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Induced Tolerance and "Homologous Disease" in X-Irradiated Mice Protected with Homologous Bone Marrow.* (23414)

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Several species of mammals can be protected against otherwise lethal doses of whole body X-irradiation by post-irradiation transfusion of bone marrow or spleen. Protection is associated with rapid recovery of the otherwise depleted or destroyed hematopoietic tissues as a result of reseeding and repopulation with cells of the transfused marrow, even if the marrow is of homologous or heterologous origin(1-6).† Mice protected against otherwise lethal doses of irradiation with homologous marrow are thereby rendered tolerant of skin grafts as well as hematopoietic tissue of the marrow donor antigenic type(7,8). Such tolerance of homologous skin and hematopoietic tissue can be abolished by introduction into the tolerant mouse of isologous lymph node or spleen cells from an unirradiated mouse of the same strain(9). The closeness of genetic relationship of the marrow donor and irradiated recipient has a great effect upon both degree of initial irradiation protection and duration of survival(8,10). In otherwise lethally irradiated mice, marrow from guinea pigs or rabbits conferred insignificant protection(8). Rat marrow gave significant but short-term protection. Any type of mouse marrow gave excellent 21-day protection, beyond which isologous marrow gave permanent protection but foreign-strain mouse marrow gave significant late mortality characteristically between the 21st or 30th day and the 60th day post-irradiation. Late mortality was more severe when the foreign strain marrow came from a donor of a different Histocompatibility - 2 genotype (A into C_{57}, Dba into A, A into CBA) than when it came from a donor of the same H-2 genotype (C_3H into CBA) as the recipient. With homologous marrow from an F_1 hybrid into the irradiated parent strain, 2 groups showed excellent pro-

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† Autologous—from same individual; isologous—from another individual of same inbred strain (presumably same genotype) or an identical twin; homologous from an individual of different genotype but of same species; heterologous—from a different species.

tection as late as 100 days post-irradiation, comparable to that obtained with isologous marrow. Two additional groups showed significant mortality, both early and late, attributable in part at least, to the use of very young mice as irradiated recipients.

The late mortality (21 to 60 days) following good early protection with homologous marrow was preceded by progressive loss of body weight. That it was not due to recovery of the host's ability to reject the homologous hematopoietic tissue was indicated by absence of hematopoietic depression before late death, and by the long duration of tolerance of tissues of the homologous marrow donor antigenic type as revealed by skin grafts. Because of this, and the absence of characteristic late mortality after good early protection with isologous marrow or F_1 hybrid homologous marrow in an irradiated parent strain, it was suggested that the late mortality might more likely be the result of the transplanted homologous hematopoietic tissue reacting immunologically against the foreign tissue antigens of its new host(8). It is well established that the homograft reaction is an immunological reaction against foreign tissue antigens mediated by lymphoid tissue elements, and that an F_1 hybrid of 2 inbred strains of mice will accept tissue transplants from either parent strain, but neither parent strain will accept tissue transplants from the F_1 hybrid. Only the lymphoid system from the same inbred strain or from an F_1 hybrid into a parent strain should not be immunologically active against the tissues of the irradiated recipient. These were the very combinations that failed to give characteristic late mortality after good early protection. Conversely, the lymphoid system of a parent strain transplanted into an F_1 hybrid should react immunologically against the F_1 tissue. The latter system provides an additional test of the theory of late mortality being the result of reaction of marrow graft against the host. While F_1 marrow in the irradiated parent should not result in such late mortality, parent marrow into the irradiated F_1 hybrid should. Such experiments constitute the basis of the present report. In addition, since each parent strain reacts against a different set of tissue antigens in the F_1 hybrid, one group of irradiated F_1 hybrids was given a mixture of half the usual dose of marrow from each of both parent strains, to see if this might give more late mortality than marrow from either strain alone. Also, since tolerance to homologous tissues can be abolished by introducing normal host type lymphocytes(9), an attempt was made to forestall late mortality with foreign-strain homologous marrow by administering also isologous marrow.

Materials and methods. Symbols used for mouse strains, and irradiation conditions were similar to those previously reported(8,11) unless otherwise stated. Mice were randomized by age and sex between treatment groups. Wherever possible an equal number of mice on each of the treatments of a given experiment were housed together in one pen. Skin grafts were performed 174 to 175 days post-irradiation. The LD_{50} for the CBA strain under the conditions of irradiation used is 600 r(11). In all of the experiments herein reported, a single dose of 770 r whole-body X-irradiation was employed, which dose is above the LD_{100}. Bone marrow was injected intravenously in a standard volume of 0.5 ml per mouse. The nucleated cell count of the suspensions used averaged 59,800 cells per cu mm (32,000 to 89,000).

Results. Fig. 1 shows that all of 60 CBA mice receiving 770 r and no marrow were dead by the 12th day. Of the 192 mice similarly irradiated but receiving any of the 4 types of marrow treatment, all groups showed excellent 21-day protection against the lethal effects of irradiation. Beyond 21 days only marrow from a foreign strain (Cb) gave characteristic late mortality. This same marrow mixed with isologous marrow gave no late mortality. Marrow from the F_1 hybrid of the irradiated strain gave protection equal to that with isologous marrow, no characteristic late mortality being observed in either case. Tolerance to grafts of homologous Cb skin was exhibited by all 10 mice so grafted that had been protected with either Cb or (Cb x CBA) F_1 hybrid marrow. Such tolerance was not exhibited by any of the 23 CBA mice so grafted that had been protected by either CBA marrow or a mixture of Cb and CBA

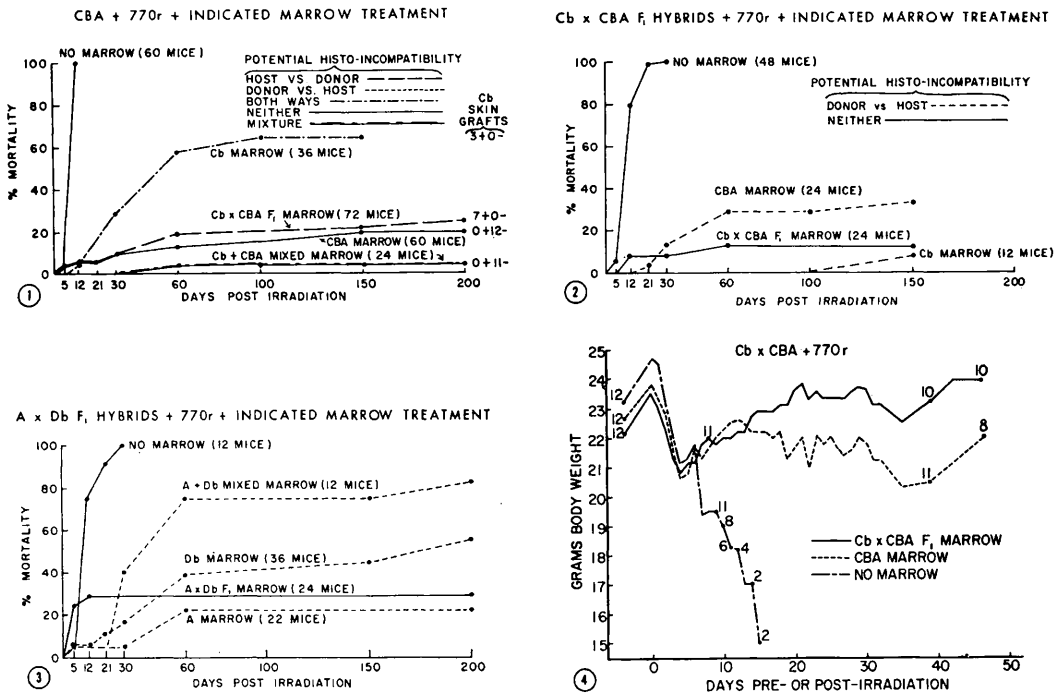

FIG. 1. Cumulative mortality in CBA mice receiving 770 r whole body irradiation followed by isologous, homologous or mixed isologous and homologous bone marrow.

FIG. 2. Cumulative mortality in (Cb × CBA) F_1 hybrid mice receiving 770 r whole body irradiation followed by isologous bone marrow or homologous marrow from either parent strain.

FIG. 3. Cumulative mortality in (A × Db) F_1 hybrid mice receiving 770 r whole body irradiation followed by isologous bone marrow or homologous marrow from either or both parent strains.

FIG. 4. Mean body wt of (Cb × CBA) F_1 hybrid mice receiving 770 r whole body irradiation followed by no bone marrow, isologous marrow, or homologous marrow from one of the parent strains. Each group consisted of 12 mice at the start. Numbers along each curve represent decreasing numbers of surviving mice.

marrow. When either the (Cb x CBA) or (A x Db) F_1 hybrids were protected with isologous marrow, no late mortality after early protection was obtained (Fig. 2, 3). The pre-5 day mortality seen in the (A x Db) mice protected with isologous marrow should not be confused with late mortality, which occurs after the 21st day. When irradiated (Cb x CBA) F_1 hybrids were protected with marrow from either parent strain, no late mortality was obtained with marrow from the Cb strain and slight late mortality with marrow from the CBA strain. However, when irradiated (A x Db) F_1 hybrids were protected with marrow from either or both parent strains, slight late mortality was obtained with marrow from the A strain, moderate late mortality with marrow from the Db strain and severe late mortality with marrow from both the A and Db strains combined. In those groups of F_1 hybrids showing slight to moderate late mortality after protection with parent marrow, many of the mice that escaped late mortality nevertheless showed body weight depression as compared to the controls receiving isologous marrow (Fig. 4). Expressed in terms of potential histo-incompatibility of donor and recipient, late mortality after good early protection against an otherwise lethal dose of irradiation was not seen in those combinations where neither the host nor the donor can potentially react immunologically against each other (CBA into CBA, (Cb x CBA) into (Cb x CBA), (A x Db) into (A x Db)), or where only the host strain can potentially react against the donor strain but not vice versa ((Cb x CBA) into CBA). It was seen in those combinations of foreign

strains where both the host and the donor strains can potentially react against each other (Cb into CBA in this report; Db into A, C_3H into CBA, A into CBA, and A into C_{57} in an earlier report)(8). It occurred in some but not all of those combinations where only the donor strain can potentially react against the recipient strain but not vice versa (Cb into (Cb x CBA); CBA into (Cb x CBA); A into (A x Db); Db into (A x Db) and both A and Db into (A x Db)). The term "potentially" is used advisedly since a host potentially capable of reacting against and rejecting the tissues of a homologous strain is rendered incapable of doing so by protection against an otherwise lethal dose of irradiation with marrow of that homologous strain, as demonstrated by the survival of skin and bone marrow. There is no evidence, however, that transplanted homologous lymphoid elements are thereby rendered incapable of reacting against the tissues of the host.

Discussion. The absence of characteristic late mortality after good early protection in irradiated CBA mice given homologous marrow from an F_1 hybrid of the CBA strain, and its presence in CBA mice protected with homologous marrow from a foreign strain (Cb), indicates that late mortality is not the result of recovery of the host's ability to react immunologically against the grafted homologous marrow elements. The CBA strain normally reacts against and rejects tissue grafts from either the Cb strain or the (Cb x CBA) F_1 hybrid, since both contain the same set of foreign antigens characteristic of the Cb strain. Yet late mortality is seen only with Cb marrow. This, together with the absence of secondary hematopoietic depression before late death, and the induced tolerance to tissues of marrow donor antigenic type as revealed by skin grafts, contradicts the concept that late mortality results from the host reacting against the marrow graft, at least under the conditions of the present experiments. In this regard it must be emphasized that the level of irradiation employed was above the LD_{100}. It is highly probable that at lower levels of irradiation some of the irradiated host's cells responsible for the homograft reaction would recover and reject homologous tissue, whether marrow or skin(11). There is one difference between the Cb and the (Cb x CBA) F_1 marrow donor that correlates well with the observed result. Lymphoid elements of the Cb strain can react against the tissues of the CBA recipient, whereas those of the (Cb x CBA) F_1 hybrid cannot.

The absence of late mortality in CBA mice protected with a mixture of Cb and CBA marrow dissociates late mortality from any immediate effect of the homologous marrow on the host, including transmission of pathogenic bacteria. Absence of late mortality in this group would appear to be related to failure of the Cb marrow to survive when administered with isologous marrow. That it could not survive in this group is indicated by rejection of Cb skin grafts in all 11 mice of this group so tested. As indicated earlier, tolerance of homologous marrow donor tissues can be abolished by subsequent administration of isologous (host type) lymphocytes(9). It would appear that tolerance can be prevented initially by simultaneous administration of homologous and isologous marrow. Implicit in these results is the concept that late mortality is related to the continued persistence and function in the irradiated host of marrow elements from a homologous donor that is capable of reacting immunologically against the recipient. The presence of characteristic late mortality in F_1 hybrids protected with marrow from some but not all parent strains is difficult to interpret. Its absence cannot be associated with the use of 2 parental strains of the same H-2 genotype, since the parent strains of both hybrids differed at the H-2 locus(12). That characteristic late mortality occurs in any group of F_1 hybrids protected with parent strain marrow is still further evidence that late mortality does not result from recovery of the host's ability to react against the grafted marrow elements, for the F_1 hybrid could not so react to begin with. But if late mortality represents an immunological reaction of the graft against the host, then it should occur in all the combinations of parent marrow into F_1 hybrid. Unless, of course, the hypothesized reaction of transplanted hematopoietic elements against the tissues of

a tolerant host does not always result in death. It is possible that if the degree of incompatibility is small enough, and the tolerant host strong enough (heterosis), the great excess of antigen and constant replacement of damaged tissue may permit the host to survive in spite of an incompatibility which when operating in the direction of host *vs.* donor would uniformly cause rejection of skin grafts. In human blood transfusion, a given blood type incompatibility which when operating in the direction of patient *vs.* donor usually gives a major transfusion reaction, will, when operating in the direction of donor *vs.* patient usually give only a minor transfusion reaction. It would seem appropriate to look toward signs in the process leading to late mortality, before the severe end-point of death itself. The fact that in some combinations of homologous donor and recipient strains not all of the treated mice show late mortality but the survivors may show a body weight depression, suggests that the "disease" in question is not always a lethal one. Impaired hair growth, atrophy of lymphoid organs and general debilitation as well as body weight depression are characteristic of irradiated mice treated with foreign strain homologous or heterologous marrow as opposed to isologous marrow, even though they may not succumb to characteristic late mortality during the 60-day post-irradiation period.

If late mortality represents the reaction of transplanted homologous or heterologous hematopoietic tissue against a tolerant host, then irradiation is not an essential component of the disease, except insofar as it is necessary to render the host tolerant. If tolerance could be achieved by other means, such a disease should be demonstrable even in unirradiated animals. This would be an important step in establishment of the etiology of the disease. Actually immunological tolerance can be achieved by introduction of homologous or heterologous tissues into unirradiated animals of several species before or shortly after birth or hatching(13). In addition, F_1 hybrid mice are normally tolerant of tissue grafts from either parent strain. Experiments on the latter system are now in progress. In the former system, Billingham and Brent(14) have observed delayed mortality. The following points of similarity between the condition they observed and that seen after irradiation protection with homologous marrow strongly suggest that the two represent the same process: a) appearance of the condition following introduction of homologous lymphoid elements or their precursors into tolerant hosts; b) delayed onset of overt manifestations; c) depression of body weight; d) lymphoid organ atrophy, and e) greater occurrence of mortality in some combinations of donor and recipient strains than in others. The possible identity of these 2 conditions, together with the absence of characteristic late mortality in irradiated mice protected with isologous or F_1 hybrid homologous marrow would prompt one to avoid use of the terms "late *irradiation* mortality" or "secondary *irradiation* syndrome." It would appear more appropriate to use the terms "homologous disease" and "heterologous disease" to designate the specific complications attributable to successful transplantation of homologous or heterologous, as opposed to isologous or autologous bone marrow or lymphoid tissue into a tolerant host. This would apply to those situations where tolerance is induced or acquired, regardless of the method, as well as where tolerance is a congenital abnormality or a genetically determined normal state. In addition to the conditions already observed in irradiated or neonatally injected animals it would include any similar conditions subsequently observed in a variety of situations, such as agammaglobulinemic or hypogammaglobulinemic individuals transplanted with lymph nodes, as well as in F_1 hybrid mice receiving bone marrow or spleen or lymph nodes from either parent strain.

Summary. CBA mice protected against an otherwise lethal dose of irradiation with isologous CBA bone marrow showed normal rejection of skin grafts from the Cb strain. Irradiated CBA mice protected with Cb or (Cb x CBA) F_1 hybrid marrow were tolerant of skin grafts from the Cb strain. Irradiated CBA mice protected with a mixture of marrow from the Cb and CBA strain were not tol-

erant of skin grafts from the Cb strain. Late mortality (21 to 60 days post-irradiation), after good early protection (21 days) against doses of whole body X-irradiation above the LD_{100}, did not occur in mice protected with isologous bone marrow (where neither the host nor the donor can react immunologically against each other). It occurred in mice protected with homologous marrow from a foreign strain (where both the host strain and the donor strain can potentially react immunologically against each other). It did not occur in mice protected with marrow from an F_1 hybrid of the irradiated strain (where the host strain can potentially react against the donor strain but the donor strain has no potential to react against the host strain). It occurred in some but not all combinations of irradiated F_1 hybrids protected with marrow from a parent strain (where the host has no potential to react against the donor strain but the donor strain can potentially react against the recipient strain).

The terms "homologous disease" and "heterologous disease" are proposed to designate the complications specifically attributable to successful transplantation of homologous or heterologous, as opposed to isologous or autologous, bone marrow or lymphoid tissue into a tolerant host.

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Production of Hyaluronic Acid in Tissue Culture by Fibroblasts Growing from Intestinal, Stomach, Liver and Kidney Explants.* (23415)

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The isolation of hyaluronic acid, and the description of its enzymatic hydrolysis by Meyer(1) brought to light the enzyme-substrate nature of the spreading reaction, first found in skin and subcutaneous tissue(2). In many tissues in which the spreading reaction was demonstrated, hyaluronic acid was found to be present and to be hydrolyzed by hyaluronidase. On the other hand, spreading factors were described which were not found to be related to hyaluronic acid and its hydrolysis, so - called hyaluronate - inactive spreading factors.

Favilli(3) and Duran-Reynals(2) demonstrated the spreading reaction in organs other than skin and subcutaneous tissue, such as stomach, intestine, and striated muscle, while no conclusion could be reached concerning spreading reaction in hepatic and renal tissue. It was, however, unknown whether the spreading reaction found in those organs was

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