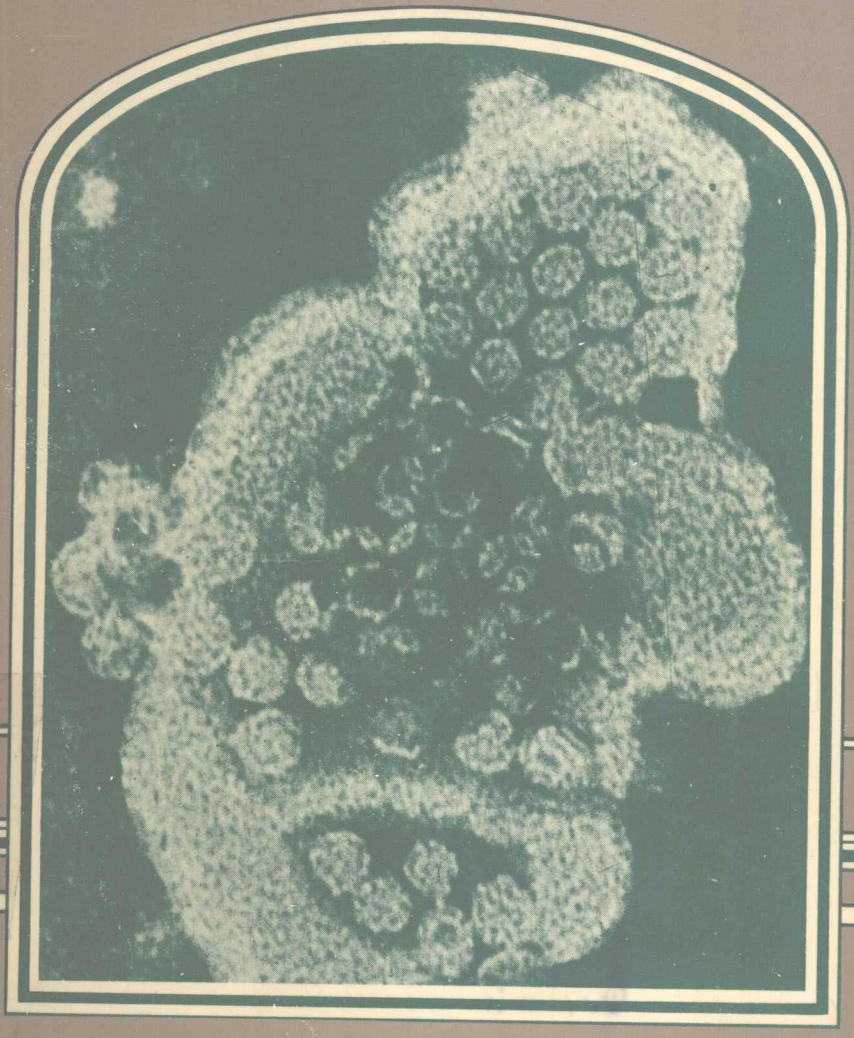
Immunological Tolerance and Enhancement

Edited by F. P. Stuart and F. W. Fitch



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Preface

The distinctions between classic immunological tolerance and enhancement began to blur in the early 1970s with the demonstration of serum blocking factors and cellular immunity in animals thought to be tolerant. Some suggested that tolerance and enhancement were part of a single spectrum. Gradually, however, as the criterion of bone marrow chimaerism was reapplied to the definition of classical tolerance, it seems again that tolerance and enhancement (or incomplete tolerance) are distinct entities. Part of the purpose of this volume is to review and reaffirm those distinctions.

Three areas of investigation that impinge both on tolerance and enhancement have developed very rapidly in the past 5 years. First is the role of I-region associated antigens within the major histocompatibility complex on the immune response and induction of enhancement and tolerance. Second and third are the role of suppressor lymphocytes and anti-receptor or anti-idiotypic responses in induction and maintenance of selective immunological suppression. Hopefully, these new areas of research will be placed in perspective and unresolved issues will be highlighted. Cancer is reviewed as an example of spontaneous tolerance or enhancement in which selective suppression of an effective response to tumour antigens is maintained, to the detriment of the host. The regimen consisting of antilymphocyte serum and donor marrow cells is reviewed as an approach to enhancement that employs both broad and narrow immunological reagents to achieve enhancement. Finally, an attempt is made to point out the areas in which basic research with small laboratory animals is likely to lead to clinical application.

Emphasis throughout the volume is on tolerance and enhancement with respect to histocompatibility or transplantation antigens rather than to haptens, serum proteins, and other antigens not directly related to tissue and organ transplantation. We intend the volume to be of interest to investigators engaged in these specific research areas, but a special effort was made to provide readable, comprehensive, and well-referenced reviews for those who are only tangentially involved with the subject matter. We hope that the volume will be helpful to immunologists and clinicians who have a keen interest in the immunobiology of selective immunosuppression and organ transplantation.

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Tolerance and the fate of the allogeneic bone marrow chimaera

W. L. ELKINS

INTRODUCTION

In 1956, Billingham, Brent and Medawar demonstrated that a state of lymphohaematopoietic (LHP) chimaerism could be induced by the injection of allogeneic bone marrow (BM) into neonatal mice, and that this chimaerism conferred specific tolerance for skin grafts from the donor strain[1]. Subsequent experiments in several laboratories revealed that LHP chimaerism could also be induced in lethally irradiated or cyclophosphamide-treated adult recipients[2–4], and in recent years we have seen the emergence of therapeutic allogeneic BM transplantation in man[5].

The existence of healthy long-term allogeneic BM chimaeras indicates that the potent responses which underlie normal resistance to allogeneic transplantation can be set aside without necessarily causing generalized immunodeficiency, and it is generally accepted that some form of immunological tolerance underlies allogeneic LHP chimaerism. Transplantation tolerance has recently been the subject of an excellent review[6], so I have elected to focus somewhat more narrowly upon the factors which influence the stability of postnatally-induced allogeneic BM chimaeras. Because of the complexity of the subject-matter, considerable effort is devoted to defining terms and identifying variables before considering the nature of tolerance in the allogeneic LHP chimaera.

VARIABLES IN THE ESTABLISHMENT OF STABLE CHIMAERISM AND TOLERANCE

In general, there is an intimate and circular relationship between the establishment of LHP chimaerism and tolerance, i.e., the establishment of the foreign LHP cells is required to induce and maintain tolerance in the host, and, over a period of time, the persistence of LHP chimaerism is generally indicative that tolerance exists. Nevertheless, immunological tolerance and stable chimaerism are not synonymous terms, and some of the variables affecting the outcome of allogeneic LHP transplantation affect primarily the establishment of chimaerism, while others may affect primarily the induction of tolerance. This distinction may be of importance in the interpretation of experiments which attempt to elucidate the nature of transplantation toler-

ance, especially where suppressor cell mechanisms are concerned. Thus there appear to be cellular mechanisms which 'suppress' the establishment and/or function of inoculated lymphocytes without regard for their specificity, i.e. suppression of chimaerism on one hand, and cellular mechanisms which suppress the function of a special sub-set of lymphocytes with specificity for antigen, as in the 'infectious' forms of tolerance, on the other. Specific examples where these considerations pertain are indicated below.

The subject of tolerance in allogeneic BM transplantation has become even more complex as we learn more about the several variables which determine its outcome. Thus, it is necessary at the outset to consider the barriers which need to be breached and the factors which determine the difficulty of this task. In the rest of this section I shall review briefly natural or genetic resistance and adaptive immunity as they relate both to host-versus-graft (HVG) and graft-versus-host (GVH) reactions.

The syngeneic barrier

The induction of syngeneic LHP chimaerism is controlled by a radiosensitive barrier, the nature of which is poorly understood. It seems, however, that exogenous HP stem cells do not compete favourably with endogenous stem cells[4,7], possibly because the latter already occupy the physiological niches in the micro-environment of haematopoietic tissue in which proliferation and differentiation is permitted. This physiological regulation is considered by some to be mediated by specialized 'managerial' cells in the stroma[8,9].

A syngeneic barrier also inhibits the incorporation of adoptively transferred lymphocytes into the functional network of the immune system[10–13]. This barrier is sensitive to radiation[10,11], is age-dependent[10], and in some cases is actively mediated by 'suppressor' T-cells[12,13]. Presumably suppression in this context has little in common with the active suppression which determines some forms of immunological tolerance, i.e. 'infectious tolerance' to use Gershon's term. The syngeneic barrier is mentioned here because it is a probable variable in some experiments dealing with adoptive abrogation of transplantation tolerance (see pp.15–19) and to illustrate the point that there are variables peculiar to the induction of LHP chimaerism which would not affect the induction of immunological tolerance to *non-cellular* antigens.

Genetic resistance to allogeneic bone marrow and lymphocytes

Engraftment of allogeneic HP cells in lethally irradiated mice is regulated by a form of resistance which is different in many respects from 'conventional' histoincompatibility. The response is variable as the strain combination is changed, but under rather well-defined genetic control. It is directed in part to non-codominantly expressed 'haematopoietic histocompatibility' (Hh) 'antigens' coded predominantly in the *H-2D* region[14–16]. It is thought to be mediated by T-independent, BM-derived cells[17], closely related to the 'natural killer cells' which can spontaneously kill certain *H-2* disparate allogeneic

lymphoma targets *in vitro*[18]. It can be overridden by increasing cell dosage.

Resistance to *neoplastic lymphocytes* derived from one parental strain is also manifest in irradiated and unirradiated F_1 hosts[19,20]. Recent work suggests that the 'antigens' which are targets of this resistance are coded in the *K* and *I* as well as the *D* region of *H-2*[20]. These observations indicate that HP stem cells are not the only cell type subject to genetic resistance.

There is also a possibility that natural or genetic resistance inhibits the establishment or function of *normal lymphocytes* in allogeneic or hemi-allogeneic hosts. It is possible that this is one reason for the failure of allogeneic thymic, fetal liver, and BM grafts to become established in children suffering from congenital immunodeficiency states in which T-cell function had been undetectable[21,22].

If there is natural resistance to normal T-cells, one might expect it to be a factor in resistance to GVHR. It has long been known that pre-treatment of adult F_1 hybrid rodents with whole body radiation or cyclophosphamide greatly facilitates the induction of GVHR[23,24,25] following the injection of P-lymphocytes. Moreover, in one study, the intensity of the proliferative GVH response of donor T-cells appeared inversely related to the degree of natural hybrid resistance to haematopoietic engraftment[26]. There is no direct evidence, however, that Hh or similar non-codominantly expressed antigens on T-cells are involved in resistance to GVHR. On the other hand, there is increasing evidence, discussed below, that idiotypic structures on GVH-mediating cells elicit an adaptive T-cell response which confers GVH resistance.

Adaptive immunity to H alloantigens

In the establishment of stable LHP chimaerism, the potent forces of *adaptive* immunity to foreign cells must also be abridged. Unlike natural resistance, the target antigens of adaptive alloaggressive responses are, so far as is known, codominantly expressed, and the primary response is radiation-sensitive and thymus-dependent. The cytotoxic T-lymphocyte (CTL) is thought to be the primary effector cell.

Furthermore, the adaptive response is *not* limited to detection of differences coded in or near the MHC complex, as currently appears to be the case for natural or genetic resistance[5,27–29]. Some of the characteristics of the natural and adaptive components of alloaggression are summarized in Table 1.1.

Depending upon immunogenetic and other circumstances, the adaptive response may occur in the host-versus-graft (HVG) or graft-versus-host (GVH) direction, and also bidirectionally[25,29]. In the case of complete MHC differences, a very high percentage (5–20%) of the T-cell repertoire is capable of responding. This response is complex, being composed of different sub-sets of cells which have followed different pathways of development and perform different roles in the response, but which also interact *inter se*, either to amplify or suppress the immune response[30–32]. There are, moreover, different loci within the MHC, the products of which preferentially stimulate functionally different sub-sets of T-cells[19,32,33]. It is thought that the intensity of allo-

Table 1.1 Distinguishing features of two systems mediating alloaggression in the mouse

	Natural	*Adaptive*
Target antigens	Hh (non-codominant)	*H-2* and '*minor*' antigens (codominant)
Hetero.-versus-homozygote response	Yes	No
Homo.-versus-heterozygote response	No	Yes
Effector cells	Bone marrow-derived nul cells, macrophages (natural killers)	T-lymphocytes
Sensitivity to anti-macrophage agents	Yes	?
Effect of lethal total body irradiation	Slight	Strong
Rejection of skin grafts	No	Yes
Elicitation of GVHR	?	Yes

aggressive responses across MHC barriers is related both to T–T collaboration and the high frequency of responsive T-cells in normal animals to antigens coded in foreign MHC.

In the absence of MHC differences, both HVG and GVHR may be elicited by so-called minor H antigens. The adaptive response to these both *in vivo* and *in vitro* is often dependent upon prior sensitization of the responder[34–37].

When we speak of tolerance in connection with allogeneic chimaerism, we usually mean specific abrogation of the adaptive response, i.e. GVHR, HVGR, or generation of CTL with specificity for the set of alloantigens by which the donor and host differ. It should be noted that 'tolerance' for allogeneic BM grafts can also be induced in the genetic resistance system[38,39], but such tolerance is not further discussed here, since so little is known about it.

Graft-versus-host reaction and graft-versus-host tolerance

So much of this review deals with tolerance of graft for host that it is appropriate that we further define the nature of GVHR. When peripheral lymphocytes are injected into an animal possessing foreign MHC determinants, and when the animal is unable to immediately reject the inoculated cells, a GVHR ensues. This can be recognized and measured either by monitoring the proliferative response of donor lymphocytes to the antigens of the host[26,32,40], or by measuring the hyperplastic response of the lymphoid organs of the host to the GVHR[25,40,41]. Obviously, the first approach is more direct, but the second is more readily subjected to quantitative analysis, and both are valid when properly controlled. The *in vitro* analogue of the first type of GVH assay is the lymphocyte reaction in mixed leucocyte culture (MLC).

T-cells of donor origin, which are specifically cytotoxic for host cells, can

be detected in the spleens of hosts undergoing GVHR[41a], and presumably these cells are important effectors of lymphoid tissue injury in the host. An *in vitro* model of this component of the GVHR is the cell-mediated lympholysis (CML) assay[33]. There is also activation of macrophages to a state of non-specific cytotoxity during GVHR[42]. Presumably this results from the elaboration of lymphokines by the responding T-cells, and several examples of 'innocent bystander' effects can be attributed to this component of GVHR[29,41].

Mortality studies are the least direct, but perhaps the most clinically relevant, of the various GVH assays. From an immunogenetic standpoint, the least equivocal of these utilizes newborn or sub-lethally irradiated adult F_1 hosts injected with parental strain lymphoid cells. Death can thus be attributed to the noxious effects of the inoculum provided the controls survive[30,43]. Mortality in *lethally* irradiated BM chimaeras is less certainly attributable to GVHR even when syngeneic controls survive. Rather it may be due to failure of immunological or haematological reconstitution.

GVHR trigger in some complex way the clinically apparent syndrome and set of lesions which may be called GVH disease (GVHD) (or, more non-committally, allogeneic disease). Environmental factors, particularly the microbial flora of the gastrointestinal tract, are important determinants of the severity of GVHD in mice[44,45]. The GVHR may also activate latent viruses, trigger autoimmune responses, and induce immunodeficiency, all of which can cause or contribute to the pathogenesis of GVHD[41,46,47]. Thus, the presence of GVHD need not indicate that the primary GVHR is occurring concurrently. The latter may simply be a relatively ephemeral trigger mechanism for a set of secondary pathogenetic processes mediated by different effectors and possessing a specificity different from that of the initiating GVHR. It follows that GVHD might develop even as the GVHR itself is subsiding and GVH tolerance is becoming established. In support of the above, considerable evidence exists that the GVHR in laboratory rodents is a self-limited process which rarely lasts longer than 2–4 weeks[29,41]. GVHD is often later in onset and longer-lasting.

The existence of GVH tolerance is revealed by removing lymphocytes from a chimaera and testing their competence to initiate GVHR or GVHD and/or to respond in MLC and CML *in vitro*. Tolerance is indicated by a diminished response to specific cellular alloantigens, while reactivity to other antigens, i.e. from a third strain, persists at the normal level. Studies of GVH tolerance based on these tests are emphasized in subsequent discussion of the cellular basis of tolerance.

Importance of the post-thymic T-cell pool

Both GVHR and HVGR to 'conventional', i.e. codominantly expressed, H-alloantigens are T-dependent responses in laboratory rodents. The capacity of foreign cells to induce tolerance in either response may be limited to an important, but imprecisely defined, degree by the number of post-thymic T-cells available to be triggered during the initial contact with antigen. Thus GVH tolerance among donor T-cells predominates over the GVHR when the graft is lacking post-thymic T-cells. The evidence for this is considered in ensuing

sections of this chapter, and mostly comes from studies with inbred rats and mice differing by MHC alloantigens. There is, unfortunately, no evidence as to the importance of post-thymic T-cells in alloaggressive responses to non-MHC alloantigens in the dog and man.

Anti-idiotypic immunity and GVH tolerance

One of the most exciting developments in modern immunobiology has been the development of Ramseier and Lindenmann's brilliant insight that the P donor cells mediating GVHR in an F_1 host might elicit a countervailing anti-receptor response from the host[48]. The specificity of this response for certain clones of attacking T-cells forms the basis for labelling it 'anti-idiotypic'.

This whole subject is reviewed by McKearn elsewhere in this volume, hence it is considered but briefly here. Several findings have emerged which are particularly relevant to the subject of this review:

1. The capacity of the adult F_1 to respond to P-lymphocytes does not depend upon prior immunization but appears within hours of a primary injection of P-cells[49].
2. The response is elicited by specifically reactive T-lymphocytes with specificity for MHC antigens of the host[50].
3. The response is mediated both by B-cells and by radiation-resistant T-lymphocytes of host origin[51,51a].
4. The anti-idiotypic effect is responsible, at least in part, for the limited duration of GVHR directed at MHC antigens[49].
5. The anti-idiotypic response is capable of preventing the development of GVHD in *irradiated* hosts provided that they are first primed with P-cells[51].

It thus seems likely that the capacity of the host to mount an anti-idiotypic response to the attacking donor T-cells is an important determinant of the onset, severity, and duration of GVHD. It follows that immunosuppressive treatment administered to a host prior to and soon after LHP transplantation is likely to aggravate GVHD if it inhibits the development of the anti-idiotypic response (or any other HVGR) more than the development of GVHR.

It also follows that the anti-idiotypic response is likely to be an important component in the development of GVH tolerance at least under some circumstances yet to be identified.

Can MHC heterozygous cells cause GVHD in irradiated homozygous recipients?

GVHR are generally thought to depend upon the activation of T-cells by codominantly expressed H antigens. However, since Shearer and Cudkowicz[39] have shown that F_1 lymphocytes can be induced to cytotoxicity by stimulation with Hh incompatible P spleen cells, it is possible that non-codominantly expressed Hh antigens in the host might trigger GVHR under certain circumstances.

It is thus worth noting that a syndrome resembling GVHD is seen when (BALB/c $\times$ C57BL/6) F_1 marrow or splenic cells from adult donors are injected into newborn mice of either parental strain[52]. The Hh barrier is

particularly strong in the case of the C57BL/6 hosts[15,16]. Other interpretations might be favoured, but it is conceivable than an F_1 anti-P GVHR occurs in response to Hh stimulation in this case. Moreover, recent studies suggest that GVHR may sometimes be elicited by non-codominantly expressed, MHC-linked antigens in lethally irradiated dogs. Storb and his colleagues encounter GVHD of unexpected severity when marrow from DLA heterozygous donors is transplanted to DLA homozygous sibs[53]. This GVHD is apparently not elicited by 'minor' alloantigens, since the latter elicit a syndrome more readily controlled by methotrexate therapy. It would be of interest to determine the T-cell dependence of these unexpected hetero-versus-homozygote responses in the dog.

Although these studies suggest that one cannot *rely* on genetic tolerance of graft for host MHC antigens to eliminate the hazard of GVHR following marrow transplants from MHC heterozygous donors to homozygous recipients, such tolerance might operate to reduce the risk of severe and acute GVHD in many donor–host combinations. Perhaps application of the CML assay, following the example of Shearer and Cudkowicz[39] will someday allow the risk of such transplants to be prospectively evaluated on an individual basis.

The 'strength' of the histocompatibility barrier

The distinction between major and minor H-antigen barriers is empirical and thus strongly influenced by the conditions and techniques of the experimental (or clinical) system. Originally, it was noted that *H-2* differences invariably caused acute rejection of skin and tumour allografts, whereas non-*H-2* differences provoked slower and more variable responses, especially to tumours[19]. Simonsen and others subsequently noted that the elicitation of GVHR depended upon differences in a single chromosomal region in each species, and that other differences elicited detectable responses only when the donor was presensitized[25,29]. Similar effects were then noted with the *in vitro* correlates of alloaggression, i.e. MLC and CML[33–37]. Hence the terms 'major histocompatibility complex' and 'strong H-antigen' have been associated with *H-2*, HL-A etc., and the other alloantigenic loci have often been designated 'minor' or 'weak'.

So far as is known, LHP chimaerism and tolerance are more easily induced both in man and mouse to minor alloantigen barriers than across the MHC[6,54,55]. Furthermore, partial MHC differences appear to be more readily transgressed by neonatal injection of LHP cells with resulting stable chimaerism than are complete MHC differences[6,56,57], and this is reflected in the ease of elimination of the precursors of specific CTL as detected in the CML assay[56,57].

There is an obvious paucity of studies in which congenic strains have been used to evaluate the capacity of restricted genetic differences to elicit tolerance. Evidence from the H-Y, and other 'minor' locus systems indicates that the apparent 'strength' of an H barrier is controlled by immune response genes of the responder as well as by the genetic locus (loci) at which the antigenic difference is determined[19,58,59]. Such findings suggest that there

may be genetic control of the capacity to be rendered chimaeric and tolerant by cells from a particular donor.

Immunological reconstitution

Alloaggressive reactions, such as GVHR, are of course not the only causes of morbidity in lethally irradiated allogeneic BM chimaeras. Successful immunological reconstitution by cells of donor origin is likely to be of critical importance to a successful outcome. Numerous workers have noted that immune function in long-established chimaeras is often defective[5,41].

A full exposition of the studies of immunodeficiency in radiation chimaeras is beyond the scope of this chapter. Most recently, however, Zinkernagel and his colleagues have shown that the thymic epithelium must share *H-2* determinants with stem cells of haematopoietic origin for the generation of T-cells capable of functioning in immunosurveillance against virus-altered lymphoid target cells[59a]. This important finding strongly indicates that even under conditions where alloaggressive reactions are controlled, haematopoietic transplants may fail to sustain life unless donor-type thymic epithelium is also transplanted, or unless the donor and patient share an MHC haplotype.

Multifactorial determination of chimaerism and tolerance

The establishment of stable LHP chimaerism depends upon the abrogation of alloaggressive GVH and HVG responses and successful immunological and haematological reconstitution. The likelihood of these happening is determined multifactorially through the interaction of the variables discussed above and subsequently. Having faced up to the complexity of our subject, we can now turn to available information concerning the nature of the immunological tolerance which underlies the health of allogeneic lymphohaematopoietic chimaeras; presumably just as does self-tolerance in the normal situation.

CLONAL SELECTION THEORY AND TOLERANCE

Models and concepts

Burnet and other proponents of the clonal selection theory have interpreted tolerance as a selective deletion of antigen-reactive cells from the repertoire of lymphocytes which is normally generated in the animal[60–62]. In this theory, tolerance for antigens or allogeneic cells has the same cellular basis as does tolerance for 'self'.

Nossal subsequently made the useful conceptual distinction between clonal deletion tolerance (in which mature, competent members of a clone are inactivated by contact with antigen acting as tolerogen) and clonal abortion tolerance (in which the members of a clone are subject to inactivation only at a relatively immature stage of differentiation)[60,61]. In the latter case, the normally responsive clone is aborted if, as in the case for some clones in

neonatal mice, most or all of its constituent cells are in this tolerance-responsive phase. On the other hand, if a portion of the clone has matured, contact with antigen will trigger immunity rather than tolerance at the level of the whole animal. Simultaneously, however, the immature, tolerance-responsive members of the clone will be inactivated as long as suitable exposure to antigen occurs[62]. Thus, tolerance might eventually develop at the level of the whole animal if the reacting component of mature cells lacked the capacity for self-renewal over a prolonged period, as might result from exhaustive sensitization or active suppression. This is a likely route to GVH tolerance in cases where a transient episode of GVHD occurs in recipients of allogeneic marrow.

Other important mechanisms of tolerance are:

1. receptor blockade, in which non-immunogeneic forms of antigen bind to the receptors of an immunocyte and thus inhibit the capacity of the cell to respond to immunogeneic forms of the antigen; and
2. activation blockade, in which the *specific* response of immunocytes is inhibited by antibody, antigen–antibody complexes, or the activity of suppressor T-cells[61].

An important distinguishing feature between clonal abortion–deletion on one hand and the blockade mechanisms on the other is the ease, rapidity, and route of recovery from tolerance. Blocked cells can recover individually to function within minutes to hours once removed from the blocking factors in their microenvironment, whereas clonal deletions are repaired more slowly by generation of fresh cohorts of reactive cells from immature precursors. The latter takes at least 4 weeks in the case of T-cells which must be processed through the thymus[63,64].

As originally conceived, clonal abortion (and deletion) resulted directly from the interaction of *antigen* and the tolerance-responsive cell. More recently an alternative mechanism has been delineated: that of idiotype suppression. When anti-idiotype serum (anti-receptor) is administered to neonatal mice, a persisting clonal deletion may result. By contrast, the same serum produces only reversible blockade when administered to *adult* mice[65].

Because of the increasing evidence, outlined above, that clones of T-cells responsive to MHC antigens may be strongly inhibited by anti-idiotypic responses, there is a strong possibility that such are at least partially responsible for the development of clonal abortions and/or deletions which underlie GVH tolerance, as well as other forms of transplantation tolerance. However, there is some preliminary evidence that anti-idiotypy is not a necessary component of tolerogenesis in LHP chimaeras (see below).

Evidence for clonal abortion in simplified systems

Recently, several basic studies have provided critical evidence for the classical clonal selectionist account of tolerance, and for the clonal abortion hypothesis in particular.

Studies by Stocker[66], and by Metcalf and Klinman[67,68] with lymphocyte cloning techniques have established that antigen is directly involved in the

induction of B-cell tolerance at the cellular level, and thus that anti-idiotypic antibody and other agents of tolerization such as suppressor T-cells are not necessary to tolerogenesis. They have also critically established the key point that immature precursors of B-cells are selectively tolerance-responsive, whether derived from neonatal spleen or adult BM.

San Fillipo, Cohen and Scott[69], have shown that pre-thymic T-cells, destined to differentiate as helper cells, can be specifically inactivated by contact with antigen *prior* to their immigration from BM to thymus. This is the only evidence to date that pre-thymic T-cells are subject to tolerance induction. Considerable evidence exists that the thymus is a critical site where the induction of self-tolerance and allogeneic tolerance generally occurs[59a,70,70a].

T-cell tolerance in neonatally induced haematopoietic chimaerism

Historically, transplantation tolerance was first induced by injection of allogeneic LHP cells into newborn mice across an MHC barrier and revealed by subsequent acceptance of donor-type skin allografts[1]. GVH tolerance was subsequently demonstrated with lymphocytes from similarly induced rat chimaeras[6,40,71]. The latter studies were the first to indicate that transplantation tolerance was a property inherent in the lymphocytes of the chimaera and was retained when the lymphocytes were removed from the environment of the chimaera.

When T-cell tolerance is demonstrated by GVH assays in which the donor cell response to host antigens is monitored directly, or when it is demonstrated by MLC or CML *in vitro*, it seems reasonably certain that we are dealing with T-cell tolerance in the adaptive immunological component of alloaggression. It seems likely that this tolerance is akin to T-cell tolerance in general, and not a curiosity of the chimaeric state.

The lymphocytes of the grown chimaera are usually at least 90% of host origin[6,63]. They can be removed from the chimaera, washed free of chimaeral serum, and tested *in vivo* or *in vitro* against a 'fresh' source of antigen for competence in GVHR, MLC, or CML. It has been found that tolerance in this case is demonstrable in the absence of chimaera serum[6,40,56,57,71–75]. Furthermore, chimaera cells mixed with normal syngeneic lymphocytes have not inhibited the responsiveness of the latter[6,72], nor are they recruited to respond by the latter[72,73]. In other words the T-cells of at least some such animals appear inert when confronted outside the chimaera with the specific set of alloantigens, but are normally responsive to alloantigens of third parties. These findings are most simply accounted for by some form of abortion–deletion tolerance among the T-cell sub-sets which would normally mediate alloaggression against the other strain.

The postulated existence of clonal abortions and deletions among the T-cells of such chimaeras is further supported by studies on recovery of specific reactivity after adoptive termination of allogeneic LHP chimaerism and tolerance. Three independent studies using chromosomal or antigenic markers to distinguish native from adoptively conferred cells have clearly indicated that the recovery of the specific native clones depends upon their

regeneration rather than upon the redemption of blocked T-cells[63,64,76]. This conclusion applies both to GVH-initiating cells[63,64], and the effectors of skin graft rejection[76].

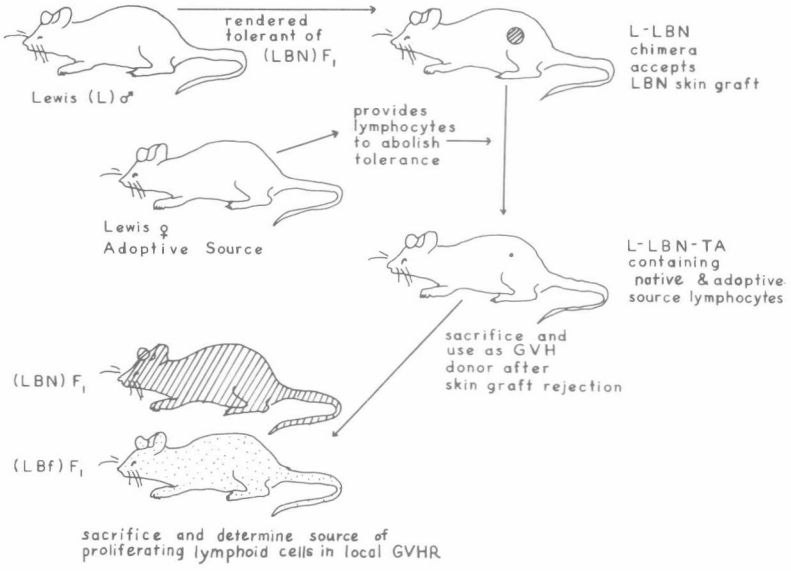

Figure 1.1 Experimental scheme to search for reversibly blocked anti-MHC lymphocytes in a neonatally induced chimaera. A neonatal male L rat (not drawn to scale) is injected at birth with (LBN)F_1 bone marrow. After it is fully grown and tolerance for a skin graft has been demonstrated, tolerance is adoptively abrogated with cells from a normal adult female. Subsequently, the original male is used as a donor of lymphocytes for local renal GVHR. The origin of reacting donor cells in the local GVHR can be determined by sex chromosome analysis

The principle of this approach is illustrated in Figure 1.1, and some data are summarized in Table 1.2. Note that different sets of donor cells are proliferating in the experimental (LBN) F_1 and third-party control (LBf) F_1 hosts during the first month after adoptive transfer. The lack of native clones responsive to BN alloantigens is indicated by the predominant response of adoptively conferred cells in the (LBN) F_1 hosts. Subsequently the native clones regenerate, but not if the chimaera had been adult thymectomized just prior to adoptive abolition of tolerance. Thus GVH tolerance in these neonatally induced chimaeras was not simply a matter of blockade; rather a specific functional capacity has been depleted from the T-cell repertoire of these rats.

The development of GVH tolerance in radiation chimaeras

Bone-marrow transplantation in inbred rodents

Adult BM contains post-thymic T-cells and their pre-thymic precursors[77–79]. As was suggested previously[29], it is likely that the GVHR which occurs following allogeneic BM transplantation is mediated mainly