A Resumé of the Current Status of the Development of Plasma-Cell Tumors in Mice

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The theme of this symposium is the relationship of the developmental process (cellular specialization) to carcinogenesis. One concept is that neoplasia results from an abnormal developmental process rather than a transformation of an established specialized cell. This concept can only be tested in developing cell systems where the precursor cells, the process of specialization, and the established specialized cell are accessible. The plasma-cell tumor system is one in which some information is available on aspects of this very complex subject, and in order to introduce this form of carcinogenesis into this symposium, an outline of the problem is presented.

TRANSFORMATION OF PRECURSOR CELLS TO PLASMA CELLS

Normal Cells

A complex system of cells is involved in immune responses (Chart 1), and it is convenient for the present discussion, if not correct, to separate cells which recognize and react to "foreignness" into two broad groups: (a) cells which apparently mediate immune reactions without secreting humoral antibody, e.g., lymphoid cells that participate in homograft and delayed hypersensitivity reactions, and (b) cells which respond to immunogens by secreting immunoglobulin molecules (i.e., plasma cells). The discussion here will be confined to the immunoglobulin-secreting plasma-cell group.

Plasma-cell development normally occurs in several basic types of locations in mammals: first, in the loose connective tissues, particularly those lining the gastrointestinal and respiratory tracts [e.g., the lamina propria of the gut, the submucosa of the intestinal villi (3, 9, 42), or in the connective tissues in general], and second, in lymphoid organs [the medullary cord regions of the lymph nodes (6, 39, 40) and the spleen]. Plasma-cell development in these locations may arise from different types of precursor cells depending chiefly on the phase of the immune response (Chart 1). For example, the first exposure to an immunogen (the primary immune response) is believed to evoke a plasmacytopoiesis in which an undifferentiated precursor is transformed to a plasma cell. A number of cell types have been implicated as precursors. Fluorescent antibody-labeling and electron microscope studies of lymph nodes have provided suggestive evidence but have not proven a fixed cell type as a precursor of plasma cells (1, 13, 16, 37, 40). Reticular cells are resident cells in both connective and lymphoid tissues; these cells have been thought to be stem cells for other cellular differentiations. A second cell...
that may be a plasma-cell precursor is a lymphocyte. The concept of the lymphocyte is a changing one, as it is becoming recognized that cells called lymphocytes include a number of different functional entities. Through the work of Gowans and colleagues (for a review see Ref. 9), it has been shown that the lymphocyte population continuously recirculates (blood vascular system \(\rightarrow\) tissues \(\rightarrow\) lymphatics \(\rightarrow\)). Some lymphocytes are removed from the circulation during responses to "foreignness" and are fixed on the targets where they perform certain functions, e.g., homograft rejections or delayed hypersensitivity reactions. Some circulating cells called lymphocytes can probably also undergo morphologic changes associated with the development of the immunoglobulin secretory apparatus (formation of a network of endoplasmic reticular membranes). These circulating lymphocytes may be derived from plasma cells that have already undergone a primary immune response. As such they would comprise a cellular "memory bank" upon which the secondary responses (amnestic response, booster effect, etc.) depend. It has not been shown conclusively that circulating small lymphocytes participate directly in primary responses. Depletion of the circulating lymphocyte population by prolonged thoracic duct drainage only slightly delays primary responses to \(\phi\times 174\) given immediately after the last day of drainage (10). However, once immunocytes are specialized to make specific an immunoglobulin, they probably can give rise mitotically to "memory cells" that persist in the organism, either in the tissues or the circulation. Circulating lymphocytes isolated from thoracic duct fluid synthesize immunoglobulin in vitro (22, 43). In particular, isolated thoracic duct lymphocytes synthesize \(\gamma\)-A-immunoglobulin in vitro. Probably circulating cells do not divide very often, but when at a later time antigen is reintroduced into the organism, these "memory cells" become localized in tissues and divide mitotically. Thus these cells by-pass the immunorecognition events and very quickly produce specific antibody.

In summary, plasma-cell development appears to involve two cell entities circulating lymphoid cells that migrate and settle down in tissues or fixed tissue cells.

**Neoplastic Cells**

With this brief and rather tentative picture of normal plasma-cell development as background, the present findings on the histologic changes in neoplastic plasma-cell development in mice (\(\text{Mus musculus}\)) will be reviewed. In this species, tumors can be induced in high frequency, thus making it possible to study some of the conditions involved in the neoplastic transformation. It should be noted that there are rare spontaneous plasma-cell tumors in mice that originate in the connective tissues in the submucosa of the ileocecal region (originally described by Thelma B. Dunn of the National Cancer Institute). These tumors appear to arise at the base of small mucosal ulcers (29). Though spontaneous plasma-cell tumors are quite rare, they appear nonetheless in greater frequency in strain C3H mice. By far the most common plasma-cell tumors in \(\text{M. musculus}\) are those induced in the highly inbred BALB/c strain. Two types of agents have been shown to induce plasma-cell tumors in BALB/c mice: (a) the solid plastics, including lucite shavings or lucite rings, onto which have been cemented Millipore membranes (25, 26), and (b) the mineral oils and mineral oil adjuvants (32, 37). Both types of agents must be introduced intraperitoneally to induce plasma-cell tumors and are not effective in other sites. The solid plastic materials are introduced once by operative procedures usually in 1- to 2-month-old animals. A tumorigenic dose of mineral oil is 3 injections (0.5 ml each) of oil each given 2 months apart. Single injections of 0.5 ml of heavy oils (e.g., Primol D) or adjuvants (e.g., complete Freund's adjuvants) are often very effective. Both types of agents stimulate the peritoneal submesothelial connective tissues, particularly in the mesentery where a chronic granulomatous tissue is formed (26, 36). There are fundamental histologic differences in the granulomatous tissue formed by the two types of agents. The mineral oils (36) are phagocytized and incorporated into macrophages. These oil-laden phagocytes adhere to each other and become organized into a tissue. This tissue is vascularized and contains a number of different cellular elements, including reticular cells, lymphoid cells, and plasma cells. Mesothelium appears to cover the mass of oil-laden phagocytes, and the tissue remains as permanent granulomatous tissue. The reaction to the solid plastics is chiefly a chronic irritative-like response that results in an increased cellularity and vascularity of the peritoneal connective tissues. From existing data on the inability to induce plasma-cell tumors in other strains of mice (26, 29), it is strongly suggested that there is a genetic basis for plasma-cell tumor susceptibility in BALB/c mice.

Plasma-cell tumors begin to appear about 6 months after the first injection of the mineral oil and continue appearing for an additional 14 months. Intermediate histologic changes have not been clearly defined. At all times the oil granuloma contains isolated plasma cells and primitive plasma cells and reticular cells. Occasionally, sections of oil granuloma taken about the time plasma-cell tumors begin to appear contain local colonies of hyperchromatic plasma cells which contain mitotic figures. As most neoplastic plasma cells are much larger (almost twice the size) of the small medullary cord plasma cells, it is particularly suggestive that some foci can be found which contain lymphoid cells (e.g., cells with large nuclei and little cytoplasm), reticular cells, small plasma cells, and large bizarre (neoplastic-like) cells.

The primary plasma-cell neoplasm is found in only two locations, the peritoneal surfaces and the superior mediastinal lymph nodes (36). Oil granuloma develops in the superior mediastinal lymph nodes coincidentally with the peritoneal oil granuloma. Thus both the superior mediastinal lymph and the peritoneal nodes contain oil granuloma long before plasma-cell tumors arise. Plasma-cell tumors, however, do not appear to arise in these lymph nodes. Neoplastic plasma cells, when found in mediastinal nodes, are seen in the least involved nodes, in the sinuses, and not in the medullary cords and cortex where oil granuloma forms, suggesting these are metastatic cells from peritoneal plasma-cell neoplasms. Tumor formation in lymph nodes in the absence of peritoneal plasmacytoma has not been observed.

Another striking example of the disparity of lymph node and peritoneal plasma cell in carcinogenesis is provided by the
peritoneal mesenteric lymph node which has not been found to be involved in mineral oil plasma tumor formation. The bone marrow also is not involved in primary plasma-cell tumor formation in mice (14). This leads to the tentative conclusion that the neoplastic transformation takes place focally in the peritoneal oil granuloma and not in lymph nodes, and further that there is some special condition in the peritoneal tissues that favors plasma-cell tumor formation.

The focal origin of plasma-cell tumor formation in cells that contact “inducer” material suggests that mineral oil might contain a chemical carcinogen that is deposited in the peritoneal tissue. No chemical carcinogen, however, has thus far been implicated. The mineral oils do not appear to contain polycyclic hydrocarbons or other UV-absorbing materials (see Ref. 29 for references). Further mineral oils with different physicochemical properties appear equally effective in inducing plasma-cell tumors (29, 32). Probably the strongest evidence against a chemical carcinogen in mineral oil is the fact that solid plastics bring about the same type of change.

Relationship of Oil Granuloma to Plasma-Cell Development

It might be appropriately asked at this point what relationship does mineral oil-induced granuloma have to normal plasma-cell development or to the immune response? It has been mentioned that plasma cells are found normally in the loose connective tissues of normal animals, i.e., whether they undergo primary response in these sites, or whether they are derived from circulating cells that “home” in these particular places. Gowans and Knight (8), for example, found that the circulating, large lymphocytes migrated in relatively high numbers into the stroma of the intestinal villi, and further that these cells resembled the resident plasma cells in these connective tissues. This might represent a replenishment of stem cells in these tissues or the arrival of “preinstructed” cells in these sites.

The γA-forming cells of the intestinal villi are of particular importance to peritoneal plasma-cell neoplasia not only because of the physical closeness of the relationship of the villi to the mesenteric tissue, but also because of an apparent physiologic similarity between the two populations of cells. It has been known for some time that the predominant heavy chain type selected in neoplastic plasma cells is the α or the heavy chain of the γA-immunoglobulins (25, 35). In a series which we recently studied, over 50% of the tumors in a single experiment were of the γA variety (Table 1). Thus, in plasma-cell neoplasia, the α chain is being selected preferentially over the other heavy chain types μ (macroglobulin), ϕ, γ, η (γ-G-related) types. The γA-immunoglobulins are associated with the special characteristic of being secreted across epithelial cells and into the lumens of various tracts (42). This form of immunoglobulin performs a special function in the immune response. A mechanism for attracting already differentiated γA immunocytes to appropriate locations or directing γA development at these sites must exist.

The mechanical effects of the bulky oil granuloma could potentially influence the mobility or alter the physiology of the intestine in such a way that the organism comes under increased exposure to the immunogens of the gut flora. In this way the solid plastic discs could be related to the oil in that both types of material influence normal intestinal function over an extensive period of time. It would have to be assumed that the primary stimulus to the immunocyte system is a special characteristic of immunogenic stimulus from the intestinal lumen; this stimulus evokes predominantly a γA type response. It is tempting to relate the spontaneous ileocelecal plasma-cell neoplasms that develop in the base of ulcers to the induced tumors, but quite clearly there is an anatomic difference in the apparent site of origin of the ileocelecal plasma-cell neoplasms. These develop submucosally and spread to the regional mesenteric lymph node in contrast to the induced tumors. It can only be concluded that too little information is available on the nature of plasma-cell development in peritoneal connective tissues. However, the important point is that, in some way, these local conditions stimulate a particular form of plasma-cell development as reflected in the predominant γA type myeloma proteins. Further, in plasma-cell neoplasia only a segment of the total plasma-cell population is involved in the neoplastic transformation.

**GENE REGULATIONS IN PLASMA-CELL DEVELOPMENT**

**Normal Cells**

One fundamental problem in cellular differentiation is the mechanism of activation of sets of genes involved in the specialization. In plasma-cell development this process is even more complex because of the alternative subsets (classes) of genes available. Plasma cells are therefore differentiated from

<table>
<thead>
<tr>
<th>Type of protein produced</th>
<th>Number of tumors</th>
</tr>
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<tbody>
<tr>
<td>γA-serum polymer</td>
<td>21</td>
</tr>
<tr>
<td>γA-halfmer</td>
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</tr>
<tr>
<td>γF</td>
<td>5</td>
</tr>
<tr>
<td>γG</td>
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</tr>
<tr>
<td>κ chain (only)</td>
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</tr>
<tr>
<td>none</td>
<td>3</td>
</tr>
</tbody>
</table>

**Total** 36

Types of immunoglobulins produced by plasma-cell tumors. The tumors were derived from a single experiment. BALB/c female mice were given three 0.5-ml injections of Bayol F spaced two months apart. These mice were also injected with foreign red blood cells at various times during the first 6 months of the experiment.
each other by the types (classes) of structural genes active in a cell. The immunoglobulin molecule is composed of two types of polypeptide chains, the light and heavy chains. Most functional immunoglobulin molecules are four-chain units consisting of two identical light chain subunits and two identical heavy chain subunits or polymers of four-chain units. The γG-related immunoglobulins are predominantly four-chain structures, while the γA are mixed polymers of the four-chain units and the γM macroglobulins are pentamers of four-chain units. In the mouse there are five basic types of heavy chains: μ, the heavy chain of the γM macroglobulins; α, the heavy chain of the γA immunoglobulins; and δ, γ, and η, the heavy chains of the three γG-related immunoglobulins, γF, γG, and γH respectively. In other mammals there are different sets of immunoglobulin genes; physiologic homologies have not yet been established among different mammals, although the various immunoglobulin chains among mammals are evolutionarily and chemically closely related to each other. In the mouse there are two light chain types, the kappa and lambda types. The kappa type chain is the predominant light chain type unit and serves as the light chain subunit for all the other heavy chain types. The lambda type chain is not well understood in the mouse, and thus far has been found only as a subunit of a macroglobulin type protein (23).

Heavy Chain Gene Selection. In plasma-cell differentiation and development one phase of the gene selection process is the activation of one light and heavy gene for protein synthesis. A particularly interesting feature of this selection is that it appears to operate within a group of genes that are not only physiologically and chemically related to each other, but also physically in close linkage (35). Through the use of serologic markers it has been possible to study the genetics of three immunoglobulin heavy chains (α, γ, and η) in the mouse. The respective genes A, G, and H are very closely linked in one chromosome region and may even be neighbors or near neighbors (17, 35). The selection process in plasma cells distinguishes among these closely linked genes. As previously discussed, in plasma-cell tumor formation the γA heavy chain gene is preferentially selected.

Immunoglobulin Specificity. It is not correct at this time to dissociate the chain selection event from a process in which an even greater chemical specificity of light and heavy chains is established; that is, the selection process does not evoke a standard chain but rather a particular variant of a group of related chain types. If one were to compare the heavy and light chains of like type from a series of different plasma-cell tumors induced in the inbred BALB/c mouse, it can be shown that, with the exception of the lambda chains, no two chains of the same class (e.g., α, φ, γ, η, and κ) (31, 53) are alike in primary structure. While these amino acid sequence differences are scattered in different positions in the chain, they are clustered in the aminiterminal segments of the respective light (30) and heavy chain types. A number of hypotheses have been proposed to explain the basis of these differences and none as yet has been conclusively established (30). It is beyond the scope of this discussion to enter into this unresolved problem other than to point out this is an additional aspect of gene regulation in plasma cells.

It is pertinent, however, to bring out several aspects of this specificity that relate to neoplasia. First, single isolated, antibody-forming cells from an individual can be shown to differ from each other in respect to the specificity of antibody produced (11, 12, 21, 28). Second, antihapten antibody molecules, e.g., γG-antibody molecules derived from genetically similar rabbits, differ from each other in primary structure just as myeloma proteins vary in structure (15). Further, it has recently been shown that a few selected myeloma proteins can combine with specific antigenic determinants (DNP hapten, pneumococcal polysaccharide, papain Fc fragments of immunoglobulins) indicating that myeloma proteins are equipped with combining sites (2, 6, 27). The specificity of antibody and myeloma protein and antibody-producing and myeloma cell appear to reflect parallels in protein product and synthesis.

Stability of Gene Selection. The mechanism for producing a specific type of immunoglobulin once it has been established in a tumor cell is an extremely stable one. Tumors transplanted for years continue to produce the same type of myeloma protein and the same types of polypeptide chains (30). This must mean that some device operates to make available a specific gene which can be stabilized in such a way that it remains uniquely available for protein synthesis during innumerable mitotic divisions. The stability of immunoglobulin formation in plasma-cell tumors reflects a basic mechanism of cellular differentiation, i.e., once a specialized cell type has evolved, more similar specialized cells can be derived from it by mitosis. It follows that the physical basis of the specialized state is replicable.

Allelic Suppression. Another aspect of gene regulation in plasma cells of general interest to the mechanism of cellular differentiation, as well as to the problem of neoplasia, is "allelic suppression." The best known prototype of this phenomenon is the singly active X chromosome effect in mammals (18, 38), in which one X chromosome is suppressed randomly in an early stage in development. As a result, the genes on this chromosome are not available for protein synthesis (4). Suppression in the case of the singly active X chromosome is associated with an apparent failure of the chromosome to uncoil after mitosis; instead, the chromosome remains in a compact state throughout interphase. Thus the DNA is replicated but is not available for transcription. When genetic markers on immunoglobulin chains became available in man, rabbit, and mouse, it was observed by a number of workers that in individual normal and neoplastic plasma cells only one of the alleles is active per cell (44, 45).

Of great interest is the fact that the immunoglobulin loci are autosomal and not on the X chromosome, indicating the presence of a special regulatory mechanism that affects immunoglobulin genes. Further, Dray (5) discovered that the allelic suppression could be specifically mediated by antiallotype antibody that was introduced into the organism during early development (19, 20). In matings between genetically different homozygous rabbits, it was observed that both alleles are expressed in the cells of F1 hybrids. However, when the dams were immunized against paternal allotype, the offspring expressed predominantly only the maternal allotype. Injection of antiallotype antiserum into newborn heterozygotes was also
effective. Allotypic allelic suppression resembled the singly active X-chromosome effect by taking place early in development and often persisting for long periods in the life of the individual. The implication is that the allele is turned off in a stem cell and remains turned off in the mitotic progeny. This also is a stable, heritable characteristic.

In summary, there are at least four characteristics of gene regulation that take place in plasma-cell development: (a) a mechanism that preferentially selects classes of immunoglobulin structural genes and coupled with this; (b) establishment of a further high degree of specificity in the light and heavy chains; (c) allelic suppression; and (d) stabilization of a program for immunoglobulin synthesis that is unaffected by mitosis. In general, these types of regulations occur in both normal and neoplastic plasma cells.

Abnormalities in Immunoglobulin Formation in Plasma-Cell Tumors

The chief type of abnormality in immunoglobulin formation found in some, but not all, plasma-cell tumors is the failure of an essential gene to be active (24, 34). Gene failures could result from a variety of organic causes, e.g., somatic mutation, chromosomal abnormalities, etc., or from some condition which affects a sequence of gene activations in such a way that the sequence of events progresses in a disorderly manner. The most common example of gene failure is represented by the group of plasma-cell tumors that make only light chains (the classical Bence Jones-producing tumors). Light chains by themselves are not believed to be functional antibodies and are also known to be pathologic for the renal tubules where they induce tubular casts. Thus cells making only light chains can, with some assurance, be regarded as pathologic forms in which the heavy chain locus has either not been turned on or, once on, has been turned off.

Two other pathologic forms resembling light chain producers in principle are the tumors that produce γA-halfmers and the "nonproducers." The γA-halfmer is a molecular form of γA-immunoglobulin that contains one light and one heavy (34) chain. These proteins do not possess the ability to assemble into four-chain monomeric units. Also, γA-halfmers like light chains are excreted in the urine. The assumption at this time is that γA synthesis in the mouse involves an additional component essential for the complete molecular form of the molecule, and that in γA-halfmer-producing tumors the essential genetic machinery is inactive just as the heavy chain locus is inactive in the light chain producers. The most extreme example of gene failures are the "nonproducers" (2). This group has not been as intensively studied; the more common forms are the established tumors which stop producing immunoglobulin.

Another related pathologic immunoglobulin producer is the tumor type which produces balanced four-chain molecules or polymers and an excess of the specific light chain subunits (34). A possible explanation for this phenotype is based on the known marked aneuploidy of BALB/c plasma-cell tumors. If, for example, the light and heavy chain genes are located on different chromosomes, then either a greater number of light chain chromosomes per cell or a lesion preferentially damag-
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