Distinctive Myeloma Globulins Associated with a New Plasma Cell Neoplasm of Strain C₃H Mice

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Experimental study of multiple myeloma and of the associated serum protein abnormalities in man has been hampered by the absence of a similar situation in experimental animals. However, plasma-cell neoplasms have recently become available in mice, and several of these have supplied experimental models analogous to multiple myeloma in man.

In the initial reports of Rask-Nielsen and Gormsen, Bichel and Dunn on the occurrence of plasma-cell neoplasms in mice, serum protein observations are not mentioned. In 1957 Potter, Fahey and Pilgrim reported the first instance of marked serum protein abnormalities, similar to those seen in multiple myeloma in man, associated with a transmissible plasma-cell neoplasm (x5563 or 5563) in an experimental animal. The plasma-cell neoplasm 5563 was found to reproduce the syndrome of multiple myeloma with osteolytic lesions, hypoalbuminemia and hyperglobulinemia in the strain C₃H mice bearing this neoplasm. Thus certain aspects of multiple myeloma became accessible to experimental study. Reports from this and other laboratories have noted additional examples of transmissible plasma-cell neoplasms in mice associated with significant serum protein alterations.

The present report concerns a new transmissible plasma-cell neoplasm, designated as 5647. This line has been under observation in our laboratories for over two years, and it is associated with serum protein abnormalities that differ electrophoretically, in the ultracentrifuge, and by other technics from plasma-cell neoplasms previously described. The serum protein changes resemble those seen in certain instances of multiple myeloma of man.

Materials and Methods

Neoplasm 5647.—This neoplasm arose in the ileocecal region of a 22 month old C₃H/He CRGL mouse that had been gonadectomized 4 months after birth. The tumor was successfully transplanted by Dr. H. Ira Pilgrim, who sent mice bearing the second generation of the tumor to the National Cancer Institute. This tumor has been maintained by carrying several parallel lines in transplant. The studies reported here were made on mice bearing transfer generations 2 to 11.

The mice employed in these studies were C₃H 1/Lw mice from the pedigreed colony of Dr. L. W. Law and C₃H/He mice obtained from the Laboratory Aids Division of the NIH. Mice were maintained in plastic cages and fed tap water and Derwood pellets ad libitum.

Transfer of 5647 was made by inoculating fragments of tumor material through a...
12 or 13 gage trochar into the subcutaneous tissues of the flank. Other procedures employing teased cell suspensions or breis prepared in Potter-Elvejhem tissue homogenizers have been unreliable. Growth of the neoplasm was detectable in most cases after 10 to 14 days. Eventually the tumor weighed 4 to 6 grams, and large tumor masses frequently eroded through the skin. Mice bearing successful tumor transplants begin to die after the 30th day, but a sharp end point of survival was not obtained. Many mice lived for extended periods of time, some as long as 70 to 90 days. Several attempts to establish an ascitic form of this neoplasm were unsuccessful.

This neoplasm was routinely inoculated into six or eight mice in each transfer. Throughout its early history, neoplasm 5647 failed to establish itself in some recipients. However, once the neoplasm was established, progressive growth without regression has always been observed. The failure to establish positive growth in all mice has been found previously with other C₃H mouse plasma-cell neoplasms.3

Detailed autopsy examinations have been performed on 20 mice bearing the 5647 neoplasm. For microscopic study all tissues were stained routinely with hematoxylin and eosin. For special histologic procedures, tissues were fixed in Zenker-formalin solution and sections were treated by the periodic acid-Schiff reagent method, the Prussian blue reaction for iron, Giemsa stain and Wilder reticulum method.

Methods of serum protein study.—Blood was obtained by cardiac puncture, allowed to clot and the serum separated after centrifugation at room temperature. Since hemoglobin has been found to migrate in the beta globulin region on electrophoresis of markedly hemolyzed samples of mouse serum, hemolyzed samples were discarded. No difference was noted between sera analyzed immediately and those stored in the frozen state. Total serum protein determinations and analytic serum protein electrophoresis were carried out as described previously.7 Both individual and pooled sera were analyzed electrophoretically and in the ultracentrifuge. Ultracentrifugal analyses were performed in a Spinco Model E ultracentrifuge at 59,780 rpm and at room temperature. Samples were diluted if necessary to approximately 1 per cent protein concentration and dialyzed against 0.20 M sodium chloride prior to analysis. The relative concentration of components was determined by planimetric analyses of enlarged patterns. All experimentally observed sedimentation coefficients are corrected to a water basis at 20 C. (S₂₀,₆).

RESULTS

Morphology

Gross.—When the neoplasm was transplanted subcutaneously, the tumor arising at the site of transplant was found at autopsy to be well circumscribed and composed of a rather firm, yellowish white tissue. Large tumor masses contained areas of necrosis. Direct invasion of adjacent tissues was occasionally observed, and rarely an adjacent axillary lymph node was enlarged. There were no consistent alterations produced in other organs by neoplasm 5647 and, except for involvement of lymph nodes, a metastasis was found in only one case, in the spleen. Amyloidosis involving the spleen and liver was noted in only one autopsy. The papain digestion technic4 was employed for examination of the osseous skeleton, but no osteolytic lesions were found. When the tumor was transplanted intraperitoneally, firm nodules of tumor tissue were found in the mesentery and on peritoneal surfaces. Though an ascitic tumor has not developed, ascitic fluid accumulating after intraperitoneal transplantation sometimes contained discrete neoplastic cells.

Microscopic.—Routine and special histologic procedures did not reveal any noteworthy effect of the tumor growth on other organs and tissues. Some increase in apparently normal plasma-cells was noted in the spleen and
lymph nodes, but a similar increase is often observed in old mice or in tumor bearing mice. The secondary centers in the spleen and lymph nodes showed mitotic figures, but this also is common in mice bearing neoplasms. The bone marrow was free of neoplastic cells, and none were found in the peripheral blood.

The tumor cells resembled normal plasma-cells in many ways (figs. 1–3). The nuclei were eccentric, and the chromatin within the nuclei was heavily stained and was found in coarse strands or masses. The cytoplasm had a purplish tint with hematoxylin and eosin staining, and the characteristic violet tint with Giemsa staining. Near the periphery of the tumor, the cells were crowded together and the cell outlines were indistinct. Giving the appearance of a syncytium, but in central areas the cells were often discrete and the cell outlines and histologic features were clearly seen. A perinuclear clear area could often be distinguished in the cytoplasm. Mitotic figures were frequent (fig. 1). Many cells contained more than one nucleus; some cells were very large and the nuclei appeared multilobulated (fig. 2). Free cells obtained from the tumor surface or after intraperitoneal implantation showed clumping of the chromatin of the nucleus, an eccentric nucleus, a clear zone in the cytoplasm, and occasional bizarre forms (fig. 3). Differential stains of the tumor cells revealed no peculiar features beyond what were observed with hematoxylin and eosin staining. Only a few reticulum fibers were noted by the Wilder reticulum method. No intranuclear bodies, such as have been described in Waldenström's macroglobulinemia in man, were found with the periodic acid-Schiff method.

**Serum Protein Changes**

*Electrophoresis.*—Electrophoretic analysis of serum from mice bearing the plasma cell neoplasm 5647 revealed striking serum protein alterations (fig. 4). Distinct peaks indicative of marked protein increases were evident in the gamma-1 globulin and beta-2 globulin regions. An increase in the protein content of the beta-1 globulin region was also seen.

The quantity of these beta and gamma globulin components depended on the amount of tumor material. As illustrated in figure 5, a progressive increase in the beta-2 globulin and gamma-1 globulin components and, to a lesser extent, in the beta-1 globulin fraction was observed with increasing tumor weight.

No certain changes in the amounts of serum albumin, alpha globulins and gamma-2 globulins were evident in mice with small tumors (table 1). However, an absolute as well as relative decrease in albumin was found in animals with neoplasms weighing 5 grams or more. The gamma-2 globulins, though normally present in lesser amounts in the mouse than in man, did not appear to be appreciably altered by the presence of the 5647 neoplasm.

*Ultracentrifugation.*—Ultracentrifugal analysis of serum from mice bearing the 5647 tumor revealed striking abnormalities. These are illustrated in figure 6. A similar analysis of normal mouse serum is included for comparison. In the normal serum three components were seen with sedimentation coefficients (S_{20, W}) of 4 S, 6.5 S and 16 S. These components comprised 81,
Fig. 1.—Plasma cell neoplasm 5467. Area near center of local tumor growth. Cells are generally detached from each other and show plasma cell characteristics, such as eccentric nuclei with densely stained masses of chromatin, and clear area in cytoplasm. Note mitotic figure on right. (Hematoxylin and eosin. 420X)

Fig. 2.—Neoplasm 5467. Some cells are very large and multinucleated. (Hematoxylin and eosin, 420X)

Fig. 3.—Cells in ascites fluid. Note eccentric position of nucleus and thick chromatin masses in nucleus, finely granular cytoplasm with pale centrosome and bizarre multinucleated cell on right. (Wright’s stain, 1500X)
Components with similar sedimentation coefficients and similar relative concentrations have been described on ultracentrifugation of normal human serum.\textsuperscript{10}

Serum from mice bearing the 5647 neoplasm contained components with the same sedimentation coefficients as in normal serum but, in addition, also demonstrated components with sedimentation coefficients of 9 S, 11 S and 13 S. These components were not evident in any of the normal mouse sera analyzed, nor were they seen on ultracentrifugal analysis of sera from mice bearing two other plasma-cell neoplasms (5563 and 70429). Also, sera from mice bearing two other reticular neoplasms (P353 and L7235) have been investigated in the ultracentrifuge, but none has shown the abnormal serum components seen with the 5647 tumor.

The components with sedimentation coefficients of 9 S, 11 S, and 13 S to-
Table 1.—Electrophoretic and Ultracentrifugal Comparison of Normal and 5647 Sera*

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<th>Electrophoresis</th>
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<td></td>
<td>Total Protein</td>
<td>Components (per cent of total)</td>
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<td></td>
<td>(Gm.%)</td>
<td>Alpha Beta-1 Beta-2 Gamma-1 Gamma-2</td>
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<tr>
<td>Normal Serum</td>
<td>6.0</td>
<td>52 20 6 13 4 5</td>
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<tr>
<td>5647</td>
<td>7.2</td>
<td>41 15 8 20.5 12 3.5</td>
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*Electrophoretic and ultracentrifugal determinations were made on each sample. Three normal serum pools and sera from three individual animals with 5647 tumors weighing 4.0, 4.3 and 5.3 grams, respectively, were analyzed separately and the results averaged.

†The sedimentation coefficient \(S_{20,w}\) represents the average of values from six serum analyses.

gather have comprised as much as 20 per cent of the total serum protein in mice bearing 5647 tumors. However, the quantity of these serum components depends upon the amount of tumor present, the quantity of the 9, 11 and 13 S serum components increasing with tumor enlargement.

Other findings.—Sera from mice bearing the 5647 tumor gave a negative Sia (water dilution) test for euglobulins. Serum cryoglobulins have not been demonstrated, and the partially purified abnormal serum proteins remained soluble in saline at 4°C. Examination of the urine from animals bearing large tumors revealed, in some instances, the presence of proteinuria. However, tests for Bence Jones protein were negative, and electrophoretic
FIG. 6.—Ultracentrifugal analysis of normal and 5647 mouse sera. Ultracentrifugation was performed at 1.2 grams per cent protein concentration for normal mouse serum, and at 1.3 grams per cent protein concentration for 5647 serum seen on the right. Photographs were taken 39 and 38 minutes, respectively, after reaching full speed (59,780 rpm).

examination revealed the urinary protein to be largely albumin. Albuminuria is a common finding in many C3H mice with no other evidence of renal disease. There was no evidence in the urine of the 5647 tumor bearing mice of a nonprecipitating Bence Jones protein, nor of urinary excretion of abnormal proteins of the type found in the serum.

DISCUSSION

The transmissible plasma-cell neoplasm (5647) described here differs from two other plasma-cell neoplasms (70429 and 5563) in strain C3H mice previously studied at the National Cancer Institute. Neoplasm 70429 was histologically undifferentiated and showed no significant serum protein changes. With neoplasm 5563 a homogeneous protein of fast gamma globulin electrophoretic mobility with an ultracentrifugal sedimentation coefficient of 6.5 S is found in the serum. Multiple osteolytic lesions filled with malignant plasma-cells may develop, and the plasma-cell neoplasm 5563 at the implantation site is notable for having a peripheral zone of edematous tissue, a soft consistency and reddish-purple color.

Rask-Nielsen, Clausen and Gormsen have recently reported two transmissible plasma-cell leukemias in mice which on serum electrophoretic examination had increased amounts of protein of gamma globulin mobility in one instance and of beta globulin mobility in the other. Their neoplasms originated in (CBA X DBA/2)F1 hybrid mice; the transplanted neoplasm was taken from enlarged pancreatic lymph nodes, and a leukemic blood picture was frequently seen. Ultracentrifugal data have not been reported.

Differences are to be expected among experimental plasma-cell neoplasms in mice if they behave as analogous tumors in man, for the neoplasm in
each patient produces individual and distinctive protein changes. Differences among the mouse plasma-cell neoplasms have been particularly evident upon detailed examination of the abnormal serum proteins in the tumor-bearing mice. Both plasma cell neoplasms 5647 and 5563 in mice have maintained their individual characteristics through repeated transfer generations. Although derived from the same cell type within the same inbred strain of mice, these tumor lines are not equivalent, and must be regarded as different entities.

Increased amounts of serum protein of beta globulin electrophoretic mobility in man, similar to that seen with neoplasm 5647, may be seen with multiple myeloma and with Waldenström's macroglobulinemia. However, the ultracentrifugal behavior of the serum proteins and the clinical findings with neoplasm 5647 do not indicate a relationship to Waldenström's macroglobulinemia. On ultracentrifugal analyses of human myeloma sera, the most common finding is a single myeloma protein with a sedimentation coefficient of about 6.6 S. However, multiple ultracentrifugal components similar to those observed in the 5647 sera are seen in the sera of some patients with multiple myeloma having myeloma proteins of fast gamma or beta globulin mobility on electrophoretic analysis.

Questions as to the number of abnormal proteins present in the sera of animals bearing neoplasm 5647, and whether these are qualitatively or quantitatively abnormal, have not been settled. Serum proteins of 9, 11 and 13 S are not seen on ultracentrifugation of normal mouse serum. However, ultracentrifugation is not a sensitive technic for detecting protein components present in very low concentration. Serum proteins normally present in very small amounts with sedimentation coefficients in this range would not be detected.

The myeloma proteins seen on serum electrophoretic analysis and the three components (9, 11 and 13 S) seen in the ultracentrifuge are thought to be related specifically to the presence of neoplasm 5647. Although these may be different individual proteins, it is possible that they represent several stages of aggregation of smaller units. It is of interest to note that the 11 S component in many of the sera examined has been present in lower concentration than either the 9 or 13 S components. Similar phenomena have been observed in several human myeloma sera containing multiple ultracentrifugal components with comparable sedimentation coefficients.

The abnormal serum proteins probably are formed in the plasma-cells of neoplasm 5647. It is possible that several proteins could be formed in different cells of the tumor. This seems unlikely, however, for it would require maintenance of a constant balance of such cells through many transplant generations. In our experience during 10 transplant generations and one and a half years of serial ultracentrifugal observations, the relative amounts of the 9, 11 and 13 S components have remained approximately the same. It is hoped that further study will shed additional light on the nature of these proteins and their relationship to the normal serum proteins.

How far the relationship will extend between plasma cell neoplasms in the mouse and in man can only be developed by further observations. These tumors in mice may be useful in the screening of chemical agents being
considered for therapeutic use in multiple myeloma in man. They may also be profitably utilized in studies of protein biosynthesis and metabolism, and of antibody regulatory mechanisms. The plasma-cell neoplasm 5563 has already been utilized to show that the serum myeloma protein is synthesized in the plasma-cell tumor.23

SUMMARY

A transmissible plasma-cell neoplasm (5647) accompanied by marked serum protein changes has been described in the mouse. The principal characteristics of this neoplasm and the associated serum protein changes have remained unchanged during two years of observation.

The transplanted neoplasm usually remains confined to the site of implantation. Osteolytic lesions and abnormal cells in the peripheral blood were not seen.

Morphologically the neoplastic cells retain many characteristics of normal plasma-cells, with clumping of the nuclear chromatin, an eccentric nucleus and a clear zone in the basophilic cytoplasm.

Serum electrophoretic analyses revealed a markedly abnormal serum protein pattern. The total serum protein was increased by two to three grams per cent in mice bearing 5647 neoplasms weighing 6 grams or more. Increased amounts of proteins with beta globulin and fast gamma globulin electrophoretic mobility were the most striking electrophoretic findings. These components comprised as much as 35 per cent of the serum proteins in animals with large neoplasms.

Serum ultracentrifugal analyses also demonstrated a markedly abnormal distribution of the serum proteins. Normal mouse serum contains components with sedimentation coefficients ($S_{20,w}$) of 4, 6.5 and 16 S. Serum of mice bearing neoplasm 5647 contained, in addition to the normal components, proteins with sedimentation coefficients of 9, 11 and 13 S. These serum components, accounting for as much as 20 per cent of the total protein, have been found only with neoplasm 5647.

The quantity of the abnormal serum components seen on electrophoretic and ultracentrifugal analyses was found to increase as the tumor weight increased.

Bence Jones protein was not found in the urine of mice bearing the 5647 neoplasm.

Neoplasm 5647 was found to differ significantly in biologic behavior and in the associated serum protein changes from two other transmissible plasma-cell neoplasms of the mouse (5563 and 70429) which have been under observation in these laboratories.

The mouse plasma-cell neoplasm 5647 resembles in several characteristics the malignant plasmacytomas in man having associated myeloma proteins of beta globulin electrophoretic mobility.

SUMMARIO IN INTERLINGUA

Un transmissibile neoplasm plasmocytic (5647), accompaniate per marcate alterationes del proteinas seral, esseva studiate in muses. Le major char-
acterísticas de este neoplasma e le associate alteraciones del proteinas seral ha remanite constante durante duo annos de observation.

Le transplantate neoplasma remane usualmente confinate al sito de su implantation. Lesiones osteolytic e cellulas anormal in le sanguine peripheric ha non essite observate.

In lor morphologia le cellulas de neoplasia retene multe características de plasmocytos normal, con aggregation del chromatina nuclear, un nucleo ec-

centric, e un zona clar in le cytoplasma basophilic.

Analyses electrophoretic del sero revelava marcate anormalitates in le proteinas. Le proteina total del sero esseva augmentate per duo a tres grammas pro cento in muses con neoplasma 5647 de pesos de 6 grammas o plus. Aug-

mentate quantitates de proteinas con le mobilitate electrophoretic de globulina beta e rapide globulina gamma esseva le plus frappante constatationes electrophoretic. Iste componentes constituite usque a 35 pro cento del proteinas sera in animales con grande neoplasmas.

Analyses per ultracentrifugation del sero etiam demonstrava marcate anormalitates in le distribution del proteinas seral. Le sero normal del mus contine componentes proteinic con coeficientes de sedimentation \((S_{20,w})\) de 4, 6, 5, e 16 S. Le sero ab muses con neoplasma 5647 contineva, a parte le componentes normal, proteinas con coeficientes de sedimentation de 9, 11, e 13 S. Iste componentes seral, representante usque a 20 pro cento del proteina total, ha essite trovate solmente in le presentia de neoplasma 5647.

Esseva constatate que le componentes anormal del sero secundo le analyses de electrophorese e de ultracentrifugation cresceva in quantitate con le augmentation del peso del tumor.

Proteina de Bence Jones non esseva trovate in le urina de muses con le neoplasma 5647.

Esseva trovate que neoplasma 5647 differe significativamente in su comportamento biologic e in le associate alterationes de proteina seral ab duo altere transmissibile neoplasmas plasmocytic del mus que ha essite sub observa-

tion in iste laboratorios sub le cifras 5563 e 70429.

Neoplasma 5647 resimila in plure caracteristicas le maligne plasmocytomas in humanos con associate proteinas de myeloma de un mobilitate electrophoretic de globulina beta.

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