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DIFFERENTIAL RADIOSENSITIVITY OF FIRST- AND
SECOND-SET RESPONSES TO ALLOGENEIC AND
XENOGENEIC SKIN GRAFTS IN SUBLETHALLY IRRADIATED MICE

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ABSTRACT

Data are presented demonstrating the differential radiosensitivity of the first and second-set responses of sublethally irradiated (670 rad) mice previously sensitized with allogeneic (H-2 difference), xenogeneic (rat) or both allogeneic and xenogeneic skin grafts: The second-set response is more radioresistant than is the first-set response; the second-set response to a xenogeneic graft remains intact during and following recovery from the effects of sublethal irradiation; while intact for a brief period following irradiation, the second-set response to an allogeneic skin graft was abrogated by sublethal irradiation (670 rad); concurrent pre-sensitization with allogeneic and xenogeneic skin grafts prevented the abrogation of the second-set response to the allogeneic graft by sublethal irradiation; the first-set response to an allogeneic graft recovered from the effects of sublethal irradiation prior to the recovery of the first-set response to a xenogeneic graft.

SUMMARY

The Problem:

A previous report demonstrated that mice sensitized with skin from a non-related mouse or from a rat retained some ability to reject subsequent specifically related skin grafts in an accelerated manner following a lethal dose of X irradiation. The present report confirms and extends these findings in sublethally irradiated mice.

The Findings:

Sublethally irradiated mice previously sensitized with rat skin rejected subsequent rat skin grafts in a normal accelerated manner at the times tested; mice similarly irradiated, but sensitized against the skin of a non-related mouse, were unable to reject subsequent grafts in an accelerated manner when tested several days following irradiation.

INTRODUCTION

A previous communication (4) presented data demonstrating in lethally irradiated, bone-marrow protected mice the differential radio-sensitivity of first and second-set responses to allogeneic and xenogeneic skin grafts. It was found that the second-set response of mice pre-sensitized with allogeneic or xenogeneic skin grafts was more radio-resistant than was the first-set response; the second-set response to a xenogeneic skin graft was more radioresistant than was that to an allogeneic graft; the converse was true with respect to the first-set response.

The present report confirms and extends these findings in sub-lethally X-irradiated (670 rad) mice.

MATERIALS AND METHODS

Twelve to 14 week old female (C57L x A) F_1 (LAF $_1$) mice were used as skin graft recipients. Skin graft donors were adult female LAF $_1$ (H2 ab), male BALB/c (H2 d) and (C3H x DBA/2) F_1 , (C3D/2 F_1), (H2 Kd) mice, and 2 - 3 week old male and female Sprague-Dawley rats. The orthotopic tail skin grafting method of Bailey and Usoma was used (1). Details of grafting and the criteria of rejection (total destruction of the engrafted tissue) have been reported previously (4). Mean survival time of the grafts and standard deviation (S.D.) are reported.

Normal values for the first and second-set responses of non-irradiated LAF $_1$ mice to (1) BALB/c skin alone, (2) rat skin alone, (3) BALB/c and rat skin and (4) BALB/c, C3D2 F_1 and rat skin have been reported previously (4) and are presented in Table I.

The mice were sensitized to BALB/c, rat, or BALB/c and rat skin by means of two consecutive skin grafts. One week following the rejection of the second graft(s), the mice received 670 rad whole body X radiation.

TABLE I

REJECTION OF ALLOGENEIC AND XENOGENEIC SKIN GRAFTS BY LAF₁ MICE¹

<u>TYPE OF GRAFTS</u>	<u>SET</u>	<u>NO. MICE</u>	<u>MEAN SURVIVAL TIME OF GRAFTS(days ± S.D.)</u>		
			<u>BALB/c</u>	<u>C3D/2F₁</u>	<u>RAT</u>
BALB/c	1st	33	12.6 ± 0.8		
BALB/c	2nd	38	6.1 ± 1.2		
Rat	1st	28			7.6 ± 0.7
Rat	2nd	29			4.4 ± 0.6
BALB/c, rat	1st	31	9.7 ± 1.2		8.3 ± 0.6
BALB/c, rat	2nd	24	5.0 ± 0.9		4.0 ± 0.0
BALB/c, C3D/2F ₁ , rat	1st	30	10.1 ± 1.6	10.1 ± 1.5	7.7 ± 1.0
BALB/c, C3D/2F ₁ , rat	2nd	10	7.1 ± 0.3	7.0 ± 0.7	4.0 ± 0.0

¹These data have been reported previously (4). Orthotopic tail skin grafts, single or multiple, were placed on 12-14 week old female LAF₁ mice. Tail skin donors were adult male BALB/c and C3D/2F₁ mice and 2-3 week old male and female Sprague-Dawley rats.

The radiation factors (250 kvp, 15 ma; HVL 1.5 mm Cu; 30 rad/min) and details of exposure were the same as previously reported from this Laboratory (2). At various intervals following irradiation (0.2, 1.0, 2.0, 4.0, 5.0, 13.0, 20.0, 30.0 and 45.0 days), groups of mice were grafted with LAF₁, BALB/c, C3D2 F₁ and rat skin. Non-sensitized mice were irradiated and grafted in a similar manner.

Preliminary observations indicated that sensitization with both BALB/c and rat skin grafts provided greater protection of the second-set response to allogeneic grafts against the effects of sublethal irradiation than did sensitization with BALB/c skin alone. A previous report (5) indicated that non-irradiated and sublethally irradiated mice previously sensitized with rat skin grafts rejected subsequent first-set allogeneic grafts significantly sooner than did their appropriate controls. It was demonstrated in sublethally irradiated mice that this effect could be abrogated by means of antisera produced against the allogeneic graft. This suggested that the phenomenon was due to a "quantitatively expanded" first-set response as specific antisera had no effect upon an established second-set response. Therefore, one group of mice sensitized against both BALB/c and rat skin received 1.0 ml LAF₁ anti-BALB/c skin graft serum intraperitoneally immediately after irradiation and just prior to grafting. The anti-serum was prepared from blood harvested aseptically from LAF₁ mice one week following the rejection of the second consecutive BALB/c skin graft. It was stored at -15 C until used.

All mice were housed 10 per cage. The diet was Purina Lab Chow, and water containing 1% Neomycin was given ad lib.

RESULTS

Allogeneic Grafts Table II

The majority of sublethally irradiated LAF₁ mice previously sensitized with BALB/c skin grafts and grafted prior to the thirteenth post-irradiation day rejected their allogeneic grafts in an apparently normal second-set manner. However, those mice grafted between the thirteenth and forty-fifth post-irradiation days showed little or no evidence of recovery of the second-set response to BALB/c skin grafts.

It should be noted that the primary response to an allogeneic skin graft recovered from the effects of the radiation prior to the first-set response to a xenogeneic graft.

TABLE II

REJECTION OF ALLOGENEIC AND XENOGENEIC SKIN GRAFTS BY SUBLETHALLY
IRRADIATED (670 RAD) LAF₁ MICE PREVIOUSLY SENSITIZED WITH BALB/c
SKIN GRAFTS¹

SENSITIZING GRAFTS	GRAFTED POST-IRRAD. DAY	NO. MICE	MEAN SURVIVAL TIME OF GRAFTS(days ± S.D.)		
			BALB/c	C3D/2F ₁	RAT
BALB/c skin	0.2	6	18.3 ± 3.1	18.2 ± 4.4	22.1 ± 2.2
BALB/c skin	0.2	10	10.7 ± 3.4 ²	10.8 ± 3.1 ³	24.4 ± 2.3
None	0.2	25	21.0 ± 3.5	21.1 ± 3.3	22.7 ± 3.5
BALB/c skin	1.0	8	11.1 ± 2.6	13.0 ± 5.0	22.5 ± 1.2
None	1.0	8	23.2 ± 2.4	22.5 ± 2.8	23.0 ± 1.7
BALB/c skin	2.0	10	10.5 ± 5.6 ⁴	10.7 ± 5.5 ⁵	19.9 ± 1.9
None	2.0	7	16.8 ± 1.9	16.1 ± 1.6	21.3 ± 1.5
BALB/c skin	13.0	5	12.8 ± 4.3 ⁶	12.8 ± 3.3 ⁷	12.8 ± 1.7
None	13.0	9	11.7 ± 0.8	11.7 ± 0.8	11.3 ± 0.8
BALB/c skin	30.0	6	10.0 ± 2.6 ⁸	11.4 ± 1.0	9.3 ± 0.9
None	30.0	10	9.3 ± 1.0	9.3 ± 1.0	6.6 ± 1.2
BALB/c skin	45.0	8	9.8 ± 2.4 ⁹	10.4 ± 1.3	9.2 ± 1.0

¹The mice were irradiated one week following the rejection of the second consecutive BALB/c skin graft.

²5/10 BALB/c grafts rejected between 7-9 days.

³5/9 C3D/2F₁ grafts rejected between 7-9 days.

⁴7/10 BALB/c grafts rejected between 6-8 days.

⁵7/10 C3D/2F₁ grafts rejected between 6-9 days.

⁶1/5 BALB/c grafts rejected at 7 days.

⁷1/5 C3D/2F₁ grafts rejected at 7 days.

⁸1/6 BALB/c grafts rejected at 6 days.

⁹2/8 BALB/c grafts rejected at 6 days.

Xenogeneic Grafts Table III

Except for a moderate impairment between the first and fifth post-irradiation days, the second-set response of sublethally irradiated mice previously sensitized with rat skin was found to be intact at the times tested (0.2, 1.0, 4.0, 5.0, and 20.0 days).

As previously reported (4), grafting irradiated mice between the second and eighth post-irradiation days was associated with an extremely high mortality (Table III). This interesting and obscure phenomenon has been reported previously where the sensitizing agents were sheep and rat RBC (3).

Allogeneic and Xenogeneic Grafts Table IV

Sublethally irradiated mice previously sensitized with both BALB/c and rat skin grafts rejected subsequent allogeneic and xenogeneic grafts in a vigorous second-set manner at the times tested (0.2 and 20.0 days). Rejection of the allogeneic grafts was significantly better than that seen in mice sensitized against BALB/c skin alone (Table II); the second-set response was intact at twenty-days post-irradiation. The injection of specific antiserum immediately following irradiation had no significant effect upon this response. In the group grafted 0.2 days following irradiation but not receiving antiserum, three isogenic grafts were rejected on the fourth day in a manner suggesting a "homo-graft response", and the remaining isografts were distinctly inflamed and friable between the fourth and sixth post-irradiation days. This phenomenon was not observed among the other groups. As previously reported (5), the nature of the pathologic changes suggested either the presence of a non-specific agent capable of increasing capillary permeability or the deficiency of a factor essential to the maintenance of capillary wall integrity.

DISCUSSION

The present data in sublethally irradiated mice support and extend the findings reported in a prior communication (4). It was demonstrated in lethally irradiated, bone-marrow protected mice, that the second-set response of mice pre-sensitized with allogeneic or xenogeneic skin grafts was more radioresistant than was the first-set response; the second-set response to a xenogeneic skin graft was more radioresistant than was that to an allogeneic graft; the converse was true with respect to the first-set response.

The foregoing data indicate that, with the methods used, the

TABLE III

REJECTION OF ALLOGENEIC AND XENOGENEIC SKIN GRAFTS BY SUBLETHALLY
IRRADIATED (670 RAD) LAF₁ MICE PREVIOUSLY SENSITIZED WITH RAT SKIN
GRAFTS¹

SENSITIZING GRAFTS	GRAFTED POST-IRRAD. DAYS	NO. MICE	MEAN SURVIVAL TIME OF GRAFTS (DAYS $\pm$ S.D.)		
			BALB/c	C3D/2F ₁	RAT
Rat skin	0.2	7	22.1 $\pm$ 3.0	22.2 $\pm$ 3.0	7.0 $\pm$ 1.4
Rat skin	0.2	10	15.4 $\pm$ 4.3	11.4 $\pm$ 4.9	4.4 $\pm$ 0.8
Rat skin	0.2	9	20.5 $\pm$ 8.7	18.3 $\pm$ 8.0	3.6 $\pm$ 0.6
None	0.2	25	21.0 $\pm$ 3.5	21.1 $\pm$ 3.3	22.7 $\pm$ 3.5
Rat skin ²	1.0	4			~ 8.0
None ³	1.0	8	~ 24.0	~ 24.0	~ 23.0
Rat skin ⁴	4.0	4			~ 5.4
None ⁵	4.0	8	~ 22.0	~ 24.0	~ 22.0
Rat skin ⁶	5.0	8			5.2 $\pm$ 1.0
None ⁷	5.0	5			
Rat skin	20.0	6	10.0 $\pm$ 1.1	9.8 $\pm$ 1.0	3.6 $\pm$ 0.7

¹The mice were irradiated one week following the rejection of the second consecutive rat skin graft.

²An extremely high mortality was associated with grafting these mice between the first and eighth post-irradiation days. 4/4 rat grafts were rejected at 8 days while all allogeneic grafts were intact at the time of death.

³8/8 lived 10 days with all grafts intact. 2/8 lived 31 days at which time all grafts had been rejected.

⁴All rat grafts were rejected and all allogeneic grafts intact when the mice died on 6th post-irradiation day.

⁵Eight mice lived 7 days with all grafts intact. Two mice lived 31 days.

⁶All rat grafts were rejected and all allogeneic grafts intact when the last mouse died on 7th post-irradiation day.

⁷All grafts were intact when the last mouse died on the 7th post-irradiation day.

TABLE IV

REJECTION OF ALLOGENEIC AND XENOGENEIC SKIN GRAFTS BY SUBLETHALLY
IRRADIATED (670 RAD) LAF₁ MICE PREVIOUSLY SENSITIZED WITH BALB/c
AND RAT SKIN GRAFTS¹

SENSITIZING GRAFTS	GRAFTED POST-IRRAD. DAY	NO. MICE	MEAN SURVIVAL TIME OF GRAFTS (DAYS $\pm$ S.D.)		
			BALB/c	C3D/2F ₁	RAT
BALB/c and rat skin ²	0.2	10	5.6 $\pm$ 1.1	5.4 $\pm$ 1.0	4.1 $\pm$ 0.5
BALB/c and rat skin ³	0.2	8	7.5 $\pm$ 2.8	6.8 $\pm$ 2.4	4.0 $\pm$ 0.5
BALB/c and rat skin	20.0	4	7.2 $\pm$ 2.9	8.7 $\pm$ 2.1	3.5 $\pm$ 0.8

¹The mice were irradiated one week following the rejection of the second set of BALB/c and rat skin grafts.

²Three isogenic grafts were rejected on the 4th day in a manner suggesting a homograft response. All other isogenic grafts were red and friable at this time.

³These mice received 1.0 ml LAF₁ anti BALB/c skin serum ip immediately following irradiation.

second-set response to an allogeneic graft remains relatively intact during the first few days following sublethal irradiation but appears to have been abrogated when tested thereafter. In contrast, except for a moderate impairment shortly following irradiation, the second-set response in sublethally irradiated mice previously sensitized with rat skin was intact at the times tested (0.2, and 20.0 days). Further, sensitization with both BALB/c and rat skin afforded relatively complete protection of the second-set response to allogeneic skin grafts against the effects of sublethal irradiation.

The implications of these observations with regard to the heterogeneity of the "immune system" have been discussed previously (4,5): These data strongly suggest the existence of independent and interdependent" cell lines or systems", each with its own spectrum and potential for reactivity; they further suggest that at least two distinct cell types are involved in the second-set rejection of an allogeneic skin graft.

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