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Unique Subpopulations of CD56$^+$ NK and NK-T Peripheral Blood Lymphocytes Identified by Chemokine Receptor Expression Repertoire[1]

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CD56, an adhesion molecule closely related to neual cell adhesion molecule, is an immunophenotypic marker for several unique populations of PBLs. Although CD56$^+$ cells derive from multiple lymphocyte lineages, they share a role in immunosurveillance and antitumor responses. We have studied the chemokine receptor expression patterns and functional migratory responses of three distinct CD56$^+$ populations from human peripheral blood. NK-T cells were found to differ greatly from NK cells, and CD16$^+$ NK cells from CD16$^-$ NK cells. CD16$^+$ NK cells were the predominant population responding to IL-8 and fractalkine, whereas NK-T cells were the predominant population responding to the CCR5 ligand macrophage-inflammatory protein-1β. CD16$^-$ NK cells were the only CD56$^+$ population that uniformly expressed trafficking molecules necessary for homing into secondary lymphoid organs through high endothelial venule. These findings describe a diverse population of cells that may have trafficking patterns entirely different from each other, and from other lymphocyte types. ***The Journal of Immunology,*** **2001, 166: 6477–6482.**

Expression of CD56 identifies diverse human PBL populations of multiple origins. Among these, NK cells were first functionally defined for their ability to recognize and kill nonself cells within the host, without requiring previous immunization (reviewed in Ref. 1). Although much remains to be learned about how NK cells function, our knowledge of these cells has increased considerably with the discovery of the killer-inhibitory receptor family of proteins, which allows NK cells to recognize cells lacking particular host-specific HLA (or MHC) molecules (reviewed in Ref. 2). Most NK cells express the FcR CD16, which may mediate Ab-dependent cytotoxicity (reviewed in Ref. 1), another known function of NK cells. However, a subset of NK cells lacks CD16 (3). This small subset also differs from the bulk of NK cells by expressing higher levels of both CD56 and CD94 (another NK-associated molecule). CD16$^-$ NKs possess activation characteristics unlike CD16$^+$ NK cells, and may thus have unique functions (3).

The other major division of the CD56$^+$ population is composed of cells from the T lineage. These CD3$^+$/CD56$^+$ cells are known as cytokine-induced killer (4, 5), or NK-T cells (due to their functional similarities to murine CD3$^+$/NK1.1$^+$ NK-T cells). The human PBL NK-T population is enriched in CD8 T cells of effector phenotype (6). Like murine NK-T cells (7), human NK-T cells contain a subset that can recognize lipid Ags presented by the HLA/MHC class I-like molecule CD1d (8).

Chemokines are a large family of small proteins involved in leukocyte trafficking (9–12). Heterogeneity of chemokine receptor expression by PBL subsets has recently been shown to contribute to tissue-specific homing of T cell subsets (13–16) (reviewed in Refs. 13 and 14).

In contrast, little is known about the homing properties of the CD56$^+$ PBL subsets. Surveys of expression patterns for individual chemokine receptors on PBLs have demonstrated the presence of several such receptors on CD56$^+$ cells. For example, CD56$^+$ cells have been variously reported to express CXC chemokine receptor (CXCR)[3] 1 (15), CCR2 and CCR5 (16), CCR7 (17), and CX3CR1 (18). However, little attempt has been made to carefully ascertain the differences in chemokine receptor expression patterns among the various CD56$^+$ subsets. In addition, many published studies have relied on in vitro activated cells (19, 20). Since the vast majority of circulating lymphocytes are not activated, we feel strongly that the repertoire of receptors expressed by fresh, resting cells is most relevant toward elucidating which receptors may be directly involved in vivo with egress from the blood.

CD56$^+$ lymphocytes are believed to be major players in immunosurveillance and antitumor responses (1, 21, 22). Thus, the mechanisms by which these cells home into tumors and areas of inflammation are of great relevance to human health and disease. To shed light on the homing potentials of CD56$^+$ subsets, we have undertaken to systematically examine the chemokine receptor repertoires of the three PBL types mentioned above: CD16$^+$ NK, CD16$^-$ NK, and NK-T. We have studied the differential enrichment of each subset after migration to individual chemokines, as well as the expression of known chemokine receptors by each

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[3] Abbreviations used in this paper: CXCR, CXC chemokine receptor; IP-10, IFN-γ-induced protein-10; ITAC, IFN-γ-inducible T cell α chemoattractant; MCP, monocyte chemoattractant protein; MIP, macrophage-inflammatory protein; SDF, stromal-derived factor; SLC, secondary lymphoid tissue chemokine.

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subset and the relationship between receptor expression and the ability to migrate toward known ligands.

We have found dramatic differences between the chemokine receptor repertoires of NK vs NK-T cells, as well as between CD16$^+$ and CD16$^-$ NK cells. These differences imply that NK and NK-T cells may have entirely different routes of circulation and trafficking patterns. We have also found that, although CD16$^+$ and CD16$^-$ NK cells are each relatively uniform in their expression of receptors, NK-T cells are extremely heterogeneous. This implies that the NK-T population may itself be further divided into previously unrecognized subpopulations.

Materials and Methods

Unconjugated mAbs

The following unconjugated mouse anti-human mAbs were used in this study: CCR1, 2D4 (IgG1; Millennium Pharmaceuticals, Cambridge, MA) and 53504 (IgG2b; R&D Systems, Minneapolis, MN); CCR2, 48607 (IgG2b; R&D Systems); CCR3 (IgG2a; Millennium Pharmaceuticals); CCR4, 1G1 (IgG1; Millennium Pharmaceuticals) and 2B10 (IgG2a; Millennium); CCR5, 2D7 (IgG2a; BD PharMingen, San Diego, CA); CCR6, 53103 (IgG2b; R&D Systems); CCR7, 7H12 (IgG2b; Millennium Pharmaceuticals) and 3D9 (IgM; Millennium Pharmaceuticals); CCR9, 96-1 (IgG1; Millennium Pharmaceuticals) and 3C3 (IgG2b; Millennium Pharmaceuticals); CXCR1, 5A12 (IgG2b; Millennium Pharmaceuticals); CXCR2, 6C6 (IgG1; BD PharMingen) and 48311 (IgG2a; R&D Systems); CXCR3, 1C6 (IgG1; Millennium Pharmaceuticals) and 49801 (R&D Systems); CXCR4, 12G5 (IgG2a; BD PharMingen) and 44716 (IgG2b; R&D Systems); CXCR5, 51505 (IgG2b; R&D Systems); CX3CR1, 1E5 (IgG1; Millennium Pharmaceuticals); Bonzo, 56811 (IgG2b; R&D Systems); L-selectin, DREG-56 (IgG1; Butcher laboratory staff) and DREG-200 (IgG1; Butcher laboratory staff). Unconjugated mAbs were visualized with biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA), followed by streptavidin PerCP (Becton Dickinson, San Jose, CA). In all cases in which more that one mAb was used for a given receptor, both mAbs behaved comparably.

Directly conjugated mAbs

The following directly conjugated mouse anti-human mAbs were obtained from BD PharMingen: CD16 FITC (3G8); CD56 PE (B-159); and CD3 APC (UCHT-1).

Chemokines

The following human chemokines were used in this study: stromal-derived factor (SDF)-1α, synthetic (from Gryphon Sciences, South San Francisco, CA) and recombinant (PeproTech, Rocky Hill, NJ); macrophage-inflammatory protein (MIP)-3β, synthetic (made by the author D.S.) and recombinant (PeproTech); secondary lymphoid tissue chemokine (SLC), recombinant (R&D Systems); IFN-γ-inducible T cell α chemoattractant (ITAC), synthetic (made by the author D.S.); IFN-γ-induced protein-10 (IP-10), recombinant (PeproTech); MIP-3α, synthetic (Gryphon); RANTES, recombinant (PeproTech); MIP-1β, recombinant (PeproTech); monocyte chemoattractant protein (MCP)-3, recombinant (PeproTech); MCP-1, recombinant (PeproTech); IL-8, recombinant (PeproTech); soluble fractalkine, synthetic (Gryphon) and recombinant (R&D Systems); monocyte-derived chemokine, synthetic (made by the author D.S.); thymus-expressed chemokine, recombinant (PeproTech). In all cases in which more than one chemokine was tested, each responded comparably with the other.

There are a large number of overlapping ligand/receptor systems (at least 12) for CCR1, CCR2, CCR3, and CCR5. The chemokines that bind one or more of these receptors include MIP-1α, MIP-1β, RANTES, MCP-1–4, Eotaxin-1–3, and hemofiltrate C-C chemokine-1, 2, and 4 (12). Thus, instead of testing each of these chemokines on the CD56 subsets (which would be extremely redundant), we included the four chemokines in the panel such that each of the above receptors is bound by two chemokines: CCR1, by RANTES and MCP-3; CCR2, by MCP-1 and MCP-3; CCR3, by RANTES and MCP-3; CCR5, by MIP-1β and RANTES.

Migration assays

Preparation of human lymphocytes, migration through 5-μm transwells, calculation of migration in percentage input, and calculation of percentage of specifically migrated cells were all performed as previously described (23, 24).

Results and Discussion

Peripheral blood CD56$^+$ lymphocytes can be divided into three distinct subpopulations (3) (Fig. 1). NK-T cells are distinguished from NK cells by CD3 expression, and comprise ~5% of total PBLs in normal healthy adult donors. These cells express TCR$\alpha\beta$ rather than TCR$\gamma\delta$; variably express CD8α and CD8β; and only very rarely express CD4 (data not shown). The CD3$^-$ NK cells can be further divided into CD16$^+$ and CD16$^-$ subsets (Fig. 1). The CD16$^+$ NK cells comprise ~5% of PBLs. The CD16$^-$ NK cells comprise ~1% of PBLs, and express higher levels of CD94 (data not shown) and CD56 than CD16$^+$ NK cells, as described elsewhere (3). In fact, the small CD56^{high} population separated from the bulk of the CD56$^+$ cells visible in the histogram (Fig. 1, *left panel*) is composed entirely of this latter population.

Enrichment of CD56$^+$ lymphocytes by migration to chemokines

Little is known about the tissue tropisms or homing receptor/chemokine receptor profiles of CD56$^+$ lymphocyte subsets. In an attempt to shed light on this issue, we designed a process to screen several chemokines on each cell type, using migration assays (Fig. 2). After compensation for spontaneously migrated cells, the process allowed us to detect enrichment (or depletion) of a given lymphocyte subtype (this method was successfully used in a previous study to detect enrichment of skin-homing cells after migration of lymphocytes to CCR4 ligands (24)).

Fresh unfractionated lymphocytes were migrated to each member of a large chemokine panel (each chemokine at optimal concentration; see Fig. 4), and the migrated populations were immunophenotyped for comparison with the starting population.

We found dramatic differences among chemokines with respect to their ability to enrich each of the three CD56$^+$ subtypes by migration (Fig. 2). SDF-1α was the only case in which each population studied was neither enriched nor depleted (with respect to the starting population) after migration to the chemokine. This is consistent with the notion that SDF-1α attracts essentially all PBLs with equal efficiency (23, 25). The role of such a universally expressed chemokine receptor in lymphocyte trafficking remains unclear.

CD16$^+$ NK cells. CD16$^+$ NK cells comprised >50% of the populations migrated to IL-8 and to soluble fractalkine, 10-fold enrichments over their representation in the starting population (Fig. 2, *upper left panel*). Migrated populations for all other tested chemokines contained depleted numbers of CD16$^+$ NK cells, with the exception of SDF-1α (as mentioned above).

NK-T cells. In contrast to the findings for CD16$^+$ NK cells, NK-T cells were only slightly enriched in the soluble fractalkine-migrated population, and were slightly depleted in the IL-8-migrated

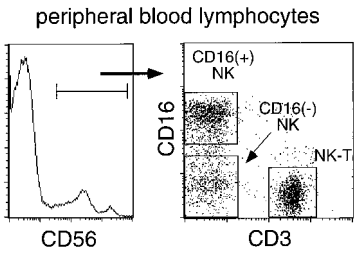

FIGURE 1. CD56$^+$ lymphocyte subsets from peripheral blood. This figure shows a typical profile for the three subsets of CD56$^+$ lymphocytes from peripheral blood of a normal healthy adult. *Left panel*, Histogram of CD56 expression on peripheral blood leukocytes gated on lymphocytes by scatter. *Right panel*, Dot plot of CD16 vs CD3 on the CD56$^+$ lymphocyte gated in the *left panel*. The square gates were the same used to define the three CD56$^+$ subsets presented in subsequent figures. The individual shown is representative of >20 donors tested.

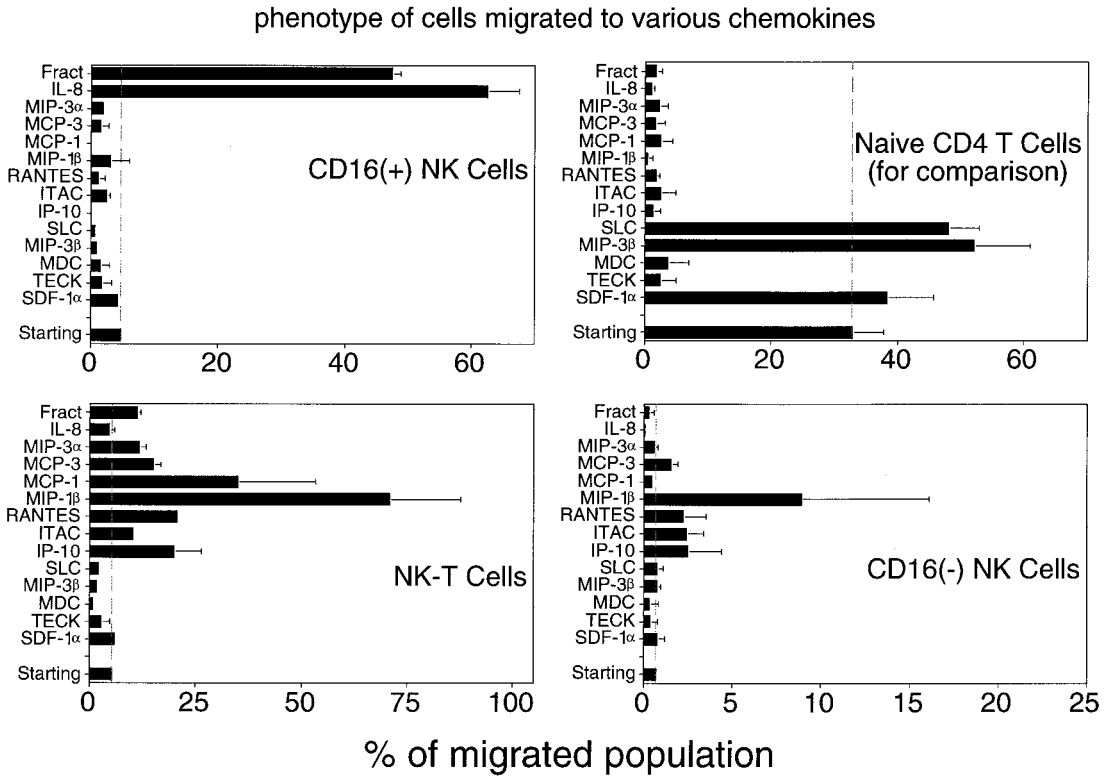

FIGURE 2. Differential enrichment of CD56$^+$ PBL subsets in migrated populations. Fresh unfractionated PBLs were allowed to migrate to completion through 5-μm transwells, as previously described (23, 24). Each chemokine was used at optimal concentration (see Fig. 4). At least three wells were used for each chemokine per donor, and eight wells without chemokine to determine nonspecific or background migration. The number of cells from each phenotype was calculated for the migrated population after staining and flow cytometry; and the number of background cells subtracted for each population to yield the specific migration. Percentage of the migrated population was then calculated for each population relative to the number of specifically migrated cells. Each plot shows the mean and SD for three different donors. Each donor was tested for response to all chemokines in parallel.

population (Fig. 2, *lower left panel*). Like CD16$^+$ NK cells, NK-T cell representation was maintained in the SDF-1α-migrated population. NK-T cells were dramatically enriched in the MIP-1β-migrated population, comprising nearly ~75% in contrast to ~5% of the starting population, a 15-fold increase. NK-T cells were also somewhat enriched in the populations attracted to MIP-3α, MCP-1, MCP-3, RANTES, ITAC, and IP-10.

CD16$^-$ NK cells. CD16$^-$ NK cells behaved much more like NK-T cells than like CD16$^+$ NK cells (Fig. 2, *lower left panel*). Although these cells comprised only <1% of the starting population, they represented ~10% of the population attracted to MIP-1β (Fig. 2, *lower left panel*). Like NK-T cells, the CD16$^-$ NKs were moderately enriched by MCP-3, RANTES, ITAC, and IP-10. Unlike the other two CD56 subsets studied, the CD16$^-$ NK cells were not depleted in the populations migrated to the CCR7 ligands MIP-3β and SLC.

For comparison, naive CD4 T cells from the same donors were also tested in this assay, in parallel with the CD56$^+$ subsets (Fig. 2, *upper right*). As previously described (24), naive CD4 T cell numbers were maintained (or slightly enriched) only in the populations attracted to SDF-1α, MIP-3β, and SLC.

Expression of receptors by CD56$^+$ lymphocyte subsets

In keeping with the migration patterns seen in Fig. 2, NK and NK-T cells differed greatly in their cell surface receptor expression.

CD16$^+$ NK cells. CD16$^+$ NK cells uniformly expressed CXCR1 and CX3CR1 at high levels, consistent with the predominance of this population in the IL-8- and soluble fractalkine-attracted populations (Fig. 3, *top rows*). CXCR4, the receptor for SDF-1α, was also present at high levels on these cells. CXCR2 and CXCR3 were present at lower levels. Chemokine receptors CCR1–7 and CCR9 were absent on this population, as well as CXCR5 and the orphan chemokine receptor Bonzo. Expression of the lymph node homing receptor L-selectin was variable.

CD16$^-$ NK cells. The IL-8R CXCR1 was absent on CD16$^-$ NK cells, and CX3CR1 was expressed at detectable, but very low levels (Fig. 3, *bottom rows*). CCR5, CCR7, CXCR3, and CXCR4 were uniformly expressed at high levels on this population. CCR1–4, 6, and 9 were not expressed, nor were CXCR2 and CXCR5. L-selectin was highly expressed on this population.

NK-T cells. In contrast to the uniform expression patterns observed among the NK populations, NK-T cells often expressed chemokine receptors in a heterogeneous or bimodal fashion (Fig. 3, *middle rows*). Like CD16$^-$ NK cells, NK-T cells expressed uniformly high levels of CCR5, CXCR3, and CXCR4. However, CCR1, CCR2, CCR6, CX3CR1, and Bonzo were heterogeneously expressed. CXCR1 expression was variable among donors, but was generally absent. Only CCR3, 4, 7, 9, and CXCR2 were consistently absent from this population.

Direct comparison of chemotactic responses among CD56$^+$ lymphocyte subsets

To comprehensively determine whether chemokine receptor expression (as detected by flow cytometry) correlates with responsiveness to chemokines, known ligands for each receptor were titered on the three CD56$^+$ subsets in migration assays. As expected, all three CD56$^+$ subsets responded equally well to SDF-

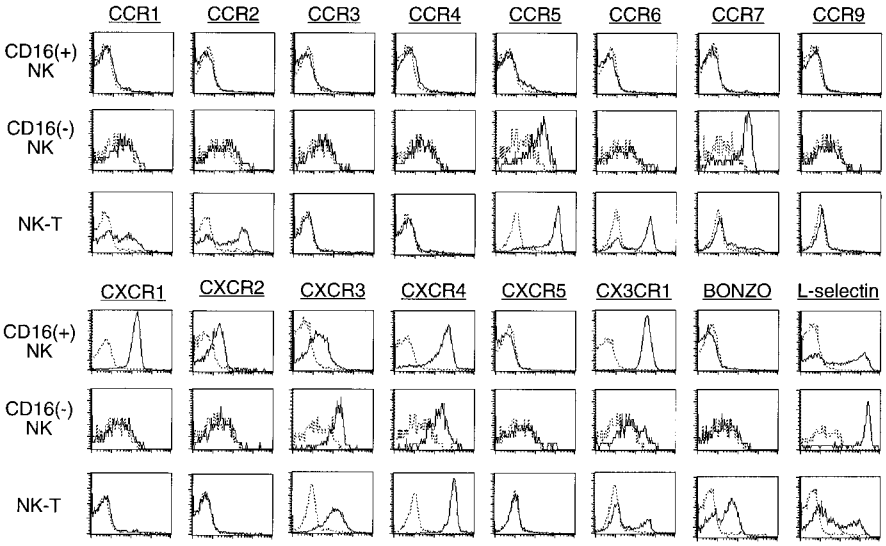

FIGURE 3. Expression of chemokine receptors by CD56$^+$ subsets. Cells were gated on each subpopulation, as shown in Fig. 1. For each FACS experiment, cells were stained with directly conjugated CD3, CD16, and CD56, and with one unconjugated chemokine receptor mAb or isotype control. *Top rows*, CD56$^+$/CD16$^+$/CD3$^-$; *middle rows*, CD56$^+$/CD16$^-$/CD3$^-$; *bottom rows*, CD56$^+$/CD3$^+$. Histograms for each chemokine receptor (solid lines) are shown overlaid on the appropriate isotype control (dotted lines). Results shown are for one donor (the same donor shown in the migration assays of Fig. 4), but are representative of 12 donors tested.

1α, and with very similar dose-response curves (Fig. 4, *upper left panel*).

CD16$^+$ NK cells. Both IL-8 and soluble fractalkine strongly attracted CD16$^+$ NK cells, but had much smaller effects on CD16$^-$ NK cells and NK-T cells (Fig. 4, filled squares). This correlates well with the high expression of CXCR1 and CX3CR1 on CD16$^+$ NK cells, but not on the other two subsets. CD16$^+$ NK cells responded moderately to the CXCR3 ligands ITAC and IP-10, in keeping with their expression of CXCR3. NK cells also responded moderately to high doses of MIP-3α, although CCR6 (the only known receptor for MIP-3α) was not detected by FACS. No appreciable migration was detected to any of the other chemokines tested.

NK-T cells. MIP-1β attracted NK-T cells much better than it attracted either type of NK cell (Fig. 4, gray triangles). However, despite the fact that NK-T cells were greatly enriched by migration to MIP-1β (Fig. 2), and expressed very high levels CCR5 (the only known receptor for CCR5 in humans; Fig. 3), MIP-1β was only a poor attractor. A reasonable explanation for this apparent discrepancy may be that MIP-1β performs poorly in the transwell assay system: it may have a shorter $t_{1/2}$ in migration medium or may adhere to plastic more that other chemokines. Thus, although the amplitude of migration to MIP-1β is poor for PBLs, this would explain why the differential effects of MIP-1β on NK-T cells vs all other PBL subsets can still be detected in the enrichment assay (Fig. 2). NK-T cells responded well to RANTES, another CCR5 ligand (Fig. 4, gray triangles). However, RANTES also interacts with CCR1, so its effects may not be entirely through CCR5. NK-T cells responded moderately to MIP-3β and SLC, although CCR7 was observable only on small numbers of NK-T cells by flow cytometry. NK-T cells responded well to MCP-2, MIP-3α, ITAC, and IP-10, in keeping with CCR2, CCR6, and CXCR3 expression on NK-T subpopulations (Fig. 4, gray triangles).

CD16$^-$ NK cells. CD16$^-$ NK cells responded most dramatically to the CCR7 ligands MIP-3β and SLC, and to the CXCR3 ligands ITAC and IP-10 (Fig. 2, open circles). Chemotactic responses of CD16$^-$ NK cells to other ligands were largely parallel to those of NK-T cells, with the exception of MCP-1. Unlike NK-T cells, CD16$^-$ NK cells responded poorly to MCP-1 (Fig. 4, open circles), and did not express CCR2, the only known receptor for MCP-1 (Fig. 3). CD16$^-$ NK cells showed little or no response to IL-8 or soluble fractalkine, which were the predominant attractants of CD16$^+$ NK cells.

Chemokine receptors provide clues to differential homing of CD56$^+$ subsets

This comprehensive analysis of the various CD56$^+$ lymphocyte subsets from human peripheral blood has demonstrated a great diversity of receptor expression, and therefore of homing potential. Recently, expression of particular chemokine receptors was found to be associated with subsets of peripheral blood CD4 T cells known to home preferentially through particular tissue types. CCR4 is expressed at high levels on CLA$^+$ memory CD4 cells, which are known to home preferentially through cutaneous sites (24). CCR9 is expressed only on a subset of $\alpha_4\beta_7$ integrin-expressing memory CD4 cells, which home preferentially through mucosal tissues (26). Neither CCR4 nor CCR9 was observed on CD56$^+$ subsets, nor did CD56$^+$ lymphocytes respond to monocyte-derived chemokine or thymus-expressed chemokine, the ligands for these receptors (respectively). Thus, CD56$^+$ subsets may not be divided into cutaneous or systemic vs mucosal or intestinal compartments like CD4 T cells (although we cannot completely exclude the possibility that very small tissue-specialized subsets may exist). In fact, many CD56$^+$ lymphocytes express both CLA and $\alpha_4\beta_7$, a combination almost never seen on CD4 T cells (J. J. Campbell, unpublished observations).

Furthermore, the two peripheral blood NK populations investigated in this study (CD16$^+$ and CD16$^-$) do not appear to have any further subdivisions at all, at least in terms of chemokine receptor expression. In this respect, their trafficking profiles may parallel more closely the cells of the innate immune system, whose migration is determined primarily by developmental processes, rather than the Ag-induced homing properties of memory or effector T cells. Like other components of the constitutive immune system in the circulation (such as neutrophils and monocytes), NK cells may be universally attracted to particular areas of inflammation. In fact, this analogy can be taken farther: The chemokine receptor repertoire of CD16$^+$ NK cells is similar to that of neutrophils, and these cells may thus be similarly attracted to sites of acute inflammation. With the exception of CCR7, chemokine receptor expression of CD16$^-$ NK cells is more like that of monocytes, and these receptors may serve to bring the cells into sites of more chronic inflammation.

The uniformity of chemokine receptor expression for NK subsets discussed above is in marked contrast to that of NK-T cells, which are subdivided into populations that express differing profiles of chemokine receptors. NK-T cells expressed diverse levels of CCR1, CCR2, CCR6, CXCR1, and CX3CR1. The number of

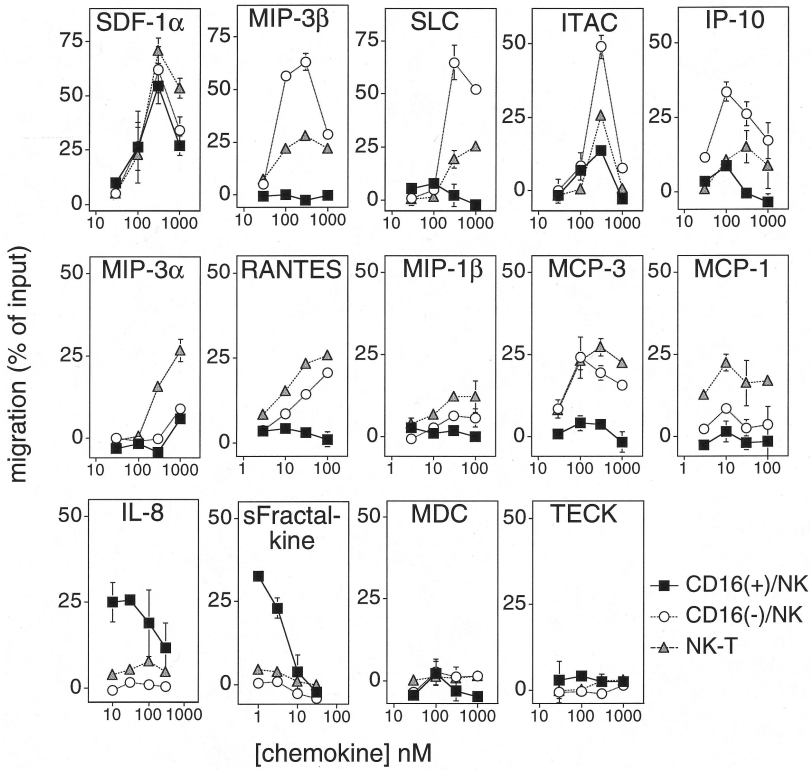

FIGURE 4. Dose response of $CD56^+$ lymphocytes to chemokines in migration assays. Lymphocytes were migrated toward chemokines, as described in the legend to Fig. 2. At least three repeat wells were performed for each concentration of chemokine, and eight wells do determine background migration (mean and SD of the three wells shown). Background migration has been subtracted from each point. ■, $CD56^+/CD16^+/CD3^-$; ○, $CD56^+/CD16^-/CD3^-$; △, $CD56^+/CD3^+$. Results shown are from one donor, with all assays performed in parallel on the same day. Results at optimal concentration are representative of those obtained from 12 donors.

high and low expressers of each of these receptors varied greatly among individuals (J. J .Campbell, unpublished observations). It remains to be seen whether these differences are related to the inflammatory state, age, or genetics of the individual. Although NK-T cells are not divided into tissue-specific subsets along the same lines as CD4 T cells (e.g., $CLA^+/CCR4^+$ skin-homing and $\alpha_4\beta_7^{high}/CCR9^+$ gut-homing subsets), their subdivisions may imply new, as yet unrecognized homing cascades to other tissues or organs. In addition, NK-T cells are the only $CD56^+$ population to express the orphan chemokine receptor Bonzo (27, 28). In some of the donors (3 of 12), the NK-T population possessed a subset with 3- to 5-fold higher CD3 expression than the other NK-T cells. For these donors, Bonzo expression was associated only with this $CD3^{high}$ population (J. J. Campbell, unpublished observations).

Homing of $CD56^+$ lymphocytes through lymphoid organs?

CCR7 has recently been shown essential for T cell homing to secondary lymphoid organs through high endothelial venules (29, 30). NK cells are not normally thought to be associated with secondary lymphoid organs, and indeed $CD16^+$ NK cells both lack CCR7 and fail to respond to CCR7 ligands. However, $CD16^-$ NK cells express high levels of CCR7, and respond very well to CCR7 ligands. These cells also express very high levels of L-selectin, which is another molecule necessary for homing through high endothelial venule (31). This small subset of NK cells may thus have a role in the events that occur within secondary lymphoid organs, and it will be interesting to see whether these cells can be found in such organs in future studies.

Measuring chemokine-induced enrichment vs direct migration within complex populations

Of all PBLs, NK-T cells were the predominant phenotype attracted by the CCR5 ligand MIP-1β (Fig. 2, *lower left panel*). MIP-1β appears to be a poorly performing chemokine for in vitro chemotaxis, in comparison with other chemokines. The dramatic effect of MIP-1β on NK-T vs other lymphocytes may thus not have been detected unless the relative enrichment numbers had been calculated (Fig. 2), demonstrating the importance of such an assay in understanding differential responses to chemokines.

Conclusions

Although $CD56^+$ lymphocytes are derived from multiple lineages, they share a functional association with immunosurveillance and antitumor responses (1, 21, 22). By understanding the pathways by which these cell types traffic throughout the body, we can begin to decipher the mechanisms by which these cells locate and infiltrate neoplasias. Such knowledge may lead to advances in therapy, allowing the design of antitumor cells with the capability of homing to and destroying cancers untreatable with current methods.

In this study, we have performed a comprehensive analysis of $CD56^+$ PBL subsets. We have found that the $CD16^+$ and $CD16^-$ NK subsets are very different with respect to each other, but are internally uniform. We have found that NK-T cells express a large number of chemokine receptors, and are internally diverse. We have found that NK-T cells are the predominant responding cell type to the CCR5 ligand MIP-1β. We have found that $CD16^-$ NK cells are the only $CD56^+$ population that uniformly expresses all of the homing molecules necessary to traffic into secondary lymphoid organs through high endothelial venule. The dramatically different chemokine receptor repertoires among these subsets may thus define entirely different routes of recirculation for these cells.

Note added in Proof. Since submission of this manuscript, the ligand for Bonzo has been identified as CXCL16 (32, 33), and Bonzo has been accordingly redesignated as CXCR6.

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