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DISSOCIATION OF SKIN HOMOGRAFT TOLERANCE AND DONOR TYPE GAMMA GLOBULIN SYNTHESIS IN ALLOGENEIC MOUSE RADIATION CHIMAERAS

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ALTHOUGH specific homograft tolerance can be readily induced in adult mice by the transfusion of allogeneic bone marrow cells into lethally irradiated recipients[1–3], the induction of tolerance in unirradiated or sublethally irradiated adult mice has proved difficult[4]. However, it has been found that sublethally irradiated adult mice can be rendered permanently tolerant to allogeneic homografts of donors sharing the same *H*-2 allele, by the transfusion of allogeneic marrow or spleen cells[5,6].

The establishment of a chimaeric state can be demonstrated by various markers for donor cells such as red cell isoantigens[7], histochemical stains[8], and chromosome markers[9]. The usefulness of serum proteins as markers had previously been predominantly limited to xenogeneic (rat–mouse) radiation chimaeras[10]. However, the recent development of methods for identifying mouse γ-globulin isoantigens[11–13] provides a direct means of analysing the host or donor contribution to serum γ-globulins in mouse allogeneic chimaeras. By this means, donor type γ-globulins were found in such chimaeras as late as 9 months after irradiation and marrow transplantation[14], chimaerism having been confirmed by the acceptance of donor type skin homografts in all but one of the mice. However, this single animal which rejected the donor skin also contained donor-type γ-globulin. Therefore, in order to investigate further this possible dissociation of tolerance, five groups of allogeneic chimaeras were set up under varying conditions, in a deliberate attempt to compare the induction of tolerance to skin homografts with the concomitant presence of donor-type γ-globulin.

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Five groups of 3- to 4-month-old male *BALB/cJ* and *DBA/2J* (both *H-2d*) mice were exposed to 500 or 840 rads of 250-kVp. X-rays given as a single dose (dose rate, 30 rads/min). One of the *BALB/c* groups had been treated with 20 mg of urethan daily for two days prior to irradiation (compare ref. 4). The mice then received intravenous injections of either adult spleen cells (36×10^6 nucleated cells), adult bone marrow cells (39×10^6 nucleated cells) or neonatal liver cells (7×10^6 nucleated cells) with or without neonatal spleen cells ($3{\cdot}5 \times 10^6$ nucleated cells). All cell injections were made within 4 h of irradiation. The cell donors were normal 3- to 4-month-old or neonatal (< 2 days old) male *DBA/2*, *CAF*1 (*BALB/cJ* × *A/J*)*F*1, or *LAF*1 (*C57L/J* × *A/J*)*F*1 mice. Tail skin grafts were made either on the following day (Groups I to III) or 1 month later (Groups IV and V). These last-mentioned two groups formed a single experiment set up under the same conditions with the exception that spleen cells were not added to the liver cells in the inoculum of Group V. Each mouse received a skin isograft, an allograft of the same genotype as the transplanted spleen or marrow cells, some a 'third party' allograft, and some a xenograft (rat). The grafts were continually examined for evidence of rejection as previously described[15]. No further treatment was given. Control groups included normal unirradiated mice grafted in similar fashion, and non-treated mice. The general procedures for preparation of cell suspensions, injections, and animal care were the same as those previously described[15].

At various times after treatment, blood was obtained from the retro-orbital sinus; the serum from each mouse was prepared individually and stored at -10° C until tested. The amount of specific donor-type γ-globulin ($7S\gamma_{2a}$)[28] was determined by the ability of the test serum to inhibit the precipitation of ^{125}I-labelled γ-globulin of the donor type by its specific mouse isoantiserum. The methods used in this inhibition assay have already been described[13]. The amount of inhibitor serum was plotted on a linear scale against the reciprocal of the percentage of labelled γ-globulin precipitated. This resulted in a straight line plot over a considerable range of inhibitor concentration. The extrapolated intercept on the axis of 1/per cent precipitated remains constant when the inhibitor serum contains γ-globulin of the same antigenic type as the labelled γ-globulin, but the slope varies directly with the concentration of this γ-globulin type in the test serum. The amount of donor-type γ-globulin in a chimaeric serum was calculated (with the aid of a LINC computer) by comparing the slope found with that of a standard serum pool of the appropriate type, and expressing the result as a percentage of that standard. The standard pool was made up of the sera from eight normal adult mice of the appropriate strain.

The results given in Table 1 show the percentages of donor-type γ-globulin for each chimaeric mouse bled on several occasions, and the fate of donor skin allografts. In each of the four experiments (Groups IV and V being an experiment) the levels of donor-type γ-globulin fell into the same general range regardless of whether the animal rejected the donor-type skin or not. Only one mouse was found not to have any detectable donor-type γ-globulin and this animal was one of the two which rejected the donor-type graft within 1 month. In each of the individual animals the level of donor-type globulin remained approximately constant for many months after the donor-type skin had been rejected. This would indicate that donor-type γ-globulin was continually being synthesized, since our preliminary investigations show the half-life of γ-globulin in these chimaeras to be the normal 3–5 days[16] or less. If γ-globulin were not being synthesized after skin graft rejection, less than 1 per cent of donor type would have remained 2 months after this rejection was complete (assuming the presence of normal donor-type γ-globulin at the time of rejection). In the final experiment (Groups IV and V) specific skin allograft tolerance seems to depend on the presence of the spleen cells in the inoculum. In Groups I and II, 'third-party' skin allografts (*A/HeJ*) were rejected at 25–28 days, and in Group III in 14–21 days. The mice of Groups IV and V rejected xenogeneic (rat) skin grafts within 14 days. Combining all the experimental groups, 34 out of 36 mice (including 10 controls) retained skin isografts for the duration of the experiment. The rejection pattern of donor-type skin allografts in Groups I, II and III (which were engrafted the day after irradiation) was characteristic of that usually seen following irradiation, that is, it was mild, and the rejection times were considerably prolonged (2–4 months). The rejection pattern of donor-type skin allografts by Group IV mice (grafted 1 month post irradiation) was more typical of the pattern of non-irradiated mice, that is, a more precise and clear-cut rejection 'endpoint' (compare ref. 15).

The relationship between acceptance of donor-type skin grafts and presence of donor-type γ-globulins is summarized in Table 2. The group to which attention should be directed is that comprising the twelve mice which rejected skin grafts but had donor-type γ-globulins. The range of levels of donor-type γ-globulins in these mice is not different from those in mice which permanently accepted skin allografts.

Table 2

Donor skin allografts	Number of mice Donor-type γ-globulin Present	Absent
Accepted	13	0
Rejected	12	1

Table 1. PRESENCE OF DONOR-TYPE γ-GLOBULIN IN THE SERA OF ALLOGENEIC MOUSE RADIATION CHIMAERAS GRAFTED WITH DONOR-TYPE SKIN

Group	Donor		Recipient		Serum bleed after skin grafting (months)	Donor-type γ-globulin (per cent of standard pool)	
	Strain	Cell inoculum	Strain	Treatment		Donor-type allografts rejected *	Donor-type allografts permanently accepted †
I	*DBA*/2	Adult bone-marrow or spleen	*BALB*/*c*	500 rads	10	0‡, 16, 30, 54	36, 42, 43, 45
					12	20, 24 d.d.	33, 33, 34, 37
II	*DBA*/2	Adult bone-marrow or spleen	*BALB*/*c*	500 rads + urethan	10	62, 66, 80	
					12	40, 50, 60	33, 50, 80
III	*CAF*1	Adult bone-marrow	*DBA*/2	500 rads	3	15‡, 21‡, 21‡, 61‡	18, 21, 31
IV	*LAF*1	Neonatal liver	*DBA*/2	840 rads	3	29, 48‡	
					5	49, 53‡	
					6	45‡, 46	
V	*LAF*1	Neonatal liver + neonatal spleen	*DBA*/2	840 rads	3		22, 23, 72
					5		38, 40, nt
					6		21, 24, 33

* Allografts rejected within 2–4 months. † Accepted > 300 days. ‡ Allografts rejected within 2 months.
Control skin grafts: rejection time of appropriate donor skin allografts in 500 rads irradiated mice without further treatment was ~50 days, and ~15 days in non-irradiated mice. d, deceased; nt, not tested.

Several other phenomena have been reported in the literature which may be of a similar nature to our findings. Rejection of donor skin grafts in xenogeneic radiation chimaeras (rat–mouse) has been described in animals in which donor-type red cells could still be detected[17,18]. Unirradiated mice or rats, rendered only partially tolerant to skin allografts by the neonatal injections of allogeneic *F*1 hybrid cells, were shown to have donor-type histocompatibility antigens in their lymphoid tissues[19,20].

Our observations indicate that the presence of donor-type immuno-globulins in allogeneic mouse radiation chimaeras is not necessarily correlated with tolerance to skin allografts from donors of the same inbred strain. This apparent paradox raises an interesting question as to the origin of the donor-type γ-globulin in the non-tolerant chimaeras.

Since the detection of donor cells in the chimaeras is based on their ability to synthesize a specific protein, it is conceivable that the donor-type γ-globulin may be produced by host cells which have received and incorporated donor messenger RNA (or DNA) which codes for donor-type γ-globulin (compare ref. 21). However, if the donor-type γ-globulins are synthesized by donor cells or their descendants, their continued presence in the face of an immune mechanism directed to another type of graft from the same donor could be accounted for by two basic hypotheses.

(1) The cell inoculum could be rejected by the same type of immune mechanism as that causing skin graft rejection, but, as it does not contain (at least in sufficient amounts) all the antigens determining histocompatibility present on the skin grafts[18–20, 22–24], tolerance to skin grafts has not been produced. It has also been suggested that, rather than inducing tolerance, the cell inoculum elicits a weak immune response and the skin graft might be more susceptible to this response than the donor haemopoietic or lymphoid cells, some of which would still persist[19,20].

(2) The rejection of skin allografts and cellular allografts depends on two different immune mechanisms, which have different radiosensitivities. Positive evidence of the existence of two basic immune mechanisms has been recorded[25], and the rejection of skin allografts has been shown to be less radiosensitive than humoral antibody formation[26,27].

The essential finding reported here is that donor-type γ-globulins may be found in allogeneic mouse radiation chimaeras without tolerance to skin homografts from donors of the same strain. To investigate this phenomenon evidence for the presence of donor transplantation antigens, transformed cells, or a differential suppression of the two types of immune reactions in chimaeric mice is being sought.

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