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IMMUNE COMPETENCE IN ^{90}SR-EXPOSED, ADULT THYMECTOMIZED AND ANTILYMPHOCYTEGLOBULIN-TREATED CBA MICE

I. Allogenic skin graft reaction

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Abstract

CBA mice subjected to either adult thymectomy, internal exposure to ^{90}Sr or antilymphocyteglobulin treatment separately, or to combinations of the three were tested for cellular immune competence using their reaction to allogenic skin grafts. Peripheral blood white cell counts did not reveal any obvious correlation between the degree of mononuclear cell depletion and the ability to accept grafts, suggesting that the particular treatments depleted specific fractions of mononuclear cells, differing in their extent of involvement in the rejection process. No single treatment alone induced a significant prolongation in the time elapsed before graft rejection. Adult thymectomy followed by appropriate antilymphocyteglobulin treatment induced severe lymphocytopenia and a profound suppression of the cell-mediated immune system, as evidenced by the acceptance of allogenic skin grafts. When applied to ^{90}Sr-preexposed mice the same treatment induced lifelong acceptance of grafts, indicating a similar, though weaker immunosuppressive impact of ^{90}Sr. Hence it was possible to significantly enhance immunosuppression in ^{90}Sr-exposed mice. This *in vivo* model should be useful when investigating the role of immunological responsiveness in radiation carcinogenesis.

Key words: Radiation biology, mice, immunology, strontium-90, thymectomy, antilymphocyteglobulin, skin-grafting.

The β-emitting radionuclide ^{90}Sr is known to be immunosuppressive (1–5) as well as carcinogenic (6–11) when incorporated into the mammalian body. The role of this immunosuppression in the oncogenic process is unclear, however. To increase our knowledge of such interactions our approach has been to severely suppress the immune system and follow the subsequent development of oncogenesis. Therefore various non-specific immunosuppressive treatments have been evaluated with regard to effectiveness, duration of effects and applicability to life-span studies of ^{90}Sr-exposed mice.

Adult thymectomy (ATx) followed by long-term treatment with antilymphocyteglobulin (ALG) causes a substantial reduction in levels of peripheral blood mononuclear cells (MNC). This is also true with ^{90}Sr, and the combined treatment is extremely efficient in this respect (1, 2).

The reduction of peripheral blood MNC counts by ALG is time restricted and may even be followed by a temporary overcompensation in number (2). After ATx, by which the main source of T-cell regeneration is removed, ALG treatment induces a pronounced and persistent T-cell deficiency which is even more profound after ^{90}Sr pretreatment (1).

Strontium-90 affects both B- and T-cells, but primarily B-cells owing to the close contact between this bone-incorporated isotope and the B- and pre-B-cells in the bone marrow. However, this loss is partly compensated for by extramedullary regeneration. The T-cells, on the other hand, which are located in the thymus, lymph nodes, spleen and blood, with only low amounts present in the bone marrow, are mainly affected by the loss of precursors located in the bone marrow. Natural killer (NK) cells are also severely depleted by radiostrontium treatment (12, 13).

In an attempt to analyse the effects of ^{90}Sr, ATx and ALG treatment on cell-mediated immune function, the method of skin allografting was applied.

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Table

Skin allograft rejection time and index (=No. of grafts rejected per group total) in male CBA mice pretreated by adult thymectomy (ATx), ^{90}Sr or antilymphocyte-globulin (ALG) or combinations of the three. Mononuclear cell (MNC) counts per µl peripheral blood at the time of grafting

Exp. group	General code	No. of mice	Pretreatments						Graft rejection time		Rejection index day 10	Mononuclear cells*
			Atx	ALG (6 mg/week)			kBq ^{90}Sr/g b.w.		Days*	Range		
				2×	4×	8×	14.8	29.6				
1	E I	11							9.6±0.3	7.5–11.5	0.60	4 510±467
2	E II	11					+		9.6±0.4	7.0–11.5	0.50	2 090±280
3	E II	11						+	9.8±0.4	7.0–11.5	0.69	1 092±136
4	E IV	8	+	+			+		11.3±0.5	8.5–13.0	0.25	555±121
5	E IV	8	+		+		+		11.7±0.7	10.0–13.5	0.33	396±84
6	E IV	8†	+	+				+	11.8±0.4	11.0–13.0	0.14	763±165
7	E IV	8	+		+			+	14.1±1.4	10.0–19.0	0.17	367±52
8	E IV	8	+			+		+	Grafts accepted**		0.00	725±121
9	E VI	10			+				10.9±0.5	8.0–13.5	0.40	2 140±266
10	E VI	10†				+			11.3±0.6	8.0–14.0	0.33	3 920±466
11	E VII	8	+	+					10.8±0.5	9.0–12.5	0.38	2 344±462
12	E VII	8	+		+				10.8±0.4	9.0–12.0	0.28	2 000±482
13	E VII	8	+			+			107.3±8.3	81.0–138.0	0.00	3 610±420
14	E VIII	11	+						9.8±0.4	7.5–12.0	0.53	4 230±490

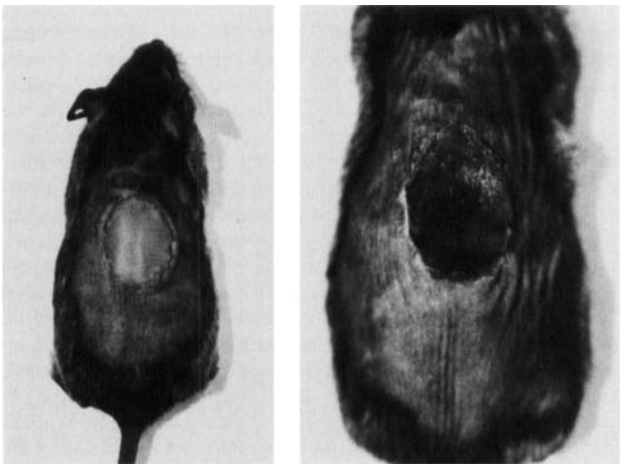
* Mean ± SE.
** No signs of rejection observed during the remainder of the animal's life, 134±39 (range 21–285) days.
† One mouse lost before grafting.

In a following report the phagocytic function of the RES and the *in vitro* mitogen responsiveness of spleen cells will be described (14).

Material and Methods

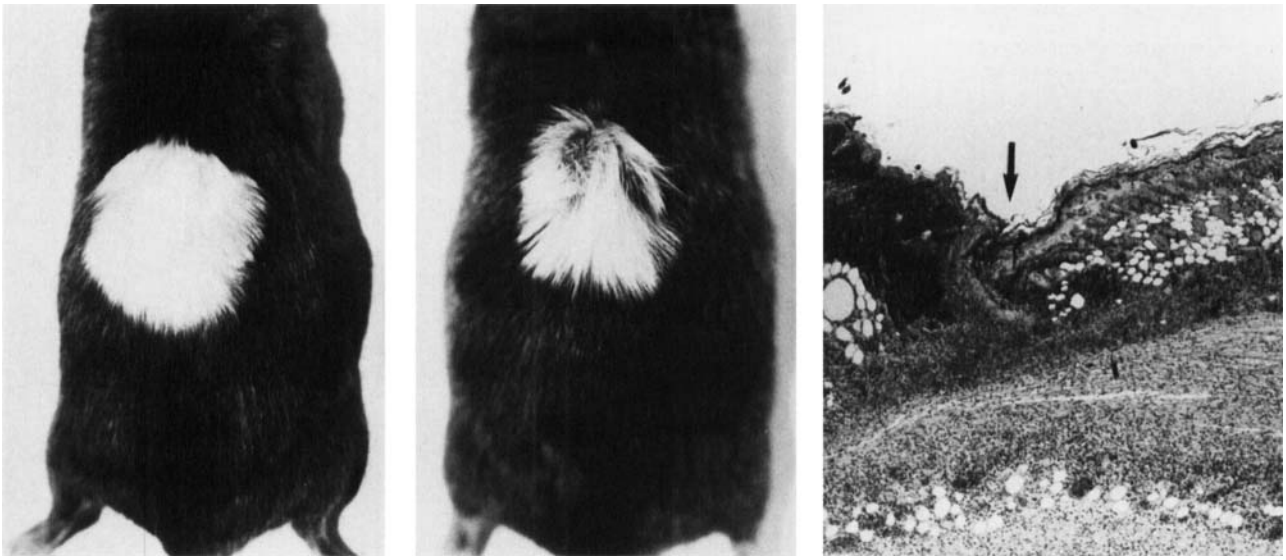
Animals and procedures. Male CBA/SU mice from our own inbred stock (origin: CBA/Ca via MRC Harwell to the Department of Genetics, Stockholm University) were used as recipients of skin-allografts after one of the following pretreatments: a) thymectomy at 60±3 days of age, b) intraperitoneal injection of ^{90}Sr (14.8 kBq (0.4 µCi) or 29.6 kBq (0.8 µCi)/g body weight) at 75±3 days of age, c) intraperitoneal injections of ALG (6 mg ALG/mouse/week for 2, 4 or 8 weeks prior to grafting) and combinations of these treatments as depicted in the Table. Non-pretreated mice were used as controls. Each group, containing a minimum of 8 mice, was coded after the principal treatment (E I–VIII) and each mouse was assigned a blind identity number. Surgical thymectomy was performed as described previously (1). ALG treatment was continued in certain of the groups for 8 weeks after grafting. For each group skin transplantations were performed on day 182 after exposure to ^{90}Sr.

A slightly modified version of the grafting technique described by Rygaard (15) was used. The graft, circular in shape and 15–20 mm in diameter, was placed in the graft bed on the dorsum of the recipient and fixed with a small amount of histoacryl (Kemi Intressen AB), an absorbable, neutral tissue adhesive (Fig. 1). The recipients were kept in individual cages during healing and observed daily. NMRI male mice, 4–5 days of age and supplied by the Anticimex breeding laboratories, Sollentuna, Sweden, were used as skin graft donors. The graft rejection time was defined as follows. Rejection time in days=1/3 (A+B+C), where A=interval between grafting and the first apparent signs of necrosis, e.g. discoloration, eleva-



a b

Fig. 1. a) Dorsal view of a non-pretreated CBA mouse illustrating the site, size and shape of a hairless skin graft from a 4-day-old NMRI mouse donor 24 h after transplantation and b) the same graft on day 9, showing typical signs of rejection with bleeding, necrosis and elevation of the graft.



a b c

Fig. 2. CBA mouse treated by ATx+ALG before skin allografting (NMRI). a) Dorsal view of well accepted graft showing full growth of white hair 40 days after transplantation. b) Same graft with clear clinical signs of rejection 100 days after transplantation. The histological picture c) reveals necrosis of grafted skin, to the right, and infiltration of macrophages and mononuclear cells. Graft junction indicated by arrow. Van Gieson ×45.

tion, stiffness and/or shrinking of the graft; B=interval between grafting and the predominance of necrosis in the graft (≥50%); C=interval between grafting and complete necrosis and discharge of the graft.

Individual blood samples were taken immediately before grafting by puncture of the orbital venous plexus. Leukocytes were stained with Türk's gentian violet and counted in a Bürker haemocytometer.

The mice were housed in conventional animal rooms in macrolon cages and given free access to a pelleted diet (Standard Feed for Rats and Mice, Astra-Ewos) and water. The room temperature was 22–24°C, the relative humidity 50 to 55% and the light/dark cycle 12/12 h.

ALG. Freeze-dried horse-antimouse-lymphocyteglobulin, raised with thymic and lymph node cells, was supplied by TNO, Rijswijk Z.H., The Netherlands. Before use it was dissolved in saline to a concentration of 30 mg/ml.

Radionuclide. Carrier-free strontium-90 nitrate in 1-N nitric acid, supplied by the Radiochemical Centre, Amersham, UK, was diluted in saline to an activity of 1.69×10^3 kBq (45.75 μCi) ^{90}Sr per ml, and was in equilibrium with ^{90}Y when injected.

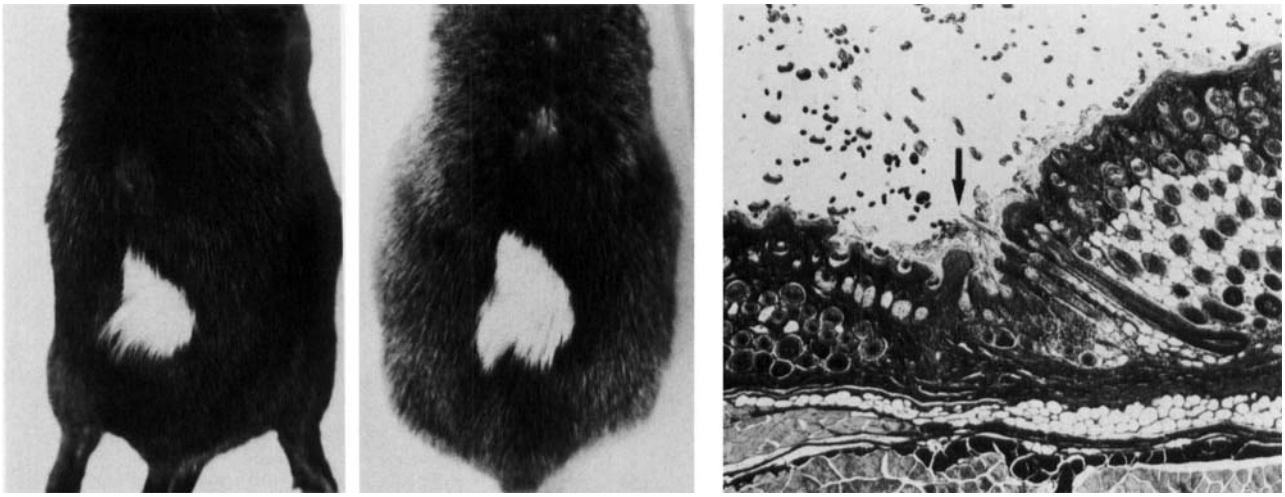
Microscopy. Skin transplants were fixed in Stieve's fluid (16). Ordinary histologic techniques were used and sections were stained according to the van Gieson's method as well as with haematoxylin and eosin.

Results

Graft rejection time. A single treatment with ^{90}Sr, ATx or ALG did not reveal any significant change in the ability of the mice to reject a primary allogenic skin graft, as compared with untreated controls (Table). However, when these treatments were combined, rejection was delayed, and the effect was significant for ATx+ALG (8x) and ^{90}Sr+ATx+ALG (8x). Thus ATx+ALG (8x)-pretreated mice (group 13) accepted the grafts readily and continued to do so for 107±8 days (mean ± SE), when rejection occurred (Fig 2a–c). Initially the ^{90}Sr+ATx+ALG (8x)-pretreated mice (group 8) accepted the grafts less readily and frequently showed incomplete acceptance; still they never showed any signs of rejection during the rest of their life (Fig 3a–c). The mean length of the observation period was 134±39 (SE) days, and the range was 21–285 days. Two animals in this group died early from malignant lymphoma, most likely a consequence of the exposure to radiostrontium.

Graft rejection index. The data on rejection index (i.e. number of grafts rejected per group total) on day 10 after transplantation reveal that there was an increasing tendency for the 4- and 8-week-long ALG treatments to delay rejection of the grafts (groups 9–10). This was not the case with ^{90}Sr or ATx when used alone (groups 2, 3 and 14). Furthermore, the ATx+ALG treatment (groups 11, 12 and 13) was even more effective in delaying rejection, an effect that was reinforced by ^{90}Sr (groups 4–8).

Mononuclear cells. The numbers of peripheral blood MNC, counted at the time of grafting, are presented in the Table. ^{90}Sr was clearly the most effective of the 3 treatments in reducing the number of circulating MNC. Although the impact of ALG was not equally obvious when used alone, it was evident in the combined treatments.



a b c

Fig. 3. CBA mouse treated by ^{90}Sr+ATx+ALG before skin allografting (NMRI). a) Representative picture of a graft that initially was only partially accepted; eventually, however, it developed a full growth of white hair and showed no signs of rejection at either 40 days or b) 100 days after transplantation. The histological picture c) reveals no signs of rejection after 150 days. Graft junction indicated by arrow with graft to the right. Note difference in hair pigmentation. Van Gieson ×45.

However, there was no simple correlation between delay in graft rejection and depletion of peripheral blood MNC.

Histology of grafts. The microscopic appearance of a skin graft undergoing rejection is illustrated in Fig. 2c. A graft with no signs of rejection reaction is shown in Fig. 3c.

Discussion

Allograft rejection is one of the standard methods used to assess cell-mediated immunological competence in experimental animals. Since rejection of a skin graft is a variable, progressive process which can be followed clinically over a number of days, the rejection time was defined as the mean value of measurements made at the beginning, middle and end of the rejection process. This recording method was particularly motivated by the prolongation and/or irregularity in development of the rejection process, as observed in some of the immunosuppressed animals.

The grafts in the ^{90}Sr+ATx+ALG (8x)-treated mice (group 8) frequently did not heal as readily and completely during the initial phase as did the grafts in the ATx+ALG (8x)-treated mice (group 13). This was probably due to reduced resistance to microbial infections in the former group, owing to extensive suppression of the immune system.

The experiment demonstrated that ATx followed by appropriate ALG treatment (6 mg ALG/mouse/week starting 8 weeks before grafting and continued for another 8 weeks) induced severe lymphocytopenia and a profound and long-lasting suppression of the cell-mediated immune system, as evidenced by the acceptance of allogenic skin grafts. When applied to ^{90}Sr-preexposed mice the same treatment induced lifelong immunosuppression, indicating a similar, though weaker immunosuppressive impact of ^{90}Sr.

The peripheral blood white cell counts did not reveal any obvious correlation between the degree of MNC depletion and the ability to accept grafts, suggesting that particular treatments depleted specific MNC-fractions, differing in their involvement in the rejection process.

The peripheral blood MNC counts confirmed our previous findings (unpublished) that ATx alone has no significant impact on the number of circulating cells, whereas ^{90}Sr as well as ALG induce a dose-related depletion of MNC. In the latter case, however, this depletion is soon compensated for by recruitment of cells (group 10 and ref. 2). We have previously shown that in ATx+long-term ALG-treated mice the depleted peripheral blood MNC population contains only a minor proportion (17%) of T-cells, which are considered crucial for transplant rejection (1).

Allograft rejection occurs when transplanted cells are exposed to sensitized host lymphocytes, macrophages and specific antibodies. The cytotoxic T-lymphocytes (CTL), which are capable of lysing histo-incompatible cells, have been implicated as the principal effector cells in this process. It has also been shown that CTL are capable of killing autologous tumour cells *in vivo* (17). Thus it seems reasonable to anticipate that immunosuppression by ATx+ALG treatment (group 13), which has proved efficient enough to abrogate acute rejection of skin allografts, may be equally as effective at permitting growth of malignant cells with antigenic properties, such as radiation-induced osteosarcomas (18, 19). Consequently, the dramatic reduction in cell-mediated immune competence induced by ATx+ALG treatment in ^{90}Sr-exposed

mice, as demonstrated in this paper, suggests that this methodology offers great promise for use in *in vivo* investigations concerning the role of immunological responsiveness in radiation carcinogenesis.

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