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Modification of Irradiation Effects Through Actively Acquired Tolerance to Homologous Bone Marrow Grafts.* (27995)

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Successful transplantation of isologous, homologous and in most instances, heterologous hematopoietic elements prolongs the life of lethally irradiated animals. Increased survival appears to depend upon partial or total repopulation of the host's depleted hematopoietic system by cells proliferating from the graft(1,2). Although 30 day survival was much improved even when marrow grafts were from homologous or heterologous animals, Barnes and Loutit(3) observed a large percentage of deaths between the 30th and 60th days in mice with such grafts. This delayed death syndrome has been referred to as "foreign bone marrow reaction," "secondary disease," "homologous disease," and "heterologous disease"(4,5). Makinodan(6) interpreted "secondary disease" as a host reaction against the proliferating foreign bone marrow antigens, *i.e.*, although radiation injury to the immune mechanism of the host makes successful transplantation of foreign marrow possible, when the host system recovers it develops an *in vivo* antigen-antibody reaction against the grafted tissue. In contrast, Trentin(7), Uphoff(8), and Barnes, *et al.*(1) suggested that the bone marrow bears the immune mechanism of the donor and that death results from an immune reaction of the grafted tissue against the host. Shaw and Vermund(9,10) in studies of homologous and heterologous (dove) bone marrow treatment of lethally irradiated pigeons, demonstrated that a similar or identical phenomenon results.

This report includes data from experiments in which the host and donor pigeons had been made immunologically tolerant in various combinations by skin graft technics of Cannon and Longmire(11).

Materials and methods. Pigeons used were domestic barn pigeons (*Columba livia*) and backcross hybrids which were derived from matings to *C. livia* of hybrids and selected backcross hybrids from crosses between *C. guinea* and *C. livia*. These hybrid birds have been sufficiently backcrossed to be considered homologous with *C. livia,* except for those loci responsible for production of one or 2 of the erythrocytic antigens B^g, C^g, and E^g derived from *C. guinea*(12). Skin grafts were made on 136 newly-hatched pigeon squabs by an adaptation of the technic described for chicks by Cannon and Longmire(11). Details of a successful method for development of actively acquired tolerance in the pigeon will appear elsewhere. Of the 136 squabs, 32 accepted and retained the original homologous skin graft and survived to an age of 8-10 months in proper condition for experimental use. Twenty-five of these 32 accepted challenge grafts of skin from the appropriate primary donor at the age of 6 months and retained them through the 10th month, indicating that actively acquired tolerance had been achieved. These birds were used in the following host-donor combinations: Group 1: The irradiated hosts were presumably tolerant of the marrow donors (9 birds—6 hosts, 3 marrow donors). Group 2: The bone marrow donors were presumably tolerant of tissue from the

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irradiated hosts (8 birds—5 hosts, 3 donors). Group 3: The irradiated hosts and marrow donors were presumably reciprocally tolerant (8 birds—4 hosts, 4 donors). Group 4: Normal animals of the same age group but not previously skin-grafted. No known state of tolerance existed between the hosts and marrow donors (15 birds—10 hosts, 5 donors). Group 5: Normal birds irradiated under the same condition as hosts in Groups 1-4, but injected with sterile saline only. (X-ray controls, 10 birds). Group 6: Unirradiated, untreated controls (10 birds).

All irradiated birds (Groups 1-5) received 2500 rads to the entire body. Bone marrow implants consisted of approximately 250 $\times$ 10^6 nucleated cells per host injected intravenously as previously described(9).

In each marrow transplant combination (Groups 1-4) the donor differed from the host by one or more erythrocytic antigen markers, the factors B^g, C^g and E^g derived from *C. guinea,* or the intraspecies antigenic factors Si and Ba detectable with phytoagglutinins, extracted from the beans, *Phaseolus limensis* and *Bandeiraea simplicifolia*(12).

Blood samples were drawn from wing veins of all birds at 4 day intervals from day 0-40, and on days 45 and 50, or until the birds died. The erythrocyte samples were tested with antisera specific for the B^g, C^g, E^g and E^1 antigenic factors and with phytoagglutinins for Si and Ba factors. Since each donor-host combination differed, the presence and approximate percentage of both donor and host erythrocytes could be determined.

The serum samples from all birds were tested for hemagglutinins against a series of erythrocyte suspensions carrying the B^g, C^g, E^g, and E^1 factors. In addition, each sample of serum from each bird was tested against its own erythrocytes obtained shortly before implantation of marrow, and against cells of the bone marrow donor. No detectable agglutinins appeared in any of these sera.

Six birds, one from each of Groups 1-6, were caged together following irradiation in an effort to minimize the influence of environmental factors. All birds were given Myzon Poultry Builder (terramycin plus vitamins and minerals) in their drinking water from 10 days prior to irradiation throughout the experimental period. Such antibiotic treatment had been previously found effective in reducing early deaths (3-5 days) presumably due to bacterial invasion as a result of radiation damage to the intestinal tract(10).

TABLE I. Mean Survival Values for Various Host-Marrow Donor Combinations.

Group	Host – donor relationship	No. of birds	Avg survival (days)
1	Host tolerant of donor	6	23.4 $\pm$ 5.6
2	Donor " " host	5	34.2 $\pm$ 4.3
3	Reciprocally tolerant	4	35.6 $\pm$ 5.2
4	No-actively acquired tolerance	10	25.3 $\pm$ 4.1
5	Irradiated controls	10	13.6 $\pm$ 1.1
6	Unirradiated "	10	— —

Results and discussion. The tolerant state of the host did not significantly alter average survival time (Table I); *i.e.,* mean survival of Group 1 (host tolerant of donor) was 25.4 days, and of Group 4 (no known tolerance) was 25.3 days. In contrast, average survival of Groups 2 and 3 was approximately 10 days longer than Groups 1 and 4. Therefore, it appears that tolerance of marrow donors to host antigens increased survival. The small numbers of birds in Groups 1-3 precluded statistical demonstration of these differences when each group was compared separately. However, when a comparison was made between combined groups in which the donors were tolerant of the hosts (Groups 2 and 3) and those in which no known tolerance existed on the part of the donors (Groups 1 and 4), there was a statistically significant difference in mean survival ($p<.05$).

Circulating cells of donor type were detectable immunologically by the 8th-12th day in all animals which received bone marrow implants (Groups 1-4). By the 28th day, all surviving pigeons in Groups 1 and 4 (host tolerant of donor and no known tolerant state respectively) showed evidence of total repopulation by donor-derived cells; *i.e.,* only erythrocytes of the donor type were detectable in the peripheral blood, Table II. There was no evidence of chimera reversal in these 2 groups. In contrast, in Groups 2 and 3, where bone marrow donors were all tolerant of the hosts, total repopulation did not occur, even in the 3 animals which lived 61, 68, and 72 days (birds #H222G_2, #H368C_2, and #3097G,

TABLE II. Representative Immunogenetic Data Based on Erythrocyte Repopulation.

Bird No. (host)	Tolerant state	Blood type* Host	Blood type* Donor	Reagents used to test RBC	Agglutination reactions of erythrocytes† Days postirradiation 0	8	16	24	28	45	60	70
H297 A_2	Host of donor	Ba+Si−	Ba−Si+	Ba-Ext	++++	+++	+++	++				
				Si-Est	0	0	++	+++	++++			
H249 J_2	*Idem.*	B^1 B^1	B^g B^1	Anti B^g	0	0	+	+++	++++	++++		
		E^g E^1	E^1 E^1	Anti E^g	++++	++++	+++	++	±	0		
3097 G	Donor of host	B^1 B^1	B^g B^1	Anti B^g	0	0	+	++	+++	+++	+++	
		Si+	Si−	Si-Ext	++++	++++	+++	++	++	++	+++	
H178 M_2	*Idem.*	B^g B^1	B^1 B^1	Anti B^g	++++	++++	+++	+++	++			
		E^1 E^1	E^g E^1	Anti E^g	0	0	++	++	++			
H222 G_2	Rec. tol.	B^g B^1	B^1 B^1	Anti B^g	++++	+++	+++	++	++	++	++	
		Si−	Si+	Si-Ext	0	±	+++	+++	+++	+++	+++	
H368 C_2	" "	E^g E^g	E^1 E^1	Anti E^1	0	0	+	+++	+++	+++	+++	+++
				Anti E^g	++++	++++	+++	+++	+++	++	++	+++
3106.75	None known	B^1 B^1	B^g B^1	Anti B^g	0	0	++	+++	++++			
		Si+	Si−	Si-Ext	++++	+++	+++	+	0			
3106.78	" "	E^1 E^1	E^g E^1	Ba-Ext	++++	++++	++	+	0	0		
		Ba+	Ba−	Anti E^g	0	±	+++	+++	++++	++++		

* Blood types given are only the erythrocytic markers which were used to differentiate the red cells of donor from the host. This does not imply that these are the only erythrocytic antigenic specificities present.

† Degrees of agglutination were as follows: Complete (4+); approx 75% (3+); approx 50% (2+); approx 25% (1+); detectable but less than 20% (±); and not detectable (0).

TABLE III. Expected Relative Survival of the 4 Irradiated, Marrow-Grafted Groups if Secondary Disease is Due Primarily to: (a) Immunologic reaction of host against grafted donor tissue (Hypothesis I); (b) Reaction of donor tissue against host (Hypothesis II); or, (c) both (Hypothesis III).

		Expected survival			
Group	Tolerance	Hypothesis I	Hypothesis II	Hypothesis III	Observed survival
1	Host of donor	Better	Poorer	Fair	Poorer
2	Donor of host	Poorer	Better	Fair	Better
3	Reciprocal	Better	"	Better	"
4	None	Poorer	Poorer	Poorer	Poorer

Table II). There was, however, no indication of chimera reversal in these birds.

Previous experience with the pigeon(9) indicates that, in this organism, at least one type of donor marrow is capable of immunologic activity of sufficient intensity to be responsible for the death of the host; *i.e.,* when heterologous dove marrow was implanted in lethally irradiated pigeons, a host-species-specific agglutinin appeared within 4 to 6 days and was sufficiently potent to result in a marked "*in vivo* agglutination." The specificity of the "agglutinin" was comparable to that anticipated if a dove (donor species) were immunized with the cells of a pigeon (host species). In subsequent studies(10) the marrow implants were delayed until 5 to 7 days after the irradiation, a "host-species-specific agglutinin" appeared 4 to 6 days after grafting and was followed by a "donor-species-specific-agglutinin," 45 days later. Both immunologic systems were thus apparently operative at different times within the same individual.

Considerable data have accumulated which support the view that immunologic reaction of the grafted donor tissue against the host plays an important role in the etiology of "homologous disease"(2,7,13,14). On the other hand, data from pigeon-dove studies (10), and earlier work by Makinodan(6), indicate that recovery of immunologic competence can and does occur in the irradiated host tissue. Furthermore, chimera reversal appears to be a frequent event in irradiated, marrow-grafted humans(15). Three hypotheses of the immunologic etiology of "secondary disease" are summarized in Table III, along with the results expected if donor or host or both are tolerant, one of the other.

The current data, along with the previously reported reversal of chimerism without "secondary death" in pigeons that had received delayed transplants of heterologous or homologous marrow(10), lead to the conclusion that "secondary disease" is primarily the result of a graft *vs* host reaction (Hypothesis II). The immunologic status of the host marrow may still be important, but the symptoms of homologous and heterologous disease (with death occurring between the 30th and 100th day) are more likely the result of the reaction on the part of the donor tissue. It is to be emphasized that results in pigeons(9, 10), and humans(15,16,17) indicate that both donor and host immunologic mechanisms are involved in the ultimate therapeutic effectiveness of bone marrow transplantation following irradiation with lethal doses.

It is of interest that 3 animals in each of Groups 1 and 3 (animals #H249J_2 and #H222G_2, for example) which survived beyond the 17th day showed indications of sloughing both primary and challenge skin-grafts. Breakdown of immunological tolerance in rats following high doses of total-body irradiation has been observed by Makela and Nossal(18) and by Stone and Owen (personal communication). In earlier studies, however, Denhardt and Owen(19) failed to find indications of breakdown of tolerance in the rat as a result of irradiation. The loss of skin homografts in 6 of 10 pigeons in this study might, however, be the result of direct radiation damage to cells of the graft and the graft-bed. Evidence for this is the fact that the birds maintained bone marrow grafts from the same donors at the time the skin-grafts were lost with no indication of chimera reversal (rejection of marrow graft).

In consideration of these data, it is suggested that chimera reversal or balanced chimerism (reciprocal donor-host tolerance) is essential for prolonged survival of lethally

irradiated animals grafted with "foreign" marrow. To achieve this end, prediction and control of such reversal or balancing will require an understanding of dynamics and interactions of both the host and donor marrow systems.

Summary. Host and donor pigeons made immunologically tolerant in various combinations by skin grafting shortly after hatching were exposed to lethal doses of irradiation at the age of 8-10 months. Homologous bone marrow transplants gave the following results:

1) The tolerant state of the host did not appreciably affect survival; *i.e.*, mean survival of Group 1 (host tolerant of donor) was 23.4 ± 5.6 and of Group 4 (no known tolerance) was 25.3 ± 4.1 days.

2) Tolerance of marrow donors for host increased mean survival by about 10 days (34.2 ± 4.3 for Group 2 and 35.6 ± 5.2 for Group 3). Differences in mean survival between combined Groups 1, 4 and 2, 3 were statistically significant ($p<.05$).

3) These data suggest that the immunologic mechanism of the donor is more important than that of the host in producing "homologous disease" in the pigeon.

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Effect of Ecdysone on Incorporation of C^{14}-Leucine into Hepatic Protein *in vitro*.* (27996)

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Previous studies(1) indicated that ecdysone, the growth and metamorphosis hormone of insects, may have an effect on mammalian cells also, even though no material yielding a positive bioassay was obtained from human tissues when methods of extraction yielding positive results in insects were used(2). Growth of mammalian cells in tissue culture was inhibited by the hormone(3,4), and a few regressions of sarcoma 180 occurred after injection of ecdysone. In a continuation of

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