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Selective Suppression of Cell Mediated Immunity by Equine Anti-rabbit Lymphocyte Serum

WE have investigated the effect of antilymphocyte serum (ALS) on the humoral antibody response, in rabbits, which readily produce large amounts of circulating antibody to well defined antigens. ALS was made by injecting a horse intramuscularly with a mixture of 2×10^9 thymus and lymph node cells from Himalayan rabbits, mixed with incomplete Freund's adjuvant. Seven days later the same number of cells, this time from white New Zealand (NZ) rabbits were given, followed after 14 days by a similar immunization. The horse was bled a week later. The same horse had also been simultaneously immunized with mouse lymphoid cells and the antimouse component was active as an immunosuppressant.

The cytotoxicity titre of the anti-rabbit component was 1 : 27 against rabbit thymocytes and 1 : 243 against lymph node cells. The ALS was heated to 56° C to destroy complement and sterilized by passing it through a Seitz filter. The normal equine serum (NES) used in the control group was handled in the same way.

The day of grafting or immunization was designated day 0, and so the preceding days were negative and succeeding days positive. Full thickness pinch ear grafts were taken from black NZ donors and placed in beds on the dorsum of the ear of white NZ recipients, and held in place by sutures. The dressings were removed on day 5, and the grafts were considered rejected when the skin became hard and scabbed over.

In the first experiment (Table 1*A*) the rabbits received a 12 day intermittent course of ALS or NES. ALS produced a striking prolongation of skin graft survival, with a mean for the group of $17{\cdot}8 \pm 0{\cdot}8$ s.e.m. days, compared with $9{\cdot}5 \pm 1{\cdot}2$ s.e.m. days for the controls. By contrast, ALS had no effect on the level of antibodies to bovine serum albumin (BSA) as measured by the indirect haemagglutination test, using the microtitre technique[1] after coupling the BSA to the sheep erythrocytes by the bisdiazotized benzidine method[2]. All rabbits treated with ALS made a normal primary response, and after another immunization a normal secondary response. All treated animals also had substantial quantities of antibodies against equine γ-globulin (EGG).

These findings were checked by giving two groups of white NZ rabbits skin grafts, as before, on day 0, but no ALS. They rejected the grafts as expected. They also made a normal primary and secondary response to BSA. The 12 day intermittent course of ALS or NES was given, starting on day 68, and a second skin graft from the same donor strain was made on day 70. The NES group rejected the grafts with a mean of $8{\cdot}25 \pm 0{\cdot}4$ s.e.m. days, but with the group treated with ALS the survival of grafts extended on average to $16{\cdot}5 \pm 1{\cdot}2$ s.e.m. days (Table 1*B*). Immunization was carried out on days 70 and 91 with ovalbumin (OA), which has no groups to cross-react with BSA or equine albumin. As can be seen from Table 1*B*, the group treated with ALS made more antibody to OA and to EGG than the group treated with NES.

Clearly in the rabbit, ALS selectively suppressed cell mediated immunity but not the production of humoral antibody, either to well defined antigens such as BSA or OA, or to antibodies against horse proteins in ALS. Although ALS is known to be ineffective in suppressing antibodies against itself[3–5] and to act "more effectively on the cellular than the humoral response" in mice[6], our results are significant in showing that this dichotomy of effectiveness is complete in rabbits.

Table 1. EFFECT OF ALS ON SKIN ALLOGRAFT SURVIVAL TIMES AND ON HUMORAL ANTIBODY PRODUCTION

Treatment	No.	Survival of skin graft (days ± s.e.m.)*	Circulating antibody, mean −log 2 haem. titre ± s.e.m. BSA† (days)			EGG (days)		
			0	14	21	28	0	21	28
(*A*)		First graft, day 0							
NES§	8	9·5 ± 1·2	0	4·1 ± 0·8	6·0 ± 0·6	10·7 ± 0·7	0	10·0 ± 0·7	10·0 ± 0·9
ALS§	8	17·8 ± 0·8	0	5·0 ± 1·2	6·4 ± 0·6	12·6 ± 1·2	0	11·0 ± 0·4	10·0 ± 0·4
			Ovalbumin‡ days						
			70	91		98	70	91	98
(*B*)		Second graft, day 70							
NES¶	15	8·25 ± 0·4	0	3·9 ± 0·4		11·3 ± 0·8	0	9·4 ± 0·4	9·0 ± 0·5
ALS¶	6	16·5 ± 1·2	0	6·7 ± 0·8		16·0 ± 1·3	0	12·8 ± 0·8	13·5 ± 1·0

* Donors: black NZ male rabbits; recipients: white NZ male rabbits.
† Immunization on days 0 and 21 with 100 mg of BSA given intravenously.
‡ Immunization on days 70 and 91 with 100 mg of ovalbumin given intravenously.
§ 3 ml./kg given subcutaneously on days −1, −2, 0, 2, 4, 6, 8 and 10.
¶ 3 ml./kg given subcutaneously on days 68, 69, 70, 72, 74, 76, 78 and 80.

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