Brief Definitive Report

HNK-1 ANTIBODY DETECTS AN ANTIGEN EXPRESSED ON NEUROECTODERMAL CELLS*

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The HNK-1 monoclonal antibody raised to the HSB-2 T cell line detects Leu-7 antigen expressed on a peripheral blood subpopulation of ~15% of lymphocytes that comprises almost all the natural killer (NK) and killer (K) cells but no other blood cell (1). Besides Leu-7, virtually all these cells carry either T cell-associated markers or the M1 antigen associated with monocytes and granulocytes (2). The biochemical characterization of Leu-7 has not been published, except for its Mr of ~110,000 (L. Lanier, personal communication cited in 3).

The distribution of Leu-7 has been studied in lymphoid organs by immunoperoxidase staining of frozen sections of spleen, tonsils, and lymph nodes where it has been specifically located within the germinal centers, while in the thymus few scattered cells were identified as HNK-1 positive (4). In bone marrow, 0.2–1.2% Leu-7-positive cells were detected in total nucleated cells (1). The presence of HNK-1-positive cells in nonhematopoietic organs has not been looked for except for the infiltration of Leu-7-positive lymphocytes in a few tumors (5).

Because we found the same distribution of Leu-7-positive cells on paraffin sections of normal lymphoid tissues as that previously observed on frozen sections, we were able to investigate the reactivity with HNK-1 of various normal tissues and tumors embedded in paraffin. The results we present here indicate that besides a discrete population of lymphocytes, the HNK-1 antibody also detects an antigen specifically associated with tissues of neuroectodermal origin.

Materials and Methods

Cell Lines. Human tumor-derived cell lines (Table I) were carried in RPMI 1640 with 10% heat-inactivated fetal calf serum. Lines MDA-MB231, MCF-7, M29, and IGR3 were donated by Dr. J. K. Youn; lines KB, MS.Rb, SA4, IMR32, and NJB by Dr. J. Belehradek; and lines HT29 and HRT18 by Dr. B. Fertil, all of the Institut G. Roussy. The placental choriocarcinoma BeWo originated from Dr. H. Sussman, and lines HT1080C and HeLaS3 from Dr. Douglas Wallace, both of Stanford University, CA. Ewing's sarcoma (ES) cell lines IARC-EW1, IARC-EW3, IARC-EW7, and IARC-EW11, and the medulloblastoma cell line IARC-186 were derived and made available to us by Dr. G. Lenoir, I.A.R.C., Lyon, France and Drs. I. Philip and T. Philip, Centre Léon Bérard, Lyon, France. Adherent cells were suspended using trypsin (1:250, 0.05%)-EDTA (0.02%) (Flow Laboratories, United Kingdom).

HNK-1 Monoclonal Antibody. The HNK-1 hybridoma was purchased from the American Type Culture Collection, Rockville, MD and grown in RPMI with 10% heat-inactivated horse serum.

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Indirect Immunofluorescence Assays. Cells were incubated with HNK-1 hybridoma supernatant (5 x 10^6 cells in 50 μl; 4°C, 45 min), washed three times, incubated with fluoresceinated goat anti-mouse IgM (Tago Inc., Burlingame, CA) at appropriate dilution (1:25 in 50 μl RPMI; 4°C, 45 min), washed three times, resuspended into 20 μl RPMI, smeared on slides, and live-examined for fluorescence under a Leitz Ortholux microscope (E Leitz, Inc., Rockleigh, NJ) equipped with the proper combination of filters for fluorescein fluorescence. Lines were scored positive when >10% of the live cells were fluorescent. Each set of experiments included a negative control with cells incubated in RPMI alone as the first step.

Immunoperoxidase Staining of Paraffin-embedded Tissues. Histological sections (3 μm) of paraffin-embedded tumors or normal tissues fixed in alcohol-formalin-acetic acid or Bouin's fixative were deparaffinized and rehydrated through xylene and graded alcohol series. Nonspecific reactions were blocked by covering the slides with a normal rabbit serum. Sections were incubated in HNK-1 supernatant (30 min, 20°C), washed in phosphate-buffered saline (PBS), pH 7.6 (10 min), then incubated with a dilution of a rabbit anti-mouse Ig antiserum (1:100, 30 min, 20°C) conjugated to peroxidase. After washing 10 min in PBS, the reaction was revealed by diaminobenzidine-H_2O_2 incubation (10 min). Sections were counterstained with hematoxylin. Negative controls (first antibody omitted or irrelevant first antibody) were included in every set of experiments.

Results and Discussion
The reactivity of the HNK-1 antibody was checked on peripheral blood mononuclear cells isolated from three normal donors. Between 12.8 and 14% of total cells were stained. After enrichment for large granular lymphocytes on a Percoll gradient, the percentage of positive cells increased to 34–43%.

A series of human tumor-derived cell lines of nonhematopoietic origin was tested by indirect immunofluorescence for HNK-1 reactivity. Results shown in Table I indicate that the cell lines derived from tumors of neuroectodermal origin were positive. These included two neuroblastomas, one medulloblastoma, one retinoblastoma, and two melanomas. Carcinomas, with the exception of the choriocarcinoma-derived BeWo, were all negative, as were sarcomas, except for three of four ES, which were positive. These data thus suggested that HNK-1 might react with an antigen related to neuroectodermal differentiation.

Our observations of paraffin-embedded lymphoid tissues from normal gastrointestinal tract and lymph nodes stained with HNK-1 were similar to those published on frozen sections (4). We could analyze the presence or absence of Leu-7 on specimens of normal and pathologic tissues embedded in paraffin. Results obtained on tumor sections (Table II) correlate with data obtained with tumor lines. Most malignancies (24 of 30) derived from neuroectoderm, of neural tube and neural crest origin, were positive. The former included astrocytoma, glioblastoma, retinoblastoma, and oligodendroglioma, while neural crest-derived tumors, such as neuroblastoma, paraganglioma, esthesioneurocytoma, neuroepithelioma, phaeochromocytoma, and carcinoid tumors, were also stained by HNK-1; one of four paragangliomas and one thyroid medullary cancer, however, were negative. Of three nerve sheath tumors probably derived from neural crest, one neurofibroma was positive, while one neurinoma and one neurogenic sarcoma were negative. Finally, one benign naevus and one of two melanomas were stained by HNK-1. Of the other tumors, including sarcomas, carcinomas, leukemias, and lymphomas, only three ES of six were HNK-1 positive.

Staining normal tissues with this same antibody provided results consistent
TABLE I

HNK-I: Reactivity with Nonhematopoietic Tumor Lines*

| Cell type          | Embryologic origin | Name      | Results |
|--------------------|--------------------|-----------|---------|
| Neuroblastoma      | Neuroectoderm      | IMR 32    | +       |
|                    |                    | NJB       | +       |
| Medulloblastoma    | Neuroectoderm      | IARC-186  | +       |
| Retinoblastoma     |                    | MS.Rb     | +       |
| Melanoma           |                    | M29       | +       |
|                    |                    | IGR3      | +       |
| Ewing's sarcoma    | Unknown            | IARC-EW1  | +       |
|                    |                    | IARC-EW3  | +       |
|                    |                    | IARC-EW7  | -       |
|                    |                    | IARC-EW11 | +       |
| Fibrosarcoma       | Mesoderm           | HT1080C   | -       |
| Liposarcoma        |                    | SA4       | -       |
| Cervix adenocarcinoma |                | Hela S3   | -       |
| Oral epidermoid carcinoma | Ectoderm | KC         | -       |
| Breast carcinoma   |                    | MCF7      | -       |
|                    |                    | MDA-MB231 | -       |
| Rectum carcinoma   | Endoderm           | HRT18     | -       |
| Colon carcinoma    |                    | HT29      | -       |
| Choriocarcinoma    | Trophoblast        | BeWo      | +       |

* Using indirect fluorescence microscopy.

TABLE II

HNK-I: Reactivity on Paraffin-embedded Tumors*

| Embryologic origin | Number tested | Number positive |
|--------------------|---------------|-----------------|
| Neuroectoderm      | 30            | 24              |
| Mesoderm           | 12            | 0               |
| Ectoderm           | 3             | 0               |
| Germ cell          | 1             | 0               |
| Ewing's sarcoma    | 6             | 3               |

* Using indirect immunoperoxidase technique.

with HNK-1 reacting with a differentiation antigen associated with neuroectodermal tissues. Nerve sheath cells were positive. In the adrenal, the neural crest-derived medulla, where phaeochromocytoma originates, but not the cortex, of mesenchymal origin, was strongly stained (Fig. 1A). In the appendix, enterochromaffin cells were the only positive cells (Fig. 1B). All other tissues tested were negative, including epithelial elements of ectodermal origin (epidermis, sweat, and sebaceous glands, respiratory epithelium of nasal cavity), the endoderm-derived lung and pancreas, connective tissues of mesodermal derivation, kidney, and all testis cells including Leydig, Sertoli, and germ cells.

It appears that the specificity of HNK-1 is not restricted to the lymphoid subset known to carry the Leu-7 antigen, but that HNK-1 also specifically stains a variety of cells with a common derivation from the neuroectoderm. Nevertheless, a few discrepancies within this pattern were noted. One choriocarcinoma cell line was stained by HNK-1. Choriocarcinomas arise form trophoblasts and are
FIGURE 1. Immunoperoxidase staining of neuroectoderm-derived tissues with HNK-1 hybridoma supernatant followed by goat anti-mouse IgM antiserum conjugated to peroxidase and revealed by diaminobenzidine. In the adrenal (A), medullary but not cortical cells are HNK-1⁺. In the appendix (B), only enterochromaffin cells are HNK-1⁺.
therefore likely to express surface antigens that can be detected on various tissues. Also, three of four ES cell lines, as well as three of six ES sections, were detected by HNK-1. The histogenesis of this bone tumor remains a matter of debate (6, 7); differential diagnosis is sometimes difficult as between ES and neuroblastoma. The observation that a percentage of ES were positive with HNK-1 could indicate a heterogeneity of derivation within the group of ES. We here suggest that HNK-1-positive ES could indeed derive from neuroectoderm.

In man, several antigens are shared by neuroectoderm-derived tumors and hematopoietic cells. Common acute lymphoblastic antigen (CALLA), first associated with acute lymphoblastic leukemia (8), has been detected on glioma (9) and melanoma (10) lines and normal kidney (11). Another leukemia antigen, defined by antibody BA2, reacts with some neuroblastomas and melanomas (12). HLA class II antigens on B lymphocytes, monocytes, and macrophage-type cells are also expressed on neuroectodermal tumors such as melanomas (13, 14). UCHT1 and Leu-4 monoclonal antibodies that react with all T cells specifically detect Purkinje cells in the central nervous system (15). By contrast, recently described antibodies directed to neuroectodermal antigens do not stain any blood cell (16, 17). Antibodies 7.51 and 7.60, directed to antigens on melanoma, neuroblastoma, retinoblastoma, and glioma, but not on lymphoma and leukemia lines, have not been reported to react with normal hematopoietic cells (18).

This paper thus constitutes the first report, to our knowledge, of a cell surface antigen present on a discrete peripheral blood lymphocyte subset with NK and K cell activities, and shared by most neuroectoderm-derived tissues. Whether HNK-1 is directed to truly identical antigens on both lineages or rather reacts with the same or cross-reacting epitopes on distinct structures on lymphocytes and neuroectodermal cells remains to be elucidated.

Because the HNK-1-defined neuroectodermal antigen is not destroyed during paraffin inclusion, the HNK-1 antibody could be of considerable clinical interest in histopathology, provided that its tissue distribution, much wider than previously believed, is recognized. Our observation, along with similar ones on other antibodies (9-11), further emphasizes that the increasing number of monoclonal antibodies reported should be carefully screened on hematopoietic and nonhematopoietic tissues before they can be presented as specific for any one cell type.

Summary

The HNK-1 antibody known to define a subpopulation of human lymphocytes with natural killer and killer cell activities was shown to detect a common neuroectodermal antigen. Most tumor lines and paraffin-embedded tumors and normal tissues of neuroectodermal origin were specifically stained by HNK-1. Lines and tissues of other derivations were negative except a trophoblastic tumor line and a percentage of Ewing's sarcomas, whose histogenesis is poorly understood. These data indicate that HNK-1 antibody could be of interest in clinical histopathology but cannot be considered as specific for a lymphocyte subset.

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