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MODIFICATION OF THE GRAFT-*VERSUS*-HOST SYNDROME BY ANTI-LYMPHOCYTE SERUM TREATMENT OF THE DONOR

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SUMMARY

Spleen and lymph node suspensions prepared from parental donor mice pre-treated with four daily anti-lymphocyte serum (ALS) injections have been demonstrated to be incapable of inducing a graft-*versus*-host (GVH) syndrome when injected into F_1 hybrid recipient mice. A single ALS injection to the parental donor renders thoracic duct lymphocytes incapable of inducing a GVH response but the activity of spleen cell suspensions is retained.

It is suggested that this observation supports the view that ALS acts mainly on circulating lymphocytes.

INTRODUCTION

The immunosuppressive properties of anti-lymphocyte serum (ALS) are being widely documented but its mode of action remains a matter of considerable speculation.

It is of particular interest to determine how ALS influences the activity of the lymphocyte. In the experimental animal, ALS administration induces a degree of lymphopenia which is not directly relatable to the degree of immunosuppression obtained. This observation has given rise to the view that ALS does not simply produce its effect by destroying lymphocytes, and various hypotheses have been put forward suggesting other more specific effects on the lymphocyte population (Woodruff, 1967).

In an attempt to determine how ALS influences the activity of lymphocytes, the effect of rabbit anti-mouse lymphocyte serum (RAMLS) on the graft-*versus*-host (GVH) syndrome has been studied. For immunopathological studies the least complex form of the GVH syndrome is that induced in F_1 hybrid recipients by the injection of parental strain lymphoid cells and the cell type responsible has been conclusively demonstrated to belong to the circulating small lymphocyte population (Gowans, Gesner & MacGregor, 1961).

Spleen and lymph node cell suspensions prepared from parental donor mice pre-treated with RAMLS have been reported to be incapable of inducing a GVH syndrome when injected into adult F_1 hybrid recipient mice (Boak, Fox & Wilson, 1967). Identical findings

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have been reported by other investigators in similar systems (Van der Werf *et al.*, 1968; Brent, Courtenay & Gowland, 1968).

The spleen and lymph node suspensions used in such experiments were prepared from organs showing histological evidence of lymphoid depletion and such suspensions could not be representative of normal spleen or lymph node cells. Experiments were therefore designed to study the activity of circulating lymphocytes following ALS injection although it is realized that such therapy may also alter the circulating lymphocyte population. The ability of thoracic duct lymphocyte suspensions from normal and RAMLS treated parental donor mice to induce GVH syndrome in neonatal F_1 hybrid recipients was compared.

MATERIALS AND METHODS

Experimental plan

Spleen and thoracic duct suspensions were prepared from normal C57BL and RAMLS-treated C57BL parental donor mice and injected intraperitoneally at various dose levels into neonatal (DBA × C57BL) F_1 hybrid recipients.

The resultant GVH response was assayed by killing litters on the 8th day after injection and calculating spleen ratios relative to litter-mates injected with syngeneic F_1 hybrid spleen cells (Simonsen, 1962).

Animals

Adult male C57BL/6J donor mice and 1–8-day-old (DBA × C57BL/6J) F_1 hybrid recipients bred in The Jackson Laboratory, Bar Harbor, Maine, were used in all experiments.

Antiserum

Antiserum was raised in adult white New Zealand rabbits (Gray *et al.*, 1966) and its ability to prolong skin allograft tested as described previously (Boak *et al.*, 1967).

The antiserum was injected as a single 0·25-ml intraperitoneal dose into mice 24 hr before their thoracic ducts were cannulated or they were killed for spleen cells.

Cell suspensions

Thoracic duct cell suspensions were prepared (Boak & Woodruff, 1965) from normal or RAMLS-treated C57BL mice which were cannulated in batches of twelve. Lymph was collected at room temperature in Medium 199 with 20 units of heparin, 2000 units of penicillin and 2·5 mg of streptomycin per millilitre. Lymph was pooled after 12 hr of collection, washed in Medium 199, and resuspended in that media to the desired cell concentration. Spleen cell suspensions were prepared in Medium 199 (Woodruff & Symes, 1962).

Viability was assessed, using 0·05% trypan blue, and cell suspensions of less than 85% viability were not utilized. Smears of injected cell suspensions were stained with Wright's stain. Cells were injected intraperitoneally in a volume of 0·05 ml, the needle entering the abdomen *via* the thigh muscles to avoid leakage.

Histology

When mice were killed, spleen was fixed in 10% formalin and sections subsequently cut and stained with haematoxylin and eosin.

TABLE 1. Spleen indices of littermate neonatal (DBA × C57BL) F_1 hybrid recipients of C57BL parental spleen cells

Category	No.	Spleen indices (relative spleen weight treated)/ (relative spleen weight F_1 hybrid control)	Mean ± 1 SD
(1) Normal spleen cells 10×10^6	6	1·32, 1·72, 1·8, 1·72, 2·34, 2·6	1·92 ± 0·47
(2) RAMLS treated spleen cells (1 day) 10×10^6	8	1·22, 0·9, 1·51, 1·29, 2·45, 2·39, 1·4, 1·04	1·53 ± 0·58
1 *vs* 2 $0{\cdot}4 > P > 0{\cdot}2$			

TABLE 2. Spleen indices of littermate neonatal (DBA × C57BL) F_1 hybrid recipients of C57BL parental thoracic duct cells

Category	No.	Spleen indices (relative spleen weight treated)/ (relative spleen weight F_1 hybrid control)	Mean ± 1 SD
Normal thoracic duct cells			
(1) 10×10^6	5	2·13, 2·36, 2·43, 2·54, 2·68	2·4 ± 0·2
(2) 5×10^6	6	1·6, 1·68, 1·7, 2·7, 2·96, 3·1	2·4 ± 0·6
(3) $2{\cdot}5 \times 10^6$	6	1·5, 1·6, 1·9, 2·04, 2·79, 3·2	2·2 ± 0·7
RAMLS treated thoracic duct cells (1 day)			
(4) 10×10^6	12	0·83, 0·89, 0·90, 0·92, 0·94, 0·98, 1·02, 1·02, 1·09, 1·04, 1·06, 1·22	0·99 ± 0·1
1 *vs* 2 $0{\cdot}5 > P > 0{\cdot}4$			
1 *vs* 3 $0{\cdot}5 > P > 0{\cdot}4$			
1 *vs* 4 $P < 0{\cdot}001$			
2 *vs* 3 $0{\cdot}5 > P > 0{\cdot}4$			
2 *vs* 4 $P < 0{\cdot}001$			
3 *vs* 4 $P < 0{\cdot}001$			

RESULTS

Spleen cell suspensions prepared from normal adult parental donor mice produced a marked splenomegaly in neonatal F_1 hybrid recipients. Ten million spleen cells from parental donor mice pretreated with a single i.p. injection of RAMLS induced a splenomegaly which did not differ significantly from that induced by normal cell suspensions (Table 1).

Thoracic duct cell suspensions from normal parental donor mice caused splenomegaly in neonatal recipients when injected at dose levels of 2·5, 5 and 10×10^6 cells. No splenic enlargement was noted following the injection of 10×10^6 thoracic duct cells from RAMLS-treated mice (Table 2).

Due to great variation in the lymph volumes obtained from individual mice, no accurate comparisons can be made between the output of normal and RAMLS-treated mice. Pooled collections, however, indicated that the cell content of lymph from RAMLS-treated mice was reduced to approximately 10% of that obtained from normal mice. A single injection of RAMLS induced a lymphopenia of 5–20% of normal levels, and no morphological difference was detected between cell populations from normal or RAMLS-treated mice.

DISCUSSION

Repeated injection of RAMLS over a period of 4–6 days renders spleen and lymph node parental donor cells incapable of inducing a GVH syndrome in F_1 hybrid recipients (Boak *et al.*, 1967; Brent *et al.*, 1968; Van der Werf *et al.*, 1968) but this property may be recoverable (Van der Werf *et al.*, 1968). Such recovery may be related to the maturation of cells to replace lymphocytes destroyed by ALS, but in technically difficult experiments Brent and his colleagues have successfully restored competence to spleen cells from ALS-treated animals by removing their coating of rabbit globulin (Brent *et al.*, 1968).

A single RAMLS injection renders circulating lymphocytes incompetent to induce a GVH syndrome, but this is not true for spleen cells (Tables 1 and 2). More prolonged ALS administration may be necessary to coat, inactivate or kill lymphocytes resident in spleen or lymph nodes. Ledney and van Bekkum have demonstrated that the ability of spleen cells from ALS-treated parental donors to induce a GVH syndrome in F_1 hybrid recipients decreases with each ALS injection but that only after the fourth daily injection was there failure to induce a GVH reaction (Ledney & van Bekkum, 1968). It is, therefore, possible that, as other investigators have suggested (Levey & Medawar, 1967; Denman, Denman & Holborow, 1967; James, 1968), RAMLS may act directly on circulating lymphocytes and the 'inactivation' of spleen and lymph node cells may be secondary to depletion due to their replenishing the circulating lymphocyte pool.

A single injection of RAMLS induces a lymphopenia which is reflected in a low cell count in collected lymph. Miller and his colleagues have noted a reduction in lymphocyte output from cannulated mice treated with a similar antiserum but later observed no such reduction with a different antiserum (Martin & Miller, 1967). A profound lymphopenia within 4 hr of injection (Woodruff & Anderson, 1963; Gray *et al.*, 1966) probably reflects rapid destruction and possibly sequestration of lymphocytes (Anderson, James & Woodruff, 1967). The remaining circulating lymphocytes cannot induce a GVH syndrome in F_1 hybrid recipients because they have been inactivated (Levey & Medawar, 1966), immunologically competent small lymphocytes have been selectively killed (Anderson *et al.*, 1967) or following their injection into the F_1 hybrid either fail to gain access to lymphoid organs (Martin & Miller, 1967) or simply die as may have happened had they remained in the donor animal.

Coating of the graft (Guttmann *et al.*, 1967) in the F_1 hybrid as represented by the histocompatibility antigens of the DBA parent could play no part in these experiments.

These studies, considered in the light of other reported results, suggest that circulating lymphocytes may be more susceptible to the effect of ALS administration than are lymphocytes resident in spleen and lymph nodes. Further evidence to support this concept was obtained by investigating the effect of administering ALS to the F_1 hybrid recipient at various intervals before and after parental strain lymphoid cell injection (Boak *et al.*, 1968).

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