DIFFERENTIATION STAGES OF HUMAN NATURAL KILLER CELLS IN LYMPHOID TISSUES FROM FETAL TO ADULT LIFE*

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The differentiation pathway of natural killer (NK)[1] and killer (K) cells is controversial. There is some evidence suggesting they are of T cell lineage, whereas other data indicate that they are of monocyte/macrophage lineage (1, 2). These previous studies demonstrated that T cell or myeloid antigens were expressed on operationally defined NK and K cell populations, but no unique set of differentiation antigens were defined. We have recently identified an HNK-1 differentiation antigen that is expressed on virtually all human granular lymphocytes with NK and K cell function (3). These HNK-1^+ cells in blood expand after birth as a function of age and sex (4). Two distinct phenotypes of HNK-1^+ cells were identified that might represent different stages in NK cell differentiation (5). Thus, the majority (>60%) of blood HNK-1^+ cells expressed a myeloid antigen (M1) but lacked any T cell antigens (i.e., $HNK^+T3^-M1^+$), contained abundant cytoplasmic granules, and exhibited a high level of NK activity. On the other hand, the minority (<40%) of blood HNK-1^+ cells and almost all bone marrow HNK-1^+ cells expressed the T cell antigens (e.g., T3 and T8) but lacked the M1 antigen (i.e., $HNK^+T3^+M1^-$), had few cytoplasmic granules, and exhibited low NK function.

In this study, we examined possible differentiation stages of HNK-1^+ cells from all available lymphoid compartments (blood, spleen, lymph node, thymus, bone marrow, and fetal liver) to systematically characterize their phenotype, morphology, and NK cell function. The results of HNK-1^+ cells examined from fetal to adult life indicated that there were at least three distinct stages of NK cell differentiation ($HNK^+T3^-M1^-$, $HNK^+T3^+M1^-$, and $HNK^+T3^-M1^+$) and that they are selectively distributed among lymphoid tissues.

Materials and Methods

Cell Preparations. Human adult spleen, lymph node, and thymus were obtained from patients undergoing splenectomy for trauma, diagnostic lymph node biopsies, and partial thymectomy to facilitate cardiac surgery, respectively (6). Adult bone marrow was obtained from resected ribs in patients undergoing thoracic surgery. Single-cell suspensions were prepared as described previously (7).

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[1] *Abbreviations used in this paper:* FACS, fluorescence-activated cell sorter; FITC, fluorescein isothiocyanate; K, killer; NK, natural killer; RITC, tetramethylrhodamine isothiocyanate.

Human fetal tissue samples were obtained through the University of Alabama at Birmingham Tissue Procurement Service from medically approved abortions. Single-cell suspensions were prepared from the fetal liver, bone marrow, spleen, and thymus within 1–2 h after removal from the uterus. Erythrocytes were eliminated either by water-shock lysis or by Ficoll-Hypaque gradient centrifugation (3). Cord blood was obtained from neonates at full-term birth. Mononuclear cells from both cord blood and adult blood samples were separated by the Ficoll-Hypaque gradient centrifugation (3). All cell preparations were placed in RPMI 1640 medium supplemented with 10% heat-inactivated fetal calf serum plus 50 μg/ml gentamycin.

Immunofluorescence Assay. Cell surface antigens defined by monoclonal antibodies were enumerated by either direct or indirect two-color immunofluorescence assays (5). A monoclonal IgM antibody, HNK-1 (Leu-7; Becton-Dickinson & Co., Sunnyvale, CA) was used at a concentration of 10 μg/ml for indirect immunofluorescence while fluorescein isothiocyanate (FITC)- or tetramethylrhodamine isothiocyanate (RITC)-conjugated HNK-1 antibody was used at a concentration of 50 μg/ml for the direct assay (5). For indirect two-color immunofluorescence, other monoclonal antibodies of IgG isotype were used in combination with the IgM HNK-1 antibody. A panel of OKT monoclonal antibodies, T3, T8, T4, T6, T9, T10, M1, and Ial (8–13), were obtained from Ortho Pharmaceutical, Raritan, NJ. A monoclonal anti-HLA-A,B,C common determinant antibody (PA 2.6) was a gift from Dr. A. J. McMichael of the University of Oxford, Oxford, England (14). Secondary antibodies were FITC- or RITC-conjugated goat anti-mouse μ, γ1, γ2a, and γ2b heavy chain antibodies, which were generously provided by Dr. W. E. Gathings of the University of Alabama in Birmingham.

Fluorescence-activated Cell Sorting. Subpopulations of mononuclear cells after immunofluorescence staining were analyzed and separated with a fluorescence-activated cell sorter (FACS IV; B-D FACS Systems, Becton-Dickinson & Co., Mountainview, CA) as described previously (5).

NK Assay. NK cell function was examined by a ^{51}Cr-specific release assay using K562 target cells. The method and formula for calculating specific cytolysis have been described previously (3). The lytic units were calculated from the result of a dose-response curve of the effector cells. One lytic unit was defined as the number of effector cells required to achieve 30% lysis of 10^4 target cells.

Phagocytic Function and Peroxidase Staining. Phagocytic functional activity was tested by phagocytosis of heat-killed yeast particles (15). Peroxidase staining was performed according to the technique of Kaplow (16).

Results

Fetal Expression of HNK-1 and Other Differentiation Antigens in Lymphoid Tissues. The HNK-1 antigen was expressed on only a small fraction (<0.2%) of the nucleated cells recovered from the liver, bone marrow, and thymus (Table I) or from a single spleen (data not shown) from 13 to 17 wk-old fetuses. T cell antigens, T3, T8, T4, and T6 were identified on <1% of fetal liver and bone marrow cells, whereas these T cell antigens were found on most fetal thymocytes (Table I). The majority (>50%) from cells from fetal liver and bone marrow expressed the myeloid antigen M1 (Table I). The other antigens T9, T10, HLA-DR or HLA-A,B,C were also expressed at high levels of nucleated cells (20–40%).

Co-expression of these antigens on fetal HNK-1^+ cells from liver and bone marrow was then examined with a two-color immunofluorescence assay (Table II). The majority (>70%) of HNK-1^+ cells lacked any of the other surface antigens tested. The remaining HNK-1^+ cells (<30%) co-expressed some mature T cell antigens T3 and T8, but lacked T4, T6, and T9 antigens and lacked the myeloid antigen M1.

The fetal cells were then examined for surface and cytoplasmic expression of HNK-1 antigen. The HNK-1^+ cells were sorted with the FACS after staining with FITC-

TABLE I

Surface Antigen Expression Identified by Monoclonal Antibodies in Lymphoid Tissues from Fetuses, Neonates, and Adults

Tissues (number tested)	Percent cells identified by monoclonal antibodies									
	HNK-1	T3	T8	T4	T6	T9	T10	M1	HLA-DR	HLA-A,B,C
Fetuses (13–17 wk)										
Liver (2)	~0.2	0.3	0.05	0.1	0.05	ND	ND	~50	ND	ND
Bone marrow (6)	~0.1	0.4	0.1	0.2	0.1	38 (21)‡	23 (18)	54 (17)	~30	43 (14)
Thymus (2)	<0.05	74 (62–86)	85 (76–94)	80 (69–91)	56 (44–68)	ND	ND	ND	<1.0	ND
Neonates										
Bone marrow (2)	0.1	ND	ND	ND	ND	ND	ND	ND	ND	ND
Blood (4)	0.3	73 (15)	9 (5)	43 (8)	~2.0	~20	~20	24 (11)	8 (5)	>95
Thymus (2)	<0.1	30 (23–37)	63 (50–76)	<95	ND	7	>95	0.2	0.1	ND
Adults (15–40 yr)										
Bone marrow (4)	0.8	5.8	5.0	5.6	<1.0	~20	11 (3)	ND	ND	100
Blood (6)	14 (6)	59 (11)	16 (6)	48 (16)	0	0	~15	25 (12)	26 (13)	100
Spleen (5)	10 (8)	37 (13)	25 (11)	20 (8)	0	~2	~10	12 (7)	22 (4)	100
Lymph node (3)	0.6	61 (9)	8.5	56 (21)	0	~2	~10	5	3	100
Thymus (3)	0.1	55 (3)	75 (4)	80 (11)	90 (5)	10 (3)	~60	1.7	23 (7)	ND

* Not done.

‡ Numbers in parentheses indicate either the range of values for two observations or the standard deviations (SD) for three or more observations. SD of >2% were not represented to simplify the table.

TABLE II

Co-expression of Other Surface Antigens on HNK-1$^+$ Cells in Lymphoid Tissues from Fetuses, Neonates, and Adults

Tissues (number tested)	Percent HNK-1$^+$ cells expressing:								
	T3	T8	T4	T6	T9	T10	M1	HLA-DR	HLA-A,B,C
Fetuses (13–17 wk)									
Liver (2)	29 (21–37)‡	8	0	0	0	ND§	0	3	25 (22–28)
Bone marrow (6)	18 (13)	4	0	0	0	ND	0	5	20 (9)
Neonates									
Blood (4)	77 (11)	51 (9)	19 (3)	0	0	ND	11 (3)	42 (7)	100
Thymus ‖ (2)	80	82	>80	1.0	5	ND	0	ND	>90
Adults (15–40 yr)									
Bone marrow (4)	>95	86 (20)	18 (11)	0	0	ND	3	32 (14)	100
Blood (6)	23 (20)	26 (7)	8 (3)	0	0	~80	72 (18)	24 (7)	100
Spleen (4)	64 (13)	25 (6)	6 (3)	0	0	~50	35 (5)	41 (4)	100
Lymph node (2)	78 (74–82)	44 (42–46)	25 (21–29)	0	0	ND	10 (7–12)	5 (2–7)	100
Thymus (2)	82 (77–86)	81 (79–82)	>90	<3.0	7	ND	0	ND	ND

* An accurate enumeration of T10 antigen expression was always difficult because the staining intensity of this antibody ranges from low to high degrees.

‡ Numbers in parentheses indicate either the range of values for two observations or the SD for three or more observations. SD of less <2% were not represented to simplify the table.

§ Not done.

‖ Thymic HNK-1$^+$ cells derived from both neonates and adults had a minor but specific phenotype. Most of the cells expressed the helper cell antigen (T4) and few expressed the T6 or T9 antigens.

HNK-1 antibody and then restained after fixation on slides with RITC-HNK-1 antibody. Two types of HNK-1$^+$ cells could be identified in fetal liver and bone marrow. About 80% were relatively small (10–15 μm), whereas a minority were quite large (>15 μm) (data not shown). None of these HNK-1$^+$ cells contained detectable cytoplasmic HNK-1 antigen.

Neonatal Expression of HNK-1 and Other Differentiation Antigens in Lymphoid Tissues and Cord Blood. The proportion of HNK-1$^+$ cells was also very low in neonates, comprising <1% of cord blood and bone marrow cells (Table I). In contrast to fetal HNK-1$^+$ cells that generally lacked other differentiation antigens, the majority (>80%) of HNK-1$^+$ cells in blood and thymus of neonates co-expressed the pan-T cell antigen (T3) and suppressor T cell antigen (T8). About 18% blood HNK-1$^+$ cells and 80% of HNK-1$^+$ thymocytes expressed a helper T cell antigen (T4) (Table II). In addition, almost all HNK-1$^+$ cells expressed HLA-A,B,C antigen, whereas 42% of blood HNK-1$^+$ expressed HLA-DR antigen. Very few neonatal HNK-1$^+$ cells expressed the immature T antigen (T6), the transferrin receptor (T9), or the myeloid antigen (M1). The cytoplasmic expression of the HNK-1 antigen in cord blood lymphocytes was weak.

HNK-1 Antigen Expression in Adults Among Lymphoid Tissues. The proportion of HNK-1$^+$ cells was much higher in the blood and spleen of adults relative to neonates and fetuses (Table I). This confirms our previous observation that there is a unique postnatal expansion of HNK-1$^+$ cells (4). Moreover, the distribution of HNK-1$^+$ cells is selective, being highest in adult blood (14%) and spleen (10%), but comprising <1% of nucleated cells in the lymph node, thymus, and bone marrow (Table I).

We have previously described two distinct phenotypes of HNK-1$^+$ cells in adult blood (5). The majority (>60%) of HNK-1$^+$ cells were HNK$^+$T3$^-$M1$^+$, whereas the minority (<40%) were HNK$^+$T3$^+$M1$^-$. These reciprocal relationships were confirmed in this study and appeared to be valid in other tissues as well. Thus, the majority of

HNK-1$^+$ cells from adult bone marrow, lymph node, and thymus co-expressed the T3 and T8 antigens, but very few co-expressed M1 antigen (Table II). Blood and spleen HNK-1$^+$ cells, on the other hand, had the highest proportion of M1 antigen expression and the lowest proportion of T cell antigen expression (T3 and T8). Only HNK-1$^+$ cells from the adult thymus expressed a high proportion (>90%) of helper T cell antigen (T4) just as was observed in neonatal thymus (>80%). The immature T cell antigen T6 and the transferrin receptor T9 were not expressed on HNK-1$^+$ cells from adult, neonatal, or fetal lymphoid tissue. HLA-DR antigen expression on adult HNK-1$^+$ cells was selectively distributed, being highest on splenic HNK-1$^+$ cells (41%) and lowest on HNK-1$^+$ cells from lymph node (5%). When the cytoplasmic expression of HNK-1 antigen was examined, some differences were observed among lymphoid compartments. Adult blood HNK-1$^+$ cells all contained a diffuse distribution of HNK-1 antigen throughout the cytoplasm but not in the nucleus. HNK-1$^+$ cells from bone marrow exhibited a weaker stain of cytoplasmic HNK-1 antigen or lacked a detectable amount of antigen altogether.

Distinguishing Morphological Features of HNK-1$^+$ Cells at Different Stages of Differentiation. As a group, HNK-1$^+$ cells from adult blood are a homogeneous population of medium-sized granular lymphocytes (3). We have also shown that they can be subdivided into two groups: (*a*) HNK$^+$T3$^-$M1$^+$ with abundant cytoplasmic granules that are efficient cytotoxic cells and comprise the majority of blood HNK-1$^+$ cells, and (*b*) HNK$^+$T3$^+$M1$^-$ cells with sparse granules that have a relatively low level of

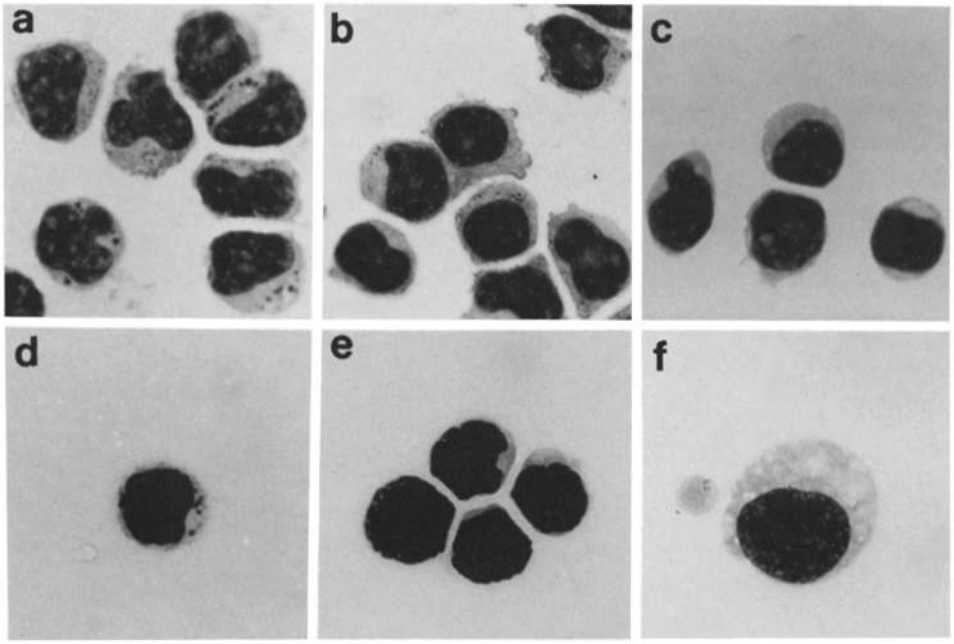

FIG. 1. The morphological appearance of HNK-1$^+$ cells from adult blood, bone marrow, and fetal lymphoid tissues. FACS-sorted HNK-1$^+$ cells from adult blood (a), bone marrow (b), and fetal bone marrow (d, e, and f), including sorted HNK-1$^-$ cells from adult blood (c), were stained by May-Grünwald-Giemsa method (× 1,000). Three types of fetal HNK-1$^+$ cells with distinct morphology are represented in d, e, and f.

NK function and comprise a minority of blood HNK-1^+ cells (5). In this study, we extended these observations by examining the morphology of FACS-purified HNK-1^+ cells from other lymphoid compartments using the May-Grünwald-Giemsa stain (Fig. 1). Compared with adult blood HNK-1^+ cells (Fig. 1 a), cord blood and adult bone marrow HNK-1^+ cells had fewer cytoplasmic granules (Fig. 1 b). In fact, they are quite similar to the minor population of adult blood HNK$^+$T3$^+$M1$^-$ cells. These cells may represent a more immature form of the HNK-1^+ cells. More than 98% of the HNK-1^- cells sorted by the FACS from the above sources lacked cytoplasmic granules (Fig. 1 c). In the 13–17-wk fetuses, three distinct morphological types of HNK-1^+ cells could be identified: (*a*) a minor population (2–10%) with the same granular appearance as blood and bone marrow (Fig. 1 d), (*b*) a majority of HNK-1^+

TABLE III

Demonstration of Spontaneous Killer Activity against K562 Using FACS-purified HNK-1^+ Cells from Fetal Bone Marrow

Cell fraction	Target/effector ratio	Percent ^{51}Cr-specific release*	
		13 wk fetus	16 wk fetus
Unfractionated bone marrow cells‡	1:200	2.0	3.0
	1:100	4.4	4.5
	1:50	3.9	3.5
	1:25	2.4	3.0
	1:12	2.9	3.1
FACS-purified HNK-1^+ cells§	1:10	11.1	15.3
	1:5	7.7	9.8
	1:2.5	5.0	6.0
	1:1.2	4.1	4.5
	1:0.6	2.5	3.0

* In these studies, the killer activity was determined after an 18-h incubation to demonstrate the maximum activity.

‡ By FACS analysis, the nucleated bone marrow cells in 13- and 16-wk fetuses were 0.06 and 0.1% HNK-1^+, respectively. However, these cells did not exhibit significant killer activity with a clear dose-response correlation.

§ FACS-purified HNK-1^+ cells that were >96% pure by fluorescent microscopy showed significant NK activity.

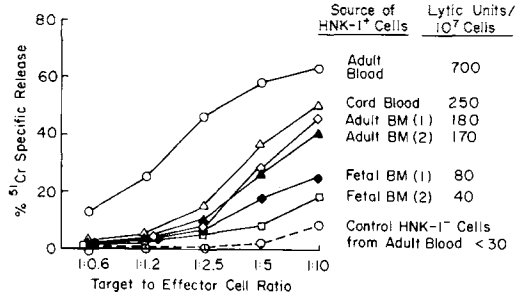

FIG. 2. A comparison of the cytotoxic capabilities of FACS-purified HNK-1^+ cells from adult blood, bone marrow, neonatal cord blood, and fetal (15 and 17 wk) bone marrow. Spontaneous killer activity against K5623 target cells were measured in a 6-h incubation assay using FACS-purified HNK-1^+ cells. As a control, adult blood HNK-1^- cells, which contained less than 0.2% HNK-1^+ by fluorescent microscopy, are shown in parallel.

cells (70–80%) that were small to medium size with a narrow cytoplasm and no granules (Fig. 1 e), and (*c*) a small proportion (15%) of giant HNK-1$^+$ cells with a large nucleus and a broad neutrophilic cytoplasm (Fig. 1 f). These latter cells are probably the same large cells noted in the fluorescence assay. All of these fetal HNK-1$^+$ cells were negative for peroxidase staining and were incapable of phagocytosing yeast particles.

NK Cell Functional Activity in the Fetus. The spontaneous killing activity against K562 target cells was determined for unfractionated nucleated bone marrow cells from 13- and 16-wk fetuses and for FACS-purified HNK-1$^+$ cells from those same bone marrows (Table III). Although unfractionated whole bone marrow cells contained up to 0.1% HNK-1$^+$ cells, they did not exhibit a significant killing activity with a dose-response correlation even at a 1:200 target/effector cell ratio. In contrast, purified HNK-1$^+$ cells from both of the fetal bone marrows exhibited cytotoxic capability with a clear dose-response correlation. The NK activity of purified HNK-1$^+$ cells was confirmed in another 13-wk fetus (data not shown) and in two cases of 15- and 17-wk fetuses (see Fig. 2).

Comparison of NK Cell Cytotoxicity for Purified HNK-1$^+$ Cells From Different Sources. The NK cell functional capability was compared in FACS-purified HNK-1$^+$ cells from different sources. The objective was to correlate function with surface antigen phenotype. The maximal NK cell functional activity was in adult blood HNK-1$^+$ cells, the majority of which were HNK$^+$T3$^-$M1$^+$ (Fig. 2). HNK-1$^+$ cells from cord blood and adult bone marrow exhibited a reduced functional capability with about one-third the activity of adult blood HNK-1$^+$ cells (Fig. 2). Almost all of these HNK-1$^+$ cells had the phenotype HNK$^+$T3$^+$M1$^-$. Minimal functional activity was observed in HNK-1$^+$ cells purified from fetal bone marrow and was <5% of that obtained with adult blood. The majority of these fetal HNK-1$^+$ cells had a phenotype of HNK$^+$T3$^-$M1$^-$.

Discussion

These results demonstrated an excellent correlation of NK cell function with cellular morphology and phenotypic antigen expression, because all granular lymphocytes examined in adult, neonates, and fetal lymphoid tissues expressed the HNK-1 antigen and because virtually all NK cell function resided in this cell population. The validity of these observations is strengthened by similar correlations in other species, as granular lymphocytes in mice and rats are known to possess NK cell function (17–19). In humans, spontaneous cytotoxic function has been demonstrated using enriched populations of granular lymphocytes (20, 21). Various combinations of cell surface markers have been demonstrated on these cytotoxic granular lymphocytes as well as related fractions of cells (Tγ and null cells) (22–26). All of these surface markers, however, had overlapping representation on other functional subpopulations of lymphoid cells, thus making it difficult to purify the entire population of granular lymphocytes. Only the HNK-1 differentiation antigen is expressed exclusively on granular lymphocytes (3). Functional characteristics of these FACS-purified cells have so far demonstrated that they possess both NK and K cell functions and that they exhibit little or no proliferative response to mitogens (phytohemagglutinin, concana-

valin A, and pokeweed mitogen) or to alloantigens (27). The cytotoxic efficiency of these HNK-1^+ cells can be boosted with interferon (28).

At least three subsets of HNK-1^+ cells were defined in this study having different surface phenotypes: $HNK^+T3^-M1^-$, $HNK^+T3^+M1^-$, and $HNK^+T3^-M1^+$. These three populations of HNK-1^+ cells probably represent different stages of differentiation because they correlate well with distinct features of cellular morphology, cytotoxic functional capability, and distribution in adult, neonatal, and fetal lymphoid compartments. A proposed model of HNK-1^+ cellular differentiation is shown in Fig. 3. Recently, a minor population of lymphocytes was demonstrated to simultaneously express the T3 and M1 antigens. These cells constitute 30% of the Tγ cell fraction in blood, have low levels of NK function (26), and represent <5% of bone marrow HNK-1^+ cells (5). These cells might represent a transitional stage of NK cells between that represented by the $HNK^+T3^+M1^-$ and $HNK^+T3^-M1^+$ cells.

The $HNK^+T3^-M1^-$ cells represent the earliest definable stage of HNK-1^+ cellular differentiation. These cells were only identified in the 13–17 wk-old fetuses, where they constituted the majority (>70%) of fetal HNK-1^+ cells. These early HNK-1^+ cells comprised only a small proportion of nucleated cells but they had several unique morphological features. About 15% of these cells were very large and lacked granules. The majority of cells (70–80%) were small agranular lymphocytes, whereas only a small minority (~10%) were granular lymphocytes. Although these studies could not identify the maturational stages of these cells, it is reasonable to speculate that the agranular lymphocytes represent precursors of the granular lymphocytes. This possibility was supported by the finding that up to 20% of HNK-1^+ cells from the bone marrow of children and adults were small granular lymphocytes, but these cells were infrequent (<5%) in all other adult lymphoid compartments (unpublished observation).

NK cell function has not been previously demonstrated in the human fetus using unfractionated lymphoid cell populations (29). Even in this study, there was virtually no detectable NK activity using fetal bone marrow cells. However, when the 0.1% of HNK-1^+ bone marrow cells was purified with the cell sorter, the purified HNK-1^+ cells exhibited a low level of NK function, thus confirming the functional correlations with HNK-1^+ cells and NK function in adults. It is conceivable that the NK cell function was due to the granular HNK-1^+ fetal cells, but there were insufficient numbers of cells to further separate granular and agranular HNK-1^+ fetal cells. These data in humans are also supported by studies in mice demonstrating the fetal liver and bone marrow cells contain the precursors of NK cells (30).

The $HNK^+T3^+M1^-$ cells probably represent an immature form of NK cells. These

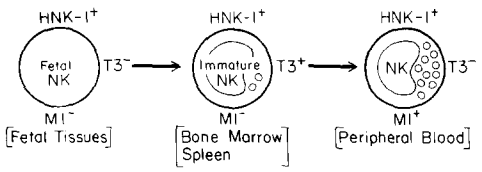

FIG. 3. A scheme of a possible differentiation pathway of human NK cells identified by the HNK-1 antibody.

cells have sparse cytoplasmic granules and relatively low levels of NK cell function (5). Only a small proportion (20–30%) of fetal HNK-1$^+$ cells express the T3 antigen, but the vast majority (77–80%) of neonatal HNK-1$^+$ cells are T3$^+$ (Table II). In adults, this subset of cells comprised >95% of bone marrow, and ~80% of HNK-1$^+$ cells from the thymus and lymph nodes. Only a minority of blood HNK-1$^+$ cells had this immature phenotype.

The most mature form of HNK-1$^+$ cells have the HNK$^+$T3$^-$M1$^+$ phenotype. These cells have the highest level of NK cytotoxicity and the largest number of cytoplasmic granules (5). They were found predominately in adult blood and spleen. Their levels were low in neonatal blood and they were not found in any fetal tissues. The distribution of these cells correlated precisely with the NK cell functional activity among these lymphoid compartments (e.g., high in adult blood, low in bone marrow or cord blood). NK cell activity in humans and in other species has been previously described as being the highest in blood and spleen, but lowest in thymus, lymph node, and tonsil (31).

These studies of granular lymphocyte differentiation provided additional evidence that human NK cells may have a unique lineage of differentiation, rather than a variant of T cell or myeloid cellular differentiation. The HNK-1 antigen was expressed in the fetus without any co-expression of T cell antigens on most cells; HNK-1$^+$ cells lacked peroxidase activity, phagocytic activity, and did not express the DR and M1 antigens found on myeloid cells. The NK cell functional activity found in the purified population of fetal HNK-1$^+$ bone marrow cells at 13 wk gestation even precedes the acquisition of cytotoxic function of T cells that has been demonstrated at 18 wk gestational age (32). The granular HNK-1$^+$ lymphocytes were quite low in number in all lymphoid compartments until childhood. They then expanded as a population through adulthood, a period when the thymus gland is undergoing involution. Although some HNK-1$^+$ cells expressed mature T cell differentiation antigens (T3 and T8), we did not detect an immature T cell antigen (T6) on these cells. Finally, the most mature subset of HNK-1$^+$ granular lymphocytes lacked T cell antigens altogether.

The differentiation schema proposed for granular HNK-1$^+$ lymphocytes is entirely compatible with the available data. However, confirmation of this must be made by demonstrating that these are sequential stages or by demonstrating a switching of phenotypes in vitro with cultured HNK-1$^+$ cells. In this regard, we have established cultured cell lines of HNK-1$^+$ granular lymphocytes, all of which have the HNK$^+$T3$^+$M1$^-$ phenotype (unpublished data). This uniform pattern of antigen expression supports the proposed model of NK cell differentiation and may provide a valuable tool for determining if these subsets of HNK-1$^+$ cells can be induced to switch their maturational stage.

Summary

Virtually all human granular lymphocytes expressed the HNK-1 differentiation antigen when examined in lymphoid compartments from adults, neonates, and fetuses. The HNK-1$^+$ cells were distinguishable into three subsets having distinct antigenic phenotypes: HNK$^+$T3$^-$M1$^-$, HNK$^+$T3$^+$M1$^-$, and HNK$^+$T3$^-$M1$^+$. Thus,

>70% of the HNK-1$^+$ cells from 13–17 wk fetuses (<0.2% of nucleated cells) lacked T cell antigens (e.g., T3, T8, T4, and T6) and the M1 myeloid antigen. Morphologically, the HNK$^+$T3$^-$M1$^-$ cells consisted of three different types: small granular lymphocytes (<10% of HNK-1$^+$ cells), agranular small lymphocytes with a narrow rim of cytoplasm (70–80%), and agranular giant cells (>15 μm) with considerable neutrophilic cytoplasm (15%). The purified fetal HNK-1$^+$ cells exhibited a low level of cytotoxicity against K562 target cells. On the other hand, almost all of HNK-1$^+$ cells in neonatal tissues as well as adult bone marrow, lymph node, and thymus, exhibited the HNK$^+$T3$^+$M1$^-$phenotype, contained sparse cytoplasmic granules, and had an intermediate level of NK functional activity. Only adult blood and spleen contained a majority of mature HNK-1$^+$ cells. These cells had an HNK$^+$T3$^-$M1$^+$ phenotype, abundant cytoplasmic granules, and maximum NK function. We propose that human NK cells may generate from a separate cell lineage and that they alter their phenotype, morphology, and functional capability during differentiation.

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