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Expression of CD161 (NKR-P1A) Defines Subsets of Human CD4 and CD8 T Cells with Different Functional Activities

Tsuyoshi Takahashi, Sussan Dejbakhsh-Jones, and Samuel Strober

A subset of T cells in human peripheral blood express CD161 (NKR-P1A) receptors that are primarily associated with NK cells. In the current study we isolated blood T cell subsets according to the expression of CD161 and examined their contents of naive, central memory, and effecter memory cells and their capacities for proliferation, cytokine secretion, and natural cytolyis. We found that CD4\(^+\)CD161\(^-\) and CD8\(^+\)CD161\(^-\) subsets contained predominantly naive T cells that secreted high levels of IL-2 after in vitro stimulation, and CD4\(^+\)CD161\(^{int}\) and CD8\(^+\)CD161\(^{int}\) subsets contained predominantly effecter and central memory T cells that secreted high levels of IFN-\(\gamma\) and TNF-\(\alpha\). All of these subsets showed vigorous proliferation after stimulation in vitro, but none had NK lytic activity. Unexpectedly, the CD8\(^+\)CD161\(^{+}\) cells contained an anergic CD8\(^+\)CD8\(^{\text{dim}}\)-CD161\(^{\text{high}}\) T cell subset that failed to proliferate, secrete cytokines, or mediate NK lytic activity. The Journal of Immunology, 2006, 176: 211–216.

In studies of mice, the cell surface receptor NK1.1, a member of the NKR-P1 family, is expressed on almost all NK cells and on a subset of T cells, NK T cells (1, 2). Almost all NK1.1\(^+\) T cells recognize their TCR ligands in association with the Ag-presenting molecule CD1d, and the majority of NK1.1\(^+\) T cells in the thymus, liver, and secondary lymphoid tissues express the invariant V\(\alpha\)4J\(\alpha\)8 TRC (3–7). Subsets of NK1.1\(^+\) T cells are CD4\(^+\), CD8\(^+\), or CD4\(^-\)CD8\(^-\). In the thymus, blood, and secondary lymphoid tissues, NK1.1\(^+\) T cells represent 1–2% of all T cells; in the liver and bone marrow, they represent 20–40% of all T cells (8, 9).

In humans, the NKR-P1A receptor (CD161), another member of the NK-R-P1 family, is also expressed on almost all NK cells and on a subset of \(~25\)% of blood T cells (10). CD161 is expressed predominantly on T cells with the memory phenotype (CD45RO\(^+\)) (10, 11). Only a small minority of human CD161\(^+\) T cells are NK T cells with the invariant TCR (V\(\alpha\)24V\(\beta\)11), because the latter cells represent no less than 1% of all T cells in the lymphoid tissues and liver (12, 13). Although, CD161 is expressed on the minority of human blood T cells, CD161\(^+\) T cells expressing either CD4 or CD8 represented the majority of T cells from the epithelial and lamina propria layers of the human duodenum and colon (14). The latter cells contained few, if any, V\(\alpha\)24V\(\beta\)11 invariant NK T cells and secreted IFN-\(\gamma\) and TNF-\(\alpha\) without IL-4 after in vitro stimulation. An abundance of CD161\(^+\) T cells was also found in the liver and intestinal epithelial cells of the jejunum; the majority of the latter cells were CD161\(^+\)CD8\(^+\) T cells (15). CD161 is also expressed on human monocytes and dendritic cells (16). Engagement of CD161 on the cell surface using an appropriate mAb induced the production of IL-1\(\beta\) and IL-12 by monocytes and dendritic cells, respectively (16).

Although both human NK cells and a subset of human T cells express the CD56 marker, only a small minority of CD161\(^+\) T cells also express CD56 (10, 17). Although CD56\(^+\)CD8\(^+\) T cells have been shown to have natural cytolytic activity against the K562 and Raji cell lines (18), it is not clear whether CD161\(^+\)CD8\(^+\) T cells have natural cytolytic activity or the capacity to proliferate and secrete cytokines after stimulation via the TCR.

In the current study we isolated human peripheral blood T cell subsets according to their expression of CD161 and examined their extended surface receptor phenotype for their contents of naive, central memory, and effecter memory cells and their capacities for proliferation, cytokine secretion, and natural cytolyis. We found that the CD4\(^+\)CD161\(^{int}\) and CD8\(^+\)CD161\(^{int}\) T cell subsets secreted more IFN-\(\gamma\) and TNF-\(\beta\) than the CD161\(^-\) subset after in vitro stimulation via the TCR, and that the latter subset secreted more IL-2. All these subsets showed vigorous proliferation after stimulation, but none had natural cytolytic activity. Unexpectedly, we found a subset of CD8\(^+\)CD161\(^{\text{high}}\) T cells that was anergic and failed to proliferate, secrete cytokines, or mediate natural cytolytic activity.

Materials and Methods

Abs and chemical reagents

FITC-conjugated mAbs recognizing human CD4 (RPA-T4), CD8 (RPA-T8), CD45RA (HI100), CD45RO (UCHL1), and TCR\(\alpha\beta\) (T10B9.1A-31) were purchased from BD Pharmingen, and FITC-anti-V\(\alpha\)24 mAb (C15) was purchased from Beckman Coulter. PE-conjugated mAb against CD161 (191B8), PE-Texas Red-conjugated mAb against CD8\(^\beta\) (2S7.8.5HT), and CD62L (DREG56) were purchased from Beckman Coulter. Allophycocyanin-conjugated anti-CD4 (RPA-T4), CD8 (RPA-T8), CD28 (CD28.2), and CD56 (B159) mAbs were purchased from BD Pharmingen. Biotin-conjugated anti-V\(\beta\)11 mAb (C21) was purchased from Beckman Coulter. Streptavidin-Texas Red was purchased from Caltag Laboratories. Biotin-conjugated anti-CCR7 (3D12) mAb, biotinylated mouse anti-rat IgG2a, and streptavidin-PE were purchased from BD Pharmingen. Anti-CD3 (UCHT1) and CD28 (CD28.2) mAb for T cell stimulation were purchased from Beckman Coulter. Anti-human CD4 and CD8 magnetic beads were purchased from Miltenyi Biotec.

Cell preparation and flow cytometry

Peripheral blood was obtained from healthy donors after obtaining their informed consent at the Stanford Blood Center. PBMCs were isolated by

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Ficoll-Hypaque density centrifugation (Amersham Biosciences). For surface marker analyses, three- to four-color immunostaining and flow cytometry were performed using standard techniques and equipment (LSR and FACSVantage cytometers; BD Biosciences).

**Cytokine bead array analyses**

For CD4\(^+\) CD161\(^−\), CD4\(^+\) CD161\(^++\), CD8\(^+\) CD161\(^−\), CD8\(^+\) CD161\(^++\), and CD8\(^+\) CD161\(^+++\) subsets, CD4 or CD8 Immunomagnetic bead-enriched cells were stained with CD4 or CD8 and CD161 and sorted by a FACSVantage apparatus (BD Biosciences). The purity of the sorted cells was >95%, as judged by reanalysis of sorted cells by FACSVantage. Sorted cells (5 \(\times\) 10\(^5\)) were cultured in duplicate wells coated with anti-CD3 mAb (10 \(\mu\)g/ml overnight at 4°C) containing 200 \(\mu\)l of tissue culture medium with soluble anti-CD28 mAb (5 \(\mu\)g/ml) in RPMI 1640, 10% human AB serum, 2 \(\mu\)M L-glutamine, 100 \(\mu\)g/ml penicillin, 100 U/ml streptomycin, and 50 \(\mu\)M 2-ME. After 24 h, 100 \(\mu\)l of cell-free supernatant was collected from each well of the 96-well plates and frozen at -80°C until cytokine analysis. Cytokines were detected with the cytokine bead array kit (BD Pharmingen) according to the manufacturer’s protocol, using beads coated with a mAb against a single cytokine (IL-2, IL-4, IL-5, IL-10, IFN-γ, or TNF-α). Samples were collected and analyzed with FACSscan and CellQuest software (BD Biosciences). To evaluate the cytokine contribution from V\(α\)24\(^+\) invariant NKT cells, CD4\(^+\) CD161\(^−\) V\(α\)24\(^+\) or CD4\(^+\) CD161\(^++\) V\(α\)24\(^+\) cells were sorted by the FACSVantage apparatus, and cytokine production was compared with CD4\(^+\) or CD4\(^+\) CD161\(^++\) cells, respectively.

**Proliferation assay**

Sorted T cells (5 \(\times\) 10\(^3\); >95% purity) were cultured with anti-CD3 mAb (10 \(\mu\)g/ml; coated) and soluble anti-CD28 mAb (5 \(\mu\)g/ml) in 200 \(\mu\)l of complete medium in round-bottom, 96-well plates in triplicate wells. The cells were incubated at 37°C in an atmosphere containing 5% CO₂ for 72 h. For the final 16 h of incubation, 1 \(\mu\)Ci of \(^{3}H\)thymidine (PerkinElmer) was added to each well, and the incorporation of \(^{3}H\)thymidine was determined by liquid scintillation counting (BETA PLATE; Wallac). In some experiments, 100 U/ml human rIL-2 (Chiron) was added to the culture medium.

**Cytotoxicity assays**

A total of 5 \(\times\) 10\(^3\) \(^{51}Cr\) (Amersham Biosciences) labeled K562 or Jurkat cells was used as target cells and cultured with various numbers of effector cells (95% purity) in 200 \(\mu\)l of culture medium in 96-well round-bottom microtiter wells. The cultures were incubated at 37°C in an atmosphere containing 5% CO₂ for 72 h. For the final 20 h of incubation, 1 \(\mu\)Ci of \(^{51}Cr\) was added to each well, and the incorporation of \(^{51}Cr\) was calculated by liquid scintillation counting (BETA PLATE; Wallac).

**Statistical analyses**

Differences in mean cytokine production and proliferation among T subsets were analyzed using the two-tailed Student’s t test. A value of \(p < 0.05\) was considered statistically significant.

**Results**

**Human T cell subsets defined by CD161 expression**

PBMCs from 21 normal individuals were analyzed by immunofluorescent staining and multicolor flow cytometric analysis to define subsets of CD4 and CD8 T cells based on the expression of CD161. As shown in Fig. 1, staining for CD4 vs CD161 separated two subsets of CD4\(^+\) cells: a major subset (mean, 77 ± 5%) of CD4\(^+\) CD161\(^−\) cells and a minor subset (mean, 23 ± 2%) of CD4\(^+\) CD161\(^++\) cells (contained in boxes I and II of left panel). Staining of CD161 also defined three subsets of CD8\(^+\) cells (hereafter referred to as CD8\(^+\) cells): a major subset of CD8\(^+\) CD161\(^−\) cells (mean, 79 ± 9%), a minor subset of CD8\(^+\) CD161\(^++\) cells (mean, 9 ± 1%), and a minor subset of CD8\(^+\) CD161\(^+++\) cells (mean, 11 ± 2%; contained in boxes I, II, and III of the right panel of Fig. 1, respectively). The mean percentage of total CD4\(^+\) T cells among all lymphocytes was 32 ± 2%, and that of total CD8\(^+\) T cells was 19 ± 2%. A discrete population of null-staining CD8\(^+\) cells that expressed intermediate levels of CD161 was observed also (Fig. 1, arrow). However, on subsequent analysis, the latter cells were found to be CD3\(^−\) CD56\(^+\) NK cells (data not shown).

Fig. 2 shows the extended phenotypic analysis of gated CD4\(^+\) CD161\(^−\) and CD4\(^+\) CD161\(^++\) subsets that were contained in boxes I and II, respectively, of Fig. 2a. Both subsets were made up of T cells (>99% TCR\(αβ\) CD28\(^+\)) that contained 1–2% CD65\(^−\) T cells and <1% V\(α\)24\(^+\) V\(β\)11\(^+\) invariant NK T cells (Fig. 2, b and c). The subsets differed in their content of naive and memory cells, because the CD4\(^+\) CD161\(^−\) cells were almost all memory cells (97% CD45RO\(^+\)) and the CD4\(^+\) CD161\(^++\) cells were a mixture of naive cells (63% CD45RA\(^+\)) and memory cells (26% CD45RO\(^+\)). Among the CD4\(^+\) CD161\(^++\) CD45RO\(^+\) cells, about half expressed the typical central memory phenotype (45% CD62L\(^−\) CCR7\(^+\)) (Fig. 2b). Approximately 85% of the CD4\(^+\) CD161\(^−\) cells were CD62L\(^+\) CCR7\(^+\) cells that included naive and central memory cells, and 9% were either CD62L\(^−\) or CCR7\(^−\) (Fig. 2b).

Fig. 3 shows the extended phenotypic analysis of gated CD8\(^−\) CD161\(^−\), CD8\(^−\) CD161\(^++\), and CD8\(^−\) CD161\(^+++\) subsets that are contained in boxes I, II, and III of Fig. 3a. Almost all subsets were T cells (93–99% TCR\(αβ\)). The subsets again differed in their content of memory T cells; although most of the CD8\(^−\) CD161\(^−\) cells were naive (83% CD45RA\(^+\); 13% CD45RO\(^+\)), the majority of CD8\(^−\) CD161\(^++\) (76% CD45RO\(^+\)) and CD8\(^−\) CD161\(^+++\) (93% CD45RO\(^+\)) were memory T cells. Almost all the latter cells were effector memory cells, because <1% expressed the CD62L\(^−\) CCR7\(^+\) central memory phenotype. Although cell subsets stained brightly for CD8α, the expression of CD8β on the CD161\(^++\) subset was reduced (61% CD8\(^β\)high) compared with the expression of CD8\(^β\) on the two other subsets (98–99% CD8\(^β\)high).

Less than 5% of the CD8\(^−\) CD161\(^−\) or CD8\(^−\) CD161\(^++\) subsets expressed CD56, and <0.1% of all three subsets was invariant V\(α\)24\(^+\) V\(β\)11\(^−\) NK T cells (Fig. 3). Reduced expression of CD28 was found on CD8\(^−\) CD161\(^++\) cells (85% CD28\(^−\)) compared with CD8\(^−\) CD161\(^−\) and CD8\(^−\) CD161\(^+++\) cells (>98% CD28\(^−\)).
Cytokine secretion of T cell subsets defined by CD161

To compare the functional activities of the CD4⁺CD161⁻ and CD4⁺CD161int T cell subsets from normal PBMCs, the two subsets were sorted as described in Fig. 1, and sorted cells were stimulated in vitro with anti-CD3 and anti-CD28 mAbs. After 24 h, supernatants were harvested from the wells, and cytokine concentrations were determined using a microbead assay. Fig. 4 shows the mean concentrations from triplicate wells for the six cytokines secreted by either CD4⁺CD161⁻ or CD4⁺CD161int T cells. There was a statistically significant increase in the concentrations of IFN-γ (p = 0.0002), TNF-α (p = 0.035), IL-4 (p = 0.046), IL-5 (p = 0.024), and IL-10 (p = 0.0029) in the supernatants from CD4⁺CD161int cells compared with those from CD4⁺CD161⁻ cells. In contrast, the concentration of IL-2 was higher in the supernatants from CD4⁺CD161⁻ cells compared with CD4⁺CD161int cells, but the difference was not significant (p = 0.32).

FIGURE 2. Extended phenotype of CD8⁺CD161⁻ and CD8⁺CD161int T cell subsets. CD8⁺ T cell subsets were gated as shown in a, and one-color analysis of staining for various receptors are shown in b and c. Percentages in each profile show the positive staining of cells above isotype control backgrounds. In addition, gated cells in a were analyzed for two-color staining for CCR7 vs CD62L and Vα24⁺ vs Vβ11 in b and c. The percentages of Vα24⁺Vβ11⁺ cells are shown in boxes and represent invariant NK T cells. A representative sample of at least six analyses is shown.

FIGURE 3. Extended phenotype of CD8⁺CD161⁻, CD8⁺CD161int, and CD8⁺CD161high T cells subsets. CD8⁺ T cell subsets were gated as shown in a, and one- or two-color analysis was performed in b–d, as described in Fig. 2. A representative example of at least six analyses is shown.
This pattern was consistent with the predominance of memory T cells in the CD4<sup>+</sup>CD161<sup>int</sup> subset and the increased representation of naive T cells in the CD4<sup>+</sup>CD161<sup>−</sup> subset. Because CD4<sup>+</sup> V<sub>α</sub>24<sup>+</sup>V<sub>B</sub>11<sup>+</sup> invariant NK T cells are known to express CD161 and produce large amounts of Th1- and Th2-type cytokines, we compared the cytokine production of CD4<sup>+</sup>V<sub>α</sub>24<sup>+</sup>CD161<sup>int</sup> T cells with that of CD4<sup>+</sup>CD161<sup>−</sup> T cells; we observed no significant difference between them (data not shown).

We investigated the in vitro cytokine secretion of sorted CD8<sup>+</sup>CD161<sup>−</sup>, CD8<sup>+</sup>CD161<sup>int</sup>, and CD8<sup>+</sup>CD161<sup>high</sup> T cell subsets also using the gating thresholds shown in Fig. 1. None of the sorted CD8<sup>+</sup> T cell subsets secreted detectable levels of IL-4, IL-5, or IL-10. Fig. 5 summarizes the cytokine secretion only for IFN-γ, TNF-α, and IL-2. Interestingly, the CD8<sup>+</sup>CD161<sup>high</sup> subset failed to secrete detectable levels of the latter three cytokines, whereas cytokine secretion was >400 pg/ml for all three using CD8<sup>+</sup>CD161<sup>−</sup> cells (IFN-γ, p = 0.0016; TNF-α, p = 0.034; IL-2, p = 0.017). The CD8<sup>+</sup>CD161<sup>int</sup> subset secreted higher levels of IFN-γ and TNF-α than the CD8<sup>+</sup>CD161<sup>−</sup> subset (IFN-γ, p = 0.042; TNF-α, p = 0.049). In contrast, the CD8<sup>+</sup>CD161<sup>−</sup> subset secreted significantly higher levels of IL-2 than the CD8<sup>+</sup>CD161<sup>int</sup> subset (p = 0.037). This pattern was again consistent with the predominance of memory T cells in the CD161<sup>int</sup> subset and increased representation of naive cells in the CD161<sup>−</sup> subset.

### Proliferation of T cell subsets defined by CD161

Sorted subsets of CD4<sup>+</sup> and CD8<sup>+</sup> T cells were assayed for their capacity to proliferate after stimulation with anti-CD3 and anti-CD28 mAbs. As shown in Fig. 6, mean [<sup>3</sup>H]thymidine incorporation of triplicate wells of CD4<sup>+</sup>CD161<sup>−</sup> and CD4<sup>+</sup>CD161<sup>int</sup> cells was similar to that of total CD4<sup>+</sup> T cells and in the range of ~4 × 10<sup>4</sup> cpm (CD4<sup>+</sup>CD161<sup>−</sup>, p = 0.066; CD4<sup>+</sup>CD161<sup>int</sup>, p = 0.69). Among CD8<sup>+</sup> T cell subsets, CD8<sup>+</sup>CD161<sup>−</sup> and CD8<sup>+</sup>CD161<sup>int</sup> cells had mean [<sup>3</sup>H]thymidine incorporation in the range of 2–3 × 10<sup>4</sup> cpm (p = 0.067). However, sorted CD8<sup>+</sup>CD161<sup>high</sup> T cells failed to incorporate [<sup>3</sup>H]thymidine above background levels of ~0.3 × 10<sup>4</sup> cpm, and the mean value was significantly decreased compared with that of CD8<sup>+</sup>CD161<sup>−</sup> cells (p = 0.0033). Addition of 100 U/ml human rIL-2 to the latter cultures failed to increase [<sup>3</sup>H]thymidine above background (p = 0.0033; Fig. 6).

### Presence of cytolytic granules and lack of spontaneous cytolytic activity of CD8<sup>+</sup> T cell subsets

NK cells express high levels of intracellular cytolytic granules, including perforin and granzyme A, and these granules contribute to the spontaneous cytolytic activity of the NK cells on selected target cells (19). In additional studies we compared the subsets of

![FIGURE 4](http://example.com/f4.png)  
**FIGURE 4.** Cytokine production of sorted CD4<sup>+</sup>CD161<sup>−</sup> and CD4<sup>+</sup>CD161<sup>int</sup> T cell subsets. Sorted subsets were incubated for 24 h in the presence of anti-CD3 and anti-CD28 mAbs, and supernatants were harvested. The concentration of each cytokine was determined by a bead array flow cytometric assay. Bars show the means, and brackets show the SEs of triplicate determinations from a single individual. Data are representative of five independent experiments. *, p < 0.05; **, p < 0.01 (by Student’s t test).

![FIGURE 5](http://example.com/f5.png)  
**FIGURE 5.** Cytokine production of sorted CD8<sup>+</sup>CD161<sup>−</sup>, CD8<sup>+</sup>CD161<sup>int</sup>, and CD8<sup>+</sup>CD161<sup>high</sup> T cell subsets. Sorted subsets were stimulated with anti-CD3 and anti-CD28 mAbs, and supernatants were collected after 24 h. Bars show the mean, and brackets show the SEs of triplicate determinations from a single individual. Data are representative of five independent experiments. *, p < 0.05; **, p < 0.01 (by Student’s t test).

![FIGURE 6](http://example.com/f6.png)  
**FIGURE 6.** Proliferation of sorted CD4<sup>+</sup> and CD8<sup>+</sup> T cell subsets after stimulation with anti-CD3 and anti-CD28 mAbs. [ ] Mean (±SE) [<sup>3</sup>H]thymidine incorporation of triplicate wells of the indicated sorted subsets; ■, mean [<sup>3</sup>H]thymidine incorporation when rIL-2 (100 U/ml) was added to cultures of CD8<sup>+</sup>CD161<sup>high</sup> T cells. Data are representative of three independent experiments. **, p < 0.01 (by Student’s t test). The proliferation of CD4<sup>+</sup> T cell subsets was compared with that of whole CD4<sup>+</sup> T cells, and the proliferation of CD8<sup>+</sup> T cell subsets was compared with that of CD8<sup>+</sup>CD161<sup>−</sup> T cells.
CD8⁺ T cells distinguished by the expression of CD161 for their capacities to mediate cytolysis of NK cell-sensitive target cell lines and for their levels of intracellular cytolytic granules. As shown in Fig. 7, ~97% of gated NK cells (CD8dimCD161⁺) expressed high levels of perforin, as judged by intracellular staining, and ~24% of CD8⁺ CD161⁻ T cells showed positive staining for intracellular perforin. Although ~57% of CD8⁺ CD161⁺ cells and 93% of CD8⁺ CD161high cells stained positively for perforin, the peak channel of fluorescence was ~10-fold lower than that of NK cells (Fig. 7). Similarly, 94% of NK cells stained positively for intracellular granzyme A, but 29% of CD8⁺ CD161⁻ T cells showed positive staining. Approximately 56% of CD8⁺ CD161⁺ and 88% of CD8⁺ CD161high T cells stained positively for granzyme A, and the peak channel of fluorescence of the latter subset was similar to that of NK cells. Thus, the highest levels of cytolytic granules in the CD8⁺ T cell subsets were found in the CD8⁺ CD161high subset.

We tested the three CD8⁺ T cell subsets for their capacities to kill two NK cell-sensitive target cell lines, K562 and Jurkat. As shown in Fig. 8, sorted NK cells (CD3⁺CD56⁺) showed vigorous cytolysis of both the K562 line (~55% specific lysis) and the Jurkat line (~18% specific cytolysis) at an E:T cell ratio of 10:1, as judged by the ⁵¹Cr release assay, after a 4-h incubation period. The percent specific cytolysis fell to ~35 and 10%, respectively, in the K562 and Jurkat lines at an E:T cell ratio of 5:1. In contrast, none of the three sorted CD8⁺ T cell subsets (CD161⁻, CD161⁺, and CD161high) was able to lyse the target cell lines, and specific lysis was ~2% at E:T cell ratios of 20:1, 10:1, and 5:1 (Fig. 8). Next, we examined the effect of IL-2 preincubation on the lytic activity of CD8⁺ CD161⁻ T cells. CD8⁺ CD161⁻ T cells as well as CD8⁺ CD161⁻ T cells were cultured in cRPMI with IL-2 (1000 U/ml) for 2 days and used as effector cells. None of the populations exhibited NK lytic activity (data not shown).

**Discussion**

CD161 has been reported to be expressed on a minority of human CD4⁺ and CD8⁺ T cells and on almost all NK cells (10). However, it is not clear how subsets of T cells defined by the expression of CD161 on the cell surface differ from each other with regard to their functions or their extended surface receptor phenotypes. The goal of the current study was to elucidate the characteristics of human T cell subsets based on CD161 expression and to determine whether CD161⁺ subsets shared some of the functional capacities of NK cells.

Flow cytometric analysis of human T cells showed that among CD4⁺ T cells, a major population of CD4⁺ CD161⁻ and a minor population (~23%) of CD4⁺ CD161⁺ cells could be defined, whereas among CD8⁺ T cells, a major population of CD8⁺ CD161⁻ and two minor populations of CD8⁺ CD161⁺ (~9%) and CD8⁺ CD161high (~11%) cells were defined. Extended phenotypic analysis showed that the CD4⁺ CD161⁺ cells were composed almost entirely of CD45RO⁺ memory T cells, and ~45% of the latter cells expressed the central memory (CD45RO⁺ CD27⁺ CCR7⁺) phenotype (20). The CD4⁺ CD161⁻ cells were a mixture of naive and memory cells at about a 2:1 ratio. The cytokine secretion pattern of the sorted CD4⁺ CD161⁻ and CD4⁺ CD161⁺ cells was studied after activating the cells in vitro with anti-CD3 and anti-CD28 mAbs. The CD4⁺ CD161⁺ cells secreted significantly higher levels of IFN-γ, TNF-α, IL-4, IL-5, and IL-10 than the CD4⁺ CD161⁻ cells. Because CD4⁺ memory T cells secrete higher levels of these cytokines than CD4⁺ naive T cells (21), the different cytokine patterns appeared to reflect the different balances of naive and memory T cells in the CD4⁺ CD161⁻ and CD4⁺ CD161⁺ subsets. In contrast, the vigorous secretion of IL-2 by the CD4⁺ CD161⁻ subset was an expected result of the contribution of naive T cells (21). We did not compare the cytokine secretion patterns or other functions of purified CD4⁺ CD161⁺ CD45RO⁺ memory T cells to those of CD4⁺ CD161⁺ CD45RO⁻ memory T cells, and the differences in the two subsets of memory T cells are the subject of continuing investigation.

In similar studies we compared the extended phenotypes of the gated CD8⁺ T cell subsets, and found that ~80% of the CD8⁺ CD161⁻ cells expressed the CD45RA⁻CD45RO⁺ CD62L⁻ CCR7⁻ naive T cell pattern, whereas ~90% of the CD8⁺ CD161high cells expressed the CD45RA⁻CD45RO⁺ CD62L⁻ CCR7⁻ effector memory cell pattern (20). The CD8⁺ CD161⁻ cells contained a mixture of memory and naive T cells, and only ~7% expressed the CD62L⁻ CCR7⁻ phenotype of naive or central memory T cells. Thus, in both CD4⁺ and CD8⁺ T cells, the CD161⁻ subset contained a majority of naive T cells, whereas the CD8⁺ CD161⁻ subsets contained predominantly effector memory T cells, and the CD4⁺ CD161⁻ subset contained a mixture of central and effector memory cells. More than 97% of the CD8⁺ CD161⁻ and CD8⁺ CD161⁺ cells expressed high levels of CD8β, but only ~60% of CD8⁺ CD161high T cells expressed high levels. The remaining CD8⁺ CD161high cells, which expressed the CD8β⁻/low phenotype, expressed as high levels of CD8α as the other CD8⁺ T cell subsets.

Although the majority of NK1.1⁺ (NK1.1⁺) T cells in mice are invariant NK T cells that express the Vα14Jα8 TCR α-chain
(8, 9), <1% of all the human T cell subsets that we studied expressed the Vα24Vß11 TCR of invariant NK T cells regardless of the level of expression of CD161. These results are consistent with previous studies showing that invariant NK T cells are found in <1% of human peripheral blood T cells (12). Nevertheless, almost all human invariant NK T cells express CD161 (12), and as expected, Vα24Vß11 invariant NK T cells were not detected (≤0.02%) in CD4+CD161− and CD8+CD161 T cells, but were present in the range of 0.1–1% in CD4+CD161hi and CD8+CD161hi T cells. Invariant NK T cells were not detected in the CD8+CD161high subset. We did not attempt to identify noninvariant CD161+ NK T cells that recognize CD1d in the current study. A previous study showed that these noninvariant NK T cells account for the majority of CD1-reactive NK T cells in the human bone marrow and can suppress the MLR (22).

Studies of the cytokine secretion pattern of sorted CD8+ T cells showed that none produced detectable IL-4, IL-5, or IL-10 after in vitro stimulation with anti-CD3 and anti-CD28 mAbs. As in the case of CD4+ T cells, the CD8+CD161int subset secreted significantly higher levels of IFN-γ and TNF-α than the CD8+CD161− subset, whereas the CD8+CD161− subset produced significantly higher levels of IL-2. Again, the pattern reflected the balance of naïve and memory T cells. Surprisingly, the CD8+CD161hi subset failed to produce any of the three cytokines. Although the CD4+ T cell subsets and the CD8+CD161− and CD8+CD161int subsets all proliferated in response to in vitro stimulation with anti-CD3 and anti-CD28 mAbs, the CD8+CD161high subset failed to proliferate above background levels. The lack of cytokine secretion and proliferation indicated that the CD8+CD161hi subset was anergic. Anergy could not be reversed by adding IL-2 to the cultures. In a previous study, CD8α+CD8β− cells were reported to have a restricted CD3R3 spectratype compared with CD8α−CD8β+ cells and were considered to be an oligoclonal population of terminally differentiated memory cells (23). The current results indicate that these cells are part of a larger subset of anergic CD8+CD161high cells that contain a mixture of CD8β+ and CD8β− cells. We have not yet determined whether the larger subset is oligoclonal.

Finally, we compared the abilities of the sorted CD8+ T cell subsets to kill the NK cell-sensitive target cell lines K562 and Jurkat. In control experiments, sorted NK cells showed vigorous cytolytic activity, but none of the sorted CD8+ T cell subsets had killing activity despite the detection of high levels of intracellular perforin and granzyme A granules in the CD8+CD161high subset.

In conclusion, the expression of CD161 can be used to define T cell subsets, in particular, an anergic CD8+CD161high subset that expresses an effector memory phenotype. The latter subset may contain terminally differentiated effector cells that have lost the capacity for proliferation and cytokine secretion. In general, the expression of CD161 on human CD4+ and CD8+ T cells appeared to be a marker of central and/or effector memory cells, as judged by the surface phenotype and cytokine secretion patterns, and, unlike mouse T cells, contained only a small minority of invariant NK T cells.

Disclosures

The authors have no financial conflict of interest.

References