Histol Histopath (1989) 4: 137-152

Histology and Histopathology

Ultrastructural analysis of HNK-1$^+$ cells in human peripheral blood and lymph nodes

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Summary. HNK-1 positive (HNK-1$^+$) cells in human peripheral blood and lymph nodes were comparatively analysed by means of immunohistochemistry and immunoelectron microscopy. In peripheral blood, the HNK-1$^+$ cells were grouped into large granular lymphocytes (LGLs), small lymphocytes and intermediate forms, all of which had many fine cytoplasmic processes. Except for smooth-surfaced lymphocytes, they could not be distinguished from helper/inducer T (OKT4/Leu3a) cells and suppresssor/cytotoxic T (OKT8/Leu2a) cells. In double staining, HNK-1$^+$T3$^-$ cells and HNK-1$^+$T3$^+$ cells could not be clearly distinguished in terms of morphology, although the former contained many LGLs. The HNK-1$^+$ cells in the lymph nodes accumulated in the light zones of the germinal centers (GCs). These cells were small to medium-sized lymphocytes with few electron-dense granules and exclusively co-expressed helper/inducer T cell antigens (HNK-1$^+$T4$^+$). Their cytoplasmic projections were interwoven with those of the follicular dendritic cells which trap immune complexes for a long duration. These configurations suggest that HNK-1$^+$T4$^+$ cells in GCs are engaged in an immunological regulation of germinal center cells. On the other hand, large blastic HNK-1$^+$ cells were scattered outside the GCs and some of them were in the process of mitosis. Furthermore, HNK-1$^+$LGL-like cells with a few large electron-dense granules were rarely seen. These observations indicate that the HNK-1$^+$ cells in the lymph nodes may proliferate outside GCs and differentiate into LGLs with a strong natural killer function.

Key words: HNK-1$^+$ cells, Large granular lymphocytes, Germinal centers, Lymph nodes

Introduction

The monoclonal antibody HNK-1 reacts with natural killer (NK) and killer (K) cells (Abo and Balch, 1981) which are morphologically characterized by large granular lymphocytes (LGLs) (Timonen, 1980). In addition to being found in the peripheral blood, HNK-1$^+$ cells have been microscopically discovered in the spleen, lymph nodes, tonsils and lymphoid tissues of the gastro-intestinal tracts (Banerjee and Thibert, 1983; Hsu et al., 1983; Mori et al., 1983; Poppema et al., 1983; Ritchie et al., 1983; Si and Whiteside, 1983; von Gaudecker et al., 1984; Shioda et al., 1984). In these lymphoid tissues the HNK-1$^+$ cells were shown to accumulate in the germinal centers (GCs), especially in the light zones. GCs are known to be a major depot of memory B cells (Thorbecke and Lerman, 1976; Terashima et al., 1977). In the light zones of GCs, follicular dendritic cells (FDCs) which trap antigen-antibody complexes for a long duration are distributed (Nossal et al., 1968). Furthermore, HNK-1$^+$ cells have been reported to co-express T cell antigens in the peripheral blood and lymphoid tissues (Abo et al., 1982; Poppema et al., 1983; Porwit-Ksiazek et al., 1983; Pizzolo and Chilosi, 1984). The relationship between the HNK-1$^+$ cells in the peripheral blood and those in the lymphoid tissues has not been clarified. This paper deals with a comparative analysis of the HNK-1$^+$ cells and/or T cell antigen positive cells in the human peripheral blood and lymph nodes by means of double staining techniques in light and electron microscopy. In particular, we wanted to define the morphological properties of the HNK-1$^+$ cells accumulated in the GCs and to clarify the close relationship between the HNK-1$^+$ cells and the follicular dendritic cells (FDCs) in the light zones of the GCs.

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Materials and methods

Peripheral blood

The mononuclear cells (MNCs) were fractionated from the peripheral blood of five healthy adults with the method of Ficoll-Hypaque (Pharmacia) gradient centrifugation. For surface expression of the HNK-1 antigen,

the MNCs were fixed in a periodate-lysine-paraformaldehyde (PLP) fixative (McLean and Nakane, 1974) at 4°C for 30 minutes. The cells were suspended in 0.01M phosphate buffered saline (PBS, pH 7.4) and then made to adhere to glass slides with a cytospin centrifuge (Shandon Southern Products). For cytoplasmic expression of the HNK-1 antigen, cytospin preparation of the MNCs was made before PLP-fixation. For immunoelectron microscopy the MNCs were fixed in the same fixative.

Lymph nodes

Lymph nodes were extirpated from patients with gastric or colonic cancer and gastric ulcer, and checked for absence of cancer metastasis. They were fixed in the PLP fixative at 4°C for six hours. For light microscopy, the fixed lymph nodes were embedded in an O.C.T. compound (Ames) in liquid nitrogen and were sectioned into thicknesses of 4 μm with a Cryostat (Leiz). For electron microscopy, the PLP fixed tissues were sliced 40 μm thick with a Microslicer (Dosaka E.M., Japan).

Immunohistochemical staining

The fixed cells and tissues were incubated in 5mM periodic acid (Sigma)/PBS for 20 minutes for inhibition of endogenous peroxidase (Isobe et al., 1977). After washing they were incubated overnight in monoclonal antibodies of OKT (Ortho Diagnostics) and Leu (Becton Dickinson) series. The first antibodies were followed by peroxidase-labelled secondary antibodies including anti-mouse IgG goat IgG and anti-mouse IgM goat IgG (Tago Laboratories) for four hours. The labelled peroxidase was visualized with diamino-bentizin (DAB). For electron microscopy the sections were postfixed in 1% Osmium tetraoxide and embedded in an Epon Araldite mixture following serial dehydration with alcohol.

Double stainings for light microscopy

After inhibition of endogenous peroxidase, the cyrostat sections and cytospin-prepared cells were incubated overnight in a mixture of HNK-1 antibody (mouse IgM) and anti-human T cell antibodies (OKT3, OKT4, OKT8, Leu1, Leu2a, Leu3a; mouse IgG). Having been washed three times in PBS they were reacted simultaneously with alkaline phosphatase (ALPase)-labelled anti-mouse IgM goat IgG (KPL) and peroxidase-labelled anti-mouse IgG(Fc) goat IgG (Cappel). They were incubated at room temperature in an ALPase reaction medium (Naphtol AS-BI phosphatic acid, Fast blue RR salt; Sigma) in the presence of 1 mM levamisole for 15 minutes and subsequently incubated with DAB-H_2O_2 solution for five minutes. HNK-1 positivity was marked by blue-colored ALPase reaction products in contrast to brown DAB reaction products for T cell antigens.

Double stainings for electron microscopy

The mononuclear cells of the peripheral blood fixed in PLP solution were dipped in the HNK-1 antibody and anti-T cell monoclonal antibodies as described above. Colloidal gold-labelled anti-mouse IgM goat IgG (E.Y. Laboratories) and peroxidase-labelled anti-mouse IgG(Fc) goat IgG (Cappel) were used as secondary antibodies. DAB reaction was performed for detection of the labelled peroxidase.

Results

Light microscopical findings in double staining

1) Peripheral blood

The HNK-1, OKT3/Leu1, OKT4/Leu3a-, and OKT8/Leu2a-positive cells were represented as HNK-1$^+$, T3$^+$, T4$^+$ and T8$^+$ cells respectively.

A majority of the HNK-1$^+$ cells (accounting for about 80%) were medium-sized lymphocytes with an eccentric, kidney-shaped nucleus and conspicuous cytoplasm. The blue granules of ALPase reaction products were observed on the cell surface or in the cytoplasm. The remaining 20% of the positive cells were small lymphocytes with a round nucleus. Half of the HNK-1$^+$ cells were HNK-1$^+$T3$^-$ and the other half HNK-1$^+$ T3$^+$. The HNK-1$^+$T3$^-$ cell group consisted of medium-sized lymphocytes with a reniform nucleus as well as of small lymphocytes with a round nucleus. This group accounted for about 20%. The HNK-1$^+$T3$^+$ cell group with a granular positivity in the cytoplasm, especially in the Golgi area, included medium-sized lymphocytes and small lymphocytes (Figs. 1a, 1b).

2) Lymph nodes

In the lymph nodes a majority of the HNK-1$^+$ cells accumulated in the secondary follicles, especially in the light zones of the GCs. A few positive cells were also observed in the coronas and primary follicles. Outside the lymph follicles a small number of the positive cells were scattered in the paracortex and medulla. The T4$^+$ cells were predominantly found in the paracortex and were scattered in the GCs, especially in the light zones, T8$^+$ cells, but fewer than T4$^+$ cells, were also observed in the paracortex. In the GCs they were much smaller in number than the T4$^+$ cells. This T4$^+$ cell predominance was even more remarkable in the hyperplastic GCs.

In the double stainings almost all of the HNK-1$^+$ cells in the GCs co-expressed helper/inducer T cell antigens. Outside the GCs the HNK-1$^+$ T4$^+$ cells and HNK-1$^+$ T8$^+$ cells were scattered in various numbers. HNK-1$^+$T3$^-$ cells were occasionally recognized in the vessels and capsules as well as in apposition to the medullary sinuses (Figs. 2a, 2b).

Ultrastructure of the HNK-1$^+$ cells in the peripheral blood

More than 50% of the HNK-1$^+$ cells showing electron-dense DAB reaction products on the plasmalemma were

medium-sized lymphocytes, 8 to 10 μm in diameter. They had an eccentrically located reniform nucleus with clumped heterochromatin and conspicuous cytoplasm abundant in fine cytoplasmic processes measuring about 350 to 500 nm in length. The cytoplasm contained well-developed Golgi apparatus and a number of coated vesicles and multivesicular bodies as well as many electron-dense granules. The ultrastructural profiles of the HNK-1$^+$ cells agreed with those of large granular lymphocytes (LGLs). Most of the coated vesicles, multivesicular bodies and coarse granules were located in the vicinity of the Golgi apparatus and some had gathered in the peripheral cytoplasm (Fig. 3). The characteristic coarse granules were parallel tubular arrays (PTAs) measuring up to 1 μm in diameter, and consisted of microtubular structures (Figs. 4a, 4b). They were occasionally clustered or fused together, and their tubular structures were often blurred (Fig. 4c). Some round, membrane-bound electron-dense granules, up to 400nm in diameter, with condensed granular matrices were also scattered through the cells (Fig. 4d).

Twenty percent of the HNK-1$^+$ cells were small lymphocytes with a round heterochromatin-rich nucleus and many cytoplasmic processes. Golgi apparatus, coated vesicles, multivesicular bodies and electron-dense granules were poorly developed (Fig. 5).

The transitional forms between typical LGLs and small lymphocytes were also confirmed, accounting for about 30% of all HNK-1$^+$ cells. Their irregular outline, reniform nuclei and abundant cytoplasm were similar to those of typical LGLs; however, no PTAs were found and some small round granules were found scattered (Fig. 6).

Fig. 2. A germinal center of the lymph node in double staining. (a) HNK-1$^+$ cells (blue) have accumulated in the light zone of the germinal center. (GC). Numerous T4$^+$ cells (brown) are found in the interfollicular area and paracortex. (b) High power view of Fig. 1a. Most HNK-1$^+$ cells in the GC have also reacted with Leu 3a antibody (HNK-1$^+$ T4$^+$). HNK-1 & Leu 3a, (a) × 160; (b) × 400

Fig. 3. Ultrastructure of an HNK-1$^+$ LGL in the peripheral blood. DAB reaction products are visible on the cell surface. Note the low N/C ratio, the reniform nucleus, numerous cytoplasmic organelles and electron-dense granules, and many cytoplasmic processes. HNK-1, × 5,000

Fig. 4. Ultrastructure characteristics of the electron-dense granules of the HNK-1$^+$ LGLs. (a and b). Parallel tubular arrays (PTAs). Note electron-dense tubular structure. (c). PTAs are occasionally clustered and fused together, and their tubular structures have often disappeared. (d). Electron-dense granules with condensed granular matrices. HNK-1, (a, b and d). × 12,000; (c) × 8,000

Fig. 5. Ultrastructure of an HNK-1$^+$ small lymphocyte in the peripheral blood. The cell shows a high N/C ratio, few cytoplasmic organelles and granules, and many fine cytoplasmic processes. HNK-1, × 6,000

Fig. 6. An HNK-1$^+$ transitional form in the peripheral blood. The cell is very similar to the LGL (Fig. 4) except for a few electron-dense granules. HNK-1, × 4,000

Fig. 7. Ultrastructure of a T4$^+$ small lymphocytes in the peripheral blood. The cell shows a smooth outline, a high N/C ratio, a round nucleus and poorly developed cytoplasmic organelles. A Gall body (arrow) is occasionally seen. OKT4, × 6,000

Fig. 8. Ultrastructure of a T4$^+$ medium-sized lymphocyte in the peripheral blood. Note the morphological similarity with the HNK-1$^+$ cell in Fig. 6. OKT4, × 4,000

Fig. 9. Ultrastructure of two T8$^+$ small lymphocytes in the peripheral blood. The morphology of the right cell with an uneven cell surface is characteristic for half of the T8$^+$ cells. The left T8$^+$ cell shows a smooth outline and poorly developed organelles. OKT8, × 4,000

Fig. 10. Ultrastructure of a T8$^+$ medium-sized lymphocyte. Note morphological similarity with the HNK-1$^+$ transitional form (Fig. 5) and the T4$^+$ medium-sized cell (Fig. 8). OKT8, × 4,000

Fig. 11. Ultrastructure of an HNK-1$^+$T3$^+$ small lymphocyte in the peripheral blood. (a and b) DAB reaction products and dotted gold particles are found on the cell membrane. The cell shows a high N/C ratio, an uneven cell contour and a few cytoplasmic organelles. HNK-1 & OKT3, (a) × 7,000, (b) × 20,000

Fig. 12. LGLs in ultrastructural double staining with the HNK-1 and OKT 3 antibodies. Note resemblance between the HNK-1$^+$ T3$^+$-LGL (a) and the HNK-1$^-$T3$^+$-LGL (b) HNK-1 & OKT3, × 3,000 & × 12,000

Fig. 13. Ultrastructure of HNK-1$^+$ cells in the light zone of the germinal center of the lymph node. Five HNK-1$^+$ cells are small- to medium-sized lymphocytes with a few cytoplasmic organelles and lacking large electron-dense granules. HNK-1, × 2,500

Fig. 14. HNK-1$^+$ cells in the light zone of the germinal center. The cytoplasmic projections of the positive cells show interlocking with those of the follicular dendritic cells near the labyrinth structure (LS). HNK-1, × 4,000

Fig. 15. A large HNK-1$^+$ blast in the deep paracortex of the lymph node. The positive cells show a conspicuous cytoplasm and a round euchromatic nucleus with a prominent nucleolus. Few cytoplasmic organelles and granules are seen. HNK-1, × 3,000

Fig. 16. A mitotic figure of the HNK-1$^+$ cells in the paracortex of the lymph node. The cell shows many fine cytoplasmic processes. HNK-1, × 3,000

Fig. 17. An HNK-1$^+$ LGL-like cell in the medullary sinus of the lymph node. Note some large electron-dense granules and an uneven cell surface. HNK-1 × 4,000

Fig. 18. Low power view of the T4$^+$ cells in the light zone of the germinal center. The T4$^+$ cells are small- to medium-sized lymphocytes which cannot be distinguished from the HNK-1$^+$ cells in the GC (Fig. 13). Leu3a. × 2,000

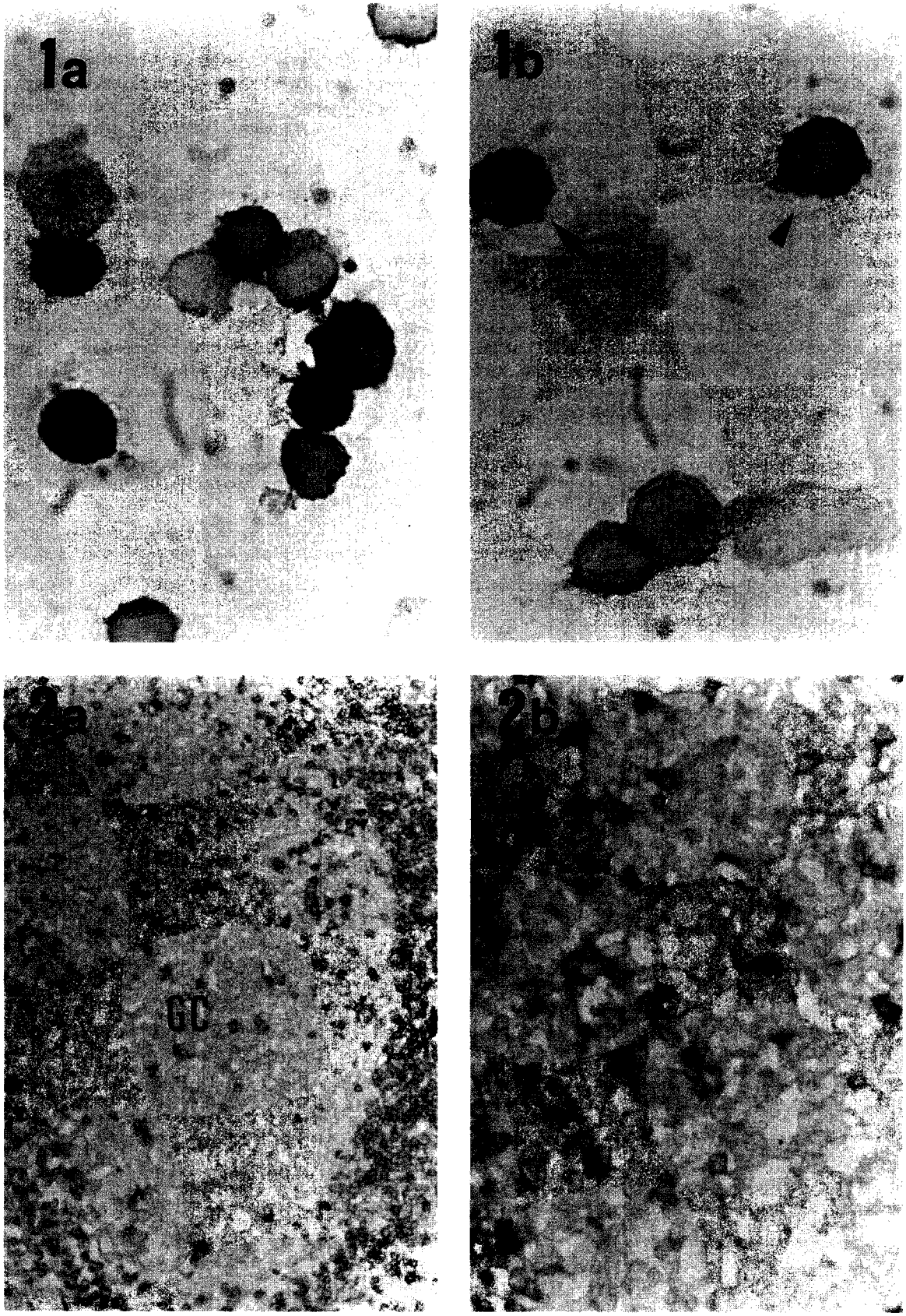

Fig. 1. Mononuclear cells in the peripheral blood stained with the double (immunoperoxidase and immunoalkaline phosphatase) method. (a). The medium-sized lymphocyte with a reniform nucleus is an HNK-1^+ $T3^-$ (dark blue). Other small lymphocytes with a round nucleus are HNK-1^- $T3^+$s (brown); × 1,000 (b). Note HNK-1^+ $T3^+$ (arrow) and HNK-1^+T3^- (arrow head) small lymphocytes. HNK-1 & OKT3, × 1,000


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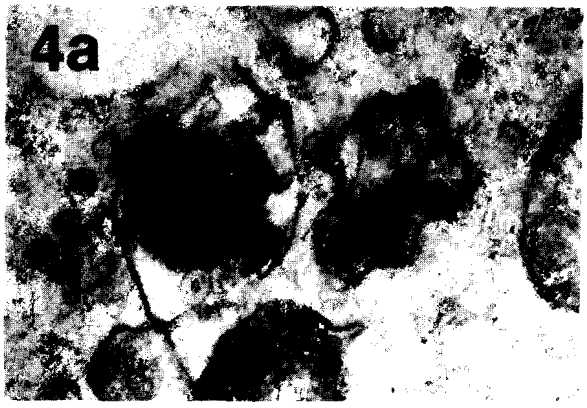
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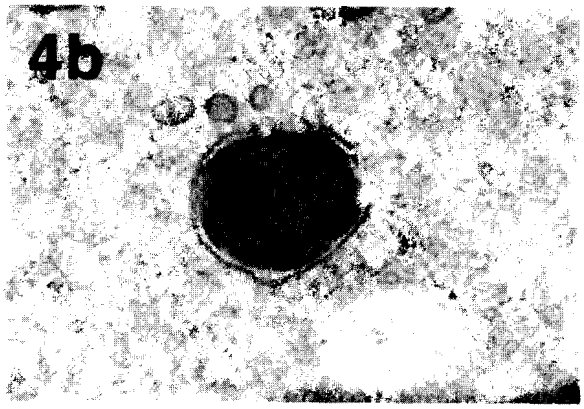
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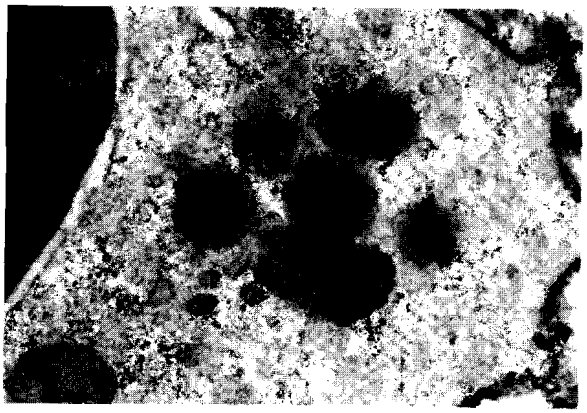

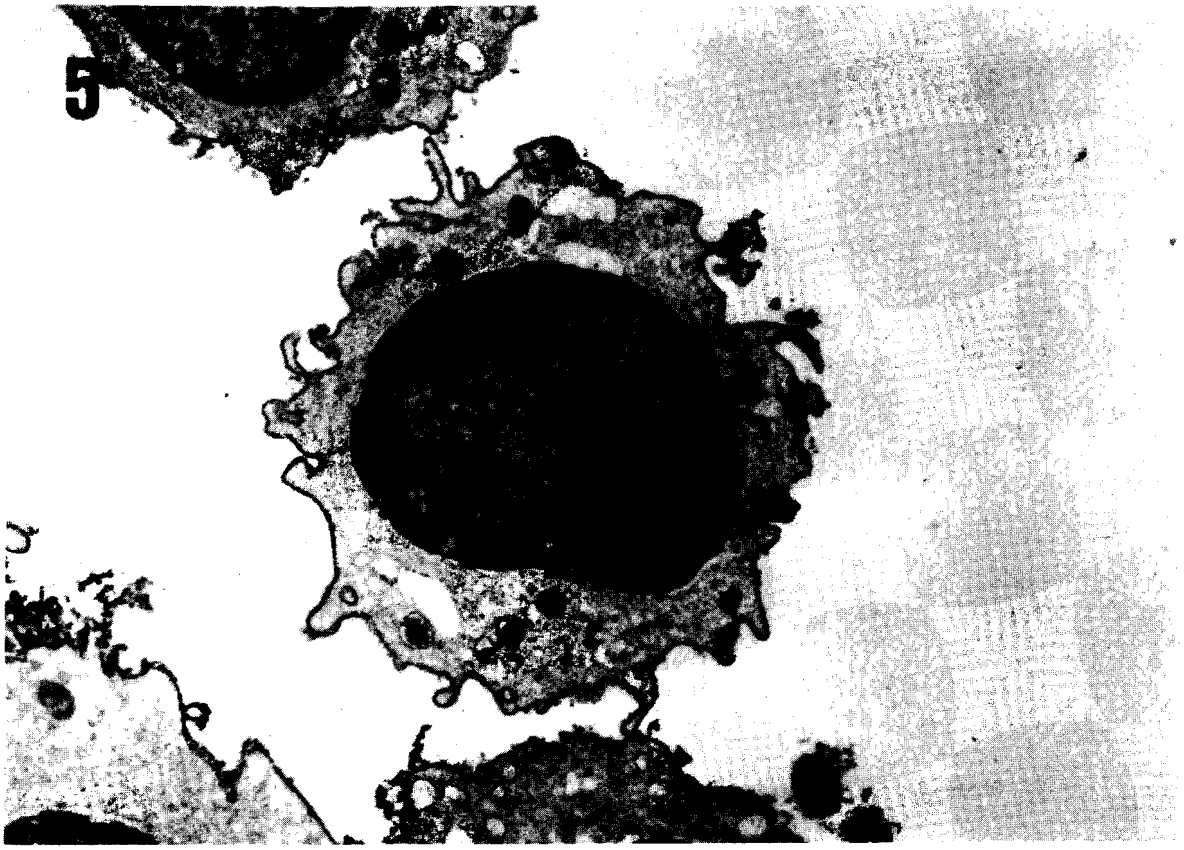
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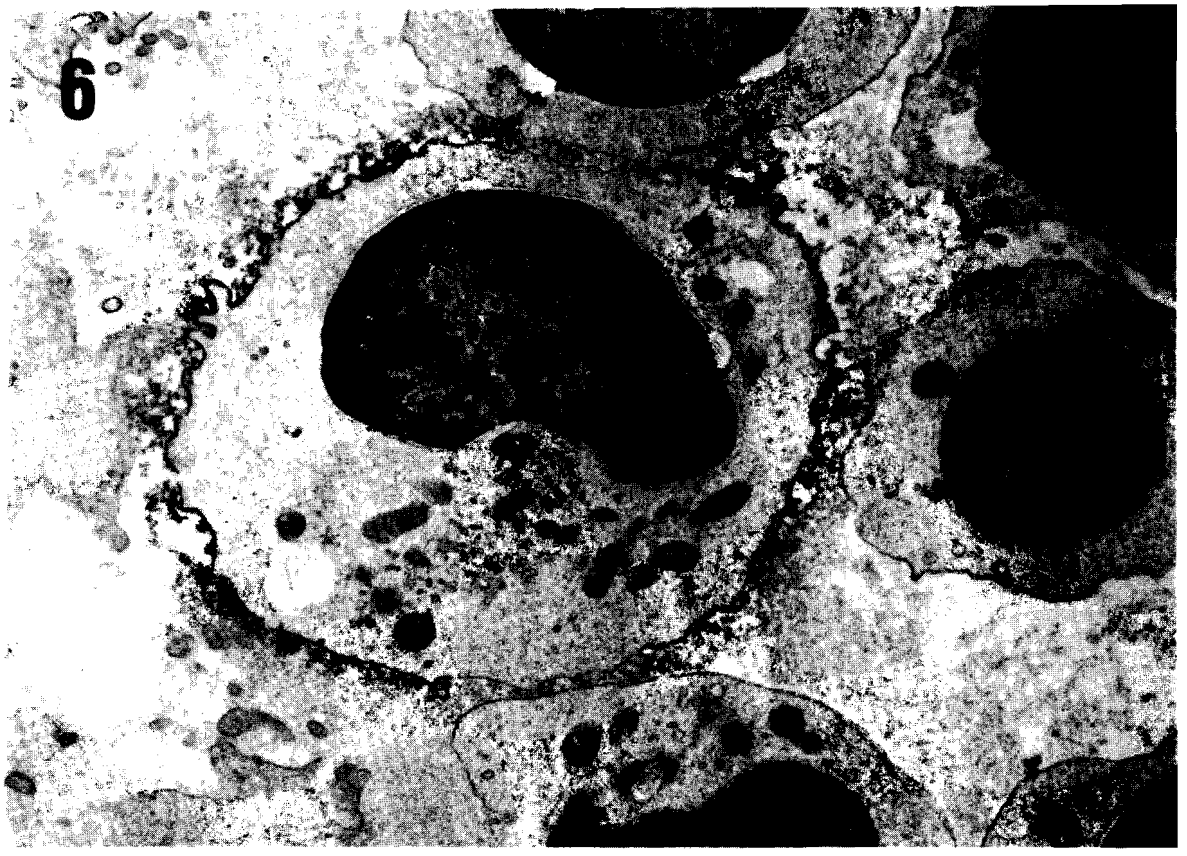
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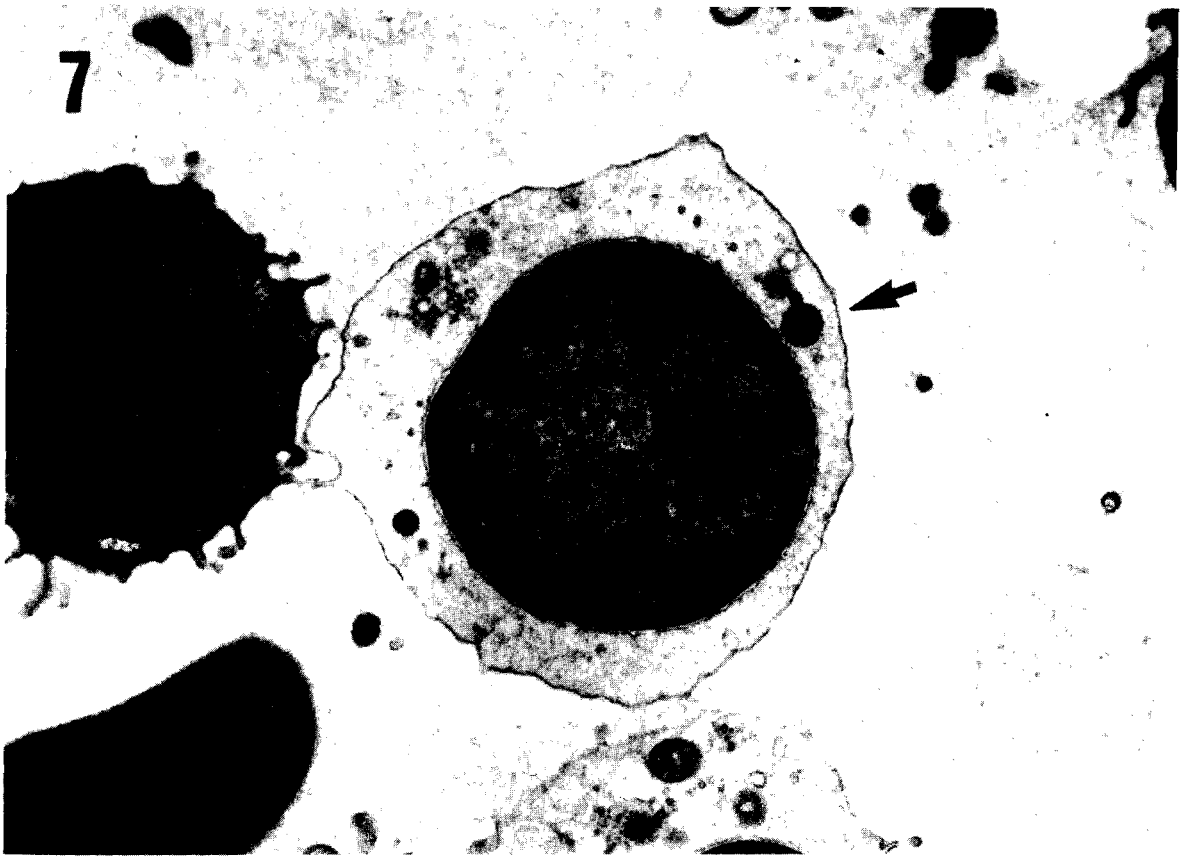
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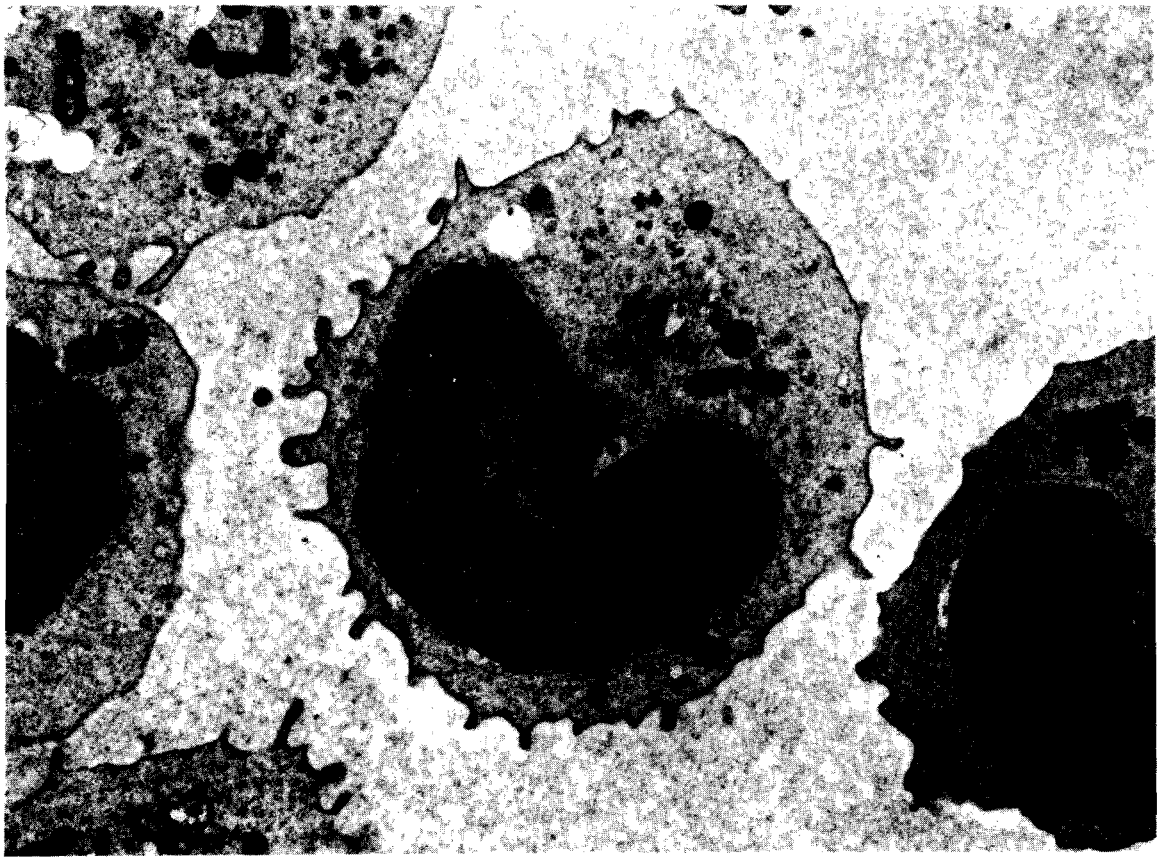

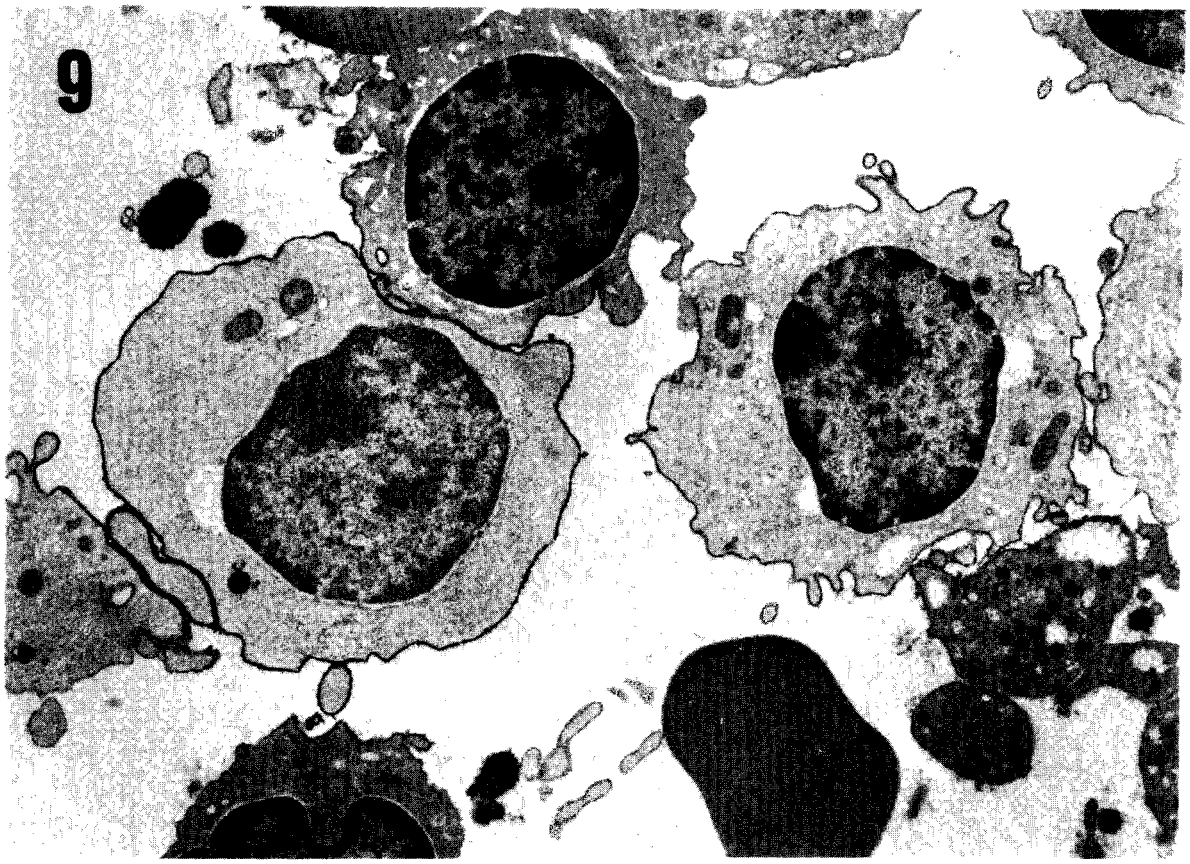
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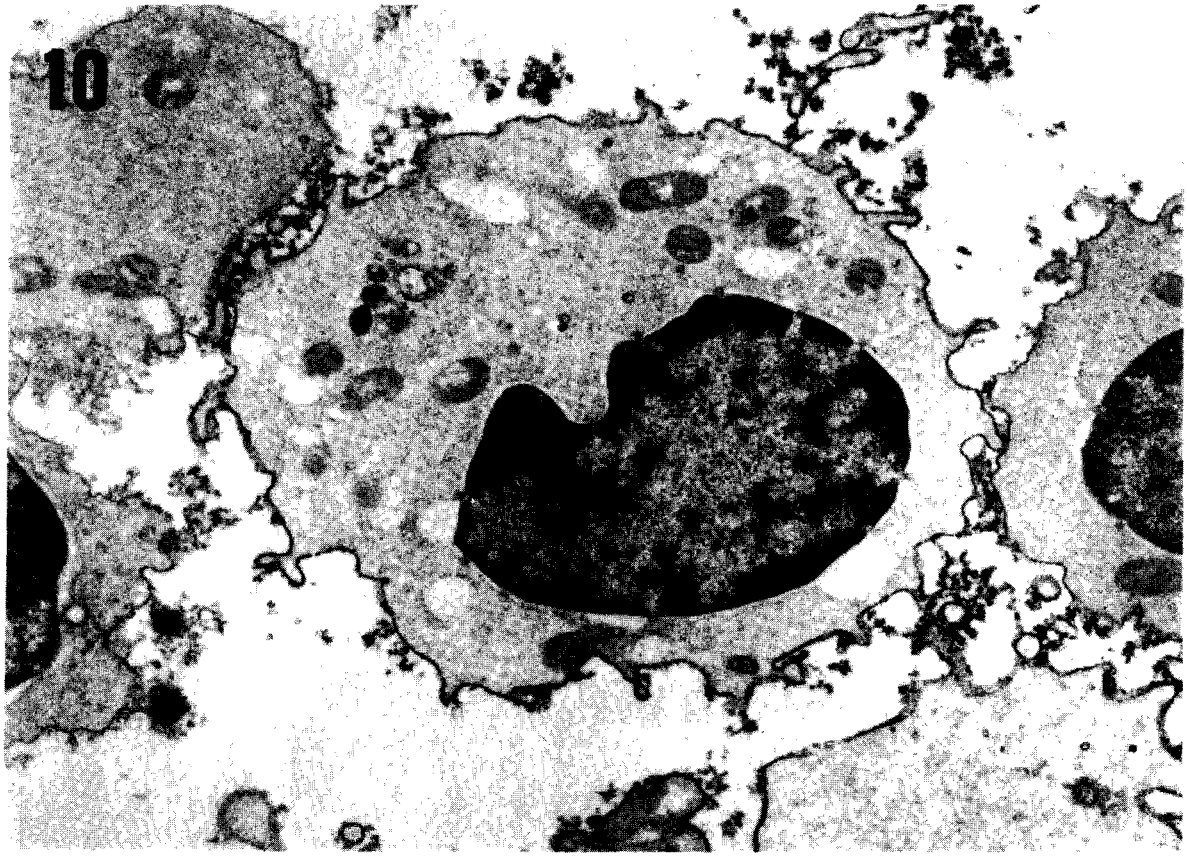
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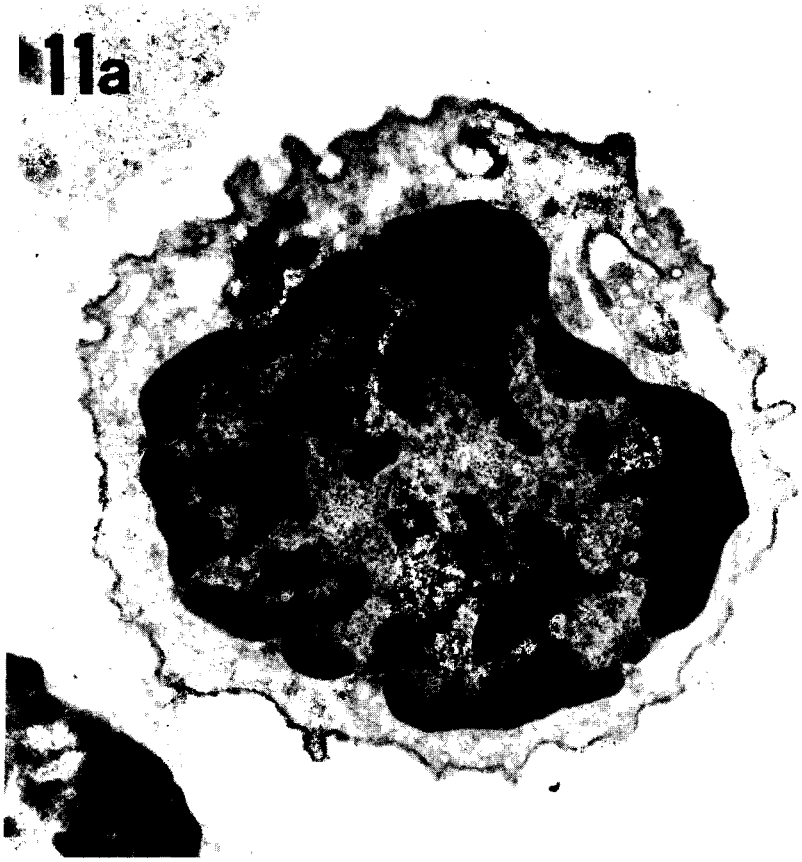
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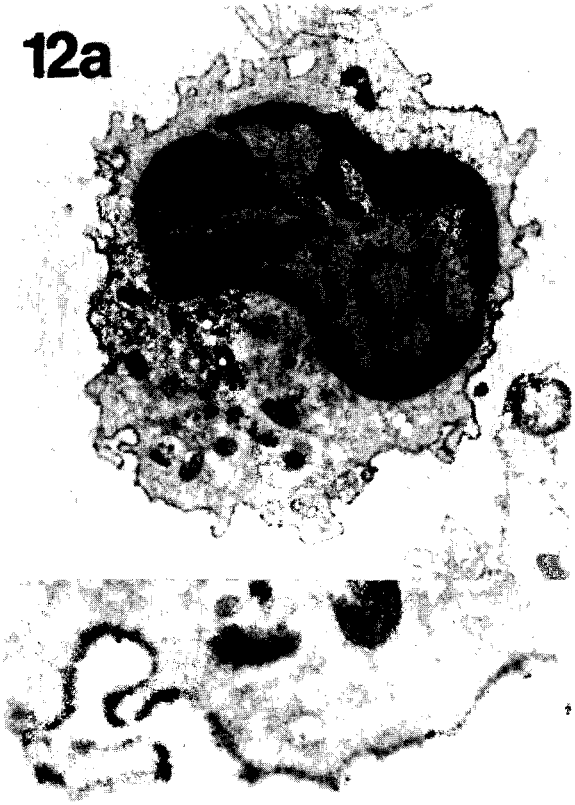
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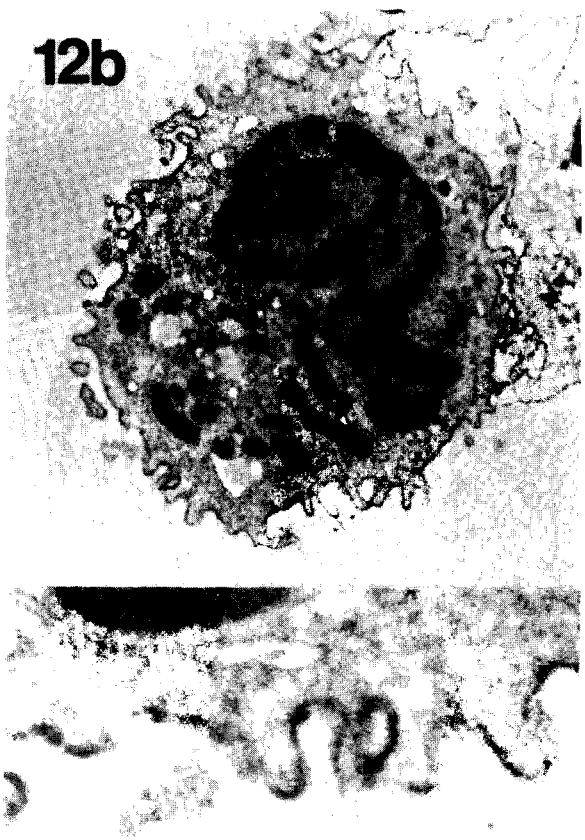
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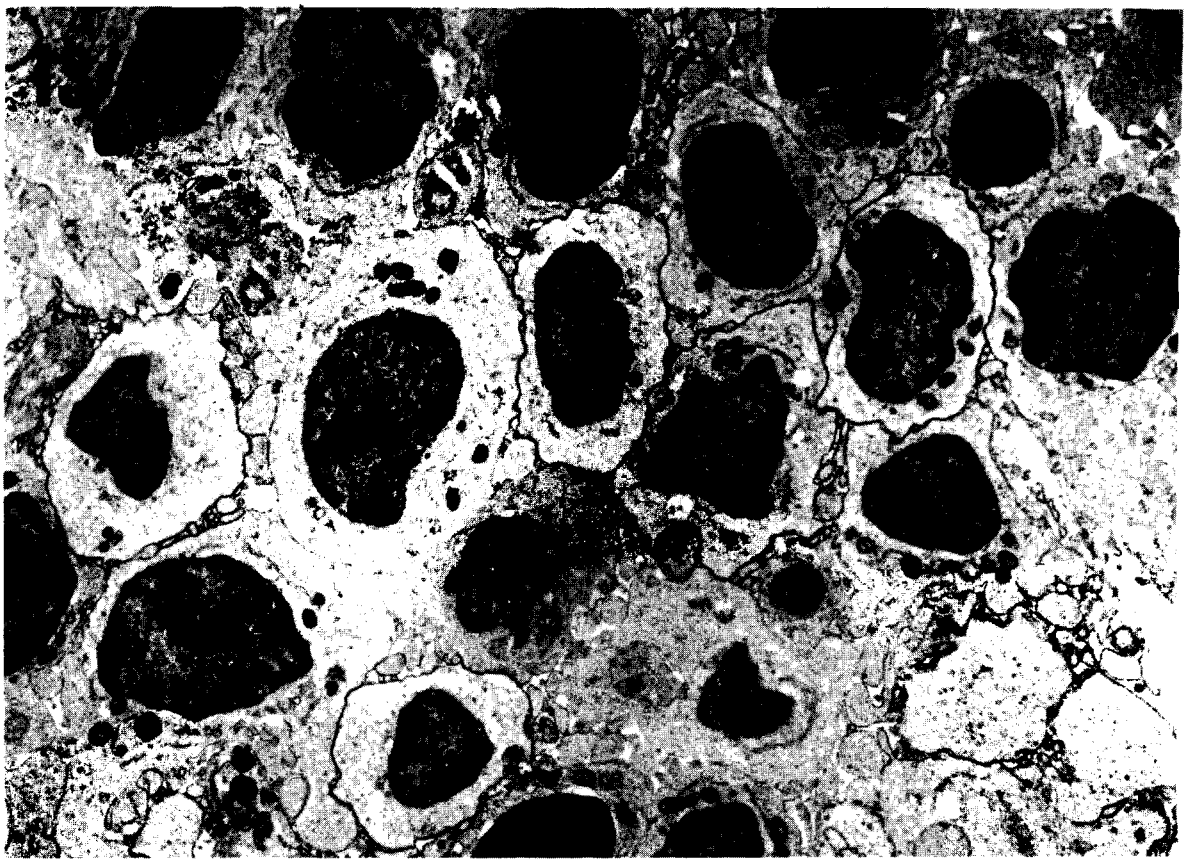

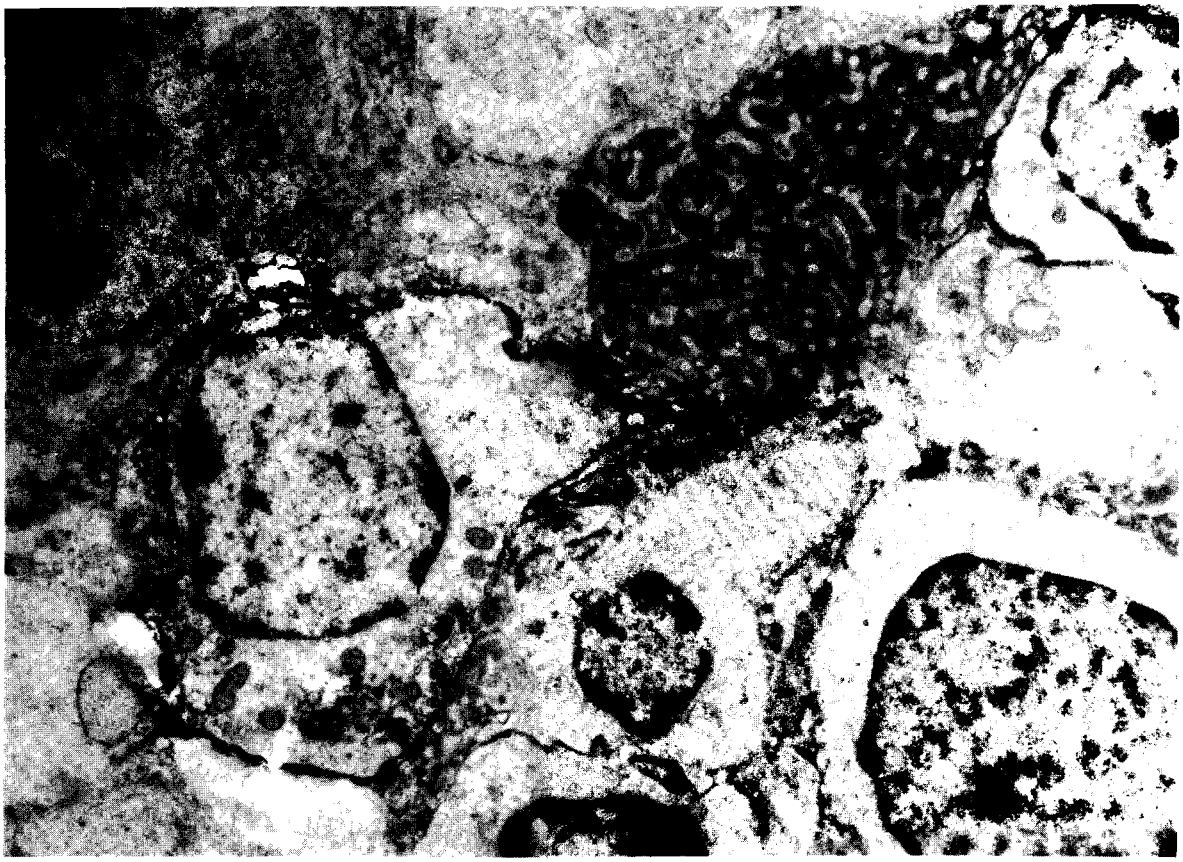

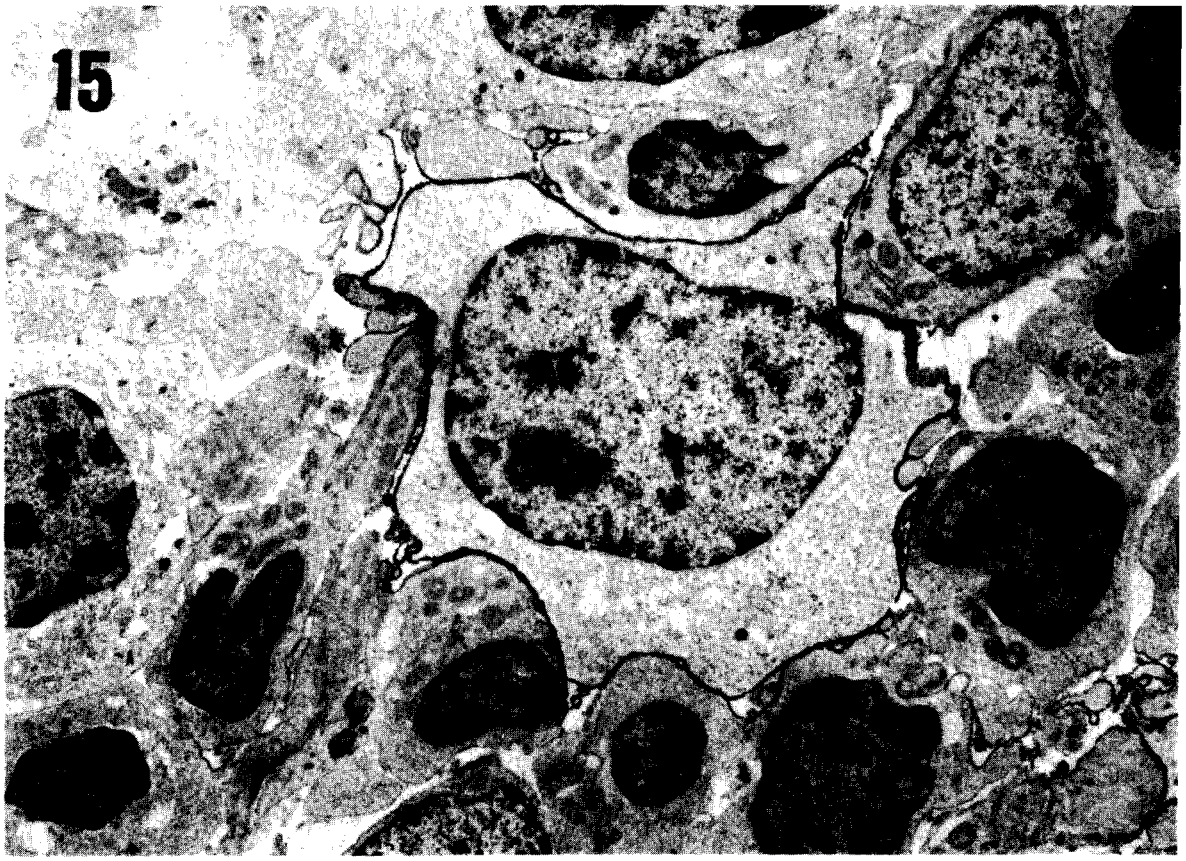
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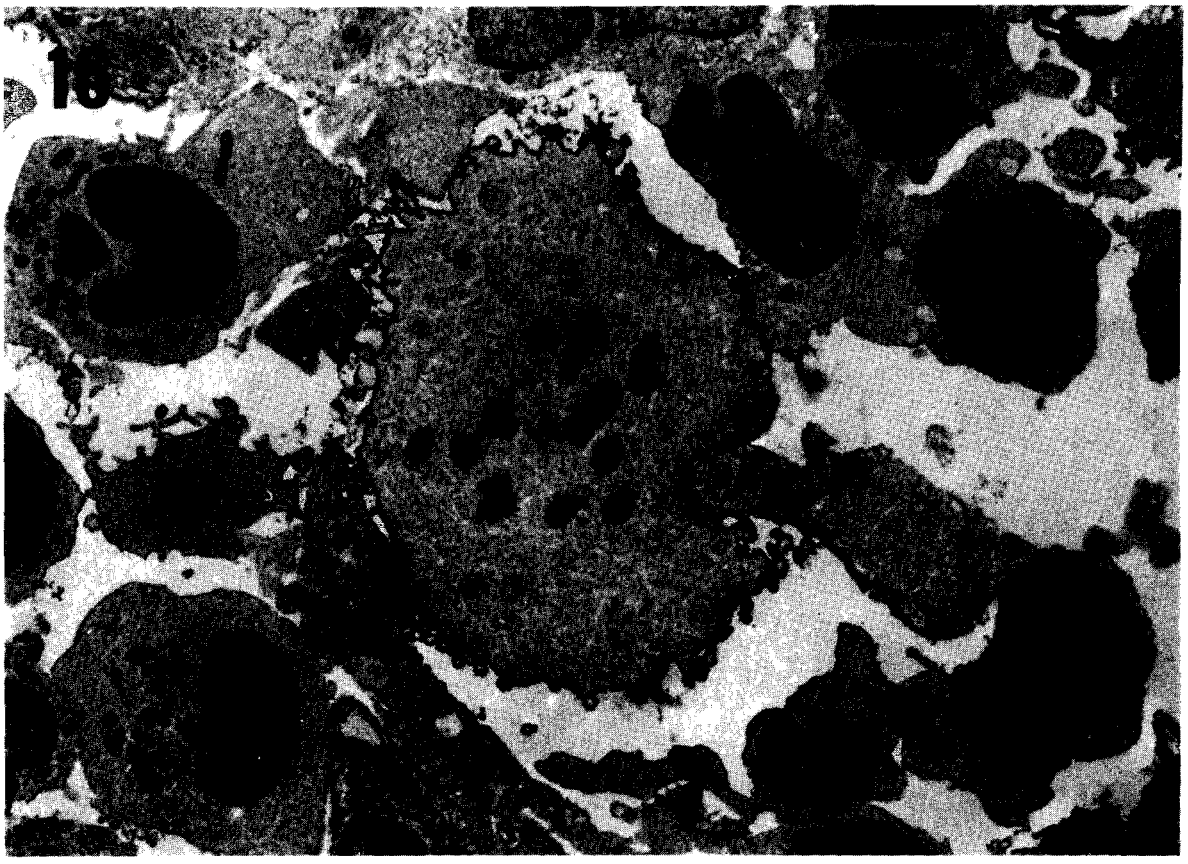
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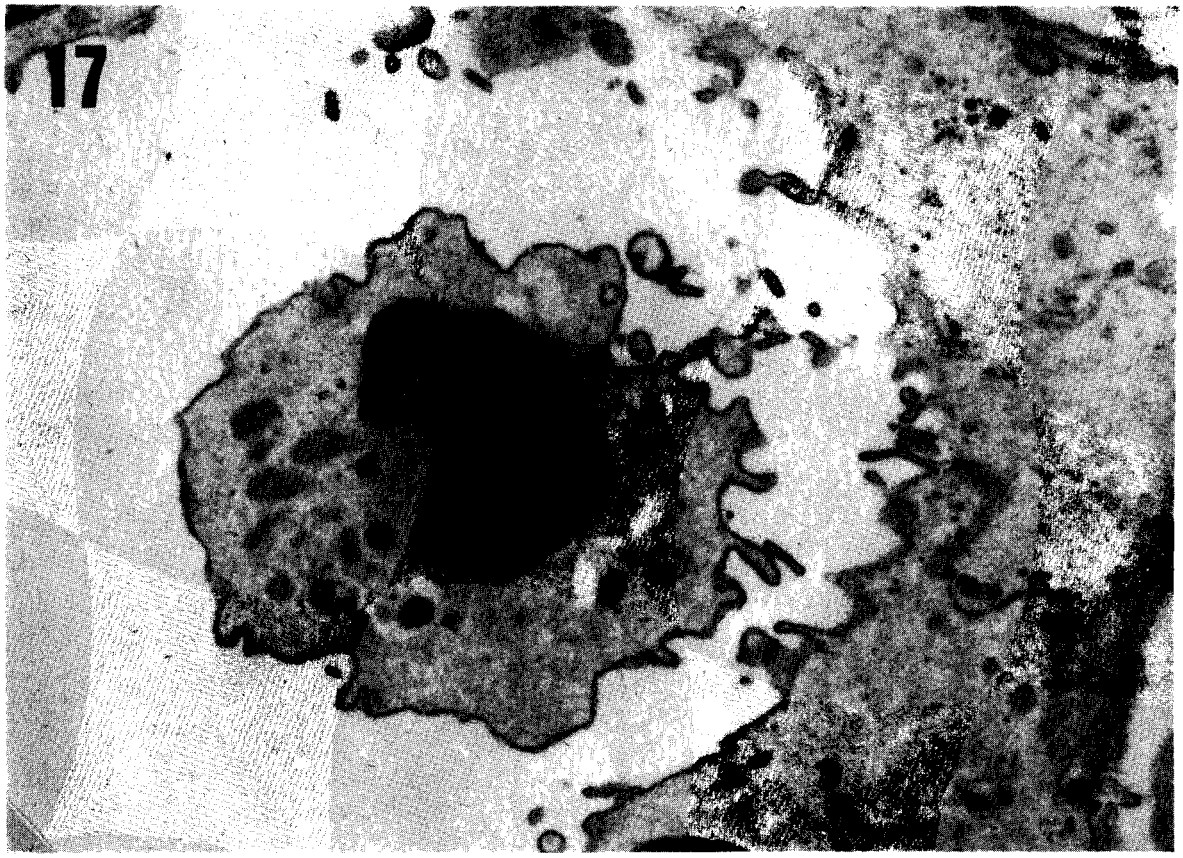
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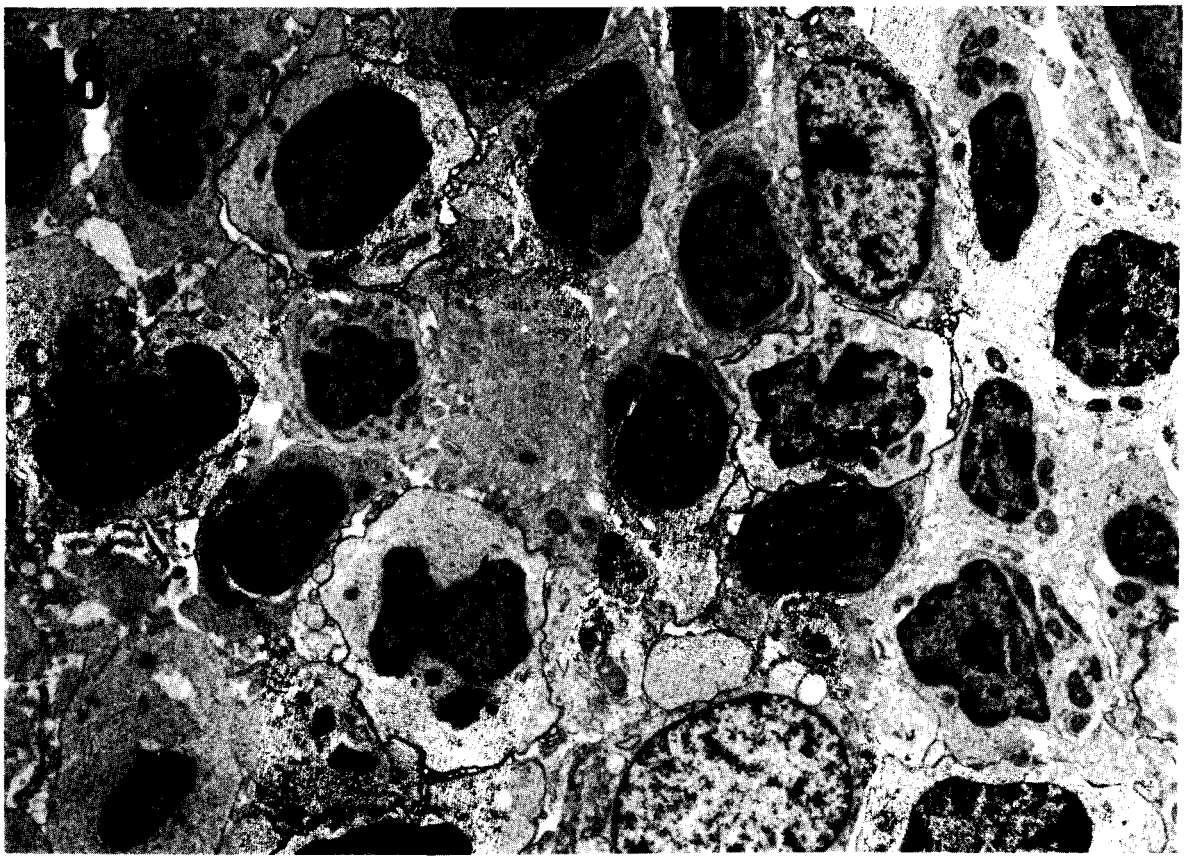
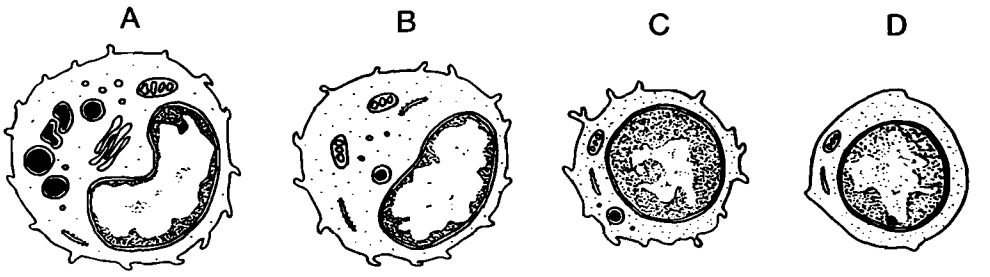
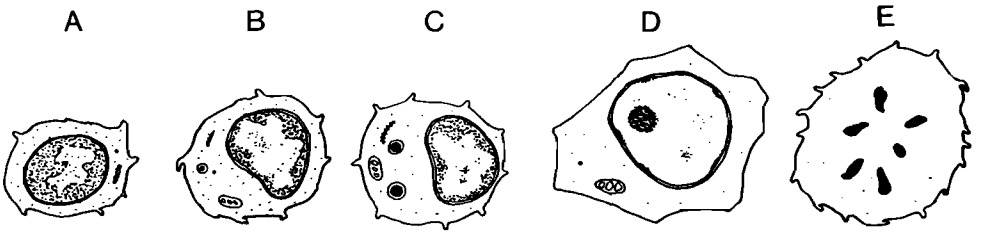




	A	B	C	D
HNK-1⁺	+++	++	+	−
T4⁺	−/+	+	++	+++
T8⁺	+	++	+++	++

Table 1. A schema for frequency of appearance of HNK-1⁺, T4⁺ and T8⁺ cells in the peripheral blood with a function of the cell types varing in cell size and development of cytoplasmic organelles and cytoplasmic processes. The HNK-1⁺ cells are divided into three types: LGLs (A), transitional forms (B) and small lymphocytes (C) with many cytoplasmic processes. Both the T4⁺ cells and the T8⁺ cells generally include these three types and also contain smooth-surfaced lymphocytes (D).



	A	B	C	D	E
Germinal Center	+++	++	−	−	−
Paracortex	+	+	−/+	+	+
Medulla	+	+	−/+	+	+

Table 2. Frequency in appearance of various HNK-1⁺ cells in the germinal center, paracortex and medulla of the lymph nodes. The HNK-1⁺ cells in the GCs are small-to medium-sized lymphocytes (A and B) poor in cytoplasmic organelles and granules. Outside the GCs, the HNK-1⁺ cells are also seen blastic forms (D), mitotic figures (E) and LGL-like cells (C).

Ultrastructure of the T cell subsets in the peripheral blood

Most T4$^+$ cells and a minority of the T8$^+$ cells were smooth-surfaced small lymphocytes with a heterochromatin-rich, round nucleus. There were few cytoplasmic organelles and electron-dense granules. A few small round granules and Gall bodies were occasionally found (Figs. 7, 9). The Gall bodies, which consisted of moderately electron-dense matrices, could not be recognized in the HNK-1$^+$ cells. A small number of T4$^+$ cells and a majority of the T8$^+$ cells were small to medium-sized lymphocytes with many cytoplasmic processes, similar to the HNK-1$^+$ cells (Figs. 8, 10). T8$^+$ cells had more cytoplasmic processes, cell organelles and electron-dense granules than T4$^+$ cells. LGLs were also contained in both subjsets. Therefore, there was no distinct morphological differences on a per cell basis between the HNK-1$^+$ cells and T cell subsets, except for smooth-surfaced lymphocytes.

Ultrastructural double stainings in the peripheral blood

HNK-1$^+$T3$^+$ cells were identified by the presence of dotted gold particles (HNK-1$^+$) and DAB reaction products (T3$^+$) on the cell-surface plasmalemma. Most HNK-1$^+$T3$^+$ cells were transitional forms between LGLs and small lymphocytes, while some were typical LGLs. A small number of the HNK-1$^+$ T3$^+$ cells were irregular-surfaced small lymphocytes, poor in cytoplasmic organelles and electron-dense granules (Figs. 11, 12a). Among the HNK-1$^+$T3$^-$ cells, irregular-surfaced small lymphocytes with few granules were occasionally found in addition to LGLs and transitional forms. A comparison of the HNK-1$^+$ T3$^-$ cell group with the HNK-1$^+$ T3$^+$ cell group shows that LGLs were mostly included in the former. However, the two groups could not be individually distinguished when merely morphologically examined. The HNK-1$^-$ T3$^+$ cell group was composed mainly of smooth-surfaced small lymphocytes, and included occasionally LGLs and transitional forms (Fig. 12b). Morphological differentiation between the HNK-1$^+$ cell group and HNK-1$^-$ T3$^+$ cell group could not be made either, although only the latter contained smooth-surfaced lymphocytes.

Ultrastructure of the HNK-1$^+$ cells in the lymph nodes

In the GCs the HNK-1$^+$ cells were small- to medium-sized lymphocytes with a round or notched heterochromatin-rich nucleus. Golgi apparatus, coated vesicles and cytoplasmic granules were poorly developed (Fig. 13). No typical LGLs could be observed in the GCs. Some HNK-1$^+$ cells in the GCs showed cytoplasmic processes interlocked with those of the follicular dendritic cells (FDCs) near the labyrinth structures (Fig. 14). Outside the GCs most HNK-1$^+$ cells were found to be small- to medium-sized lymphocytes, similar to the GCs. Some large lymphoblastic cells also reacted with the HNK-1 antibody. These HNK-1$^+$ blasts were characterized by a large euchromatic nucleus with a prominent nucleolus, copious cytoplasm poor in organelles, and a smooth cell contour (Fig. 15). They were scattered in or near the medullary sinuses with some HNK-1$^+$ blastic cells with many cytoplasmic processes being in the process of mitosis (Fig. 16). In a few instances, the HNK-1$^+$ medium-sized lymphocytes with a few large electron-dense granules could be confirmed near or in the medullary sinuses. They were similar to the HNK-1$^+$ transitional forms or LGLs in the peripheral blood (Fig. 17).

Ultrastructure of the T cell subsets in the lymph nodes

Both T4$^+$ cells and T8$^+$ cells in the GCs were small- to medium-sized lymphocytes with a heterochromatin-rich round nucleus and organelle-poor cytoplasm. T4$^+$ and T8$^+$ cells could not be distinguished from the HNK-1$^+$ cells in the GCs merely on the basis of morphological profiles (Fig. 18). Outside the GCs, large blastic T4$^+$ or T8$^+$ cells, very similar to the HNK-1$^+$ blastic cells, were scattered, in addition to small- to medium-sized lymphocytes. The T cell subsets and the HNK-1$^+$ cells in the lymph nodes could not be individually differentiated, as was the case in the peripheral blood.

Discussion

In the present immunocyto-histochemical investigation, attention was focused on comparative analysis of the HNK-1$^+$ cells in the peripheral blood and lymph nodes.

In the peripheral blood the HNK-1$^+$ cells were so heterogeneous in morphology that they could be divided into three groups: large granular lymphocytes (LGLs), intermediate forms and small lymphocytes, all of which had many cytoplasmic processes. The HNK-1$^+$ cells and T cell subsets could not be distinguished individually only on the basis of their morphological aspects, except that the T cell subsets included smooth-surfaced lymphocytes with few cytoplasmic organelles. The morphological analogy between the HNK-1$^+$ cells and T cell subsets supports the finding of Abo et al. (1982) that the HNK-1$^+$ cells co-expressed T cell antigens in the maturation stage of NK cells. Abo et al. (1983) have reported that the HNK-1$^+$T$^-$M$^+$ cells (mature NK cells) had a high level of NK cytotoxity and more cytoplasmic granules than the HNK-1$^+$T$^+$M$^-$ cells (immature NK cells). However, in our immunoelectron microscopic double staining we could not clearly define the relationship between the ultrastructural pattern of the granule depelopment and the expression of the HNK-1 and T cell antigens. These findings indicate a broader morphological spectrum within the HNK-1$^+$ cells, as schematically illustrated in Table 1, than that described by Abo et al. (1982) and Marana et al. (1984, 1985). It is also interesting that the T8$^+$ (suppressor/cytotoxic T) cells in the peripheral blood have been found to have more cytoplasmic processes and electron-dense granules than the T4 (helper/inducer T) cells (Bosen and Hokland, 1982; 1982; van der Weij et al., 1983; Kockman et al., 1984). This morphological characteristic of the T8$^+$cells

suggests a close relationship to the HNK-1$^+$ cells in terms of their cytotoxic function.

It has already been reported that the HNK-1$^+$ cells accumulate in the germinal centers (GCs) of the secondary follicles in the lymph nodes (Banerjee and Thibert, 1983; Hsu et al., 1983; Mori et al., 1983; Poppema et al., 1983; Ritchie et al., 1983; Si and Whiteside, 1983). In our investigation, as illustrated in Table 2, we found that the numerous HNK-1$^+$ cells in the GCs do not resemble LGLs, but have the appearance of small- to medium-sized lymphocytes with few electron-dense granules. It has been previously described that NK cell activity in human lymph nodes was lower than in the blood and spleen (Antonelli et al., 1981). Based on these observations, we suggest that the HNK-1$^+$ lymphocytes in the GCs are not related to natural killer (NK) and killer (K) cell functions. In the double staining technique of this study and others' (Poppema et al., 1983; Porwit-Ksiazek et al., 1983; Pizzolo and Chilosi, 1984), the HNK-1$^+$ cells in the GCs exclusively co-expressed helper T cell antigens. Therefore, it cannot be completely excluded that they were in an immature stage of the NK cell differentiation pathway, as described by Abo et al. (1983). It interested us to find that the cytoplasmic processes of the HNK-1$^+$ cells in the light zones of the GCs were interlocking with those of the follicular dendritic cells (FDCs), which are well known to trap antigen-antibody complexes for a long duration (Nossal et al., 1968; Klaus et al., 1980; Mandel et al., 1980; Tew et al., 1984). This characteristic finding suggests than the HNK-1$^+$ cells in the GCs may play a role in the immunological regulation of the germinal center cells regarded as memory B cells (Thorbecke and Lerman, 1976; Terashima et al., 1977). Moreover, the HNK-1$^+$ cells may mediate the suppressor function against B cell proliferation when activated with immune complexes, as Tilden et al. (1983) reported.

The presence of mitotic activity and the blastic form of the HNK-1$^+$ cells in lymph nodes indicate that the lymph nodes are a proliferation site of the HNK-1$^+$ cells. Since HNK-1$^+$ blastic cells are not found in GCs, in contrast to the paracortex and medulla, HNK-1$^+$ cells may not proliferate inside but outside GCs. Very few numbers of the LGL-like lymphocytes were seen outside GCs, especially in or near the medullary sinuses, suggesting that the HNK-1$^+$ cells differentiate into LGLs in the lymph nodes. Lymph nodes may therefore be a source of the HNK-1$^+$LGLs, which have a strong natural killer function, in the peripheral blood.

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Accepted October 1, 1988