cells did. Finally, the naive T cell marker CXCR4 was more highly expressed on KIR+ than on

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**FIGURE 1.** KIR+ CD4 T cell numbers in healthy donors. KIR expression frequency on peripheral blood CD4 T cells determined by staining with a mix of KIR Abs (EB6, GL183, DX9, FES172). Together, the KIR Abs covered a wide range of KIR: KIR2DL1, KIR2DL2, KIR2DL3, KIR2DS1, KIR2DS2, KIR2DS4, and KIR3DL1. Newborn samples were drawn from UCB; adults were random healthy blood bank donors age 18–70 years. Frequencies were calculated by subtracting background staining with isotype control Abs (average 0.03 ± 0.02% (±SD) of CD4 T cells) from KIR staining. Calculated frequencies were: UCB (n = 7) mean 0.00 ± 0.05%; adults (n = 30) mean 0.20 ± 0.15%; 90-year-old donors (n = 10) mean 0.98 ± 0.89%.

**FIGURE 2.** Surface markers of KIR+ and KIR- CD4 T cells. PBMC were stained with CD3, CD4, KIR Abs combined with Abs to markers of T cells (A), NK cells (B), memory cells (C), and Abs to chemokine receptors (D). Histograms were gated on KIR+ CD3+CD4+ (KIR+) or KIR- CD3+CD4+ (KIR-), live lymphocytes were selected using forward light scatter/side light scatter gates. Data are from a single healthy donor, representative of six. Surface marker of interest (thick line) and isotype control (dotted line) are indicated.
KIR⁺ CD4 T cells. In conclusion, the KIR⁺ CD4 T cell population appeared biased toward effector memory Th1 cells and largely devoid of Th2 cells.

**Effector memory cells frequently express KIR**

Overall, the best markers to distinguish KIR⁺ from KIR⁻ CD4 T cells were CD244, CD27, CD28, and CD57. Therefore, we determined how well these markers predicted KIR expression for the CD4 T cell population. Approximately one-tenth of CD28⁻ or CD57⁺ cells expressed detectable KIR at their cell surface (Fig. 3A). Compared with the entire CD4 T cell population, of which 0.2% expressed KIR, this was a roughly 50-fold enrichment. Of note, the Abs used detect only a subset of surface expressed KIR and therefore provide an underestimate of the true number of KIR⁺ cells. Thus, a sizeable fraction of late-stage memory CD4 cells expressed KIR.

**CD4 T cells express a distinct KIR repertoire**

KIR genes are expressed stochastically and independently from each other. As a result, individual cells express a seemingly random selection of the KIR genes available in the genome, resulting in a diverse repertoire of specificities.

The KIR repertoire of CD4 T cells was compared with that of NK cells. To this end, the four KIR Abs thus far used in combination were now used separately in FACS experiments (Fig. 4). Due to the great diversity in KIR genotypes, the KIR repertoires of NK cells usually differ between individuals (33). For this reason, lymphocyte subsets were only compared within the same individuals. In all donors examined, the KIR repertoire of CD4 T cells differed markedly from the NK cell KIR repertoire (Fig. 4A).

For example, the EB6 Ab, specific for KIR2DL1 and KIR2DS1, did not stain CD4 T cells detectably, whereas the NK cells in the same sample did stain with this Ab. Thus, peripheral blood CD4 T cells display KIR repertoires that are distinct from that of NK cells.

CD28⁻ CD4 T cells have been reported to play a role in rheumatoid arthritis and acute coronary syndromes (17, 34). As these cells frequently expressed KIR (Fig. 3), we compared the KIR repertoire of CD28⁻ to that of CD28⁺ CD4 T cells by FACS (Fig. 4B). Again, the EB6 Ab did not stain either population detectably. In all donors, the GL183 epitope present on KIR2DL2, KIR2DL3, and KIR2DS2 dominated the KIR repertoire of the CD28⁻ cells, whereas the CD28⁺ cells displayed a broader repertoire of KIR. Therefore, KIR repertoires differed between CD28⁻ and CD28⁺ CD4 cells.

**KIR⁺ CD4 T cells need not express KIR2DL4 or an inhibitory self-HLA-specific KIR**

The available KIR Abs have a number of limitations. First, they cover only a subset of KIR. Second, some Abs (EB6, GL183) do not discriminate between activating and inhibitory KIR. Third, at least one Ab (DX9) does not detect every allele of its ligand (KIR3DL1; Ref. 35). To overcome these limitations, KIR⁺ CD4 T cell clones were isolated from an adult healthy donor, and KIR expression was determined by RT-PCR (Fig. 5A).

We focused our attention on 10 KIR⁺ CD4 T cell clones and five KIR⁻ control clones from a single donor (Fig. 5A). A number of observations were noteworthy. First, the putative HLA-G receptor KIR2DL4 (36), expressed by all NK (24) and most KIR⁺ CD8 T (37–39) cell clones expanded in vitro, was expressed by only two of ten KIR⁺ CD4 clones (Fig. 5A). In clones containing detectable KIR2DL4 transcripts (Fig. 5B), KIR2DL4 was found expressed at the cell surface (Fig. 5C). Thus far, surface expression of KIR2DL4 protein has been detected only on CD56bright and on IL-2-activated NK cells (40, 41). Second, a self-specific inhibitory NK receptor was not obligatory for the KIR⁺ CD4 cells. The donor carried two HLA class I alleles that are known ligands for
inhibitory KIR: HLA-Cw3 and HLA-Cw4, which are ligands for KIR2DL2 and KIR2DL1, respectively. Yet, none of the clones expressed KIR2DL1, and only one clone of ten expressed KIR2DL2. Furthermore, an Ab to the broadly HLA class I specific inhibitory receptor ILT-2 stained two clones (clones 3 and 5), and none of the clones stained with Abs to NKp30, NKp44, NKp46, CD94, or NKG2D (data not shown). Finally, nine of ten clones expressed KIR2DS2, suggesting that the KIR

TCR diversity of KIR⁺ CD4 T cells
KIR⁺ CD8 T cells of healthy donors and KIR⁺ CD28⁻ CD4 T cells from rheumatoid arthritis patients are polyclonal, but do have a restricted TCR repertoire (13, 38, 39). The ten KIR⁺ clones examined carried at least five different TCR (Fig. 5A). However, four of ten KIR⁺ clones carried TCR-β chain V22. Two TCR-β chain V22⁺ clones in this panel (clones 7 and 9) expressed distinct TCR and KIR, but the other two (clones 3 and 5) expressed identical TCR and KIR genes (Fig. 5D), demonstrating that the latter two represented a clone that had divided at least once in vivo. Thus, the data indicate that KIR⁺ CD4 T cells display an oligo-

Th1 biased cytokine profile of KIR⁺ vs KIR⁻ CD4 T cells
The absence of Th2 marker CCR4 from KIR⁺ CD4s (Fig. 2) suggested that these cells were biased toward a Th0/Th1 phenotype. To test this hypothesis, cytokine production profiles of in vitro

FIGURE 5. KIR and TCR gene expression in KIR⁺ CD4 T cell clones. A, Ten KIR⁺ and five KIR⁻ CD4 T cell clones from a single donor were analyzed for KIR expression by RT-PCR. KIR RT-PCRs were performed for KIR present in the genome of the donor. In all cases, KIR expression detected by Ab (data not shown) matched the KIR genes found expressed by RT-PCR. Control samples included cDNA from LAKs from the same donor, as well as LAKs from a KIR transgenic (KIR tg) and a control mouse (non tg). Cloned KIR cDNAs (2DL1, 3DL3) were also tested. TCR-β chain variable gene segment usage was determined by FACS using TCR-β chain variable-specific Abs. Of the TCR-β chain V22⁺ clones, TCR-α chain variable gene segment usage was determined by RT-PCR. n.d., Not determined. B, KIR2DL4 RT-PCR on cDNA from selected clones (clones 4, 5, and 7) and LAKs from PBMC of the same donor (+). Control templates were water (−) and cDNA from LAKs of a nontransgenic control (−/−) and a KIR transgenic (+/+) mouse (25). C, FACS staining of clones 4, 5, and 7 with a KIR2DL4-specific (thick line) or isotype control (thin line) Ab. D, The TCR CDR3 regions of two pairs of clones expressing identical TCR-α chain and TCR-β chain variable gene segments were sequenced and aligned.
expanded KIR+ and KIR− CD4 T cell bulk cultures were compared (Fig. 6A). The in vitro expansion was necessary to obtain large enough numbers of purified responder cells. Upon stimulation with plate-bound anti-CD3, both CD4 subsets produced similar amounts of IFN-γ (Fig. 6A). In contrast, the production of Th2 cytokines IL-4, IL-5, and IL-10 was markedly lower in the KIR+ CD4 cells. IL-17 is produced by a subset of Th1/Th0 cells (42), but only KIR− CD4 cells produced significant amounts of this cytokine (Fig. 6A). Thus, the KIR+ CD4 T cell population included mainly Th1 cells unable to produce IL-17.

KIR+ CD4 T cells contain HLA class II-restricted recall Ag-specific cells

The specificity of KIR+ TCR-αβ+ CD4 T cells is unknown. Because KIR+ CD4s expressed memory markers, they might also specifically recognize recall Ags. To test this hypothesis, equal numbers of in vitro expanded KIR+ and KIR− CD4 T cells were stimulated with autologous irradiated PBMC loaded with bacterial, fungal, or viral recall Ags. Both CD4 subsets contained cells that proliferated in response to a number of these recall Ags, CMV lysate in particular (Fig. 6B). The response to CMV lysate could be inhibited by Abs to HLA class II and CD4, but not by an anti-HLA class I Ab (Fig. 6C). Thus, the KIR+ CD4 T cell population contained HLA class II-restricted memory cells specific for CMV Ags.

Discussion

We investigated the frequency, surface phenotype, cytokine profile, and Ag specificity of KIR+ CD4 T cells in healthy individuals. In contrast with previous reports, low numbers of KIR+ CD4 T cells were found in the peripheral blood of nearly every healthy adult, and this number increased with age. These cells were strongly enriched for effector memory cells that produced mainly IFN-γ. Furthermore, a subset of KIR+ CD4 T cells recognized recall Ags in an HLA class II-restricted fashion.

The repertoire of KIRs expressed by KIR+ CD4 T cells differed markedly from that of NK cells. In contrast with NK cells (24) and in agreement with reports on KIR+ CD28− CD4 T cells (9, 14), the majority of KIR+ CD4 T cells did not express KIR2DL4 or a self-HLA specific inhibitory NK receptor. Most strikingly, the expression of KIR2DL1 and KIR2DS1 was extremely rare on CD4 T cells. KIR2DL1 and KIR2DS1 specifically bind HLA-C group II molecules such as HLA-Cw4 (43). However, the HLA type C of our donors was diverse and could not easily explain the absence of KIR2DL1/KIR2DS1 from CD4 T cells. Alternatively, it is conceivable that CD4 T cells lack one or more transcription factors required for the expression of these receptors. Indeed, the promoters of KIR2DL1 and KIR2DS1 lack a binding site for YY1 present in most other KIR promoters, and may therefore be regulated differently from the other KIR genes (our unpublished observations). Finally, the KIR repertoire may be skewed depending on T cell Ag specificity or affinity (44). Clearly, CD4 T cells and NK cells do not obey the same rules for KIR expression.

The KIR+ CD4 T cells shared a number of features with previously described KIR+ CD8 T cells. First, both populations accumulate with increasing age (12). Second, both are enriched for effector memory cells (11, 12, 39), characterized by the absence of surface expression of CCR7, CD27, and CD28 as well as the presence of CD57 on the cell surface (45). In contrast with KIR+ CD8 T cells (12) and NK cells, however, KIR+ CD4 T cells did not show expression of the IL-2/IL-15 receptor β-chain (CD122, data not shown). This finding fits the observation that IL-15 stimulates CD8 but not CD4 memory cells (46). Thus, for all TCR-αβ T cells, an effector memory surface phenotype is predictive of KIR expression.
pression, suggesting that KIR play an important role in shaping both CD8 and CD4 effector memory cell responses.

The KIR4 CD4 T cells from healthy donors described in this report share several similarities with the CD28CD4 T cell population in rheumatoid arthritis patients (16). CD28CD4 T cells in rheumatoid arthritis patient PBMC also expressed CD57 (16) and KIR (13). Moreover, the KIR repertoire expressed by CD28CD4 T cells in rheumatoid arthritis patients and healthy donors was comparable: the cells rarely expressed the E6B (KIR2DL1, KIR2DS1) marker, preferentially stained with the GL183 (KIR2DL2, KIR2DL3, KIR2DS2) Ab (13), and not all KIR+ cells expressed KIR2DL4 (9, 14). Finally, the predominant cytokine produced by both KIR+ populations is IFN-γ (47). However, there were a number of significant differences as well. First, unlike CD28− cells from rheumatoid arthritis patients (16), KIR+ CD4 cells from healthy donors did not proliferate in response to autologous PBMC. This was also true for CD28− KIR+ CD4 T cell clones isolated from a healthy donor (data not shown). Furthermore, KIR+ CD4 T cells did not contain intracellular stores of perforin (data not shown), contrary to rheumatoid arthritis patient KIR+ CD4 T cells (15). In addition, rheumatoid arthritis patient CD28− CD4 cells are reportedly more likely to express KIR2DS2 than are CD28− CD4 cells from healthy controls (9). Finally, a substantial subset (40% on average) of KIR+ CD4 cells in healthy donors was CD28+. Thus, KIR+ CD4 T cells from rheumatoid arthritis patients may represent an expanded or modified subset of KIR+ CD4 T cells also present in healthy donors.

Tumor- and virus-specific KIR+ CD8 T cells have been described (38, 48, 49), but the Ag specificity of KIR+ CD4 T cells was unknown. We now show that at least some KIR+ CD4 T cells proliferate in response to CMV Ags. This finding is in line with the fact that CMV-specific CD4 T cells have high precursor frequencies and tend to lack CD28 (50–52). However, the proliferative response of the KIR+ CD4 cells did not exceed that of the KIR− population, nor did their IFN-γ production (data not shown). This was unexpected, as the percentage of memory cells in the KIR+ population was much lower than in KIR− cells. One way to reconcile these data would be to postulate that only certain pathogens induce KIR+ memory cells. Given the late stage memory phenotype of KIR+ CD4 T cells and their accumulation with age, it is conceivable that KIR+ CD4 T cells arise through chronic exposure to mostly latent pathogens, such as large DNA viruses like EBV, CMV, and HSV, and mycobacteria. Recognition of viral Ags by CD4 T cells classically occurs indirectly via presentation in the context of MHC class II. Alternatively, activating KIR may recognize viral Ags on the surface of infected target cells directly, analogous to the recognition of a mouse CMV-encoded MHC class I-like molecule by the activating NK receptor Ly49H (53, 54). This would not be detected in our assays because we used noninfectious CMV lysate to stimulate KIR− CD4 T cells. Another clue to the Ag specificity of KIR+ CD4 T cells stems from the CD28− is the CD57+ phenotype of the majority of these cells. Expanded populations of such CD4 T cells have been reported in rheumatoid arthritis (16), acute coronary syndromes (34), multiple sclerosis (55), Crohn’s disease (56), and Wegener’s granulomatosis (57). It is tempting to suggest that KIR2DS2 on CD28− CD4 T cells plays an important role in the recognition of CMV in health and disease. In short, KIR may regulate CD4 T cell responses to chronic pathogens as well as to endogenous Ags.

What is the function of KIR on CD4 T cells? Inhibitory KIR on CD4 cells may, in analogy with CD8 cells (39, 58, 59), enhance survival of memory T cells, possibly via increased resistance to activation-induced cell death, and may also be involved in peripheral tolerance by inactivating otherwise autoreactive cells. Activating KIR can costimulate CD4 T cells (13, 18, 60), and may thereby recruit low-affinity T cells to the CD4 T cell response. This would effectively expand the Ag-specific T cell repertoire. This beneficial mechanism may come at a dear price, as activating KIR may unleash the autoreactive potential of T cells with a low affinity for self Ags.

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