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Specific Allogeneic Unresponsiveness in the Adult Host—Present-Day Experimental Models

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THE FEASIBILITY of inducing allogeneic unresponsiveness in adult animals as well as in fetal life or in the newborn[1,2] was heralded by the classic studies of Main and Prehn[3] in 1955, with the induction of tolerance to skin allografts across non-H-2 barriers in irradiated mice reconstituted with allogeneic bone marrow. This experiment marked the onset of a long-term intensive effort to apply similar methods to the transplantation problem in a wide variety of mammalian species. The approaches used for this purpose range from chemical immunosuppression[4] to conditioning regimens with various alloantigens and alloantibodies,[5-7] to the study of idiotypic antibodies.[8] A number of other studies have aimed at optimizing the generation and balance of host suppressor cells,[9] and altering the immunogenicity of transplantable tissues in vitro.[10]

In this context, three principal categories of experimental models have evolved for the induction of specific states of allogeneic unresponsiveness in the adult host. The first, involving the combined use in rodents of immunosuppressive agents (such as ALG or ATG), prospective donor strain cells or antigens, and/or bacterial adjuvants, was described originally by Michie and Woodruff,[2] Lance and Medawar,[11] Monaco and associates,[12-14] and Brent, Kilshaw, Pinto, and their associates.[5-7] A second model was based upon a combination of total body irradiation and host reconstitution with allogeneic, syngeneic, and eventually, stored autologous marrow, as described by the NYU-Cooperstown Group,[15-17] Jirsch and associates,[18] and Dittmer, Bennett, and Moran.[19-21] The third approach, which has recently aroused increasing interest, is fractionated total lymphoid irradiation, as pioneered by Henry Kaplan for the treatment of Hodgkin's disease and applied to transplantation by Strober, Slavin, and associates.[22-25]

It is interesting that all three models share a number of characteristics, and probably aim at the same general host mechanisms. In each instance, some form of immunosuppression is required for adequate preparation of the host; bone marrow transplantation (i.e., the implantation of immature hemopoietic cells into a pretreated host milieu) is a prerequisite for optimal results; the outcome is exquisitely dependent upon the precise sequence and timing of each step in the procedure; and suppressor cells have been implicated as the key mediator of the observed effects by a number of investigators, including Brent et al.,[26] Wood and Monaco,[9] Dittmer, Moran, and Bennett,[21] and Strober et al.[22] Taken together, the data suggest that the broad principles listed by Murray et al.[27] over two decades ago for successful application of the models to clinical problems may already have been met in experimental animals, and will soon be tested widely in humans. As noted by Murray et al.,[27] "The original requirements for adaptation of the experimental design of irradiation, marrow and homografts to man appear to be a heavy dose of X-irradiation to the entire host to destroy its immune mechanism, a source of hemopoietic cells capable of self-reproduction and a subsequent graft."

This goal expressed by Murray et al.[27] has thus far been achieved with greatest success in

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the canine species, largely through the development by the Bassett Hospital, in Cooperstown, N.Y., of a unique colony of selectively bred lines of dogs of precisely known DLA-A,B,C, and D genotypes,[15] whose restricted genetic pool has also permitted the assessment and quantitation of the biologically active non-DLA histocompatibility antigens that also condition bone marrow and organ allograft survival.[15,16] As a portion of these studies,[15,16] prospectively selected Cooperstown dogs underwent supralethal total body irradiation, and were reconstituted with allogeneic marrow cells obtained from a genotypically DLA-identical donor. The use of bone marrow from such donors regularly resulted in engraftment and complete reconstitution of the host without any evidence of graft-versus-host disease GVHD.[15] These data were in marked contrast with the results in dogs obtained from more outbred colonies, and constituted a key difference permitting a routine and relatively effortless extension of the system of Main and Prehn to the canine species. For this purpose, dogs reconstituted with allogeneic marrow were given a kidney allograft obtained from the marrow donor and their own kidneys were removed. Permanent allograft survival ensued in every instance without any further treatment.[15,16] The technique has been shown to induce specific unresponsiveness to allografts of skin, kidney, heart, lung, pancreas, liver, and small intestine obtained from the marrow donor, while retaining the host's capacity to reject fully any tissue or organ allografts obtained from other sources.[15]

Further studies have demonstrated that the specificity of the induced unresponsiveness was broadened, and the latent period between bone marrow reconstitution and kidney transplantation could be shortened if the recipient was given a kidney allograft within 12–15 hr after transplantation of bone marrow.[15,16] One of the key conclusions derived from this model was that replacement of immature hemopoietic cells into the host's irradiated milieu could trigger an evanescent cycle of selective cell replication recapitulating the events of immunologic ontogeny, with particular reference to the generation of a temporary suppressor-cell preponderance in the rapidly proliferating cell populations. The demonstration by Alter et al.[28] that this phase in human subjects is associated with a transient fetal erythropoiesis and the production of newborn-type gamma chains, as well as the report by Haot and associates[29] of the reappearance of fetal- and newborn-type lymphoid cells in irradiated mice, have provided further support to this notion. A logical corollary to this hypothesis was that once a cell milieu favorable to induction of unresponsiveness was established, it might actually not be necessary to use allogeneic bone marrow, but that immature marrow cells of autologous origin could be equally effective in the implementation of unresponsiveness. In a series of experimental tests of this possibility, bone marrow was removed from prospective allograft recipients and was stored while each dog underwent a standard course of supralethal total body irradiation. The animals were reconstituted with their own marrow, and were given a kidney allograft obtained from a genotypically DLA-identical donor.[17] This method was associated with the development of long-term allogeneic unresponsiveness to renal allografts with no requirement for further treatment in 27 of 44 (61%) of the dogs given their kidney transplants within 12–28 hr after bone marrow replacement.[17] The resulting unresponsiveness was specific for the kidney donor, and did not generally extend to other organs from the same dog, with the possible exception of the pancreas.[30] The successful establishment of unresponsiveness required genotypic DLA identity of donor and recipient, and was clearly mediated by one or more populations of immature or precursor stem cells present in the retransplanted autologous bone marrow. The phenomenon was also exquisitely time-dependent; in order to achieve optimal results, at least one cycle of replication by the retransplanted cells in the host's irradiated milieu was needed before exposure of the recipient to

renal alloantigens.[17] Adaptation of autologous marrow to this model also had the additional virtue of eliminating the risks of GVHD complications associated with the use of allogeneic marrow in other canine populations.[17]

One of the intriguing features of the experimental models described here has been the observation that neither the canine experience[15, 17] nor the results of Bennett, Dittmer, and Moran[19-21] in a very similar system using heart transplantation in irradiated rats given syngeneic marrow, have reported success rates in inducting allogeneic unresponsiveness exceeding 75% of animals treated in similar fashion. This factor has introduced an ominous caveat to consideration of such models at the clinical level. It also suggests that the methods used heretofore may not have been adequate to stimulate the generation of a temporary preponderance of suppressor cells in *all* recipients, possibly as a consequence of variability in the persistence of immunologically competent postthymic cells in the treated host and/or the inoculum of autologous marrow returned to the recipient after irradiation. An alternative possibility is the continued survival of varying concentrations of immature precursors of such cells in the marrow of some of the dogs.

The first effort directed at control of this variable has consisted of exposing the peripheral blood of prospective recipients to a 5-week course of extracorporeal irradiation (ECIB), a technique long known to selectively ablate the host's small recirculating lymphocyte population.[11] For this purpose, the neck vessels of the recipients were cannulated, and blood was passed through a radioactive cesium source 5 times weekly for 4–5 weeks, until a total dose of 20,000–30,000 rads was given. This dose produced a profound lymphopenia, but had no ill effect upon erythrocytes or their precursors. Such treatment also had no detectable effect upon the capacity of marrow obtained from treated animals to repopulate these dogs after supralethal total body irradiation.

An alternative technique consisted of exposing the stored autologous marrow scheduled to repopulate a given recipient to methylprednisolone (MPd) and DNase for 30 min prior to reinfusion into such dogs. This approach was prompted by the finding of Tutschka, Santos, and their associates[12] of a differential and highly selective effect of MPd against lymphoid cells, in contrast with other hemopoietic stem cells. Preliminary results indicate that either treatment may provide some significant improvement (up to 80%) in the capacity of irradiation and autologous marrow replacement to establish in the canine host a milieu conducive to the development of adult allogeneic unresponsiveness in dogs. This result did not, however, provide any information on the nature or identity of the

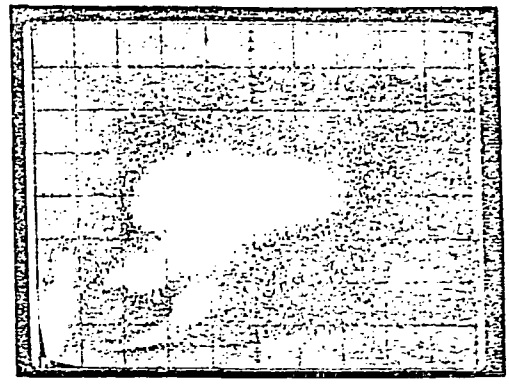

Fig. 1. Distribution of cell clusters in normal canine bone marrow.

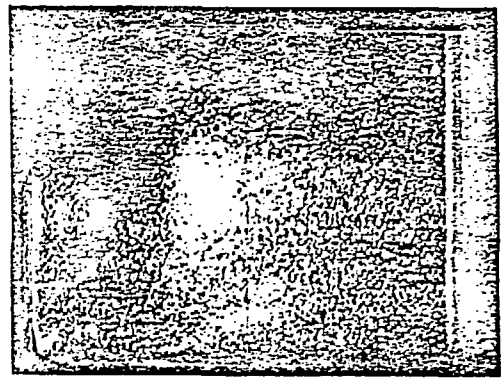

Fig. 2. Alterations in bone marrow cell populations after in vitro chemoseparation by methylprednisolone.

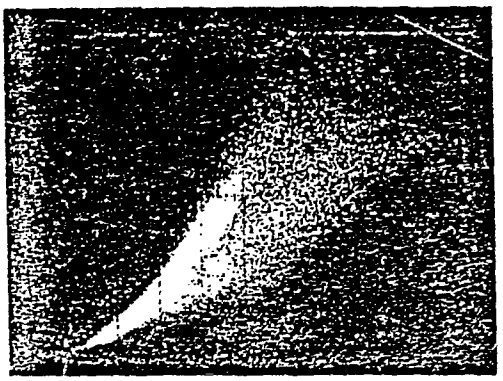

Fig. 3. Appearance of normal bone marrow cell populations after purification by the Ficoll-Iron method.

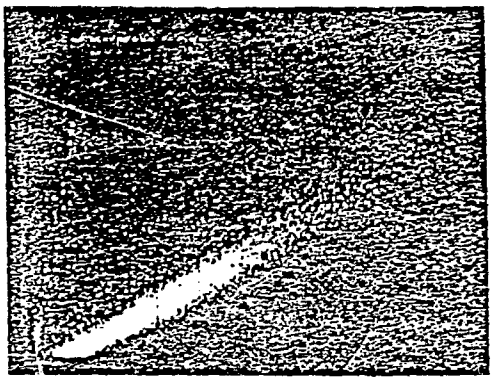

Fig. 4. Alterations in Ficoll-Iron-purified bone marrow populations after in vitro chemoseparation by methylprednisolone.

cells that appear to modulate allograft responsiveness under these experimental conditions. Further data on this important question were sought through a serial analysis of bone marrow cell samples at various intervals before and after ECIB and/or MPd treatment. The samples were studied in an Ortho 50-11 cell sorter, using green fluorescence for DNA in the X axis and red fluorescence (for RNA) or a narrow forward scatter (for cell size) for the Y axis. Whole marrow cell suspensions and Ficoll-iron purified preparations of marrow cells, which were predominantly of the lymphocytoid and monocytoid series, were also studied in this manner. The appearance of normal canine bone marrow is shown in Fig. 1. Normal marrow cells appear to be distributed in six main groups or clusters. Three of the clusters correspond to myeloid cells at various stages of differentiation; a smaller cluster of normoblasts and small lymphocytes appears in the far left aspect of this photograph. The fifth and sixth clusters are located slightly below and to the left of the principal cluster of myeloid cells. The fifth and sixth clusters are most likely monocytoid in origin and are particularly rich in cells corresponding to the size and shape of canine blood monocytes. As shown in Fig. 2, these two clusters are eliminated after chemoseparation of bone marrow cells with MPd.[12] Parallel results were obtained with a 5-week course of ECIB.[31] After treatment of normal marrow by the Ficoll-iron method, the predominant cell populations remaining in the suspension were of mononuclear origin. As shown in Fig. 3, such cells could be divided into a lower portion rich in DNA and an upper cluster containing larger cells and a greater concentration of RNA. Addition of MPd to such preparation resulted in complete elimination of this population of larger mononuclear cells (Fig. 4).

The disappearance of a particular population of mononuclear cells from the cell clusters in adult bone marrow upon treatment with ECIB or MPd, and the association of such disappearance with increased success in the induction of allogeneic unresponsiveness, may be interesting with regard to further studies of the precise role of the monocyte and/or macrophage in the modulation of adult immunologic reactivity. The apparent improvement in the capacity to develop allograft tolerance observed after elimination of clusters of such cells from the host's immunologic armament may also be useful in the development of new techniques for the induction of this type of unresponsiveness in the adult host.

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