Experimental Plasmacytomas in Relation to Human Multiple Myeloma

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ABSTRACT

Among animal models of plasma cell tumors, that induced in BALB/c mice by means of intraperitoneal injections of oils remains the most reproducible and the most intensively studied.

The BALB/c oil-induced and transplantable plasmacytomas resemble human myeloma in their ability to produce a monoclonal immunoglobulin or Bence Jones protein. Bence Jones type nephrosis in BALB/c mice closely mimics its human counterpart. There are also similarities in background of immunodeficiency and in antigen-binding affinity of monoclonal immunoglobulins, as well as in interesting interrelationships with malignant lymphomas. Unlike the BALB/c tumor model, human myeloma is, however, principally a skeletal disease, not a gut-oriented or peritoneal plasmacytoma. The intriguing presence of intracisternal type A virus-like particles in BALB/c plasmacytoma cells and their absence in human myeloma is another major difference between the two forms of growth.

The pathogenesis of human myeloma remains obscure but the availability of experimental plasmacytoma models offers a means of systematically analyzing events leading to the neoplastic transformation of antibody-forming cells.

Introduction

In addition to man, several mammals were observed to develop plasma cell tumors spontaneously. For example, the multiple myeloma of the dog resembles human myeloma and features osteolytic lesions, monoclonal gammapathy and Bence Jones type nephropathy. Multiple myeloma has been also observed in a cat. Two plasmacytomas have been described in the Syrian hamster: one arose in the neck and the other in a mesenteric lymph node. These spontaneous tumors constitute uncommon though fascinating events. Plasma cell tumors can be induced in a predictable fashion in mice, particularly the BALB/c strain. The reader is referred to Potter's comprehensive review of experimental plasma cell tumors of mice.

The aim of this presentation is to outline the characteristics of induced plasma cell tumors of mice, particularly the BALB/c
TABLE I

Plasmacytomas of Mice

<table>
<thead>
<tr>
<th>Tumor Model</th>
<th>Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>&quot;Plasma Cell Leukemia&quot; in Street Strain Mice</td>
<td>1951</td>
</tr>
<tr>
<td>Rask-Nielsen, R. and Gormsen, H.</td>
<td></td>
</tr>
<tr>
<td>Ileocecal Plasmacytomas of C3H Mice</td>
<td>1954</td>
</tr>
<tr>
<td>Dunn, T.B.</td>
<td></td>
</tr>
<tr>
<td>Induced Peritoneal Plasmacytomas (BALB/c Mice)</td>
<td>1960</td>
</tr>
<tr>
<td>Potter, M. and Robertson, C.L.</td>
<td></td>
</tr>
<tr>
<td>Plasma Cell Tumors in NZB and NZBX BALB/c Mice</td>
<td>1966</td>
</tr>
<tr>
<td>Werner, N. et al</td>
<td></td>
</tr>
<tr>
<td>Pleomorphic Lymphomas in SJL/J Mice</td>
<td>1969</td>
</tr>
<tr>
<td>Murphy, E.D.</td>
<td></td>
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</tbody>
</table>

strain, and relate these to the problem of human myeloma.

Plasmacytomas of Mice

The more important experimental plasma cell tumor models of mice are listed in table I. Although the reader's attention will be drawn to the oil-induced peritoneal plasmacytomas of BALB/c mice, a brief outline of other models of plasmacytic and related lymphoreticular growths is in order.

The "plasma cell leukemia" in Street strain mice described by Rask-Nielsen and and Gormsen\(^9\) in 1951 has the morphologic features of reticulum cell sarcoma with plasmacytic differentiation. In later studies, (CBA X DBA/2) F1 hybrids, including mice injected with a variety of cell-free material, developed a high incidence of "plasma cell leukemia."\(^{21}\) Several of the transplanted reticular and plasmacytic neoplasms transplanted by Ebbesen and Rask-Nielsen\(^9\) were associated with "paraproteinemia" and amyloidosis.

Thelma Dunn\(^6\) reported in 1954 that several mice had an inflammatory lesion of the cecum. In some of these lesions, plasmacytic neoplasms arise in the ileocecal region and in relation to the cecitis. A later survey\(^7\) indicated that ileocecal plasmacytomas were commoner in C3H mice than in other strains and that they occurred more frequently in old mice.

Murphy\(^{15}\) described in 1963 a new inbred strain of mice (SJL/J) with a high incidence of reticulum cell neoplasms. Fifty percent of these neoplasms were associated with "paraproteinemia." Unfortunately, the production of monoclonal immunoglobulin has been unstable following transplantation of these tumors. Fujinaga et al\(^{11}\) transmitted "SJL/J disease" to BALB/c mice with cell-free extracts. They succeeded in producing reticulum cell neoplasms but not plasmacytomas.

Plasmacytomas are successfully induced by intraperitoneal injection of oily substances in BALB/c and NZB mice as well as in (BALB/c X NZB) F1 hybrids.\(^{18}\) The NZB strain is also genetically susceptible to the development of nephritis and autoimmune phenomena. The oil-induced plasmacytomas are by far the most widely studied model of immunoglobulin-producing experimental tumors. Primary and transplanted BALB/c plasmacytomas are under active investigation in our laboratory. They are considered to be a dependable and readily available source of tumor that strikingly resembles human myeloma.

Oil-Induced Plasmacytomas of BALB/c Mice

In an experiment designed to test the survival of allogeneic grafts as well as the behavior of M.T.A. virus, Merwin and Algire\(^{12}\) implanted estrogen-treated BALB/c mice with Millipore diffusion chambers containing C3H mammary tumor. Six months later, the BALB/c recipients developed hemorrhagic ascites owing to per-
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iteonal plasma cell tumor or fibrosarcoma.8 Empty chambers, leucite discs or borings also caused the development of plasma cell tumors.14 Subsequently, Potter and Boyce19 reported that plasma cell tumors could be induced by means of intraperitoneal injections of mineral oil alone or adjuvant mixtures. A variety of oils have been tested since. More recently, pristane (2,6,10,14-tetramethylpentadecane) was found to be an extremely potent inducer of plasmacytomas in BALB/c mice.1

The experimental model of oil-induced plasmacytomas has been described in detail elsewhere.18 This model has been extensively exploited to demonstrate the chain of events leading to the synthesis of specific classes and types of immunoglobulin. The same model has been used to study the effect of hereditary and hormonal influences on the induction of plasma cell tumors. Since the majority of oil-induced plasmacytomas are immunoglobulin-forming tumors, the role of intestinal bacteria and bacterial antigens on the differentiation of plasmacytomas has also been extensively investigated.

MORPHOLOGIC FEATURES

The sequence of events following the intraperitoneal injections of mineral oil or pristane starts with an acute inflammatory reaction which first covers the peritoneal surfaces and surrounds oil droplets. No plasma cells or lymphocytes are noted at this stage. At a later period, from one to three months after the initial injection of the oil, individual or small clusters of plasma cells begin to form within the oil granulomas. From five months to eight months following the injection of the oil, larger clusters of plasma cell precursors are observed. Such aggregates are particularly noted under the diaphragm and over the surface of the liver. Ultimately, these granules grow into small tumors which become autonomous plasmacytomas that can be readily transplanted. Primary or induced plasmacytomas may vary in size from one mm to two or three cm in greater diameter. Transplantable plasmacytomas grow into BALB/c hosts or BALB/c hybrids to attain a size of two to three cm at which point they frequently ulcerate or kill their host as a result of bowel strangulation or of Bence Jones type nephrosis.

Bence Jones type nephrosis with typical hard, fragmented or laminated casts are seen in animals bearing κ or λ polypeptide-producing tumors. Metastases are not seen as a rule in primary or induced plasma-

| TABLE II |
| Neoplasms Found in BALB/c Mice Following Intraperitoneal Injection of Adjuvant |
|---------------------------------|---------------|---------------|---------------|---------------|
|                                 | Con- | Ex- | Germ-free | Germ-free | Ven- | tion- |
| Plasma cell tumor               | 2    | 24  | 28         |               |     |       |
| Pleomorphic reticulum cell sarcoma | 16  | 3   | 4          |               |     |       |
| Monomorphic reticulum cell sarcoma | 7   | 1   | 1          |               |     |       |
| Mixed reticulum cell sarcoma    | 2    |     |            |               |     |       |
| Lymphocytic neoplasm            | 1    |     |            |               |     |       |
| Pulmonary adenoma               | 2    |     |            |               |     |       |
| Myoepithelioma                  | 1    | 1   | 1          |               |     |       |
| Uterine adenoma                 | 1    |     |            |               |     |       |
| Reticular hyperplasia           | 3    |     |            |               |     |       |
| (no tumor)                      |     |     |            |               |     |       |
| Total                           | 33   | 31  | 40         |               |     |       |

After McIntire and Princker: Immunology 17:481, 1969.
cytomas but do develop in animals bearing large transplanted plasmacytomas.

Plasmacytoma cells often resemble reticulo-

ulum cells except for their basophilic and pyroninophilic cytoplasm. Seldom do they appear to be frankly plasmacytic. Trans-

Figure 1. Electron micrograph of portion of Pristane-induced plasmacytoma cell illustrating a large number of intracisternal type A particles. Some of these appear to be budding from the endoplasmic reticulum lining the cisternae. Uranyl acetate and lead citrate, ×51,000.
planted plasmacytomas are even more ana­plastic looking than primary plasma cell tumors. On electron microscopy, plasmacytoma cells generally display an abundance of rough endoplasmic reticulin as well as free polyribosomes.

**Type A—Intracisternal Particles**

Whereas the normal lymphoid tissue of the BALB/c mouse may contain type B or C RNA viruses with well-formed nucleoids, one does not readily observe type A particles in either lymphocytes or plasma cells. In contrast to the lack of type A intracisternal particles within reactive plasma cells, these particles are found in abundance within the cisternae of plasmacytoma cells. In figure 1 is illustrated the intracisternal clustering of type A particles within a BALB/c pristane-induced plasmacytoma cell. It appears that type A particles are markers for plasmacytoma cells although their significance is far from known. These particles are non-infective; they lack a nucleoid and are poor in RNA. They are known to be rich in DNA-polymerase which is thought to represent a defective tumor virus enzyme. It should be emphasized that human myeloma cells are free of type A particles. Furthermore, there have been no consistent or well-documented observations of any type of virus within human myeloma cells.

**Monoclonal Immunoglobulins**

As in human myeloma, the majority of oil-induced primary plasma cell tumors are associated with production of an immunoglobulin and/or of a light-chain polypeptide. The majority of immunoglobulins produced have been monoclonal IgA. There is also a marked predominance of \( \kappa \) over \( \lambda \) polypeptides. Some of the monoclonal immunoglobulins produced by BALB/c plasmacytomas as well as in human myeloma cases were found to have antigen-binding properties. The electrophoretic serum patterns of seven transplantable plasma cell tumors are shown in figure 2. A distinct M component is revealed in the upper three patterns: HOPC-1 and RPC-5 produce, respectively, an IgG \( \kappa \) or \( \kappa \) monoclonal protein; TEPC-138, an IgM \( \kappa \). MOPC-321 produces \( \kappa \) polypeptide alone and its serum electrophoretic pattern shows no M component; its urine contains Bence Jones protein. RPC-20 produces \( \lambda \) polypeptide alone. Its serum as well as its urine electrophoretic patterns revealed an M component. NP-2 is a “non-producer” and shows a normal pattern. MOPC-315 is an IgA producer; no M component is apparent on this electrophoretic pattern.

The monoclonicity of the class of immunoglobulin produced by these BALB/c transplantable plasmacytomas is revealed in the immunoelectrophoretic studies illus-
Figure 3. Immunoelectrophoretic thin-gel agarose tracings of sera of normal mouse and of two BALB/c mice (RPC-5 and HOPC-1) bearing an IgG-producing monoclonal protein.

trated in figure 3. Compared to control serum, both RPC-5 and HOPC-1 demonstrate the characteristic "scooping-out" of the IgG arc which set them apart from the diffusely spread-out and even arc of the polyclonal IgG of the control serum.

Effect of Bacterial Environment

McIntire and Princler\textsuperscript{12} reported that 60 to 80 percent of conventional and ex-germ-free BALB/c mice develop plasma cell tumor following the injection of mineral oil into the peritoneal cavity, whereas germ-free mice develop a high incidence of lymphoreticular neoplasms of a type more primitive than plasma cell tumors. These findings of McIntire and Princler are summarized in table I. The results suggest the importance of microbacterial flora in the development and differentiation of plasma cells in a genetically susceptible host.

Genetic Make-Up

The BALB/c mouse appears to be uniquely susceptible to the development of oil-induced plasma cell tumors. This genetic susceptibility was further studied by observing the incidence of induced plasma cell tumors in a variety of hybrid mice. Among the various combinations tested, only the (NZB X BALB/c) F1 hybrid appears to be highly susceptible to induction of plasma cell tumors. The NZB mouse itself has a relatively high incidence rate of 20 to 30 percent. This could probably be raised if it were not for the susceptibility of this strain to early death from nephritis and autoimmune disorders.

Comparison with Human Multiple Myeloma

The morphologic characteristics of human multiple myeloma have been described in detail elsewhere.\textsuperscript{2} There are certain obvious similarities between the experimental plasmacytomas and human myelomas: Both forms are usually associated with production of monoclonal immunoglobulins and of Bence Jones polypeptides. When the latter are produced in mice, the kidneys develop a Bence Jones type nephrosis which is strikingly similar to its human counterpart. Amyloidosis is not a common development in oil-induced plasma cell tumors of BALB/c mice. It was, however, frequently observed by Ebbesen and Rask-Nielsen\textsuperscript{9} in association with their sublines of murine "plasma cell leukemia." As in human myeloma, there is an interesting interrelationship with reticulum cell sarcoma which is particularly demonstrated in the spectrum of lymphoreticular neoplasms induced in germ-free mice\textsuperscript{12} and in the pleomorphic lymphomas of SJL/J mice.\textsuperscript{15}

Although the pathogenesis of human myeloma is obscure, there appears to be a background of hypogammaglobulinemia and immunodeficiency which is particularly evident in the "non-secretory" forms of myeloma.\textsuperscript{3} The BALB/c mouse model may offer a similar example of immunodeficiency. There is an initial modest rise in serum immunoglobulins following the three customary intraperitoneal oil injections. Several months later, the oil-treated
mice develop a relative hypogammaglobulinemia that ushers the production of plasma cell tumors.

There are also some marked differences between the experimental mouse plasmacytomas and human myelomas. The type A intracisternal particles have been consistently observed in oil-induced as well as in transplanted plasmacytoma cells but not in myeloma cells. Granulomas and other states of chronic inflammation are generally absent in the clinical period preceding human myeloma. Hormonal and genetic influences appear to be unimportant or of obscure significance in man. Finally, human myeloma is principally a skeletal disease and, as such, it is more akin to the canine or feline myeloma than to the BALB/c oil-induced peritoneal plasmacytomas.

References