IMMUNODEFICIENT ANIMALS FOR CANCER RESEARCH

Edited by
Stephen Sparrow

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MRC Laboratory Animals Centre,
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Preface

Since 1961 when Miller demonstrated that neonatal thymectomy of mice prolonged the survival of allogeneic skin grafts, the immunodeficient animal has played a major role in our understanding of immunity. It has also made xenografting possible. The contribution of these animals to cancer research cannot be ignored.

The occurrence of the mutant gene *nu* in the mouse and the subsequent discovery of its effect on the thymus added new impetus to work on xenografting. The nude mouse has, in the past decade, become the most widely used mouse mutant overshadowing other immunodeficient animal models.

In organising the symposium 'Immunodeficient Animals for Cancer Research' an attempt was made to review the use of the many different animal models, to identify their limitations and to suggest ways of overcoming some of the limitations. The answers may well lie with the geneticists and their manipulations rather than the surgical techniques that Miller initiated but there seems little doubt that immunodeficient animals will continue to have a profound effect on our understanding of cancer.

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Immunodeficient animals

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The idea of implantation of human tumours into experimental animal hosts is not new. It is, in some senses, an extension of the many ways in which transplantation of animal tissues and organs to the human species has been attempted as replacement therapy. The object in relation to tumour xenografting is to prolong the life of the tumour in such a way that it can be further studied or its susceptibility to attack determined.

In recent years such explorations have become more feasible with the discovery of a function for the thymus in relation to the development of immunological responsiveness. It seems that this organ produces in vertebrates a population of T lymphocytes without which the capacity to resist infection or, more artefactually, tumour xenografts is usually reduced. Most work has been done in mammals and the purpose of this brief review is to determine what we can predict about various kinds of immunologically incompetent rodents in relation to their capacity to support the growth of tumour xenografts.

The immune response is usually taken as a property of vertebrate animals which has two defining characteristics. First, it represents a specific and often prolonged response to antigenic stimulation and, second, it is specifically anamnestic in that responses to second or subsequent contacts with an antigen may be quicker or more substantial than the response to first contact with the same antigen. There is some circularity in this definition which, perhaps, arises from the biologically strange stimuli often adopted by immunologists to evoke their favourite response. Often a non-living antigen is given once and the response to this apparently acute stimulus is followed over a period of days. In such circumstances it is not certain whether the immune response observed represents a continuation of a response which requires the persistence of the antigen or whether the response proceeds by recourse to some molecular memory independent of the presence of the antigen itself. Equally the quicker and larger responses which often follow 'recall' injections of antigen can be thought of as either due to augmentation of an ongoing response or evocation of a quiescent but specific memory or both. Whilst such considerations may be deemed irrelevant to much of the activities of immunologists, responses to many tissue and organ grafts clearly involve persistent antigen and its role in relation to the course of the response observed is not easily predicted on any *a-priori* grounds.

A distinction is often drawn between cell-mediated and humoral immunity, recognising two apparently different modes of response based on effector cells and effector antibody molecules respectively. The demonstration that, *in vitro*, sensitisation of target cells by specific antibody may facilitate attack by other-

wise non-specific killer cells tends to blur the absolute distinction between the two modes of response. It should, however, be remembered that K cells perhaps do not operate *in vivo* and *in vitro* in the same way—it is difficult to be sure. But, putting aside for the moment such considerations, the distinction between cellular and humoral immunity relates also to the belief that cellular mechanisms are concerned with rejection of cellular targets and that, whatever the significance of antibody molecules, they are insufficient in themselves to do the same. Such considerations arise largely from studies of skin homograft rejection in which it has been demonstrated, with some dissenting opinion, that lymphoid cells but not humoral isoantibody are effective. The collateral finding that T lymphocytes *in vitro* seem capable of exercising specific cytotoxicity reinforces the belief that in the homografted animal a comparable mechanism is exercised to bring about the demise of the foreign graft.

In the present context the question is as to whether the ground rules just stated for homografts apply to xenografts. The answer is probably yes, with the proviso that xenografts may be more sensitive to rejection by antibody molecules alone than are homografts (Baldamus *et al.*, 1973). It should also be remarked that graft rejection can occur in many living organisms which have no recognisable specific immunity. This suggests that recognition and repulsion of foreign material need not necessarily require the whole weight of the immunological apparatus. Such considerations may be particularly germane to xenografts in which it is easy to envisage physiological incompatibilities between graft and host which will result in graft failure.

For all these uncertainties, if there is a basis for prediction of the suitability of a particular group of animals to act as hosts for malignant xenografts it is probably on their putative numbers of T lymphocytes which are to be estimated numerically in various ways.

For example adult CBA mice which have been thymectomised and irradiated with 850 rad prior to injection of 5×10^6 syngeneic bone-marrow cells have about 15–20 per cent of the normal numbers of T lymphocytes 30 days later (Doenhoff, 1970). This count was made by the use of an anti-Thy 1 antiserum or by determining the numbers of cells responding to phytohaemagglutinin. The two estimates were similar. Furthermore it was shown that T cells present were derived—as far as could be determined—one-third from the host animal and two-thirds from the bone-marrow donor. Apart from finding that bone-marrow transference carries with it post-thymic T cells and that at least some T cells or their precursors can survive very heavy doses of irradiation, there are two further problems. First, the immunological response of these 'T' cell-deprived mice is much less than 15 per cent of normal (Leuchars, 1971) and, second, it is difficult to see where the 30×10^6 or so cells that are present have come from anyway. Fewer than 1 per cent of the injected bone-marrow cells, that is $< 5 \times 10^4$ cells are Thy positive and yet there seems to have been generation of 2×10^7 such cells over a 30-day period. It could be that Thy 1 positive cells derive from Thy negative post-thymic precursors or, alternatively, it must seem that T lymphocytes have a considerable capacity for generating T lymphocytes outside the thymus.

Whatever the answer to this minor lymphocyte conundrum the T cells of deprived mice do not seem to be equivalent to those in normal mice. Thus T cell

quantitation *per se* may not help to give a predictive basis for xenograft acceptance. It can, however, be predicted that higher radiation to the host, T cell removal from the bone-marrow inoculum, or reduction in numbers of bone-marrow cells injected should all reduce the numbers of T lymphocytes in the resulting animals. It should be remarked *en passant* that strains of mice differ in the proportion of T lymphocytes in their marrow and thus not all kinds of syngeneic radiation chimaera will be equivalently T cell deprived by what are logistically comparable schedules of treatment.

Nude mice are said completely to lack T lymphocytes in that their thymus does not function properly during foetal life. It seems that the organ develops properly for about 15 days and comes to contain the large basophilic stem cells which in an appropriate thymus undergo proliferation and differentiation to form thymocytes. In nude mice this further step does not occur and the nude animals therefore lack post-thymic T lymphocytes. It has however been claimed, and it is widely believed at the present time, that there are stem cells in the bone-marrow and probably spleen of mice which are destined to go to the thymus and have been referred to as prothymocytes (Haran-Ghera *et al.*, 1978).

The evidence for this view is largely that there are various attributes of thymocytes, particularly the presence of a terminal transferase enzyme which is found on some bone-marrow cells (Baltimore *et al.*, 1976). This finding is consonant with the view that the enzyme property is retained by the putative prothymocyte when it reaches the thymus. The other evidence is that a variety of thymocyte characteristics can be induced in bone-marrow cells by incubation with various agents – for example, thymosin or cyclic AMP. The authors of these experiments suppose that the induction is simply precocious revelation of characteristics which would have appeared when the 'prothymocytes' reached the thymus. Others have argued that there is an accumulation of prethymic cells in nude mice because they are unable to go to the thymus and enact their differentiational destiny (Loor, 1977). Arguments such as these are persuasive, but the demonstration that selective removal of these precursor cells engenders a bone-marrow which is specifically incapable of repopulating the thymus when injected, after such an insult as total body irradiation, has yet to be made.

I prefer to believe that prothymocytes have no existence independent of general haematopoietic precursor cells, but this is my prejudice and I could be wrong. The point about all these considerations of the T cell status of immunodeficient mice is that there are very considerable disagreements of opinion among the experts as to how to estimate it and what do the estimates mean. If prothymocytes do exist in nude mice and if they are readily inducible to form functional T lymphocytes then they represent a further complication which the xenografter may eventually have to take into consideration.

Thus within certain limits the best way of determining whether a particular human tumour will or will not grow in a particular kind of deprived mouse is to try it, bearing in mind that failure is not necessarily due to exercise of a specific immunological defence mechanism. It is more important that whatever animal is used in xenografting it should be sufficiently robust to withstand the grafting procedure and should, in addition, be so housed as to facilitate its survival free from pathogenic infection.

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2
Inherited immunological defects in laboratory animals

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INTRODUCTION

The immune system of mammals has evolved as one of the most important protective mechanisms against infection by parasites, bacteria and viruses, and possibly also as a protection against malignant clones arising as a result of somatic mutation, that is as a mechanism for immune surveillance. It is an extremely complex system, as might be expected in view of the very great range of different parasitic microorganisms that may attack an individual, and it is clear that within any one species there is great genetic diversity overlying the normal immunological characteristics of the species. This genetic variation takes three forms. First, there is a wide range of polyorphism at certain genetic loci such as those concerned with alloantigens. Second, many aspects of the immune system are clearly under polygenic control. Third, there are pathological mutations which would not normally survive in natural populations unless there are strong forces acting to maintain them (as is found in the case of the lethal *t* alleles). The aim of this paper is to review the nature and extent of genetic variation in the immune system in laboratory mammals, and to consider how these mutants and variants may be used in research both with regard to clarifying the role of the immune system in protection against cancer, and with a view to improving the success of xenografts of human and other tumours.

Normal Variation in the Immune System and the Production of Defective Individuals

The great range of genetic variation in the immune system probably arises from the very close struggle between host and parasite during evolution with a genetic change in the host conferring resistance to one class of organisms quickly being countered by genetic changes in the parasites. A wide range of polymorphisms at alloantigen, immune response and other genetic loci are known, which probably arose under the influence of such selective forces. These are summarised in Table 2.1. Little is known about the exact function of many of these loci. Even the function of the H-2 complex of the mouse, which has been studied intensively for more than 40 years, is still obscure, though it is known to influence a wide range

Table 2.1 Examples of some polymorphic genetic loci influencing the immune system of the mouse

Gene symbol	Name and comment
Alloantigen loci	
H-1 to H-38	Histocompatibility loci. The H-2 locus or complex is particularly polymorphic, and has a profound influence on many aspects of the biology of the species.
T, t^x	The 'tailess' or brachyury complex. Many alleles in five complementation groups. Recessive homozygotes usually die *in utero*. Complex may influence cell surface alloantigens essential for normal development.
Ea-1 to Ea-8	Erythrocyte alloantigens, function unknown.
Ly-1 to Ly-8	Lymphocyte alloantigens, function known.
Ia-1 to Ia-3	I-region associated antigens form part of the H-2 major histocompatibility complex.
Tla	Thymus leukaemia antigen. This is a complex locus with at least three haplotypes and is linked to the H-2 complex.
Thy-1	Theta governs an alloantigen specific to thymus-derived lymphocytes and related cells.
Mls	Mouse minor MLC-stimulating locus (formerly M locus), associated with mixed lymphocyte reaction activity.
Lad-1 to Lad-4	Lymphocyte activating determinants. Several loci within the H-2 complex concerned with the mixed lymphocyte reaction.
Ig-1 to Ig-4	A set of loci determining variation in the constant chain of the immunoglobulin molecule, that is immunoglobulin allotypes.
Virus susceptibility†	
Fv-1 to Fv-2	Determines susceptibility to Friend leukaemia virus.
Rgv-1, 2	Determines resistance to Gross virus.
Rv-1, 2	Determines susceptibility to Rauscher leukaemia virus.
Mlv-1	Determines susceptibility to murine leukaemia virus.
Mtv-1, 2	Governs induction of mammary tumour virus.
Other loci	
Hc	A locus governing a C5 complement polymorphism.
Ir-1 to Ir-5	Several loci controlling immune responses. These may be linked to the H-2 complex (Ir-1) or may be unlinked (Ir-2).
Ss	A locus within the H-2 complex controlling a serum protein now known to be the C4 component of complement.

†It is not clear to what extent the loci in this category should be regarded as being of 'immunological' interest.

of characteristics including immune responses, life-span and some aspects of reproductive behaviour (Klein, 1975). Whether its role in controlling cell-surface antigens is purely coincidental, or whether these antigens are a vital part of the functioning of this genetic complex is not clear. However, it is known to be associated with resistance to some oncogenic viruses with different haplotypes conferring different levels of resistance (Tennant and Snell, 1968).

Defective individuals can arise in two main ways, as illustrated in figure 2.1. First, they can arise by the chance association of normal polymorphic alleles which interact or combine to produce a defective phenotype, or, secondly, they may arise as a result of a pathological mutation which influences the immune system. Many such mutations affect the immune system as a secondary or 'pleiotropic' effect of the mutation. For example, pituitary dwarf mutants have a defective cellular immune system presumably because normal development of the thymus depends on a normal output of pituitary hormones.

In the case of polygenic characters a 'threshold' mode of inheritance can give rise to defective individuals. In this case, there is an underlying continuous variation for a character determined by many genes, but above (or below) a certain threshold level the extreme individuals are defective.

Many other aspects of the immune system are controlled by polygenic systems, that is they are under the genetic control of many different loci, though these loci have not yet been individually identified. Evidence for such complex genetic con-

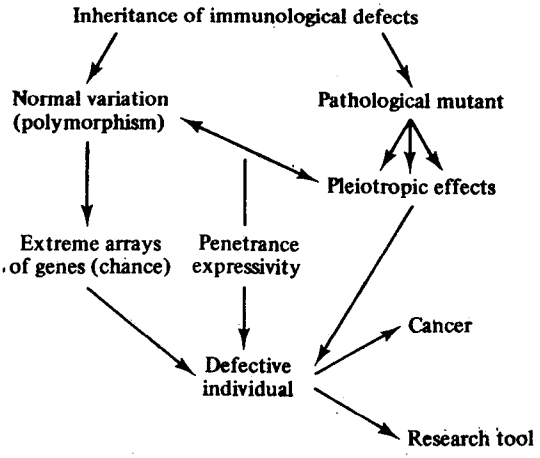

Figure 2.1 Mode of inheritance of immunological defects. Such defects can arise via the chance association of extreme arrays of 'normal' polymorphic alleles (shown on the left) or as a result of pathological mutations. The latter will have a number of pleiotropic effects which may affect various parts of the immune system. The penetrance and expression of these pathological mutations may be influenced by normal polygenic variation and environmental factors. Once a defective individual has arisen, it may give information about the biology of cancer, or it may be useful as a tool in research, for example as a medium for growing xenografts.