

Absence of Alkaline Phosphatase in Rat Thymic Lymphoma Induced by Murine Leukemia Virus¹

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SUMMARY

Alkaline phosphatase activity has been determined by a histochemical assay on thymic lymphomas induced by murine leukemia virus in C57BL mice and W/Fu rats. Approximately 60% of the former and none of the latter tumors showed this activity in the plasma membrane of the tumor cells. The possibility that the enzyme "activation" represents expression of a derepressed cell gene rather than of a viral gene is discussed.

INTRODUCTION

An association of APase² activity with thymic lymphoma cell membranes has been thoroughly documented in recent years (3, 8, 11, 13). The enzyme is present in both "spontaneous" thymic tumors and those induced by a variety of physical, chemical, and viral agents (3, 8, 9, 11). A recent study from this laboratory comparing the biochemical characteristics of APases of several normal organs with those of thymic lymphomas of C57BL mice suggests that the appearance of the enzyme may result from derepression of a cellular gene rather than the expression of a viral gene (9). The tumors used in that study were induced either by a chemical carcinogen or a virus extracted originally from a 6-mercaptopurine-induced tumor (2) and serially passaged in C57BL mice. Recently, we have used neonatal injection of this virus to induce tumors in W/Fu rats. These thymic tumors, unlike those induced in mice, are consistently APase negative, a finding which is in accord with the hypothesis that the enzyme is coded for by a cellular rather than a viral gene.

MATERIALS AND METHODS

The virus passage was maintained by intrathymic injection into 3- to 20-day-old C57BL mice of approximately 0.02 ml of a centrifuged and filtered (0.45- μ porosity) extract of thymic tumor tissue (20% homogenate in phosphate-buffered saline). A similar preparation was used to induce tumors in newborn

W/Fu rats by intrathymic injection. All treated animals received 100 R whole-body X-irradiation 1 week postinjection. The tumors induced in these rats were morphologically identical to those described by Okano *et al.* (12).

Histochemical assay of APase on frozen sections of tumor was performed by the method of Mannheimer and Seligman (10) as modified by Lagerlöf and Kaplan (8). The appearance of the histochemical preparations for APase was similar to that described by Lagerlöf and Kaplan (8). The reaction occurred on the plasma membrane of the tumor cells and was quite variable in intensity. In tumors demonstrating no APase activity, the appearance of the cells was no different from cells incubated in buffer without substrate and dye being present.

RESULTS AND DISCUSSION

The incidence of tumors in mice obtained with the serially passaged virus is given in Table 1. It is probable from the consistently high tumor incidence in mouse passage Generations 2 through 6 that a high level of virus is being maintained in this passage. Susceptibility of the W/Fu strain of rats to the murine leukemia viruses has previously been reported from several laboratories (4, 5, 7), and the 100% tumor incidence

Table 1
Incidence, mean latent period, and APase activity of virus-induced thymic lymphomas in C57BL mice

Mice received intrathymic neonatal injections of a filtered centrifugate of tumor homogenate (0.02 ml of supernatant from a 20% homogenate in phosphate-buffered saline) followed by 100 R whole-body X-irradiation 1 week later. The original tumor arose in a mouse treated neonatally with 6-mercaptopurine (2).

Passage generation	Tumor incidence	Mean latent period (mo.)	Intensity of histochemical stain for APase ^a					No. positive/no. tested
			0	1+	2+	3+	4+	
1	11/23 (48%)	8.5	Not tested					
2	26/29 (90%)	4.0	Not tested					
3	114/122 (93%)	4.5	11	3	2	6	0	11/22
4	48/54 (89%)	4.5	8	9	2	6	1	18/26
5	28/29 (97%)	5.0	6	3	1	1	0	5/11
6	50/50 (100%)	4.2	9	5	4	6	1	16/25
Totals			34	20	9	19	2	50/84

^a The intensity of staining was assigned an arbitrary rating of 0 to 4+.

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² The abbreviation used is: APase, alkaline phosphatase.
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found in these experiments (Table 2) is similar to that reported by Geering *et al.* (4).

Table 1 also shows the APase activity of virus-induced thymic lymphomas in C57BL mice for the 3rd through 6th passage generations. It is apparent that neither the frequency nor the intensity of the membrane-associated reaction in tumor cells is correlated with the length of time that the virus has been passaged. The frequency of positive tumors is, however, lower than that found in chemically induced lymphomas (9) while being similar to that seen in X-ray-induced tumors (8).

Table 2

APase activity in thymic lymphomas of rats given neonatal injections of murine leukemia virus

Newborn W/Fu rats were given intrathymic injections of extract of mouse tumor as prepared for mouse injection (see Table 1).

Source of virus	No. of tumors/ no. injected ^a	Mean latent period (mo.)	APase activity (No. positive/ no. tested)
7th mouse passage generation	10/10	4.0	0/10
8th mouse passage generation	7/7	4.1	0/7

^a Number surviving to weaning.

The absence of APase activity in the tumors induced by this virus in rats is strikingly demonstrated by the data of Table 2. Not 1 of the 17 tumors tested had any activity other than that present in cells lining blood vessels or other nontumor cells. Three control W/Fu thymuses were examined for APase activity, and again none was found.

That many surface changes occur in cells as a result of neoplastic transformation involving different causative agents (viruses, chemicals, etc.) has now been amply demonstrated (reviewed in Ref. 6). These include the induction of tumor-specific transplantation antigens, new surface antigens detected by cytotoxic or fluorescent antibody methods, and derepression of a cell-coded gene, that for the thymus leukemia antigen (1), a phenomenon which, like the activation of APase, also occurs in thymic lymphomas. Whether or not the appearance of APase activity in thymic lymphoma cells is intimately related to the transformation process remains unknown. That it may be merely an incidental occurrence, not necessarily associated with such transformation, is supported by the present data. In addition, the close relationship of the enzyme in tumor to that found in normal spleen and embryo thymus of the mouse (9) implicates a cell-coded rather than a virus-coded gene in its specification. It may then be postulated that in the complex virus-host cell relationship leading to malignant transformation in the mouse thymus, fortuitous

activation of the APase gene occurs. In the rat, on the other hand, such an activation does not take place. Alternative interpretations of these data are possible, of course, and cannot be ruled out at the present time. It is possible that APase is essential for thymic lymphoid neoplasia in the mouse but not in the rat. However, the finding that approximately 40% of these tumors lack APase activity (Table 1) mitigates against this hypothesis. Another possible interpretation is that a viral APase gene is expressed during transformation in the mouse cell but not in the rat cell. We have no direct evidence for or against this hypothesis at present.

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