Variation in Histology and Growth Characteristics of Transplantable Marek’s Disease Lymphomas¹

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ABSTRACT

A comparative study was made of the histology and growth characteristics of three different Marek’s disease virus-induced transplantable lymphomas. These lymphomas were developed previously in related inbred chicken lines G-B1 and G-B2. The UG1 lymphoma was developed by serial i.m. passage in G-B1 chickens, and the UG2 and UG4 lymphomas were developed similarly in G-B2 chickens. While all three lymphomas grow progressively and cause rapid death in syngeneic hosts, differences in pathogenicity exist. For equivalent passage levels, the mean time to death of syngeneic chickens inoculated with 10⁶ lymphoma cells was 10.8, 12.8, and 16.3 days postinoculation for UG1, UG2, and UG4, respectively. Histological features examined at the light microscopic level included tumor necrosis, muscle invasion, mitotic activity, and presence of heterophils (comparable to mammalian neutrophils). The UG2 lymphoma was characterized by a high degree of necrosis during all stages of growth. This feature was least pronounced in UG4 lymphomas, which generally grow to a much larger size than UG1 or UG2 lymphomas. Vascular invasion was a feature of UG2 lymphoma cells in skeletal muscle and may account for the necrosis. The UG2 cells, which are somewhat larger than UG4 cells, occasionally contained cytoplasmic vacuoles. While the number of heterophils was highest in early stages of UG2 tumors, the role of these cells is unclear. The findings provide the basis for utilizing the transplantable lymphomas as a model to study mechanisms underlying variable pathogenicity of malignant tumors.

INTRODUCTION

MDV is an oncogenic herpesvirus capable of causing high mortality in chickens as a result of lymphoma formation in visceral organs (10). Primary MD lymphomas frequently occur in the kidneys, gonads, liver, and spleen of infected chickens. By serial in vivo passage of MD tumor cells in syngeneic hosts or immature allogeneic hosts, a number of transplantable MD lymphomas have been developed (14). Although many MD lymphomas appear to have very limited transplantability in syngeneic hosts (2), some acquire a highly pathogenic nature during serial transfer, an indication that selection for clones of cells with the potential for immune escape and rapid metastatic spread has occurred. During development of 3 different transplantable MD lymphomas in 2 inbred lines of chickens, we observed distinctive characteristics in the rate of tumor growth and latency between tumor cell inoculation and host death. In the present study, a light microscopic examination of these 3 lymphomas was made to determine the relationship between histological features and growth characteristics of the tumors. The histology of metastatic lesions found in visceral organs of birds bearing transplantable lymphomas in the pectoral muscle was also studied.

MATERIALS AND METHODS

Animals. Chickens from related inbred lines G-B1 and G-B2 and F₁ offspring from a cross of the 2 lines were used. These 2 lines differ for major histocompatibility complex (MHC) genes. MHC genotypes are B¹/B¹, B¹/B², and B²/B² for lines G-B1 and G-B2, respectively. Each line is over 95% inbred and nearly all within-line skin transplants are permanently accepted. Graft rejection that does occur is chronic and begins after 30 days postgrafting. Chickens from both lines have a normal degree of immune competency. All birds were maintained in isolation chambers with filtered air under positive pressure.

Transplantable Lymphomas. The MD transplantable lymphomas used in this study, which are designated in accordance with a standard nomenclature system (14), included MDCT-UG1, developed in line G-B1 birds, and MDCT-UG2 and MDCT-UG4, developed in line G-B2 birds. These lymphomas, henceforth abbreviated to UG1, UG2, and UG4, were derived from primary T-cell tumors induced by the Conn-B strain of MDV. Passage procedures used in developing UG1 and UG2 and identification of their T-cell origin have been described previously (2). The UG4 lymphoma also was developed in this laboratory in the same manner. These lymphomas are histocompatible for MHC antigens as well as most minor transplantation antigens present in the line of origin.

Progressively growing transplantable lymphomas were removed aseptically and processed into cell suspensions described previously (13). To induce the lymphomas used for histological analyses, approximately 2 x 10⁶ cells were inoculated i.m. into the pectoral muscle of 3- to 4-week-old recipients. Differences in pathogenicity of the 3 transplantable lymphomas were compared by determining the mean time to death of syngeneic hosts after inoculation with the same numbers of cells from equivalent passage levels.

Histological Evaluations. The histology of the transplantable lymphomas was studied in 5 separate experiments. In Experiment 1, UG1 (passage 27) and UG2 (passage 31) lymphomas were obtained from syngeneic parental line hosts. In Experiment 2, G-B1 x G-B2 F₁ hosts were used for both UG1 (passage 29) and UG2 (passage 33). The UG4 lymphomas (passage 35) from syngeneic G-B2 birds were examined in Experiment 3. Direct comparisons of UG2 and UG4 lymphomas from syngeneic hosts were made in Experiments 4 (passage 42 of UG2 and passage 44 of UG4) and Experiment 5 (passage 90 of UG2 and passage 83 of UG4).

Evaluations were made of each transplantable MD tumor at 1- to 2-day intervals during development, beginning on Day 4 or 5 PI. For each experiment, 2 to 4 lymphomas of the same tumor line were harvested on the same day. Lymphomas obtained for histological examination were fixed in 10% buffered formalin. For Experiments 1, 2, and 3, the tissues were embedded in paraffin. For Experiments 4 and 5 they were embedded in glycol methacrylate (8). For the latter 2 experiments, liver, spleen, and kidney tissues were examined in addition to the breast muscle lymphomas of each animal. Tissue sections 2 μm thick were cut,

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³ The abbreviations used are: MDV, Marek’s disease virus; MD, Marek’s disease; MHC, major histocompatibility complex; PI, postinoculation.

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mounted on glass slides, and stained with hematoxylin and eosin. The sections were coded and scored for 4 criteria: degree of necrosis; muscle invasion; mitotic activity; and infiltration of heterophils (polymorphonuclear granulocytes comparable to mammalian neutrophils). Subjective scores used for the 4 criteria ranged from 0 to 3, with 0 representing absence of the characteristic and 1, 2, and 3 representing low or mild, intermediate or moderate, and high or severe, respectively. In initial experiments, mitotic cell counts were made to provide validity to the subjective scores for mitotic activity.

Statistical Analyses. Lesion data were transformed by taking the square root of the sum of the lesion score plus 0.5. Mean times to death were calculated by the Student's t-test. The findings for the 4 histological criteria evaluated are summarized in Table 2. Some distinctive histological features of the lymphomas appeared to be more pronounced during early or late stages of development; therefore, the data obtained for lymphomas up to Day 7 and after Day 7 PI are presented separately.

RESULTS

The mean times to death after i.m. inoculation of cells from the 3 transplantable lymphomas are shown in Table 1. For comparable passage levels, UG1 was clearly the most pathogenic, and UG4 was the least pathogenic. However, all 3 lymphomas caused 100% mortality in syngeneic hosts that were inoculated with $10^6$ cells.

The histological lesion scores assigned to UG1 and UG2 lymphomas obtained from syngeneic parental line birds (Experiment 1) were very similar to the scores assigned when these lymphomas were taken from F$_2$ hosts (Experiment 2). This finding indicated that the histological differences in the tumors were a property of the tumor cell lines and not due to host-cell response differences between G-B1 and G-B2 birds. With respect to lesion score for each transplantable lymphoma, the results were found to be essentially the same for all 5 experiments. Therefore, the data were pooled for statistical analysis. The findings for the 4 histological criteria evaluated are summarized in Table 2. Some distinctive histological features of the lymphomas appeared to be more pronounced during early or late stages of development; therefore, the data obtained for lymphomas up to Day 7 and after Day 7 PI are presented separately.

A unique characteristic of the UG2 lymphoma was the high degree of necrosis that occurred in both early and late stages of growth (Table 2). The UG4 lymphomas, which grew to a much smaller size than UG1 or UG2 lymphomas before host death occurred, generally had the least amount of necrosis. UG1 tumors had histological features similar to those of UG2 tumors in skeletal muscle. These tumors were composed of large cells with pleomorphic nuclei and indistinct cell borders (Figs. 1 and 2). Some cells within the UG2 tumor contained cytoplasmic vacuoles (Figs. 2 and 3). Cells of the UG4 lymphoma (Figs. 4, 5, and 6) were smaller with more distinct cell borders.

The mean score for muscle invasion was lowest for UG4 lymphomas that were obtained prior to Day 8 PI. This was reflected by the clinical observation of a 1- or 2-day-longer latent period between time of inoculation and appearance of a palpable tumor for UG4 as compared to UG1 and UG2. With the exception of early stage UG4 lymphomas, which tended to be multinodular (Fig. 6), muscle invasion scores were generally high, reflecting the diffuse infiltration of muscle by tumor cells (Fig. 3).

The number of heterophils (Fig. 2) found in the lymphomas harvested prior to Day 8 PI seemed to be correlated with the degree of necrosis. This relationship did not exist for lymphomas harvested later (Table 2).

Vascular invasion was a feature of UG2 lymphomas in skeletal muscle (Fig. 3), but metastatic tumors in kidney and liver were equally prevalent in both UG2- and UG4-inoculated chickens. The metastatic lymphomas generally resembled the skeletal lymphomas except that necrosis was not a striking feature in the UG2 metastatic sites. Heterophils were present in UG2 and UG4 metastatic lymphomas.

DISCUSSION

The results of this study showed that transplantable MD lymphomas, derived from primary tumors initiated by the same strain of MDV, have distinctive growth and histological features. This variability extended to MD lymphomas developed in the same inbred line of chickens (i.e., UG2 and UG4). That such variability would exist between the transplantable lymphomas is not surprising. Considerable evidence exists for genetic instability of tumor cells, and neoplastic progression is probably the result of sequential selection of variant subpopulations of transformed cells (9). Phenotypic diversity can also occur in metastatic tumors which originate from a single transformed cell (5). In a previous study with chickens, variant cell populations with the potential for organ-specific metastasis were selectively derived from an in vitro-cultured MD lymphoblastoid cell line (12).

The essential findings of this study provide the basis for utilizing transplantable MD lymphomas as a model to study mechanisms underlying variable pathogenicity of malignant tumors. Since lymphomas from a number of passage levels of each line have been cryopreserved, it will also be possible to perform comparative studies relating biological diversity with tumor passage level. For example, by using cryopreserved UG4
cells we found that the latent period between tumor cell inoculation and host death decreased by about 3 days between passages 33 and 71. However, histological features measured in the present study (Experiments 4 and 5) did not differ significantly between early and late passages. These observations suggest that an evaluation of other cellular changes that accompany increased virulence may be worth pursuing.

The reason for the apparent marked differences in cell types that predominate in UG2 and UG4 lymphomas is not clear, but additional studies utilizing enzyme histochemistry and electron microscopic techniques may help explain these differences. The presence of heterophils in the tumors may be due to lymphokines released as a result of host-tumor interactions and/or as a reaction to cell necrosis. An interesting finding was the high degree of necrosis uniquely associated with the UG2 lymphoma. Because the necrosis occurs as early as 3 days PI of UG2 cells, this lesion is not considered a consequence of host reactivity to minor histocompatibility antigens. The UG2 lymphomas rarely grow to the same size as UG1 and UG4 lymphomas, probably because of the high rate of tumor cell death. It is possible that this may be associated with vascular invasion by tumor cells that occurs when this lymphoma grows in skeletal muscle, which could limit the blood supply required by proliferating lymphoma cells. A related observation with hepatocarcinomas in guinea pigs suggested that one tumor cell line underwent regression due in part to ischemic necrosis secondary to widespread microvascular injury (3).

Chick kidney cells infected with the GA-5 strain of MDV, when inoculated into the pectoral muscle, can cause late-occurring lymphomas (4) which are highly necrotic. However, it seems unlikely that the necrosis in UG2 lymphomas is due to a cytolytic infection by MDV associated with the tumor cells. In vitro-assays (2) for virus in the UG2 lymphoma cells have indicated that few, if any, productively infected cells are present in the tumor cell population. The possibility that UG2 tumors may elicit the production of a necrotizing factor similar to an endotoxin-induced tumor necrosis factor (1) cannot be ruled out at this time.

Differences in the size attained by the 3 transplantable MD lymphomas as well as degree of necrosis may be due to differences in the production and release of a tumor angiogenesis factor (7) or an angiogenesis inhibitor (8). Since the growth of solid tumors is dependent upon their releasing a soluble factor that elicits capillary proliferation, studies designed to quantitate angiogenic properties of the lymphomas may indicate why they have different growth and histological characteristics.

Recent findings from a separate study are relevant to the differences in pathogenicity observed between UG1 and UG2 lymphomas. Effective protection against the lymphomas in syngeneic hosts was obtained by immunization with in vitro-cultured lymphoblastoid cells derived from MD lymphomas. However, the immunity to UG2 lymphomas was significantly more pronounced than immunity to UG1 lymphomas (11). Further comparative studies with the transplantable lymphomas may provide new information regarding the control of tumor cell proliferation.

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