Seventh International Workshop on Immune-deficient Animals

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Abstracts from the
Seventh International Workshop on Immune-deficient Animals

Introduction
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Immune-deficient animals are widely utilized in many areas of basic and applied biomedical research. The Seventh International Workshop on Immune-deficient Animals held at The Jackson Laboratory in Bar Harbor, Maine focused on recent advances resulting from experimentation with such animals. Results of this experimentation have provided insights into mechanisms underlying the development and regulation of the mammalian immune system in normal and pathologic states. Our understanding of mechanisms underlying heritable human immunologic diseases has been advanced through the study of experimental animals bearing single gene mutations that perturb the immune system. While certain of these mutations are considered as homologues for specific human diseases, the major value of the immunologic mutants are as tools with which to dissect the mammalian immune system. An alternate approach for the investigation of immunological mechanisms has been the development of mice bearing disrupted genes whose products are critical to normal immune system development or function. While the occurrence of spontaneous mutations opens new doors into the function of previously unknown genetic loci, the targeted disruption of specific genes facilitates the precise determination of the roles of these genes in the functioning of the immune system.

Immune-deficient animals have also been valuable tools for the study of mechanisms of resistance to infectious diseases. While such animals have been used for many years in the study of bacterial and viral diseases, recent work has focused on their use as hosts for diseases caused by protozoa, fungi, and nematode parasites. Advances in cancer research have also been facilitated by the use of these animals. Although congenitally athymic nude mice have been used for many years as hosts for the growth of human malignant cells, it has not been possible to grow normal human lymphoid cells in such mice. In contrast, the growth of normal human lymphoid cells in mice homozygous for the severe combined immunodeficiency (scid) mutation has facilitated investigation of the interaction of malignant cells with tumor infiltrating lymphocytes. Moreover, scid/scid mice that harbor normal human lymphoid cells can be infected with HIV, thus providing an invaluable animal model for AIDS research. The following abstracts summarize work with immune-deficient animals in a number of areas including basic immunologic research, assessment of mechanisms of resistance to infectious diseases, evaluation of factors underlying host resistance to neoplasia, and the study of acquired immunodeficiency syndrome.

We greatly acknowledge the support from the National Institute for Allergy and Infectious Diseases (AI 31415) and from the following organizations: Accurate Chemical and Scientific Co., Amgen Inc., Bristol Myers Squibb Pharmaceutical Research Institute, Burroughs Wellcome Co., Connaught Laboratories Inc., Connaught Laboratories Ltd., The Dupont-Merck Pharmaceutical Co., Genentech Inc., Glaxo Inc. Research Institute, The Jackson Laboratory, R.W. Johnson Pharmaceutical Research Institute, Merck Research Laboratories, Miles Inc., Minnesota Mining and Manufacturing Co., Warner Lambert Co., Sandoz Research Institute, G.D. Searle and Co., Serono Laboratories, The Upjohn Co., and The Wellcome Foundation.

Correlation between P-glycoprotein expression and multidrug resistance in human tumor xenografts
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Multidrug resistance is a critical subject in cancer treatment. Various mechanisms confer multidrug resistance in tumor cells. A human multidrug resistance gene (MDR1) encodes a membrane associated protein, P-glycoprotein (P-Gp). P-Gp excretes drugs outside the cells, resulting in decreased drug accumulation in the resistant cells. Solid tumors often show natural resistance to drugs. The mechanisms are still unclear in vivo.

We examined chemosensitivity to three drugs (ADR, VCR, and VLB) on 48 human tumor xenografts in nude mice, using a clinically equivalent dose. We evaluated MDR1 gene expression in human tumor xenografts by Northern blot analysis and reverse transcription PCR (RT-PCR) assay. We used the indirect immunohisto-
chemical technique with anti-P-Gp monoclonal antibody (C219) to estimate cellular P-Gp production in the tumor.

A total of 23 cell lines showed drug resistance to the three chemical agents. Six of the 23 cell lines showed low level or heterogeneous expression of the MDR1 gene by RT-PCR, while Northern blot analysis detected no apparent expression. Five of the 23 cell lines demonstrated both MDR1 expression and P-Gp production. One cell line showed apparent MDR1 gene expression without P-Gp production. Nine tumor cell lines showed no apparent MDR1 gene expression. The other eight cell lines revealed neither MDR1 gene expression nor P-Gp production. The results suggest that P-Gp has a role in multidrug resistance mechanism in certain kinds of tumor cell lines. The alternative mechanism may contribute to multidrug resistance in tumor cells.

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Regression of a murine fibrosarcoma cell transfected with the hIL-2 gene in SCID and SCID-beige mice

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Local secretion of various cytokines appears to protect mice from tumor engraftment (1,2). The methylecholanthrene-induced fibrosarcoma CMS-5 is a rapidly growing tumor when transplanted subcutaneously into BALB/c mice. Its hIL-2 producing counterpart, N2A/IL2/CMS5, regresses in BALB/c mice. Regression appears to be mediated by CD8+ T cells and is accompanied by immune memory. Transplantation of CMS-5 and N2A/IL2/CMS5 into B- and T-cell deficient SCID mice gives similar results, suggesting that in addition to CD8+ mature T cells another or several other cell types are involved in effecting the IL-2 induced tumor regression. Since SCID mice have normal levels of functional NK cells, an attempt was made to determine whether or not these cells could be one of the possible cell types mediating the N2A/IL2/CMS5 regression. This is a possibility since these tumors did not regress in NK cell deficient scid-bg mice treated with anti-asialo-GM1 antibody. While the identity of the effector cell in SCID mice has not been resolved, it was established that these cells do not express memory. Histological evaluation of the sites of tumor regression in N2A/IL2/CMS5 engrafted SCID mice revealed an inflammatory response that was lymphoid in nature. We conclude that cytokine gene transfected tumor cell regression is mediated by a non-T cell, non-B cell, and possibly an NK cell or other ASGM1 positive cell in the SCID mouse.

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Role of pituitary-ovarian hormones in initiation and growth of malignant ovarian granulosa cell tumors in genetically susceptible mice

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Ovarian tumors occur in approximately two percent of Caucasian women and are the most lethal of the gynecologic cancers. Tumors of the granulosa cells within follicles comprise 10 to 15 percent of these cases and are bimodally distributed with respect to age of onset. The initial age cluster occurs in young girls between infancy and menarche (juvenile onset) while the second occurs in mature adult and post-menopausal (adult onset) women. Both subsets of granulosa cell tumors manifest a substantial incidence of malignancy.

Human epidemiologic and laboratory experimental induction studies have provided circumstantial evidence that pituitary gonadotropins (lutecinizing (LH) and follicle stimulating hormones (FSH)) have a role (or roles) in granulosa cell tumorigenic processes, although mechanisms have remained elusive. We have identified a heritable model for juvenile onset granulosa cell tumors in SWR inbred mice. These tumors develop spontaneously in one or both ovaries between 24 and 42 days of age as pubertal ovarian maturation takes place. Tumorigenesis is controlled by a small number of genes that have segregated in a set of 14 recombinant inbred strains (designated SWXJ) derived from SWR and SJL mice. These genes include: a) granulosa cell tumorigenesis (Gct) on Chromosome 4, b) an androgen metabolism gene of uncertain location, and c) a genomic imprinting locus of uncertain location. Our studies have used these genetically susceptible mice in concert with mice carrying additional unique mutant genes to obtain insight about pituitary-ovarian hormones in primary and metastatic disease.

To pursue these studies, a stock of mice doubly homozygous for the mutant genes severe combined immune-deficiency and hypogonadal (scid/scid, hpg/hpg) was developed as a host for allogeneic ovarian tissue grafts. These doubly homozygous mice are severely immunodeficient and lack serum pituitary gonadotropins
and ovarian sex steroids. Experiments were conducted by grafting genetically tumor-susceptible ovaries or tumor tissue beneath the left kidney capsule of hosts. Four weeks thereafter, necropsies were performed to obtain data on tumor incidence, neoplastic growth, and histological appearance as a function of genotype, age, hormone treatment, and tumor tissue status. Tumors formed only in susceptible ovarian grafts placed in scid/scid, +/+ hosts, and not in scid/scid, hpg/hpg hosts.

The combination of phenotypes characteristic of the scid and hpg mutant genes represents a powerful experimental tool that answered four critical questions posed by the genetically determined ovarian juvenile-onset granulosa cell tumor biology. The concept of combining mutant genes to address specific questions is applicable to investigations of other tumor systems, including parallel studies of human and murine tumors, with limits established only by investigator originality.

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Effects of HIV infection in human fetal thymus in the SCID-hu mouse
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Individuals infected by the human immunodeficiency virus type 1 (HIV-1) exhibit progressive depletion and severely reduced function of peripheral CD4+ helper T lymphocytes. A variety of mechanisms have been proposed to account for the observed loss of CD4+ T cells, including virally mediated cytopathic killing of infected cells and deleterious infection of T-cell precursors. Even though in vitro data support both these hypotheses, it has been difficult to demonstrate or analyze these processes in vivo. We have previously shown that human fetal thymus/liver implants in SCID-hu mice support infection by HIV-1 (JR-CSF). In this study, thymus implants were inoculated with primary patient isolates of HIV (SM, TY, or EW) or with molecular HIV clones (JR-CSF or 2.4). Over time, the implants were harvested and analyzed by ELISA, flow cytometry (FACS), and immunohistochemistry for expression of viral antigens and changes in overall thymic profile. In all cases, thymic p24 levels increased in a time-dependent manner, indicating productive infection of some thymic elements. In the case of patient isolates, FACS and immunohistochemical analysis showed that, concomitant to the increase in p24, thymus cellularity was greatly reduced, reflecting the nearly complete ablation of thymocytes of the CD4-8- and CD4-8+ phenotypes. CD4-8+ thymocytes and CD4-8+ cells remained as the principal lymphoid components of infected thymi. Moreover, thymic lobes were shrunken, with cortical regions appearing extremely compressed and practically devoid of thymocytes. Expression of viral antigens could be localized in several cell types, including thymocytes and thymic stromal elements. These observations support the notion that the spread of HIV infection to the thymus can lead to the elimination of a majority of developing thymocytes, and thus preclude regeneration of the peripheral T-cell pool in HIV-infected individuals.

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Status of Ig and TCR genes in non-transformed B- and T-lineage cells of SCID mice
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SCID mice lack functional lymphocytes because they carry a mutation that impairs rearrangement of immunoglobulin (Ig) and T-cell receptor (TCR) genes. Rearrangement of TCRd, but not 6 and 8 genes was routinely observed in DNA of SCID thymocytes and thymocyte hybridomas. TCRd gene rearrangements appeared to involve Dd1, Dd2, and Jd1 elements only; rearrangement of elements upstream of Dd1 (e.g., Vd1) was not observed and transcripts corresponding to fully-assembled TCRd genes (VDJd or VDJd'Jd) were not detected in RNA from SCID thymocytes. These findings suggest that Dd1, Dd2 and Jd1 may be among the first TCR gene elements to undergo recombination and that SCID T-lineage cells are developmentally arrested during or shortly after this stage of differentiation.

SCID B-lineage cells are arrested at the pro-B stage (B220-57 IgM-). D_H elements undergo rearrangement but fully-assembled VDJ_H transcripts are not detected and pre-B cells (B220-57 IgM+) fail to develop. Interestingly, the introduction of a functionally rearranged 6 transgene into the SCID mouse genome results in normal numbers of "pre-B cells" (B220-57 IgM-), but no B cells (B220-57 IgM+). However, the pre-B cells in such 6 transgenic scid mice (sc/sc, 6 mice) do not show clear evidence of IgL(k) gene rearrangement as is the case for pre-B cells from heterozygous scid littermates with and without the 6 transgene (sc/+, 6 and sc/+ mice).

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The SCID mouse as a model for the virus-host relationship: Immune regulation of viremia and maternal-fetal virus transmission
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We have investigated lactate dehydrogenase-elevating virus (LDV) infection in SCID mice. LDV infects
been LDV-infected for several months prior to conception, but became much more distinct after growth.

Host reaction was not noticeable after the first 6 days of growth. However, in immunocompetent mice, the graft growth did not stop on the 6th day. In the case of immunosuppressed mice, the grafts stopped growing with obvious host reaction on the 6th day after transplantation. In the immunocompetent mice, the tumor pieces were then implanted into the renal capsule (SRC) as the cancer transplantation region.

The esophageal cancer cell line Eca109 was transplanted subcutaneously to nude mice and a solid tumor was formed. The tumor pieces were then implanted into SRCs of the three groups of mice. The immunosuppressed mice had been pretreated with 200 mg/kg of cyclophosphamide before the transplantation. The growth curves showed that in the immunocompetent mice, the grafts stopped growing with obvious host reaction on the 6th day after transplantation. In the immunosuppressed mice, the graft growth did not stop on the 6th day but continued growing for an additional 2 to 4 days. Host reaction was not noticeable after the first 6 days of growth, but became much more distinct after growth.

The results showed that immunosuppressed mice can replace nude mice, which are expensive and for which care is inconvenient. It also showed that transplantation in SRC can replace the subcutaneous method, which requires a lengthy experimental period. Therefore, immunosuppressed mice with tumor grafts in SRC may be the optimal subjects for quick screening of anti-esophageal cancer drugs.

Promoting engraftment of the human B-cell lineage in SCID mice by human interleukin-6

B.A. Croy, S. Williams, and R.B. Bankert

Interleukin-6 (IL-6) is a lymphohematopoietic growth factor that enhances the survival of stem cells and promotes differentiation of the B-cell lineage. Murine IL-6 does not bind to the human IL-6 receptor. To determine whether hIL-6 could promote engraftment of human peripheral blood leukocytes (hPBL) in C.B17-scid mice, hPBL were pretreated ten minutes with 4ng hrIL-6 (R & D Systems, Minneapolis, MN) or PBS and then administered intraperitoneally. One group of mice receiving treated cells also received repeated intraperitoneal injections of 4ng hrIL-6 (nine injections every 8 hours followed by 11 injections every 12 hours). Engraftment was assessed by measurement of hlg (ELISA) in serum from the SCID-hu mice. All mice receiving PBL treated with hrIL-6 displayed hlg. In comparison to control animals, the mice receiving multiple doses of hrIL-6 had twice the level of hlg in their sera within 3 weeks following engraftment and this difference was sustained. These data suggest that the levels of IL-6 in SCID mice are not optimal for engraftment of hPBL and that exogenous hrIL-6 can stimulate the human component within the SCID-hu chimera. These promising results have encouraged us to cross C57BL/6-scid/scid-
bg/bg mice (1) to C57BL/6-hIL-6 transgenic mice (line Ld46) (2) to obtain improved recipients for studies of hPBH and human tumor/TIL engrafment.

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Otitis media in SCID mice due to infection with an atypical pseudomonas bacteria (Fox Chase SCID™)

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Mice homozygous for the scid mutation (scid/scid) are deficient for immune functions mediated by T and B lymphocytes. Due to the immune-deficiency of this mouse, it is susceptible to infections with many pathogens. Few reports have dealt with specific infections such as Pneumocystis carinii classified as immunodeficient mutant scid mice. Am. J. Pathol. 136:1173-1186.

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TCR expression during early neonatal development in euthymic and athymic (nu/nu) mice is restricted to the intestinal lamina propria

Bernard de Geus, Lex Nagelkerken, and Jan Rozing

Intestinal intra-epithelial lymphocytes (iIEL) form a heterogeneous population of T cells of which more than 50 percent differentiates independent of the thymus. This thymus independent iIEL population is composed of a population of T cells expressing the TCRgd and a T-cell population expressing the TCRαβ. Both populations express only the CD8α chains. It has been suggested that another cell population which is CD8α⁺ but CD3⁺ and TCR⁺, and is also present in the intestinal epithelium, forms a precursor population of the thymus independent iIEL. However, our results indicate that this hypothesis is false, since these CD3⁺ and TCR⁺ CD8α⁺ cells are present in high numbers in the intestinal epithelium of C.B17-scid/scid mice and are absent at the same site in 2-7 day-old neonatal mice of euthymic as well as of athymic (nu/nu) origin. Furthermore, the localization of TCR positive cells in the same 2-7 day-old neonatal
animals suggests that the intestinal lamina propria may be important in the differentiation of iIEL, since TCR cells in these animals are only located in the intestinal lamina propria. These TCR cells are CD8a and Thy-1.

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Animal models of normal and leukemic human hematopoiesis by transplantation into immune-deficient mice
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A complete understanding of the organization of the human hematopoietic stem cell hierarchy and of the molecular events regulating the stem cell developmental program has been hampered by the absence of suitable in vivo stem cell assays. Perturbations in the normal stem cell program leading to neoplastic growth as the result of aberrant expression of key regulatory genes are also difficult to study because leukemic cells are difficult to grow in culture. Recent advances in the transplantation of human cells into immune-deficient mice provide an unprecedented opportunity to study human hematopoiesis—both normal and abnormal—in the context of a small animal model. Our animal model was designed to reflect as close as possible the current approaches of bone marrow transplantation using intravenous injection of adult bone marrow into conditioned recipient mice. We have evidence that the murine microenvironment can support human stem cells and the provision of human growth factors can stimulate high level multi-lineage engraftment. In addition to normal cells, we have engrafted mice with cells obtained from patients with pre-B acute lymphoblastic leukemia (ALL) at different stages of their disease. All of the samples from patients at relapse grew rapidly and disseminated widely in the mice, while cells obtained from patients at diagnosis grew poorly if at all. This indicates that there is a correlation between growth in the SCID mouse and clinical outcome. These results establish that the SCID mouse is a powerful model to examine the biologic characteristics of the growth of human leukemic cells. It should also serve as an important system to test various therapeutic strategies targeted against drug-resistant leukemic cells. The combination of this in vivo model with high efficiency gene transfer methods will provide an important system to test the genetic alterations involved in leukemic progression.

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Differential in situ expansion of tumor-infiltrating lymphocytes (TIL) following adoptive immunotherapy of tumor-bearing immunologically-deficient mice is not sufficient to ensure a successful outcome to therapy

Robert Evans, Sonya J. Kamdar, and Theodore M. Duffy

C57BL/6J mice bearing the MCA/76-9 immunogenic sarcoma received combination therapy consisting of a single intraperitoneal injection of cyclophosphamide (CY) and adoptive immunotherapy (AIT) in the form of an intravenous injection of tumor-sensitized T cells. These were derived from spleens of tumor-bearers or mice immunized against the tumor cells, as well as from progressing or regressing tumors (tumor-infiltrating lymphocytes (TIL)). The use of positively-selected immune Thy-1 T cells or TIL for AIT resulted in a consistent pattern of permanent tumor regression in all experiments, despite the fact that these T-cell sources contained preponderantly either CD4 or CD8 T cells. Using congenic B6.PL.Thy-1 mice immune T cells or TIL, it was shown that 8 days after AIT, more than 95 percent of the TIL were donor in origin. When TIL were expanded for 6 to 8 days in rIL-2, resulting in populations comprised of more than 95 percent CD8 TIL, and then used for AIT, it was seen that more T cells were required to induce permanent regression compared with unexpanded TIL. It was also seen that the incidence of permanent regressions became variable. Tumor regression induced by AIT was associated in all cases with a differential expansion of CD4 and CD8 TIL between days 5 and 9 after AIT with a parallel modulation of Thy-1, CD4, and CD8 antigen expression. These findings were confirmed by Northern hybridization analysis of RNA extracted from whole tumor tissue, as well as from positively selected Thy-1, CD4, and CD8 TIL. AIT was shown to result in a pronounced time-related increase in Ly-2, Ly-4, IL-2 and IFNγ mRNA levels supporting the view that AIT results in a differential activation of the immune and cytokine network at the tumor site. However, expansion of TIL in situ appeared to be only a prelude to the induction of permanent tumor regression. When tumor-bearing immunological mutant mice, nude (B6nu) and rhino (B6hr), received AIT, a similar degree of in situ expansion of CD4 and CD8 TIL occurred. Permanent tumor regression was not seen despite the evidence for amplification of immune responses in these mice. It was found that TIL isolated from the treated immunological mutant mice were cytotoxic in both an in vitro and in vivo assay; there was an increased responsiveness of spleen cells to stimulation with Con A, as measured by the production of IL-2; and there was an increase in class II-MHC expression by tumor-associated macrophages. These and other findings
Insights into mast cell development and function derived from analyses of mice carrying mutations at W/C-kit or Sl/MGF (SCF) loci

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Mast cell development is a complex process that results in the appearance of phenotypically distinct populations of mast cells in different anatomical sites. Mice homozygous for mutations at the W or Sl locus exhibit several phenotypic abnormalities, including a virtual absence of mast cells in all organs and tissues. Recent work indicates that W encodes the c-kit tyrosine kinase receptor, whereas Sl encodes a c-kit ligand that has been designated stem cell factor (SCF), mast cell growth factor (MGF), kit ligand (KL), and Steel factor. In vitro and in vivo studies indicate that recombinant SCF can induce the proliferation of both immature and mature mast cell populations and can induce the development of both connective tissue-type and mucosal mast cells. In addition, SCF can induce mast cell maturation and heparin synthesis. In addition to their value in identifying the products encoded at the W and Sl loci and defining the role of these products in mast cell development, W or Sl mutant mice also have been useful for the analysis of mast cell function. W/W mice can be locally and selectively reared of their mast cell deficiency by adoptive transfer of in vitro-derived immature mast cells of congenic +/+ origin. Analysis of biological responses in mast cell-deficient W/W or Sl/Sl mice, the congenic normal (+/+), mice, and W/W mice locally and selectively reared of their mast cell deficiency by adoptive transfer of mast cells of congenic +/+ origin, indicate that mast cells are essential for the expression of IgE-dependent cutaneous responses and certain other cutaneous inflammatory responses. This also indicates that TNF-α of mast cell origin represents an important mediator of the leukocyte infiltration which occurs during some of these reactions. However, mast cells appear to make no detectable contribution to the expression of T-cell dependent contact sensitivity responses in the skin. In addition, even though systemic activation of mast cells can induce anaphylactic responses associated with tachycardia, changes in pulmonary mechanics, and death, some anaphylactic responses can occur by mechanisms which are apparently completely independent of the mast cell.

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Malignant behavior of human tumors in immunodeficient mice

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The malignant potential of human tumors in nude, triple deficient bg-nu-xid and SCID mice was investigated. When tumor lines (one melanoma, two colon, one kidney, and two ovarian carcinomas) were injected subcutaneously, all the mice produced local tumors with no significant differences in growth rate. A few spontaneous metastases to the lung were observed in SCID mice. Following the intravenous injection of A375 melanoma cells, bg-nu-xid mice showed a higher number of lung colonies compared to SCID and nude mice. Similarly, the intrasplenic injection of HT-29 colon carcinoma resulted in a higher number of liver colonies in bg-nu-xid mice compared to nude and SCID mice. These findings indicate that the SCID and bg-nu-xid mice may offer some advantage to study the malignant behavior of human tumor xenografts, and this may depend on the route of tumor cells injection and tumor cells type.

The use of immunodeficient mice for studying human hematopoietic neoplasms

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Immunodeficient SCID and bg-nu-xid mice have been described as recipient for normal and transformed hematopoietic cell engraftment. We found that the injection of human acute leukemia cells into immunodeficient mice resulted in serially transplantable leukemic cell lines (2 myeloid and 3 lymphoid) that retained their original cytological features, immune surface antigens, and molecular characteristics. Among these leukemias, of particular interest was the observation that the intravenous injection of a human T-lymphoblastic lymphoma in bg-nu-xid mice resulted in systemic massive lymphomatous involvement with infiltration of bone marrow and various organs including spleen, liver, kidney, lung and meninges.
These findings confirm that immunodeficient mice can be used to propagate and establish human hematopoietic neoplasia. The stable features of these tumor lines and their ability to disseminate in a fashion that resembles the clinical picture of the original disease makes these animal models unique for studying the biology and therapy of human leukemias.

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The Institute of Laboratory Animal Resources: A source of information on animal models

Dorothy D. Greenhouse

For 40 years, the Institute of Laboratory Animal Resources (ILAR) has assisted the biomedical research community by developing guidelines for the care and use of laboratory animals and providing information on animal models and sources of animals. ILAR is a part of the Commission on Life Sciences, National Research Council (NRC), National Academy of Sciences. The Academy is a private, nonprofit organization, created by congressional charter in 1863 to serve as an official advisor to the federal government on matters of science and technology. In 1916 the Academy founded the NRC as its principal operating agency. Nearly all substantive tasks of the NRC are carried out by carefully balanced committees of recognized scientific experts in the field of study. ILAR's ability to convene such committees has led to national recognition and acceptance of its reports.

Several aspects of ILAR's work are of particular interest to the participants of the Seventh International Workshop on Immune-Deficient Animals. The first is the report Immunodeficient Rodents: A Guide to Their Immunobiology, Husbandry, and Use. Prepared by the eight-member Committee on Immunologically Compromised Rodents and 16 invited participants, this report is aimed primarily at scientists new to the field of immunology. Following an introduction to immune function and immunodeficiency, the report addresses the genetics, pathophysiology, husbandry, and reproduction of each strain or stock of rodent known to have a spontaneously arising deficiency in immune function. The husbandry and mating systems for maintaining these models are also presented. The report was published in 1989 and is available for sale from the National Academy Press.

ILAR's most frequently used ongoing service is the Animal Models and Genetic Stocks Information Program. ILAR has developed a computerized database of commercial and investigator-held colonies of both commonly and less commonly used laboratory animals. Staff annually responds to hundreds of requests for information on sources of animals for laboratory investiga
gation, appropriate animal models for studying human diseases and normal biologic phenomena, and animal care and treatment. On behalf of the International Committee on Standardized Genetic Nomenclature for Mice, ILAR assigns and distributes laboratory registration codes used to identify specific substrains and sublines of genetically defined rodents and rabbits and provides information on nomenclature.

Also relevant to this workshop is the quarterly journal ILAR News, which contains articles of interest to both biomedical and laboratory animal scientists. The journal pays special attention to providing information useful to institutional animal care and use committees and to scientists presenting protocols to these committees. It also contains inserts comprised of ILAR committee reports, individually authored documents, or bibliographies on alternatives to the use of live vertebrates. These inserts are a rapid, cost-effective means of disseminating information not readily available elsewhere. ILAR News is distributed free to more than 4,300 individuals and libraries worldwide.

To obtain a laboratory registration code or information on animal models, or to subscribe to ILAR News, write ILAR, National Research Council, 2101 Constitution Avenue, N.W., Washington, D.C. 20418. Tel: 202/334-2590; Fax: 202/334-1687.

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Depletion of CD4+ cells in MHC class II deficient mice

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The normal development of mature CD4+ T lymphocytes requires their interaction with major histocompatibility (MHC) encoded class II molecules in the thymus. These highly polymorphic molecules are present on thymic cortical epithelial cells and their engagement with the aβ T-cell receptor on immature thymocytes is thought to result in the positive selection of CD4+ T cells and their ultimate export to the periphery. We have used the technique of gene targeting in embryonic stem cells to disrupt the MHC class II Aβ gene, and from these cells, have generated mice which lack cell surface expression of MHC class II molecules. Immunohistochemistry of frozen thymic sections and flow cytometry of peripheral lymphocytes confirmed that disruption of the Aβ allele has led to a null phenotype with respect to MHC class II antigens. Analysis of these animals reveals that they are depleted of CD4+ T cells in lymphoid tissues and deficient in cell mediated immune responses, thereby providing genetic evidence for the requirement of class II molecules in the maturation and function of this T-cell subset.
The role of Kit on murine development

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The murine mutation dominant white spotting (W) is in the proto-oncogene, c-kit. The receptor tyrosine kinase encoded by this gene has pleiotrophic effects on murine development including hemopoietic cells, melanocytes and germ cells. More recently, characterization of a factor that stimulates the growth of mast cells (MGF/SLF/KL) and that binds the Kit receptor has led to the identification of the SI gene product. Phenotypic analysis of W and SI mice has unequivocally proven the role of the receptor tyrosine kinase-mediated signaling in murine development in embryonal and postnatal life. However, previous studies have not specified which stage of these three lineage cells is functionally required.

Anti-Kit monoclonal antibodies were established by immunizing cultured mast cells which express Kit on their surface. Mast cells of W/W mice, which cannot express Kit due to a mutation at a splice donor site which results in deletion of exons including the transmembrane domain, were used as a negative control. These antibodies were used not only as a marker for Kit* cells, but also as an antagonistic blocker capable of controlling Kit function in vivo. First, we identified at least two distinct Kit-dependent processes during melanocyte precursors in the mesodermal layer, which eventually leads to the melanocyte entry into the epidermal layer and the other during melanocyte activation along with the hair cycle in postnatal life.

Second, in gonadal tissues during postnatal development, the survival and/or proliferation of the differentiating type A spermatogonia is dependent on c-kit, but the antibody administration had no significant effect on the oocyte maturation and ovulation.

Finally, in hemopoietic progenitor cells, after Kit* cells were removed from adult bone marrow cells, hemopoietic progenitor cells reactive to IL-3, GM-CSF, or M-CSF and also those which give rise to spleen colonies in irradiated recipients disappeared almost completely. To investigate the role of Kit in the hemopoiesis in vivo, we injected the antibody against Kit. As early as 2 days after the injection, almost all hemopoietic progenitors disappeared from the bone marrow. These results provide direct evidence that Kit is an essential molecule for constitutive intramarrow hemopoiesis, especially for the self-renewal of hemopoietic progenitor cells at various stages of differentiation. The roles of Kit on hemopoiesis in fetal stage and on lymphocyte generation will also be discussed.

Abnormal thymocytopoiesis in viable moth-eaten (me') mutant mice

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Mice homozygous for the viable moth-eaten (me') allele manifest immunodeficient and autoimmune disorders. Premature thymic involution occurs at four weeks of age in homozygous me' mice, and their bone marrow prothymocytes fail to repopulate the thymus of irradiated recipients following intravenous transfer. However, me'/me' bone marrow cells are capable of generating normal numbers of donor-origin thymocytes following intrathymic adoptive transfer. Based on these observations, we have suggested that me'/me' bone marrow prothymocytes may fail to migrate to and/or enter into the thymus of adoptive recipients. To investigate this, we have developed an assay system to study the homing patterns of prothymocytes following intravenous injection. In this assay system, bone marrow cells from Ly5.2 donors are injected intravenally into irradiated Ly5.1 congenic recipients. At various times after reconstitution, the spleen, thymus, or bone marrow were recovered and the cells adoptively transferred intrathecal into irradiated secondary Ly5.1 congenic recipients. The homing pattern of prothymocytes to the tissues of the primary recipients was assessed by quantitating the number of donor-origin (Ly5.2') thymocytes generated by each of the tissues in the secondary recipients. Our results demonstrated that me'/me' prothymocytes differed in their kinetics of seeding and in their tissue distribution in adoptive recipients as compared to that of wild type prothymocytes. Furthermore, we observed that at least some me'/me' prothymocytes are able to home to the thymus of irradiated recipients, but fail to proliferate and differentiate into thymocytes in the absence of an "accessory" cell or factor provided by normal bone marrow cells. However, the homing of me'/me' prothymocytes to the thymus of irradiated recipients, even in the presence of the "accessory" cell or factor, is not as efficient as that observed by wild type bone marrow prothymocytes. These results suggest that the abnormalities in thymocytopoiesis in me'/me' mice may in part result from environmental or "accessory" cells or factors, as well as from intrinsic genetically determined defects in prothymocytes.
HIV infection of hu-PBMC-SCID mice

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The development of animal models for HIV infection which can be used for the testing of therapeutic agents and vaccines is of utmost importance in the battle against AIDS. One recently described model is the human peripheral blood mononuclear cell reconstituted SCID mouse (hu-PBMC-SCID) (1). In this system, SCID mice are injected intraperitoneally with 20 x 10^6 human donor PBMC which migrate to engraft in lymphoid tissues of the mouse. These engrafted cells are then susceptible to infection with HIV (2). We have sought to further characterize human reconstitution and subsequent HIV infection of SCID mice to establish a baseline on which to evaluate the effects of antiviral and immune modulating interventions.

We have looked at the significance of reconstitution variables such as age of mice at reconstitution, number of donor cells, EBV status of donor, type of cells used for reconstitution, and variation between individual donors. Reconstituted mice produce human immunoglobulin at levels about one log lower than that seen in normal human serum, but produce soluble CD8 and IL-2R (CD25) at levels considerably higher than those seen in normal human serum. We have examined cell surface phenotype of reconstituted cells, proliferative responses of reconstituted cells, and histological appearance of lymphoid tissues. In HIV infected SCID mice, we have examined the route of administration of virus, titer of virus required for infection, optimum time for infection after reconstitution, optimum time for recovery of virus, recovery of virus from various tissues (using culture, direct p24 assay, and PCR), and quantitation of recovered virus. Preliminary results of protection studies using HIV-specific cytotoxic T-lymphocyte clones show that such CTL can be used in vivo in mice reconstituted with cells from MHC-matched donors to alter the course of HIV infection. We are also assessing the feasibility of therapeutic intervention using pharmaceutical agents.

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Peyer's patch cells reconstitute the peripheral and mucosal immune systems of SCID mice

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Previous reports have suggested that murine Peyer's patches contain a selected pool of lymphocytes characterized by a high frequency of IgA precursor cells and expression of Peyer's patch specific homing receptors. In an effort to further understand the lymphocytic composition and immunologic potential of murine Peyer's patches, DBA/2 Peyer's patch cells were transferred intravenally to immunodeficient SCID mice. Using this system we assessed the ability of Peyer's patch cells to reconstitute the immune system in SCID mice as measured by serum Ig levels, lymphoid organ composition, and immunohistological analyses. Results indicate that Peyer's patch cells phenotypically and functionally reconstitute both a normal peripheral and mucosal immune system in SCID mice. Serum IgA, IgG, and IgM levels appear normal within 4 weeks of reconstitution and remain so for up to one year. The observed levels and isotypic profiles of serum Ig, as well as antibody from antigen-specific TI or TD responses, is indistinguishable from that of normal DBA/2 and BALB/c mice. The cellular reconstitution of the spleen, lamina propria, and lymph nodes also appears normal within 8 weeks of reconstitution. Each lymphoid organ contains the expected ratio of T and B cells organized into characteristic tissue-specific structures. Taken together, these results suggest that removal of cells from the Peyer's patch microenvironment alleviates the apparent isotope restriction and homing properties previously attributed to these cells and, that the lymphoid microenvironments into which these cells subsequently migrate clearly dictate both the composition and organization of lymphocytes within it. Although the reconstitution of the peripheral lymphoid tissues appears normal, analyses of thymii from these animals revealed a markedly different cellular composition and organization than that observed in normal thymus. The thymi of Peyer's patch reconstituted SCID mice contain three lymphocytic populations. By day 84 post transfer, approximately half of the cells in the thymus are Thy1", IL2R", Qa-2", CD4", and CD8" cells. The phenotype of these cells is consistent with that previously described for endogenous immature SCID thymocytes. The remaining cells are TCR aβ", donor derived cells which express either the helper (CD4", CD8") or cytotoxic (CD4" CD8") phenotype characteristic of mature peripheral T cells. No double positive donor derived cells were detected.

Clearly, the SCID mouse is an invaluable model for the study of immune system reconstitution, lymphocyte
Changes induced by nonshivering thermogenesis in macrophage populations and in NK cells of nude mice

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Exposure of individually caged mice to 22°C induces in nude mice profound physiological alterations in comparison with nude and haired littermates exposed to 28°C (catecholamines, 5'-deiodase, thyroid hormones, development of thermogenic mitochondria in brown fat). A long-term exposure (3 to 6 weeks), associated with a marked hyperplasia of the thermogenic tissue, provoked an increase in the percentage of phagocytosing and of MAC-1+ peritoneal macrophages. Also blood professional phagocytes increased their phagocytic performance. However, the density of FcR, MAC-1, F 4/80 and la markers decreased on peritoneal macrophages, among which large cells predominated in cold-exposed nu/nu. During 2, 3, and 6 weeks exposure, the percentage of MAC-1+ cells gradually increased in bone marrow and spleen; MAC-3+ cells gradually increased in the spleen; and a sharp rise occurred in FcR+ cells in all cold-exposed nude mice in bone marrow, spleen, and lymph nodes.

Also spleen NK cell activity (cytotoxic test) was higher in the nudes during the long-term exposure, and so were the numbers of asialo-GM1+ cells in different tissues.

Since some of the changes described (phagocytosis, NK activity) occur also after a short-term (6–24 hrs) exposure to 22°C, it is likely that catecholamines have an important role in the cold-adaptation in nude. The changes in another hairless mutant (BFU mice, not immunodeficient) are much less pronounced.

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Immunological defects of nu/+ heterozygotes

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It was verified in an improved CFU/s assay that hematopoietic stem cells of the bone marrow are reduced both in nu/nu and nu/+ mice (compared to +/+, C57BL/10ScSn). Thymus weight is also reduced in nu/nu mice compared to +/+, the reduction being more pronounced in males (1–2 months of age).

In BALB/c mice, the antibody-forming cells (AFC) in draining lymph nodes 9 days after immunization with a thymus-dependent antigen (hen egg lysozyme in incomplete Freund's adjuvant), were reduced by two orders in nu/nu, compared to +/+ . Nu/+ mice displayed an extreme variability and their AFC counts were spread over the whole range from one to 5 x 10^3 AFC per 10^6 cells. The same antigen applied in a soluble form 10 days prior to immunization produced a clear-cut tolerance (depression of AFC by two orders) and only a very moderate decrease in AFC in nu/+ mice. On the other hand, identical reactivity of +/+ and nu/+ mice to Concanavalin A and E. coli lipopolysaccharide was observed in the in vitro spleen cell assay. The results corroborate the assumption that the phenotypic expression of the nude gene is variable in heterozygous animals.

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Reduction of lymphokine-activated killer cell activity by the introduction of the beige gene into SCID mice

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The adoptive immunotherapy of cancer using lymphokine-activated killer (LAK) cells has been intensively investigated, but the lineage of LAK cells is poorly understood. Hasui reported that the SCID (T- and B-cell deficiency) had a high level of LAK cell activity, which implied that the majority of LAK activity exerted by blood or spleen lymphocytes might be attributable to IL-2-activated NK cells. But there is evidence that immature T cells are present in SCID mice. It is possible that LAK effector cells may be generated from some immature T cells of congenitally immunodeficient mice.

To further clarify whether the LAK cells come from NK cells or immature T cells, we introduced the beige gene (NK cell deficiency) into the SCID mice and observed the influence of the beige gene on the LAK activity of scid mice. The introduction of the beige gene into SCID mice was carried out by successive crossings and intercrossings, starting from C57BL/6N-bg females (f) and C.B17-scid males (m). After the beige gene was introduced into SCID mice and the new stock of scid/
scid-bg/bg mice showed a combined immunodeficiency of T, B, NK cells, we further applied the new stock of scid/scid-bg/bg mice and scid mice to analyze the source of the precursor of LAK cell.

The proliferation of splenocytes of scid/scid-bg/bg mice could not be induced by addition of rIL-2 (1,100 ± 612.34 cpm at IL-2 500μl/ml vs. 10,523.07 ± 1107.30 cpm for C.B17-scid mice and 3,910.12 ± 816.34 for control BALB/c mice, p<0.01). Morphological studies of incubated cells with IL-2 did not show a clonal proliferation of lymphocyte-like cells, but did show a large amount of macrophages and mast cells. They also showed a low LAK cell activity (5.80 ± 5.50, vs. 97.7 ± 13.6 for C.B17-scid mice at IL-2 500μl/ml; E/T 100.1, 3 days, p<0.01). Importantly, we have shown that rIL-2 cultured splenocytes of scid/scid-bg/bg mice did not give augmented cytotoxic activity against NK-resistant P815 cells with an increase in the incubation days or in a dose-response study. The reduction of LAK activity in the scid/scid-bg/bg mice was obviously related to the introduction of the beige gene which causes an NK cell deficiency. This implied that the LAK activity in SCID mice may have a strong relationship with the NK cell.

This is the first time that we directly demonstrated the relationship between LAK cells and NK cells by introduction of the beige gene into SCID mice. The introduction of a specific, known immune-deficient gene into various strains of immunodeficient mice would help us to understand the influence of the induced gene (bg gene) on the LAK cell activity. These methods also deliver a new and powerful way to analyze the source of highly potent cytotoxic effector cells of LAK cells for adoptive immunotherapy of cancer in experimental animals and humans.

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The study of adult nasopharyngeal mucosa xenograft and dinitrosopiperazine (DNP) carcinogenesis

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Adult nasopharyngeal mucosa from 44 cases of chronic nasopharyngitis diagnosed by pathology was transplanted into 59 nude mice. The mice were divided into two groups—the control group (13 nude mice) was given transplants without any treatment, and the experimental group (46 nude mice) was subcutaneously injected with 15mg/kg of DNP twice a week, for 6 to 15 weeks beginning 10 to 16 days following transplantation. In the experimental group, the xenografts in 27 nude mice were examined after 18 to 37 weeks of transplantation, and survived in 20 mice. The survival rate was 74.1 percent. Seven out of 20 of the surviving epithelia showed focal hyperplasia and dysplasia, and four carcinomas in situ and four early infiltrating carcinomas showed carcinomatous changes. The carcinoma incidence was 40 percent. In the control group, the xenografts in seven nude mice were examined after 8 to 35 weeks of transplantation, and survived in six mice. The surviving epithelia did not develop dysplasia and carcinomatous changes. The results suggested that DNA may induce the adult nasopharyngeal epithelia into precarcinomatous lesions and carcinomatous changes in nude mice. According to the results, the authors analyze the factors related to carcinomatous changes of nasopharyngeal epithelia.

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Acute human-versus-mouse graft-versus-host disease in normal and immune-deficient mice

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Recent reports of persistent engraftment of human lymphocytes in hereditary immune-deficient SCID and bg-nu-xid mice have raised the question why these animals did not develop graft-versus-host disease (GvHD). We postulated that this was due to lack of human specific lymphocyte cytokines in the recipient mouse tissues.

To overcome this problem we transplanted large numbers of human peripheral blood lymphocytes (PBLs) (2 x 10⁷ cells per gram body weight and more), assuming that these numbers would produce enough human lymphokines to support proliferation of human reactive T-cell clones. The SCID mice were conditioned with 400 mg/kg of cyclophosphamide, the bg-nu-xid mice with 7–9 Gy total body irradiation (TBI). Under these conditions all mice developed acute GvHD. Similarly, acute GvHD occurred in newborn normal BCBA mice and in 3-week-old xid mice following high dose TBI and grafting of large numbers of human PBL. The clinical manifestations and the histopathology of this xenogeneic acute GvHD are quite different from those of allogeneic GvHD. The former is primarily confined to the hematolymphoid tissues and locations adjacent to accumulations of proliferating lymphoblasts, such as the peritoneal cavity in case of intraperitoneal transplantation. The discordant xenogeneic GvHD is specifically induced by human T-lymphocytes and can be abrogated by treatment with anti-human T-cell serum.

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Prediction of clinical antitumor effect based on Clinically Equivalent Dose preestimated by animal scale-up

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In our previous reports, we have emphasized that it is very important to use Clinically Equivalent Dose (CED), previously called Rational Dose (RD), to reproduce or predict the clinical antitumor effect of a drug by human tumor/nude mouse model. CED is a dose for mice pharmacokinetically equivalent to the clinical dose. In order to determine the CED, we need not only experimental but clinical data on pharmacokinetics and maximum tolerated dose (MTD). Therefore, it is impossible to determine the CED of a new drug on the preclinical stage. As a new trial to overcome this difficulty, we attempted to preestimate the CED on new drugs in animal scale-up procedure. Practically, assuming two clinical antitumor drugs to be “new drugs,” we preestimated their CEDs and examined the antitumor effects of these drugs at CEDs on human tumor xenografts.

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Comparative study on Pneumocystis carinii infection in SCID and nude mice

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Pneumocystis carinii is an opportunistic agent causing severe pneumonia in immunocompromised patients, notably those with AIDS. Since SCID and nude mice also spontaneously develop severe pneumonia with immunosuppressants, they can be considered useful as animal models for the research of P. carinii infection in such patients. In this study, we attempted to elucidate the characteristics of P. carinii infection in SCID mice by comparing experimental infection in SCID with that in nude mice.

Twenty-five C.B17-scid and BALB/cA- nu females maintained in our institute under SPF conditions were intranasally inoculated with 0.9 x 10^5 P. carinii cysts. After inoculation, live weights were measured each week, and day of death was recorded. In another experiment, lungs were collected each month post-inoculation and the number of P. carinii in the lungs was calculated by staining with toluidine blue O. Histological examinations of formalin-fixed lung sections were also performed.

Weight loss after infection in SCID mice was delayed compared to nude mice. Moreover, the survival rate in SCID mice was much higher than that in nude mice. Only one out of 25 SCID mice died 20 weeks after infection, compared to 13 out of 25 nude mice (52 percent). These results were reproduced in another experiment using 15 of each type of mouse.

In contrast, the number of lung cysts was higher in SCID mice than in nude mice. A histological examination showed that both types of mice developed P. carinii pneumonia typical of immunodeficient animals, and showed small numbers of infiltrating cells and extensive multiplication of protozoa. However, infiltration of lymphocytes into the lungs of SCID mice was less significant than in nude mice. These results show that the SCID mouse paradoxically survived longer than the nude mouse despite a higher degree of P. carinii multiplication. It is suggested that host cellular responses are responsible for impairment of lung function in P. carinii pneumonia rather than multiplication of the organism itself.

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Feline immunodeficiency virus infection of SCID mice engrafted with feline tissues: A murine model for HIV drug testing

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Animal models of Human Immunodeficiency Virus (HIV) infection are essential for preclinical evaluation of potential antiretroviral drugs and vaccines. SCID mice engrafted with human tissues and infected with HIV are useful models because they utilize the actual human pathogen in a host amenable to large-scale drug testing. However, public health concerns regarding accidental infection of laboratory workers with HIV restrict this model to relatively few laboratories. Feline Immunodeficiency Virus (FIV) is a lentivirus that induces immunodeficiency in cats by depleting CD4-bearing peripheral blood lymphocytes (PBL), thereby inverting the CD4:CD8 ratio. Chronically-infected cats become susceptible to a wide variety of opportunistic pathogens, suggesting that FIV-infected cats are useful models for the immunopathogenesis of HIV-1 infection in humans. Furthermore, FIV is not infectious to humans and responds to reverse transcriptase-targeted drugs similarly to HIV. Therefore, SCID mice engrafted with feline tissues and infected with FIV would be valuable in preclinical screenings of potential AIDS drugs.

SCID mice were anesthetized and surgically implanted in the mammary fat pad with sections of feline thymus and lymph node, then given intraperitoneal injections of feline liver, bone marrow, peripheral blood lymphocytes, and spleen lymphocytes. Two weeks after implantation, mice were injected intraperitoneally with...
7 x 10^6 FIV-NCSU1-infected feline PBL. Ten mice were given Retrovir™ (Azidothymidine, AZT) at 125 mg/kg/day in the drinking water beginning 24 hours prior to virus challenge and continuing for 2 weeks. Two weeks post infection, mice were sacrificed and implants were analyzed for FIV proviral DNA by PCR. Grafting efficiency was determined by PCR amplification of feline-specific sequences of the c-fes protooncogene from the blood and spleen.

Feline cells were detected in 23 out of 28 mice (82 percent) in either peripheral blood or spleen lymphocytes. The number of mice positive for FIV (summarized below) indicates a lower frequency of detection of FIV provirus in AZT-treated animals as compared to untreated.

| Number of Mice FIV Positive by PCR | Untreated | AZT Treated |
|-----------------------------------|-----------|-------------|
| Thymus Implant                    | 11/17 (65%) | 2/10 (20%) |
| Lymph Node Implant                | 11/17 (65%) | 4/10 (40%) |
| Both Implants                     | 8/17 (47%)  | 0/10 (0%)  |

Hybridization intensities of FIV-positive samples in which equal amounts of DNA amplified by PCR were compared to determine relative levels of provirus in each sample. Comparison of five untreated mice with five AZT-treated mice showed a significant reduction in provirus burden associated with AZT treatment. These data suggest that the FIV-infected SCID-fe mouse is a safe, realistic murine model for HIV antiviral therapy.

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**Helminths infection in SCID mice**

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The course and characteristics of several helminths infections in SCID (C.B17-scid/scid) mice and in normal C.B17 mice were compared. More *Taenia taeniaeformis* cysticerci were recovered from SCID mice, in which almost no eosinophil and macrophage infiltration nor necrosis of the hepatic cells around the liver cyst were observed. Rapid asexual propagation of *Echinococcus multilocularis* cysts was observed in SCID mice intraperitoneally injected with cyst suspension. Numerous but mostly sterile fluid-filled cysts of *E. granulosis* were recovered from the abdominal cavities of SCID mice 10 months after intraperitoneal injection with hydatid sand. Higher intestinal worm recovery and a longer period of parasitism of *Trichinella pseudospiralis* were observed in SCID mice than in normal mice. Non-specific acute phase serum protein and intestinal eosinophilia response were observed in both the *Trichinella*-infected SCID and normal mice. However, more muscle larvae were recovered from the SCID than from the normal mice. Unexpectedly, fewer worms were recovered from SCID mice than from the normal mice in primary infection with *Schistosoma mansoni*, but no evidence of acquired immunity to the reinfection was observed in the SCID mice. Experiments were also carried out to examine the possible role of the SCID mouse as an alternative definitive host for *Taenia crassiceps, E. multilocularis,* and *Angiostrongylus cantonusis.* Underdeveloped tapeworms of *T. crassiceps* were recovered from the intestine 12 days postinfection from prednisolone-treated SCID mice inoculated orally with cysticerci but not from the untreated SCID mice. The protoscoleces of *E. multilocularis* failed to establish in the intestine of the SCID mice and were expelled 1 day postinfection. A high mortality rate was observed in SCID mice infected with *A. cantonensis* at around 30 days postinfection. In the few mice that survived longer, *A. cantonensis* young adult worms failed to migrate to the lungs, which is the final predilection site in its natural rat host. On the whole, our results showed that there is not much difference between SCID and normal C.B17 mice in the ability to serve as an alternative definitive host for the tested helminths.

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**Increased antitumor activity of 5-fluorouracil by I-leucovorin and interferon on human colon carcinoma xenograft transplanted into nude mice**

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We have investigated the modulated antitumor activity of 5-fluorouracil (5-FU) by I-leucovorin (LV) and recombinant human interferon a-2a (IFN) against human colon carcinoma xenograft (Co-4) serially transplanted into nude mice. 5-FU showed dose-dependent antitumor activity on Co-4 and a significant combination antitumor effect was obtained, when 200 mg of LV per kg was administered intraperitoneally on -1 and 0 hours before 5-FU (90 mg/kg, intraperitoneally) treatment. The
modulated antitumor effect of 5-FU was dependent on the dose of LV and correlated with the increment of the thymidylate synthase (TS) inhibition. When IFN was administered subcutaneously in a schedule of qdx14 at doses of $6 \times 10^3$, $6 \times 10^4$ and $6 \times 10^5$ IU/mouse, IFN alone indicated the antitumor activity in a dose-dependent manner. The combination treatment of 5-FU (60 mg/kg intraperitoneal, qdx3) and IFN (6 $\times 10^6$ IU/mouse subcutaneously, qdx14) showed an additive antitumor effect on Co-4 without any changes of TS inhibition. These results showed that the modes of action of 5-FU modulator are diverse and a human tumor xenograft nude mouse system would be useful in evaluating these modes of action.

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Orthotopic xenotransplantation of human lung cancer in the lungs of nude mice and experimental observation of invasion and metastasis

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With the purpose of establishing a model for orthotopic xenotransplantation of human lung cancer in nude mice, we first made the methodological experiment replacing lung cancer cell suspension by 1 percent Methylene Blue solution. The success rate was 26/32. Then we implanted human lung giant cell carcinoma cell strain into the lung of BALB/cA nude mice by the same via bronchial procedure. We observed the tumorigenicity, local invasion, and metastatic pattern of orthotopic xenografts. When implanting $2.0 \times 10^5$ tumor cells per animal, tumorigenicity was 0/4; when implanting $1.0 \times 10^5$, it was 1/5; when implanting $2.0 \times 10^5$, it was 7/18. The dissemination of xenografts within airway (2/8) and invasion to diaphragm (5/8) were discovered. Lymphatic, vascular, and seeding metastasis all occurred in tumor-bearing animals. The pathohistological, ultrastructural features and expression of Vimentin confirmed by immunohistochemistry were consistent with the parent human lung giant carcinoma. These results indicate that the tumor cell strains grow autonomously when implanted orthotopically and the invasive and metastatic pattern of xenografts more closely imitates the clinical manifestation of lung cancer patients than the subcutaneous counterparts. It suggests that the implant site of the host affects the biological behavior of the xenografts. The same Vimentin expression implicates the similarity between the microenvironment of human and nude mice lungs. Nude mice lungs can be used as a better model for study of invasion, metastasis, and experimental therapy of lung cancer.

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Point mutation and its stability of ras oncogene in human neoplasms and tumor xenografts

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We have examined the active c-Ki-ras gene by point mutation at the twelfth codon in human neoplasms and tumor xenografts, using the polymerase chain reaction and oligonucleotide hybridization methods. Point mutations at the twelfth codon of the Ki-ras gene were detected in 20.3 percent (15/74) of the primary human neoplasms and 19.3 percent (11/57) of the human tumor xenografts. Xenografts of human pancreas carcinomas revealed high frequency (83.8 percent) of the point mutation as well as the primary pancreas carcinomas (80 percent). The mutation of GGT (Gly) to GAT (Asp) was most frequent in the tumor xenografts (63.3 percent) as well as the primary human neoplasms (73.3 percent). The twelfth codon of Ki-ras showed no discrepancy between the original human neoplasms and their xenografts in 16 (84.2 percent) of the 19 cases. Two of the 19 cases revealed point mutation of GAT (Asp) in the xenografts despite having had no mutation in the original tumors. The other one case showed point mutation of GAT (Asp) in the original tumor despite an absence of mutation in the xenograft. These results suggested that point mutation at the twelfth codon of Ki-ras gene was stable in human neoplasms and their tumor xenografts.

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SCID and bg-nu-xid mice implanted with human fetal thymus and liver: A comparison

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We implanted human fetal (17–19 gw) thymus and liver under the renal capsule of 56 SCID (C.B17-scid/scid) mice and nine bg-nu-xid mice. Histological examination revealed that implants of human fetal thymus and liver grew in SCID (examined after 1 and 3 months) and in bg-nu-xid mice (examined after 1 month) into a structure comparable to normal fetal thymus but with interspersed areas of myeloid hematopoiesis. No difference between the appearance of the implant in SCID and bg-nu-xid mice at 1 month post-implantation was observed. Human and mouse serum immunoglobulin levels were measured 1 month after the implantation of tissue from the same fetus. Human IgG was detected in most of the implanted SCID mice and in all implanted bg-nu-xid

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mice, whereas human IgM was detected in few of the implanted SCID mice but was absent in all of the implanted bg-nu-xid mice. Growth of the implant was not affected by the presence of leakiness (>50 g/ml mouse IgG) in SCID mice, and leakiness was not induced in most non-leaky SCID mice following implantation. In contrast, most implanted bg-nu-xid mice showed a marked increase of mouse IgG production, suggesting that mouse B-cell function in bg-nu-xid mice was enhanced by the implantation of human fetal thymus and liver. We conclude that unirradiated bg-nu-xid mice do not seem to offer an apparent advantage over unirradiated SCID mice as recipients for human fetal thymus and liver. In addition, the hypogammaglobulinaemia in bg-nu-xid mice is normalized following implantation of human fetal thymus and liver.

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**Nude mouse model for development of radiolabeled monoclonal antibodies to treat human lung cancer**

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Lung cancer continues to be a serious public health problem despite an increased understanding of the biology of the tumors. Presently, lung cancer is the leading cause of cancer-related deaths among both men and women in the United States with approximately 142,000 new cases diagnosed each year and about the same number of deaths. Survival rates have not improved in 20 years, even though there have been advances in categorization of lung tumor types.

Currently, lung cancers can be categorized on a clinical basis into two broad groups, small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC). SCLC comprise about 25 percent of all lung cancers, and are frequently metastatic at diagnosis; they are, however, more responsive to chemotherapy than are the NSCLC. Therefore, treatment for SCLC has been chiefly chemotherapy, whereas treatment for NSCLC has been primarily surgery and radiation. A small percentage of NSCLC have been identified that share neuroendocrine features with SCLC and may respond to chemotherapy. Although there have been advances in diagnosis as well as in chemotherapy and radiation therapy, patient survival rates are poor. Current strategies are inadequate.

Chemotherapy is not sufficiently specific for neoplastic cells; toxicity kills patients either directly, or indirectly via suppression of their immune system which leads to patients’ susceptibility to infections and death. There is heterogeneity in tumors; not only in initial lesions, but there is evolution of cell types during metastatic progression leading to multidrug resistance. More specific treatments as well as diagnostic techniques are needed.

Newer diagnostic methods based on monoclonal antibody (MOAB) panels, immunohistochemistry, flow cytometry, and even molecular probing may ultimately lead to “individual” patient management. Although there probably are no unique tumor specific antigens, there is preferential expression of certain antigens by particular tumors; for example, neuroendocrine tumors including SCLC and neuroblastomas express CD56 and CD57 antigens as defined by MOABs from the fourth Human Leucocyte Differentiation Antigen Workshop. Through use of panels of MOABs, individual tumors may be phenotyped very specifically, and in the future, appropriate MOABs may be selected for targeted therapy.

One example of in vivo testing of a candidate MOAB for future patient use is provided by anti-NKH1. Anti-NKH1 not conjugated to either an immunotoxin or radioisotope has very limited efficacy in inhibiting the growth of an unusual SCLC line, SHP-77, which has biochemical features of an SCLC “classic,” but histological features of an SCLC “variant.” It is especially useful for emphasizing the plasticity of human tumors which evolve as they metastasize during therapy.

To test potential therapeutic efficacy of anti-NKH1 (Coulter Immunology, athymic nude mice (Harlan Sprague Dawley) were injected with SHP-77 cells in each of two sites on the abdomen. When visible tumors appeared, mice were divided into two groups. One group was injected intraperitoneally weekly with anti-NKH1 murine MOAB in RPMI 1640 cell culture medium. The other group received RPMI 1640 alone. Tumors were measured weekly. The anti-NKH1-treated group of mice had xenografts that grew slightly more slowly and were phenotypically different as defined by a MOAB panel, but were histologically indistinguishable in H and E sections compared with controls. These results show treatment of mice with anti-NKH1 must be started early after tumor cell inoculation and possibly could be given more frequently than once a week to inhibit tumor growth. More importantly, however, this model emphasizes that for future extrapolation to patient treatment, unconjugated MOAB treatment is not effective in eliminating tumors. Results of in vitro studies with SHP-77 are similar.

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**High human IgG levels in the SCID mouse reconstituted with human splenic tissues from patients with gastric cancer**

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We have implanted normal peripheral blood lymphocyte (PBL) from healthy donors and splenic tissues from patients with gastric cancers into the SCID mouse, demonstrating that the SCID mouse with splenic tissue can produce a high level human immunoglobulin G (IgG). The normal PBLs at numbers of $10^7$ and $10^9$ per mouse were implanted intraperitoneally, and three splenic tissues with a diameter of 3 x 3 x 3 mm from patients with gastric cancers were inoculated subcutaneously into the bilateral backs of the mouse. On 2, 4, 6, and 8 weeks after the inoculation the mice were sacrificed, and the human IgG was assessed by ELISA method. The SCID mice containing the splenic tissue revealed a high level human IgG from 2 weeks after the inoculation and approximately 2 mg IgG per ml was observed 8 weeks after the implantation, while the levels of IgG in mice treated with PBLs were limited. Since the half time of the extrinsic human IgG was 10.2 days, the high level human IgG in the SCID mice was supposed to be produced by human plasma cells in the splenic tissue from the patients with gastric cancer. This model was thought to be adequate in evaluating the human immunological functions in vivo.

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Antitumor activity of human lymphoma-reactive T cells against disseminated Burkitt's (Daudi) lymphoma in SCID mice

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Human Burkitt's lymphoma (Daudi) cells grow as progressive disseminated tumors in SCID mice after either intravenous or intraperitoneal injection (1). These cells are lysed efficiently in vitro by human V59/Vd2 T cells which recognize the groEL homolog on the Daudi cell surface (2). The in vitro culture of normal peripheral blood mononuclear cells (PBMC) with irradiated Daudi cells results in selective outgrowth of the V69/Vd2 T-cell subset. We studied the in vivo antitumor activity of these highly cytotoxic cell populations against Daudi lymphoma in a SCID mouse model. Normal human PBMC were incubated for 2 weeks with irradiated Daudi cells in complete RPMI 1640 with 10 percent fetal calf serum. The resulting cell populations (PBMC-Daudi) contained 42 percent V69/Vd2 T cells and showed 21 percent specific lysis of $^{51}$Cr labelled Daudi cells at 1.5:1 E:T ratio in a 4-hour $^{51}$Cr release assay. Groups of SCID mice were injected with 1) $10^5$ Daudi cells alone, either intraperitoneally or intravenously; 2) $10^5$ Daudi cells and $10^7$ PBMC-Daudi, intraperitoneally or intravenously through separate routes; and 3) $10^7$ Daudi cells and $10^7$ unstimulated PBMC, intraperitoneally or intravenously through separate routes. All animals injected with Daudi cells alone or Daudi cells and unstimulated PBMC developed disseminated tumors (in the kidneys, ovaries, adrenals, spinal cord, bone marrow, and other organs) and their survival was significantly shorter than that of mice injected with Daudi cells and PBMC-Daudi ($p<0.0001$ for both intraperitoneal and intravenous routes). Similar results were obtained when PBMC-Daudi was administered intraperitoneally 4 days before or 4 days after Daudi cells. Some of the PBMC-Daudi treated mice are still alive at 6 months with no signs of tumors. The above data suggest that the SCID mouse model can be used for studying in vivo antitumor effects of human tumor-reactive lymphocytes.

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SCID thymus: A model of T-cell development in an early stage

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Lymphoid stem cells migrate into the thymus where they develop to be mature T cells in the ordered manner. Since immature thymocytes, CD4+ CD8- (DN) cells do not express T-cell antigen receptor (TCR), it is assumed that TCR may not be responsible for developing pathway from DN to CD4+CD8- (DP) cells. To examine this issue, we used SCID thymocytes in which TCR are not expressed on their cell surface because of abnormal or defective rearrangement of TCR genes. Expression of cell surface molecules on the SCID thymocytes was determined by staining with monoclonal antibodies (mAb). The majority ($\approx$90 percent) of SCID thymocytes were Thy-1+, JlId, IL-2RA+, B', CD2', CD3', CD4', CD8', and TCR+. IL-2RB and CD2 were co-expressed on a minor population of the thymocytes. This phenotype is similar to embryonic thymocytes on day 13 of gestation which are considered to be the most immature thymocytes. Among the SCID thymocytes, IL-2RB- cell population showed a good responsiveness to IL-2 in vitro but IL-2RB+IL-2RA- did not, while both cells produced IL-2 by culture with PMA and A 23187. These characteristics are not different from those of DN embryonic
2PBL thymocytes in normal mice. However, IL-2RB'A+ embryonic thymocytes were found to be different from SCID thymocytes with the same phenotype in the following ways. 1) During co-culture with our established thymic stromal cell clone, TNC-R3.1, embryonic IL2RA+ cells lost IL-2RA expression; and 2) In the same culture, the cυIL-2RB' were able to differentiate into DP cells. Such changes were not observed in the SCID thymocytes. The importance of TCR for differentiation and proliferation of immature thymocytes will be discussed.

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Treatment with rat stem cell factor affects thymocyte progenitor cells

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Recombinant rat stem cell factor (rrSCF164), a form of the c-kit ligand, has numerous developmental effects on a multitude of stem/progenitor cells particularly important to the erythroid and myeloid lineages. However, the relevance of the kit-ligand to lymphoid cell development is less clear since c-kit-ligand deficient, Sl/Sld mice and c-kit defective, W/Wv mice have apparently normal peripheral B and T lymphocytes. We have recently shown that the cellularity of 12-week-old Sl/Sld thymi is reduced by approximately 50 percent when compared with age- and weight-matched normal F1 +/+ littersmates and certain IL-7 responsive B-cell precursors have enhanced growth in the presence of rrSCF and IL-7. In the present study, we have analyzed thymocyte progenitor cells (cells able to repopulate the thymus of adoptive recipients following intravenous injection) to determine if rrSCF affects their development in vivo. A quantitative in vivo assay for thymocyte progenitors has been used to assess relative numbers of thymocyte progenitor cells in rrSCF164PEG-treated and saline-treated rat bone marrow and spleen. Donor/host determinations were made using the RT-7.1 and 7.2 allotypic pan lymphocyte antigens to distinguish M520 rat (donor) cell regeneration in irradiated BUF rats (host). Time course and cell dose studies revealed no significant differences in thymocyte regeneration resulting from transplantation of bone marrow cells from either saline-treated rats or rats treated for 7 days with rrSCF164PEG. In contrast, splenocytes from rrSCF164PEG-treated rats were significantly (p<0.05) more effective in regenerating the thymus than splenocytes from saline-treated rats. These data suggest that rrSCF164 treatment in vivo induces an increase in either the number or activity of spleen cells able to migrate to and colonize the thymus of an irradiated recipient. Future studies will be directed towards comparison of these induced splenic thymocyte progenitors with normal bone marrow or spleen thymocyte progenitors.

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Comparison of resistance to Pseudomonas aeruginosa infection among nude, SCID, and drug-induced immunodeficient mice using biological response modifier S(BRM)

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Infections are continuously changing, and these changes are especially marked in cases of bacterial infections. Infections by highly contagious bacteria are decreasing, while those by bacteria considered as weakly toxic or nonpathogenic are increasing. These are known as opportunistic infections. We investigated the relative importance of the various component factors of the vital defense mechanism in the elimination of foreign matter and studied the mutual relation between the host and microorganisms on the basis of an analysis of comparative importance. At present, we are performing experiments on opportunistic infections in compromised hosts and the role of phagocytes against the causative bacteria in such cases using an experimental Pseudomonas aeruginosa infection system. We have also performed a comparative investigation on activators which show non-specific, rapid, and strong effects on the vital defense mechanism in the early stage of infections in compromised hosts with SCID, nude, and drug-induced immunodeficient mice. SCID mice and BALB/c mice pretreated with Cyclophosphamide (60mg/kg, intravenously for 3 days) all failed to survive the challenge of Pseudomonas aeruginosa M-24 (2 x 107 CFU intraperitoneal). The survival rate of BALB/c mice pretreated with Ciclosporin A (30mg/kg, subcutaneously for 7 days) and nude mice was 40 percent and 100 percent. Phagocytic activity of abdominalis cavity cells was high in nude, BALB/c mice, SCID mice.

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Retrovirus-induced immunodeficiency in the mouse

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Susceptible strains of mice infected with the mixture of murine leukemia viruses (MuLV), termed LP-BM5, develop progressive lymphoproliferation and profound immunodeficiency, a syndrome designated MAIDS. The disease-inducing agent, a replication defective virus, encodes a Pr60\textsuperscript{gag} protein that behaves like a "superantigen," stimulating marked T-cell activation. Targets for infection and expression of the defective virus include macrophages, B cells, and CD4\textsuperscript{+} T cells with expression in B cells being crucial for T-cell activation. The consequences of T-cell activation include production of cytokines with the spectrum of genes expressed varying with time after infection. At one week, IFN-\gamma, IL-2, -4, -5, and -10 are produced without restimulation by mitogen or antigen. Thereafter only low levels of spontaneous production are detected, but stimulation with mitogen results in high level production of IL-4, -5, and -10 with impaired production of IL-2 and IFN-\gamma. Levels of IL-10 mRNA expression also increase at these later times, as do levels of serum IgE. The latter findings suggest that Th2-type helper cells are preferentially activated in vivo in the later stages of disease. As IL-10 impairs cytokine expression by Th1 cells and CD8\textsuperscript{+} T cells and IL-4 and -5 stimulate B cells, activation of Th2 cytokines may explain impaired T-cell responses to infectious agents (T. gondii, cytomegalovirus) or conventional antigens as well as chronic B-cell activation. The central role ascribed to cytokine dysregulation in the pathogenesis of MAIDS is supported by the finding that treatment of mice with cyclosporin A retards the development of B- and T-cell dysfunction and lymphoproliferation. Analyses of disease resistance exhibited by some mouse strains was found to be mediated by genes both within and outside the MHC. MHC influences are dominated by class I genes, their effect being to direct potent CD8\textsuperscript{+} T-cell responses capable of ridding the host of the defective virus genome.

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Differentiation of M-CSF-independent macrophages and glucan-induced granuloma formation in osteopetrotic (op/op) mice

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Osteopetrotic (op/op) mice are characterized by a generalized skeletal sclerosis due to failure of bone resorption and remodeling resulting from a defect of osteoclasts. The op mutation is within the coding region of the macrophage-colony-stimulating factor (Csf\textsubscript{m}) gene. In the mutant, peripheral blood monocytes, macrophages in various visceral organs, and osteoclasts in the bone are markedly reduced. However, we found ultrastructurally immature tissue macrophages in many organs and tissues. In co-cultures of normal mouse bone marrow cells with fibroblast cell lines prepared from the lung of the op/op mice, we confirmed a defective differentiation of mono-
Epidermal growth factor receptor gene alteration in human neural tumor xenografts in nude mice

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Tumors of the nervous system showed various histological and biological features. While the proper meaning of the features is still unclear, it is considered that various mechanisms, including activation of oncogenes, may affect carcinogenesis and progression of tumors. Primary neural tumor specimens limit fine analysis because of their small size. The human tumor/nude mouse system provides useful tools for studying human neoplasm. We have reported that some glioma xenografts showed structural and functional alterations of the epidermal growth factor receptor (EGFR) gene. We studied 20 lines of human neural tumor xenografts including glioma, ependymoma and schwannoma. We examined the structure of the EGFR gene by Southern blot analysis.

We estimated the expression of the EGFR gene by Northern blot analysis and reverse transcription-polymerase chain reaction (RT-PCR) methods. Three of 15 glioblastoma cell lines showed amplification and rearrangement of the EGFR gene. RT-PCR analysis demonstrated aberrant transcripts of the EGFR gene with deletion (800 bp) at the extracellular domain coding region in the three cell lines. One of three malignant schwannomas also revealed EGFR gene amplification, while the cell line showed no rearrangement. Four tumors overexpressed mRNA of the EGFR gene. The other six tumors (two ependymomas and four glioblastomas) expressed the EGFR gene moderately without gene amplification. Our results suggest that various EGFR gene alterations were associated with neoplasms of the peripheral nervous system as well as the central nervous system.

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Pleiotropic effects of the scid mutation

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The immune-deficiency in SCID mice results from their inability to correctly rearrange their immunoglobulin in T-cell receptor genes. However, the basic defect in SCID mice is a DNA repair gene which affects the ability of the mice to repair double-strand DNA breaks. Although this defect affects all tissues equally, lymphoid tissues are severely affected since their normal function depends on the use of this repair gene. The effect of the scid mutation on many tissues make this mouse a unique recipient for studies requiring transfer of hematopoietic cells. Thus, SCID mice are ideal recipients for analyzing lymphoid progenitors. The increased radiation sensitivity also makes sublethally irradiated SCID mice excellent recipients for myeloid progenitors despite the fact that this lineage exhibits no detectable defect in these mice. In addition, sublethally irradiated SCID mice offer many advantages for studying autoimmune diseases, such as graft-vs-host disease (GvHD). GvHD is particularly interesting since the disease in these recipients is markedly different from that observed in other mice. The differences in pathology may be due to the absence of host lymphocytes which are potential targets for GvHD. Host lymphocytes may also be required to initiate some components of the GvH reaction. In any case, the extremely low numbers of lymphocytes in SCID mice makes it feasible to investigate cellular interactions and the role of different lymphocyte subpopulations in the pathogenesis of many autoimmune diseases.

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Epstein-Barr virus-induced lymphoproliferative disorders in SCID-hu chimeric mice

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EBV B95-8 infection of SCID mice reconstituted with PBL from human donors is followed 20–40 days later by development of invasive and rapidly lethal peritoneal tumors of human B-cell origin. Histopathologic studies indicated that tumors were composed of plasmacytoid immunoblasts, with well-defined areas of necrosis, and Southern blots showed that tumors carried EBV DNA. Analysis of Ig gene rearrangements and EBV terminal repeat (TR) sequences demonstrated that SCID/hu tumors were oligoclonal. Southern blot analysis also showed that the EBV genome in tumors resultant from B95-8 infection of SCID/hu mice was episomal, whereas spontaneous tumors arising in SCID mice reconstituted with PBL from EBV-seropositive donors possessed both episomal and linear, replicating EBV genomes.

Cellular and viral gene expression in SCID/hu tumors broadly paralleled patterns seen in LCL rather than Burkitt lymphoma cells, except that CD20 and CD23 expression was down-regulated in tumor biopsy cells, and, most notably, that EBV latent gene expression may also be down-regulated. EBV-specific T-cell recognition was found to correlate with levels of EBV EBNA-2 and LMP expression by tumor cells and autologous LCL.

The data suggest that down-regulation of EBV latent gene expression in tumor cells, and consequent reduction in vulnerability of EBV-specific T-cell recognition, may further contribute to pathogenesis in immunosuppressed patients with impaired T-cell function.

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SCID mice as models for human parasitic diseases

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Parasitic diseases represent an enormous economic burden in many tropical and subtropical countries. Research into basic pathophysiology of these diseases has been hampered by the fact that many human parasites are not transmissible to mice. We are developing SCID mice as models for two parasitic diseases of global significance.

Human lymphatic filariasis is a major public health problem in a number of developing countries, where there are an estimated 100 million cases, caused by the organisms *Wuchereria bancrofti* and *Brugia malayi* (3). We have shown that subcutaneous injection of infective L3 larvae into SCID mice recapitulates the human disease, in that: (a) the parasites home to lymphatics where they cause lymphatic dilation and retention of lymph; and where their presence causes inflammatory changes including lymphangitis and lymphadenitis; and (b) immunological reconstitution of SCID mice prior to injection of infective larvae prevents the establishment of infection, whereas a normal, functional immune system is unable to cure established infection. An observation that is puzzling is that 'leaky' SCID mice appear to be resistant to infection. There is evidence in the literature that these mice generate a very limited repertoire of T- and B-cell clones, and it would appear unlikely that any one of the oligoclonal clones would be filarial specific.

The nematode parasite *Onchocerca volvulus* is responsible for the second largest incidence of preventable blindness in the world. *O. volvulus* is an obligate human parasite and its study has been difficult due to an inability to maintain it outside the human host. Over the past year, we have been successful in transplanting Onchocercomata containing live adult *O. volvulus* worms into immunodeficient C.B17 scid/scid (scid) or athymic mu/mu (nu/nu) rats. Live, motile worms containing viable mf are present in Onchocercomata recovered from SCID mice or nude rats for up to 20 weeks, establishing a novel animal model for future investigation of the biology of *O. volvulus*.

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Selective reconstitution of T-cell subsets in SCID mice

Jorg Reimann

Adoptive transfer of purified CD4+CD8+ T cells from thymus, spleen, or lymph node of congenic (BALB/c, C.B17) or semi-allogeneic (dm2), but not completely allogeneic (C57BL/6) adult donor mice selectively repopulated the respective T-cell compartments of spleen and peritoneal cavity in young SCID recipient mice. Engraftment of T cells 3 to 5 months post-transfer was assessed by different reconstitution parameters: cytofluorographic analyses, histological examinations, in vitro establishment of donor-derived CD3+CD4+CD8+ T-cell lines from transplanted SCID mice, and repeated serial transfers of donor-type T-cell populations through young SCID recipients. Repopulated CD4+ T cells repopulated splenic and peritoneal cavity, but not lymph nodes or thymus of SCID recipients. Transfer of limiting numbers of purified CD4+CD8+ T cells (10^6 to 10^5 cells per mouse) led to partial and selective reconstitution of T-dependent splenic areas of SCID recipients. Only a
small fraction of injected CD4+ T cells is apparently selected for engraftment by the SCID mouse environment. CD4+ T cells obtained from the spleen of transplanted SCID mice displayed in vitro proliferative, A4-restricted self-reactivity, but no evidence for autoimmune lesions was found in vivo in CD4+ T cell-transplanted SCID mice. Extensive in vivo expansion of donor-type CD4+ T cells was observed in the course of repeated passages of CD4+ T cells through young SCID recipients for more than one year. Attempts to antigen-specifically activate transplanted CD4+ T cells in SCID hosts were unsuccessful.

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Genetics and functional studies of minor histocompatibility loci

Derry C. Roopenian, Larry E. Mobraaten, Sheau-Chiann Wang, Greg Christianson, and David Higgins

There are many control points at which a given stimuli can have profound effects on both qualitative and quantitative aspects of an ensuing immune response. To better understand these control points, we used the minor histocompatibility (H) antigen system because it is a model system pertinent not only for transplantation immunity but also auto and tumor immunity. We found previously that two minor H loci H-3 and H-4 are genetically complex and can be subdivided into two gene types (1,2). One type encodes products that stimulate MHC class I-restricted cytotoxic T cells (Tc) and the other encodes products that stimulate MHC class II-restricted helper T cells (Th). Thus, the nature of the minor H locus gene product is a control point that dictates which T-cell subset will be stimulated. We produced new mouse strains that carry a crossover between the above two gene types in order to assess the characteristics of immunity that each type of gene product would elicit. Tc were not detected when the mice were primed with spleen cells expressing the Tc-defined antigens alone; Th stimulation was required. Thus, stimulation of the Th subset during immunization was a control point that profoundly influenced the Tc immune response. Moreover, skin allograft rejection across Tc-defined antigen barriers proceeded quite inefficiently, and in many cases not at all. The type of immunizing cell also heavily influenced the Tc response since the requirement for Th was less pronounced when the primary immune stimulus was semipurified dendritic cells or skin allografts. These results indicated the nature of the antigen presenting cell also is a control point for the Tc response. Skin allografts solely across a Th-defined antigen barrier were retained indefinitely. Thus, Th in the absence of Tc did not lead to effective skin graft rejection. Allografts bearing both Th and Tc-defined antigens were rejected rapidly, as were Tc-defined antigen-disparate grafts after priming across Th + Tc antigen barriers. Thus, Th are not necessary proximal effectors of rejection, but contribute to the rejection process by facilitating the differentiation of precursors into mature Tc. We discuss these results in the context of control points involved in transplantation, auto- and tumor-immunity.

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Production of pathogen-free immune-deficient animals

Robert J. Russell

Today, animals are produced in barrier buildings and isolator housing systems to prevent infection with adventitious viruses, bacteria, Mycoplasma sp., fungi, and parasites. Pathogenic agents of special concern in immune-deficient mice include Sendai virus, mouse hepatitis virus, Staphylococcus aureus, Pseudomonas aeruginosa, and Pneumocystis sp. Contaminated animal stocks and strains are rederived by the use of hysterectomy and embryo transfer techniques. Rederived animals are then maintained in either barrier buildings or isolators. Because of potential contact with the animal care staff, barrier building produced animals may harbor commensal and other nonpathogenic bacteria that can interfere with the research utilization of immune-deficient animals, e.g., athymic nude, scid, and bg-nu-ld. Therefore, immune-deficient animals are produced and maintained in flexible film isolators free from direct human contact until they are delivered to investigators. Isolators are designed and operated to provide total animal protection and maintain animals free of unwanted organisms. Isolator techniques and procedures include complete sterilization of the flexible film isolator system prior to animal population; HEPA filtration of supply and exhaust air; sterilization of all supplies and equipment in contact with the animals; and complete separation of animals from direct human contact. Isolator raised animals are therefore better protected from pathogens, potential pathogens, opportunistic agents, and commensal organisms than barrier raised animals. Frequent (monthly) health monitoring of immune-competent and immune-deficient animals from the isolator colonies provides continued assurance regarding their health status. Animals are transported to the investigator in filtered shipping containers in pathogen-free, environment-controlled vehicles. These systems assure the
Comparative evaluation of congenitally athymic and euthymic rat strains bred and maintained at different institutes

I. Euthymic rats

H.-J. Schuurman (Study Coordinator), E.B. Bell*, K. Gartner†, H.J. Hedricht‡, A. Kornerup Hansen§, B.C. Kruijt§, P. DeVrey‡, R. Leyten©, S.J. Maeder**, R. Moutier††, U. Mohnle‡‡, J. Vankerkom§§, K. Harlan Sprague Dawley, Inc., Frederick, Maryland

We performed a comparative evaluation of the immune status, focused on the T-cell system, of euthymic rat strains in which the nude mutation had been introduced. This evaluation was initiated to enable a correct comparison when the extent of the T-cell system in the nude mutation in these strains is assessed. We sampled 12 groups of euthymic rats, from 10 institutes, at 1½-2 months and 6 months of age. We analyzed body, spleen, and thymus weight; antibody response and delayed-type hypersensitivity response to ovalbumin immunization; and (immuno)histology of spleen, lymph nodes, and lymphoid tissue along the gastrointestinal tract. In the spleen morphometric analysis was done of the periarteriolar lymphocyte sheath in spleen (using the antibody R73 recognizing the αβ-T-cell receptor) and of the red pulp (using the antibody ED2 recognizing red pulp macrophages). For almost all parameters tested, statistically significant differences between the groups (origin of the animals) was observed. A cluster analysis on the basis of body weight, spleen weight, and morphometric data of spleen did not yield clusters with a different composition among animals from individual groups. Based on the antibody response to ovalbumin, clustering revealed groups of "fast-and-high," "slow-and-low," and "intermediate" responders. The various groups differed in terms of location within these clusters, i.e., the speed and extent of the immune response depends on the background euthymic strain.

Considering the microbiological status assessed by serology, a variation was found both in post-infection rates at entrance in the study, and in primo-infection associated with a rise in antibody concentrations during the study. These states showed no negative effect on anti-ovalbumin reactivity. Rather, the response in primo-infection to Rat Corona virus, Sendai virus, and Pneumonia virus of mice was the highest in animals clustered as "fast-and-high" responders to ovalbumin.

II. Athymic rats

We performed a comparative evaluation of the immune status, focused on the T-cell system, in congenitally athymic rat strains. We sampled 15 groups of animals from 11 institutes around the world at 1½-2 months and 6 months of age. The analysis included body and spleen weight; antibody response and delayed-type hypersensitivity response after immunization with ovalbumin; and (immuno)histology of spleen, lymph nodes and lymphoid tissue along the gastrointestinal tract. Morphometric analysis was done for αβ-T-cell receptor-bearing cells in spleen tissue as a measure of the periarteriolar lymphocyte sheath; it was also done for splenic red pulp using the antibody ED2 recognizing red pulp macrophages. For almost all variables analyzed, statistically significant differences between the groups were observed. The extent of αβ-T-cell receptor-bearing cells in the spleen increased with age. The functioning of these cells in immunological responses can be questioned, because an immune response to ovalbumin was invariably absent. But secondary follicles with germinal centers, reflecting T-cell-dependent B-cell reactivity, were observed in lymph nodes and Peyer's patches (up to 40 percent and 75 percent, respectively, depending on the group), with a higher prevalence in older animals. A cluster analysis on the basis of body and spleen weight and composition of spleen compartments did not yield clusters with a different profile in regard to the animals' group of origin. The data presented are useful when comparing studies performed with various athymic rat strains at different institutes.

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Xenotransplantation of human spleen in SCID mice

Kazou Shimamura, Yuko Yanagida*, and Sonoko Habu†

Several populations of human hematopoietic cells are able to survive and differentiate in SCID mice. In an attempt to analyze human hematopoietic cells in the mice, survival and homing of human spleen cells transplanted in the mice were studied. Fragments of human spleen tissues were subcutaneously transplanted in SCID mice. Sections of the transplanted spleen fragments and regional lymph nodes were examined immunohistochemically using antibodies against human T and B cells, 2–6 weeks after the subcutaneous transplantation. In addition, immunofluorescence analysis with FACScan was performed with cell suspensions from the regional lymph nodes. We identified human T, B and plasma cells in spleen tissue transplants 6 weeks after the subcutaneous transplantation. Human T cells and plasma cells, but not B cells, were present in regional lymph nodes. However, proliferation of the T cells in paracortical T-cell zones could not be identified.

These data suggest that cell-to-cell interactions, which are essential for lymphocyte proliferation in lymph nodes, were absent between human lymphocytes and murine lymphoid tissues.

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A nude mouse model for the study of human tumor xenograft invasion and metastasis

Mogens Spang-Thomsen and Nils Brüiner

The nude mouse with human tumor xenografts has proven to be of major importance as a tool in many aspects of cancer research. Generally, human tumor xenografts grow well-circumscribed without local invasion or distant metastases in the animals. The lack of expression of these essential malignant phenotypic characteristics represents a strong limitation of the model in the study of the biology of cancer. We introduce a new model for the study of invasion and metastasis of human tumor xenografts. The model is based on a substrain of nude mice, nu/nu-META/BOM™, in which a number of human tumor xenografts reproducibly are local invasive and form distant metastases. Tumors hitherto examined include fibrosarcoma, breast, prostate, ovary, and small cell lung cancer xenografts. A diversity in invasive and metastatic potential was found, suggesting that the expression of these biological characteristics is not only dependent on the host but also determined by tumor factors. The nu/nu-META/BOM™ mice thus allow tumor xenografts to express their differential malignant potential. The model extends the fields of potential applications for the use of tumor xenografts in basic cancer research. Studies of tumor characteristics can be related to the invasive/metastatic capacity of the tumors as a highly significant endpoint. The mechanisms involved in the processes of invasion and metastasis can

Malignant xenografts: A molecular cross-talk at the host-tumor interface

Bernard C. M. Sordat

Advances in heterotransplantation of human tumors to immune-deficient hosts have made it possible to directly evaluate the contribution of multiple factors, including oncogenes, to tumor take, growth, and progression. Besides the respective effects of dominant and/or recessive genetic lesions, there are likely to be epigenetic influences on the emergence and temporal expression of tumorigenic, invasive and metastatic phenotypes. Significant progress came from the introduction of orthotopic xenografting procedures allowing to reflect more closely the clinical behavior and the histopathological growth patterns proper to the tumors of origin. Documented examples are human colorectal, renal cell, skin, pancreas, and bladder carcinoma, as well as melanomas growing at ortho-versus heterotopic sites in nude mice. Factors from the host tissue microenvironments have been shown to modulate the malignant phenotype. Reciprocal interactions between tumor cells and host matrices may manifest by changes in invasive and metastatic pattern, cell growth requirements and induction of matrix-remodeling enzymatic activities, especially plasminogen activators and collagens. Resident growth factors bound to matrix constituents, cytokines, proteolysis activators and inhibitors may be found and stored in tumor stroma and may contribute in vivo to the extent of cell invasion, including tumor-induced angiogenesis. Orthotopic xenografting has considerable interest both when investigating the genetic events at proposed stages of carcinoma progression and when evaluating in vivo the cell-cell and cell-matrix molecular cross-talks in the course of malignant invasion.

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be studied in vivo, and the model offers a means to investigate the effect and mechanisms of action of new anti-invasive and -metastatic drugs.

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Immunomodulation of soluble Qa-2 levels in mice
Piotr Tabaczewski and Iwona Stroynowski

Qa-2 proteins are members of the murine major histocompatibility complex (H-2) molecules. These polypeptides, like classical H-2 antigens, consist of heavy chains associated with β2 microglobulin and are presumed to fold into HLA-A2-like structures. Qa-2 antigens can be recognized by T-cell receptors and function as guiding molecules for allogenic cytotoxic reactions. They can also transduce signals leading to the proliferation of cells on which they are expressed. Thus, the Qa-2 antigens appear to perform a dual role as mediators of the expansion and/or deletion of specific subsets of cells in the immune system. In contrast to the classical H-2 antigens, which exist only as membrane spanning proteins, the Qa-2 antigens are attached to the cell surfaces by glycosylphosphoinoside (GPI) lipid anchors. They exist also as actively secreted forms due to alternative splicing of their transcripts. Both of these forms are expressed in ConA stimulated mouse splenocytes in vitro and both give rise to soluble Qa-2 polypeptides found in culture supernatants. To determine the relevance of these findings to the in vivo system we have screened mouse sera for soluble Qa-2. Using ELISA method to discriminate secreted form from GPI derived form of Qa-2, we have estimated that the total circulating Qa-2 level in adult, healthy C57BL/6 mice is of the order of 100 ng/ml. The actively secreted form constitutes less than half of the total soluble Qa-2. It appears that different cell subsets contribute to the Qa-2 pool in serum since all the C57BL/6 mutants that demonstrate severe dysfunctions of immunological cell systems (beige, nude, SCID, moth-eaten) contain Qa-2 in blood, albeit at different levels. The level of soluble Qa-2 is age dependent: it is lowest at birth, reaches maximum at 3–5 months of age and then declines. The amount of soluble Qa-2 found in serum is strongly enhanced in polyI:C injected mice and is decreased in hydrocortisone treated animals, suggesting dependence on immunomodulation. We hope that these studies will help to clarify the physiological function(s) of Qa-2 molecules in immune responses in vivo.

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Chemo- and endocrino-therapy of human breast carcinoma xenografts in the dormant or exponential growth phase
Tooru Takeuchi, Tetsuro Kubota, Yoshinori Yamada*, Jun-ichi Koh†, and Masaki Kitajima

The breast carcinoma cell in the dormant stage is one of the most important problems in post-operative patients after mastectomy. To elucidate a rationale to treat breast cancer in the dormant stage, we have developed a new treatment model using human breast carcinoma xenografts in nude mice. Three human breast carcinoma xenografts, MCF7, R-27, and Br-10, were implanted into female nude mice, and the treatment was initiated with or without the previous estradiol (E2) stimulation. In the case of exponential growth phase, 5 mg of E2 per kg was administered intramuscularly on Day 0 and the treatments were initiated when the tumors started the exponential growth. In the case of the dormant stage, the treatments were initiated on Day 1, and the E2 stimulation was performed on Day 28, when the treatments were completed. Mitomycin C, which was given intraperitoneally at a dose of 6 mg/kg, was effective on the xenografts in the exponential growth phase but ineffective on tumors in the dormant stage, while tamoxifen pellet (2.5 mg/mouse) was effective against tumors both in the exponential and growth phase, suggesting the preventive effects of tamoxifen for E2 stimulation. This model seems to be useful in developing the adjuvant therapy of breast cancer.

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A newly developed hexamethylmelamine derivative (SAE 9) with an activity of aromatase inhibitor
Hirokazu Tanino, Tetsuro Kubota, Tooru Takeuchi, Yoshinori Yamada*, Jun-ichi Koh†, Suguru Kase, and Masaki Kitajima

The effect of hexamethylmelamine (HMM) and SAE 9 (SAE) was evaluated in terms of aromatase inhibition, the antitumor activity on P388 cells in vitro, and human breast carcinoma xenografts (R-27, T-61 and MX-1) serially transplanted into nude mice. SAE showed the same degree of aromatase inhibitory effect with 1:10 molar concentration of CGS16949A, while 50 percent inhibitory concentration of SAE was almost equivalent to that of HMM. These effects suggested that SAE might be a promising agent against estrogen receptor positive human breast carcinomas. When SAE and HMM were

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applied for the human tumor xenografts in nude mice, the maximum tolerated doses were estimated to be 100 and 75 mg/kg/day postoperative for 4 weeks, and the dose limiting toxicity was estimated to be the acute neural disturbance. HMM has suppressed the growth of MX-1 and T-61 completely, and R-27 was also sensitive to this agent. Although the antitumor activity of SAE was not superior to that of HMM in the administration method tested, the analogous compounds of HMM with an aromatase inhibition activity would be promising agents with a unique mode of action including direct antitumor like HMM and aromatase inhibiting effects against estrogen receptor positive human breast carcinomas.

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**Xenotransplantation of human cancers into SCID mice**

Yoshito Ueyama, Kouji Urano*, Yasuyuki Ohnishi*, Rieko Saegusa, Yuko Kato, Norikazu Tamaoki, and Tatsuji Nomura*

One-hundred and forty-nine human cancers were simultaneously transplanted to SCID mice and nude mice. These included 41 breast carcinomas, 16 colon carcinomas, 14 esophageal carcinomas, 14 ovarian carcinomas, 10 hematopoietic tumors, five kidney carcinomas, and four pancreatic carcinomas. Overall successful transplanation rate of human cancers to SCID mice was 36.9 percent (55/149), while that of nude mice was 16.8 percent (25/149).

The successful transplanation rates of each kind of cancer to SCID mice were: 8/41 (19.5 percent) in breast carcinoma; 6/16 (37.5 percent) in colon carcinoma; 13/14 (92.9 percent) in esophageal carcinoma; 8/14 (57.1 percent) in ovarian carcinoma; 1/10 (10.0 percent) in hematopoietic tumors; 1/5 (20.0 percent) in kidney carcinoma; 3/4 (75.0 percent) in pancreatic carcinoma; and 15/48 in the other carcinomas. On the other hand, the successful transplanation rates for nude mice were: 4/41 (9.8 percent) in breast carcinoma; 1/16 (6.3 percent) in colon carcinoma; 9/14 (64.3 percent) in esophageal carcinoma; 2/14 (14.3 percent) in ovarian carcinoma; 0/10 (0.0 percent) in hematopoietic tumors; 1/5 (20.0 percent) in kidney carcinoma; 2/4 (50.0 percent) in pancreatic carcinoma; and 6/49 in the other carcinomas. All kinds of carcinomas showed higher successful transplanation rates in SCID mice than in nude mice. In particular, successful transplanation rates between ovarian carcinomas and colon carcinomas differed significantly (P<0.05).

*These results demonstrate the important role B-cells play in the rejection of xenografts, because SCID mice are depleted with B-cell functions in addition to T-cell functions with which nude mice are depleted. These results also show the usefulness of SCID mice for xenotransplantation of human cancers.

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**Role of natural antibodies in rejection of discordant bone marrow grafts**

D.W. van Bekkum, W. Huppes, and J. Paulonis

Recent reports of persistent engraftment of human lymphocytes and myeloid cells in hereditary immune-deficient SCID and bg-nu-xid mice have raised the question: Why have all attempts to graft human cells into artificially immune-suppressed normal mice failed so far? In the present study we provide evidence that this difference is due to the absence of Natural Antibodies (NA) in the mutant mice. We demonstrate that human PBL can be grafted in normal mice immune-suppressed by heavy doses of total body irradiation, provided the transplant is performed when the recipients lack natural antibodies in their serum, e.g. as in newborn normal mice, in mice treated with anti-mouse IgM antibody from birth, and in 3-week-old B-cell deficient CBA/N mice. In all cases, large numbers of human PBL were required.

The assays employed for NA in the serum were agglutination of human erythrocytes and FACS analysis with fluorescent anti-mouse Ig of human erythrocytes and mononuclear cells following exposure to mouse serum. NA titers appeared to vary when target cells from different human donors were used. The relationship between this phenomenon and blood group types is presently under investigation.

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**T but not B cells are required for the clearance of murine malaria parasites from SCID mice**

H.C. van der Heyde, S. B. Mannino, J. Manning, B. M. Greene, and W. P. Weidanz

In previous studies using B-cell deficient and athymic mice we demonstrated that acute blood-stage infections with the murine malaria parasite P. chabaudi adami (pca) were resolved by T-cell dependent immune mechanisms independent of antibody. Adoptive transfer studies with fractionated T cells from immune donors as well as malarial antigen-specific T-cell lines and clones into athymic recipient mice indicated that CD4⁺,CD8⁺, T H1
cells played an essential role in immunity to *pca*. However,
the use of nude recipients in these studies did not allow us to rule out the participation of B cells in the immune response to this parasite. Accordingly, we have begun to study the role of T cells in immunity to acute *pca* infection in SCID mice.

Unreconstituted control SCID mice infected with *pca* parasitized erythrocytes developed parasitemia which oscillated between 5 percent and 40 percent during a 2-month period. To determine if T cells alone were capable of allowing SCID mice to resolve acute infections with *pca*, groups of SCID mice were grafted with unfracti-

onated spleen cells from immunologically intact but uninfected (normal) donors, or from donor mice rendered immune by repeated *pca* infection. Other groups received splenic cells from which the macrophage-like cells were removed by plastic adherence and B lymphocytes by a Magnetic Activated Cell Sorter (MACS). B cells were labelled with a-B220 and a-IA^d^ prior to positive selection by the MACS. The resulting preparation was determined by flow cytometry to be >90 percent T cells with <3 percent B cells. 1 x 10^7^ unfracti-

onated and T-cell enriched spleen cells were transferred into groups of SCID and nude mice. Cells from normal donors were injected into SCID recipients only. All mice, as well as a group which did not receive cells, were infected with 1 x 10^7^ *pca* parasitized erythrocytes. The resulting parasitemia was monitored at subsequent intervals of time by enumerating parasites in blood films stained with Giemsa. SCID and nude mice that had received unfracti-

onated spleen or T cells from immune donors cleared their infections by day 23 (parasitemia < .1 percent). SCID mice that received cells from normal donors also suppressed their parasitemia to < .5 percent by day 40. While all grafted mice displayed a patent infection on challenge with *pca*, the peak parasitemia in mice grafted with immune cells was significantly less than seen with cells from normal donors. These results indicate that B cells are not required for clearance of acute *pca* blood-stage infections.

To determine if T cells activated during acute infection were able to suppress patent parasitemia, SCID mice were grafted with cells from three groups of donors: normal, immune and donor mice sacrificed at peak parasitemia. Recipient SCID mice that received cells from immune donors cleared their infections by day 14. In contrast, SCID mice that had received cells from infected or normal donor mice had parasitemia of prolonged duration. SCID mice engrafted with unfracti-

onated or T-cell enriched spleen cells activated by infection suppressed their parasitemia to about 10 percent by day 14, whereas parasitemia in recipients of cells from normal donors increased >20 percent at the same time. These results suggest that exposure to the infecting agent relative to the time of cell isolation has a marked influence on how the recipient animal responds to the subsequent infection. The SCID model provides a unique opportunity to investigate these differences further.

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**Immunodeficient animals as models for opportunistic infections**

P. D. Walzer

Congenitally immunodeficient mice and other animals have been used as experimental models for infectious diseases since the 1970s. It had been thought that these animals would be particularly useful for studies of organisms such as *Pneumocystis carinii* and *Cryptosporidium*, which are difficult to cultivate and of low virulence. However, studies of *P. carinii* pneumonia in athymic or nude (nu) mice have generally been disappointing. Problems have included the lack of reproducibility among different investigators, the presence of latent *P. carinii* infection in the animal colonies which can persist for long periods of time, and the increased susceptibility of these mice to infection with other opportunistic pathogens. More recent attention has focused on mice homozygous for the severe combined immunodeficiency (scid) mutation, and these animals are gaining increasing popularity as models of pneumocystosis. Factors contributing to the use of SCID mice include improved animal housing and breeding facilities, new *P. carinii* inoculation procedures, and cellular depletion and reconstitution techniques. SCID mice offer useful targets for *P. carinii* treatment with antimicrobial drugs or with immunomodulators. SCID mice may be limited by the level of pneumocystosis which can be achieved and by their susceptibility to infection with other agents.

Current mouse models of *P. carinii* pneumonia use organisms derived from mice as the source of infection. The greatest need in this area of research is to develop an experimental model in mice using *P. carinii* derived from humans. The picture is complicated by the fact that most human *P. carinii* specimens available for study contain small numbers of viable organisms or are infected with HIV or other opportunistic pathogens. Other experimental needs of mouse models include development of approaches to mimic the immune or inflammation responses to *P. carinii* in the lungs which appear to play an important role in the pathogenesis of pneumocystosis in humans; evaluation of the influence of other respiratory viruses and other organisms on the development of *P. carinii* pneumonia; and identification of specific factors which promote the development of extrapulmonary spread of *P. carinii*.

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Human immune response to mouse erythrocyte antigens demonstrated in SCID mouse-human chimera

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SCID mice engrafted with human PBLs (hu-PBL-SCID) represent a potentially important small animal model for the study of human immune function. Attempts to generate human humoral immune responses in the hu-PBL-SCID mouse to exogenous antigens have had limited success and have raised questions about the functional capacity of human lymphocytes in the SCID environment. We demonstrate here that human PBLs, engrafted into SCID mice, are able to produce antibodies specific for host erythrocyte proteins. Greater than one-third of our hu-PBL-SCID mice were Coomb's positive, had hemagglutinating in their sera, or had decreased hematocrits or increased erythrocyte sedimentation rates. Ten percent of the mice had all of these symptoms indicative of an ongoing immune hemolytic anemia. The appearance of human anti-mouse erythrocyte antibodies in the sera was observed as early as 2 weeks and as late as 10 weeks after engraftment of human PBLs. Specific human anti-mouse erythrocyte antibodies were detected by Western blot analysis and recognized several different erythrocyte proteins. Furthermore, the pattern of reactive bands in the Western blots varied between mice engrafted with PBLs from the same donor. On the basis of these reactivity patterns we conclude that human peripheral B cells retain the ability to respond to specific antigens within the SCID environment, and that the engraftment of B cells is random and oligoclonal with respect to reactivity against mouse red cell epitopes. The limited ability of human lymphocytes in SCID mice to respond to exogenous antigens may be related to the selection and expression of host reactive B cells immediately following the engraftment process.

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CD5, T independent type 2 responses and X-linked immune deficiency

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Mice expressing X-linked immune deficiency cannot respond to repeating unit thymus-independent type 2 (TI-2) antigens, nor do they have B cells with the surface molecule CD5 (ly-1). Our recent finding that B cells activated by slg cross-linking express CD5 while those activated by LPS (TI-1) or a cognate interaction with T cells (TD) do not, provides an explanation for these xid defects. We propose that when surface Ig is cross-linked on xid B cells, activation is aborted: B cells enlarge and show increased CD44 but fail to express CD5. Unlike normal B cells, anti-Ig activated xid B cells die within three days of stimulation. Thus, while xid B cells can respond to TD stimulation, TI-2 antigens produce clonal abortion. B cells with specificity for common TI-2 antigens will be deleted, resulting in an altered xid Ig repertoire. This model provides an explanation for the observation that expression of xid results in an abrogation of autoantibody production.

Whether the failure to express surface CD5 is critical to this abortive activation or is a distal event is not known. In fact, the underlying defect in xid mice is still unknown. Our favored hypothesis is that xid B cells lack the ability to make or respond to an autocrine viability (e.g. IL-10) or growth factor. As a result, xid B cells are extraordinarily dependent on T cell-derived factors.

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Some biological characteristics of highly metastatic human carcinoma in nude mice

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Transfection of high-molecular-weight genomic DNA from a highly metastatic human lung carcinoma (PG) grown in nude mice was shown to confer the metastatic phenotype on NIH 3T3 recipient cells. A DNA library was constructed with DNA from one of the transformed cell lines, l-3-1, screened with human repetitive sequence, and its subclone, pLC-2, showed transforming activities on NIH 3T3 cells. The transformed cells produced fast-growing fibrosarcomas after subcutaneous injection into nude mice. The positive signals were obtained both on chromosomes of PG cells and TF-87210 cells, by chromosomal in situ hybridization using 1-3-1 and pLC-2 DNA as probes. pLC-2 sequence was compared with Genebank, and no significant homology was revealed, suggesting that a new transforming sequence, which is associated with the human metastatic tumor cells, has been identified.

Laminin receptors (LN-R) were isolated from PG tumor cells. The monoclonal antibodies to LN-R (McBj) could markedly inhibit the attachment, spread, migration, and proliferation of PG cells in vitro, and experimental metastasis of PG cells in nude mice. The monoclonal antibodies to LN-R are definitely useful to study the interaction between tumor cells and extracellular matrix, and the mechanisms of cancer metastasis.
LAK cells isolated from healthy people were used to inhibit experimental metastasis of PG cells in nude mice, and showed effective results.

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Growth and metastatic behavior of human tumors xenografted in nude and SCID mice

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We investigated the growth characteristics and metastatic behavior in nude and SCID mice of three human tumor cell lines and a human colon carcinoma previously passed in nude mice.

The three human tumor lines included a bladder carcinoma (T24B), a malignant melanoma (RPMI 7931) and a breast cancer (MDA-MB-43 5). Adult (7–10 weeks) NMRI nu/nu and C.B17-scid mice were inoculated with $5 \times 10^5 - 5 \times 10^6$ tumor cells subcutaneously. Comparable take rates, latent periods, and growth rates of implanted tumors were observed in nude and SCID mice for each of the cell lines tested. At the time of autopsy, which varied from 6 to 11 weeks after tumor inoculation, a significantly higher incidence of lung metastasis was discovered in SCID mice (96 percent) than in age-matched nude mice (28 percent, total $P < 0.01$). The difference is especially distinct for the bladder carcinoma and melanoma cell lines, which developed lung metastasis in 19 out of 19 SCID mice (100 percent), but only in three out of 26 nude mice (12 percent). The metastatic nodules were mainly distributed in the peripheral area of both left and right lungs. No evidence of metastasis in lymph node or any other organs was found. The intermediate differentiated human colon carcinoma, which was low invasive and non-metastatic during serial passages, developed metastasis in neither SCID nor nude mice. In vitro NK cell activity of adult NMRI nu/nu and scid mice was tested against YAC-1 mouse lymphoma cells. SCID mice demonstrated a slightly higher NK activity than nude mice, but the difference is not statistically significant ($P > 0.05$).

Our results suggest that the xenografted human tumor lines grow equally well in nude and SCID mice, though SCID mice suffer more severe immunodeficiency. However, since the intrinsic metastatic capacity of human tumor cells is fully expressed in SCID mice, they may provide an advantageous model for studying human tumor metastasis.

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Studies on SDI chemosensitivity patterns of surgically resected tumors before and after transplantation into athymic nude mice

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We studied chemosensitivity patterns of surgically resected tumors before and after transplantation into athymic nude mice using the succinate dehydrogenase inhibition (SDI) test. Tumor specimens were obtained from patients with gastric, colorectal and mediastinal cancers. One $10^3$ dissociated viable cells obtained through mechanical and enzymatical treatment were incubated with four anticancer agents for 4 days under serum-free conditions. Test results for rectal and mediastinal cancer specimens showed that the most sensitive agents to tumors immediately following resection were identical to those sensitive to the tumors after the transplantation into nude mice. The most sensitive drugs shown by the in vivo test using nude mice also coincided with the in vitro results.

In contrast, a change in sensitivity pattern was found for a gastric cancer specimen. While all four agents tested were ineffective when the SDI test was applied on the fresh specimen, three out of the four agents were effective to the tumor after transplantation.

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Metastatic behavior of murine and human cancer cells in nude mice

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An experimental model of hepatic metastasis was established in BALB/c athymic nude mice (nu/nu) and their immunocompetent littermates (nu/×). Three weeks following intrasplenic injection of $5 \times 10^3$ cells of colon-26, a syngeneic murine colon carcinoma, numbers of hepatic foci were counted. All the nu/+ mice developed hepatic metastases, whereas no metastasis was observed in nu/nu mice, in which natural killer (NK) activity was significantly higher than that of nu/+ mice. Suppression of NK activity by the administration of anti-asialo GM1 antibody increased the number of hepatic metastases both in nu/nu/ and nu/+ mice. It was also found that the intraperitoneal injection of a streptococcal preparation (OK-432), a biological response modifier, inhibited metastasis in nu/+/ mice.

The metastatic behavior of human carcinomas in athymic nude mice will also be discussed.

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Research on human tumor-killing cells derived from peripheral blood and tumor tissues

Jie Zheng, Bingxue Zhang, and Bing-Quan Wu

Mononuclear cells were isolated from peripheral blood, spleen, and tumor tissues through single density gradient or double discontinued density gradient centrifugation. The isolated lymphocytes were cultured in vitro with recombinant human interleukin-2 and became LAK cells or activated TILs. LAK cells, either from healthy donors or cancer patients, showed similar cytotoxicity in vitro and in vivo, on autologous as well as allogeneic fresh tumor cells and cultured tumor cell lines. LAK cells could be propagated up to $2 \times 10^3$-fold expansion and exhibited the same biological activities. TILs from various human cancers showed strong lytic effects on autologous fresh tumor cells, but weak lytic effects on allogeneic tumor cells. LAK cells were able to inhibit PG (a highly metastatic human cancer cell line) cells to colonize in host lungs after tail vein injections. Phenotypic analysis demonstrated that LAK cells from different cancer patients were not uniform in percentages of OKT3, OKT4, and OKT8 cells, but for TILs OKT3 cells became predominant in the process of cell proliferation in vitro. There was OKT4/OKT8 ratio inversion in both LAK cells and TILs after long-term culture in vitro. LAK cells and TILs have been further proven as two different kinds of tumor-killing effectors with different antitumor spectra, although they may share some common biological and morphological characters.

The breeding of T, B, and NK cell immunodeficient PBI/3-xid-bg-nu mice

Pinren Zhong

Starting in 1979, we bred PBI/1 nude mice, T and NK cell-combined immunodeficient congenic C57BL/6PBI-bg-nu mice non-congenic (615 x B6) PBI/2-bg-nu mice. We used PBI/2-bg-nu mice as donor mice, and CBA/N mice with low B cell function as receptors. Through cross-backcross and back cross-intercross systems and test-mating, we introduced bg and nu genes from PBI/2-bg-nu mice, and successfully bred T, B, and NK cell triadic immunodeficient PBI/3-xid-bg-nu mice.

It has been proven that the PBI/3-xid-bg-nu mice are nude, have no thymus and have prolonged blood coagulation. These characteristics are specified of their parents.

The NK cell activity of PBI/3 xid-bg-nu mice is equivalent to that of PBI/2-bg-nu mice, but is significantly lower than that of PBI/1-nu mice. Con A-induced proliferation in PBI/3-xid-bg-nu mice is lower than that in 615/PBI inbred mice, and equivalent to PBI/1-nu mice.

The EAC cell percentage and LPS-induced proliferation in PBI/3-xid-bg-nu mice are significantly lower than PBI/2-bg-nu mice. The SmIg percentage in PBI/3-xid-bg-nu mice is about 2/3 of that in PBI/2-bg-nu mice.
