Immunological and Ultrastructural Characterization of True Histiocytic Lymphoma in the Northern Pike, *Esox lucius* L.¹

James S. Thompson² and Anja A. I. Kostiala

University of Helsinki, Departments of Limnology, Viikki, SF-00710 Helsinki [J. S. T.], and of Bacteriology/Immunology, Haartmaninkatu 3, SF-00290 Helsinki [A. A. I. K.], Finland

Abstract

An epidemic of a malignant neoplasm occurs in northern pike, *Esox lucius* L., from the Åland Islands of Finland. The neoplasm is morphologically similar to other pike hemic tumors reported in other areas of the world. Pike normal tissues showed evolutionary conservation with the mammalian intermediate filament proteins cytokeratin, desmin, vimentin, neurofilament protein, and glial fibrillary acidic protein; tumor cells are positive for vimentin, suggesting that the neoplasm is of mesenchymal origin. Hemic tissue mononuclear cells undergo polyclonal stimulation by the known mammalian T- and B-lymphocyte mitogens phytohemagglutinin P, concanavalin A, tuberculin-purified protein derivative, and lipopolysaccharide W; pike tumor cells are nonreactive. Pike normal hemic tissue mononuclear cells are variously positive for surface and cytoplasmic immunoglobulins, using rabbit anti-pike immunoglobulin antibodies to defined cellular constituents. In recent years, fish antibodies should be developed. It is with this in mind that we undertook these first investigations into the basic immunology and tumor cell ultrastructure of northern pike to derive a basis for proper classification of the neoplasm. Studies of the dynamics of fish populations can also shed light on the effects of disease mortality.

Materials and Methods

Maintenance of pike in aquaria, preparation of media and cell suspensions, harvesting of tissues, and techniques for demonstration of pathlogy, ultrastructure, Slg and Clg, SRBC rosettes, IF, mitogenic proliferation, ANAE, lysozyme, AAT, as well as age of pike were previously reported (1-9).

Results

Tumors. The tumors were usually located on the posterior half to two-thirds of the trunk and often regressed during summer. By light microscopy they arose in the dermis; the adjacent muscle tissue was invaded and often destroyed by undifferentiated mononuclear cells (1, 2). No metastases were noted, although rare cases of a leukemia-like condition were observed; in one, Ficoll-Isopaque-separated mononuclear cells from head kidney and blood displayed counts of 75 and 180 million cells/ml, respectively, while typical counts for these two respective organs varied around 10 to 20 and 5 to 7 million cells/ml (1).

Cell Culture. At +20°C all cultures died by 30 days. In two cases after 4-mo growth at +4°C, a stable population of apparently malignant cells at 1 to 2 million cells/ml was observed (1, 2).

EM. The tumor consisted of masses of closely apposed, variably sized mononuclear cells with an absence of epithelial and connective tissue features and presence of occasional primary lysosomes and perinuclear IF strands and numerous lipid droplets (1, 4, 9). All results with head kidney and tumor, using anti-AAT and anti-lysozyme antibodies, were negative, as was an EM search for viral particles in cultured tumor cells (1, 2, 4).

IF. Typical epithelial, muscle, connective, glial, and neural components of various tissues exhibited keratin, desmin, vimentin, GFAP, and NFP reactivities, respectively (1, 6). Tumor cells were negative for keratin, NFP, GFAP, and desmin, but positive for vimentin. In sodium dodecyl sulfate-polyacrylamide gel electrophoresis and immunoblotting of tumor, a positive reaction was found with polyclonal anti-vimentin antibodies compatible with a vimentin-positive molecule of 58,000 daltons.

Mitogens. The basic metabolic rate of the tumor was much higher than that of hematic tissues and greater in FCS than in APP; head kidney was higher in APP (1, 3). Maximal stimulation in all hematic tissues was usual with 2.5 μg/ml of phytohemagglutinin P, 12.5 μg/ml of concanavalin A, 100 μg/ml of PPD in APP, and 33% of each concentration of PPD in FCS; responses to lipopolysaccharide W were less clear-cut (1, 3).

¹ Presented at the "XIVth Symposium of the International Association for Comparative Research on Leukemia and Related Diseases," October 8-12, 1989, Vail, CO.
² To whom requests for reprints should be addressed.

The abbreviations used are: SRBC, sheep red blood cells; AAT, α-antitrypsin; ANAE, α-naphthyl acetate; APP, autologous pike plasma; BNAE, β-naphthyl acetate; Clg, cytoplasmic immunoglobulin; EM, electron microscopy; FCS, fetal calf serum; GFAP, glial fibrillary acidic protein; IF, intermediate filament(s); IgM, immunoglobulin M; NFP, neurofilament protein; PPD, tuberculin-purified protein derivative; Slg, surface immunoglobulin; THL, true histiocytic lymphoma; WCT-3, monoclonal anti-carp IgM antibodies.
Responses of 4- and 5-day cultures were similar as were those of normal and tumorous pike. The tumor cell response in FCS and APP was much lower than that of tumor pike lymphocytes, which was as good as that from normal pike. Optimal proliferation indices of tumor and normal pike lymphocytes were similar in FCS; differences were noted in APP (1, 3).

**Slg and Clg.** The polyclonal anti-winter flounder IgM antibodies and monoclonal anti-rainbow trout IgM antibodies were negative in Ouchterlony immunodiffusion or indirect immunofluorescence (1, 7). In CIg assays an optimal dilution of 1:4000 was optimal for Slg and Clg. Hemic tissue mononuclear cells showed 90 to 95% reactivity, whereas the tumor was negative (1, 7).

**Polyclonal Anti-Northern Pike IgM Antibodies.** These antibodies reacted strongly in Ouchterlony immunodiffusion against normal and tumor pike sera. In studies on Slg, hemic tissue but not tumor mononuclear cells were agglutinated. A dilution of 1:4000 was optimal for Slg and Clg. Hemic tissue mononuclear cells showed 90 to 95% reactivity, whereas the tumor was negative (1, 7).

**WCT-3.** These antibodies cross-reacted with pike IgM by indirect immunofluorescence. An optimal dilution in Slg assays of 1:1000 stained 40 to 45% of hemic tissue mononuclear cells. In Clg assays an optimal dilution of 1:40 gave 33 to 56% reactivities in hemic tissues; the tumor was negative for Slg and Clg (1, 7).

**Rosettes.** The proportion of mononuclear cells forming SRBC rosettes was low in all tissues: <5% in hemic tissues and 0 to 1% in tumor (1, 5).

**ANAE.** Hemic tissues demonstrated 3 to 57% and tumor 86% diffuse NaF-sensitive esterase reactivities, respectively. Tumor demonstrated 2% NaF-resistant dot-like ANAE activity; hemic tissues were virtually negative. Weaker diffuse reactions were noted when BNAE was used (1, 7).

**Populations.** Young males and females were rare among tumorous versus normal pike. Tumor was not observed in pike of either sex under 3 yr of age, but affected primarily males 5 to 6 yr of age. The sex ratio (male/female) was 2.5/1 in tumorous pike and 1.1/1 in normal pike; mean lengths-at-age were similar (1, 8).

**Discussion**

Our results show that a neoplasm of Åland pike resembles esocid lymphomas from other areas of the world (1, 2). In this study, we investigated the presence of IF in pike normal and tumorous tissues to determine the tissue of origin of the neoplasm (1, 6). Our results revealed a tissue type-specific expression of IF as seen in other species and lend support for the idea of evolutionary conservation of IF (1, 6). The lack of cytokeratin, desmin, GFAP, and NFP and presence of vimentin activities in tumor suggest that it is a mesenchymal neoplasm and is probably hemic cell derived (1, 6).

The presence of mitogen-responsive lymphocytes and lack of anatomical compartmentalization of lymphocytes in pike were demonstrated, agreeing with studies in other species (1, 3). Thymocytes, however, could not be tested due to the involution of thymus in the present adult fish. Our data agree with earlier findings that mitogenesis is dependent on the dose of mitogens and species source of serum (1, 3). Mitogen reactivity of tumorous pike hemic tissue mononuclear cells was as good in APP as in FCS and as good or better as that in normal pike, possibly due to the presence of nonmetastatic tumors, and suggesting that tumorous pike lymphocytes are both normal (1, 3) and also may not constitute the normal cellular counterpart of the tumor cell.

Our data in pike using polyclonal anti-northern pike IgM antibodies also show that practically all hemic tissue mononuclear cells were positive in Slg and Clg assays, agreeing with findings in other species that most fish lymphocytes, including thymocytes, are membrane fluorescent with polyclonal antibodies to IgM (1, 7). Our results with WCT-3 also agree with other findings and demonstrate cross-reactivity between carp and pike immunoglobulin; however, the WCT-3 may only react with specific isotypes of pike immunoglobulin which may not be present on all cells (1, 7).

The diffuse NaF-sensitive ANAE staining pattern in the tumor cells was reminiscent of that noted in human monocytes and their progeny (1, 7). The demonstration of nonspecific esterase activity with both ANAE and BNAE rules out the presence of lipases (1, 7). The low numbers of SRBC rosettes noted in lymphocytes and tumor cells and of dot-like NaF-resistant ANAE patterns reminiscent of human and anuran T-lymphocytes suggest that these two assays cannot be used as T-cell markers in the present pike (1, 5, 7).

The percentages of mitogenically reactive lymphocytes, SRBC rosettes, or dot-like ANAE may have been influenced by the low in vivo temperatures at which pike were maintained in aquaria, or to crowding, trauma, fright responses, behavioral changes, or seasonal or circadian rhythms (1). The virtual lack of mitogen proliferation in tumor was most likely due to loss of reactivity during malignant transformation or the presence of non-T- or -B-lymphocytes or nonlymphoid cells (1, 3). The lack of demonstrable Slg and Clg in tumor cells virtually rules out a B-cell lymphoma or immunoglobulin-producing plasmacytoma, but not that an undetectable immunoglobulin-like molecule may be present on the surfaces of pike T-cells (1, 7). The tumor cells are morphologically similar to large cells reminiscent of head kidney monocytes and expressed an ANAE pattern typical of human monocytes. However, a T-lymphocytic origin of the tumor cells cannot be totally excluded because of a lack of suitable T-cell markers in Esox. In fact, polyclonal anti-immunoglobulin antibodies may react with peripheral T-cells, presumably due to carbohydrate cross-reactivity; thus, the tumor cells may be aberrant T-cells which do not bear such determinants (1, 7). The results of lysozyme and AAT assays are inconclusive in the present pike, possibly due to inopportune time for demonstration, absence or antigenic differences as noted in other species, or to a fickle appearance as often noted in human histiocytic disorders (1, 4).

Our results obviate the need for marker profiles of hemic cells in Esox and other fish. While the T-cell origin of the neoplasm is possible, the absence of desmosomes, tight junctions, large, uniformly electron-dense cytoplasmic granules, and rough endoplasmic reticulum and presence of lysosomes and multitudes of lipid droplets are suggestive of a neoplasm of histiocytic derivation versus an epithelial, granulocytic, or plasmacytic tumor. Sinusoidal infiltration of tumor cells to hemic tissues or liver was never observed, and thus the neoplasm may be a piscine analogue of THL originating in a skin tissue histiocyte (1, 4).

That young pike and females are less susceptible to THL than older males suggests that the onset of maturity may be a prerequisite for development of THL (1, 8). The difference in sex ratio between tumorous and normal pike may be due to physiological differences between sexes and/or to varying contact rates during spawning (1, 8). No true seasonal periodicity was observed. The greater survival of females suggests that males contract the disease more often (1, 8). THL does not presently appear to be a major factor affecting Åland pike populations. The high prevalence (up to 10% or more) suggests
that the mortality is fairly low; the rarity of the leukemia-like condition suggests that it could occur as a rapid but seldom noted terminal event or as a rare sequel to THL, which is contrary to the high incidence of leukemia in some pike populations, e.g., 11% in pike from the polluted Fox River in Illinois (1, 8).

Chemical pollution is clearly not the cause of THL, since the tumor occurs in pike living in very clean waters (1, 2). Although no evidence currently exists, a virus is suspected on the basis of pathology and occurrence of THL and demonstration of retrovirus and reverse transcriptase in North American pike lymphoma (1, 2, 8). The solitary nature of the pike, except at spawning time, and the appearance of tumor mainly on sexually mature fish suggests that spawning would be ideal for transfer of the presumptive virus between individuals, especially if sperm is the transporting agent; furthermore, the shallow water in the spawning areas would tend to concentrate the virus (1, 8). Parasite vectors are not likely involved, although the high salinities present in the Åland Islands may introduce chronic stress factors which could contribute to a general lack of resistance to disease (1, 2, 8).

References

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*Cancer Res* 1990;50:5668s-5670s.

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