The Neoplastic Cell Type in Lymphoreticular Neoplasms of the Northern Pike, *Esox lucius* L.

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SUMMARY

The morphological characteristics of the cells of lymphoreticular tumors in the northern pike (*Esox lucius* L.) are described. The study was based on light microscopy of sections and imprints subjected to selected staining and histochemical procedures and on electron microscopy of thin sections.

Neoplastic cells from tumors in different individuals as well as within a given tumor were remarkably uniform, highly undifferentiated, and atypical in form. They tended to align themselves in contact with reticulin fibers or capillary walls and were interspersed with histiocytes which often contained phagocytosed nuclear remains.

Nuclei of neoplastic cells were round or slightly indented, with finely dispersed chromatin and a single, fairly large nucleolus. The cytoplasm was moderately abundant and smoothly basophilic and pyroninophilic, except for a juxtanuclear region occupied by a Golgi complex and centrioles. The juxtanuclear regions gave a weakly positive periodic acid-Schiff reaction and a strongly positive acid phosphatase reaction. A few fat droplets were generally present in the cytoplasm.

Free ribosomes, partly in polyribosomal aggregates, were abundant, and mitochondria were numerous. The endoplasmic reticulum was not highly developed, as in typical plasma cells, but occasionally showed configurations suggestive of some protein-secretory activity.

Morphologically, the neoplastic cells seem best classified as atypical hemocytoblasts (stem cells) or immunoblasts. Although limited transmission experiments suggest the possibility of viral etiology, virus particles have not been visualized by thin-section electron microscopy.

INTRODUCTION

The prevalence and the histopathology of lymphoreticular neoplasms in northern pike (*E. lucius* L.) in Ireland have been described (4). In most tumor-bearing pike, the lesions are conspicuous as large, external globular tumors on many parts of the body and most often involve the jaws and oral cavity (5). This report further describes the morphological characteristics of the neoplastic cells.

MATERIALS AND METHODS

Cytological examination by light microscopy was based on sections of formalin-fixed spontaneous tumors from 4 freshly killed pike and on air-dried imprints from fresh tumors excised from 2 pike. Sections and imprints of the kidneys of normal pike were also examined. Frozen sections were used for Oil Red O stains, and paraffin-embedded sections were used for hematoxylin and eosin, Giemsa, methyl green-pyronin, Wilder reticulin, and periodic acid-Schiff stains. Air-dried imprints were stained by routine Wright's and peroxidase methods. Formol vapor fixation was applied to imprints before subjecting them to the periodic acid-Schiff reaction (with and without prior diastase treatment) and methyl green-pyronin staining. Formalin-acetone fixation was applied before staining with the acid phosphatase method of Rosales et al. (7).

For electron microscopy, material from spontaneous tumors of 3 pike and induced tumors in the thymus and a gill bar of 1 pike were fixed in 3% cacodylate-buffered glutaraldehyde, postfixed in phosphate-buffered 1% osmium tetroxide, and embedded in Epon. Sections were cut with glass knives on an LKB microtome and stained with lead citrate. Micrographs were taken with a Siemens-Elmiskop I at 60 kV.

RESULTS

Light Microscopy. The neoplastic cells were remarkably uniform in size, shape, and staining properties (Figs. 1 and 2). The majority were in the size range of hematopoietic reticular cells, or hemocytoblasts, and gave virtually no evidence of differentiating along any specific cell line. Necrosis of individual tumor cells, as well as fairly large foci of necrotic tissue, was quite commonplace. Fibrosis did not accompany the necrosis. In some areas, there was a distinct tendency for the neoplastic cells to adhere to and align themselves along the reticulin fibers and capillaries (Fig. 3). Phagocytosis of nuclear debris, presumably derived from dead tumor cells, was much in evidence. The phagocytes had single small, oval nuclei characteristic of normal histiocytes,
and their intracytoplasmic hematoxylinophilic particles were typical of the tingible bodies of Flemming. Binucleate or multinucleate tumor cells were not observed. Mitoses were moderately numerous in some tumors or in limited areas within a given tumor but were rare in other areas.

Nuclei of the atypical neoplastic stem cells generally contained a single, moderately large nucleolus, and the nuclear chromatin was finely dispersed. The nuclear membrane was moderately coarse and only infrequently appeared infolded. No periodic acid-Schiff-positive or other intranuclear inclusions were noted.

The cytoplasm was moderately abundant, bearing about the same ratio to the nucleus as in normal hemocytoblasts. In imprints stained with Wright's method the cytoplasm was smoothly and intensely basophilic except for a fairly prominent, nonstaining area generally eccentrically located at the broadest part of the cytoplasm and often adjacent to a slight nuclear indentation (Fig. 4). The cytoplasmic basophilia in preparations made with Wright's stain corresponded closely with the cytoplasmic basophilia in preparations made with Wright's stain corresponded closely to the pattern of intense cytoplasmic pyroninophilia in the methyl green-pyronin-stained imprints (Fig. 5). Specific granules such as peroxidase granules, Russell bodies, or azurophilic granules were lacking. Imprints subjected to the periodic acid-Schiff reaction, either with or without prior diastase digestion, gave a rather diffuse, weakly positive reaction in the juxtanuclear areas, which were nonstaining in Wright's preparations. The juxtanuclear areas also gave strongly positive reactions for acid phosphatase, and with this method they showed a fine to moderately coarse granular composition (Fig. 6). In the imprints, many of the atypical stem cells from one of the tumors contained 1 to 3 or 4 clear, nonstaining vacuoles in their cytoplasm. These vacuoles corresponded in size, number, and distribution to Oil Red O-positive droplets in the cytoplasm of tumor cells in frozen sections (Fig. 7). In the other tumor studied in imprints, only an occasional tumor cell contained such vacuoles, but when present, they generally occupied most of the cytoplasm. In the 2nd imprinted tumor, the acid phosphatase-positive zones within the cytoplasm were less prominent than in the 1st tumor. With these two exceptions, there were no significant differences among the cells from the 6 tumors examined.

Electron Microscopy. Many features of the neoplastic cells noted under light microscopy were observed in electron micrographs. The neoplastic cells again showed a fairly uniform appearance and had round or slightly indented nuclei with finely dispersed chromatin and a distinct nucleolus. The nucleus had many nuclear pores (Fig. 8); the nucleolus appeared fairly loose in structure. The cytoplasm had numerous free ribosomes, in some cells partly aggregated into polyribosomes (Fig. 9). The endoplasmic reticulum was rather inconspicuous in some cells, but in others there were several long, narrow cisternae arranged concentrically around the nucleus or along the cell membrane (Fig. 10). The multitude of mitochondria was striking. They were fairly large and had tubular cristae; this seems to be a feature common in many species of fish (Figs. 9 and 10). Close to the nucleus, frequently near an indented part, a discrete Golgi complex and 2 centrioles could be observed (Fig. 11);

these apparently corresponding to the juxtanuclear areas which were seen under the light microscope to be nonstaining in Wright's stain preparations. Some cells contained a few multivesicular and dense bodies occasionally with crystalloid inclusions (Fig. 9).

The ultrastructure of cells of the experimentally induced tumors was similar to those of the spontaneous neoplasm with the additional feature that in many of the cells of induced tumors fibrillar areas were seen, often in a juxtanuclear position (Fig. 12). The significance of such areas is not known, although similar structures are described in a variety of animal cells following viral infection (2). Virus particles have not been seen in the sections. However, preliminary transmission experiments have resulted in the reproduction of the neoplasm in 5 out of 6 adult healthy pike, which were given i.p. injections of a homogenous tumor that had been passed through a 0.22-μ Millipore filter (6). This suggests that a viral or mycoplasmal agent may in fact be an etiological factor.

CONCLUSIONS AND DISCUSSION

It seems most appropriate to designate these tumors as a stem cell type of lymphoreticular neoplasm, taking into consideration all the available morphological criteria, the positive signs of marked nuclear and cytoplasmic immaturity, the lack of evidence of differentiation along a specialized blood cell line, and the absence of any evidence for any appreciable phagocytic activity on the part of the neoplastic cells. A search for signs of functional activities of the tumor cells, such as production of abnormal serum proteins, is being undertaken. Should evidence of a specific protein product be obtained, a revision of the classification would perhaps be required. It seems entirely possible that individual examples of these tumors might have functional activity, while others might not. Such a situation exists for the African (Burkitt) lymphomas (1) in man, among which some individual examples have been found to produce γ-globulin in tissue culture (3), while others apparently do not. This illustrates a certain degree of diversity that is rather commonly observed within most morphological groupings of neoplasms. Often it is an exceptional variant within a group of related neoplasms that gives the strongest clue to the origin of the entire group. For this reason, it will be of interest and importance to study as many of the pike tumors as possible, in the finest detail.

In this study, we examined sections and imprints of the kidneys of normal northern pike for the purpose of identifying a cell type acceptable as a normal counterpart of the neoplastic cells. We were not successful in finding a precisely comparable cell, because the most immature cells in the normal hematopoietic tissue in the kidney lacked the prominent juxtanuclear acid phosphatase-positive areas; they also lacked the intracytoplasmic vacuoles so commonly seen in the neoplastic cells. In the absence of reports of thorough hematological studies of this species, it seems that a profitable approach towards more precise identification of the cell of origin would be: (a) to study the hematopoietic tissues of pike that had received antigenic stimuli, as in the study of
Maire F. Mulcahy, Gösta Winqvist, and Clyde J. Dawe

the bluegill by Smith et al. (8); or (b) to study the hematopoietic tissues of ostensibly normal pike from epizootic areas, in hope of encountering a preneoplastic stage of the disease, characterized by a hyperplastic condition of the cell line from which the neoplasm emerges. If it proves possible to transmit the disease consistently with ultrafiltrates, this second type of approach will become much more feasible.

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REFERENCES


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