Mouse plasmacytoma: an experimental model of human multiple myeloma

KLARA GADÓ,* SANTIAGO SILVA,° KATALIN PÁLÓCZI,* GYULA DOMJÁN,# ANDRAS FALUS@*

*National Institute of Hematology and Immunology, Budapest, Hungary; °Microbiology and Tumor Biology Center, Karolinska Institute, Stockholm, Sweden; #Department of Medicine and Geriatrics, Faculty of Health Sciences, Semmelweis University, Budapest; @Cell- and Immunobiology, Department of Genetics, Faculty of Medicine, Semmelweis University, Budapest, Hungary

Background and Objectives. There is no ideal animal model for human multiple myeloma (MM). All the models resemble the human disease in some respect, but none of them fulfils all the criteria of a perfect animal model.

Evidence and Information Sources. The pristane oil (2,6,10,12-tetramethylpentadecane)-induced mouse plasmacytoma (MPC) model is the most widely used and accepted model and has provided the most data on plasmacytomagenesis so far. This model gives the opportunity to study the role of c-*myc* dysregulations, the mechanisms leading to cytogenetic changes involving Ig genes, the role of chronic inflammatory factors, the role of interleukin-6 (IL-6), insulin-like growth factor-I, prostaglandins, as well as signal transduction pathways in the neoplastic process. Therapeutic agents have been succesfully tested. Although MPC growth is usually restricted to the peritoneal environment, intraperitoneal injection of MPC cell suspensions can reproduce the disseminated characteristics of the human disease in recipients. The IL-6 transgene and knockout models are important tools for clarifying the role of IL-6 in the pathogenesis of MM. Transgenic mice and retroviral gene transfer facilitate the study of oncogenes in neoplastic transformation. Spontaneous development of plasmacytomas in C57BL/ KaLwRij aging mice has several advantages, mainly because the disseminated growth, the typical bone lesions and renal involvement resemble, in part, the human disease. Furthermore, this model has already proved useful in studies on the effect of bisphosphonate in the treatment of bone disease in MM. The severe combined immunodeficiency (SCID) mouse model is also very attractive. A disseminated-like disease can be reproduced in this model. Multiple osteolytic bone lesions and bone marrow involvement are generated, and conventional drugs applied in the treatment of human multiple myeloma have proven to be effective. Nevertheless, the immune system of SCID mice basically differs from that of a MM patient.

Perspectives. Taken together, all these models have contributed to our understanding of MM, but demonstrate the opportuness of developing a more appropriate model of the human disease.

Key words: multiple myeloma, experimental plasmacytoma, tumorigenesis, animal model, transgenic mice

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Correspondence: Andras Falus Ph.D., Professor, Nagyvárad tér 4, Budapest, 1089, Semmelweis University, Faculty of Medicine, Department of Genetics, Cell and Immunobiology, Hungary.
Phone: international +36-1-2102929 - Fax: international +36-1-3036968 - E-mail: faland@dgci.sote.hu

Human multiple myeloma (MM) is a currently incurable B-cell malignancy that accounts for approximately 10% of human hematopoietic malignancies and 1% of all human cancers. MM often originates from a common premalignant condition called monoclonal gammopathy of undetermined significance that affects 1% of the adult population. The clonal B-cell abnormality in MM affects precursors of terminally differentiated B-cells. MM is characterized by long-lived plasma cells that, as a rule, have suffered somatic hypermutation, antigen selection and IgH switching in the germinal center.[1] The characteristic clinical features of the disease are a high production of monoclonal immunoglobulins or immunoglobulin fragments, anemia, bone pain, pathologic fractures, susceptibility to infections, renal impairment and hyperviscosity of the blood.

There is a plethora of experimental *in vitro* and *in vivo* studies on human MM.[2] It has been clearly shown that interleukin-6 (IL-6) is a key growth and survival factor for myeloma cells, as well as a major morbidity factor for patients with MM.[3] There is strong evidence for both an autocrine (in myeloma cells) and paracrine source of IL-6 induction (from bone marrow stromal cells).[4]

There are similarities in the tumor biology and phenotype between human MM and mouse plasmacytoma (MPC). It is known that MPCs, like their human counterpart, are Ig producers and also dependent on IL-6 for growth *in vitro* and *in vivo*. The genetic abnormality characterized by Ig/myc translocations, invariably present in almost all MPCs, is occasionally observed in < 5% of MM. Nevertheless, there is some evidence that c-myc is overexpressed in MM.[5] The most frequent karyotypically detectable translocation in MM involves chromosome 14q32IgH switch regions and occurs in 10% to 40% of tumors. The illegitimate IgH translocations involve diverse but not random chromosome partners i.e. 11q13 locus (30% of these cases) or 4p16.

There is agreement that the lack of good experimental models of MM hampers the full understanding of the development of the disease.[6] The development of appropriate animal models to study the biology of MM would

undoubtably help to clarify the mechanism of activation of pre-switched plasma cells in premalignant monoclonal gammopathy, the sub-clonal expansion and isotypical divergence, the relevance of viral infections such as Kaposi's sarcoma-associated herpes virus (KSHV) disease[6] and would also benefit the development of therapeutic approaches.

An experimental animal model of MM must fulfil the following criteria:

- it must reproduce the characteristic features of human MM;
- it must produce similar clinical symptoms and laboratory abnormalities as those of human MM;
- and basic MM therapy must be effective in an animal model and *vice versa*.

There are several experimental mouse models that have features resembling the human disease in some respect, but none of them seems to fulfil the criteria detailed above. Consequently, we thought that a review of the most relevant murine models with an evaluation of the results achieved following their application and an assessment of their limitations, may be a first step in helping to develop a tailor-made animal model for studying human MM.

Main features of mouse plasmacytoma

Cytogenetics

Virtually all MPCs carry chromosomal translocations or other rearrangements juxtaposing either the *c-myc* or the *Pvt-1* genes to one of the three immunoglobulin loci. This displacement brings the *c-myc* gene under the control of the Ig locus. As a result, *c-myc* no longer obeys its normal regulatory pathways and is not downregulated when the cell is programmed to leave the cycling compartment, and thereby prevents the cell from entering the G0 stage.[7,8]

The most frequent or typical translocation leads to the juxtaposition of *c-myc* on chromosome (chr) 15 to the IgH locus on chr 12. The t(12;15)(IgH/*myc*) translocation is found in 90% of pristane-induced plasmacytomas.[9]

A less frequent type of MPC-associated translocation (also called variant), results in the juxtaposition of either IgLκ gene on chr 6 or IgLλ on chr 16 to the *c-myc*/Pvt-1 locus on chr 15.

In t(12;15) carrying tumors, usually 5' sequences of one IgH switch region face 5' regions of *c-myc* in a head-to-head position.[10] As a rule, 5' constant-region sequences of the κ or λ light chain are transposed to the 3' Pvt-1-*myc* region in a head-to-tail orientation. This has led to the suggestion that switch recombinational machinery may be involved in the process of illegitimate recombinations.[11] Nevertheless, recent studies suggest that MPC carrying t(12;15) translocations may develop from immature/mature B-cells and not from differentiated plasma cells.[12]

There is no doubt that the Ig/*myc* translocation is an early initiating step in the genesis of MPC. The clonal origin of the tumors suggests that, beside *c-myc* activation, additional genetic changes may be required to generate autonomous tumors.[13]

Immunoglobulin production

Sixty per cent of the MPCs produce IgA. IgG producers have also been described. In contrast, IgM producers are rare.[11]

Propagation and growth of MPCs

Role of interleukin-6

Interleukin-6 (IL-6) has been reported to be a major growth factor for myeloma cells and may play a role in promoting the survival of malignant plasma cells. Intraperitoneal injection of pristane induces production of IL-6 and plasmacytoma in mice. Pristane does not induce plasmacytoma in IL-6 knockout (IL-$6^{-/-}$) mice, suggesting that IL-6 is required for this process.[14] Lattanzio *et al*. studied the role of IL-6 in mouse plasmacytoma. In the pristane model, IL-6-deficient BALB/c mice were protected against tumor development. In the absence of IL-6, there were never signs of uncontrolled proliferation of either normal B-lymphocytes or tumor cells.[15]

The induction of MPCs by pristane is favored by the presence of IL-6 and other growth factors in the granulomatous microenvironment. It is conceivable that, during the course of IL-6-induced expansion of polyclonal plasma cells, Ig/*myc* translocation may occur in one or few clones that will finally generate monoclonal or more rarely, oligoclonal plasmacytomas. Alternatively, the compartment of plasma cell precursors may contain pre-neoplastic cells already carrying Ig/*myc* translocations that suffer malignant transformation in the presence of IL-6. In either case, plasmacytomagenesis will require the BALB/c genetic background.[16]

Role of prostaglandin E2

Prostaglandin E2 (PGE_2) has been shown to have a number of regulatory effects on B-cell activities. The major biological effect is to inhibit B-cell proliferation and activation. PGE_2 also inhibits the proliferation of B-cell lymphomas.

Prostaglandins can be detected at high levels in the oil granuloma and are most likely produced by macrophages and fibroblasts. Since PGE_2 stimulates IL-6 production in macrophages and other inflammatory cells, it becomes a relevant factor during MPC genesis. If high ambient levels of IL-6 are required for MPC development, then inhibition of PGE_2 synthesis could play an important role in shaping the permissive environment of MPC formation.[11,17,18,19] Such a condition can be achieved, for example, by the continous administration of anti-inflammatory drugs, such as indomethacin, that inhibit plasmacytoma development in pristane-treated hypersusceptible BALB/cAn mice.[20,21] The effects of prostaglandins on T-cells may also have an important role.[22] A surveillance hypothesis for explaining indomethacin inhibition is that as small clones of plas-

ma cells attain sufficient size and antigen production, they are recognized by the immune system, and cytotoxic T-lymphocytes are generated to destroy the clones. The presence of a high level of PGE_2 abrogates this immune process, allowing the cells to escape and increase in size to a point at which the cytotoxic T-lymphocyte response is ineffective.

The role of PGE_2 in the human MM cytokine network was examined in short-term cultures of bone marrow from patients with MM.[23] It was shown that PGE_2 was produced concomitantly with IL-6 and IL-1. Indomethacin significantly inhibits IL-6 but not IL-1 production. Introduction of exogenous PGE_2 reverses this inhibition or even stimulates IL-6 production. An IL-1 receptor antagonist (IL-1RA) also significantly inhibits PGE_2, IL-6 production and myeloma cell growth. These results indicate that induction of IL-6 by IL-1 is related to PGE_2 in the bone marrow of patients with MM. Consequently, by hampering the PGE_2/IL-1/IL-6 pathway it might be possible to inhibit myeloma cell proliferation.

Role of insulin-like growth factor-1 and transforming growth factor-β

Insulin-like growth factor-1 (IGF-1) has been found to stimulate the proliferation of human myeloma cell lines but it does not influence normal B-cells. IGF-1 also possesses an anti-apoptotic effect on myeloma cells as it was demonstrated to protect myeloma cells against dexamethasone-induced apoptosis.[24] Human myeloma cell lines and MPC lines express IGF-1 receptor. Thus the availability of IGF-1 in the oil granuloma environment may play a critical role during MPC development. Since macrophage IGF-1 production is stimulated by PGE_2, the low levels of PGE_2 following indomethacin may limit the availability of IGF-1.[21]

Transforming growth factor-β (TGF-β) is known to play a negative role in tumor development. Though PCTs produce TGF-β *in vitro*, they are refractory to TGF-β-mediated growth inhibition and apoptosis because of the lack of functional TGF-β receptor.[11,25,26]

Signal transduction pathways

IL-6 induces signaling through the activation of signal transducers and activators of transcription (STAT) proteins. Stat3, a member of the STAT family, was found to be constitutively activated in bone marrow mononuclear cells from patients with MM. Activation of Stat3 was demonstrated to be essential for the survival of myeloma cells and to contribute to the pathogenesis of MM by preventing apoptosis.[27] An important step in the malignant progression of murine plasmacytomas is the transition from dependence on IL-6 to a state of IL-6 independence. Rawat *et al.*[28] found that Stat3 was constitutively activated and phosphorylated in IL-6-independent but not in IL-6-dependent cell lines and concluded that the constitutive activation of Stat3 is associated with the acquisition of an IL-6-independent phenotype. Another potential growth signaling pathway is insulin-like growth I (IGF-I). The IGF-I receptor (IGF-IR) was found to be expressed in 7/8 MM cell lines.[29] Downstream of IGF-IR, insulin receptor substrate 1 was phosphorylated leading to the activation of phosphatidylinositol-3'-kinase (PI-3K). PI-3K regulated two distinct pathways. The first included Akt and Bad, leading to an inhibition of apoptosis; the second included the mitogen-activated protein kinase (MAPK), resulting in proliferation. Insulin receptor substrate was found to be either constitutively or IGF-I-dependently activated in all plasma cell tumors. Biological relevance was demonstrated by the fact that expression of a dominant-negative mutant of IGF-IR in plasma cell lines strongly suppressed tumorigenesis *in vivo*.[30] It is also known that IL-6 activates the signal transducer gp130. Signal transduction of gp130 involves the Janus tyrosine kinases (JAK) JAK1, JAK2 and Tyk2 and then the downstream effectors comprising stat3 and MAPK pathways. De Vos *et al.*[31] evaluated the effects of the JAK2 inhibitor tyrphostin AG490 on MM cells and found that AG490 suppressed cell proliferation and induced apoptosis in IL-6-dependent MM cell lines. Concordantly, JAK2 kinase activity, ERK2 and stat3 phosphorylation were inhibited suggesting that chemical blockade of the gp130 signaling pathway at the level of JAK could be a relevant therapeutic approach.

Genetic susceptibility

The great majority of standard laboratory inbred strains are highly resistant to the induction of MPCs by pristane. Only the BALB/c and NZB strains are susceptible. Susceptibility to MPC induction varies among the BALB/c substrains.[32] As described elsewhere, Potter *et al.* reported that about 60% of pristane-treated BALB/cAnPt develop MPC; in contrast only 10% of BALB/cJ are susceptible.[33] Resistance is dominant over susceptibility in F1 hybrids between BALB/cAnPt and the resistant non-BALB/c strains. This observation indicates that susceptibility may have a genetic basis.[9,34,35]

Studies on recombinant inbred (RI) strains suggested that multiple genes on chromosomes 1 and 4 may each contribute with a small effect in plasmacytomagenesis. Furthermore, at least three loci located at the distal part of chromosome 4 may contain genes controlling resistance[33, 36] (*and Mock, B. personal communication*). The mechanism by which these genes control susceptibility and resistance is not known. These mouse chromosome 4 regions share linkage homology with human chromosomes 9p21, 1p32 and 1p36.[35]

Pristane induction of plasmacytomas

Although spontaneous MPCs occur rarely in mice they can be induced with a variable incidence in BALB/c mice by repeated intraperitoneal injections of pristane.

Histopathology and diagnosis

Pristane oil first induces a chronic inflammatory granuloma (oil granuloma = OG) in the peritoneal cavity. About 120 days after the first pristane injection, development of ascites can be observed. The diagnosis of MPC

is established following the finding of 10 or more characteristic tumor plasma cells/field in the ascitic fluid. Plasmacytoma cells can be identified by their large size, abundant basophilic cytoplasm, a well developed endoplasmic reticulum and Golgi apparatus, together with an eccentric kidney-shaped non-pyknotic nucleus with a clear perinuclear zone. The mouse plasmacytoma cell, like its human counterpart, secretes Ig, continues to cycle and is derived from B-cells that are completing or have completed heavy chain isotype switching.

Effect of the environment

Pristane-treated BALB/cAn mice develop plasmacytomas with a 60% incidence provided they are housed under conventional conditions. A dramatic reduction in tumor incidence is observed when the pristane-treated BALB/cAn mice are kept in viral specific pathogen-free (SPF) conditions.[37]

Testing therapeutic agents in the pristane model

In 1966, Takura *et al*. showed that daily injections of cortisol inhibited paraffin oil induction of MPCs.[38] Thompson and VanFurth demonstrated that hydrocortisone prevented the migration of mononuclear cells into the inflammed peritoneal cavity, but that it did not reduce the existing population of peritoneal macrophages. Their results suggest that pristane-induced adherent peritoneal cells are critical for the establishment of a conditioned environment, and cortisol treatment should abrogate the conditioning effect.[39] As already mentioned, Potter *et al.* demonstrated that the non-steroidal anti-inflammatory agent indomethacin strongly inhibited the development of MPC formation in pristane-treated BALB/cAnPt mice.[20, 21] It is known that the biochemical targets of this inhibitor are the cyclo-oxygenase enzymes, COX-1 and COX-2.

Main advantages of the pristane model

As already mentioned, pristane-induced mouse plasmacytomas share a number of common features with human multiple myeloma. This experimental model gives the opportunity to study the role of *c-myc* dysregulations, the mechanisms leading to cytogenetic changes involving Ig genes, the role of chronic inflammatory factors, the role of IL-6, insulin-like growth factor-I, prostaglandins, as well as signal transduction pathways in the neoplastic process. Therapeutic agents, e.g. melphalan, corticosteroids, have been succesfully tested.

Primary pristane-induced MPCs are OG-dependent, thus they can be successfully transplanted only in recipients that have been pretreated (primed) with intraperitoneal pristane. Usually, after several passages *in vivo*, the OG dependence is lost, and the tumors can be transplanted into unprimed animals. Consequently, primary MPCs can be regarded as a neoplastic process restricted to the peritoneal cavity. In order to overcome this limitation, long-term cultured plasmacytoma cell lines can be injected intraperitoneally or intravenously into irradiated BALB/c mice.[40] Following this artificial metastatic spreading process, plasmacytoma cell homing can be detected in nearly all tissues, after latent periods ranging between 14-69 days. The lung and liver are the most frequently involved organs. Among intravenous recipients, approximately one-half became emaciated, had ruffled fur, and frequently displayed hind leg paralysis, suggesting spinal compression. Animals uniformly exhibited loss of weight. The detection of tumor cells within the bone marrow suggested a previously unobserved similarity to MM. Notably, intraperitoneal or intravenous injection of an IL-6-dependent cell line results in a minimal disease pattern in irradiated only mice. However, pristane treatment of the recipients prior to tumor cell injection favors the artificial metastatic process, most likely due to the IL-6 and other growth factor enrichment created by the OG cells.

Disadvantages

The typical clinical features of MM: bone lesions, anemia, and renal impairment, have not been described in the mouse model. Unlike MM, in which the bone marrow is affected and malignant plasma cells disseminate, MPCs are localized to the peritoneal cavity. Furthermore, in MM there is paracrine production of IL-6 by stromal cells in the bone marrow microenvironment whereas OG-activated macrophages, and presumably Th2 cells, appear to be the main source of IL-6 in MPC.[41] Therapeutic agents that have been examined in the mouse model (e.g. indomethacin) were shown to be ineffective at curing the human disease. On the other hand, there are only few data about the effect of standard MM therapy drugs on MPC.

Another major difference is the rare observation of Ig/c-myc type translocations in MM, which contrasts with their almost universal presence in MPCs.[40,42]

Transgenic models

Plasmacytomas have also been experimentally generated in mouse transgenic models. As a rule, DNA-constructs generated by coupling gene sequences with specific or universal promoters and/or enhancers are microinjected into fertilized 1-2 day old mouse eggs that are then surgically implanted into surrogate mothers. The majority of the offsprings will incorporate the exogenous gene into their germ line and can be used to breed mouse transgenic lines. The transgene expression can be directed or restricted to specific cell lineages. Untreated transgenic mice carrying either Eμ-v-abl, Eμ-IL-6 or Eμ-bcl-2 were reported to develop MPCs with variable incidences.[11,43]

Eμ-IL-6 transgenic mice

The observation that IL-6 is one of the main paracrine growth factors detected in the bone marrow of MM patients and also in the OG microenvironment during MPC induction, led to a search for transgenic mice models carrying IL-6 facsimile constructs. An Eμ-IL-6/C57BL/6 transgenic line was created by Kishimoto and

associates by introducing human IL-6 cDNA, under the transcriptional control of the murine major histocompatibility complex class (H-2Ld) promoter, into fertilized eggs of C57BL/6 donors.[44] The original Eμ-IL-6/C57BL/6 transgenic model showed high blood concentrations of human IL-6 and a 120- to 400-fold increase in IgG1 and developed polyclonal plasmacytosis affecting mainly the thymus, lymph node, and kidney. Nevertheless, no plasmacytoma development was observed. However, introduction of a BALB/c genetic background into IL-6 transgenic mice resulted in the appearance of monoclonal transplantable plasmacytomas carrying t(12;15) translocations.[16] These pieces of evidence indicate that IL-6 may contribute to plasmacytomagenesis, provided the BALB/c genetic background is present.

The role of IL-6 in progressive kidney damage observed in patients in terminal stages of MM (myeloma kidney), was studied in MT-I/IL-6 transgenic mice. MT-I/IL-6 transgenic mice carry a fusion between mouse metallothionein-I (MT-I) gene promoter and human IL-6 cDNA, that is constitutively expressed in the liver. The cytokine is detected in the blood together with a polyclonal hypergammaglobulinemia. MT-I/IL-6 transgenics die between 12 to 20 weeks of age. Post-mortem histologic examination reveals an increased number of megakaryocytes in the spleen and bone marrow, and IgG plasmacytosis in the spleen, lymph nodes, and thymus. The distinguishing feature of MT-I/IL-6 transgenics is the development of extensive tubular damage that reproduces the damage observed in the kidney in terminal stages of myeloma.[45]

Eμ-v-abl transgenic mice

V-abl is a truncated form of the c-abl gene and is a highly active non-membrane protein tyrosine kinase. A v-abl transgene was placed under the control of an Ig heavy chain enhancer (Eμ-) and the SV40 promoter. Beginning at 70 days of age, approximately 60% of the Eμ-v-abl transgenic mice develop MPCs but not B-cell lymphomas[46] indicating that the pathogenic expression of the transgene is confined to differentiated plasma cells. MPCs develop predominantly in lymphoid tissues, mesenteric nodes, intramural intestinal lymphoid tissue, and the bone marrow. Nearly 80% of the Eμ-v-abl MPCs bear a rearranged *c-myc* gene. Moreover, the crossing of Eμ-v-abl mice with Eμ-c-*myc* transgene carriers favors MPC development with short latent periods in the double transgene F1 progenies, but the mice do not develop B-cell lymphomas. These observations indicate that the expression of the Eμ-v-abl transgene alone is insufficient to generate plasmacytomas.

Eμ-bcl-2 transgenic mice

Transgenic mice bearing an Eμ- enhancer activated bcl-2 transgene develop malignant lymphomas[47] and Ig-secreting MPCs, although with a low incidence (3-15%).[48] Nearly 50% of the MPCs were shown to carry clonal Ig/*c-myc* rearrangements.[49] The backcrossing of a Eμ-bcl-2 transgene into the BALB/c strain background, followed by pristane treatment of transgene carriers, resulted in an increased incidence of MPC (>60%) already after two backcrosses. Typical t(12;15)(IgH/myc) translocations are present in all the Eμ-bcl-2/BALB/c plasmacytomas examined.[13] These observations indicate that *myc* and bcl-2 can co-operate during *in vivo* lymphomagenesis. This synergistic activity was confirmed using double transgenic mice. Eμ-bcl-2/Eμ-myc mice showed hyperproliferation of pre-B and B-cells and developed tumors much faster than single Eμ-myc transgenic mice.[49,50]

Advantages and disadvantages

Transgenic mice bearing oncogene(s) targets for expression in specific tissue have been useful for clarifying how specific oncogenes participate in normal cell differentiation and for the understanding of the oncogenic pathways to malignancy. The monoclonality of B-cell derived tumors in Eμ-v-abl and Eμ-bcl-2 transgenic strains helped to clarify the occurrence of additional genetic alterations that co-operate with the transgene during lymphomagenesis. Indeed, most MPCs from Eμ-v-abl, Eμ-bcl-2 and Eμ-IL-6 transgenic mice were shown to carry *c-myc* rearrangements. The Eμ-IL-6 transgenic mouse is a useful model for studying the role of IL-6 in the pathogenesis of MM. Nevertheless, transgenic mouse strains are extremely labor intensive to create.

Models using retroviral gene transfer

The successful transfer of retroviral gene(s) into hematopoietic stem and progenitor cells can ensure that the majority of the recipient mice will carry the transferred gene in all their hematopoietic lines.

Infection of pristane-conditioned mice with retroviruses or facsimile constructs containing retroviruses usually leads to so-called *accelerated* plasmacytogenesis.[11] Some of these artificial viruses introduce two oncogenes (e.g. c-*myc* + Ha-ras, v-*myc* + v-raf1, or v-abl + c-*myc*). MPCs that develop following the introduction of viral constructs containing *c-myc* oncogene usually do not carry translocations of the Ig/*myc* type.

ABL-MYC, a murine retrovirus that encodes the v-abl and c-*myc* oncogenes, was constructed from Abelson virus in order to assess the biological consequences of co-expression of these genes in lymphoid cells.[51] A plasmacytoma incidence of up to 100% is achieved following ABL-MYC infection. Pristane treatment prior to the infection shortened the latent period of plasmacytomagenesis. The majority of the MPCs induced by pristane + ABL-MYC are IgM-producers; in contrast, in the absence of pristane, IgA-secreting tumors are predominantly observed. Histopathologic analysis of ABL-MYC-infected mice showed foci containing transformed plasma cells as early as 14 days after infection. These results confirmed the reported synergism between v-abl and c-*myc* in B-cell transformation.

The use of J-3, a facsimile construct carrying v-*myc* sequences provided the opportunity to test the onco-

genic spectrum of a dysregulated v-*myc in vivo*. It was shown that the J-3 virus infection of pristane conditioned BALB/cAn mice provoked MPC development in 20.5% of mice already 42 to 117 days after virus injection. J-3 + pristane-induced MPCs produced IgA or IgM; alternatively more than two Ig classes and occasionally non-secretory tumors were identified. The lack of Ig/*myc* translocations in the J-3 tumors indicated that v-*myc* may have obviated the need for endogenous activated *c-myc*. Not all retroviruses that contained v-*myc* sequences were able to induce MPCs. This suggests that the hybrid MH2/MC29 v-*myc* gene in the absence of a functional raf gene has special plasmacytomagenetic properties.[52] A low yield of MPCs (15-20%) with shorter latent periods can also be achieved in BALB/cAn mice infected with Abelson murine leukemia virus 20 to 40 days after pristane injection; MPCs start to develop already 52 days after virus infection and 93 days after pristane injection. Almost 100% of pristane + Abelson MPCs have either t(12;15), t(6;15) or t(15;16) chromosomal translocations.[11, 53]

The retroviral gene transfer model also allowed investigation of the role of cytokine genes overexpressed in hematopoietic tissues.[43] Stable cytokine levels of ng/mL are achieved for at least six months after retroviral transfer to hematopoietic stem and progenitor cells. Mice transplanted with IL-6 vector-transduced bone marrow cells developed a clinical syndrome resembling human Castleman's disease (a rare, benign lymphoproliferative disease characterized by fever, anemia, hypergammaglobulinemia, elevation of acute-phase proteins in the serum, and polyclonal collections of lymphocytes and plasma cells in the lymph nodes). IL-6 production in lymph nodes from patients with Castleman's disease has been reported. However, none of the mice developed a malignant or clonal process resembling human MM. Autocrine or paracrine production of IL-6 leads to a state of chronic hyperproliferation but no acute neoplastic transformation.

Hilbert used the retroviral model to examine the role of T-cells in plasma-cell tumor development. By using a myc, raf-containing retrovirus, J3V1, to induce plasmacytomas in normal BALB/c mice, he demonstrated that T-cells induce terminal differentiation of transformed B-cells into mature plasma cell tumors.[54]

Advantages and disadvantages

Injecting retroviruses after the pristane inoculum has several experimental advantages. MPCs can be induced rapidly and the requirement for chromosome 15 translocation is circumvented. These tumors can be useful for defining secondary biochemical changes that are critical for MPC development and, finally, the role of other oncogenes and dysregulated genes in plasma cell development can be explored.[52] The disadvantage of this model, compared to the transgenic models, is the coexistence of a mixed cell population, composed of virus-expressing transfected cells and non-transfected normal cells.[43]

Spontaneous development of plasmacytomas in aging mice of the C57BL/KaLwRij strain

Patients receiving maintenance immunosuppressive treatment (MIST) were shown to be at increased risk of developing early malignancies, often of cells of the immune system. Some clinical studies indicated an age-related increase in the incidence of plasma-cell disorders, in particular in that of MM.

Aging C57BL/KaLwRij mice are known for their susceptibility to develop monoclonal gammopathies, with symptoms similar to those manifested in humans.[55-57] In addition, an old mouse can occasionally be found with an excessive homogenous immunoglobulin component (H-Ig) in the serum, which progressively increases in concentration while the other Igs decrease. When investigating the bone marrow of such an animal, typical myeloma cells showing monoclonal expansion were found by morphologic and immunocytochemical examinations. Skeletal radiography revealed osteoporosis with occasional osteolytic lesions, mainly in the ribs, femora, and tibiae. This spontaneous mouse MM (mMM) appeared in C57BL/KaLwRij mice older than 2 years, with a frequency of about 0.5%.[55] The majority of MM found produced H-Ig of the IgG isotype with κ light chain. The only other isotype represented was Ig-D-κ.

The C57BL/KaLwRij mMM can be propagated by intravenous transfer of bone marrow cells into syngeneic recipients. A 100% *take* is achieved in the first and in subsequent transfer generations. Spleen cells, too, can be successfully used for transplantation, especially when donor mice show advanced mMM.

Different mMM lines maintain the properties of the original mMM. The amount of the mMM H-Ig component can exceed 2 g/dL. In close correlation with the paraprotein concentration, the numbers of mMM cells in the bone marrow increase and the neoplasia eventually spreads to other organs. The red pulp of the spleen is most frequently affected. Lymph nodes, and gut- and bronchus-associated lymphoid tissues are involved to a minor extent. Occasionally the characteristic picture of a *myeloma kidney* can be observed. 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 mMM appear ill, and have bristling hair. Some of them show signs of hyperesthesia and, on rare occasions, epileptic seizures can be observed. In advanced stages, anemia with a low hematocrit is often seen. While the percentages of plasma cells in the bone marrow (femur) of normal mice and mice of comparable age with benign monoclonal gammopathy are approximately 0.2 and 0.5%, respectively, the primary MM-bearing mice show variable but higher figures from 5 to more than 50%.

Garrett *et al.* modified this model by establishing and subcloning a cell line from the murine myeloma. Mice

inoculated intravenously with the cultured cells predictably developed an identical disease to the mice injected intravenously with fresh bone-marrow-derived myeloma cells, including monoclonal gammopathy and radiologic bone lesions. Mice became hypercalcemic and the bone lesions were characterized by increased osteoclast activity. Because of the known number of inoculated tumor cells, this could be a more accurate model for determining the mechanism of osteoclast activation caused by myeloma cells.[56]

The most significant difference between the rodent plasmacytomas and the mMM in aging C57BL/KaLwRij mice concerns the presence of *c-myc* rearrangements in the neoplastic cells. MPC-like Ig/*c-myc* translocations are only occasionally observed in mMM of C57BL/KaLwRij mice.

This model has already proved its usefulness in studies on the effect of bisphosphonate in the treatment of bone disease in MM, on immunoregulation, and on the possibilities of immunologic treatment of MM after a stage of minimal residual disease has been achieved by chemotherapy. Radl *et al*. examined the effect of APD-bisphosphonates on bone destruction of MM mice. It was demonstrated by radiography and histologic investigation that treatment with APD-bisphosphonate protected the mice against a loss of bone to a significant extent. It seemed that treatment with bisphosphonates not only diminished the bone destruction by the MM but also led to the formation of new bone in already affected bone tissue.[58]

Dallas *et al*. examined the effect of an orally potent amino bisphosphonate, ibandronate, on this model. Ibandronate significantly reduced the occurrence of osteolytic bone lesions in myeloma-bearing mice, but it was not effective in preventing animals from developing hind limb paralysis and did not prolong survival of myeloma-bearing C57BL/KaLwRij mice. Ibandronate did not reduce the tumor burden as assayed by serum IgG2b level.[59]

The therapeutic potential of interferon-α (IFN-α) and melphalan was confirmed in C57BL/KaLwRij mice bearing disseminated 5T33 myeloma. Combinations of IFN-α and melphalan were found to be additive in their inhibitory effects on myeloma cell growth and significantly increased median survival duration of tumor-bearing mice.[60]

The effectiveness of anti-idiotypic treatment of mice with 5T2 MM residual disease was demonstrated. Since the serum idiotype concentration in MM is far too high for anti-idiotype antibodies to reach the target cells, reduction of the MM to a minimal residual disease was performed by treatment with cyclophosphamide. Intravenous treatment with anti-5T2 MM idiotype antibodies resulted in prevention of myeloma growth in most animals.[61]

Advantages and disadvantages

This model resembles MM with respect to disseminated disease, the characteristic bone lesions and renal involvement and laboratory findings. The effects of therapeutic agents such as bisphosphonates, interferon-α, and melphalan have been demonstrated. The disadvantage of this model is the low frequency of spontaneous tumorigenesis.

Using the SCID mouse as a model of multiple myeloma

The severe combined immunodeficiency (SCID) mouse provides an attractive model for the study of a variety of human tumors *in vivo*. Successful engraftment of normal or neoplastic lymphoid populations in SCID mice provides a valuable model for studying normal or malignant cell behavior.[6,62-65]

SCID mice injected with primary human MM cells

Yaccoby *et al*.[6] studied the fate of primary myeloma cells following inoculation in SCID mice (SCID-hu). They observed that myeloma bone marrow from 80% of patients readily grew in reconstructed SCID-hu mice. The SCID-hu mice inoculated with bone marrow cells from patients with myeloma developed typical manifestations of the disease such as plasmacytosis, high levels of monoclonal Igs, and severe bone resorption. The authors suggested that SCID-hu mice provide a hospitable environment for reproducible growth of primary myeloma cells.

SCID mice injected with human plasma cell leukemia cell line, ARH-77

Alsina *et al.* used the human plasma cell leukemia cell line, ARH-77, as a model for human MM.[65] ARH-77 cells injected intravenously into irradiated SCID recipients showed disseminated growth and expressed IgGκ. The mice developed hypercalcemia, lytic bone lesions and hind limb paralysis. Histologic examination showed infiltration of myeloma cells in the liver and spleen and marked infiltration in vertebrae and long bones, with loss of bony trabeculae and increased numbers of osteoclasts. The serum and marrow plasma levels of IL-6, IL-1, and TGFα were low compared with those in control mice, although ARH-77 cells produced IL-6.

SCID mice injected with human MM cell line KPMM2

In this model, endogenous IL-6 from SCID mice was ineffective at eliciting growth of the established human MM cell line KPMM2; these cells achieved autonomous growth through their autocrine secretion of IL-6.[66] The etiopathology in this disease model is consistent with that of human MM. When more than 3×10^6 KPMM2 cells were injected intravenously, tumors developed in all mice and were predominantly localized in their bone marrow. Tumors were also apparent in the lymph nodes, but absent from other organs. Immunostaining of cell surface antigen CD38 revealed that myeloma-derived cells represented more than 40% of the femoral bone marrow cells in the advanced stage of tumor progression. Further histologic analysis showed that the bone marrow

was largely occupied by plasmablastic cells and bones had developed osteolytic lesions at multiple sites. There was an increase in ionized plasma calcium. M-protein was detected in the serum within 10 days after transplantation, and this correlated with the tumor progression. Between 30 to 40 days after the transplantation the mice started to show a rapid and severe loss of body weight together with hind leg paralysis and fatigue. Finally, the mice died within a week. A single injection of 0.2 mg humanized anti-IL-6R antibody into mice one day after tumor transplantation substantially suppressed the elevation of serum M-protein and development of the tumor-associated abnormalities and significantly increased the life-span of tumor-bearing mice.

Agonist anti-human gp130 transducer monoclonal antibodies

One reason for the difficulty in getting a SCID model of MM might be the myeloma cell growth dependence on gp130 interleukin-6 transducer-activating cytokines. Because murine gp130 cytokines do not activate human gp130 transducer, it is necessary either to implant human stromal cells producing these cytokines or to inject human gp130 cytokines, in particular IL-6. Another difficulty is that human IL-6 binds to murine IL-6R and activates murine gp130 transducer. As a consequence, human IL-6 may induce the toxicities reported for IL-6 *in vivo*, mainly an inflammatory and cachectic syndrome. Rebouissou *et al*. have developed a SCID model of human MM, taking into account the myeloma cell growth dependence on gp130 cytokines and avoiding IL-6 toxicities. They used agonist anti-human gp130 transducer monoclonal antibodies (MoAbs) to grow myeloma cells.[67] These antibodies did not bind to murine gp130 or activated murine cells and, as a consequence, did not induce IL-6-related toxicities. Agonist anti-human gp130 transducer MoAbs have a 2-week half-life *in vivo* when injected into the peritoneum. The agonist antibodies made possible the *in vivo* growth of exogenous IL-6-dependent human myeloma cells as well as that of freshly explanted myeloma cells from a patient with secondary plasma cell leukemia. Tumors occurred 4 to 10 weeks after myeloma cell grafting and weighed 3 to 5 g. They grew as solid tumors in the peritoneal cavity and metastasized to the liver, pancreas, spleen, and intestine. Tumor cells were detected in the blood and bone marrow. Tumor cells grown in SCID mice had kept the phenotypic characteristics of the original tumor cells and their *in vitro* growth required the presence of IL-6 or agonist anti-gp130 MoAbs.

Testing therapeutic agents in the SCID model

Using three human tumor-cell lines of hematopoietic origin (CCRF-CEM, Raji, HS-Sultan), disseminated tumor cell growth was established in SCID mice. This allowed the *in vivo* effects of four chemotherapeutic agents (daunorubicin, idarubicin, ifosfamide, etoposide) to be examined.[68] Furthermore, inoculation of human myeloma cell lines subcutaneously into SCID mice was shown to produce a suitable model for evaluating the *in vivo* effect of anti-human IL-6R and IL-6 on myeloma cell growth.[69] In the S6B45 myeloma cell line human IL-6 acts as an autocrine growth factor. SCID mice received subcutaneous injection of $1x10^7$ S6B45 cells from *in vitro* culture. The resulting solid tumor was further passaged *in vivo*. The recipient mice were inoculated subcutaneously with 40 mg of freshly *ex vivo* isolated solid tumor. The intraperitoneal administration of 10 doses of 100 μg of anti-human IL-6R antibody PM1 at 48 h intervals, starting 24 h after the tumor inoculation, strongly inhibited the growth of S6B45 cells. Tumor growth inhibition was also observed after administration of anti-human IL-6 antibody MH166 using the same procedure as for PM1.

Advantages and disadvantages

This model shows a close resemblance to the human disease as far as concerns the development of multiple osteolytic bone lesions, disseminated disease, and the involvment of bone marrow. While IL-6 does not seem to play a role in ARH-77-injected SCID mice, in the case of human MM cell line KPMM2A-injected SCID mice, anti-IL-6R antibody proved to be effective. The effects of conventional drugs applied in human multiple myeloma have also been proven.

Since ARH-77 is an EBV-infected B-cell line, the validity of these results needs to be tested with MM cell lines.

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KG: conception, design, drafting, article revision, final approval. SS: conception, design, drafting, article revision, final approval. KP: conception, design, drafting, article revision, final approval. GyD: conception, design, drafting, article revision, final approval. AF: conception, design, drafting, article revision, final approval.

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Conflict of interest: none.

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