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ANIMAL MODEL OF HUMAN DISEASE

Multiple Myeloma

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ANIMAL MODELS of spontaneous and induced plasmacytomas in some inbred strains of mice have proven to be useful tools for different studies on tumorigenesis and immunoregulation.[1-3] Their wide applicability and the fact that after their intravenous transplantation, the recipient mice developed bone lesions, led in some instances to an incorrect use of the term myeloma for these neoplasms.[4] They originate and grow as extramedullary plasmacytomas, mostly without involvement of the bone marrow. Therefore, they cannot be compared with the most frequent plasma cell malignancy in humans, the multiple myeloma (MM). A more appropriate model for MM is available in aging mice of the C57BL/KaLwRij strain.

Biologic Features

Aging C57BL/KaLwRij mice develop frequently benign B cell monoclonal proliferative disorders (benign monoclonal gammopathy, BMG).[5] In addition, an old mouse occasionally can be found with an excessive homogeneous immunoglobulin component (H-Ig) in the serum, which progressively increases in concentration while the other immunoglobulins decrease. When investigating the bone marrow of such an animal, typical myeloma cells showing a monoclonal expansion are found by morphologic and immunocytochemical examinations (Figure 1). On radiographs of the skeletons, osteoporosis with occasional osteolytic lesions are found, mainly in the ribs, femora, and tibiae.[6] This spontaneous MM appears in C57BL mice older than 2 years, with a frequency of about 0.5%. This value is derived from the screening for H-Ig in the sera by agar electrophoresis according to Wieme.[7] This means that MM with only Bence Jones protein production, some MM with fast-moving H-Ig, and nonsecretory forms of MM could have been missed.

The majority of MM found so far produced H-Ig of the IgG isotype with kappa light chains (Table 1). The only other isotype represented was one IgD-k. Occasional observations of mice with an IgM H-Ig component of a high concentration are not included because the underlying disease resembled more that of human Waldenström's macroglobulinaemia than MM.

The C57BL MM can be propagated readily by intravenous transfer of bone marrow cells into recipients of the same strain. A "take" of this neoplasm approaching 100% is achieved in the first and in subsequent cell transfer generations. This contrasts sharply with the limited clone survival which has a maximum of three to four transplantation generations in the case of benign monoclonal gammopathy in the same mouse strain.[8] Spleen cells also can be used successfully for transplantation, especially when donor mice are taken with an advanced MM. Different MM lines maintain properties of the original MM. MM growing slowly and with little bone destruction tends to retain these characteristics in subsequent transplantation generations. Some sublines gradually may develop a more aggressive growth with greater bone destruction and excessive Bence Jones proteinuria, however. While most of the transplanted MM lines show osteolytic lesions of the bones, one subline (5T14) developed an osteosclerotic form of MM. The development

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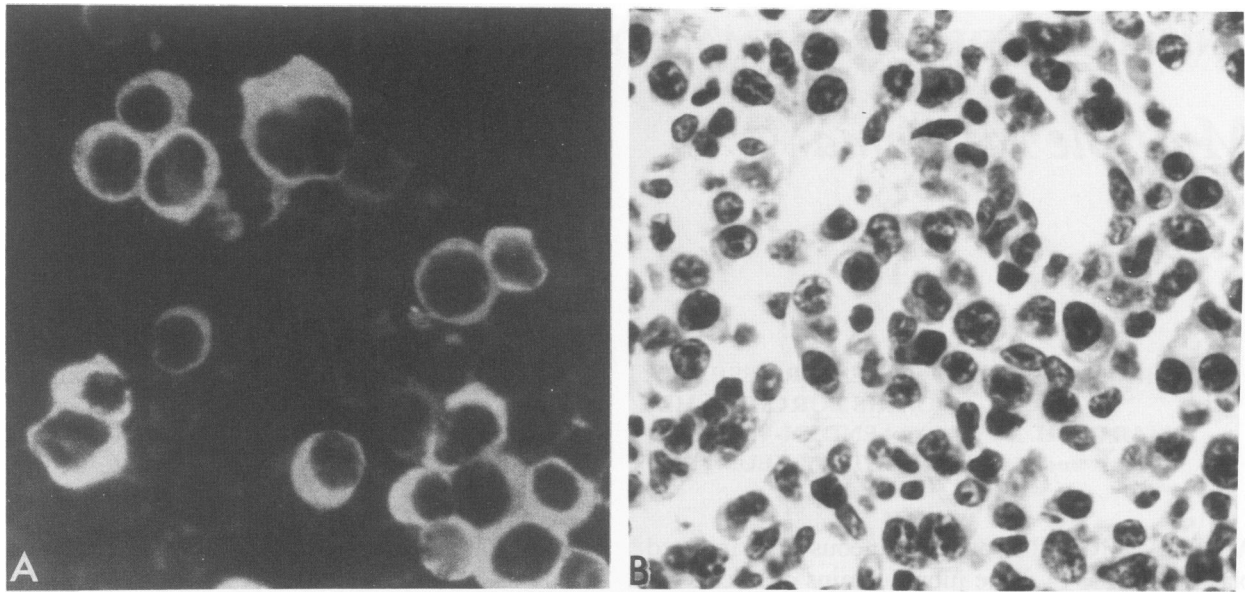

Figure 1A—Bone marrow cells of the primary 5T2 MM as examined on cytospin preparation by immunofluorescence. **B**—Histologic picture of sternum from the same mouse showing an area of atypical plasmacytoid cells. Note pleomorphic excentric nuclei with clumped chromatin or with margination of chromatin and large nucleoli. The cytoplasm is amphophylic, variable in quantity, and frequently contains a pale staining paranuclear zone. HPS, ×800

of individual transplanted MM lines can be monitored by detection of the MM H-Ig component by agar electrophoresis, immunoelectrophoresis, or by more sensitive techniques using isotype or idiotype specific antisera (Figure 2).[9] The amount of the MM H-Ig component can reach values exceeding 2 g/dl. In close correlation with the paraprotein concentration, the numbers of MM cells in the bone marrow increase and the neoplasia eventually spreads to other organs. Some recipient mice develop neurologic complications, such as paraparesis or paraplegia or central symptomatology, due to neoplastic growth around the spinal cord or into the meninges of the brain. Spontaneous fracture of one of the long bones is an exceptional finding. Mice with advanced MM appear ill, and have bristling hair. Some of them show signs of hyperaesthesia and, on rare occasions, epileptical seizures can be observed. In advanced stages, anemia with low haematocrit values is often seen.

While the percentage of plasma cells in the bone marrow (femur) of normal mice and mice with BMG of comparable age are approximately 0.2 and 0.5%, respectively, the primary MM-bearing mice show higher but variable figures, ie, from 5 (5T2) to more than 50% (5T13). The cytologic pattern varied among different lines, from normal-looking plasma cells (5T7) to abnormal cells with (plasma)blastlike appearance (5T14). The finding of pleomorphic plasma cells in diffuse pattern and/or nest forming was not pronounced in some 5T MM and contrasted with a clear-cut picture of an advanced MM on cytospin preparations investigated by immunofluorescence.

Most data on the transplanted form of MM were obtained from the longest known MM, the 5T2 MM.[8-11] After intravenous transplantation, the typical 5T2 MM cells initially can be found only in the bone marrow of the recipient. Their distribution pattern generally is characterized by multifocal areas of

Table 1—List of Documented 5T MM in Aging C57BL/KaLwRij Mice

5T MM number	Isotype	Trans-planted	Generation	Growth	Remark
5T2	IgG2a-k	+	27	Moderately progressive	Antibody specificity known
5T7	IgG2b-k	+	8	"Smoldering MM"	
5T8	IgG2a-k	−	2		Lost
5T13	IgG2b-k	+	3	Moderate	
5T14	IgG1-k	+	12	Aggressive	Different sublines
5T21	IgD-k	+	11	Mainly spleen	Atypical
5T33	IgG2b-k	+	8	Moderate	
5T41	IgG3-k	+	2	Moderate	
5TF	IgG3-k	+	3	Moderate	Spontaneous in F1 (C57BL × CBA); discontinued

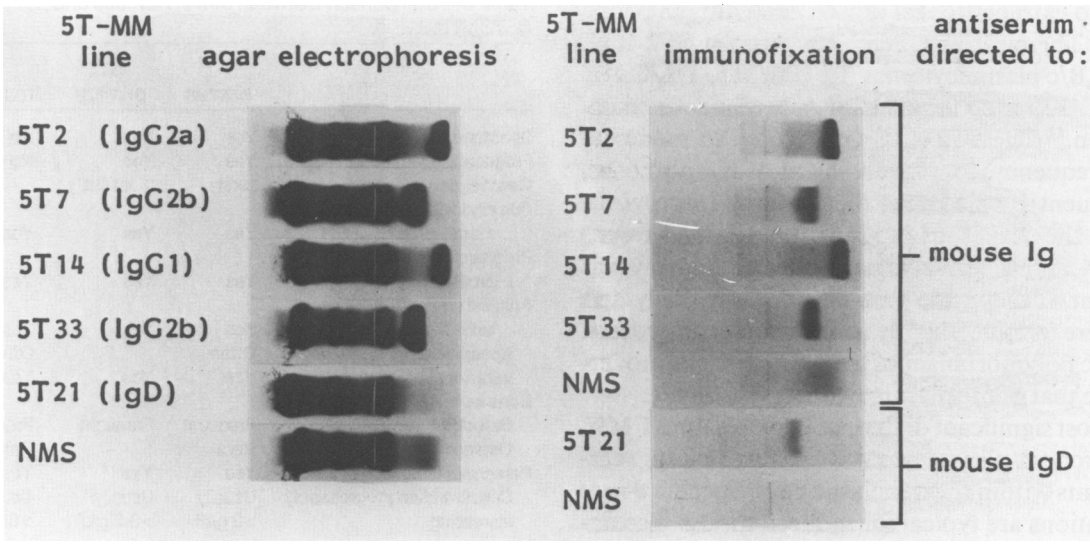

Figure 2—Example of serum pattern of five 5T-MM lines by agar electrophoresis (Wieme) and immunofixation. NMS, normal mouse serum of C57BL origin.

diffusely spread pleomorphic plasmacytoid cells, with no distortion of the architecture of the bone marrow space. With the progression of the disease, the MM cells occupy most of the bone marrow space and largely replace the normal hematopoietic tissue. When osteolytic disease is present, a focal destruction of cortical bone and a decrease in the amount of trabecular bone is seen. The cortical destruction is most extensive where blood vessels pass through the cortex. Further progression leads to the extension of the growth of the MM outside of the bone, ie, into the surrounding muscles, into the vertebral canal, into the meninges from the skull or all three. The red pulp of the spleen is affected most frequently. Lymph nodes, and gut- and bronchus-associated lymphoid tissues are involved to a minor extent. Occasionally the characteristic picture of a "myeloma kidney" was observed.

The cytologic pattern of individual transplanted lines varies slightly among different generations. The 5T2 MM cells generally are moderately to poorly differentiated, with the exception of some cases, mainly in the early generations, that show a greater extent of differentiation. Ultrastructural studies performed on several samples from different generations showed cells characterized by the presence of clumped chromatin, an appreciable amount of rough endoplasmic reticulum, and a high nuclear/cytoplasmic ratio. Incongruity between the immature looking nucleus and the more differentiated cytoplasm was typical. Double-nucleated cells are a frequent finding. Distinct nucleoli often are seen within the nucleus.

The intratumor heterogeneity of 5T2 MM indicated by morphologic examination was confirmed in cell separation experiments using the velocity sedimentation technique and flow cytometric analysis.[9] The 5T2 MM cells were shown to have an aneuploid DNA content and to consist of subpopulations with differences in size, proliferation capacity, and expression of their membrane idiotype.

Comparison with Murine BALB/c Plasmacytoma and Louvain rat Immunocytoma

In contrast with MM in the C57BL mice, the plasma cell tumors in BALB/c mice or in rats originated either after induction or spontaneously, as local plasmacytomas. They were not located in the bone marrow but most frequently in the peritoneal connective tissue. Probably they originated from gut-associated lymphoid tissue, as indicated by the fact that most BALB/c plasmacytomas (60%) secrete IgA and most rat immunocytomas form IgE (infestation of gut by parasites).[12,13] This is in contrast with the origin of human and C57BL MM (mainly IgG isotype), which may originate from B cells of the systemic immune system. In addition to the differences in the primary form, some distinct differences remain when the neoplasms are propagated by transplantation in syngeneic hosts. If propagated intravenously, the BALB/c plasmacytoma cells can also be found in the bone marrow and lytic bone lesions may develop. However, advanced cases with tumor growth into the periost showed osteoplastic changes. After intraperitoneal transplantation, the 5T MM differs from BALB/c plasmacytoma in that the mice develop ascites only exceptionally and the MM cells disseminate fre-

quently to extraperitoneal organs, including the bone marrow. The clearest differences between 5T2 MM and BALB/c plasmacytomas (MOPC 315, TEPC 183 and RPC 20) were found after subcutaneous transplantation.[11] The 5T2 MM showed low to moderate "take" frequency, low involvement of lymph nodes, and frequent involvement of the bone marrow. In contrast, the "take" of the BALB/c plasmacytomas was 100% and the involvement of bone marrow was infrequent. If they were present, however, the lymph nodes were involved highly in the neoplastic process. Cytologic polymorphism of the 5T2 MM was moderate, while that of the plasmacytomas was severe.

The most significant difference between the 5T MM and the rodent plasmacytomas concerns *c-myc* rearrangements within the neoplastic cells. Specific *c-myc* translocations are typical for induced mouse plasmacytomas and rat immunocytomas (and also for human Burkitt's lymphoma)[14] but they are exceptional in the human MM. The presence of *c-myc* rearrangements was tested in the different 5T MM by Southern blot analysis using a probe for the third exon of the human *c-myc* gene. A rearrangement of the *c-myc* gene was found only in later generations of some of the 5T2 MM sublines, but in none of the other 5T MM (manuscript in preparation).

Comparison with Human Disease

The major features of the primary and transplanted mouse 5T MM compared with human MM are given in Table 2. Even when taking into consideration the large spectrum of individual cases within human disease and the limited number of the primary 5T MM observed so far, it can be concluded that the features are essentially the same in both species. Some minor differences, eg, the paraprotein concentration, result from differences in magnitude of the organ and cellular components within the immune system of humans and mice. The observation that, in contrast to man, the 5T MM cells are often present in the mouse spleen is not contradictory since the mouse spleen is a bone-marrowlike, ie, hematopoietic, organ, whereas the human spleen is part of the secondary lymphoid tissues that are of the lymph-node type. The question of how far the properties of 5T MM change during their propagation *in vivo* for several generations is difficult to answer and deserves a careful study. It is obvious that some aspects of MM (eg, the cause) can be studied only in its primary form while the transplanted form may be useful for other aspects.

Usefulness of the Animal Model

The 5T MM model already proved its usefulness in studies on the effect of the bisphosphonate APD in the treatment of bone disease in MM,[10,11] on immunoregulation,[15,16] and on the possibilities of immunologic treatment of MM after the stage of a minimal residual disease is achieved by chemotherapy treatment.[11,17]

Table 2—Comparison Between MM in Humans and C57BL/KaLwRij Mice

	Human	5T primary	5T transplanted
Spontaneous origin	Yes	Yes	No
Frequency age-related	Yes	Yes	No
Genetic predisposition	Likely	Most likely	
Primary localization in bone marrow	Yes	Yes	Yes
Progressive monoclonal proliferation	Yes	Yes	Yes
Atypical ("myeloma") plasma cells	Yes	Yes	Yes (>)*
Aneuploidy	Often	?	Often
Intraclonal heterogeneity	Yes	Yes	Yes
Bone lesions			
Osteolytic	Frequent	Frequent	Frequent (>)
Osteosclerotic	Rare	–	Rare
Paraproteinemia	Yes	Yes	Yes
Concentration progressively	Usually	Usually	Usually
Increasing	>2 g/dl	>0.5 g/dl	>0.5 g/dl
Most frequent isotype	IgG	IgG	IgG
Excess production of Bence Jones protein	Often	?	Observed
Depression of other Ig	Yes	Yes	Yes
Anemia	Yes	Yes	Yes
Myeloma kidney	Often	?	Rare (5T2) ? (others)

* (>), more pronounced in transplanted form.

Availability

Mice of the C57BL/KaLwRij strain are available at the REP TNO Institutes in Rijswijk, The Netherlands.

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