Malignant Lymphomas Arising in Peyer's Patches and Other Organs of Untreated NZO/B1 Mice

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SUMMARY

The pathology of all types of lymphoreticular cancers in untreated NZO/B1 mice of the original colonies is presented. Overall, there were 37/451 (8.2%) animals with malignantlymphomas, 50% of the tumors primary in Peyer's patches of the intestine. Peyer's patch lymphomas spread to other abdominal lymphatic organs but did not involve the liver or extend beyond the abdomen, as did cytologically similar tumors primary in other sites. Cytological classification of the primary lymphomas showed 59% overall were of mixed cell type, 18% histiocytic type, and 10% lymphocytic type. Of the Peyer's patch lymphomas, 78% were of mixed cell type and the remainder were histiocytic. The sex incidence of lymphomas was equal, but lymphomas of lymphocytic type were found only in females and occurred late in life. The anatomic pattern of lymphoma metastasis resembled that for other types of cancer with direct extension, regional, and distant metastasis; there were only 2 cases with leukemia-like disease. The possible significance of Peyer's patch lymphomas for etiological studies is noted.

INTRODUCTION

This paper reports on malignant lymphomas of all types found in the NZO/B1 strain and particularly deals with malignant lymphomas arising in Peyer's patches of the intestine. Peyer's patch tumors are rare in experimental animals, and the NZO/B1 strain of mice is unusual in showing, without treatment, a considerable incidence of this form of primary lymphoreticular cancer. While the sporadic observation of primary malignant lymphomas of Peyer's patches has been mentioned in the past (9, 10, 14), the microscopic anatomy of these tumors has not been previously described in detail. Much of the work in this long-term investigation of the importance of inheritance in oncogenesis was initiated by the late Dr. Franz Bielschowsky, and some of his unpublished data are included in this paper.

MATERIALS AND METHODS

This report is based on a histopathological study of 451 untreated NZO/B1 mice, including 40 breeder females from which 1 to 3 litters had been taken, 217 virgin females, and 194 males. The origin, natural history, and some of the metabolic abnormalities characteristic of the NZO strain have been described in previous papers (1, 2). The animals were housed in zinc-plated cages 23 x 15 x 13 cm, in rooms thermostatically controlled at 70 ± 2°F; they were given tap water to drink and fed ad libitum with the diet already described (1). The mice studied include animals that were taken from the pedigreed breeding nucleus at intervals during the years 1953 to 1967, from generations F18 up to F76, together with some groups of animals which had been set aside as untreated controls for various carcinogenesis and biochemical experiments. Most animals were killed when moribund, but some were killed at arbitrary times for histological comparisons; only a few were found dead.

Full autopsies were performed, and tissues were fixed in Zenker-formol or formol-0.9% NaCl solution for histological examination; sections were stained with hematoxylin and eosin, periodic acid-Schiff, methyl green pyronin, Laidlaw's reticulin, and other standard methods when appropriate.

In classifying the lymphoreticular cancers, the following definitions were used (12): the term MCT for a pleomorphic tumor in which both histiocytes and lymphocytic elements showed cellular and nuclear atypia and were represented without appreciable preponderence of either cell type and the term HCT for those tumors in which histiocytes predominated, with or without some lymphocytic elements.

A scattering of plasma cells was often seen in malignant lymphomas, particularly those of the mixed cell type, and these tumors closely resembled the so-called reticulum cell sarcoma type B of Dunn (5, 6) or the Hodgkin's-like tumor that has been described in SJL/J mice (6, 9). HCT corresponded to the reticulum cell sarcoma type A of Dunn (5).

RESULTS

Histopathological Features of the Malignant Lymphoreticular Cancers. A total of 37 mice were found to

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have 39 primary lymphomas (Table 1), 2 of the males each having 2 independent primary tumors of Peyer's patches. Twenty-seven females (10.1%) and 10 males (5.2%) were affected, but the preponderence of lymphomas in females was of only borderline statistical significance (0.10 < p < 0.05).

Of the total 39 malignant lymphomas, 23 (59%) were classified as MCT (Fig. 10) occurring at ages 381 to 757 days, 8 (21%) were HCT (Fig. 6; 479 to 712 days), and 4 (10%) were of lymphocytic type (Figs. 9 and 11; 412 to 754 days). One female mouse had an unusual mast cell tumor of the thymus (546 days). There were 2 cases of disseminated leukemia-like disease, 1 of lymphocytic type (712 days), and another unclassifiable (564 days). Lymphocytic lymphomas were found only in females, but this was not statistically significant (p = 0.2).

The overall distribution of the primary lymphomas which could be localized according to anatomic site was: Peyer's patch, 20 (51%); abdominal lymph node, 10 (25%); spleen, 3 (8%); thymus, 3 (8%); and peripheral lymph node, 1 (2.5%).

**Peyer's Patch Lymphomas.** The primary lymphomas of Peyer's patches were solitary in 15 of the 18 mice affected, and they constituted one-half of all malignant lymphomas in both sexes of the NZO strain. The Peyer's patch lesions ranged in size from a barely perceptible enlargement (Fig. 2), up to large tumor masses almost 1 cm in diameter, and they differed in their growth pattern. Some grew toward the lumen (Fig. 4), occasionally with ulceration of the overlying epithelium; in other cases, the tumor grew outwardly, usually on the antimesenteric surface, with irregular expansion of the gut wall (Fig. 5). In the latter cases, circumferential expansion between tissue planes was observed, and the mucosa of the gut lumen retained its normal folded pattern (Fig. 3). The lumenal pattern of growth was more frequent. Some of the larger tumors produced significant narrowing of the intestinal lumen (Fig. 4) so that partial obstruction must have occurred, although no direct observation of functional obstruction was made. The tumors grossly appeared as opaque whitish masses both externally and on section. In 2 cases, the tumors were so large that the Peyer's patch was completely obliterated.

Microscopically, the Peyer's patch lymphomas occupied the mucosa and submucosa, and some of them extended in varying depth into the muscular layers (Fig. 1), sometimes as far as the subserosa. The majority of the tumors were separated from the lumen of the gut only by a single layer of tall columnar cells, and in some instances normal or hyperplastic glands and crypts were incorporated in the tumor mass (Fig. 5). Lymphocytes of relatively normal appearance were often seen at the periphery of the tumor, and plasma cells sometimes occurred in small numbers. However, Russell's bodies were not demonstrable with the periodic acid-Schiff stain in the plasma cells associated with these tumors. Most of the cancers were of pleomorphic cytology, and the cells that were most atypical could be classified as histiocytes or reticulum cells (Fig. 6). These cells had large nucleoli which were often acidophilic, distinct nuclear membranes, and usually there was an optically clear zone between the nucleoli and the nuclear membranes. Frequently, the nucleolus was not completely surrounded by this optically clear zone but was in 1 part of its circumference contiguous with the nuclear membrane. Occasionally, the cells were binucleate and resembled Reed-Sternberg cells (Figs. 7, 8, and 10). The abundance of lymphocytes varied, and many of those present had atypical, irregular, and indented nuclei. Malignant lymphomas of the lymphocytic type (Figs. 9 and 11) were rare, and none were found in the small intestine.

**Anatomic Pattern of Tumor Extension.** The necropsy records and sections of the lymph nodes, bone marrow, and viscera were studied to ascertain the pattern of anatomic extension of the lymphomas. The proportion of primary tumors which metastasized was 75% in males and 50% in females; however, this difference was not statistically significant.

Of the 20 primary lymphomas of Peyer's patches, 11 (55%) were limited to the intestine. In the remainder which did show extension and metastasis, the abdominal lymph nodes (mesenteric, portal, and retroperitoneal groups) were involved in every case, the spleen was involved in 2/9 mice and the pancreas in 1 case; extension of the neoplastic disease beyond the abdomen was not observed. The limited anatomic spread and the high percentage of cases in which the cancer was confined to the site of origin probably reflect the relatively low-grade malignancy of the lesions and the practice in our laboratory of frequently inspecting the animals, which usually were killed soon after declining weight or other signs of ill health were detected, and the tumors were presumably still at an early stage of development. A remarkable feature of the series was the complete absence of liver involvement in any of the cases in which lymphoma originated in Peyer's patches (Fisher's p = 0.01), particularly as the liver was often involved (Fig. 11) when lymphomas originated in abdominal lymph nodes or spleen, even although most of the lymph node tumors and those in Peyer's patches were cytologically identical.

### DISCUSSION

From the data provided by Dunn and others on inbred strains of mice (5), the incidence of lymphomas late in life in

### Table 1

<table>
<thead>
<tr>
<th>Histological type</th>
<th>MCT</th>
<th>HCT</th>
<th>LCT</th>
<th>Totals</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>9</td>
<td>2</td>
<td>0</td>
<td>11</td>
</tr>
<tr>
<td>Peyer's patches</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td>10</td>
</tr>
<tr>
<td>Abdominal lymph nodes</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>1</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Thymus</td>
<td>0.5</td>
<td>0.2</td>
<td>0.1</td>
<td>0.8</td>
</tr>
<tr>
<td>Total in 451 mice</td>
<td>15</td>
<td>10</td>
<td>6</td>
<td>27</td>
</tr>
</tbody>
</table>

* a LCT, lymphocytic cell type.
* b Totals include 2 disseminated lymphomas (1 LCT, 1 unclassified) and 1 mast cell thymic tumor in females and 1 MCT in peripheral lymph node in a male.
* c Two male mice were judged to have 2 independent primary Peyer's patch tumors each; for all other types there was one primary tumor in each animal.
NZO mice seems higher than in C3Hf mice and approximates that reported in AHR-HRBC, AWA–WBAC, and HR (hairless) mice. The NZO strain has a very much lower lymphoma incidence than reported for strains AKR, C58, C57L, C57BL, Rb, or SJL/J mice (5, 9–11). In comparison of different inbred strains, the pathological types of lymphoma must be considered as well as population statistics: for example, our data indicate a higher overall incidence of lymphoma in NZO mice compared with the data of Deringer and Dunn on A mice (6), but the A mice had developed tumors of lymphocytic type with an incidence of about 4% by 200 days, when NZO animals were still tumor free. Also, in the NZO strain, tumors of the thymus or tumors of lymphocytic type occurred late in the period of lymphoma risk (500 to 800 days) and thus did not obey the generalization that lymphocytic and thymic tumors in mice usually occur relatively early, while HCT and MCT occur late (5), which was true, however, for strain A as 1 example.

Comparison of the morphology of lymphomas in untreated NZO mice with observations recorded in untreated mice of other strains and in humans shows some contrasts and some similarities. Leukemia, most often of lymphocytic type, appears to be common in mice generally (5) but was rare in NZO animals, in which tumors of lymphocytic cell type accounted for only 10% (4/39) of the total, and there were only 2 cases of leukemia-like disseminated disease. The dominant cytological forms of lymphoma found were MCT (25/39, 64%), and HCT (which might be regarded as a more advanced stage of MCT) accounted for 8/39 (21%). In NZO mice, the histological pattern and occurrence of cells resembling Reed-Sternberg cells often gave a picture similar to the Hodgkin’s-like disease noted by several workers in other strains, most particularly in SJL/J mice (6, 7, 9, 10). Even in man, however, cells indistinguishable from Reed-Sternberg cells have been reported occasionally in MCT and malignant lymphomas of the poorly differentiated lymphocytic type (13). The rare observation of such cells in murine malignant lymphomas of mixed cellular composition is, therefore, insufficent evidence to consider these tumors as necessarily being similar to, or identical with, Hodgkin’s disease in man. Most of the lymphomas in our material were found at a relatively early pathological stage, and it would be possible to regard the few cases of leukemia observed either as representing different diseases or later stages of invasion and bloodborne dissemination, particularly as bone marrow was not involved in any other cases.

The principal primary sites for lymphoma in NZO mice were the abdominal lymph nodes and Peyer’s patches. The abdominal lymph nodes (particularly the mesenteric, pancreatic, and portal nodes) have been noted as a major site for primary lymphoma in other strains of mice (5) as well as rats (3, 8), but tumors primary in Peyer’s patches seem to be rare and the observation that nearly half of the lymphomas in NZO mice occurred at this site is therefore of some interest. MCT (reticulum cell cancer type B) originating in Peyer’s patches has been described by Murphy (10) in old, untreated C57L mice. Peyer’s patch lymphomas were found in 2/168 mice of the inbred MA strain by Tomatis and Goodall, (14) but in the untreated F1, F2, and F3 descendants from mothers given DMBA during pregnancy there was a 5-fold increase in the incidence of this lesion. Peyer’s patches were sometimes the site of reticulum cell cancers also in untreated SJL/J mice (9). Our data strongly support the interpretations of Dunn and Murphy that MCT (or type B reticulum cell cancers) may originate in the Peyer’s patches of the intestine (5, 6).

In view of the possible immunological significance of Peyer’s patches, the existence of an inbred strain of mice in which a high incidence of malignant lymphomas of this organ can be observed is of interest. The microanatomy of Peyer’s patches (4), which allows relatively direct exposure of their lymphoid cells to carcinogenic chemical or viral agents in the intestinal contents, may make etiological studies of intestinal lymphomas fruitful. The relative histopathological uniformity of Peyer’s patch lymphomas and their origin in an unusual site could suggest that this form of malignant lymphoma may be a disease entity which differs in etiology or pathogenesis from lymphoreticular cancers originating in other organs or tissues.

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Fig. 1. Malignant lymphoma of Peyer's patch, same as Fig. 3. The epithelial lining and the glands superficial to the tumor are intact. The muscularis propria is completely replaced by the tumor and no longer recognizable. H & E, X 100.
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Fig. 2. Cross-section of small intestine of male NZO mouse, 429 days. A Peyer's patch is evident on the right. Careful study of cytological detail showed areas suspiciously like malignant lymphoma, but the evidence was inconclusive. H & E, × 25.

Fig. 3. MCT. The entire wall is involved from the epithelial lining to the serosa. NZO virgin female mouse, 721 days. H & E, × 25.

Fig. 4. MCT of Peyer's patch growing into and obstructing the intestinal lumen. This was a single tumor in the intestine, and an identical lymphoma was found in the mesenteric and peripancreatic lymph nodes, with invasion of the pancreas. NZO virgin female mouse, 555 days. H & E, × 25.

Fig. 5. MCT of Peyer's patch. The tumor grows in an extraluminal direction. It was a single tumor in the intestine with an identical picture seen in the mesenteric lymph nodes. Atypical cells resembling Reed-Sternberg cells similar to those illustrated in Figs. 8 and 10 were evident. Breeder female NZO mouse, 559 days. H & E, × 25.
Fig. 6. MCT. Tumor of Peyer's patch at high magnification; a mesenteric lymph node showed an identical picture. Numerous mitoses were evident. Male NZO mouse, 571 days. H & E, X 1500.

Fig. 7. MCT. Mononuclear histiocytes with nuclear features resembling those of Reed-Sternberg cells are evident. Note the abundant cytoplasm and the large nucleolus in the cell on the right, slightly above center. In this mouse, the disease was disseminated to involve in addition to the abdominal lymph nodes, the spleen, thymus, and many extraabdominal lymph nodes. Virgin female NZO mouse, 563 days. H & E, X 150.

Fig. 8. Same case as Fig. 7. A binucleated histiocyte resembling a Reed-Sternberg cell is evident near the lower margin of the illustration. H & E, X 150.

Fig. 9. Malignant lymphoma, well-differentiated lymphocytic type. This relatively uncommon malignant lymphoma, primary in the spleen, is shown for comparison with those illustrated in Figs. 6 and 10. Virgin female NZO mouse, 412 days. H & E, X 1500.
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Fig. 10. MCT involving mesenteric lymph node. To the left, the capsule is completely infiltrated by the cancer. The mixed cellular composition in which both the histiocytes and lymphocytes are represented without appreciable preponderence of either cell type is readily evident. To the right, a binucleated cell resembling a Reed-Sternberg cell is present (arrow). This cell is shown at higher magnification in the inset. Malignant lymphoma was limited to the abdominal nodes in this case. Virgin female NZO mouse, 546 days. H & E, x 40; inset x 1200.

Fig. 11. Malignant lymphoma, well-differentiated lymphocytic type, involving the liver. In this case (same as Fig. 9), the entire spleen was replaced by the lymphocytic proliferation, sparing only small foci of intervening red pulp. This histological picture was uncommon in the NZO strain of mice and entirely different from that observed in their Peyer's patches. Virgin female NZO mouse, 412 days. H & E, x 600.
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