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Immunoglobulin-Producing Tumors and Myeloma Proteins of Mice

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I. INTRODUCTION

The tumors in mice thus far associated with immunoglobulin production are the plasmacytomas and lymphomas. These represent neoplastic derivatives of various cell types within the immunocyte system of cells; the immunoglobulin produced may be secreted or bound to the cell surface or both (Table 1).

The most widely studied immunoglobulin-producing tumors in mice are the plasmacytomas. These tumors appear to arise from cells specialized in such a way that they can produce only a single molecular type of immunoglobulin. It is generally accepted that normal plasma cells are similarly specialized and that the tumors by their great proliferative potential are amplifications of individual cell types within the immune system. The plasmacytomas thus are an extremely useful means for studying the individual components in the vastly heterogeneous immunocyte system of cells.

Plasmacytomas are of monoclonal origin on the basis of the homogeneous immunoglobulin that each tumor produces. Nearly every plasmacytoma cell of independent origin is restricted to producing only a single species of immunoglobulin molecule, i.e., molecules that all contain the same types of light and heavy polypeptide chains. It cannot be stated categorically that each tumor or normal immunocyte produces only a single type of immunoglobulin molecule because there are now a few exceptions among both normal (184, 201) and tumor (201, 207, 291) immunocytes where at least two different classes of immunoglobulins are produced. These special cases are discussed in the sections on tumors of immature plasma cells and immunoglobulin synthesis. While the greatest majority of plasmacytomas are indeed monoclonal when appraised by immunoglobulin production, they nonetheless themselves originate within normal clones. The abnormal proliferative property probably develops only within a few cells of a clone.

TABLE 1. *Immunoglobulin-producing tumors in mice*

Normal Immunocyte from which Tumor Originates	Immunoglobulin Production		Pathological Designation of Tumor
	Cell surface	Secretory	
B lymphocyte	+	−	Lymphocytic neoplasm
T lymphocyte	+*	−	Lymphocyte neoplasm†
Immunoblast	?	+	Plasma cell leukemia, reticulum cell neoplasm type B‡
Plasma cell	+§	+	Plasmacytoma (myeloma)
Lymphoplasmacyte	?	+	Lymphoma

B lymphocyte = bone marrow-derived lymphocyte; T lymphocyte = thymus-derived lymphocyte.

* Immunoglobulin on the surface of T lymphocytes is very difficult to demonstrate (83, 185). † Neoplasms of T lymphocytes (commonly called leukemias in the mouse) are the most common lymphoreticular neoplasms in the mouse. Receptor immunoglobulin has not yet been demonstrated on these tumors. ‡ Reticulum cell neoplasm type B in the Dunn classification (58, 61) is a pleomorphic tumor containing mixed cell types. Some of the cells may be large dendritic macrophages that are associated with reticulum fibers. It has not been shown that dendritic macrophages themselves produce immunoglobulin. The source of immunoglobulin associated with these tumors presents problems that are discussed in the text. § Some plasmacytomas have been shown to have immunoglobulin on their surface (263, 275).

The plasmacytomas represent only one group of immunoglobulin-producing tumors. Lymphomatous neoplasms in mice (reticulum cell sarcomas, plasma cell leukemias) are other pathologic forms that have been associated with immunoglobulin production. These tumors appear to be derived from precursors of plasma cells; unlike the plasmacytomas, immunoglobulin production is not consistently associated with tumors of these morphologic types. There is potentially a third class of tumors associated with immunoglobulins; these are tumors of cells that have only immunoglobulin receptors on their surface and do not possess the potential for secreting immunoglobulin. Very little is currently known about such tumors although a few lymphoid tumors have recently been described in man (201) and mouse (263) that have immunoglobulin receptors on their surface.

It is a remarkable and fortunate experimental fact that plasma cell tumors can be induced in umlimited numbers in the highly inbred BALB/c strain of mice by relatively simple procedures such as the intraperitoneal injection of mineral oil.

Although the plasmacytomas are relatively easily produced, the process by which they evolve is very complex. A number of factors have been described; foremost among these is the unique genetic susceptibility of the inbred BALB/c strain of mice. The genetic basis of susceptibility has not been worked out and remains one of the intriguing problems in this field. A second essential factor in plasmacytoma formation is the abnormal peritoneal environment—the anatomic site of plasmacytoma formation. An abnormal peritoneal environment can be created by implantation of large solid plastic materials or injection of mineral oils. There has been also speculation about the possible role of viruses in plasma cell

tumor formation based on the finding of intracisternal type-A particles in virtually every plasma cell tumor so far examined by electron microscopy.

Plasma cell tumors have been most useful in providing a source for large quantities of homogeneous immunoglobulin, since most of the tumors are relatively easy to transplant in syngeneic hosts. The transplants obtain massive size, approaching one-third of the body weight, and large quantities of immunoglobulin can be isolated from the serum, ascites, or urine of these mice. Tumor transplant lines are usually quite stable and maintain the continuous production of the characteristic immunoglobulin through many generations (215). The oldest immunoglobulin-producing tumor (X5563) has been in nearly continuous passage since 1957 (216) and still produces the same immunoglobulin.

Many of the first uses of homogeneous immunoglobulins concerned classifying various forms of immunoglobulins by chemical and serological methods. The discovery of antigen-binding activity of a few mouse myeloma proteins has stimulated many studies on the immunochemistry of "homogeneous antibody." Myeloma proteins with antigen-binding activity are "antibody-like" and probably resemble an individual species of immunoglobulin that might be found in a population of molecules that bind the same antigen (antibody).

The study of immunoglobulin synthesis has been greatly facilitated by the availability of murine plasma cell tumors. Much of the early work dealt with the assembly of the immunoglobulin molecule, whereas recent investigations, though far from resolved, have been more concerned with the problem of the immunoglobulin messenger RNA. In an exciting series of investigations Scharff and his colleagues have developed selective in vitro cloning procedures based on specific immunoglobulin production and have discovered a high rate of development of cell types with defective programs of immunoglobulin synthesis.

A. Abbreviations

1) Plasmacytomas. Plasmacytomas are designated by a prefix (which usually contains PC to indicate plasma cell tumor), an accession number, and occasionally a letter that indicates a transplant subline. Prefixes may indicate the agent used to induce the tumor or the name of the investigator in whose laboratory the tumor was induced; for example, MOPC104E = mineral oil-induced plasmacytoma 104, transplant subline E. Common prefixes:

Adj	Adjuvant
MO	Mineral oil
TE	Tetramethylpentadecane
HO	7*n* hexyloctadecane
SA	*Salmonella* associated
M	Merwin
D	Dunn
S	Sanford
Mc	McIntire

Y	Yancey
G	Goldstein
MS	Moriwaki

M. Cohn and associates, Salk Institute, have 2 series of plasmacytomas designated the S (Salk) and J series; they do not use PC in the prefix.

The first transplantable plasmacytoma was designated by the experimental accession number X5563 and has retained the name.

Scharff has designated the nonproducing line of MPC11 as NP2.

2) Immunoglobulins. Immunoglobulin (Ig) molecules are usually classified according to the heavy-chain subunit. Two nomenclatures exist for the mouse, one originated by Fahey et al. (72, 73) and the other by Potter and Lieberman (219, 220).

Class		Heavy Chain	
Potter	Fahey	Potter et al.	Fahey et al.
IgM	IgM	μ	μ
IgA	IgA	α	α
IgF	Igγ1	θ	γ1
IgG	Igγ2a	γ	γ2a
IgH	Igγ2b	η	γ2b
IgJ606	IgG3		

3) Immunoglobulin subunits

Proteolytic fragments: Fab = fragment with antibody activity, Fc = crystallizable fragment. The proteolytic fragments were originally isolated from antibody, corresponding fragments can be derived as well from immunoglobulins that have no known antigen binding activity.

Polypeptide chains: H = heavy chain, L = light chain.

Polypeptide chain segments: V = variable, C = constant. Each chain has a V and C segment designated V_H, C_H, and V_L, C_L. Greek letter designations for chain classes can be used in place of H or L.

4) Immunoglobulin genes. The complete designation for an immunoglobulin gene is Ig followed by the symbol for the immunoglobulin polypeptide segment followed by the class symbol: e.g. Ig C_H A. C_H genes are A, F, G, H, M, and J606. C_L genes are K (kappa), L1 and L2 for the two lambda types.

Allotypic antigenic determinants assigned to polypeptide segments are designated by a superscript arabic numeral: e.g. Ig $C_H A^{12, 13, 14}$.

Strains of mice that are congenic for immunoglobulin genes are designated by symbols that include the parent strain, strain source of new immunoglobulin gene(s), number of introgressive backcrosses (BC), and number of homozygous brother-sister matings (F); e.g. BALB/c.C57BL/Ka Ig $C_H BC_{20} F_2$.

5) Light-chain classes: κ = kappa, $\lambda 1$ = lambda 1, $\lambda 2$ = lambda 2.

6) Mineral oils: Bayol F, Primol D (Humble Oil, Rahway, N.J.), Drakeol 6VR (Pennsylvania Oil).

7) M component. Homogeneous immunoglobulin found in high concentration in serum or other body fluid; may be of tumor or normal cell origin.

8) Antigens

MuLV = murine leukemia virus
G = Gross
G_{IX} = Gross antigen controlled in part by a gene in the 9th linkage group of the mouse
GCSA = Gross cell surface antigen
gs = group-specific antigen of MuLV
PC.1 = plasma cell alloantigen
MSPCA = mouse plasma cell tumor antigen
θ = theta antigen

9) Proteins, protein hormones

BSA = bovine serum albumin
MUP = major urinary protein complex in the mouse
ACTH = adrenocorticotropic hormone
FSH = follicle-stimulating hormone
LH = luteinizing hormone
TSH = thyroid-stimulating hormone

10) Chemicals

DNP = dinitrophenyl
TNP = trinitrophenyl
SDS = sodium dodecylsulfate
DOC = deoxycholate
AcU = 5-acetyluracil

11) Amino acids

C	cysteine (Cys)	F	phenylalanine (Phe)
H	histidine (His)	K	lysine (Lys)
I	isoleucine (Ile)	R	arginine (Arg)
M	methionine (Met)	Y	tyrosine (Tyr)
S	serine (Ser)	W	tryptophan (Trp)
V	valine (Val)	D	aspartic acid (Asp)
A	alanine (Ala)	N	asparagine (Asn)
G	glycine (Gly)	B	asp or asn
L	leucine (Leu)	E	glutamic acid (Glu)
P	proline (Pro)	Q	glutamine (Gln)
T	threonine (Thr)	Z	Glu or Glx
PCA	pyrrolidone carboxylic acid		

12) Cell types

B cell = bone marrow-derived lymphocyte
T cell = thymus-derived lymphocyte

I. PLASMACYTOMAS

A. Induction of Plasmacytomas

Merwin and Algire (165) were the first to induce plasmacytomas in BALB/c mice. They were studying the long-term survival of allogeneic tissue (C3H mam-

mary tumor tissue) inside Millipore diffusion chambers that had been implanted intraperitoneally in BALB/c mice. After 6 months the BALB/c mice developed hemorrhagic ascites that was caused by plasmacytoma or fibrosarcoma that had developed in the subperitoneal connective tissues. Merwin and Redmon (166) demonstrated later that empty chambers, Millipore discs (17.5 or 21 mm diam), or rough-edged plexiglass (Lucite) borings (1 mm diam) also induced plasmacytomas. More tumors were induced with the larger (21 mm) discs and borings. Few or no tumors were induced with smooth Lucite rings, Lucite fragments, or small discs. The large discs and borings caused considerable irritation and chronic inflammation. Merwin and Redmon suggested the irritative inflammatory reaction was an important factor in plasma cell tumor development. The fibrous chronic reactive tissue developed over most of the contiguous peritoneal surfaces. Fibrosarcomas arose in the capsules covering the discs or chambers while plasmacytomas developed elsewhere on the peritoneal surfaces.

Recently Anderson (5) has induced plasmacytomas with Lucite discs and noted, as did Merwin and Redmon, that the large discs often eroded the gut wall and in extreme cases the edge of the disc could be found in the gut lumen. These reactions apparently occurred without suppurative peritonitis, although many adhesions developed. Merwin and Redmon (166) also noted that inbred BALB/c mice appeared uniquely susceptible, since discs implanted in other strains induced only a few (if any) plasma cell tumors.

After the observations of Merwin and Algire (165), other agents that induce plasma cell tumors in BALB/c mice were found. The first was a staphylococcal-adjuvant mixture (225) that contained one part heat-killed *Staphylococcus* and one part incomplete Freund's adjuvant [8.5 parts Bayol F and 1.5 parts Arlacel A (mannide monooleate)]. This mixture was used by Lieberman et al. (138) to induce ascites in mice in order to produce quantities of antibody (138, 140). The staphylococcal-adjuvant mixture also induced extensive peritoneal adhesions and a chronic inflammatory response. Lieberman et al. (140) subsequently induced plasmacytomas in BALB/c mice with this adjuvant material.

In a search for the active component in the adjuvant, Potter and Boyce (213) found that the mineral oil alone induced plasmacytomas. A few tumors were induced with a single injection of 0.5 ml of the mineral oil Bayol F; three 0.5-ml injections of mineral oil spaced 2 months apart induced plasmacytomas in 40–60% of females so injected (206, 213). A number of light and heavy pure white mineral oils were active, e.g. Bayol F, Drakeol 6VR, Primol D, and other USP grade oils sold commercially (5, 206). Mineral oils contain large numbers of straight-chain, branched-chain, and ring-structured saturated hydrocarbons. Only a few individual components are available in pure form, but among those tested with the same regimens used for mineral oils all have been as active or more so than mineral oil alone; this includes pristane (2,6,10,14-tetramethylpentadecane), phytane (2,6,10,14-tetramethylhexadecane), and 7*n* hexyloctadecane (5, 6). Usually the latent period for pristane is shorter than with mineral oil (Fig. 1). In preliminary experiments *n*-hexadecane was also tried but found to be toxic and could not be evaluated properly. Carcinogenic polycyclic hydrocarbons in trace quantities are apparently not the active components, as they are not present in the oils nor in

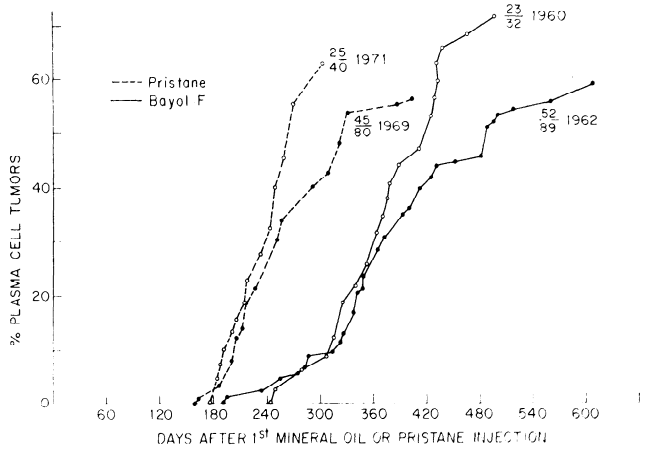


FIG. 1. Incidence of plasma cell tumors in BALB/c mice treated with three 0.5-ml intraperitoneal injections of pristane (broken line) or Bayol F (solid line) from four different experiments; injections were given bimonthly. These experiments were selected because the mice were carefully followed for tumor incidence. Fractions indicate actual number of plasma cell tumors over total number of mice injected. Time of appearance of plasma cell tumors was plotted from the day the mice received the 1st injection (30–90 days of age). Pristane-injected mice developed plasma cell tumors sooner and more precipitously than did mineral oil-treated mice. Plasma cell tumors did not usually begin to appear until 30–60 days after the 3rd injection of oil. The greatest number develop within the 1st year, but others continue to form during the 2nd year.

the pure alkanes (206, 233) that are active inducers. It may be concluded that many different alkanes induce plasma cell tumors.

Several characteristics of the mineral oils and branched-chain alkanes are relevant. Wilner et al. (302) synthesized 7*n* hexyloctadecane and several other straight- and branched-chain alkanes in a search for a more effective chemically defined immunologic adjuvant. They found straight-chain alkanes C_{10}–C_{18} were highly irritating to the skin of guinea pigs, but that this adverse property could be overcome by using branched-chain compounds of roughly similar molecular weight. They compared a variety of branched-chain compounds, including both pristane and 7*n* hexyloctadecane, to Drakeol 6VR as adjuvants. They found that pristane and 7*n* hexyloctadecane, for example, were superior or equal to Drakeol 6VR as an immunological adjuvant by sustaining high titers of antibody over long periods of time. (The test oils and Arlacel A were mixed with antigens to make water-in-oil emulsions; for plasma cell induction the unmixed oil or hydrocarbon is injected directly.)

Pristane was first isolated in 1917 by Tsujimoto (see ref 18) from basking shark livers. Subsequent studies have shown its origin is probably biogenic as 1–3% of the body fat of several species of marine copepods is pristane (27). Pristane has also been found in a variety of mineral oils. Avigan et al. (18) isolated 2–9 μg pristane/g of human serum or liver and 52 μg/g in skin; phytane was also observed but in lower concentrations. It was suggested that pristane may enter the organism

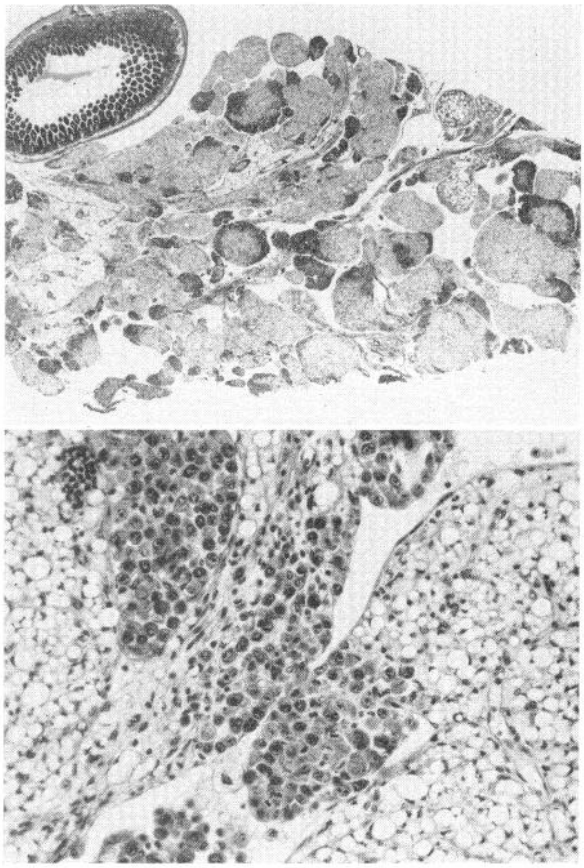
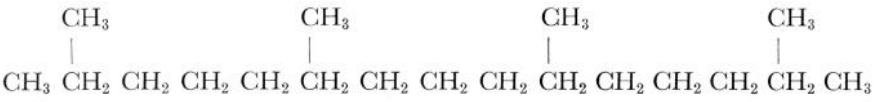


FIG. 2. Peritoneal oil granuloma in a primary plasmacytoma (hematoxylin and eosin stain). *Upper:* photomicrograph showing extensive mesenteric oil granuloma. Intensely stained plasmacytoma tissue is irregularly distributed on some of peritoneal surfaces. [10×] *Lower:* high-power photomicrograph of a single area from same section showing plasmacytoma tissue on peritoneal surface of oil granuloma. [165×]

via skin absorption. O'Neill et al. (187) have also identified pristane in human skin, and Boitnott et al. (28) have repeatedly isolated mineral oil from human tissues. These compounds, however, have not been implicated in human plasma cell tumor formation.



2,6,10,14-tetramethylpentadecane (pristane)

Plasma cell tumors arise in the peritoneal oil granuloma and appear to require this environment for development as they are not observed elsewhere nor have they been induced by subcutaneous injections of mineral oil (223) (Fig. 2). Intraperitoneal mineral oil evokes the formation of an oil granuloma on peritoneal surfaces, abdominal wall, diaphragm, mesentery, peripancreatic regions, and retroperitoneally (223). This tissue begins to develop within a few days after oil injection. The main bulk of the oil granuloma consists of oil-laden histiocytes that adhere to

each other, attach to peritoneal surfaces, and become vascularized from the mesenteric vessels beneath. The tissue varies in the amount of reactive components; some oil granulomas contain many focal collections of lymphoid cells and occasional small focal areas of granulocytic proliferation containing primitive and mature cells in the series. Often the oil granuloma contains only a few scattered lymphoid cells between the histiocytes. Plasma cells with a normal morphology (a small eccentric, rather pyknotic, nucleus) are often observed lying between histiocytes or long blood vessels. Further, they are often found admixed with lymphoid cells in areas containing many lymphocytes. Focal proliferations of plasma cells appear to arise in similar locations. Dividing plasma cells usually are hyperchromatic and their nuclei are larger and less uniform than normal. Plasma cells in tumors are usually characterized by their relatively large size and hyperchromicity with hematoxylin and eosin stain. Many of these cells have multilobed nuclei or double nuclei. A perinuclear clear zone (hof) is a characteristic feature. Incipient histologic stages of plasma cell tumor development have been reported to develop in mice 4 months after the first injection of oil; others have been found in mice autopsied at various times after they had received a complete series of mineral oil injections (223). That plasma cell tumors may actually develop in the peritoneal fluid has not been ruled out, although direct evidence for this has not yet been obtained. Ascitic origin is suggested by histologic study of primary tumors where the plasmacytomatic process is seen to be confined almost exclusively to peritoneal surfaces (Fig. 2), suggesting primary origin in the fluid and subsequent multiple seedings of tumor cells on various loci. The vast majority if not all mice with large bizarre plasma cells (resembling those seen in neoplasms) in the peritoneal fluid also have infiltration of the connective tissues. Invasion of the peritoneal connective tissue by plasmacytoma is associated with peritoneal bleeding, which in some cases develops precipitously and may kill the mouse. Interestingly, although plasma cell tumors apparently do not develop in other strains injected with mineral oil, the oil granuloma that develops in these other strains is quite similar in morphology to that observed in BALB/c mice.

The only other tissues consistently containing oil granuloma are the superior mediastinal lymph nodes that drain the peritoneum. Plasmacytomas metastasize to these nodes but do not appear to develop in them (223); i.e. isolated cells are found in the sinuses rather than the medullary cords. Further, plasma cell tumors have not yet been observed to develop in the bone marrow cavities or other lymph nodes.

B. Factors Influencing Plasmacytoma Induction and Incidence

1) General. A basic technical problem in studying factors influencing plasma cell tumor induction in BALB/c mice is establishing the true incidence of tumors. Essentially incidence is defined as the number of plasma cell tumors obtained divided by the total number of mice alive after all the mineral oil treatments are given.

Groups of 30–40 female BALB/c mice (average size of an experimental group) are difficult to follow over long periods, for often individual mice die rather sud-

denly and unexpectedly of pneumonitis and other conditions during the course of an experiment. If this happens overnight or on a weekend, the mouse is autolysed before any diagnosis can be made from tissue sections. In order to establish an incidence, mice must be examined at least 3 times weekly for signs of illness (weight loss) and the development of ascites (bloody). All mice with ascites should be tapped and smears made to detect abnormal cells. Mice with a nonbloody ascites that contains no malignant cells can be returned to their cages and re-examined in several days when deemed necessary. Although the diagnosis can be made from smears containing numerous characteristic cells, confirmation by tissue section is advisable in all cases where determination of incidence is critical. Maintenance of mice in closed colonies could potentially aid in reducing the number of deaths due to infections. In our laboratory, where mice are brought in and out, we routinely treat mice with aureomycin in the drinking water to control pneumonitis when it appears (206). Since considerable time is involved in an incidence experiment it is important to carefully select a group of mice that have been successfully acclimatized to the animal room and probably have developed a good natural immunity to the common flora in the room. Female mice are used preferentially because they can be housed in groups of eight; male mice under these conditions fight and kill each other.

An incidence of 60% of plasmacytomas is a good yield of tumors in an average experiment (see Fig. 1). However, other factors, many not well understood (e.g. immunization, infections, stress, natural immunization, etc.), influence plasma cell tumor development and affect incidence and hence experiments must be carried out with adequate controls.

2) Genetic. Plasma cell tumors have been induced in high incidence in only two inbred strains, BALB/c (166, 206) and NZB (294). Relatively few other strains have been studied: DBA/2, A/He, A/LN, C57BL/He, C57BLKa, C3H/He, and SWR (166). An occasional plasma cell tumor has been found after mineral oil injections or Millipore diffusion chamber implantation in members of these strains (166, 206, 307). The restriction of plasma cell tumor susceptibility to a few specific inbred strains of mice suggests that specific genes are involved in plasma cell tumor development. None of these have yet been identified. An interesting experimental problem in the future will be to find how genetically determined factors influence plasma cell tumor development.

First-generation (F_1) hybrids of BALB/c and other strains develop plasmacytomas after injection of mineral oil (81, 206, 294). The incidence is usually low and depends on the contribution of the plasma cell tumor-resistant parent. We reported plasmacytomas in 4 of 32 (BALB/c × AL/N)F_1, 0 of 82 (BALB/c × DBA/2)F_1, 4 of 25 (BALB/c × C57BL/Ka)F_1, and 2 of 40 (BALB/c × NH)F_1 (206). Probably several genes are involved in susceptibility, and the different strains may contribute susceptibility as well as resistance factors to the hybrids. Noting the unusually high incidence of 60% plasmacytomas induced in (BALB/c × NZB)F_1 (81) hybrids, Warner (294) suspected that NZB itself might be susceptible to mineral oil induction. Warner (294) treated NZB mice with three injections of medicinal parrafin at 2, 3, and 4 months of age and obtained an incidence of 35%

plasmacytomas (personal communication). It is likely that strain NZB is as susceptible to plasma cell tumor development as BALB/c but that the incidence is usually reduced because the mice die intermittently from other causes. The discovery of the susceptibility of NZB mice is an important new finding. These mice develop autoimmune disease (63, 164, 268), and also 4 of 20 NZB/B1 mice 9–11 months old were found to have developed lymphomas spontaneously. Two of these were lymphoid or plasmacytic in morphology; these were both associated with immunoglobulin production. Further, NZB mice that develop autoimmune hemolytic anemia also have splenomegaly associated with hyperplasia of reticulum cells and plasma cells.

The possible association of genetically controlled factors that regulate the immune response with plasmacytoma development is suggested by the finding of plasma cell tumors in both NZB and BALB/c strains of mice. Common characteristics of immune responses in the two strains may provide a clue. Staples and Talal (268) noted that NZB and BALB/c mice, unlike other strains studied, did not become tolerant to ultracentrifuged human gamma globulin; instead both strains actively produced antibody. The enhanced immune responses were attributed to different mechanisms. Others have observed exaggerated responses in BALB/c mice to specific antigens (20, 34). Further, BALB/c mice treated with antithymocyte serum develop severalfold more plaque-forming cells in response to the pneumococcus type III polysaccharide than is observed in other strains.

3) Hormonal factors. Takakura et al. (276–279) have studied the role of hormones; their findings have been reviewed and discussed by Hollander et al. (102). Using heavy mineral oil (Bayol 355 or Primol D), differences in response of males and females were investigated. In one experiment plasmacytomas developed in 57% of male BALB/c mice but only in 26% of female mice. When the mice were gonadectomized, the incidence was reduced to 29% in males and was raised to 61% in females. Females treated with 0.1 mg testosterone 5 times a week during the period of plasma cell induction and during the latent period developed an incidence of 88% plasmacytomas in less than a year. The high incidence and short latent period indicate testosterone played an important role in the development of plasmacytomas.

It is not clear as yet how testosterone affects plasma cell tumor formation. Takakura et al. (279) have provided evidence that transplantable plasmacytomas can be stimulated to grow more rapidly with 0.1 mg testosterone daily, suggesting that testosterone during induction directly stimulated growth. It may be argued that testosterone could affect plasma cell tumor development indirectly by interfering with the function of the thymus. For example, testosterone inhibits radiation-induced leukemogenesis in the C57BL/Ka mouse by causing thymic atrophy (113).

The effects of progesterone and estradiol on plasma cell tumor development have also been studied by Takakura et al. (279); progesterone (0.01 mg/day) and estradiol (.01 mg/day) inhibited plasma cell tumor development. In a separate study Takakura et al. (276) have also shown that continuous cortisol treatments (0.1–0.5 mg 5 times a week during induction and latent period) profoundly inhibit plasma cell tumor development in both males and females. They noted that

cortisol suppresses inflammatory responses in the peritoneal connective tissues, inhibiting the development of lipogranuloma, and in extreme cases reduces the granuloma to pinpoint whitish lesions. The number of inflammatory cells in the ascites was also greatly reduced, and the life span of mice was increased. Hollander (102) suggested that cortisol might act by suppressing the inflammatory response in the peritoneum. The possibility that cortisol acts more centrally by reducing available lymphoid cells has not been ruled out. It has been known for some time that cortisone induces a severe thymic cortical atrophy characterized by a loss of cortical small lymphocytes; it has been found, however, that the remaining medullary thymic lymphocytes are immunocompetent. Cohen and Clayman (44) found that hydrocortisone given in a single large dose of 2.5 mg/mouse depresses the humoral immune response to sheep red blood cells (RBC) by inhibiting B lymphocytes. It may be speculated that the prolonged cortisone treatment used by Takakura et al. (276) reduced plasma cell tumor formation by reducing the availability of precursor cells.

Several curious findings developed from the study of the influence of pituitary hormones on plasma cell tumor development (277, 278, 309). Mice given 3 bimonthly intraperitoneal injections of Bayol 355 were treated with daily subcutaneous injections of various glycoprotein hormones: ACTH, bovine TSH, porcine FSH, or ovine LH. Bovine serum albumin (0.5 mg/day) was used as an inactive protein control. Plasma cell tumor incidence was high in both untreated controls (49/60) and albumin-treated mice (17/20), intermediate in ACTH-treated mice (8/30), but very low in TSH- (2/20), FSH- (2/20), and LH- (3/20) treated mice. Subsequent studies on the effects of bovine growth hormone and bovine prolactin demonstrated that growth hormone accelerated plasma cell tumor formation and gave an incidence comparable with that observed in mice receiving only the oil injections. Several controls were used: albumin, 0.5 mg/day; bovine serum glycoprotein, 0.1 mg/day; chondroitin sulfate, 0.1 mg/day; and carboxymethylcellulose, 0.5 mg/day. None of these significantly reduced the incidence of plasma cell tumors.

As yet there is no explanation for the results. However, Hollander et al. (102) suggest that some form of immune reaction appears to suppresss plasma cell tumor development. They noted that treated mice all developed characteristic oil granulomata but that the peritoneal fluids of mice treated with TSH, FSH, and LH and the steroid progesterone contained numerous mastlike cells. [Excellent illustrations of those cells appear in the review by Hollander et al. (102).] The mastlike cells appeared to have a phagocytic function and some cells were observed with phagocytized tumor cells. It was also found that some mice had plasma cells in their peritoneal fluids, but these tended to disappear over a period of weeks. In addition, evidence of the development of antibodies cytotoxic for plasma cells was observed in these mice as well as in mice treated with oil alone.

A highly tentative explanation offered by Hollander et al. (102) for the apparent specificity of some of these glycoprotein hormones is that the hormones induce antibodies that cross-react with antigens on the tumors.

4) Immunosuppressants. Mandel and DeCosse (152) have studied the effect of

rabbit antimouse thymocyte serum (ATS) on plasmacytoma development. An increased incidence of plasma cell tumors was observed when ATS was given over a period of 4 months beginning with the 1st of 3 monthly injections of mineral oil. The incidence and rate of plasmacytoma development were both increased over that observed in controls. In the same study thymectomy or a single exposure to 250 r of total-body X irradiation did not alter the incidence or rate of tumor development. Prolonged treatment with cyclophosphamide or azathioprine reduced drastically the plasma cell tumor incidence.

Evidence has also been obtained to show that the intraperitoneal injection of mineral oil (35, 120) or the subcutaneous implantation of plastic cylinders (244) (a condition possibly simulating the intraperitoneal implantation of a large plastic disc) depresses immune responses. Chakrabarty and Friedman (35) have shown that the intraperitoneal injection of emulsions of incomplete Freund's adjuvant interferes with immune response to intraperitoneally injected sheep RBC. They postulate the oil causes a local blockage of the macrophages. Kripke and Weiss (120) and Saal et al. (244) imply the foreign materials affect immune responses occurring at sites other than where the oil or plastic has been deposited.

5) *Antigenic environment.* McIntire and Princler (158) injected 45 germfree (GF) BALB/c mice and 45 ex-GF BALB/c AnN (removed at 1–2 months of age from GF tanks) with autoclaved Bayol F and produced only 2 plasma cell tumors in the GF mice and 24 plasma cell tumors in the ex-GF mice. This reduced number is not a reliable incidence value because many mice were lost during the experiments; however, the result does reflect a striking reduction in plasma cell tumor development and has been confirmed in subsequent experiments by McIntire (personal communication). Many of the GF BALB/c mice were immunized successfully during the progress of the experiments to DNP-ovalbumin, hemocyanin, or ferritin. The 2 plasma cell tumors developed in mice that had not been immunized. While the GF mice did not have a high incidence of plasmacytomas, they did develop an increased number of reticular neoplasms (none was associated with the appearance of high levels of unique immunoglobulins). The absence of a bacterial flora apparently reduced the incidence of tumors. Presumably the microbial flora of the gastrointestinal and respiratory tracts provide a continuous source of natural antigens (50) that stimulate the production of plasma cells.

C. Immunoglobulins in Plasmacytoma Development

The predominant heavy-chain class of immunoglobulin expressed in plasma cell tumors is IgA (Table 2). In at least four separate studies so far reported, the incidence of IgA-forming tumors is roughly 60–65%. In multiple myeloma in man the ratio of IgG/IgA is roughly 60/30 (for refs see ref 210). The proportion of plasmacytomas making IgA does not reflect the relative concentration of IgA among the immunoglobulins in serum. For example, in the serum of BALB/c mice the IgA is less than 15% of the total immoglobulin; further, these serum IgA levels are relatively low (0.7–0.8 mg/ml) in comparison with other strains (21, 23, 24). Serum IgA levels are believed to be maintained by lamina propria plasma cells

TABLE 2. *Immunoglobulin classes in the mouse expressed in plasma cell tumors*

Immunoglobulin	BALB/c Normal Serum Levels[a], mg/m	Distribution of Heavy-Chain Classes in Myeloma Protein				
		Merwin (166)	Cohn (45)	McIntire (158)	Potter[b] (208)	Grey (88)
IgM	0.8		0	2	0	0
IgA	0.7	20	53	29	66	120
IgG						
γ (γF)	2.4		9	2	10	19
γ (γG)	1.4	3	3	4	7	10
γ (γH)			6	5	11	11
γ (γJ606)	0.1					1
κ	[c]	2	3	4	6	
λ	[c]		0		2	
Two-chain IgA	[d]		0		0	
Nonproducer		2	41	5	9	
Total		27	122[e]	51	111	161

[a] From Barth et al. (21). [d] 111 consecutively transplanted tumors. [c] κ:λ ratio = 97:3 (159). [d] We have observed 6 two-chain IgA proteins over the years. [e] A number of cases have not been typed.

(23, 24, 96). The preponderance of IgA-producing plasmacytomas in the BALB/c mouse suggests that cells differentiated to make IgA immunoglobulins are more disposed to undergo neoplastic transformation than other plasma cells in the organism; this could be due to: *1*) an inherent susceptibility of cells differentiated to make IgA immunoglobulin; or *2*) the preferential predilection of cells differentiated to make IgA to migrate into the peritoneal tissues, where the neoplastic change appears to develop. IgA-forming cells in the normal mouse are distributed chiefly in the lamina propria of the gastrointestinal and respiratory tracts (50). Mandel and Asofsky have shown that circulating thoracic duct cells in mice are capable of IgA synthesis (150). Craig and Cebra (51) in the rabbit demonstrated with immunofluorescence that Peyer's patches were an enriched source of IgA-producing cells that also possessed a special efficiency after intravenous injection to home into the lamina propria of the gut. Growing evidence suggests that antigenic sensitization from antigens entering via the gut or respiratory tracts involves IgA-committed cells that circulate and later home into other lymphoreticular tissues (96). Should the peritoneum and the peritoneal oil granuloma possess a microenvironmental cue for attracting cells differentiated to make IgA then the preponderance of IgA-producing plasmacytomas in the mouse can be explained.

Changes in immunoglobulin levels during mineral oil-induced plasmacytoma development have been only briefly studied. Talal et al. (280) noted mice given Bayol F (4 injections, 0.5 ml each, 2 months apart) developed hypogammaglobulinemia and only after the 12th–16th week was there evidence of hyperimmunoglobulinemia. However, this was detected by immunoelectrophoretic changes. Immunized mice treated with Bayol F developed hypergammaglobulinemia (280). Yamada et al. (302) studied changes in total serum globulins (from stained cellulose

acetate strips) after 3 injections of Primol D. In BALB/c and strain A mice, but not DBA, C3H, and C57BL mice, the globulins increased steadily over a 4-month period. Kripke and Weiss (120) compared immune responses of BALB/c and C57BL mice during mineral oil treatment and found that BALB/c mice developed lower amounts of anti-T-2 phage antibody than did C57BL mice. Some evidence was provided to show that established plasma tumors grew better in mineral oil-treated BALB/c mice, but further work on this subject needs to be done.

D. Viruses: Intracisternal Viruslike Particles, Viral Antigens

Every spontaneous and oil- or plastic-induced plasmacytoma so far examined by electron microscopy contains intracisternal type-A viruslike particles (53). These particles bud from the rough endoplasmic reticulum and may be seen in all stages of development (53); they have an outside diameter between 70 and 100 mμ (122) and consist of two concentric electron-dense membranes surrounding a central zone that is usually quite electron lucent. In some transplanted plasmacytomas and tissue-cultured cells, the number of particles per cell may be extremely abundant, i.e. hundreds of particles can be observed in one cell (125, 198).

The intracisternal type-A particle associated with plasmacytomas is apparently different from the intracytoplasmic A particles associated with mammary tumors in mice. Howatson and McCullough (106) were the first to note these viruslike particles in plasymacytomas. They examined the X5563 plasmacytoma of C3H/He CRGL origin and found viruslike particles in the Golgi region. Subsequently Dalton et al. (53) examined induced BALB/c plasmacytomas and other C3H plasmacytomas and found two types of particles: the intracytoplasmic A particle and the intracisternal A particle. Some of these mice carried the mammary tumor virus (MTV). Electron-microscopic studies of BALB/c plasmactyomas in which the MTV was excluded revealed the presence of only intracisternal A particles. Dalton and Potter (52) induced plasmacytomas in BALB/c mice carrying the mammary tumor agent; 17 plasmacytomas contained the intracisternal A particles, while 7 others contained in addition the intracytoplasmic A particles. Those containing the intracytoplasmic A particles were found also to have type-C particles associated with the cell membrane. The intracytoplasmic A particles resembled those observed in mammary tumors; however, no mature B particles with electron-dense nucleoids were observed in the plasmacytomas.

In independent studies Wivel et al. (304) have reported that normal BALB/c lymphoid cells from the thoracic duct that contain endoplasmic reticulum (possible plasma cells themselves) also contain intracisternal A particles and a type-C particle. This finding indicates that normal BALB/c cells contain the particles prior to injections of mineral oil. Also, intracisternal type-A particles have been found in tumors of other cell types, e.g. neuroblastoma, sarcomas (305), and epithelial tumors induced by methylcholanthrene (122).

Kuff et al. (125) have isolated intracisternal A particles from plasmacytomas by subjecting microsome fractions to mechanical shear in the presence of optimal concentrations Triton X-100. The particles were then concentrated in sucrose

density gradients and analyzed. Although the fraction contained some minor contamination with microsomal membranes, the material contained 80% protein, 15% phospholipid, 5–6% RNA, and no DNA. About half of this RNA was believed to be contributed by the viruslike particle. Injection of highly purified fractions of intracisternal A particles into newborn BALB/c and C3H mice failed to induce plasmacytomas (125) or leukemias. Kuff et al. (122) isolated from intracisternal A particles a major structural protein (mol wt near 70,000 daltons) by solubilization with 1% SDS and mercaptoethanol at pH 7.1. The major protein component gave a characteristic band in acrylamide-gel electrophoresis. Rabbit antisera prepared to the protein were specific. Using complement fixing or precipitin tests carried out in the presence of 0.01–0.1% SDS the intracisternal A structural protein was identified in extracts of murine plasmacytomas, neuroblastomas, methylcholanthrene-induced epidermal carcinomas, two fibroblast tissue-culture lines (NCTC-4700 and 4953), L1210 leukemia, and the Ehrlich ascites carcinoma. Preparations of the MTV and Rauscher and Moloney leukemia viruses were negative. Highly purified intracisternal A particles have been examined for the presence of MuLV antigens and these have not been found (122).

The constant presence of intracisternal A particles has suggested to many that a virus may be implicated in plasma cell tumor development. Newborn mice injected with extracts of plasmacytomas do not develop leukemia (297) nor plasmacytomas (125). This assay method, however, may not be considered evidence against the viral role in plasma cell tumor development because other essential factors may not have been satisfied. First, induced plasmacytomas develop in the peritoneal connective tissues and may require a granulomatous tissue environment. Second, the particles may represent evidence of viral infection but lack infectious properties themselves because they are incomplete forms.

The relationship of the intracisternal A particle to the MuLV has not been established but the lack of sharing of common antisera suggests that the A particle is not a defective form of the MuLV or a precursor. This situation does not exclude the possibility that plasmacytomas of BALB/c mice also carry viruses related to the MuLV group. Watson et al. (297) have reported that both extra- and intracellular virus particles isolated from tissue culture-adapted plasmacytomas contain the leukemia virus group-specific antigen.

The cell membrane particle was a C-type particle, one that resembles those associated with leukemia viruses in many species. RNA isolated from the viruses in the plasmacytomas had similar properties to MuLV RNA (297).

The observations of Watson et al. (297) have been confirmed in part by Stockert et al. (270) and Herberman (95), who have observed antigens associated with the Gross MuLV on plasmacytomas maintained in vivo. Stockert et al. (270) tested six transplantable myelomas for two antigens—the GCSA, detected by a cytotoxicity test using a C57BL anti-AKR leukemia antiserum; the G_{IX} (also a cell surface antigen found on normal lymphoid cells), detected by a cytotoxicity test using a W/Fu rat anti-Gross passage A-induced W/Fu rat leukemia. Of six BALB/c plasmacytomas tested one was GCSA positive, G_{IX} positive; one was GCSA positive, G_{IX} negative; three were GCSA negative, G_{IX} positive; and one was negative

for both. The G_{IX} antigen is not found on normal BALB/c lymphoid cells but does appear on cells infected with murine leukemia virus. The reason why the antigens, both of which are associated with MuLV, vary independently is not clear. The data however, do support the findings of Watson et al. (297).

Tissues from old normal BALB/c mice (94) and tissue-culture lines derived from normal BALB/c embryos have been repeatedly shown to possess antigens or "tissue-culture" infectious viruses related to the MuLV group; further, these viruses can be induced in negative cultures by bromodeoxyuridine (1, 146).

Whether one of the viruses in the MuLV group is responsible for the neoplastic transformation is an unresolved question and awaits the development of an experimental system to prove that it is or is not an essential factor.

E. Karyological Changes

The large size of neoplastic plasma cells may be related to aneuploidy and hypotetraploidy. Yosida et al. (314) examined 16 transplantable plasmacytomas and found that 13 had hypotetraploid chromosome numbers; 1 had a hypertetraploid stem-line modal number, 1 had a hypotriploid number, and 1 was hyperdiploid (314). As the tumors were progressively transplanted, the range of chromosome numbers that was usually quite variable in early transfer generations became more tightly clustered around a modal number. Many of the tumors contained marker chromosomes and these varied from tumor to tumor. Schubert et al. (254) have also found abnormal chromosome numbers and have noted that clones derived from these tumors have more restricted numbers and tend to cluster around a modal number. Yosida et al. (313), in a recent study of primary oil-induced plasmacytomas, again found that about half of the primary tumors were hypotetraploid and the other half were aneuploid around the diploid number. Bimodal chromosome numbers with peaks in the near-diploid, near-tetraploid range have been observed in some tissue-culture lines (254).

It is possible that the factors contributing to aneuploidy and tetraploidy in the BALB/c plasmacytomas may also play a role in the carcinogenic process in these cells. This speculation is based on the high frequency of abnormal chromosome numbers in transplantable and primary plasmacytomas and the large cellular size of most primary plasmacytomas. However, Moriwaki et al. (175) have observed that a diploid plasmacytoma (MSPC1) continuously gave rise to new diploid and tetraploid variants during 50 transfer generations. In the first 10 transplant generations the frequency of tetraploid cells fluctuated from 0 to 100% in different individual hosts. Total-body irradiation of the host did not increase the incidence of tetraploid cells. In general the shift appeared to be from diploid to tetraploid. The findings suggest the karyotype in plasma cells is unstable and continuously produces new variants containing new chromosome rearrangements; many of these variants are apparently unstable and degenerate during transfer. The evidence then would appear to support the notion that chromosome instability is a continuous property of the neoplastic cell and not an exclusive characteristic of the transformation process itself.

F. Spontaneous Ileocecal Plasmacytomas

The most common spontaneous plasmacytomas in mice develop in the ileocecal lamina propria; these tumors are associated with mucosal ulcerations and submucosal inflammation (59, 203). The connective tissue surrounding these ulcerations contains hyperplastic plasma cells, plasma cell infiltrates, and plasma cell infiltrates that invade the muscularis layers (59). Interspersed with many normal plasma cells are large bizarre plasma cells similar to those observed in tumors. Advanced tumors metastasize to the mesenteric node (59, 203). Dunn (58) first observed these tumors at the National Cancer Institute and found they occurred predominantly in C3H/He mice, with an estimated incidence of 1% of old C3H mice. Factors increasing the incidence are not known. A lamina propria plasmacytoma of the stomach has been described by Dunn (59). Pilgrim (203) has systematically studied the ileocecal regions of 125 old C3H/He CRGL/Pi mice. Of these cecums 69 appeared normal on gross inspection, but on microscopic examination they were found to have mucosal ulcers without evidence of inflammation or plasmacytoma development. Of the 56 that appeared abnormal on gross inspection, 55 were associated with mucosal ulcers, 26 of these with inflammation and 28 with plasmacytoma observed microscopically. The diagnosis of plasmacytoma was based on the presence of bizarre plasma cells in the ileocecal region. The tumors, however, did not metastasize nor were they transplantable. Three of 28 mice with plasmacytomas also had type-B reticulum cell sarcomas in adjacent lymphoid tissues. Ileocecal plasmacytomas in general have been difficult to transplant; only 5 have been successfully established: the IgG-producing X5563 (216), the IgA-producing X5647 (71), the SPC1 and the DPC1 [tumors all of C3H/He origin (59, 62, 215)], and the YPC1 tumor [which arose in (BALB/c × A)F_1 hybrids (310)].

G. Comments on Plasmacytoma Development

Induced and spontaneous plasmacytomas develop in different anatomic sites, the peritoneal connective tissues (or even the peritoneal space) and the lamina propria of the gut, respectively, which suggests that different sets of factors influence the two forms of tumor development.

In spontaneous plasmacytomas the ulceration of the ileocecal mucosa and prolonged inflammatory reactions occurring in the lamina propria itself may be important factors.

In induced plasmacytomas local factors may again play an important role. An unusual characteristic of the induced tumors is that roughly 60% of them produce IgA-class immunoglobulins, which suggests that the tumor cells are in some way related to the normal IgA-producing lamina propria population even though the tumors do not develop in the lamina propria. These peritoneal connective tissues and the lamina propria are not in direct anatomic continuity. Several experimental facts bear on the possible relationship of the two sites: *1*) normal IgA responses develop chiefly in response to antigens that enter through the gut and

respiratory tracts (96) (the absence of a microbial flora in germfree mice greatly reduces the induced plasma cell tumor formation); *2*) IgA-producing cells found in the lymphoreticular tissues (e.g., spleen and mesenteric lymph node) that arise from sensitization with antigens entering via the gut or respiratory tract probably do so by leaving the sensitization site and entering the circulation. A hypothesis on the origin of peritoneal IgA-producing plasmacytomas is that they are derived from normal precursors sensitized by antigens that entered via the gut or respiratory tracts. Progeny of these sensitized cells entered the circulation and the peritoneum. Their relative abundance in the peritoneum may be due to factors that attract IgA-differentiated cells. The supply of these cells can be apparently reduced by treatment with cortisone or other agents known to reduce the supply of B cells. Also precursors of plasma cells can possibly be increased, as indicated in the experiments of Mandel and DeCosse (152), by inhibiting the thymus-derived T cells with ATS. Presumably ATS or ALS (antilymphocyte serum) enhances antibody production by selectively eliminating a population of thymus derived cells that suppress B-cell responses (20).

Factors related to the neoplastic transformation itself are more obscure. Some of these are no doubt inherited genetically determined influences, which may control the growth and proliferation of plasma cells. The role of viruses (related to the murine leukemia virus complex) that are transmitted along with the germ-line genome of the mouse is also suggestive. Possibly cells that produce large amounts of virus may be more prone to develop neoplastic transformation. The evidence clearly shows the ubiquitous presence of intracisternal A particles in all plasmacytomas. The continuous presence of viruses acting in concert with factors increasing plasma cell proliferation may increase the chances for developing somatic mutations affecting growth regulation. Pathological factors related to the abnormal environment of the granulomatous peritoneum contribute an important influence that permits the transformed clones to attain a dangerous population density. One can only speculate as to what these factors might be, but such influences as the obstruction of the passage of fluid through the peritoneum (brought about by the oil- or plastic-induced granulomatous changes) might cause cells to remain too long in an adverse environment. Should the abnormal peritoneum exert a selective force in encouraging cells to proliferate, this alone may allow defective mutants to develop into tumors.

III. TUMORS OF IMMATURE CELLS IN IMMUNOCYTE SERIES

The immediate precursors of the plasma cells are the immunoblasts (immunoglobulin-secreting cells with a lymphoid morphology) and the lymphocytes (nonsecreting thymus- and bone marrow-derived lymphocytes) that do not secrete immunoglobulin but nonetheless produce immunoglobulin on their cell surfaces. Tumors of these cell types present certain special problems related to the detection and characterization of the immunoglobulin. Only recently has attention been focused on these potentially interesting tumors, and data are still being formulated. Much of the discussion is centered about specific model systems that have been

evaluated for immunoglobulin production. There is an extensive literature on the pathogenesis of lymphocytic and reticulum cell neoplasms (the pathological designation often used to describe tumors of possible immature immunocyte origin) that is not covered in this review since few attempts have been made to relate these neoplasms to the immune system until recently.

A. Nonsecreting Tumors

It is now well accepted that there are at least two populations of lymphocytes in mice (174, 227, 281) that are associated with the immune system: the thymus-derived lymphocytes (T cells) and the bone marrow-derived lymphocytes (B cells). Both forms are believed to have immunoglobulin receptors on their surface (83, 185). B cells, which have the most readily demonstrable surface immunoglobulin (185, 201), can develop into mature immunoglobulin-secreting plasma cells (183). By contrast immunoglobulin receptors have been difficult to demonstrate on the surface of T cells (83, 185) and are probably present in much less amount than on B cells (185). The receptor immunoglobulin on the surface of B cells contains μ-chains. This has been shown by Nossal et al. (185), using highly sensitive radioimmunolabeling techniques, and indirectly by Lawton et al. (128), who have demonstrated that administration of goat antimouse μ-chain antiserum to newborn germfree mice greatly suppresses the normal development of germinal centers, plasma cell development, and immunoglobulin formation.

Very few systematic studies of lymphocytic neoplasms (leukemias) in the mouse have been made in order to demonstrate immunoglobulin receptors. Most lymphocytic neoplasms in the mouse are of thymic origin and carry the theta antigen on their cell surface (201). No immunoglobulin has yet been demonstrated on neoplasms of T cells, since this immunoglobulin is very difficult to demonstrate even in normal cells, but it may become possible in the future to show its presence. If this could be done it might then be possible to show the functional individuality of lymphocytic neoplasms as has been demonstrated for plasmacytomas. Among several transplantable leukemias tested, Shevach et al. (263) demonstrated with a rabbit antimouse kappa-chain antiserum an immunoglobulin of the surface of one tumor by direct fluorescence microscopy. This tumor (RBL3) was a Rauscher virus-induced lymphocytic neoplasm that was induced in strain C57BL/6.

B. Secreting Tumors

These tumors originate from immunoblasts and have a lymphoid or a lymphoplasmacytic morphology. In addition some of them have been called reticulum cell neoplasms and in particular reticiulum cell neoplasm type B or Hodgkin's-like lesion in the Dunn classification (58, 61). This designation raises some confusion because reticulum cell neoplasms in the strict sense should be reserved for tumors associated with reticulum fibers, e.g. tumors derived from the dendritic macrophages of lymphoreticular tissues.

As in the SJL/J disease, reticulum cell neoplasms have been associated with abnormal immunoglobulin production. The basis for this immunoglobulin formation is not established and it has not been shown that neoplastic dendritic macrophages in these tumors produce the immunoglobulin. (Other explanations are discussed in sect. III, *B2.*)

Rask-Neilsen and Gormsen (235) were the first to recognize the plasmacytoid morphology in lymphomas in mice and they subsequently demonstrated that many of these tumors were able to produce immunoglobulin (231). Collectively these tumors have been designated plasma cell leukemias by Rask-Nielsen and coworkers. Included among these are the IgM-M component-producing tumors of lymphoplasmacytes (39, 239). In man these tumors are lymphomatous tumors associated with macroglobulinemia (Waldenstrom's macroglobulinemia).

1) Plasma cell leukemia (Rask-Nielsen). Plasma cell leukemia[1] is a term used by Rask-Nielsen and coworkers (66, 235, 236) to describe a group of neoplasms that are biologically different from the peritoneal and ileocecal plasmacytomas just described. First, these tumors fall into the lymphoma group in that they appear to develop in the lymph nodes, spleen, or other lymphoreticular tissues rather than the peritoneal connective tissues. Second, they are associated with abnormal production of immunoglobulins but on a less regular basis than plasmacytomas. Third, they are not a morphologically homogeneous group of tumors as are the plasmacytomas but rather a mixed group containing several forms. Fourth, plasma cell leukemias are often associated with amyloid production, which the plasma cell tumors are not.

The original Rask-Nielsen and Gormsen classification, to which they have adhered throughout, was based almost entirely on morphology. Essentially it was based on the concept that many of the tumors contained cells that resembled plasma cells and on the consideration that these different forms could be arranged into a graded series (grades I–IV) in which the most differentiated tumor types would contain differentiated types (grade I) and the most undifferentiated would contain undifferentiated cell types (grade IV). Since the 1951 publication and with the availability of the description of the morphologic and biologic characteristics of ileocecal and peritoneal plasmacytomas, Rask-Nielsen and Gormsen have recognized they do not have tumors of differentiated plasma cells similar to the plasmacytomas. Moreover, the neoplastic nature of the original plasmacytomas was not established. The current classification consists of grades II–IV. Neoplasms of grades II and III contain predominantly plasmacytoid or lymphoid cells with morphologic characteristics that have some plasma cell features, grade III would be more lymphoid than plasmacytoid, and grade IV is undifferentiated and is called a reticulosarcoma.

Lymphomas very similar to those described by Rask-Nielsen have been ob-

[1] *Plasma cell leukemia* was originally introduced in 1951 at a time when *leukemia* in mice was a general term for lymphoma, leukosis, or leukemia. Rask-Nielsen has continued to use it, although it is not meant to indicate all the tumors have leukemic blood pictures. However, Rask-Nielsen et al. (39, 237) have also repeatedly noted that many of the plasma cell leukemias are associated with the appearance of neoplastic cells in the peripheral blood.

served commonly in many strains in mice. However, in most studies the relationship of these tumors to immunoglobulin-producing cells has not been established. The importance of the work of Rask-Nielsen and colleagues is that they have related certain lymphomas in mice to immunoglobulin-producing cells.

A) SPONTANEOUS AND INDUCED PLASMA CELL LEUKEMIAS. In an initial study in 1951 (235) done with Street mice three groups were studied: *1*) 1912 untreated mice, among which 6 plasma cell leukemias were found between 14 and 18 months of age; *2*) 1229 mice that received an intrathymic injection of a low dose of a polycyclic hydrocarbon (carcinogen), among which 5 plasma cell leukemias and 1 plasmacytoma were observed; and *3*) 1350 mice that received large doses of carcinogen, among which 5 plasmacytomas were observed. This was the first report of neoplasms related to plasma cells in mice.

In 1956 Rask-Nielsen and Gormsen (236) extended their search for similar tumors in strains DBA/1, DBA/2, C3H, C57B1, CBA, and hybrids including (DBA/2 × CBA)F_1 and again injected 9,10-dimethyl-1,2-benzanthracene into various lymphoid organs. The overall spontaneous incidence of neoplasms called plasma cell leukemias was 0.5–4.3% among 1925 mice, whereas the incidence was increased to 2.3–13% in treated mice. In 1958 Rask-Nielsen (230) studied the effects of grafting CBA and DBA/2 thymuses into (CBA × DBA/2)F_1 hybrid mice; they observed that old (18 months or older) (CBA × DBA/2)F_1 hybrids in both grafted and control groups developed "leukemias," over half of which were "plasma-cell leukemias" grades II–IV. Thymic grafts from DBA/2 or CBA parents did not increase the incidence of leukemias, but the injection of leukemic tissues that had been killed by irradiation did increase the incidence of leukemias.

Attempts were made to transmit plasma cell leukemias with cell-free extracts. Preparations derived from tumors arising in (CBA × DBA/2)F_1 hybrids and

TABLE 3. *Incidence of leukemia in BALB/c females injected with subcellular materials (66)*

Source of Subcellular Fraction	Age When Injected, days	Total No. Mice	Number of Leukemias		Plasma Cell Leukemia, %	Age of Appearance, months
			Plasma Cell	Other		
CBA × DBA/2*	1–7	19	12	0		6–27
	30	24	15	0		17–25
DBA†	1–7	26	21	1		13–27
	30	35	14	3		9–25
BALB/c‡	1–7	66	38	1		14–27
	30	22	8	4		18–26
Total		192	108	9	56.2	
None (controls)		70	10	6	14.2	10–28

* Transplantable leukemic tissues from spontaneous cases; 4 different tumors used. † DBA/2 mice (without leukemia) that had been inoculated with (and rejected?) (CBA × DBA/2) hybrid leukemias. ‡ BALB/c leukemias were used.

BALB/c were injected into neonatal and newborn mice (Table 3). An overall incidence of 56% plasma cell leukemia was observed in the injected mice versus 14.2% in the controls (66).

Ebbesen and Nielsen (64) studied seven immature plasma cell leukemias with the electron microscope and found a few percent of the cells in each of the tumors contained intracisternal A particles (64), the same type of viruslike particle associated with induced plasmacytomas (52, 53). No type-C particles were observed.

B) PARAPROTEINEMIA. Many of the plasma cell leukemias have been associated with paraproteinemia (40, 231, 237–240), a term that is used to mean abnormal production of immunoglobulin and that may include monoclonal or polyclonal components. Essentially two groups of tumors have been characterized—those arising in (CBA × DBA/2)F_1 (234) and those arising in BALB/c (240). Of the 213 grade II–IV leukemias observed in (CBA × DBA/2)F_1 hybrid series, 96 were studied pathologically and serologically; 50 of the cases were plasma cell leukemias (35 grade III and 15 grade IV) and 23 of these were associated with paraproteinemia (monoclonal or polyclonal increases of immunoglobulin in the serum. Among the remaining 46 neoplasms, 11 were reticulosarcomas and 1 of these was associated with paraproteinemia. The 23 cases with paraproteins were typed serologically, usually by immunoelectrophoresis; 19 were IgG, 2 were IgA, and 2 were IgM. Several of the transplant lines were further studied because of interesting biological characteristics; these are summarized in Table 4. Line 45 produced an IgM component and resembles human Waldenstrom's macroglobulinemia (39, 239, 288). During serial transplantation the plasma cell leukemias often tended to revert to reticulosarcomas and also lose the capacity to produce immunoglobulin (234).

When plasma cell leukemias from this study in BALB/c mice were examined serologically 44 of the 95 cases showed evidence of paraproteinemia on agar-gel electrophoresis and immunoelectrophoresis (240). On the basis of the immunoelectrophoretic arcs alone the paraproteinemias were typed: 9% were γF, 55% were γG, 34% were γH, and 2% were γM. No IgA components or Bence Jones proteins were observed. Nineteen of the lines were transplanted and 14 produced a similar protein. However, the lines often lost their protein-producing properties, and for this reason further immunochemical characterization of the abnormal immunoglobulins was not possible.

The line RN 140 of BALB/c origin (240) has been carried in transplant for many generations and continues to produce an IgM-M component. This line grew as a lymphoma.

C) AMYLOID. In amyloidosis, fibrillary protein materials that characteristically stain with Congo red and possess polarization birefringence are deposited in various tissues. Amyloid is related to the discussion of immunoglobulin-producing tumors because amyloid deposition has been associated clinically with proliferative disorders of plasma cells (188). Although amyloid occurs spontaneously in mice (60) it can be readily induced by a variety of means (for review see refs 43, 267), the most common of which is the injection of casein solutions and the intraperitoneal injection of Freund's adjuvants (55, 228, 229). The chemical composition of

TABLE 4. *Transplantation characteristics of representative (CBA × DBA/2)F_1 plasma cell leukemias (Rask-Nielsen)*

Line	Morphology*	Mode of Growth	Leukemia	Type	Paraprotein, mg/ml	Stability‡	Amyloid	Ref
20	PC leukemia III, reticulosarcoma	Generalized lymphoma	+	γG	27	8	None	231, 237
45	PC leukemia III, with lymphocytoid PC, reticulosarcoma	Generalized lymphoma	+	γM		8–10		39, 239
75	PC leukemia III			γG	30	Stable		239
28	PC leukemia III			γA		?	+	238
37	Reticulosarcoma			γG†		Varies†	+	232
68	PC leukemias II, III, IV	Generalized lymphoma of transplant lines established	+	γG, (Varries)		Unstable Varies	3 lines + 4 lines −	65

+ = positive; − = negative.

* First entry is morphology in early transfers; second is morphology of later transfers. † γG paraproteinemia developed only in mice with rapidly growing tumor; in those with slower growing tumor amyloidosis developed. ‡ Number of continuous transfer generations during which paraproteinemia was observed.

murine amyloid has been recently studied in clinical amyloidosis by Glenner et al. (77), who demonstrated in man that some amyloid deposits contain fragments of immunoglobulin light chains (78). The immunoglobulin nature of the murine amyloid has not yet been established despite preliminary results that suggested that it might be immunoglobulin (77, 109).

Although amyloidosis has not been associated with either primary nor transplantable plasmacytomas (see sect. v*D*) it has been associated with reticulum cell neoplasms and the plasma cell leukemias (65). Ebbesen and Rask-Nielsen (65) described amyloidosis and paraproteinemia in transplant lines derived from a single plasma cell leukemia that arose in a 29-month-old (CBA × DBA/2)F_1 female mouse.

The original plasma cell leukemia (grades II and III) was transplanted through three transfer generations that required 17, 14, and 11 months to develop. In all three of these transfers the sera of the mice contained a single band electrophoretically. At the fourth transfer amyloidosis was also present and at this time the tumor grew more rapidly, killing the host in 20–40 days; seven sublines were then established.

In three of the transplant lines amyloid regularly appeared while in four others

it was rarely or not at all seen. The amyloid deposits occurred around the follicles in the spleen in the glomeruli of the kidneys (65); in some lines the amyloid deposition was restricted to either the kidneys or the spleen. Only two of the sublines were associated with paraproteinemia. Amyloidosis also occurs with other transplantable reticulum cell sarcomas. Wanstrup et al. (292) have shown a variety of unusual host reactions to a transplantable reticulum cell sarcoma, including amyloidosis. The plasma cell leukemias thus provide experimental systems that link amyloid with immunoglobulin-producing cells.

The studies of Rask-Nielsen, Gormsen, and Ebbesen indicate that many lymphomas in mice, particularly those in BALB/c, are in fact either themselves tumors of immunoglobulin-producing cells or stimulate immunoglobulin production (paraproteinemia) in some way not yet understood. The candidate cells involved are the precursors of plasma cells, i.e. circulating lymphocytes (derived from bone marrow), immunoblasts, and lymphoplasmacytes (in particular the IgM-producing types). Biologically tumors of these cells tend to proliferate in the lymphoreticular tissues and hence produce pathologically tumors of lymph nodes, spleen, and other lymphoreticular tissues. A viral origin has been suggested by the finding that more of these tumors appear in mice injected with cell-free materials; however, unlike other tumor virus systems these tumors have not developed earlier in life than the spontaneous forms.

2) SJL/J disease. Murphy (177) described a new inbred strain of mice (SJL/J) in which over 80% of the mice between the ages of 8 and 24 months (avg 13 months) develop nonthymic lymphomas. Most of these are pleomorphic or mixed-cell types commonly called type-B reticulum cell neoplasms by Dunn (58); a few are type-A histiocytomas (155) in the Dunn classification and lymphocytic neoplasms. Type-B reticulum cell neoplasms are among the most common types of tumors in old mice of many different strains, appearing usually in the lymphoreticular tissues of mice that are 18 months or older (61). The unusual feature of the SJL/J reticulum cell neoplasms is their regular and early appearance. Anatomically the first proliferative changes in SJL/J mice develop in the mesenteric lymph node and occasionally in Peyer's patches; later other lymph nodes and the spleen are involved. Siegler and Rich (264), in their study of the sequential histogenesis of the lesion, noted the early (beginning around 22 days of age) preneoplastic proliferation of large cells in the centers of cortical follicles in the mesenteric lymph node. The proliferating cells resembled "in size, pattern of growth and staining characteristics the reticular cells of germinal follicles" (264). These cells were regularly associated with macrophages that contained nuclear debris (tingible bodies). Cells containing tingible bodies are associated with germinal centers and are believed to reflect a high rate of cell death in rapidly proliferating germinal center cells. The proliferating cells in the centers of the cortical follicles continued to expand in numbers and increased the size of the lymph nodes to approximately 5 times normal. An important distinguishing characteristic about these abnormal expanding follicles was the relative absence of a cuff of perifollicular lymphocytes (264).

By 264 days of age many of the mice were considered to have neoplastic changes in their lymph nodes. About this period the follicles expanded and en-

croached on each other, ultimately destroying the architecture of the nodes. Siegler and Rich suggested that the "tumor" cell in this process is a "reticulum" cell. They illustrated that the cell has a poorly differentiated cytoplasm with a scant amount of rough endoplasmic reticulum. These cells are also capable of wrapping their outer cell membranes around bundles of collagen fibrils.

The aspect of the pathologic change in the SJL/J disease most difficult to understand is the granulomatous "degenerative" changes that occur in association with the proliferation. These consisted of: *1*) evidence of cell death, *2*) clumping or agglutination of tumor cells and plasma cells in follicles, *3*) giant cell formation, *4*) plasma cell hyperplasia surrounding and intermingled with the follicular areas, and *5*) fibrosis. These changes were believed to be due to the high rate of cell death of proliferating tumor cells, which in turn triggers a granulomatous reaction. The plasmacytosis that accompanies the process was considered to arise from either *1*) differentiation of the reticular cells or *2*) host reaction to the dying cells.

A variety of factors have been studied for their effects on incidence of lymphomas in SJL/J mice, including sex (92, 243), thymectomy (92), splenectomy (92), gonadectomy (92), total-body X irradiation (243), carcinogenic hydrocarbons, and superinfection with Gross leukemia virus (243). These results are summarized in Table 5, where it may be seen that various ablative procedures do not drastically reduce the incidence of the disease. Lymphocytic neoplasms were induced in SJL/J mice by Gross leukemia virus, total-body X irradiation at birth, or ingestion of 7,12-dimethylbenzanthracene (Table 5).

TABLE 5. *Factors influencing lymphomas and lymphocytic neoplasms in SJL/J mice*

Experimental Procedure	No. Mice	Sex	Lymphoma*		Leukemia†		Ref
			%	Mean age, days	%	Mean age, days	
None	131	F	70	388			243
	69	M	25	334			243
	55	F	78	380			92
	17	M	71	348			92
	51	F, M	73	480			155
Thymectomy	15	F	66	356			92
Splenectomy	17	F	65	450	7		92
Gonadectomy	26	F	61	350	8		92
Gonadectomy	30	M	57	347	3		92
DMBA‡	47		15	157	74	146	92
DMBA, Thymx	30		7	162	60	170	92
X irrad (175 r × 4)							
At 30 days	27		52	391			243
At birth	34				65	179	243
SJL/J extract	23		52	343			243
Gross leukemia virus	19				68	170	243
Graffi leukemia virus	37				100	63	243

* Reticulum cell neoplasm type B. † Lymphocytic neoplasm. ‡ 7,12-Dimethylbenzanthracene administered by gastric instillation once per week × 10.

The question of a viral influence on SJL/J disease has been investigated, with conflicting results. Yumoto and Dmochowski (316) reported type-C particles in SJL/J reticulum cell sarcomas, whereas Tkaczevski and Wanebo (284) found a few intracisternal type-A particles in transplanted reticulum cells but rarely in primary tumors. However, Fujinaga et al. (76) have attempted to transmit the disease with cell-free extracts to BALB/c mice. They succeeded in producing reticulum cell neoplasms (chiefly type A, i.e. histiocytomas) and other changes by intraperitoneal passage of an extract to 1- to 14-day-old SJL/J or BALB/c mice. Whether these workers have or have not transmitted reticulum cell neoplasms with cell-free materials will require further evaluation.

The SJL/J reticulum cell lymphomas, like other reticulum cell neoplasms in mice, are somewhat difficult to establish in transplant. Wanebo et al. (290) successfully grew 6 continuous lines from 16 advanced cases; McIntire and Law (155) grew 12 from 16 tries; and Haran-Ghera et al. (92) grew 7 of 12 lines attempted. Murphy (178) estimates 25–50 % of the tumors can be established. When the tumors grow they usually develop as lymphomas; i.e. the recipients develop large lymph nodes and spleens but little if any local growths. Often the first-generation transplants take considerable time to develop, and when this extends to many months it becomes difficult to distinguish the transplant from a new primary. During serial transplantation, tumors accelerate and produce lymphomas in shorter intervals; also the lines converge histologically to a more monomorphic reticulum cell type (178). It has been suggested the tumors are highly antigenic in the isogeneic host (178, 264). Carswell et al. (33) have demonstrated tumor-specific immunity by immunizing with heavily irradiated cells from 5 transplant lines in the 3rd–21st transfer generation. Carswell et al. (33) have attempted unsuccessfully to induce immunity to the primary disease, whereas Murphy (178) has obtained suggestive evidence that immunization with transplantable tumors may immunize against the primary disease. Wanebo et al. (290) report that primary and transplanted SJL/J mice lack the gs and TL antigens.

A) PARAPROTEINEMIA IN SJL/J DISEASE. Paraproteinemia has been associated with the SJL/J disease in two independent studies (155, 290). Wanebo et al. (290) serially bled 31 nontumorous mice each month starting at various ages from 5 to 14 months until they developed enlarged lymph nodes and spleen; 24 of the mice developed lymphomatous reticulum cell neoplasms during this period and 21 of these had abnormalities relating to the immunoglobulins. There were two essential types of abnormalities: *1*) an "anomalous" fast IgG component, which in immunoelectrophoretic analysis was evident by a bowing in the fast gamma arc (this region electrophoretically was anodal to the origin in the alpha or beta region); and *2*) monoclonal-like components. Of the 21 mice 7 had the anomalous fast gamma band alone, 11 had the monoclonal components, and 3 had both. The fast component was found to be γF or γ1. It is not clear yet from available data what this component represents. (Note: the author has observed several γF myeloma proteins with "fast" electrophoretic mobilities; thus these could represent separate monoclonal components.) Wanebo et al. (290) studied separately 46 other sera with abnormal proteins. McIntire and Law (155) made a similar

TABLE 6. *Paraproteinemia in SJL/J disease*

Condition	No. Paraproteinemia/Total	Monoclonal Components						Anomalous fast Fγ		Ref
		F	G	H	G + H	2 Components	Total (%)	Only	+M comp.	
With tumor	19/33	11	1	4	5	3	19 (57)			155
With tumor	34/34	13			6	5	24 (*)	7	3	290
Without tumor	12/12	3			2	1	6 (*)	5	1	290
With tumor	21/24						11 (87)	7	3	290

* Sera with paraproteinemia were selected for this study.

study of 33 sera. The results of these studies are summarized in Table 6. Aside from the anomalous fast gamma component both groups found M components. It is of interest that Wanebo et al. (290) found abnormalities in mice without evidence of overt disease.

However, the monoclonal-like components illustrated in these publications are not typical of those observed in plasmacytomas in that many of them are electrophoretically heterogeneous [see Fig. 15 of McIntire and Law (155), cases 1, 3, 5, 6, 7]. This may represent an unusual immunoglobulinopathy—one in which a selective population of immunocytes (i.e. cells producing IgG in lymph nodes) is involved. Possibly the clones occur against a generally expanded background and represent an extreme form of plasma cell hyperplasia in which there may be one or more greatly expanded clones. It is essential to have more rigorous evidence for monoclonality than has been presented; this should include serological (idiotypic) or chemical evidence, e.g. V-region subclass amino acid sequence data, to prove that one and not several clones are involved.

3) Other forms in PBA and NZB strains. Recently Bailey et al. (19) have described another inbred strain (PBA) that also develops a very high incidence of lymphoreticular neoplasms and an associated plasmacytosis much like the SJL. Abnormal immunoglobulin changes have not yet been reported in these PBA mice; however, they do have a diffuse hypergammaglobulinemia.

Mellors (164) has studied 20 NZB/B1 mice 9–11 months old and found 4 of them had lymphomas; 2 of these were reticulum cell tumors without paraproteinemia and 2 were lymphomas with a lymphocytic or plasmacytic morphology that is associated with paraproteinemia. These tumors were transplanted and the abnormal proteins were found in mice bearing the transplants. The lymphomas contained many bizarre plasma cells and also Russell body cells and may represent a true plasmacytoma. It is of interest that Warner (294) has reported the induction of plasmacytomas in mineral oil-treated NZB mice.

C. Comments

There are many similarities among the plasma cell leukemias and the SJL/J lymphomas. The one characteristic relevant to the present discussion is their

association with immunoglobulin production. The basic question that remains unresolved with both experimental systems concerns the origin of the immunoglobulins. There appears to be more than one type of origin.

First, it is clear that some of the lymphomas are indeed tumors of immunoglobulin-producing cells. Among these are the tumors of lymphoplasmacytes that are counterparts of the Waldenstrom macroglobulinemias. Another group may be tumors of the most primitive immunoglobulin-differentiated cells in the lymph nodes, the immunoblasts. It may be speculated that immunoblasts are usually lymphoid in morphology but also have reticulum cell characteristics. Since these cells are differentiated, the immunoglobulins they produce are homogeneous. Examples of neoplasms with both reticulum cell and plasmacytic morphology have been observed very rarely in man (186).

A second explanation for immunoglobulin production with the lymphomas and reticulum cell neoplasms is that the tumor cells themselves do not produce the immunoglobulin but rather the immunoglobulin is formed by reactive cells. Wanstrup et al. (292) have provided evidence that transplanted reticulum cells neoplasms in mice evoke a variety of reactive responses, including plasma cell hyperplasia. The polyclonal immunoglobulin changes in SJL/J disease and plasma cell leukemias also suggest this explanation. Further, it has been shown that the SJL/J lymphomas are highly antigenic in isogeneic situations (33, 178).

One of the intriguing aspects of the reticulum cell neoplasms and plasma cell leukemias is their pathogenesis. Here very little direct information is known. In SJL/J, NZB (164), and hybrid mice with chronic allogeneic disease (11, 257) unusual immunogenic stimuli may provoke a sequence of events in which the immune system is hyperstimulated. It might be argued that an excessive amount of immunogen is made available to antigen-sensitive cells, which leads to stages of hyperplasia of antigen-sensitive plasma cell precursors and then neoplasia. Plasma cell hyperplasia, for example, has been noted in chronic allogeneic disease (11). Although specific immunogens for reactions such as these have not been identified, viruses have been suggested as a possible antigenic source (153). It is of interest that in species other than the mouse several viruses are known that are associated with plasma cell hyperplasia and hypergammaglobulinemia. These are the well-known Aleutian mink disease (204, 303) and African swine fever virus (189). In Aleutian mink disease some of the hypergammaglobulinemia is associated with extremely high titers of complement-fixing antibodies to viral antigens (153), and also M components appear late in the disease (204).

Alternatively, excessive stimulation of the antigen-sensitive system may develop from faulty transport and localization of immunogen. For example, it is known that the reticulum cell neoplasms are associated with old age in mice (except the SJL/J), and recently Hanna et al. (91) have noted that old mice prone to develop reticulum cell neoplasms also have a defect in antigen clearance and antigen localization. Although the two phenomena—defective antigen clearance and localization and the development of reticulum cell neoplasms—were not directly correlated on a statistical basis in the study, the suggestion nonetheless is interesting and should be followed with analysis of other systems, particularly the

SJL/J disease. Possibly the primary abnormality in SJL/J disease resides in dendritic macrophages and the paraproteinemia for unexplained cause is a secondary consequence.

IV. PROPAGATION AND GROWTH OF PLASMACYTOMAS

A. Transplantation

Since transplantable plasmacytomas are used for many purposes by workers in various disciplines, it is helpful to summarize here some of their characteristics. The primary plasmacytoma that originates in peritoneal connective tissues is usually transplanted subcutaneously by the trocar method. As a rule 2–5 fragments of minced tumor are loaded into a 2.5-inch beveled 13-gauge trocar fitted with a smooth obturator that protrudes 2 3 mm. The trocar is introduced in the inguinal region and the fragments are delivered in the axillary region. First-generation transplant lines may appear as early as 3 months but in many other cases growth does not commence for 6–14 months after transfer. Individual nodules of primary plasmacytomas may be dissected out and transplanted under the renal capsule (205). Using this technique it has been possible to find different protein-producing transplant lines in the original primary host (205). In most of the cases adequately studied by serologic and biochemical methods it has been shown that the types of different protein-producing lines are derived from a clone; i.e. sister variants may include one line that produces excess L chains, and another that is balanced, or a line that produces only L chains, and another that produces two-chain IgA containing the same light chain. (217). Most lines obtained by this method represent the sort of variation obtained, for example, by Scharff and colleagues with tissue-culture cloning. Despite earlier suggestions (205) that more than one primary plasmacytoma can be isolated by this method, no two different primary plasmacytomas have yet been isolated from the same primary host. This phenomenon is usually explained as being due to the rarity of finding two clones with the same growth potential. Thus when a plasmacytoma becomes manifest in a mouse the tumor that provokes it has already outstripped other potential clones in the population. Primary transplantation by the intraperitoneal route with cells obtained from ascites has not been used routinely in our laboratory, as the incidence of takes has been very low.

Plasmacytomas are maintained in serial transplant by subcutaneous passage of tumor fragments with the trocar method. This has proved to be the most reliable means for maintaining continuous immunoglobulin production by a line. During serial transplantation some tumor lines may lose the property of immunoglobulin production, and in our experience this occurs more frequently in lines that are transplanted intraperitoneally. Intraperitoneal transplantation of cells teased from subcutaneous grafts or of cells that have been found free in the peritoneal cavity is effective in evoking new grafts. Intraperitoneally transplanted cells tend to invade the peritoneal connective tissues and may evoke a hemorrhagic

ascites. Thus intraperitoneally transplanted tumors are useful for the collection of large volumes of ascites fluid containing immunoglobulin. When it is desirable to obtain large quantities of a mycloma protein the subcutaneously transplanted line is converted temporarily to an ascites form. However, the basic stem-line tumor is maintained in continuous serial subcutaneous passage. Plasma cell tumors may be converted to fairly stable ascites forms but these rarely attain the cellular density associated with such ascites tumors as the Ehrlich ascites tumors or some of the lymphocytic neoplasms (15, 123). Fakhri and Hobbs (74, 75) have studied the growth rate of a stable γG (γ2a)-producing ascitic form of X5563. The tumor grew logarithmically between 4 and 8 days after the intraperitoneal injection of 1×10^6 cells and attained population sizes of 1×10^8 cells (74). During this period the serum level of the X5563 myeloma protein increased linearly; cell populations of 1×10^8 peritoneal tumor cells produced levels of 4 mg/ml of the myeloma protein (75). Individual mice that have formed an ascites associated with intraperitoneal transplantation may contain 5–15 ml of ascites that contain 1–20 mg of myeloma protein per milliliter, depending on the tumor.

Very recently we (224a) have found that primary plasmacytomas can be established rapidly in transplant by intraperitoneal injection of primary ascites tumor cells to mice that have received one or two 0.5-ml injections of mineral oil (given 1 month apart). We have established by this method 15 of 15 tumors using 10^5 cell doses; the lowest effective cell dose thus far has been 10^2 cells. During the time of observation most of the tumors have not developed in controls not treated with mineral oil. Several of the tumors have maintained an apparent dependence on the mineral oil-conditioned peritoneum for several transfer generations before acquiring the ability to grow in normal mice. In the first few transfers most of the tumors have produced immunoglobulin; further, all the cell lines derived from the same primary host have produced the same immunoglobulin, indicating the tumors that developed in the mineral oil-conditioned mice were of donor origin. These results indicate that the mineral oil-conditioned peritoneum has the ability to permit tumor cells to proliferate or provides a selective milieu for proliferation.

B. Colony Formation in Spleen

Bergsagel and Valeriote (25) studied the growth characteristics of the AdjPC5 plasmacytoma by estimating the number of spleen colonies after intravenous injection of cell suspensions. They found a linear relationship between the number of spleen colonies and the cell dose injected. Taking into consideration an estimate of the fraction of the population injected that actually reached the spleen, it was estimated that 4.1% of the cells in a given colony were capable of forming colonies themselves in reinjection into new hosts. A doubling time of 20 ± 4 hr was estimated for division in vivo. The basis for the difference between dividing and nondividing cells is not known.

A "spleen-cloning" method by inoculating 10^3–10^6 cells intravenously into young adults has been reported by Horibata and Harris (105); 10–14 days later

well-isolated nodules were dissected out to be retransplanted. Laskov and Scharff (127) cloned MPC11 by intravenous injections of 10^4–10^5 cells from cell suspensions filtered through 100-mesh stainless steel screen; 1–5 colonies per spleen were detected 2–3 weeks after injection. Parkhouse (personal communication) has been unable to "spleen-clone" the MOPC104 plasmacytoma from a solid tumor; however, he has been successful in obtaining clones from ascites cells of this tumor in the same general dose range given above.

C. Preservation of Plasmacytomas by Freezing

Most established lines of plasmacytoma may be successfully frozen (206) and preserved in liquid-nitrogen storage banks (115) without loss of viability or the capacity to produce immunoglobulin. Finely minced tumor fragments are placed in media containing either 10–20% glycerin or 10–20% DMSO (freshly prepared and tested for toxicity and shown to be nontoxic to cells). The tumor preparations are placed in vials, which are then frozen at a controlled rate until a temperature of approximately −20 C is obtained. The critical temperature is between 0 C and −10 C, where freezing occurs. During this time the temperature is reduced by 1 C/min to prevent the formation of ice crystals within the cells.

D. Tissue Culture

Many plasmacytomas have now been successfully established in vitro, using a variety of techniques, media, and supplements: X5563 (202); MOPC21, AdjPC5, S63, S194, (105), MOPC315, McPC603, MOPC870 (200), MOPC173 (190), MOPC31B (182), and MPC11 (127). Most of the tumors have been grown as suspension cultures and lack the ability to stick to glass. A few tumors have been adapted in vitro directly but others have required passage back into mice (105, 127, 200). The MPC11 tumor was cycled 3–6 times before stable in vitro lines were established. The cloning efficiency of various cultures differs, as may be expected (127). The cells that grow in suspension and do not attach to glass can be cloned by placing them in soft agar. Horibata and Harris (105) have succeeded in cloning cells directly on washed agar with a cloning efficiency of 1–3%. Coffino and Scharff (41) have developed highly efficient agar-cloning methods using soft agar with an underlayer containing contact-inhibited 3T3 BALB/c fibroblast feeder cells. The MPC11 tumor grown in this fashion has a cloning efficiency of approximately 90% (M. Scharff, personal communication).

Park et al. (193) have established four plasmacytomas in primary culture by overlaying dissociated cells onto feeder layers containing mouse renal tubules. They obtained from each of the four tumors colonies that were round and in which the cells were not contact inhibited. Reinoculation of these colonies into mice caused tumor formation. It was shown, however, that the colony-forming tumor cells had special metabolic requirements and that their maintenance required the use of fresh medium daily and a hitherto unrecognized factor in 10% human serum

and fresh medium 1066 that was not glutamine. Subsequently Park et al. (194) identified the factor as ascorbic acid. The requirement was apparently unique for plasma cell tumor stem cells, since granulocytic stem cells did not require ascorbic acid for colony formation. In all, four plasmacytomas were studied: MOPC104E, AdjPC5, MOPC460, and MOPC46B. From 0.7 % to 1.2 % of tumor cells in a solid AdjPC5 tumor were capable of forming colonies using this method. This is in fairly close agreement with the estimates made by using the spleen-cloning method, which was 4.1 % (25) for the same AdjPC5 tumor.

It must be remembered here that Park et al. (193, 194) were working with cells derived from in vivo passaged tumors and not from cells adapted to in vitro conditions. Scharff (personal communication) has obtained very high (ca. 90 %) cloning efficiency with adapted tissue-culture lines of plasmacytomas.

Paraf et al. (190) have found a most unusual morphologic variant in the mouse plasmacytoma MOPC173. They selected cells that attached to glass and discarded the free-floating cells. After 2–4 months an established fibroblastic line was obtained, and from this line a new morphologic variant arose called the epithelioid line. The two lines differed in that the fibroblastic line was not contact inhibited while the epithelioid line was contact inhibited. Both lines produced immunoglobulin and both lines during early transfer generations (up to 30–60 passages in vitro) were tumorigenic on reinoculation into mice. However, after the 60th transfer the epithelioid line was treated with trypsin in vitro for a number of passages and it reverted to the fibroblastic line and again became tumorigenic. Here is an example of a tumor line that manifests in vitro different morphologic phenotypes that are correlated with the ability to induce tumor formation on reinoculation in mice.

Most of the tumors that have been adapted in vitro can produce tumors on inoculation in vivo (105, 127, 190, 200). A number of lines have changed in vitro and lost the capacity to produce heavy chains (42, 105, 200) or heavy and light chains (42). This property may be due to the accumulation of "mutants": i.e. producers of only light chains or nonproducers during adaptation in vitro (see sect. IX*C* for further discussion). Several tumors producing antigen-binding myeloma proteins have been adapted in vitro (105, 200) and some of these have continued to produce a complete immunoglobulin.

E. Commonly Used Transplantable Plasmacytomas

Transplantable plasma cell tumors are frequently used as a source of homogeneous immunoglobulin. Since many tumors continue to produce the same immunoglobulin for many years, there are now in use tumors that have been widely distributed. A list of commonly used plasma cell tumors and the immunoglobulins they produce is given in Table 7.

In the mouse two morphologic tumor types produce IgM myeloma proteins. The MOPC104E (154) originated in a mineral oil-treated BALB/c mouse and is a plasmacytoma. The tumor RN 45, which arose spontaneously in a (DBA/2 × CBA)F_1 hybrid, is a "plasma cell leukemia" (39) and behaves in transplant as a

TABLE 7. *Immunoglobulins produced by commonly used transplantable plasmacytomas*

Heavy-Chain Class	Light-Chain Type		
	κ	λ1	λ2
M (μ)	McPC774*	MOPC104 λ1 (9, 154)	
	TEPC183 (196)		
A polymer (α)	MOPC460 (111)		
	TEPC15 (221)		
	MOPC167 (221)	J558 (298)	MOPC315 (69)†
	MOPC320 (219)†		
	AdjPC6A (217)		
A Two-chain (α)	MOPC47A (141, 217)		
	AdjPC6C (141, 217)		
	MOPC88 (141, 214)		
F (φ)	MOPC21 (219)		
	MOPC70 + κ (82, 217)		
	MOPC300 (219)†		
G (γ)	X5563 (C3H) (216)	HOPC1 (298)	
	AdjPC5 (213)		
	MOPC173 (222)		
	LPC1 (222)		
H (η)	MPC11 (73)		
	MOPC141 (222)		
	MOPC195 (222)		
G3 (γ3)	J606 (88)		
None	MOPC41 (82)	RPC20 (9)	
	MOPC46B (49, 161)		
	MOPC321 (160)		
	MOPC47B (217)		

* Maintained by K. R. McIntire. † Of BALB/c.C57BL Ig C_H Bc, F origin; see Table 15.

reticulum cell neoplasm. This tumor is a lymphoma and more closely resembles a Waldenstrom macroglobulinemia than do the plasmacytomas.

F. Chemotherapy

The growth of plasmacytomas can be inhibited dramatically by several compounds that appear to have a growth-inhibiting or a killing effect on the tumor cells (4, 80, 299, 300). Among the alkylating agents *N*,*N*-di-2-chlorethylaniline (aniline mustard) produces dramatic effects. Goldacre and Whisson (80) treated the AdjPC5 plasmacytoma with aniline mustard and produced remarkable regressions of massive plasmacytoma growths, some of which were up to a third of the total body weight (7 g). Sections of the highly vascularized tumor 3 hr after beginning treatment did not initiate new tumor growths, indicating the powerful effect of this drug on tumor viability (80).

Whisson and Conners (299) mentioned experiments that showed mice with

drug-regressed tumors are immune to reinoculation 100 days later. The full details on this immunity were not described, although it was stated that by 23 days the reinoculated tumor had grown in only 4 of the 12 mice inoculated. We (J. Walters and M. Potter, unpublished results) repeated this exciting observation with AdjPC5 with roughly similar results. Apparently treatment with the alkylating agent does not interfere with the development of tumor immunity. Whisson and Conners (300) investigated the killing effect of a number of derivatives of aniline mustard as well as several other related compounds and found that if the para position on the aniline group was not free the compound was virtually inactive. They found, for example, that the *p*-methyl- and *p*-fluoroaniline mustards derivatives were inactive. These workers suggest that these tumors have the ability to convert aniline mustard to *p*-hydroxyaniline mustard, which is 5 times more cytotoxic than the parent compound (300).

Teller et al. (282) and Abraham et al. (4) have studied the effect of several drugs including cyclophosphomide, 5-fluorouracil, and aniline mustard on various plasmacytomas. These workers utilized intraperitoneal transplantation of the ascites form of the tumors. They obtained prolonged survival times with a variety of drugs, including 5-fluorouracil, aniline mustard, cyclophosphamide, chlorambucil, and nitrogen mustard. Treatments were administered 10 days after tumor implantation. The difference in the results obtained by Goldacre et al. (80) and by Teller et al. (282) and Abraham et al. (4) in terms of total survival may be due to modifications of the tumor cell when adapted to the ascites form.

V. PATHOLOGICAL AND PATHOPHYSIOLOGICAL CHANGES ASSOCIATED WITH PLASMACYTOMAS

In this section pathological changes associated with transplantable plasma cell tumors and their products are considered.

A. Bone Lesions

In man the malignant plasmacytomas (multiple myeloma) usually produce multiple osteolytic lesions as a consequence of their predilection for growing in the bone marrow cavities. As previously discussed, experimental plasmacytomas in mice develop in the peritoneal connective tissues.

The primary induced plasmacytomas have not produced osteolytic lesions (119). Subcutaneously transplanted plasmacytomas metastasize to the bone marrow and induce osteolytic lesions but only when the tumor has remained in the host 90 days or more (119, 216). Osteolytic lesions are most frequently found in the proximal tibia, neck of the humerus, distal femur, ramus of the mandible, and vertebras. Similar and more extensive lesions are produced by intravenous injection of cell doses of 10^5–10^6 cells (119). Lesser numbers of inoculated cells 10^3–10^4 cells produced fewer lesions. Osteolytic lesions result from intramedullary tumor expansion to the cortex, probably by interfering with the dynamics of bone forma-

tion (119). When plasmacytomas contact periosteum they produce osteoblastic bone formation (119). Osteolysis appears to result from the local effects of the growing tumor rather than from a disturbance in bone metabolism.

B. Renal Lesions

Tumors producing large quantities of κ- and λ-light chains as well as those making the two-chain IgA proteins usually induce the formation of "myeloma kidney," which consists of a kidney filled with intratubular esoinophilic casts (48, 62, 156). Myeloma kidneys have been observed in mice with both primary plasmacytoma and transplants. The process may be extremely extensive and can virtually destroy both kidneys. Myeloma kidney may develop asymmetrically in mice, involving only one kidney or one half (upper or lower half) of a kidney (156). McIntire et al. (157) have isolated the tubular casts from myeloma kidneys and shown that they consist of mostly denatured light chains. The casts were solubilized by treatment at very high pH and the light chains were identified by serologic methods.

C. Bence Jones Proteinuria

Bence Jones proteinuria, as defined in man, is the urinary excretion of immunoglobulin light chains that occurs in association with multiple myeloma. In the mouse the Bence Jones proteins include κ- and λ-light chains and the two-chain IgA protein (156).

The Bence Jones proteins in mice do not have the same thermal solubility properties as their counterpart in man; that is, they characteristically do not go back into solution at 100 C (70). The urinary Bence Jones proteins in mice are excreted in variable quantities ranging from 1 to 51 mg/ml. Many tumors produce large quantities of protein and are useful sources of immunoglobulin light chains for chemical analysis. Other light chain-producing plasmacytomas produce only small quantities of Bence Jones protein in the urine. The urine containing Bence Jones protein contains insoluble amorphous precipitates, some of which is immunoglobulin that may be solubilized by mercaptoethanol. Bence Jones proteins may be separated from normal mouse urinary protein (MUP) by ion-exchange chromatography at pH 5.5 on DEAE-cellulose (206).

D. Amyloidosis

Amyloidosis in mice bearing transplantable plasmacytomas has been sought by many investigators and not found. R. A. DeLellis (personal communication) has examined over 50 plasmacytomas from this laboratory and found no evidence of amyloid associated with them. Lehner et al. (129) have reported finding amyloidosis associated with the transplantable AdjPC5 plasmacytoma. These workers identified the amyloid fibrils as about 12 mμ wide. Most of the amyloid was asso-

ciated with the tumor but some was found in other tissues. The Congo red test was weakly positive and the birefringence and dichroism were negative. The amyloid appeared about 15 days after transplantation and remained only in small amounts. DeLellis (personal communication) was unable to obtain amyloid in AdjPC5 line maintained here at NIH. Amyloid has been found to be associated with the plasma cell leukemias (65, 232). (See sect. III*B* for further discussion.)

E. Effects on Induced Antibody Formation, IgG Catabolism

Smith et al. (266) immunized mice carrying the IgG-producing X5563 plasmacytoma with sheep RBC and measured the hemolysin response after 5–7 days. In mice with large tumors there was a marked depression of the primary hemolysin response. By contrast, mice bearing large tumors that received two injections of antigen did not demonstrate a depression in their hemolysin titers after 7–21 days.

Zolla et al. (318) have repeated this experiment with several other BALB/c plasmacytomas, also using sheep RBC as antigen and the number of plaque-forming cells in the spleen as an assay. They included in their study a nonproducing plasmacytoma NP2 derived by Scharff from MPC11 by in vitro cloning procedures (245) and found that this tumor also suppressed the primary response to sheep RBC. Zolla et al. (318) also showed that spleen cells from tumor-bearing mice were able to restore immune responses to lethally irradiated non-tumor-bearing mice. This result suggested that the inhibiting effect required the presence of the tumor. The explanation for this interesting result is not known.

Humphrey and Fahey (107) studied the catabolism of IgG immunoglobulin in the presence of transplanted plasmacytoma and found that the synthesis of normal immunoglobulin was greatly accelerated. A normal half-time of 4.6 days changed to 1.8 days in mice with transplants.

VI. ANTIGENS ASSOCIATED WITH PLASMA CELLS AND PLASMA CELL TUMORS

Four types of antigens have been associated with plasma cell tumors: *1*) "differentiation" antigens (e.g. PC.1 and MSPCA), which are found on both normal and various types of neoplastic plasma cells and distinguish plasma cells from lymphocytes; *2*) immunoglobulin antigens, which are associated with the membranes of plasma cells and have so far been related to the constant-region parts of the immunoglobulin molecule; *3*) plasma cell tumor-specific antigens, which are unique to a specific tumor; and *4*) antigens related to viruses (e. g. GCSA, G_{IX}) and the antigen associated with the structural protein of the intracisternal A particle (these antigens have been previously discussed in sect. II*D*).

A. Differentiation Antigens

Takahashi et al. (272, 273) immunized DBA/2 mice with γF (γ1)-producing MOPC70A plasmacytoma cells of BALB/c origin. In this donor-recipient com-

bination there are apparently no genetic differences in genes governing histocompatibility-2 antigens (the major histocompatibility antigen in the mouse) or in the γF immunoglobulins (173). DBA/2 mice respond to this immunization by producing cytotoxic antibodies that are directed to an antigen on plasma cells. The antiserum identifies all BALB/c plasmacytomas and also some normal plasmacytes (plaque-forming cells). Using the Jerne plaque-assay system it was found, for example, that the antiplasma cell antiserum (anti-PC.1) and complement admixed with spleen cells from immunized mice greatly reduced the number of IgG-releasing cells but was virtually ineffective against IgM-releasing cells. This result suggests that only a portion of the immunoglobulin-secreting cell population can be destroyed by the cytotoxic antibody and complement. It is not clear yet whether the cells differ because of an absolute difference in the presence or absence of PC.1. The PC.1 antigen is not a strict plasma cell antigen as it apparently is found on liver, kidney, and brain (272). PC.1 is an alloantigen found on the normal plasma cells from strains C3H/An, C3H/He A, A/TL$^-$, A SW, AKR, AKR-H-2^b, BALB/c NZB, MA, RF.101, SJL/J, PL, CE, and BALB/c.C57BL/Ka Ig C_H (BALB/c-2); PC.1 is not found on cells from strains C3H^f/Bi, C57BL/6, C57BL H-2^k, C57BL/TL$^+$, C57BL/LyA1, C57BL/LyB1, B.10D$_2$, H21, H2G, C58, CBA-T6, DBA/2.129, and I (272). The gene controlling PC.1 behaves in appropriate crosses as a single Mendelian dominant; no linkage to H-2 or several other loci has been found (110, 272). The PC.1 antigen is not found on lymphocytes or lymphocytic neoplasms (275); it is apparently a characteristic that develops in plasma cells late in their maturation.

There have been several attempts to produce antisera to mouse plasma cells in rabbits. Watanabe et al. (296) prepared rabbit antisera to extracts of six different plasmacytomas. The tumors were homogenized in a tissue grinder, centrifuged lightly to remove the debris, and then recentrifuged at 105,000 *g*; the resulting sediments were used to immunize the rabbits. The antisera produced were tested by a fluorochromasia cytotoxicity test and found to react with both lymphocytic and plasma cell antigens. After absorption with lymphocytes each of the antisera was cytotoxic for all of the plasmacytomas. The antiserum to MOPC104E appeared also to have a unique specificity (idiotypic?) since absorption with other plasmacytomas did not remove the cytotoxic antibodies to MOPC104E.

Takahashi et al. (274) have identified another plasma cell-differentiation antigen with rabbit antisera that they have called mouse-specific plasma cell antigen (MSPCA). They immunized their rabbits with carefully washed ascites tumor cells from MOPC70A, MOPC104E, and MPC67. The antisera so prepared also reacted with lymphocytes and therefore prior to use were absorbed with BALB/c mouse thymocytes. The absorbed antisera were tested for their ability to kill various cells, using the trypan blue staining method as an index of cytotoxicity, and also for their ability to eliminate plaque-forming cells (PFC) and rosette-forming cells (RFC) from spleen suspensions from immunized mice. Two types of antisera were obtained. The antiserum to MOPC70A identified immunoglobulin determinants that were found on MOPC70A and another γ1-producing tumor but not on six other plasmacytomas tested that produced immunoglobulin of classes other than

γ1. The rabbit antisera to MOPC104 and MPC67 (an IgA κ-producing tumor) were directed to an MSPCA found on all of the plasma cell tumors. The anti-MSPCA antiserum eliminated 22–52% of the IgM and 23–45% of the indirect cells in the plaque-assay studies. The tissue distribution of MSPCA was not clearly established; however, the specificity of the antigen was based on its absence from mouse lymphocytes and all rat cells.

B. Immunoglobulin

As mentioned in the studies just described with rabbit antisera to mouse myeloma proteins, there was evidence from the anti-MOPC104E antiserum prepared by Watanabe et al. (296) of an idiotypic determinant on MOPC104E cells and also from the studies of Takahashi et al. (275) that rabbit antisera to MOPC70A identified an immunoglobulin determinant related to γ1-producing cells. Takahashi et al. (275) have investigated the ability of rabbit antisera to myeloma proteins to produce cytotoxic effects on plasmacytoma cells. Among 8 plasmacytomas tested with 22 class-specific rabbit antisera with various specificities, it was found that antisera with specificity for γ1 and κ were cytotoxic for the two γ1-producing tumors tested.

Princler and McIntire (see ref 275) have studied two other γ1-producing tumors and found they were sensitive in cytotoxic tests to anti-κ and anti-γ1 antisera, whereas an additional eight other tumors producing other classes of Ig also tested were negative.

Using MOPC70A cells Takahashi et al. (275) have been able to show that, after incubation of the cells with anti-κ antiserum in the absence of complement, their sensitivity to lysis by complement added at a later time was suppressed. This effect was temperature dependent and did not occur at 0 C. This finding suggests that the loss of the immunoglobulin on the surface of the MOPC70A cells may resemble the loss of the TL antigen from lymphocytes, a phenomenon called antigenic modulation. During this process a cell surface antigen can be resynthesized. Paraskevas et al. (191), using hybrid antibody as a probe in a reverse immune cytoadherence test, have been unable to detect immunoglobulin on the surface of plasmacytoma cells, including at least one γF-producing tumor (192).

C. Tumor-Specific Antigens

There have been several reports on the development of immunity to transplanted plasmacytomas in BALB/c mice. Whisson and Connors (299), as described, induced immunity to the AdjPC5 plasmacytoma by regressing large tumors with aniline mustard and challenging the mice 100 days later. Moriwaki et al. (175) induced immunity to the MSPC1 plasmacytoma of BALB/c origin by immunization of BALB/c mice with tumor sonicates. The mice were challenged with limiting doses of tumor cells in 30 days and a large percentage (over 60%) were immune.

Paraf et al. (190) found that mice injected with epithelioid MOPC173 tissue-culture lines with cells from the 60–90th passage, which did not induce tumor formation, did provoke the development of immunity to challenge with the in vivo MOPC173 line. However, when the experiment was repeated with cells from the 90th–130th tissue-culture passage of the epithelioid line, less immunity was found. This interesting work needs to be further investigated. Lespinats (135, 136) has immunized BALB/c mice with BALB/c plasma cells from four different tumors that were inactivated with 10^{-3} M sodium iodoacetate. Antisera obtained from immunized animals were tested by an indirect fluorescent-antibody test (136). Cells isolated by the Madden and Burk (149) procedure and shown to be viable were incubated with antiserum for 15 min at 37 C and then after washing were stained with fluorescein isothiocyanate-conjugated rabbit antimouse globulin diluted 10-fold. The cells were then examined under a fluorescent microscope for the presence of staining at the cell surface, which usually appeared as fluorescent dots around the surface. The fluorescence index was the proportion of negative cells in a sample exposed to normal mouse serum minus the proportion of negative cells in the sample exposed to the test antiserum divided by the proportion of negative cells in the normal sample; an index above 0.3 was considered positive. These workers demonstrated that using these criteria humoral antibodies were produced to four different plasmacytomas. They further showed by absorption tests or by using different plasmacytomas as targets that some of these antisera were specific for individual tumors. In one study they were able to demonstrate that a high fluorescence index was also associated with resistance to challenge by the isologous plasmacytoma. Mandel and DeCosse (151) studied the effect of a heterologous antithymocyte antiserum on mice bearing plasmacytomas and found that the antiserum accelerated the growth of the plasmacytomas. The antiserum in this case acted as if it were an enhancing antibody. They interpreted this result as indicating that the tumor contained antigens responsible for the slow growth of the tumor in syngeneic hosts.

D. Comments

There is considerable evidence that plasmacytomas possess some form of tumor antigen; however, the studies have not been pursued to the point where the nature of the antigenic determinants has been established. Immunoglobulin integrated into the cell surface may be a possible contributing factor. The γF-producing cells appear to be unusual in this respect. It will be important in the future to utilize anti-idiotypic sera in these studies, and this may require usage of homologous sera.

VII. MYELOMA PROTEINS: STRUCTURE

As a preface to the next three sections dealing with structure, antigen-combining activity, and synthesis of immunoglobulins derived from murine plasma-

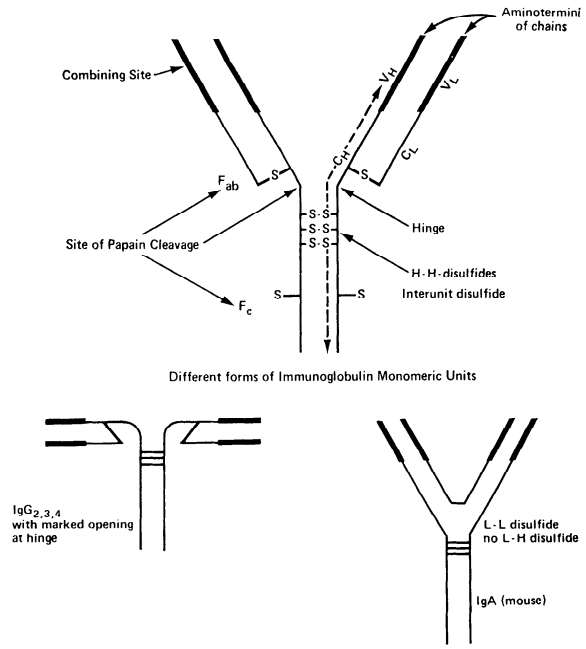


FIG. 3. Scheme of four-chain immunoglobulin monomer.

cytomas, a general sketch of the immunoglobulin molecules is included for orientation (Fig. 3). The reader is referred to past reviews for detailed references (57, 171, 206).

The completed functional immunoglobulins that are secreted from immunoglobulin-producing cells are composed of four-chain (polypeptide) molecular units. The chains are called H or heavy (mol wt ca. 50,000) and L or light (mol wt ca. 23,000). In a single molecule the two L chains are identical and the two H chains are identical. The vertebrate organism produces a great variety of immunoglobulins that have been categorized into classes and subclasses based on common molecular, antigenic, and chemical properties. (The molecules have also been broken down into classes based on common properties between chains.) The three major classes are IgG, IgM, and IgA. The IgG immunoglobulins remain in the circulation and tissues as single four-chain units, while the IgM four-chain units are formed into a polymer that in mouse and man is a pentamer; the IgA units form polymers that may have two, three, or even more four-chain units. All polymers appear to be formed outside the cell, as discussed below, by linking four-chain units to each other by covalent disulfide bonds.

Each four-chain unit has three globular domains that can be artificially separated by proteolytic digestion (papain, trypsin, pepsin). Two of the fragments are similar and are called Fab (fragment, antibody) because each one carries one antigen-combining site. The third fragment was named Fc (fragment, crystallizable) because in the rabbit it could be easily crystallized. Each Fab is composed of a complete light chain and roughly half (the amino terminal half) of a heavy chain.

The Fc is composed of two heavy-chain fragments derived from the carboxyl-terminal half of the chains. The proteolytically derived fragments retain much of their native structure and function; the univalent Fab, for example, combine with antigen as effectively as they do when they are situated in the four-chain unit. In the native intact molecule the three fragments have some mobility. The flexible part of the molecule is called the hinge region, and the angle in the hinge in different immunoglobulins orients the combining sites on the Fab in different spatial relationships to each other. If the four-chain immunoglobulin molecule is thought of as a cross-link between two macromolecules, then it can be imagined that the angle of rotation in the hinge will sterically limit the cross-linking functions. A molecule with 180° between the two combining sites (a "T") is more effective in certain cross-linking functions than one with only 90° (a "Y").

Each immunoglobulin polypeptide chain is subdivided into two polypeptide segments under separate genetic control. Immunoglobulin light chains (ca. 214–218 amino acids) have V_L polypeptide that begins at the amino-terminal end and is about 100–110 amino acids in length. The C_L is the carboxyl-terminal segment. For heavy chains (ca. 420 amino acids) the V_H is approximately the same length as in the light chain, but the C_H is 3 times as long as the C_L.

The division of C and V polypeptides evolved from the comparative study of the primary structures of related immunoglobulin molecules. For example, the κ-type light chains from one haplotype in one species all have a similar amino acid sequence in the carboxyl-terminal half of the chain, while each chain differs from the others by amino acid variations in the amino-terminal half of the chain. The extent of possible variations in one species is not known.

The most feasible model to date for explaining the genetic control of a highly

TABLE 8. *Classes and subclasses of immunoglobulins in mice*

Major Class	Form or Subclass*	No. 4-Chain Units/ Molecule	Disulfide Bonds/4-Chain Unit			Heavy Chain		Ref
			No. L-H	No. H-H	Location relative to H-H	Mol wt	C-term amino acid	
IgM (19S)	None	5	2			55,000		260
IgA (7, 9, 11, 13S)	A_1(BALB/c)	1, 2, 3, 4	0	3		54,000	Tyr	2, 3
	A_2(NZB)	1, 2, 3, 4	2					89
						40,000 (47A)		258
						46,000 (6C)		179
	2-Chain (BALB/c)	1/2	1	0			Gln	258
IgG (7S)	F (γ1)	1	2	3	Close			271
	G (γ2a)	1	2	3	Distant			226
	H (γ2b)	1	2	4	Distant			226
	G3 (γ3)	1	2			50,000 (est)	None obt.	88

* Symbol used originally by Fahey et al. (72, 73) in parentheses.

heterogeneous system of chains, each with the same constant segment and a different variable segment, is by hypothesizing one gene for each segment of the chain. No other examples of polypeptide chains controlled by two different gene elements have been described; in fact, the dogma of genetics that prevailed for some time held that one gene controlled one polypeptide chain.

In common with other mammals the mouse (*M. musculus*) has three major classes of serum immunoglobulins: *1*) the 19*S* IgM macroglobulins (72, 154, 260), *2*) the polymeric (71, 72) IgA immunoglobulins, and *3*) the IgG immunoglobulins (72, 73). An IgE class is suspected in mice but a myeloma protein of this class has not yet been reported. Two of the major classes, IgA and IgG, contain additional forms or subclasses. The IgA immunoglobulins in man have been subdivided into A_1 and A_2 types (87) based on the presence (A_2) or absence (A_1) of L-H disulfide bonds. In the inbred BALB/c strain of mice the predominant form is the A_1; in strain NZB the A_2 has been reported (89). There is also a pathological IgA form in the mouse—the two-chain IgA (141, 179, 214, 217), which thus far has been produced only by neoplastic plasma cells. There are four subclasses of IgG in the mouse. A summary of the basic characteristics of mouse immunoglobulins is given in Table 8.

A. Two-Chain IgA

The two-chain IgA form is probably pathological since it has thus far only been found to be the product of certain plasmacytomas. Six tumors originating in our laboratory (AdjPC6C, MOPC47A, MOPC88, MOPC4G, MOPC116, and MOPC287) have been found that make this form of IgA (179, 214, 217). The two-chain molecules have sedimentation coefficients of 3.9*S*, and after release from tumor cells they accumulate in the serum and urine (217). Lieberman et al. (141) have prepared potent precipitating antisera to these proteins by immunization of strain AL or A/He mice. These antisera possess two types of activities: one directed to the unique specificity located on the Fab of the molecule and the other directed to a determinant common to all two-chain molecules. Using antisera specific for the two-chain IgA common determinant, an extensive survey of body fluids of normal BALB/c was made, including thoracic duct fluid, milk, and urine concentrates, for the presence of two-chain molecules and none were found (141).

Several lines of evidence from structural studies also suggest these proteins are abnormal. First, the molecular weights of the α-chains have been determined (Table 8) and found to be 40,000 for MOPC47A (258) and 46,000 (179) for AdjPC6C. Both are below the estimated weight of α-chains isolated from polymeric IgA molecules (54,000–55,000) (179, 258, 287). This suggests that part of the α-chains of two-chain molecules is deleted. This is supported by other evidence. Abel and Grey have shown BALB/c polymeric IgA have tyrosine as a carboxyl terminal (2). The MOPC47A α-chain, however, has glutamine (258). Peptide map studies (179), as well as the number of half-cystine residues per chain, indicate the two-chain molecules lack a segment of the polypeptide sequence. This probably

is a large deletion involving 90 amino acids (179) but its exact location is not known. An unexpected characteristic of two-chain IgA molecules is the presence of an L-H disulfide bond that is lacking in BALB/c IgA polymers (179). This bond, which forms intracellularly (179, 245), may result from the availability of a free Cys-SH group that normally participates in an H-H disulfide bridge.

The AdjPC6A and 6C (two-chain IgA) transplant lines were originated from the same primary host (217) and share the same idiotypic antigenic specificity (141, 179), which suggests that the 6A and 6C cells were derived from the same clone. If so, it is possible the 6C line developed as a mutant of the 6A line (217). It will be of great interest to determine if all the two-chain α-chains possess a similar defect or whether they are all individually different.

B. IgG

There are four subclasses of IgG in the mouse (see Table 2). Of the four varieties the γG and γH forms are the most closely related. Serologic similarities were first detected with heterologous antisera (73). Tryptic peptide maps of the Fc of these proteins contain 12 peptides that cochromatograph (222), suggesting extensive areas of sequence identity. Recently, Preval et al. (226) have sequenced peptides around the H-H disulfide bridges of MOPC173 γG and MOPC141 γH and found that there are regions of marked sequence variation as well as regions that show interesting homologies (Table 9). It was found that the L-H disulfide bridges on γG and γH heavy chains were closer to the amino-terminal end (226) and resembled L-H linkages in the human γ2, γ3, and γ4 subclasses. By contrast in the γF proteins the L-H bridge was located closer to the carboxyl-terminal end and in fact was quite close to the region where the H-H bridges were found (271). Murine γF resembled the human γ1 proteins in this respect. It was also determined that the γG proteins contained three H-H disulfide bridges while the γH proteins contained four. Preval et al. (226) have been able to demonstrate striking sequence differences between γG and γH proteins, although these proteins appear to have common specific characteristics. This suggests that despite the species-specific characteristics shared by these two chain types considerable further evolution has taken place in common region genes in the mouse species that have in effect differentiated the two classes. Comparisons of γG and γH molecules from different heavy-chain linkage

TABLE 9. *Partial amino acid sequences of C_H polypeptides around H-II disulfide bridges*

MOPC21	F (ϕ)							Asp	Cys	Gly	Cys	
MOPC173	G (γ)		Gly	Pro	Thr	Ile	Lys	Pro	Cys	Cys		
MOPC141	H (η)	Ile	Asn	Pro	Cys	Pro	Pro	Cys	Lys	Glu		
MOPC21 (cont.)		Pro	Cys	Ile	Cys	Thr	Val	Pro	Glu	Val	(271)	
MOPC173 (cont.)		Pro	Pro	Lys	Cys	Pro	Ala	Pro	Asn	Leu	(226)	
MOPC141 (cont.)		Cys	His	Lys	Cys	Pro	Ala	Pro	Asn	Leu	(226)	

groups in the mouse may give further insight into the extent of evolutionary divergence of immunoglobulin genes within a species. Myeloma proteins from genomes (other than BALB/c) can be obtained by inducing plasma cell tumors in congenic strains such as BALB/c.C57BL Ig C_H and BALB/c.AL Ig C_H (219).

Grey et al. (88) found among 161 sera tested one myeloma protein that did not type with any known class of immunoglobulins; this myeloma protein, designated J606, was subsequently found to be a unique subclass of IgG and was then designated IgG3. Molecular weight of this protein was estimated to be approximately 150,000. Normally IgG3 molecules are present in mouse serum at very low concentration (0.1–0.2 mg/ml). IgG3 proteins do not fix complement and are believed to be efficiently transported across the placenta and effectively concentrated in the fetus.

C. Immunoglobulin Genes

According to the currently widely accepted dogma the immunoglobulin molecule (four-chain unit) is controlled by four structural genes: two for the light chain V_L (variable polypeptide segment, light chain) and C_L (constant polypeptide segment, light chain) and two corresponding genes V_H and C_H for the heavy chain. It is convenient to describe the varieties of genes within the mouse (and even more specifically the BALB/c mouse) according to this classification (Table 10).

The genes controlling the constant heavy-chain polypeptides C_α, C_γ, C_η, C_ϕ in the mouse are tightly linked on one chromosomal region (93, 97, 143, 219, 220). Crossing over of immunoglobulin genes in this locus has not been observed under experimental conditions (98, 143). For this reason there are in the mouse characteristic linked sets of C_H genes (heavy-chain linkage groups) and the inbred strains have been typed according to these characteristic sets. It is of interest that evidence for crossing over in the C_H region has been obtained from studies on wild mice (144). Wild mice (*M. musculus molossinus*, Kyushu, Japan) carry an unusual chromosome that contains at least five C_H genes and appears to have evolved from an unequal

TABLE 10. *Immunoglobulin structural genes in the mouse*

Constant Heavy Chain C_H	Variable Heavy Chain V_H	Constant Light Chain C_L	Variable Light Chain V_L
C_μ	Several subclasses	C_κ	V_κ (over 18 subclasses)
C_α*		$C_{\lambda 1}$	
C_γ or $C_{\gamma 2a}$*		$C_{\lambda 2}$	V_λ (probably all variants single subclass)‡
C_η or $C_{\gamma 2b}$*			
C_ϕ or $C_{\gamma 1}$†			
$C_{\gamma 3}$			

* Antigenic (allotypes) polymorphisms have been identified (see Table 15). † Electrophoretic (allotypes) polymorphisms. ‡ Subclasses are based on amino acid sequence differences in amino-terminal Cys 23 peptide.

crossing over of two heavy-chain linkage groups that are found independently in various inbred strains of mice (139).

D. C_L

The two major types of C_L (κ and λ) are found in the mouse. The predominant form is kappa. Estimates on the prevalence of κ-subunits in normal mouse immunoglobulins based on serological identification (Mancini test) suggest that 97% of normal serum immunoglobulins have κ-subunits (159). Three C_κ regions have been sequenced and differences have been reported; these are most likely due to technical problems rather than genetic polymorphisms or isotypes (82, 172).

The number of C_λ genes per haplotype in the mouse is not yet firmly established. It appears certain, however, from available sequence data that there are two C_λ in the species *M. musculus*, $C_{\lambda 1}$ and $C_{\lambda 2}$. The major C_λ form is $C_{\lambda 1}$, which has been found now in 10 of the 11 λ-chains sequenced. This gene governs the sequence of the λ-chain from position 111 to the COOH terminal. Thus far the 10 $C_{\lambda 1}$ proteins have been established by either complete or partial sequences (7, 298). $C_{\lambda 2}$ has been isolated from the MOPC315 IgA myeloma protein (256). The MOPC-315 λ-chain differs from the MOPC104E $\lambda 1$-chain in at least 28 positions between 111 and the COOH terminal.

The question of whether the BALB/c genome contains two C_λ genes or isotypes (256) or whether $C_{\lambda 1}$ and $C_{\lambda 2}$ represent allelic differences develops from the fact that the MOPC315 plasmacytoma (the source of the $\lambda 2$-protein) was induced in a special backcross mouse (69). This backcross line was initiated from a cross between BALB/c AnN and C57BL/Ka (219). The F_1 progeny were mated to BALB/c AnN, and thereafter backcross mice carrying the C57BL/Ka immunoglobulin heavy-chain linkage group were selected and mated to BALB/c. During development of this introgressive backcross, mice were tested for their ability to develop plasma cell tumors by mineral oil treatment (219). The MOPC315 arose in a mouse at the 7th backcross generation; this mouse was known to be heterozygous for the BALB/c and C57BL heavy-chain linkage groups but undoubtedly other C57BL/Ka genes were present. The MOPC315 myeloma protein was IgA in class and carried the three allotype markers $A^{12, 13, 14}$ (69) of BALB/c. The light-chain subunit was unusual, however, and did not resemble the BALB/c κ- or λ chains by the tryptic peptide map technique (69). Goetzl and Metzger (79), during a study of the structure of the MOPC315 L chain for another purpose, noted that the carboxyl terminal was Leu. The partial sequence of MOPC315 has been determined and the light chain has been found to have many homologies and identities with MOPC104, indicating it is clearly a λ-chain (256). Whether the gene that directs this sequence is of BALB/c or of C57BL/Ka origin is not clear. It is possible that the C57BL/Ka λ-chain locus could be linked to the heavy-chain locus and introduced onto the BALB/c genome during the introgressive backcrossing. On the other hand, the unusual MOPC315 L chains could represent an isotype much as the OZ and Kern isotypes in man (8, 101). Because of the absence

of a serological means for identification or a means for selectively studying lambda light chains from normal immunoglobulins by structural means the question of whether or not mice have two nonallelic loci ($C_{\lambda 1}$ and $C_{\lambda 2}$) remains open.

E. $V_L(V_\lambda, V_\kappa)$

In the mouse as in man the V polypeptides that associate with C_λ are completely different from those that associate with C_κ. In the mouse the 97:3 κ:λ ratio correlates well with the number and variety of V_κ and V_λ polypeptides. Many varieties of V_κ polypeptides have been isolated and described whereas only a few varieties of V_λ have been isolated.

1) V_λ. In an early study of the mouse λ-chains MOPC104E and RPC20, Appella et al. (9, 10) noted that the two chains, though derived from independently induced tumors, had indistinguishable amino acid compositions and partial amino acid sequences. The complete primary structure of these two mouse λ-chains has now been published by Appella (7), including the basic methodology for peptide isolation and recovery; he found the sequences of the two proteins were identical. Weigert et al. (298) independently obtained the partial sequences of eight λ1-chains, including RPC20 and MOPC104E (Weigert used as the source of the 104E protein a tissue-culture cell line of MOPC104E called XP8). Six of the 10 proteins had identical sequences that were the same as the unique sequence found by Appella for MOPC104E and RPC20. Weigert et al. (298) and Appella (7) obtained one difference for the RPC20 protein at position 50: Ile and Leu, respectively. There appears to be no biological basis for this difference as yet and its cause remains unexplained.

There are several interesting features of the mouse λ-chains. First, if they were to be arbitrarily subgrouped by the amino-terminal Cys 23 peptide they would all be identical and fall into the same subgroup. Second, the number of variations found among the 10 sequences reported is minimal. Only 5 positions of the V_λ have variations: 25, 32, 50, 52, and 97. One of the chains with variations (S176) (Table 11) differs from this basic MOPC104E sequence by 1 residue, another (2020) by 2, and a third (S178) by 3. The MOPC315 V_λ sequence differs from MOPC104E in at least 8 positions between the amino terminal and residue 98 (Table 11). This is the largest number of sequence variations found between any two λ-chains and may indicate that gene controlling the MOPC315 V_λ evolved considerably away from BALB/c MOPC104E V_λ.

The V_λ sequences, then, have only minimal variations and many identities. The phenomenon has been interpreted by Weigert et al. (298) to reflect the basic mechanism of immunoglobulin variation. Essentially they postulate that a single V_λ gene is inherited and that this gene in different somatic cells undergoes random mutations that are selected sequentially. These mutations could develop in parallel or in series in a dividing stem-line population. According to a germ-line theory it might be argued that the mouse V_λ genome contains only a few genes.

The most important characteristic of the $V_{\lambda 1}$ in the mouse is that it is a model for "minimal variation" among immunoglobulin chains. Most workers now agree

TABLE 11. *Amino acid sequence variations in mouse $V_{\lambda 1}$ and $V_{\lambda 2}$ chains*

Lambda Chain	Amino Acid Position Where Alternatives have Been Observed													Ref
	16	25	32	38	50	52	54	62	87	94	95	97	98	
MOPC104E, RPC20* (λ_1)	Glu	Ser	Ser	Val	Ile	Gly	Asn	Ala	Ile	Tyr	Ser	His	Trp	7
S104†, XP8†, 2061, J558, J698, HOPC1 (λ_1)														298
RPC20‡ (λ_1)					Leu									298
S176 (λ_1)		Asn												298
2020 (λ_1)		Thr	Gly											298
S178 (λ_1)		Asn				Asn						Arg		298
MOPC315§ (λ_2)		Asn		Ile			Ser							79
MOPC315 (λ_2)	Gly			Ile			Ser	Val	Met	Phe	Arg		Phe	256

* RPC20 in vivo line, NIH. † S104 is a different tumor than MOPC104E and XP8 is a tissue-culture line from MOPC104E. ‡ RPC20 line maintained at Salk Institute originally obtained from MIH. § Goetzl and Metzger (79) also found Ser in position 36 of MOPC315.

that there are multiple genes in each of the V-region loci but not on how many. The mechanism of the genetic and biochemical basis for minimal variations remains as an outstanding unresolved problem in immunology; it can be explained presently by either the somatic or germ-line theories but the arguments until recently have been constructed from very little available data. As described in the following section, evidence for minimal variations has now been found within a κ-subgroup in the mouse by McKean et al. (160). Analysis of several of these systems may provide the basis for a biochemical hypothesis of the origin of variation. It should be kept in mind that variations in amino acid sequence in immunoglobulin V polypeptides are not entirely random and they appear to have preferential localization in regions of the chain that have been called hypervariable regions by Wu and Kabat (306).

2) V_κ. The complete or nearly complete sequences have been determined for 6 mouse κ-chains: MOPC41 and MOPC70E (82), MOPC21 (172), MOPC63, MOPC321, and TEPC124 (160) (Tables 12, 13). The V polypeptides may differ in length, as was originally found by Gray et al. (82). The MOPC41 and 21 (82, 172) chains, for example, contain 214 residues, whereas the others have 218 residues.

The difference is accounted for by an additional 4 residues between positions 27 and 28 in the MOPC41 numbering system. V_κ polypeptides in man (103) also differ by 4–6 amino acids at this site. The biologic origin of the difference is not known. One possibility is that an ancestral V_κ mammalian gene duplicated and one of the duplicates lost 4 residues via a deletion or gained 4 or more by an insertion; both forms were subsequently incorporated into the genomes of many mammalian species including man and mouse. The region near position 27 is of further biologic importance because it is one of the hypervariable region in κ-chains (306).

McKean et al. (160) have obtained the partial sequences of 3 proteins with

TABLE 12. *Partial amino acid sequences of four mouse V_κ polypeptides*

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
321	D	I	V	L	T	Q	S	P	A	S	L	A	V	S	L	G	Q	R	A	T	I	S	C
70	D	I	V	L	T	Q	S	P	A	S	L	A	V	S	L	G	Q	R	A	T	I	S	C
41	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	L	G	E	R	V	S	L	T	C
21	N	I	V	M	T	Q	S	P	K	S	M	S	M	S	V	G	E	R	V	T	L	T	C

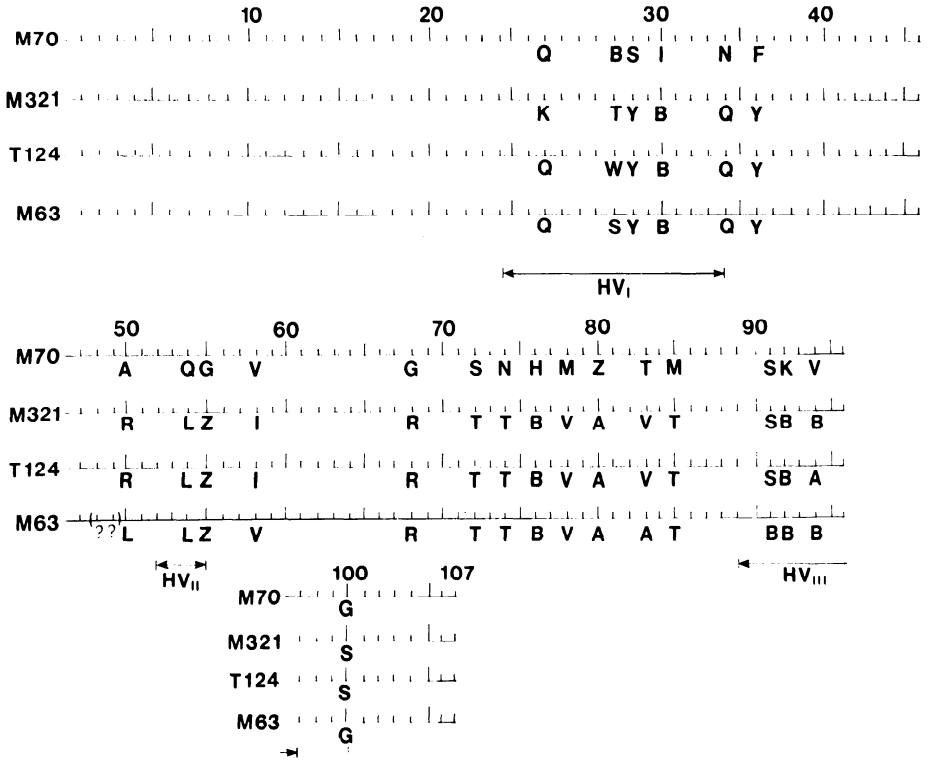
	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46
321	R	A	S	K	S	V	B	T	Y	G	B	S	F	M	Q	W	Y	Z	Z	K	P	G	Z
70	R	A	S	Q	S	V	B	B	S	G	I	S	F	M	N	W	F	Z	Z	K	P	G	Z
41	R	A	S	Q					B	I	G	S	L	S	B	W	L	Z	Z	G	P	B	T
21	R	A	S	Q					N	V	V	T	Y	V	S	W	Y	Q	Q	K	P	E	Q

	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71
321	P	P	K	L	L	I	Y	R	A	S	N	L	Z	S	G	I	P	A	R	F	S	G	S	G	S
70	P	P	K	L	L	I	Y	A	A	S	N	Q	G	S	G	V	P	A	R	F	S	G	S	G	S
41	P	P	I	K	R	L	Y	A	T	S	S	L	B	S	G	V	P	K	R	F	S	G	S	R	S
21	P	P	K	L	L	I	Y	G	A	S	N	R	Y	T	G	V	P	D	R	F	T	G	S	G	S

	72	73	74	75	76	77	78	79	80	81	82	83	84	85	86	87	88	89	90	91	92	93	94	95	96	97	98	99	100
321	R	T	B	F	T	L	T	I	B	P	V	Z	A	B	B	V	A	T	Y	F	C	Z	Z	S	B	Z	B	P	W // S
70	R	T	D	F	S	L	N	I	H	P	M	Z	Z	B	B	T	A	M	Y	F	C	Z	Z	S	K	F	V	P	W // G
41	R	T	D	Y	S	L	T	I	S	S	L	E	S	E	D	F	V	D	Y	?	C	L	Q	Y	A	S	S	P	W // G
21	R	T	D	F	T	L	T	I	S	S	V	Q	A	E	D	L	A	D	Y	H	C	G	Q	G	Y	S	Y	P	Y // G

321, McKean et al. (160); 70, 41, Gray et al. (82); 21, Milstein and Svasti (172).

TABLE 13. *Mouse κ-chain variable region sequence comparison*



The sequences of MOPC321, TEPC124, and MOPC63 were determined by McKean et al. (160).

the same amino-terminal sequence as MOPC70E (82). These proteins have the additional 4 residues between positions 27 and 28 (as in MOPC70) in the MOPC41 numbering system (82). The differences among the proteins in this group are given in Table 13. The MOPC70E differs from these 3 new proteins in at least 21 positions, clearly indicating that extensive differences may be found among proteins sharing the same amino-terminal sequence up to position 23. The 3 new proteins have minimal differences; MOPC321 and TEPC124 differ at only 3 positions, while MOPC63 differs from MOPC321 and TEPC124 at 8 positions. Considered as a group the MOPC321 and TEPC124 proteins resemble the mouse $V_{\lambda 1}$ subgroup described by Weigert et al. (298) in that they have only minimal variations among the members. The variations appear to occur in roughly the same regions of the κ-chains as in the λ-chains and correspond to the location of hypervariable regions as proposed by Wu and Kabat (306).

Many partial sequences of mouse V_κ polypeptides have also been determined; most have been done with the automated amino acid sequencer (104) (Table 14). With the exceptions of a single-chain AdjPC9 (199) that has an NH_2-terminal PCA and MOPC21 that has Asn (172), the other V polypeptides reported so far have either Asp or Glu at the amino terminal. Possibly the alternatives Asn and Asp at the amino terminal are not due to differences in the nucleic acid triplets but to chemical changes after the molecules have been secreted from the cell. Further evaluation of this problem in light of the recent findings of Milstein and Svasti (172) is now necessary.

The partial sequences of 31 V_κ polypeptides have been reported up to the first cysteine residue at position 23 (Table 14). Any 2 of these sequences can differ in as many as 60% of the positions (e. g. compare 773 and 167); most differences were considerably less. Among the first 23 positions only 2 remain invariant: Gln 5 and Cys 23 (Table 14). From 2 to 5 different amino acids were found at the other positions in the different polypeptides (Table 14); these were distributed throughout the polypeptide. Two of them, for example, occurred at positions previously considered invariant, e. g. types McPC600: Ile 5, TEPC15: Ser 16.

The amino-terminal sequence data on 31 proteins derived from different tumors reveal extensive variations. The important point concerning many of these unusual sequences (e. g. prototypes 773, 674, 603, 15, 167, and others) is that they have been isolated from different mice. It is difficult to conceive that variants such as these, which occur in parts of the molecule that have not as yet been implicated in antigen binding, would recur by chance. The most plausible explanation is that each of these sequences is controlled by a separate germ-line gene. The same argument may not apply to the minimal variations observed in $V_{\lambda 1}$ and the V_κ MOPC-321 subgroup, which could arise at the somatic level by a mechanism not yet elucidated.

F. C_H

Allotypes for only C_H genes have been demonstrated in the mouse (97, 142–144, 219, 220, 222) (Table 15). Most of the phenotypic variants are antigenic and

TABLE 14. *Amino-terminal sequences of 31 mouse κ-chains of BALB/c origin*

Prototype	No. Chains	1									10									20			23
773	2	E	T	T	V	T	Q	S	P	A	S	L	S	M	A	I	G	E	K	V	T	I	S	C
M29	1	E	N	V	L	T	Z	S	P	A	I	M	S	A	S	P	G	E	R	V	T	M	T	C
T29	1	E	V	V	L	T	Z	S	P	A	I	M	S	A	S	L	G	L	R	V	S	M	S	C
47	1	E	V	V	M	T	Q	T	P	L	S	L	A	V	(S)	L	G	( )	Z	A	(S)	( )	( )	( )
674	2	D	V	V	M	T	Q	T	P	L	T	L	S	V	T	I	G	E	P	A	S	L	S	C
LI	1	D	I	V	M	T	Q	S	P	S	S	M	Q	A	S	I	G	E	K	V	T	I	S	C
157	1	D	I	V	M	T	Q	S	Q	S	F	M	S	T	S	V	G	D	R	V	S	V	T	C
603	3	D	I	V	M	T	Q	S	P	S	S	L	S	V	S	A	G	E	K	V	T	M	S	( )
15	2	D	I	V	M	T	Q	S	P	T	F	L	A	V	T	A	S	K	K	V	T	I	S	C
467	2	D	V	L	M	T	Q	T	P	L	S	L	P	V	(S)	L	G	D	E	A	( )	I	S	C
173	1	D	I	Q	M	T	Q	T	T	S	S	L	S	A	S	L	G	D	( )	V	T	I	( )	( )
31C	2	D	I	Q	M	T	Q	S	P	A	S	L	S	A	S	V	G	E	R	V	T	I	T	C
41	1	D	I	Q	M	T	Q	S	P	S	S	L	S	A	S	L	G	E	R	V	S	L	T	C
149	1	D	I	Q	M	T	Q	S	P	B	Y	L	S	A	S	V	G	E	T	V	T	I	T	( )
600	1	D	I	Q	M	I	Z	S	P	S	S	M	F	A	S	I	G	D	Z	V	S	I	S	C
46	2	D	I	V	L	T	Q	S	P	A	T	L	S	V	T	P	G	D	S	V	S	L	S	C
70	4	D	I	V	L	T	Q	S	P	A	S	L	A	V	S	L	G	Q	R	A	T	I	S	C
167	2	D	I	V	I	T	Q	B	E	L	S	D	P	V	T	S	G	E	S	V	S	I	T	C
21	1	N	I	V	M	T	Q	S	P	K	S	M	S	M	S	V	G	E	R	V	T	L	T	C
9	1	PCA	(I	V	L)																			
Total 20	31																							

No. of chains = number of chains with similar sequence from positions 1 to 23.

Tumor Source of Prototype V_κ Polypeptide	Other Tumors With Similar NH_2-Terminal Sequence	Ref	Tumor Source of Prototype V_κ Polypeptide	Other Tumors With Similar NH_2-Terminal Sequence	Ref
McPC773	MOPC265	104	MOPC173		104
MOPC29		207	MOPC31C	RPC23	104
TEPC29		207	MOPC41		82
MOPC47		207	MOPC149		104
McPC674	McPC843	104	McPC600		207
LPC1		104	MOPC46	MOPC172	207
MOPC157		207	MOPC70E	MOPC321, TECP124, MOPC63	82, 104
McPC603	MOPC384, McPC870	104	MOPC167	MOPC511	104, 207
TEPC15	HOPC8	104	MOPC21		172
MOPC467	MPC37	104	AdjPC9		199

can be identified by specific homologous antisera that are prepared by immunizing one strain of mouse with an appropriate immunoglobulin from a genetically different source (98, 139). The typing of mouse immunoglobulins is discussed in detail elsewhere (100, 219, 220); the available polymorphic forms of myeloma proteins are listed here in Table 14. Some of these proteins are derived from rare tumors that arose in other strains, but most have come from congenic stocks of mice in which genes controlling C_H polypeptides have been introgressively backcrossed onto BALB/c (219) or hybrids. Plasma cell tumors have been induced in these stocks, thus providing tumors that produce immunoglobulins controlled by genes other than those in BALB/c. It is a well-established fact that the phenomenon of allelic exclusion affects immunoglobulin structural genes (93, 100, 295); that is, in a given normal plasma cell only one of the two alleles is functional. In a plasma cell tumor

TABLE 15. *Prototype myeloma proteins that carry allotypic markers for different polymorphisms*

C_H gene	BALB/c, C3H/HE		BALB/c.C57BL IgC_H		AL/N, NZB, BALB/c.AL IgC_H	
	Myeloma	Marker	Myeloma	Marker	Myeloma	Marker
G (γ2a)	AdjPCX5563	$G^{1,6,7,8}$		2	GPC8	$4G^{6,7,8}$
H (γ2b)	MOPC141	$H^{9,11}$	MOPC352*	$H^{9,16}$	GPC7	$Ig^{1,5}$ $Ig^{3.3}$†
F (γ1)	MOPC21	F^{fast}	MOPC300*	F^{slow}		F^{fast}
A (α)	TEPC15	$A^{12,13,14}$	MOPC320*	A^{15}	AL3	A^{13}

BALB/c.C57BL/Ka IgC_H congenic strain was developed by 20 consecutive introgressive backcrosses of the chromosome segment carrying the C57BL/Ka Ig C_H region onto BALB/c. During development of this strain, tumors were induced at various backcross generations; in addition backcrossed mice were mated to each other to produce mice homozygous for the C57BL/Ka IgC_H region.

* Tumor arose in progeny of a BALB/c.C57BL Ig C_H mouse that was homozygous for 5 C57BL heavy-chain linkage group (219). † Marker system used here is described in ref 220 except for GPC7, which is described in ref 295.

TABLE 16. *Summary of available V_H polyeptide sequences*

	1					10									20									30								
MOPC173*	E	V	K	L	L	Q	S	G	G	P	L	V	Q	L	G	G	S	L		K	L	S	C	A	A	S	G	F	D	F	S	R	Y	W	M
MOPC167**	E	V	K	V	V	Q	S	G	G	G	L	V	P	S	G	G	S	L†																	
MOPC511‡	E	V	K	L	V	Z	S	G	G	G	L	V	Z	P	G	G	(	)	L	K†															
McPC603**	E	V	K	L	V	Q	S	G	G	G	L	V	Q	P	G	G	S	L		(	)	L†													
TEPC15, HOPC8**	E	V	K	L	V	Q	S	G	G	G	L	V	Q	P	G	G	S	L		(	)	L†													
MOPC21A§	D	V	Q	L	V	Q	S	G	G	G	L	V	Q	P	G	G	S	(M)		K	L	S	C	A	A	S	G	F†							
MOPC406§	D	V	K	L	L	Q	S	G	G	G	L	V	Q	P	G	G	S	L		K	L	S	C	A	A	S	G	F†							

( ) = residue was recovered but not identified. * Bourgois et al. (29). † End of determined sequence. ** Sequences determined by L. Hood and M. Potter (unpublished data). ‡ Sequence determined by S. Rudikoff et al. (243a). § Tentative sequences determined by J. D. Capra and J. M. Kehoe (unpublished data).

all the cells express only the one allele. Thus in plasma cell tumors arising in mice heterozygous for polymorphic forms of immunoglobulins only one of the two alleles is operative. Warner et al. (295) have demonstrated this phenomenon in three plasmacytomas induced in (NZB × BALB/c)F_1 hybrids.

The GPC7 plasmacytoma produced a γG myeloma protein that was directed by the heavy-chain gene of NZB origin, and the GPC8 plasmacytoma produced a γG myeloma protein that was directed by the BALB/c heavy-chain gene (296). The GPC5 tumor produced a γH myeloma protein of NZB origin that was unusual in that it carried two allotypic determinants, one that was usually associated with γG and the other with γH. Herzenberg et al. (100) speculated that the unusual combination might have evolved by a somatic crossing over. An alternative possibility is that all γH molecules directed by the NZB heavy-chain gene carry both determinants and that the two determinants became associated in the germline gene.

G. V_H

Very few sequences have been published for V_H polypeptides (see Table 16). In the mouse many V_H polypeptides are unblocked and this has permitted the determination of the amino acid sequence from the amino-terminal end on the sequencer. Bourgois and colleagues (29) sequenced the MOPC173 V_H polypeptide and found that it has many homologies to the Eu polypeptide in man. A list of other partial sequences determined is given in Table 16.

H. Comments

The data currently suggest an immunoglobulin structural gene locus will be a complex locus (i.e. containing multiple genetic elements dealing with the same function). Each locus, i.e. "kappa" and "lambda" (possibly λ1 and λ2) and "heavy," contains a C gene and a set of V genes. There must be considerable variability at such loci, for the number and types of genes appear to differ between species. That is, during evolution the number of genes at a locus may change; further, the genes within the locus in any two species may differ so much that it is difficult to demonstrate homologies between species. Striking species characteristics of *Mus* (in contrast to human) are the large number of variations in amino acid sequence in the amino-terminal Cys 23 peptide of V_κ polypeptides and the limited number of V_λ polypeptide variations. Human λ-chains, however, in contrast to those in *Mus* have many variations, some of which are demonstrable in the Cys 23 amino-terminal peptide.

Two theories for the mechanism that produces variations are the multiple-gene theory and the somatic-mutation concept (46). The evidence in the mouse strongly indicates that many genes are involved. The question remains whether somatic mutations are superimposed on multiple genes to account for further diversity or whether in fact there is a single germ-line gene for each unique V polypeptide.

VIII. ANTIGEN-BINDING MYELOMA PROTEINS

Since 1967 there has been a concerted effort to find antigen-binding myeloma proteins; as a result approximately 5% of myeloma proteins in mice have been shown to combine with specific antigens (Table 17) (207–209).

Antigen-binding myeloma proteins in mice are usually discovered by screening procedures in which myeloma proteins are tested for their ability to precipitate, agglutinate, or bind any of a variety of antigens or haptens. The most economical and most widely used screening method is the micro-Ouchterlony precipitin method. Other methods have been employed, including a spectral-shift assay (69) and the Farr assay method (283). In 1967 Cohn (45) reported the results of a screening test in mice in which he identified the first active myeloma protein in the mouse, an IgA protein (S63), which precipitated with the pneumococcus C polysaccharide. Since 1967

TABLE 17. *Antigen-binding myeloma proteins*

Specificity	Natural Antigen*	Other Antigens*	Myeloma Proteins (Ig Class)
Nitrophenyl		DNP-, TNP-derived proteins	MOPC315 (α, λ2), MOPC460 (α, κ)
Phosphoryl-choline	Lactobacillus 4	Pneumococcus C ps	S63, S107, TEPC15, HOPC8
	Trichoderma	*Ascaris* extract	McPC603, MOPC167, MOPC511 (all α, κ)
α1→ 3-linked glucose		B1355 dextran (Leuconostoc)	MOPC104 (μ, λ1), J558 (α, λ1)
β1→ 6-linked galactose	Milling wheat, hardwood bedding extract	Arabinogalactan, gum ghatti protein-*p*-azophenyl β-D-galactoside	J539(α) SAPC10 (α, κ) TEPC191B (α, κ)
N-acetyl-*d*-glucosamine		BGG-*p*-azophenyl-β-*N*-acetyl-D-glucosamide, *S. aureus*, teichoic acid, streptococcal group A ps	S117 (α)
N-acetyl-D-mannosamine		*S. weslaco* lps, *E. coli* 031 lps	MOPC406 (α, κ)
α-Methyl-D-galactose	*Proteus mirabilis* sp2 lps	*S. tel aviv* lps, *S. tranoroa* lps	MOPC384 (α, κ)
α-Methyl-D-mannoside	*Proteus mirabilis* sp2 lps	*S. tel aviv* lps, *S. tranoroa* lps	McPC870 (α, κ)
Undetermined	*Mima polymorpha* lps		TEPC48 (α, ?)
		Levan, inulin	J606 (γ3, κ)
		H. influenzae B ps	SAPC15 (?, λ)
Protein?	Wheat extract		TEPC521 (α, ?)
		Salmonella antigen	MOPC467 (α, κ)

* Abbreviations: ps = polysaccharide; lps = lipopolysaccharide.

many new mouse myeloma proteins with antigen-binding activity to a variety of antigens have been reported (69, 88, 111, 134, 207, 209, 221, 253, 261, 289). Using screening procedures, antigens from different sources have been introduced into various screens, including: *1*) artificial antigens, (e.g. chemically substituted proteins), *2*) antigens available to an investigator from other colleagues or commercial sources, and *3*) natural antigens isolated from the environment of the mouse. It has been noted several times that myeloma protein from different tumors reacts with the same antigen and that the same antigenic determinant (hapten) is usually involved. Haptens for which there are several active myeloma proteins are nitrophenyls (69, 111) and phosphorylcholine (47, 131, 218, 221) $\alpha 1 \rightarrow 3$ dextrans (298), β-1 $\rightarrow$ 6 galactose (224, 262). Finding several myeloma proteins in different mice that bind the same antigen suggests that the antigen so identified may have some special biologic significance. The immunochemical and biologic questions relating to antigen-binding myeloma proteins are discussed in terms of the antigen or hapten (when identified.)

A. Nitrophenyl

Eisen et al. (69) screened 116 randomly assembled proteins against DNP-BγG, DNP-HSA, TNP-BγG, and TNP-HSA and found 3 that precipitated with the DNP and TNP and 1 that precipitated with TNP-substituted proteins. Schubert et al. (253) screened 240 myeloma sera of different classes against 21 different bovine serum albumin derivatives; 15 proteins precipitated with both DNP-BSA and TNP-BSA, 1 with DNP-BSA only, and 1 with TNP-BSA (Table 18). In another collection of myeloma proteins derived from consecutively established transplantable tumors (208) (including a number in ref 69), 1 precipitated with DNP and TNP and 3 precipitated with only TNP (208). Myeloma proteins that bind nitrophenyl-substituted proteins are found frequently by the precipitin screening method.

Precipitin reactions with nitrophenylated proteins must be judged with caution and should be further evaluated before drawing conclusions as to whether they resemble induced antinitrophenyl antibodies since proteins in general may bind DNP groups nonspecifically. The binding affinities for ε-DNP lysine of 15 of the myeloma proteins detected in the studies mentioned (69, 111, 253) have been determined in equilibrium dialysis and by fluorescence quenching by Eisen et al. (68). The MOPC315 protein (69) was found to have a K_A of 1×10^7 M^{-1} (4 C) and the MOPC460 (111) a K_A of 3×10^5 M^{-1} (4 C), while all the others had combining affinities below 1×10^4 M^{-1} (68). The proteins with low binding activities for ε-DNP ligands in equilibrium dialysis also did not bind DNP lysine by other criteria, e.g. by producing characteristic difference absorption spectra or fluorescence quenching. The weak reactions were probably due to nonspecific (possibly hydrophobic) interactions [see discussion by Parker and Osterland (195)] with nitrophenyl groups. Only MOPC315 and MOPC460 were considered to resemble individual molecular species in conventional induced antibody (67, 69).

1) Immunochemical studies with MOPC315 and MOPC460. Because of their relatively high binding activity for nitrophenyl groups the MOPC315 and MOPC460

TABLE 18. *BSA-derived proteins used by Schubert et al. (253) for screening 240 mouse myeloma sera for precipitating activity*

Negative		Positive
Acetanilide	7-Naphthylene	5-Acetyluracil (5Au) [19]*
p-Arsanilic acid	*o*-Nitrobenzene	2,4-Dinitrobenzene (DNP) [15]
Benzene	*m*-Nitrobenzene	Purine [7]
p-Benzene sulfonic acid	*p*-Nitrobenzene	2,4,6-Trinitrobenzene TNP [15]
p-Benzoic acid	Penicillamine	Adenylic acid [1]
p-Chlorobenzene	Phenylazobenzene	
p-Dimethylamine phenyl-sulfone	7-Quinolene	
Toluene	Estrone	

* No. in brackets is number of different myelomas that precipitated with the derivative. There were 26 active proteins in all: 1 to DNP alone, 1 to TNP alone, 10 to 5AcU alone, and 14 to various combinations (253).

TABLE 19. *Comparative properties of two IgA myeloma proteins that bind nitrophenyl derivatives*

	MOPC315	MOPC460
Light-chain subunit	λ2	κ
K_A for ε-DNP lysine: (7*S*)*	1×10^7	3×10^5
Valence of 7*S*	2*	2
Valence of Fab	1	
K_A for 2,4-dinitronaphthol	1×10^5	5×10^6
K_A for menadione (vit K_3)	5×10^5	1×10^4
Relative binding for NP derivative	2, 4, 6 ≧ 2, 4 > 2, 6 > 4†	
K_I for 5-acetyluracil caproate	3×10^4	
K_I for caffeine	5×10^4	
K_I for riboflavin	3×10^4	
K_I for adenine	2×10^3	
Ref	67, 68	67, 112

K_I and K_A are expressed as moles per liter^{-1}.

* Determined by equilibrium dialysis at 4 C. † 2,4,5 = TNP; 2,4-DNP, 2,6-DNP, 4-nitrophenyl.

myeloma proteins have been widely used as immunochemical models of homogeneous antibodies. The nitrophenyl haptens have been favorites in immunochemistry and many sensitive assays are available to measure and characterize binding. Studies relating to valence, specificity for chemically related ligands, and the structure of the antigen-combining site have been carried out with MOPC315 and MOPC-460. One of the major aims of the work not yet fulfilled is to establish a three-dimensional model of active immunoglobulin molecules by X-ray crystallography, in order to localize the antigen-combining site and to establish the segments of the L and H chains that form its boundaries. Inbar et al. (108) have made an important advance in this effort by successfully crystallizing the MOPC315 pepsin fragment.

Immunochemists usually work with IgG or IgM antibody, and the availability of the homogeneous IgA myeloma proteins raised some concern since little was known about IgA antibody. Further, it had been shown that in the BALB/c mouse IgA immunoglobulins lack L-H disulfide bonds (2, 89). Although the mouse IgA homogeneous immunoglobulins are structurally different in several respects from IgG and IgM antibodies, this has not as yet influenced the ability of these molecules to behave as "homogeneous" antibody.

Eisen et al. (67–69, 90, 111, 112, 256, 287) have studied extensively the immunochemical properties of IgA myeloma proteins MOPC315 and MOPC460. The relative binding affinities of the IgA's (7*S* monomer) and Fab's for a variety of ligands have been determined; a summary is given in Table 19. It can be seen that M315 and M460 both bind ligands with different chemical structures, e.g., the naphthoquinone menadione is bound reasonably well by M315 but weakly by M460 Further, there is considerable difference in the binding of different dinitrophenyl or dinitronaphthol compounds. MOPC315, for example, much more effectively binds 2,4-dinitrophenyl than 2,6-dinitrophenyl, as determined by fluorescence

quenching (67, 68, 287). MOPC460 has a higher affinity for 2,4-dinitronaphthol than for ϵ-2,4-dinitrophenyl lysine, while M315 is just the reverse (112). All these findings indicate the combining sites of both of these molecules, though individually specific and unique, can accommodate and bind other chemical structures. As pointed out by Eisen (68), the difference in affinity for diverse DNP compounds (e. g. 2,6-DNP vs. 2,4-DNP) rules out binding on the basis of nonspecific hydrophobic interactions. Possibly some of the binding is enhanced by the formation of charge-transfer complexes of the ligand with tryptophan residues in the site (67).

Rockey et al. (241) have studied the interaction of MOPC315 with DNP ligands in the spectropolarimeter; they observed circular dichroism bands that suggest a coupling of the DNP group and a protein chromophore (tryptophan or tyrosine) that contributes in part to the interaction in the combining site.

The inhibition of binding of ϵ-DNP lysine by MOPC315 and by unrelated compounds (5-acetyluracil caproate, caffeine, and riboflavin) was demonstrated by Eisen et al. (68). Though detectable inhibitors, these unrelated compounds were about a 1000-fold less potent than ϵ-DNP lysine.

The valence of the 7*S* MOPC315 for ϵ-DNP lysine has been a problem (69). Initially the valence was determined to be 1.2 on the basis of an incorrect molecular weight of 120,000 (69). Using the correct value of 150,000, the valence increased to 1.5, which is still below the expected 2.0 (287). It now appears that at low concentrations MOPC315 tends to be denatured. At high protein concentrations (287) 2.0 $\pm$ 0.1 sites per 7*S* molecule have now been demonstrated.

Further, the valence of Fab (mol wt 55,000) was close to 1 (287). Green et al. (84) have studied with the electron microscope the interaction of 7*S* MOPC315 with bis-(DNP-β-alanyl)-diaminosuccinate. Curious double-bar figures were observed that they interpreted as being produced by the formation of a characteristic complex that contains four 7*S* monomeric units linked together by four molecules of bifunctional hapten. The double-bar effect results from the superimposition of two Fab (on one another). They further speculate from the figures that the variable and constant regions in MOPC315 Fab form separate molecular regions (84). It has been noted that MOPC315 7*S* monomer does not agglutinate nor precipitate with polyvalent antigens (DNP-coupled red cells or proteins). This contrasts with certain 7*S* Ig molecules, e.g. the γF (γ1) homogeneous product produced by clone 9 (17), which does actively precipitate DNP_{10}-BSA (B. A. Askonas, personal communication). The difference between MOPC315 and clone E9γF is possibly due to the orientation in space of the respective combining sites. Should these form a "Y" structure, where the Fab's are more nearly parallel to each other, then cross-linking functions (e.g. agglutination and precipitation) might be sterically difficult to align. Similar observations have been made with conventional antibodies (116, 293), where it has also been observed that some 7*S* (four-chain) forms of Ig are good cross-linkers while others are not.

Because of its relatively high affinity for DNP ligands, it has been possible to label MOPC315 by affinity site with several types of reagents. Metzger and colleagues (167, 168) have labeled the MOPC315 protein with *m*-nitrobenzene diazoniumfluoroborate (NBDF). Over 90% of the label was identified on the light

chain and was specifically bound on Tyr 34 of the λ2-chain (79). The MOPC315 λ2-light chain has been sequenced in this region by Goetzle and Metzger (79). A nearly complete but still partial sequence of the MOPC315 λ-chain has now been published (256). MOPC315 varies from other λ1-type light chains in at least 9 positions between residues 1 and 98 (Table 11). Haimovich et al. (90) have affinity-labeled MOPC315 with a variety of DNP bromacetyl derivatives. The bromacetyl derivatives labeled either Tyr 34 on the λ2-light chain or lysine residue on the α-heavy chain. The specificity of labeling depends on the length of the molecules between the DNP group on one end and the bromacetyl on the other. The shorter compounds, e.g. bromacetyl ε-DNP ethylenediamine (BADE), preferentially labels Tyr 34, whereas longer compounds, e.g. bromacetyl ε-*N*-DNP lysine (BADL), preferentially labeled the lysine residue on the heavy chain (68, 90). A particularly interesting finding has been that the MOPC315 protein binds L-BADL and D-BADL equally well but only the L-BADL compounds are capable of labeling the site, presumably because when the D-BADL compound is bound the reactive bromacetyl group is not contiguous to the reactive tyrosine. Recently bis-bromacetyl derivatives have been made, and these form covalent bridges across the H and L chains (67).

Bridges and Little (30) have successfully reconstituted the MOPC315 and MOPC460 proteins from separated light and heavy chains. The IgA molecules were reduced and alkylated with iodoacetic acid, after which the chains were dissociated in 4.5 M urea-1 M propionic acid and separated by gel filtration on a Sephadex G-100 column. The reconstituted molecules were evaluated for binding activity with fluorescence quenching and found to be comparable to the parent nondissociated molecules. Heterologous molecules, e.g. 315L-460H and 460L-315H, were also prepared but little DNP binding activity was found; 315L-460H was more efficient at binding DNP L-lysine than 460L-315H. Bridges and Little (30) found unique pairs of L and H chains are required to form specific binding sites.

Sirisnha and Eisen (265) have shown that MOPC315 and MOPC460 are autoimmunogenic, as they have been able to prepare idiotypic antibodies in BALB/c mice to these proteins. The autoantibodies are nonprecipitating and are detected by radioimmunoassay methods. The interaction of the autoantibodies and the respective myeloma protein can be inhibited by DNP ligands, indicating the idiotypic determinant is at or near the site. Brient et al. (31) have prepared rabbit antisera to MOPC315 that also identify a determinant near the hapten-binding site that is inhibitable by DNP ligands.

Yamada et al. (308) have developed a plaquing method for IgA anti-DNP-producing cells. They claim (308) "almost all of the cells" in the MOPC315 tumor produce plaques.

B. 5-Acetyluracil, DNA

Schubert et al. (253) noted in their series of myeloma proteins tested with substituted proteins (see Table 18) that one of the most common activities was directed

to 5-acetyluracil BSA (5Au-BSA). Ten proteins were found that precipitated 5Au-BSA alone and nine others that precipitated 5Au-BSA and nitrophenyl derivatives. They also noted that rabbit anti-DNP antibody and MOPC315 precipitated with 5Au-BSA. However, as shown later by Eisen et al. (68), MOPC315 bound 5-acetyluracil and uracil very weakly (as determined by inhibition of binding ϵ-DNP lysine), but did bind 5-acetyluracil (5AcU) caproate. The binding activity for 5AcU caproate was 1000-fold lower than for ϵ-DNP lysine.

The ability of myeloma proteins to bind purine and pyrimidine derivatives suggested to Schubert et al. (255) that DNA might be a natural immunogen and that cells producing species of Ig that cross-react with nitrophenyl and nucleic acids were prone to neoplastic transformation during plasma cell tumor induction in BALB/c. Three proteins—J504 (DNP+, TNP+, 5Au+), S179 (5Au+ only), and S23 (DNP+, TNP+, 5Au+)—bind DNA (255). The interaction was studied by a membrane filter assay using ϕ80 DNA. Highly specific low-molecular-weight inhibitors were not identified, although several nucleoside and nucleotide inhibitors were equally able to inhibit the precipitation of DNA. Polycytidylic acid was a potent inhibitor. It was thought the combining sites on these molecules were not highly specific and further that several types of ligands could be accommodated in the sites. Possibly, too, the determinants were dependent on a secondary structure of DNA.

C. Phosphorylcholine

Eleven independently induced plasma cell tumors produce IgA myeloma proteins that precipitate with the pneumococcus C polysaccharide (PnC) (45, 47, 218, 221, 261). This antigen has choline as a constituent (32, 285), and this fact led to the important discovery by Leon and Young (131, 132) that the IgA myeloma proteins that precipitate pneumococcus C polysaccharide do so by binding phosphorylcholine or choline groups. Phosphorylcholine inhibits the precipitation of PnC by the eight myeloma proteins (132, 221), and choline inhibits the precipitation of PnC by two of the proteins (M167, M511) (132, 221). Metzger and colleagues (167) have determined the K_A's for several of these IgA myeloma proteins for phosphorylcholine in equilibrium dialysis and found the K_A's vary from 1×10^4 to 1×10^6 M^{-1} (Table 20).

Sher et al. (261) have determined the K_A's for antiphosphorylcholine-binding proteins with a membrane filter binding assay. Isolation of the proteins in pure form has depended on the use of immunoabsorbants. Chesebro and Metzger (36) have prepared a substituted Sepharose, whereas Sher et al. (261) have used Sepharose coupled with the pneumococcus C polysaccharide.

The monomeric IgA forms do not precipitate the PnC polysaccharide but do bind phosphorylcholine in equilibrium dialysis; the Fab's are also active in binding.

So far all the antiphosphorylcholine myeloma proteins are in the IgA class. The light-chain subunits for these proteins are the κ-type and are found in three of the κ-subclasses of BALB/c mice (104, 208). These subclasses are based on amino

TABLE 20. *IgA myeloma proteins that bind phosphorylcholine*

Protein	Origin	κ-Chain Amino-Terminal Sequence*	Idiotype	K_A Phosphoryl-choline,† M^{-1}	Phosphoryl-choline: Choline‡	Phosphoryl-choline: Phosphono-choline‡
S63	Cohn (45)		S63		1:560	1:90
S107	Cohn (47)		S63		1:620	1:93
TEPC15	Anderson (221)	Asp Ile Val Met Thr Gln Ser Pro Thr Phe	S63	1.9×10^5	1:550	1:95
HOPC8	Anderson (221)	Asp Ile Val Met Thr Gln Ser Pro Thr Phe	S63	1.8×10^6		
MOPC-299	Potter (221)		S63		1:430	1:50
McPC-603	McIntire (221)	Asp Ile Val Met Thr Gln Ser Pro Ser Ser	M603	2.0×10^6	1:870	1:11
MOPC 167	Potter (221)	Asp Ile Val Ile Thr Gln Asx Glu Leu Ser	M167	1.4×10^5	1:10	1:33
MOPC-511	Potter (221)	Asp Ile Val Ile Thr Glu Asx Glu Leu Ser	M511	$.14 \times 10^6$		

* Determined by Hood et al. (104). † Determined by Metzger et al. (167). ‡ Determined by Leon and Young (132).

acid sequence differences in the NH_2-Cys 23 terminal peptides (see Tables 14, 20). The MOPC511 tumor is exceptional and produces a kappa IgA myeloma protein in the serum and a Bence Jones protein of the lambda type that is excreted in the urine (207). On this basis it was thought that the subunit of the IgA serum protein was of the lambda type; however, M. Weigert (personal communication) found that the MOPC511 serum IgA myeloma protein contained predominantly a kappa-type light chain. Appella established its amino-terminal sequence in the automated amino acid sequencer and found it to be identical to the MOPC167 kappa-type light chain (see Table 14). As may be seen from Table 20, MOPC167 and MOPC-511 are inhibited by free choline.

Structural studies on the antiphosphorylcholine IgA myeloma proteins thus far are incomplete. However, these proteins have been studied serologically with highly specific anti-idiotypic sera (221). Individually specific sera are prepared by immunizing strain AL or A/He mice with the myeloma protein (143, 219, 221). Some sera require absorption with normal serum to remove antiallotype antibodies; others do not. The anti-idiotype sera are tested to a battery of over 100 myeloma sera to establish specificity (221). Lieberman and Potter (unpublished observations) have prepared antisera to over 30 different IgA myeloma proteins of BALB/c origin. Each of the 8 IgA myeloma proteins that bind phosphorylcholine-containing antigens were used for the preparation of idiotypic antisera (221). Antisera produced to MOPC167, MOPC511, and McPC603 were specific for the respective myeloma protein. Antisera prepared to any of the 5 proteins (HOPC8, TEPC15, MOPC299, S63, and S107) precipitated all 5 of the proteins regardless of the immunogen used (221). None of the other test proteins was precipitated. Further, each of the proteins effectively absorbed out all the specific precipitins in the 5 antisera regardless of whether it was the "immunogen" or not. These results indicate the proteins mentioned possess a common individual antigenic specificity,

which strongly suggests that the proteins have extensive if not complete structural identity. Recently Sher et al. (261) have found 2 new proteins with the S63 idiotype, bringing the total to 7.

Natural antigens from different sources are precipitated by these antiphosphorylcholine myeloma proteins. These include the PnC polysaccharide, polysaccharides from group O *Streptococcus* and some group H *Streptococcus* (218), *Ascaris* extract (209), *Lactobacillus acidophilus* antigen (221), and several fungi (*Aspergillus, Trichoderma, Fusarium*) (207). Natural antigens in the BALB/c environment include antigens from *Lactobacillus* and fungal antigens. *Aspergillus* and *Fusarium* were isolated from the gastrointestinal tracts of BALB/c mice and *Trichoderma* were isolated from the mouse food (207). The *Lactobacillus acidophilus* antigen is an intracellular antigen (C. W. Mills, unpublished observations). Only the McPC603 protein precipitated an antigen produced by a strain of *Proteus morganii* isolated from the BALB/c mouse (207), which suggests that the *Proteus morganii* antigen also contains a cholinelike material that 603 but not the other proteins can bind. Leon and Young noted McPC603 was most efficiently inhibited by phosphonocholine (131, 132). Possibly there is an unusual choline derivative or linkage in the *Proteus morganii* antigen.

Leon and Young (132, 133) have also found that the antiphosphorylcholine-binding myeloma proteins agglutinate sheep RBC to which human β-lipoprotein has been coupled; thus autogenous lipoprotein might be regarded as an antigen. Hyperlipidemia has not been studied in the mice bearing these tumors.

D. $\beta 1 \rightarrow$ 6-Linked Galactoses

Sher et al. (262) screened a number of myeloma sera with proteins substituted with various carbohydrate derivatives and found an IgA myeloma protein J539 that precipitated with proteins substituted with β-D-galactoside. The J539 protein was isolated on a galactoside immunoabsorbant prepared by coupling *p*-aminophenyl β-D-galactoside to Sepharose. The protein was specifically eluted with isopropylthiogalactoside. The average association constant of J539 and isopropyldithiogalactoside was 2.3×10^3 M^{-1}.

Using an extract of mouse food as a screening antigen, two other proteins (SAPC10 and TEPC191B) were identified by us that bound $\beta 1 \rightarrow$ 6-linked galactose-containing antigens (224). The active component in the mouse food extract was derived from milling wheat; other antigens also precipitated by these two proteins were arabinogalactan (larch), gum ghatti, and an extract of the hardwood bedding used in germfree cages. Gum ghatti and arabinogalactan polysaccharides have side chains of $\beta 1 \rightarrow$ 6-linked galactose (12). The di-, tri-, and tetra-$\beta 1 \rightarrow$ 6-linked galactose oligosaccharides were found to be potent inhibitors of the precipitin reactions, with the tetraose being the most potent inhibitor.

An IgA myeloma protein called SAPC10, which was produced by a plasmacytoma induced in a monocontaminated (*S. tel aviv*) germfree mouse (207), did not precipitate with antigen derived from *S. tel aviv*. A potential natural source of antigen in the environment of the germfree mouse was found to be the hardwood

bedding used in the cages in which these mice were kept. A heat extract of this bedding was prepared by autoclaving the bedding and then precipitating the extract in 75% ethyl alcohol. The TEPC191B tumor developed in a mouse fed a diet rich in wheat, which is rich in antigens with $\beta 1 \rightarrow 6$-linked galactose groups. The J539 protein (kindly supplied by M. Cohn) also actively precipitates with the same antigens (gum ghatti and arabinogalactan) as SAPC10 and TEPC191. It is assumed that these proteins form another group that reacts with a common hapten.

E. β-D-N-Acetylglucosamine

Vicari et al. (289) screened 275 myeloma sera against 14 carbohydrate-derived proteins and found one protein that precipitated with conjugates containing β-D-*N*-acetylglucosamine. In addition, this protein also precipitated with several natural antigens, including β-teichoic acids, the *Streptococcus* group A polysaccharide, and the first and third periodate oxidation and Smith degradation stages of the blood group H substance (289). The precipitation reactions were inhibited by a disaccharide β-D-GNAc (1 → 3) D-Gal.

F. α1 → 3 Dextrans

The IgM MOPC104 protein precipitates with several dextrans of microbial origin, including the preparation B1355S4 containing $\alpha 1 \rightarrow 3$ dextran from *Leuconostoc meserentoides* (isolated by A. Jeanes). This preparation contains mixed-type glucose linkages: 57% is 1 → 6, 34% $\alpha 1 \rightarrow 3$, and 9% 1 → 4 linkages. However, Leon et al. (134) found that the $\alpha 1 \rightarrow 3$ oligosaccharide series inhibits the precipitation of dextran by MOPC104. Of the various nigeryl oligosaccharides examined the most potent were nigerotriose, nigerotetraose, and nigeropentose (134); very little difference was noted for these three oligosaccharides.

Young et al. (315) have determined the association constant of MOPC104 for a series of nigeroses and found that the highest binding activity [$K \times 10^{-4}\ M^{-1} = 3.6$] was for nigerosyl $\alpha(1 \rightarrow 4)$ nigeritol; however, very little difference was noted for three other tetraoses: nigerosyl $\alpha(1 \rightarrow 3)$ nigerose, nigerosyl $\alpha(1 \rightarrow 4)$ nigerose, and nigerosyl $\alpha(1 \rightarrow 3)$ nigeritol.

Recently Weigert et al. (298) found a second IgA myeloma protein J558 that also binds $\alpha 1 \rightarrow 3$-linked dextrans. It is of great interest that the MOPC104 and J558 myeloma proteins, which possess a λ1-type light chain (298) with identical sequence, bind the same hapten. Weigert et al. suggest the anti-$\alpha 1 \rightarrow 3$ dextran-combining activity in the BALB/c mouse is probably a function of this λ-chain (46, 298).

G. N-Acetyl-D-Mannosamine

The IgA MOPC406 myeloma protein was found to precipitate with the lipopolysaccharides of *Sal. weslaco* and *E. coli* 031, which are known to contain *N*-acetyl-

D-mannosamine (147). It was then found that *N*-acetyl-D-mannosamine inhibited the precipitin reaction in agar gel (209). Rovis et al. (242), using a quantitative precipitin assay, have studied the four anomers of *N*-acetyl-D-mannosamine for their inhibitory properties in this system and found that the β-pyranoside was the most active compound, being 10 times more effective on a molar basis than the α-pyranoside, 40 times more effective than the β-furanoside, and 400 times more effective than the α-furanoside.

H. Other Antigens

Isolated myeloma proteins, usually of the IgA class, have been found that bind other antigens used in screening (see Table 17 for summary). Many of the antigens identified were found in the environment of the BALB/c mouse; that is, they can be isolated from a species in normal flora. Others were detected by screening with available antigens.

Several of the IgA myeloma proteins listed in Table 17 have been found to precipitate with polysaccharide antigens from bacterial sources. The MOPC384 and McPC870 IgA myeloma proteins precipitate with lipopolysaccharides lps (phenol aqueous-phase extracts) of *S. tel aviv, S. tranoroa, E. coli* 070, and *Proteus mirabilis* sp. 2 (of BALB/c mouse gut flora origin). The precipitation of the *S. tel aviv* lps by MOPC384 can be partially inhibited by α-methyl-D-galactoside (209), whereas the 870 reaction is not inhibitable by α-methyl-D-galactoside.

The 7*S* IgG3 J606 protein described by Grey et al. (88) precipitates with levans, particularly those containing $\beta 2 \rightarrow 6$ fructose linkages and inulin. A low-molecular-weight inhibitor has not yet been identified for this precipitation.

The IgA MOPC467 protein was previously described to precipitate with antigens from over 12 *Salmonella* serogroups as well as an antigen from *Pasteurella pneumotropica* and *Herellea vaginicola* (of BALB/c origin) (207). The antigen is destroyed by phenol extraction and trypsin digestion and is probably a protein (207). The IgA TEPC521 protein binds a pronase-sensitive antigen in wheat (224).

I. Comments

The screening of myeloma proteins for binding activity to a variety of antigens has detected a number of myeloma proteins (about 5%) that can be called functional molecules. The low number may reflect the limitations of the assay systems thus far used, which depend chiefly on multivalent immunoglobulins and polyvalent antigens. In general there is no theoretical reason why each complete four-chain immunoglobulin molecule should not have combining sites.

Evidence indicating that the activities thus far found are related to natural immune responses is suggestive but not conclusive as yet. If one could obtain a total "read-out" of the immunoglobulin gene potential independent of the influence of antigens and then sample randomly each four-chain molecule, would the number and types of activities equal those so far detected by the screening of myeloma pro-

teins? An indication that the myeloma proteins are a selected sample and hence influenced by antigen is provided by the antiphosphorylcholine group of proteins, in which there are proteins of similar and dissimilar structure that bind the same haptenic group. The anti-$\alpha 1 \rightarrow$ 3-linked glucose and anti-$\beta 1 \rightarrow$ 6-linked galactose activities are other suggestive examples. It is hypothesized here that such activities would not be encountered so frequently in the sample so far tested unless antigens were exerting a selective effect. It may be further suggested that these responses have been evoked to natural antigens derived from the microbial flora and various intermittent infections incurred during growth and maturation of the mouse.

IX. IMMUNOGLOBULIN BIOSYNTHESIS

A. General Description

Figure 4 outlines immunoglobulin biosynthesis in plasma cell tumors, which is a complex process involving many steps. Because several aspects of synthesis have been reviewed recently [e.g. immunoglobulin assembly (16, 245, 246) and carbo-

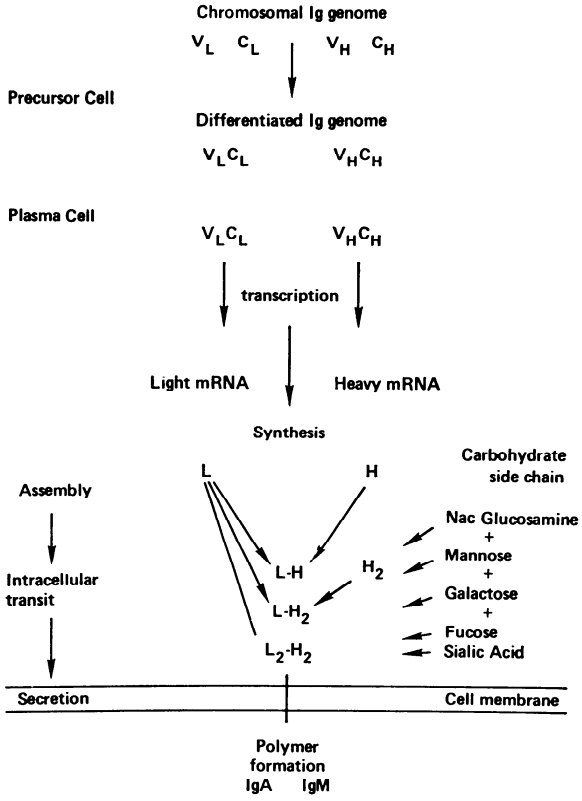


FIG. 4. Scheme of immunoglobulin synthesis.

TABLE 21. *Percent immunoglobulin synthesis in plasma cell tumors*

Plasmacytoma	Form	Time	Immunoglobulin Synthesis, % of Total Protein Synthesis			Ref
			Intracellular	Secreted	Total	
X5563 (γG)	Tissue slices	1–8 hr	13		34–52	13
RPC20 (λ)	Ascites	20–30 min			10	123
MPC11 (γH)	Tissue culture	30 min	20	100		126
MOPC31B (γF)	Tissue culture	5–120 min		50		182
MOPC46B (κ)	Suspensions from solid tumor	Short pulse	30			38
MOPC104 ($\mu\lambda + \lambda$)	Suspensions from solid tumor	Up to 1 hr	25			197

hydrate side-chain synthesis (38, 286)] the discussion presented here emphasizes the early events in biosynthesis, mainly the transcriptional process. Immunoglobulin biosynthesis in plasmacytomas probably begins with a transcriptional event that occurs in "differentiated" genomes. Presumably the differentiating events that *1)* select a set of immunoglobulin genes and *2)* trigger development of a cytoplasmic secretory apparatus (rough endoplasmic reticulum and Golgi apparatus develop in a precursor cell and are only "continued" in the neoplastic plasma cell). The differentiation is stabilized in such a way that the cell can only produce one molecular form of immunoglobulin. Most tumors produce only one type of light and heavy chain. The tumor cells may secrete only a single molecular form (i.e., a four-chain unit or a polymer of a four-chain unit) but may in addition release excesses of the light-chain subunits.

Recently there have been some exceptions to this rule in both human and mouse plasmacytomas. In man, for example, several tumors have been associated with the production of two myeloma proteins. One of the best studied cases described by Wang et al. (291) and Levin et al. (137) is a tumor that contains two cell types, one that produces only an IgM homogeneous component and one that produces an IgG component. The two M components apparently share the same V polypeptides.

It was hypothesized that IgM-producing cells differentiate to give rise to the IgG cells that produce the same idiotypic variant with a different common region. Another exceptional case has been found in the mouse MOPC511 tumor, which produces an IgA κ-type myeloma protein in the serum and a free λ-chain that appears in the serum (see ref 207). It is not known whether the same cell produces both proteins but the stability of the tumor during transplantation suggests the cells producing the two proteins are closely related, if not the same.

The plasmacytoma provides an excellent system for characterizing the process from transcription to secretion of the assembled protein, because 13–30% of intracellular synthesis in the growing cells involves immunoglobulin formation (Table 21). Immunoglobulin mRNA, transcription, the stability of the differentiated state,

and somatic cell genetics of the differentiated cell are special problems that can be approached with plasma cell tumors.

The V and C polypeptide segments in a single chain, according to the hypothesis of 2 genes: 1 polypeptide chain, are directed by separate genes (56, 57, 103). In any one species such as the mouse there are relatively large numbers of V polypeptides (104), and these are controlled by the same or a smaller number of genes. As mentioned previously it is not established whether every V polypeptide variant is controlled by a germ-line gene or whether some means for providing variations at the somatic cell level accounts for some of the variants. The sequence data with the mouse κ-chains clearly indicate many genes—i.e., over 21.

It is usually assumed that genes controlling sets of V polypeptides are in one region of a chromosome. The basis for this notion is that specific C genes are always associated with sets of V gene products.

Cλ, Cκ (103), and C_H (118) polypeptides are associated with their own set of V polypeptides, which suggests an association at the genetic level between sets of V genes and a C gene. Interchanges have not been observed between unrelated B and C groups (103).

Associations of V and C Polypeptides

Set of V polypeptides	*C gene or genes*
V_κ	C_κ
V_λ	C_λ
V_H	C_H (γ, η, ϕ, α (mouse))

Genetic data in several species, including the mouse, indicate C_H genes are closely linked in a single locus (97, 99, 143, 219, 220). The heavy-chain linkage group in the mouse contains genes controlling the constant regions of the γ, η, ϕ, and α chains (97, 99, 137, 215, 216). Limited amino acid sequence data available on V_H polypeptides have indicated that V polypeptides (presumably of the same subclasses) may interact with any of the individual C polypeptides (118). It is known from structural studies on human immunoglobulins that the same V_H polypeptide may interact with either the $C\mu$ or $C\gamma$ polypeptide (118). Details on this point are not yet available in the mouse; however, the fragmentary data available suggest the same principles will hold in the mouse as in other species.

Since individual plasma cells are highly restricted to making only one molecular form of immunoglobulin, one of the early steps in immunoglobulin differentiation must be the selective activation of four structural gene components. If the assumption is correct that related genes are clustered together or tandemly linked at specific loci then some special mechanisms of gene activation are required. Currently the process is not understood.

Immunoglobulin-chain biosynthesis could hypothetically occur by several mechanisms: *1*) two polypeptides could be synthesized by two different separate mRNA strands and these could be joined by a special enzymatic process; *2*) the covalent immunoglobulin chain could be synthesized from a single covalent mRNA that results from fusion of two strands of mRNA; or *3*) the single immunoglobulin

polypeptide chain could be synthesized from a single mRNA and a single DNA strand that has been joined at the DNA level by a specific mechanism. Since immunoglobulin chains are linked by peptide bonds and there is little precedent for a chain-joining enzyme, it is generally thought that the enzymatic peptide bond-forming mechanism is unlikely. However, several attempts to study immunoglobulin-chain biosynthesis using the "Dintzis" method (117, 130) have not produced evidence for separate growing points in immunoglobulin chains, which makes separate joining unlikely.

The genetic data and the phenotypic restrictions of sets of V polypeptides to a specific C gene strongly suggest that the joining or appropriate association of V and C genes is initiated at the DNA level, possibly as a chromosomal event.

B. Immunoglobulin mRNA Studies: Light and Heavy Polysomes

Much of the work on immunoglobulin assembly has depended on the demonstration that there are separate polysome classes in the cytoplasm of neoplastic plasma cells (245, 248, 259, 301). Polysome fractions were separated by sucrose density-gradient centrifugation, and then individual polysomes with nascent chains were precipitated with antisera specific for light or heavy chains. Shapiro et al. (259) noted that a tumor that produced only light chains contained predominantly small polyribosomes, whereas the γH-producing MPC11 tumor contained two polyribosomes classes of 190*S* and 270*S*. MPC11 cells were then labeled for 90 sec and chased with cold amino acids for 15 and 30 sec. Polyribosomes were then isolated by sucrose-gradient centrifugation from cells disrupted in 0.5% deoxycholate. The immunoglobulin chains associated with polyribosome fractions were characterized by polyacrylamide electrophoresis (in 0.1 M PO_4 buffer, pH 7.1, and 1% SDS, 0.5 M urea, and 0.1% 2-mercaptoethanol). Identification of chains was made only by electrophoretic behavior when compared with other known immunoglobulin subunits. Using the pulse-chase technique it was found that the 190*S* polysomes contained only light-chain material and that a peak of light-chain activity present 15 sec after the cold chase had virtually disappeared in 30 sec.

Heavy chains were associated in a similar way with the 270*S* polysomes for about 60 sec. It was estimated that the approximate time for L-chain synthesis was 30–45 sec and for H-chain synthesis 60–75 sec. L chains were made in excess and released into an intracellular pool; these completed chains then interacted with nascent H chains.

Independently, Williamson and Askonas (301) made similar observations on an ascites form of the IgG-producing X5563 plasmacytoma. Using preparations that contained 2–3 × 10^8 cells/ml, radioactive amino acids were added for a period of 2 min; after this the cell concentrate was treated with deoxycholate and directly overlayed on sucrose. Using this method a broad spectrum of polyribosomes was obtained. The individual sucrose gradients were then analyzed for the presence of L- and H-chain components with carefully evaluated absorbed antisera specific for light or heavy chains of X5553. The anti-light-chain antiserum was prepared by immunization with isolated L chain and absorbed with Fc. Poly-

ribosomes sedimenting near 300*S* contained heavy-chain components, while the polyribosomes that sedimented between 100 and 180*S* contained only light-chain elements. Schubert (248) performed somewhat similar studies with a tissue-culture line of MOPC21. The sucrose fractions were precipitated with a nonspecific rabbit anti-MOPC21 antiserum that precipitated with both κ-type light chains and the MOPC21 Fc fragment. The various precipitates were characterized electrophoretically on 7.5% acrylamide gels containing urea and SDS. Light-chain components were released from the light polysomes and heavy and light components from the heavy polysomes. From these studies it was hypothesized that two basic types of polysome complexes are associated with immunoglobulin synthesis and assembly, a heavier component sedimenting at approximately 270–300*S* and a lighter component sedimenting at approximately 180*S*, and that immunoglobulin biosynthesis depends on two different mRNA species.

Further support for this important conclusion has developed from other more detailed studies of the immunoglobulin assembly process. Essentially, based on the methods outlined above (pulse labeling → cell lysis in SDS → sucrose-density centrifugation → precipitation of sucrose fractions with specific antisera → identification of components radioautographically on polyacrylamide-gel electrophoresis), the following intermediates can be identified on gel electrophoresis: L, L_2, H, LH, LH_2, and L_2H_2 (15, 22, 126, 127, 245, 246). Kinetic studies have been made possible by using pulse-chase methods (22, 126, 127, 245, 246, 259).

The three major classes of immunoglobulins have been studied. Parkhouse et al. (197) have examined the assembly of the MOPC104 IgM and found H-, and L-chain precursors intracellularly and L_2H_2 but no evidence for intracellular IgM pentamers. The released L and H chains are believed to form L-H disulfides and L_2H_2 intermediates very rapidly intracellularly. In these studies on MOPC104 approximately 25% of the incorporated label went into immunoglobulin molecules (Table 21).

Pentameric IgM molecules were found outside the cell after a lag of 20–30 min. Polymerization was believed to occur close to the time of secretion and possibly in the cell membrane itself. Intracellular polymerization is apparently prevented because the "interunit" disulfide is blocked intracellularly by factors not yet elucidated. Recently Parkhouse (196) has shown that the plasmacytomas synthesizing IgA (MOPC315) and IgM (MOPC104E, TEPC183) also synthesize J chain. The J chain was synthesized in the neoplastic plasma cells and was not associated with intracellular IgM, but was apparently bonded to the immunoglobulin during the secretion process.

IgA biosynthesis in the MOPC315 tumor has been recently reported by Bevan (26). In the secreted MOPC315 IgA protein the L chains are linked covalently by L-L disulfides and there are no L-H disulfides. Bevan (26) found L_2 and H_2 intermediates intracellularly and extracellularly: H_2L_2, $(H_2L_2)_2(H_2L_2)_3$. Assembly and polymerization were believed to occur during passage through the membrane. The L-L disulfide bond formed extracellularly (26).

Mushinski (179), Scharff and Laskov (246), and Bevan (26) have studied the intracellular assembly of the two-chain IgA molecules and find that there are

disulfide-linked L-H intermediates. This form of IgA synthesis may occur because a putative deletion somewhere in the C-terminal half of the α-chain (see sect. VII*A*) frees one of the H-H disulfides for L-H disulfide-bond formation.

With IgG subclasses, intracellular L-H disulfide intermediates in assembly are often more readily demonstrable. The principle intermediates are L-H and L-H_2 disulfides. Five pathways of assembly have been hypothesized (22); two major pathways have been demonstrated:

A: $L + H \rightarrow LH$
$LH + LH \rightarrow L_2H_2$

B: $H + H \rightarrow H_2$
$L + H_2 \rightarrow LH_2$
$L + LH_2 \rightarrow L_2H_2$

The intermediates are usually linked by covalent bonds (15, 245, 246, 298, 301) or in some unusual cases by noncovalent interactions (126). A single tumor may utilize *A* (22, 182, 248) or *B* (14, 22, 126, 127) exclusively. However, generalizations are not yet possible for specific classes. Further, a single tumor may utilize both pathways. Finally, other factors may affect the assembly process and produce unusual findings. For example, Laskov et al. (126) recently studied an in vivo and an in vitro line of MPC11. The in vitro line formed L, H, H_2, LH, and LH_2 intermediates and assembled the L_2H_2 chiefly from LH_2 intermediates. An in vivo line of the tumor formed relatively large amounts of LH; however, these intermediates did not covalently link to make L_2H_2 but were actually secreted from the cell, whereafter they polymerized to make higher molecular forms. The basis for this interesting block in assembly was not established.

Recently Schubert and Cohn (250) have challenged the interpretation of 1 mRNA, 1 polypeptide chain. They examined the specific activity of membrane-bound polysomes from plasmacytomas and found two peaks of high OD260 specific activity, one associated with polysomes containing 4 ribosomes and a second with polysomes containing 11 ribosomes. They proposed that these size distributions fit the theoretical messenger RNA lengths for polypeptides of 12,000 and 36,000 mol wt and argue that immunoglobulin synthesis takes place on 4 mRNA strands: 3 strands of roughly equal size that direct the synthesis of V_L, V_H, and C_L and 1 larger strand that directs the synthesis of C_H. Kuff and Roberts (124) have isolated polyribosomes containing 2–15 ribosomes from plasmacytomas and attributed some of the heterogeneity to endogenous ribonuclease activity incurred during the isolation procedures.

Schubert and Cohn (250) examined the light polyribosome sucrose fraction for the presence of intermediate-sized polypeptides and found in four tumors (S63, S194, RPC20, and MOPC46) polypeptide components that migrated electrophoretically on polyacrylamide gels at positions where proteins of approximately 12,000 mol wt migrate. The polypeptides were precipitated immunologically with anti-light-chain antisera. Nonproducing tumors did not form similar components. Schubert and Cohn argue that the fragment could represent the V or C half of the L chain and caution against acceptance of the prevailing concept that the V_L and C_L DNA fragments are joined at the DNA level to account for the covalent L polypeptide. Quite clearly further work needs to be done on the identification of these peptide fragments before conclusions can be drawn.

C. Biologic Studies on Origin of Defective Immunoglobulin-Forming Cells

Defective immunoglobulin synthesis has been observed many times in transplantable and primary plasmacytomas. The most common manifestations of defectiveness are: *1)* the production of excessive light chains, *2)* the failure to secrete immunoglobulin, and *3)* the formation of two-chain IgA molecules. Schubert and Cohn (251, 252) have attempted to characterize several defective lines and have suggested that in some cases where no immunoglobulin is secreted intracellular blocks in the assembly process prevents formation of a secretable product (251, 252).

Defective immunoglobulin-producing cell lines often develop during adaptation of plasmacytomas to in vitro conditions. Using the established tissue-culture line of the IgH MPC11 plasmacytoma, Coffino and Scharff (41) have developed an important method for detecting variants in immunoglobulin production (Fig. 5). Essentially this method depends on the ability of isolated MPC11 cells to form microcolonies when grown on feeder layers of 3T3 contact-inhibited fibroblasts. The cloning efficiency of MPC11 cells grown in this fashion is approximately 50%. The detection of variants is achieved by overlaying 3-day cultures with a second layer of agarose; after the addition of the agarose layer, 1 ml of antiserum directed to heavy or light chains was overlayed on the agarose layers. After 24 hr of incubation, those microcolonies that produced immunoglobulin were distinguished from those that did not by the presence of a precipitate over the microcolony (Fig. 5). When colonies of MPC11 cells were evaluated for their ability to produce heavy

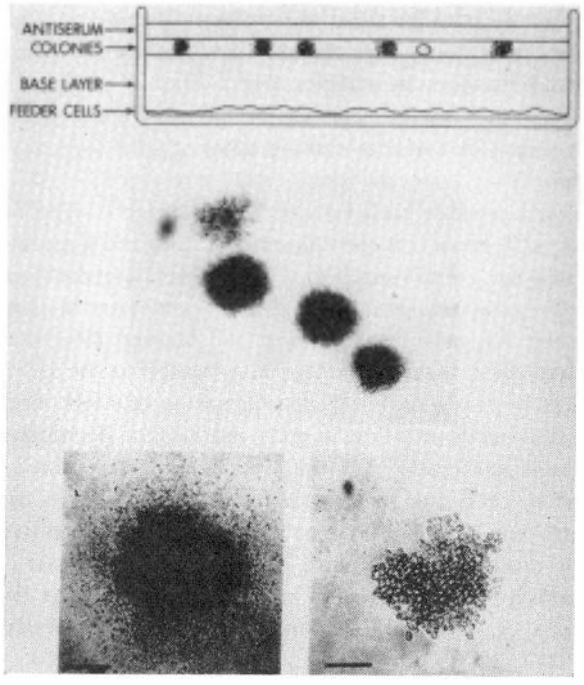


FIG. 5. Assay method of Coffino and Scharff (42) for detecting defective immunoglobulin-producing cells. At top is a schematic diagram of culture system and components. Photographs are of 6-day-old colonies that had been overlayed on *day 3* with anti-heavy-chain antiserum. *Middle photograph* shows four colonies: upper left colony is not surrounded with precipitate, while other three colonies are surrounded with precipitate. *Lower photographs* taken at higher magnification show a colony with precipitate (left) and a colony without precipitate (right). Colonies that fail to produce immunoglobulin that reacts with anti-heavy-chain antiserum are subcultures and tested for their ability to produce light chains. [From Coffino and Scharff (42).]

chains, it was found that 10 colonies of 2400 examined were negative. The negative colonies were isolated and immunoglobulin synthesis was examined. These cells all produced L chains but no longer secreted or produced any H chains. Since colonies are detected by their failure to secrete H-chain-containing Ig, some variants could possibly be nonsecreting types that have intracellular defects in assembly or in the secretion of H-chain-containing molecules. When cells producing only L chains were studied by the same technique using an anti-L-chain antiserum, 10 nonproducing colonies were isolated among 65,000 tested that failed to produce L chains. There were no reversions in either class of variants found; that is, nonproducers failed to produce L-chain lines and lines producing only L chains failed even to produce heavy-chain lines. Thus the direction of variation was H + L→ L → nonproducer. The nonproducer lines were characterized morphologically and found to contain endoplasmic reticulum, intracisternal type-A particles, and ribosomes. A fluctuation analysis has been done to determine the rates of conversion of H + L → L and L → nonproducer (42). These were found to be 1.1×10^{-3} for the conversion of H + L → L; a value was not established for L → nonproducer because of the lower rate of conversion, but it is less than 1.7×10^{-4}/cell generation. Several studies on the influence of mutagens have been described and it has been found that acridine mustard has caused a considerable increase in the number of variants (42). The high rate of appearance of cell types producing only L chains and nonproducers does not appear to resemble the classical mutation rates for point mutations; however, this suggests that some other process may account for the "failure" of the genes to remain in an active state. This finding may reflect in part an instability of the differentiated state under conditions of rapid growth in tissue culture. In addition, the presence of cell types secreting light chains only in a large tumor may account in part for the observation that some patients with multiple myeloma and some plasmactyomas associated with four-chain immunoglobulin production may also produce in addition free L chains. It must be cautioned, however, that this is not the only mechanism for the production of excessive light chains. Thus far, mutants or variants making heavy chains only have not been described in the mouse.

It is tempting to speculate on a possible basis for variants observed by Coffino and Scharff (e.g., H + L → L → 0). Here it is speculated that the gene template for the covalent mRNA chain message containing both C and V segments is transcribed from a covalent DNA gene template. Since the V and C genes are in most cases separated from chromosomes, it is postulated [Dreyer and Bennett (56)] that the C and V DNA segments have to be first interrupted and ligated at the DNA level. This may occur directly on the chromosome by the formation of a loop or it may be an extrachromosomal process resembling in part amphibian peripheral nucleus formation (170). In the hypothetical situation, the gene-differentiating event need not be recapitulated in each new cell but is preserved as long as the gene template DNA is produced. Rapid cell division or other factors may dilute the extrachromosomal DNA or destroy the template DNA, resulting in loss of immunoglobulin-synthesizing activity. As the process might be random it is expected that there would be both "L-only" and "H-only" producers; however,

"H-only" producers have not been described. If it is assumed, however, that H-chain synthesis requires free cytoplasmic L chains, then the appearance of only L-chain mutants can be explained for those cells lacking L-chain mRNA and synthesis could not make H chains.

D. Cell-Free Synthesis of Immunoglobulin Hybridization Studies

The mRNA from plasmacytomas has not yet been isolated. This is a technically difficult problem for several reasons: *1*) immunoglobulin mRNA must be distinguished from other mRNA's, e.g. nonimmunoglobulin mRNA, nuclear RNA; *2*) mRNA may be unstable; and *3*) the method requires an assay to demonstrate activity.

Namba and Hanoka (182) have attempted to isolate the mRNA for immunoglobulin chains from various polysome fractions from a tissue-culture line of the MOPC31 plasmacytoma. MOPC31B cells were pulse labeled with ^{32}P and RNA was isolated from light and heavy polysomes by the hot phenol-SDS method of Darnell.

The sedimentation profiles of the two fractions were different; both fractions contained a 4*S* and 18*S* peak, but the 200*S* polysome fraction contained in addition a 12*S* RNA species and the heavier polysome fraction contained a 22*S* RNA species. The duration of the pulse labeling prior to the extraction procedure was 20 min.

The ability of polysome fractions isolated from mouse plasmacytomas to synthesize immunoglobulin chains has been studied by several workers. Mach et al. (148) have shown that membrane-bound ribosomes from a light-chain-secreting plasmacytoma synthesized light chains in a cell-free system. Recently, Lisowska-Bernstein et al. (145) separated membrane-bound polysomes and free polysomes from the MOPC70A tumor and then used these fractions in separate systems to study protein synthesis. They found that in cell-free systems both the membrane-bound polysomes and the free polysomes were able to synthesize peptides from the light and heavy chains. They postulate that not all immunoglobulin synthesis requires that the polysomes be bound to membrane and speculate that polysomes might become detached from membranes during intracellular transit.

Very recently Stavnezer and Huang (269) have reported the synthesis of the MOPC41 κ-chain in a cell-free system containing mRNA from the MOPC41 tumor and the other components from rabbit reticulocytes. Of the total protein synthesized by the system 20–27% was MOPC41 protein, the remainder being rabbit hemoglobin chains and other proteins probably directed by other mRNA's from the MOPC41 tumor. The recovered chain and fragments were digested with trypsin and chromatographed on Dowex 1 × 2 columns, where they were compared with in vivo labeled MOPC41. All the major tryptic peptides were labeled and it was concluded that the entire chain was synthesized in vitro in the cell-free system. The mRNA was isolated from a microsomal fraction from which the nuclei had first been removed by centrifugation. The microsomal pellet was extracted with 1% SDS and then placed on a sucrose-density gradient. The fractions sedimenting between 9*S* and 13*S* were pooled and precipitated with ethanol and re-

precipitated with NaCl. Analysis of the purified RNA on acrylamide-gel electrophoresis revealed the presence of multiple components in addition to traces of 28*S* RNA and considerably more of 4*S* and 5*S* RNA. The total amount of MOPC41 κ-chain synthesized in the cell-free system was concentration dependent. Maximum synthesis was obtained with 11 A_{260} unit/ml; this value was much higher than that obtained with rabbit hemoglobin mRNA, which is considerably purer. However, these findings are very encouraging and suggest that with further refinements pure mRNA can be isolated. It is generally recognized that in vitro synthesis systems lack the essential components for reinitiation of synthesis; that is, once the ribosomes have attached to the mRNA strand they are believed to be incapable of reattaching without replenishment of initiating factors.

E. Nucleic-Acid Hybridization Studies

Several hybridization studies using nucleic acids from plasma cell tumors in mice have been reported (85, 86, 114, 121). Greenberg and Uhr (85, 86) examined the ability of 10*S*, 16*S*, and 28*S* RNA from three different plasma cell tumors to hybridize with homologous and heterologous DNA. Although full details of this study were not presented, it was found with 28*S* RNA that hybridization was greater with homologous DNA than with DNA's from the other two plasmacytomas. Since 28*S* RNA is ribosomal RNA the results of this experiment are difficult to interpret.

Kimmel (114) has attempted to obtain cytoplasmic RNA for hybridization studies. Using the tissue-culture MOPC21 line as a source of RNA, the cells were homogenized in a Dounce homogenizer and prior to RNA isolation nuclei and debris were removed by centrifugation. The supernatant of the initial fractionation was recentrifuged at 27,000 *g* for 5 min; the supernatant of this fraction contained predominantly free polysomes, while the pellet contained associated polysomes. RNA was isolated from these fractions by a cold phenol method. It has been shown earlier by Kuff et al. (123) and Kimmel (114) in these studies that a relatively large proportion of cytoplasmic RNA in plasmacytomas is associated with membranes. Most plasmacytomas have an abundant rough endoplasmic reticulum. After 30 min of labeling cells with radioactive uridine, Kimmel (114) found that the cytoplasm contained only 15% of the label and that most of this was distributed in 18*S* and 4*S*–5*S* RNA. When cells were treated with actinomycin D at low concentration (0.5 μg/ml) a greater proportion of heterodisperse cytoplasmic RNA (and presumably mRNA) was labeled. As a control source of RNA from a plasma cell line that did not produce immunoglobulin, RNA was isolated by Kimmel (114) from a nonproducer tissue-culture line designated XC1 derived from X5563. Competition experiments were then run using cytoplasmic RNA of the immunoglobulin-producing MOPC21 combined with DNA sequences that the RNA from XC1 (X5563) was incapable of binding. These approaches may provide a method for specifically isolating mRNA from plasmacytomas. In a third study Kreuger and McCarthy (121) compared RNA and DNA from four different plasmacytomas,

MOPC104E, MOPC173D, AdjPC5, and MOPC46. Total RNA was labeled by exposing primary cultures of homogenized tumor to tritiated uridine for 30 min, and no attempt was made to further fractionate the RNA. Several interesting findings were obtained. First, the hybrids of myeloma RNA with homologous DNA formed at low RNA/DNA ratios and had relatively high 50% thermal dissociation constants (as high as 81 C), thus resembling hybrids that form with unique sequences of DNA. The results suggested to the authors that they were dealing with hybridization of so-called "single-copy" rather than repetitive DNA. Second, it was observed that hybridization was more efficient in homologous situations, i.e. where the RNA and DNA were obtained from the same tumor. This was quite striking in the comparison of AdjPC5 and MOPC173D, which are both γG myeloma proteins that differ presumably only in the V region. The tempting conclusion is that the efficient homologous hybridization indicates the presence of more DNA sequences from the homologous source, and this could be accounted for by some process of gene amplification. Much remains to be done in the area of RNA/DNA hybridization in plasmacytomas; the early results indicate much is to be learned from this system.

F. Transfer RNA

Yang and Novelli (311) were the first to examine tRNA in plasmacytomas. They recovered tRNA for all 20 amino acids from a single tumor and found evidence for multiple species of tRNA's for 19 amino acids and a single tRNA species for trytophan, using reverse-phase freon chromatography. In a later study (312) Ser-tRNA species were compared in four different plasmacytomas and liver. Four Ser-tRNA fractions were found in normal mouse liver. Some of the tumors differed from each other by lacking one of the fractions; the two tumors MOPC31B and MOPC31C, which were derived from the same primary host (312), had similar Ser-tRNA profiles.

Mushinski (181) has investigated Leu-tRNA in κ-chain-producing tumors and mouse liver. Five chromatographic peaks were found on reverse-phase freon chromatography. The κ-chain-producing tumors differed from each other by the relative amounts of these fractions in much the same way as the Ser-tRNA's differed in the various tumors (312). An interesting finding, however, was that tRNA patterns from the same tumor remained stable and that the same pattern was isolated from the same tumor at different transfer generations, suggesting that each tRNA peak for a given amino acid behaved as an independent gene product. Some species of tRNA were relatively inactive in specific tumors.

It has been speculated that the availability of different tRNA's that recognize the same codons but insert different amino acids could provide a basis for antibody variability (212). No evidence has been obtained as yet to suggest that tRNA plays a role in determining variations in immunoglobulin structure. tRNA preparations from plasmacytomas that produce the MOPC149 and MOPC46 κ-chains were used as a source of tRNA in a cell-free hemoglobulin-synthesizing system (180).

These two proteins have leucine variations in the V_κ polypeptide (see Table 14), and two different Leu-tRNA profiles have been isolated from the tumors. Despite these differences both tRNA fractions effectively transferred leucine into the rabbit α-chain. The tRNA variations in plasmacytomas do not appear at present to be related to any specific protein, including immunoglobulin. The basis for their origin is not established so several possibilities need to be examined: role of viruses, heavy-chain class, somatic mutations, etc.

G. Biosynthesis of Carbohydrate Side Chains

There are now a number of publications on the biosynthesis of carbohydrate side chains on mouse immunoglobulins that have been studied using various plasma cell tumors. These studies have dealt chiefly with the IgF MOPC21 (162), IgH MPC11 (176), MOPC104E (249), IgG MOPC173 and LPC1 (247, 286, 317) tumors as well as plasmacytoma MOPC46, which produces only a κ-type light chain. The MOPC46 κ-chain (37, 38, 49, 161–163) contains a carbohydrate side chain similar in composition to that found on the IgF MOPC21 line. The carbohydrate side chain that is believed to be attached at a single site (161) contains: 3 residues of glucosamine, 4 residues of mannose, 4 residues of galactose, 2 residues of fucose, and up to 2 residues of sialic acid (163). The MOPC46 Bence Jones protein has variable numbers of sialic residues per chain and may have 0, 1, or 2 residues of sialic acid (161). The sequence of the MOPC46 side chain has not been established; however, kinetic studies have shown that specific residues are attached sequentially during the late stages of biosynthesis and secretion. Miller (169) has studied the carbohydrate compositions of the mouse immunoglobulins and many myeloma proteins. He found the basic IgG glycopeptide contained 5 residues of *N*-acetylglucosamine, 2 residues of fucose, 1 residue of galactose, and 6 residues of mannose. Neuraminic acid content varied. The composition of MOPC21 was similar to that reported by Melchers (163) for MOPC21. In general the myeloma proteins resembled the normal immunoglobulins, although some exceptions were seen. The IgG proteins appeared to have single carbohydrate chains and after pronase digestion yielded glycopeptides with molecular weights between 2800 and 3500. The IgA and IgM proteins yielded more heterogeneous glycopeptides, suggesting more than one structure. Based on the ability of glycosidases to liberate sugars from the immunoglobulins, it was concluded that the anomeric configuration of the recovered fucose and mannose was alpha and the configuration of the galactose was beta.

In the MOPC46 κ-chain the carbohydrate side chain is attached to asparagine 28, which is found in a region of hypervariability in mouse κ-chains. MOPC46, like MOPC41, does not contain the additional 4 residues between 27 and 28 (161). Although it is not known why the MOPC46 chain contains carbohydrates, it has been speculated that the V polypeptide sequence in this region forms a suitable receptor site for an aspartyl *N*-acetylglucosamyl transferase. It has also been found (286) that *N*-acetylglucosamine is covalently linked to nacent heavy chains.

Recently Choi et al. (37) and Melchers (163) have fractionated plasma cells

using a method that requires only a single centrifugation step at 70,000 *g* for 6 hr at 0 C. Tumor cells incubated with various types of radioactive precursors were broken in a Dounce homogenizer and then the homogenates were overlayered on a sucrose density gradient; after centrifugation, 5 fractions were obtained: fractions 1 and 3 contained predominantly rough and smooth membranes, respectively. Kinetic (38, 162, 163, 249) and radioautographic (317) studies demonstrated that the immunoglobulin associated with the rough endoplasmic reticulum contained glucosamine and mannose, while the protein associated with the smooth endoplasmic reticulum contained glucosamine, mannose, and galactose. The protein isolated in the cell supernatant contained more galactose residues. These sequential additions of residues were believed to occur as the intracellular immunoglobulin was being transported into different compartments of the cell. The terminal galactose and fucose residues were believed to be added as the immunoglobulin passed through the plasma membrane and the sialic residues were attached on the membrane.

Similar studies have not been made with IgA myeloma proteins in the mouse. It is known, however, that IgA myeloma proteins in man (54) contain 3 different oligosaccharide chains that are attached to different sites on the α-chain.

H. Comments

Cell-free synthesis with components derived from plasmacytomas has been achieved; however, all the components have not been isolated as pure fractions. mRNA should be accessible from plasmacytoma, but has not been isolated; this remains an important unsolved problem.

Schubert and Cohn have raised the interesting and controversial possibility that there are 4 separate mRNA's for the immunoglobulin molecule: 3 small forms for V_L, C_L, and V_H and 1 large form for C_H. This proposal is supported thus far only by measurement of polysome size of L and H polysomes—which, according to Schubert and Cohn, contain too few ribosomal units per strand—and the finding of a low-molecular-weight polypeptide corresponding to 12,000 mol wt intracellularly. Further studies are clearly needed to establish these possibilities, including the characterization of the 12,000-mol wt components. The recent demonstration of switches in biclonal gammopathies (291) and in other examples of single cells that may possibly synthesize 2 C_H classes of immunoglobulin (137, 201, 291) suggests that a mechanism for alternating C_H polypeptides probably occurs in some cells. Since differences in C_H polypeptides relate to "physiologic" differences in immunoglobulin function (e.g. complement fixation, secretion, tissue binding, transplacental transit, etc.), it is possible the regulation of the C_H genes is independent of the V_H functions. Thus different C_H genes may be activated within a clone of cells containing the same V_L or V_H functions. Although there is only a little evidence in support of this possibility, the need for further clarification is evident.

The well-known defects in biosynthesis in plasma cells (e.g. nonproducers, nonsecretors, L-chain-only producers, cells that make four-chain units and also secrete excess L chains) have been found to develop in high frequency in certain

plasmacytomas, e.g. the tissue-culture line of MPC11 (42, 126). These "mutants" do not appear to be classical mutations but rather heritable disorders of somatic cells that may be peculiar to the differentiated and neoplastic condition. Possibly they represent an instability of the differentiated state brought about by rapid cell proliferation with concomitant loss of "nonessential" functions. Possibly the "activated immunoglobulin genome" is structurally different from the inherited chromosomal immunoglobulin genome. For example, it has not been ruled out that the activated DNA is not an extrachromosomal element. This hypothetical DNA could be lost during rapid cell proliferation due to a failure of replication and the loss could account for the defective synthesis.

DNA:RNA hybridization involving components from the same tumor or different tumors are just being investigated. Preliminary evidence suggests more efficient hybrid formation with components from the same tumor, which may mean that each tumor contains not only the specific DNA template but additional copies of that template.

SUMMARY

1) The induction of plasmacytomas in the inbred BALB/c strain of mice has provided a source of large numbers of plasma cell tumors. Plasma cell tumorigenesis is influenced by a variety of factors, some of which have been identified: *a*) pathological changes in the peritoneum induced by solid plastic or oils create an abnormal tissue environment in which tumors develop; *b*) genetic factors are revealed in the unusual sensitivity of the BALB/c strain of mice (most other commonly used strains so far tested are not susceptible); *c*) antigenic environment is a factor, since germfree BALB/c mice injected with mineral oil develop a very low incidence of plasma cell tumors. The apparent preferential involvement of IgA-producing cells in plasma cell tumorigenesis, as indicated by the roughly 60% of the tumors that make immunoglobulins of the IgA class, is not explained as yet but may be due to the selective migration of IgA-producing cells into the connective tissues. A viral influence in plasma cell tumorigenesis has not been established; however, all plasmacytomas are associated with an intracisternal type-A viruslike particle and many of the tumors have been associated with the antigens associated with the murine leukemia virus system, i.e. G_{IX} and GCSA. The mechanism of plasma cell tumor development has not been explained.

2) Several types of lymphomatous tumors in mice have been associated with immunoglobulin production: the plasma cell leukemias as defined by Rask-Nielsen, the type-B reticulum cell neoplasms in strain SJL/J mice (Murphy), and lymphomas in strain NZB. The immunoglobulin produced in association with these lymphomas is of monoclonal origin in some of the cases but in others the published data suggest the immunoglobulins are of polyclonal origin. Many of the neoplasms lose the capacity to produce immunoglobulin on transplantation. However, some have been stable producers (like the plasmacytomas) and among these are a few tumors that resemble the Waldenstrom macroglobulinemias in man. The incidence

of plasma cell leukemias appears to be influenced by the injection of cell-free materials derived from other plasma cell leukemias. By contrast the SJL/J disease is spontaneous and occurs in 80–90 % of all mice in the SJL/J strain. Pathological changes are observed in the lymphoreticular tissue of SJL/J mice before the development of reticulum cell neoplasms. Viruses and genetic factors have been suggested in the development of the SJL/J disease but as yet the pathogenesis has not been worked out.

3) Most plasmacytomas can be established as transplant lines in syngeneic hosts, where they continuously produce characteristic homogeneous immunoglobulin. Exceptions are the tumors that lose the capability to secrete complete immunoglobulins and produce instead only light chains or no immunoglobulin at all. A number of plasmacytomas have been adapted in vitro and many of them continue to produce complete immunoglobulins. Colony-forming techniques both in vivo using spleen colony formation and in vitro have indicated that only 1–4 % of wells in an in vivo line are capable of colony formation. Some in vitro lines, however, have apparently become particularly well adapted and over 50 % of their cells can form colonies. The growth of most plasma cell tumors in vivo can be dramatically inhibited by aniline mustard or 5-fluorouracil.

4) Primary plasmacytomas in mice are not associated with osteolytic bone lesions as are many of the malignant plasma cell tumors in man. However, bone marrow metastases do develop during long-term growth of the tumors in transplant or by intravenous injection of tumor cells. Most of the transplantable plasmacytomas in mice that produce free light chains induce the formation of extensive renal tubular casts composed of the light-chain material. The renal lesion caused by this deposition (myeloma kidney) is similar in both mouse and man. Amyloidosis is not associated with plasmacytomas in mice but has been found in mice bearing plasma cell leukemias. Large transplantable plasmacytomas inhibit the development of the primary but not the secondary immune response to sheep RBC.

5) Several antigens have been associated with the surface of plasma cells. The differentiation antigens are PC.1 (an alloantigen) MSPCA that is detected with heterologous sera. These antigens are not found on lymphocytes; many (but not all) normal plaque-forming cells also carry these antigens. Immunoglobulin determinants have been found on the surface of some plasmacytomas, in particular those that secrete γ1 or γF myeloma proteins. Several studies suggest that plasmacytomas have tumor-specific antigens. The chemical differentiation of these antigens from immunoglobulin has not yet been done.

6) The data on the primary structure of mouse immunoglobulins have been assembled. The mouse, like most vertebrates, has the major heavy-chain classes IgM, IgG, and IgA. There are 4 subclasses of IgG in the mouse. Considerable data have been collected on the variable polypeptide sequences in the mouse. The $V_{\lambda 1}$ polypeptides show only minimal amino acid substitutions from one chain to the other and 6 λ1-chains with identical amino acid sequence have been isolated from independently induced tumors. By contrast the V_κ polypeptides show great variation. Employing an arbitrary subgrouping system based on sequence identities in the amino-terminal 23 residues, over 20 different subgroups have

been found. Nearly complete sequence analysis of 4 proteins possessing the same amino-terminal sequence for 23 residues has indicated that individual chains may have extensive (17, 18) differences between positions 23 and 109 or may have only a few differences (3–8).

7) Antigen-binding activity has been demonstrated for roughly 5% of all myeloma proteins tested. Antigen-binding activity is usually detected by screening sera for their ability to precipitate with a battery of test antigens. The antigens are usually polyvalent for specific haptens. Specific binding myeloma proteins have been identified for nitrophenyl, phosphorylcholine, $\beta 1 \rightarrow 6$ galactose, β-D-*N*-acetylglucosamine, $\alpha 1 \rightarrow 3$ glucose, and β-D-*N*-acetylmannosamine. In cases where more than one protein binds the same antigen or hapten it has been found in most cases the proteins differ in structure; however, some of the phosphorycholine-binding proteins of independent origin appear to have structural similarities if not identities.

8) The plasmacytomas have been useful systems for studying immunoglobulin synthesis. The immunoglobulin molecules are assembled on light and heavy polysome components in the cytoplasm by one of several different pathways. It has been generally assumed that two covalent mRNA strands are involved—one for the complete light chain and the other for the complete heavy chain. This assumption has not been supported with chemical data, however, and awaits confirmation by the isolation of a complete mRNA. In the meantime the assumption has been recently questioned, so further work in this area is much needed. Clones of complete immunoglobulin-producing cells give rise to cell types with defective immunoglobulin formation in vitro. This instability has been thought to be due to somatic mutation but may not be due to changes in the inherited immunoglobulin genes but rather to another mechanism.

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