(*Reprinted from Nature, Vol. 202, No. 4930, pp. 352–353, April 25, 1964*)

PRESENCE OF DONOR SPECIFIC GAMMA-GLOBULINS IN SERA OF ALLOGENEIC MOUSE RADIATION CHIMERAS

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ALTHOUGH it is well established that a re-population of host hæmopoietic and lymphoid tissues by donor-type cells occurs in lethally X-irradiated mice restored by means of injected allogeneic or xenogeneic bone-marrow cells[1], there still remain unresolved questions relative to the origin of the serum proteins, particularly the immunoglobulins in such chimeras. In 1957 Grabar *et al.*[2], using immunoelectrophoretic methods, reported the presence of rat γ-globulin in the serum of lethally X-irradiated mice previously injected with rat bone marrow-cells. Similarly, Weyzen and Vos[3] found small amounts of rat-serum proteins in mice 100 days after irradiation and injection of rat-bone marrow cells. However, Gengozian[4] and Popp and Smith[5] were unable to detect serum rat globulins in their experiments on xenogeneic (rat–mouse) bone-marrow chimeras. More recently, Grabar *et al.*[6] have re-examined this question and have shown that the sera of rat–mouse radiation chimeras "contain several rat proteins including γ-globulin, at least two α_2 globulins, one or two β_1 globulins and one β_2 globulin".

In allogeneic radiation chimeras few data are available on the origin of the immunoglobulins and other serum proteins. Shreffler[7] was able to recognize transient presence of three types of donor serum protein (transferrin, the *Ss* substance, and a pre-albumin) in allogeneic mouse chimeras.

The recent development of methods for identifying mouse γ-globulin isoantigens[8–10] provides a means for the direct analysis of the origin of circulating γ-globulins in allogeneic chimeras. In this communication we report the finding of donor type γ-globulins in such radiation chimeras as late as nine months after irradiation and marrow transplantation.

Young adult *DBA/2J* mice (3–4 months of age) received a single (otherwise 100 per cent lethal) whole-body exposure of 840 rads of X-rays (250 kVp) at a dose-rate of 30 rads/min. This was followed within a few hours by a single intravenous injection of 8×10^6 nucleated bone-

marrow cells (suspended in Tyrode's solution) obtained from the femurs of normal (*C3H/HeJ* × *DBA/2J*)F_1 (abbreviated *C3D2*F_1) mice. In some experiments the irradiated *DBA/2* mice received an intravenous injection of $5{\cdot}7 \times 10^6$ *C3H/HeJ* marrow cells plus 3×10^6 nucleated lymph-node cells from *C3D2*F_1 donors. Control groups included syngeneic chimeras (*DBA/2* mice receiving 840 rads plus an injection of 8×10^6 *DBA/2* marrow cells) and other allogeneic chimeras, that is, 840 rads-irradiated *DBA/2* mice injected with $5{\cdot}7 \times 10^6$ *C3H* marrow cells plus 3×10^6 X-radiation-inactivated (2,000 rads *in vitro*) spleen-tissue cells from *C3D2*F_1 donors.

The general procedures for preparation of cell suspensions, injections, and animal care were the same as those described previously[11]. At various times up to 9 months post-irradiation and marrow injection, blood was obtained from the retro-orbital sinus; the serum from each mouse was prepared individually and stored at $-10°$ C until tested.

Allogeneic tail skin grafts prepared by the method of Bailey and Usama[12] were engrafted on the surviving chimeras 6 weeks post-irradiation. The skin-graft donors were normal adult *C3D2*F_1 hybrid and *A/HeJ* mice. The criteria for assessing survival and rejection time of these grafts have been described in a previous report[13].

γ-Globulin-levels were determined semi-quantitatively by an end-point precipitation in Ouchterlony[14]-type agar gel double diffusion. Glass slides 2 in. × 3 in. were covered with 6·5 ml. molten agar (1 per cent 'Ionagar' in 0·05 M veronal, *p*H 8·2, and containing 0·1 per cent sodium azide). After setting of the agar, hexagonal patterns of concentric pairs of holes were made with a 15-gauge needle to which negative pressure was applied, and a plastic template. The distance between the centres of the middle and peripheral holes is 4 mm. 2 ml. of serial doubling dilutions of sera from chimeras and of standard sera were introduced into the peripheral holes, and specific antisera into the central holes. The plates were allowed to develop for 15–18 h at room temperature. Precipitation is essentially complete after 4–6 h. The γ-globulin-level is taken as the highest dilution giving a precipitation line which joins the line of 7*S* γ-globulin in one of the adjoining positions on the Ouchterlony pattern. To validate this determination, 7*S* γ-globulin-levels of sera from several chimeras were determined by inhibition of precipitation of ^{125}I-labelled 7*S* γ-globulin. The values obtained were within the two-fold range of error expected for a doubling dilution assay.

Rabbit anti-mouse-γ-globulin which precipitates strongly with 7*S* γ-globulin was produced by injection of pooled mouse (*C3H* and *C57B1/10*) 7*S* γ-globulin preparations in Freund's adjuvant, followed by booster injections without adjuvant. Specific antisera against 7*S* γ-globulin isoantigens were produced as previously described[9].

Table 1. Specific γ-Globulin Isoantigens in Serum from Allogeneic Mouse Radiation Chimeras

Chimeras: X-rayed *DBA*/2 mice (840 rads) injected with cells	Individual mouse Nos.	Time tested (months)	Specific γ-globulin level* *Iga*1	Total	*Iga*3
$C3D2F_1$ marrow	1–7	5	2–8	32	> 32
	1–7	9	1–4	8–32	8 to > 32
C3H marrow + $C3D2F_1$ lymph node	8–12	2	1–32	16–128	> 32
	8–12	6	4–32	8–128	0 to 16
	13–16	9	2–32	16–64	0–8
C3H marrow + $C3D2F_1$ thymus	17	9	8	16	0
C3H marrow + X-irrad. (2,000 rads) $C3D2F_1$ spleen	18, 19	9	4	8, 32	0, 2
DBA/2 marrow	20–24	9	0	8–32	16–32
Controls					
Non-irrad. *C3H* mice			128	> 128	0
Non-irrad. $C3D2F_1$ mice			64	> 128	> 32
Non-irrad. *DBA*/2 mice			0	> 128	> 32

* The highest dilution giving a precipitation line which joins the line of 7*S* γ-globulin. The values represent either individual mouse values or their range within each treatment group.

From the experimental data summarized in Table 1, it is evident that *Iga*1-type γ-globulins, necessarily derived from the injected cells, were present in the serum of nearly all the allogeneic radiation chimeras up to 9 months after irradiation and injection of $C3D2F_1$ marrow cells or of *C3H* marrow plus $C3D2F_1$ lymphoid cells. In nearly all chimeras, furthermore, *Iga*3-type γ-globulin, which might have come from donor or recipient cells, was found. However, there was considerable variation in both donor and total γ-globulin-levels among the chimeras. By contrast, the sera from 5 syngeneic chimeras (*DBA*/2–*DBA*/2) were negative for *Iga*1 γ-globulin, and showed high levels of *Iga*3 γ-globulin, the *DBA*/2 type.

The responses of the chimeras to skin homografts provide an additional, independent criterion for chimerism[15]. Skin grafts from normal $C3D2F_1$ and *A*-strain donors were placed on the chimeras 6 weeks after irradiation and marrow infusion. Chimeras No. 1–7 (see Table 1) all rejected *A*-strain skin grafts (generically foreign to donor and host) in 11 to 29 days, whereas $C3D2F_1$ skin (marrow donor genotype) was retained indefinitely (that is, more than 9 months) by all except one chimera (No. 4), in which case the $C3D2F_1$ skin graft was rejected at 11 days. It is of interest that this same mouse does have donor-type γ-globulin. These data indicate that the chimeras were specifically tolerant of skin homografts[11]. By contrast, the syngeneic chimeras (*DBA*/2–*DBA*/2) rejected both the *A*-strain grafts (in 15–20 days) and the $C3D2F_1$ skin grafts (in 13–25 days).

DBA/2 mice have a genetic deficiency of a protein designated *hc*[1], which migrates electrophoretically with the β-globulins and is a component of hæmolytic complement[16,17]. Since this protein is produced in *C3H* mice, its presence was looked for in the chimeras. None of the chimeras in this investigation had a detectable level of

hc[1] when tested by agar-gel precipitation with a specific antiserum which could have detected a level of 10 per cent that of *C3H*, under these conditions.

It is clear from these results that allogeneic bone marrow radiation chimeras contain circulating γ-globulin isoantigens of marrow donor origin many months after irradiation and marrow cell infusion. These γ-globulins must have been elaborated by cells originally present in the injected marrow suspension, or by their progeny. This implies that the donated γ-globulin-synthesizing cells have maintained their genetic identity in the allogeneic hosts, at least with respect to the γ-globulin marker.

It is not unexpected that all serum proteins are not suitable as markers in radiation chimeras. *Hc*[1] may now be added to the three proteins examined by Shreffler[7] as being of limited utility in following repopulation with marrow or lymph-node cells. However, a cell type which, when transplanted, yields a chimera producing one of these proteins would establish it as a site of synthesis of this protein. It is evident from the present data that the γ-globulin isoantigens offer an additional marker for following the kinetics of cell-population changes in radiation bone-marrow chimeras. Red-cell isoantigens have already been exploited for this purpose[18]. Thus, a study in which the two marker systems are used simultaneously could provide further insights on the fate and possible interrelations between donor erythroid and lymphoid cell populations in radiation chimeras.

This work was supported by U.S. Public Health Service grant *C* 4681–05, and by funds from the Bureau of Medicine and Surgery, U.S. Navy Department.

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Printed in Great Britain by Fisher, Knight & Co., Ltd., St. Albans.