

dilutions for phage inactivation and 0.1 ml. was used for assaying antibodies by the immunoadsorbent technique. The results of three repeat experiments tested by the modified phage technique (Table 1) indicate that treatment with BADL reduced the incidence of antibody forming cultures (5/17 in the experimental as compared with 16/16 in the control groups). Experiments 2 and 3 (Table 1) indicate that, unlike DNP-lysine, BADL prevented antibody production even when the antigen was applied 44 h after the removal of the affinity labelling reagent. Fig. 1 shows, moreover, that DNP-lysine had a limited effect in inhibiting antibody production to DNP by DNP-RSA, whereas BADL and BADE had a marked inhibitory effect.

Table 1. EFFECT OF BADL ON THE SECONDARY RESPONSE TO DNP INDUCED *in vitro* BY DNP-HAEMOCYANIN AND ASSAYED BY THE MODIFIED PHAGE TECHNIQUE

Expt.	Treatment of spleen donor	Treatment of spleen explants	Time of antigen challenge (h)	Incidence of reactive cultures (50 per cent inactivation)
1	Immunized	BADL in PBS	4	2/10
	Immunized	—	15	5/5
2	Immunized	BADL in PBS	24	2/4
	Immunized	DNP-lysine	24	4/4*
	Immunized	PBS, control	24	4/4
	Immunized	Medium, control	24	4/4
3	Immunized	BADL in PBS	48	1/3
	Immunized	DNP-lysine	48	3/3
	Immunized	PBS, control	48	3/3

* All these cultures responded at a low rate of antibody formation, manifested in 50–80 per cent of inactivation of the modified phage. When tested by the immunoadsorbent technique, these samples yielded an average c.p.m. of 7,207 as compared with 16,089 in the accompanying PBS control group.

Similar results were obtained in an additional experiment in which the medium samples were assayed by the immunoadsorbent technique (Table 2). Both reagents, BADL and BADE, reduced the antibody response considerably, assessed here in counts per minute.

To test the specificity of the inhibition obtained by BADL and BADE, the effect of these reagents on the response to a non-crossreacting antigen was studied. Spleen explants were obtained from mice immunized with poly-DL-alanyl-RSA. The capacity of BADE and BADL to inhibit antibody production to the poly-DL-alanine hapten in these explants was then tested. The results (Table 2 and Fig. 2) indicate that there was no

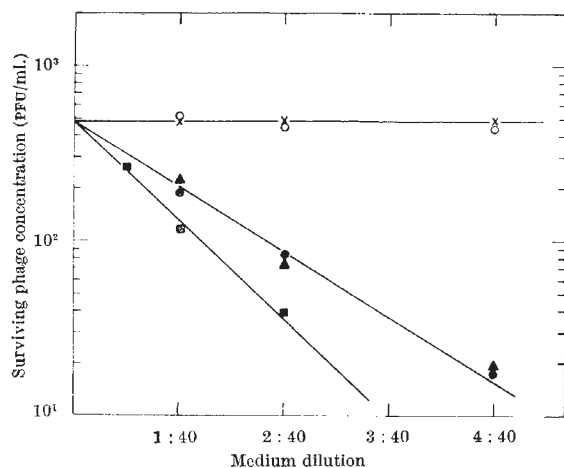


Fig. 2. The effect of BADL and BADE on antibody production *in vitro* in the poly-DL-alanyl system. The curves show inactivation of poly-D-alanyl-T₁ phage by medium from spleen explants that were induced for secondary response *in vitro* by poly-DL-alanyl-RSA. The incubation of phages and medium was for 4 h. x, Explants from normal animal, control; o, explants from primed animal without *in vitro* stimulation with poly-DL-alanyl-RSA; ●, explants incubated with PBS alone before antigen; ▲, explants incubated with BADE before antigen; ■, explants incubated with BADL before antigen.

Table 2. EFFECT OF BADL AND BADE ON THE SECONDARY RESPONSE TO DNP AND TO POLY-DL-ALANINE INDUCED *in vitro* BY DNP-HAEMOCYANIN AND BY POLY-DL-ALA-RSA, RESPECTIVELY

Group	Immunizing antigen	Treatment Antigen added <i>in vitro</i>	Inhibiting compound	Antibody response (c.p.m.)	
				To DNP	To poly-DL-Ala
1	DNP-haemocyanin	+	BADL	3,660	
2	DNP-haemocyanin	+	BADE	5,248	
3	DNP-haemocyanin	+	DNP-lysine	7,207	
4	DNP-haemocyanin	+	BADL on day 3	18,988	
5	DNP-haemocyanin	+	—	16,089	
6	—	—	—	9,144	
7	Poly-DL-Ala-RSA	+	BADL		11,100
8	Poly-DL-Ala-RSA	+	BADE		24,906
9	Poly-DL-Ala-RSA	+	DNP-lysine		15,800
10	Poly-DL-Ala-RSA	+	—		13,200
11	—	—	—		4,100

Antibodies were assayed by the immunoadsorbent technique. C.p.m. were determined on samples of pools of media from four cultures in each case.

inhibitory effect on the production of antibodies to the non-crossreacting hapten.

The results cannot be attributed to a non-specific toxic effect manifested in inhibition of antibody production because (a) BADL and BADE did not completely eliminate antibody production but reduced it to the residual levels found in cultures which were not challenged by antigen *in vitro* (Fig. 1), and (b) when the reagents were added to the explants 3 days after the antigen, no inhibition of the response could be detected (Table 2 and Fig. 1). Furthermore, the inhibitory effect of the affinity labelling compounds was found to be specific, for BADL and BADE did not block the response to poly-DL-alanine.

In summary, it is shown that BADL and BADE inhibit specifically the production of anti-DNP antibodies; and the effect, unlike that of free haptens such as DNP-lysine, persists for at least 44 h. In analogy with the affinity labelling of anti-DNP antibodies by BADL and BADE, we conclude that these reagents become covalently bound to antibody-like molecules on the cell surface of the spleen explants, which may represent the antigen recognition receptors of the antigen-reactive cells.

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Transfer of Immunity against Long Surviving Rat Skin Xenografts by Antilymphocyte Serum treated Syrian Hamsters

XENOGRAPTS, that is, grafts of which donor and recipient are members of different species, are, as a general rule, destroyed even more rapidly than allografts of similar

tissue. Until recently all procedures that were effective in prolonging the lives of allografts—for example, induction of immunological tolerance in the host, treatment of the host with immunosuppressive agents and so on—were usually ineffective for sustaining xenogeneic grafts. These empirical observations led to the suggestion that non-immunological factors, for example, syngeneic preference, metabolic shortcomings of the host and so on, may underlie the failure of xenografts to thrive^{1,2}.

The recent demonstrations^{3,4} that mice treated with heterologous antilymphocyte serum (ALS), an extremely powerful immunosuppressant, will, however, consistently retain skin grafts from donors of very remote genetic relationship, for example, rats, guinea-pigs, rabbits or even humans, made virtually untenable the belief that the failure of xenografts is attributable to non-immunological factors. The work to be reported here shows that ALS treatment effectively overcomes the Syrian hamster's resistance to xenografts; it also suggests a method for identifying the mediator(s) of xenograft rejection.

The ALS was raised in rabbits against hamster lymph node cells⁵ and had a lymphoagglutinin titre between 1:400 and 1:800 (ref. 6). Carefully fitted, full thickness, trunk skin grafts approximately 2 cm in diameter were transplanted (day 0) according to standard procedures⁷. Dressings were removed for primary inspection on the seventh day and the grafts were subsequently inspected at 2 to 3 day intervals. Their viability was assessed on the basis of outward appearance⁷, and confirmed in many instances by histological examination of biopsy specimens.

The recipients, adult hamsters of the MHA isogenic strain weighing 80–110 g, were treated with ALS (in the case of experimental animals) or normal rabbit serum (for controls) according to the following regimen: 2 ml. of whole serum was injected intraperitoneally daily from day -7 to 0 and three times per week thereafter. Consonant with the findings of others^{1,3,4} it was necessary to pretreat the xenograft recipients with ALS in order to prolong significantly the life of their grafts; this is not necessary for allografts (unpublished results of C. F. S. and W. J. Donawick).

Table 1. SURVIVAL TIMES FOR FULL THICKNESS SKIN XENOGRAFTS ON ADULT SYRIAN HAMSTERS

Host species	Donor species	Survival times of xenografts on ALS* (days)
Syrian hamster (<i>Mesocricetus auratus</i>)	Kurdistan hamster (<i>Mesocricetus brandti</i>)	71; 2 × >100
	Chinese hamster (<i>Cricetulus griseus</i>)	3 × >100
	Rat	55, 72, 78, 81, 4 × 86, 3 × 90; 14 × >100
	Mouse	5 × >100

* Panels of normal rabbit serum treated Syrian hamsters universally rejected any of the tested xenografts in <14 days.

Hamsters treated with rabbit anti-hamster lymphocyte serum routinely retained skin xenografts for 100 days or more from a variety of donor species (Table 1), including Kurdistan and Chinese hamsters, rats and mice. None of the long-surviving xenografts on hamsters showed any sign of degeneration or of the mononuclear cell infiltration suggestive of chronic host reactivity^{7,8}. In other words, although the xenografts in these experiments were observed for maximum periods of 100 days, there is every reason to believe that these grafts would have survived very much longer if ALS treatment had been continued.

Once ALS therapy was stopped, however, all the established xenografts had consistent and well defined "coasting periods", that is, periods between cessation of ALS therapy and rejection of the grafts. These proved to be independent of the length of the preceding ALS treatment; grafts of 30 days' standing coasted for as long after the cessation of ALS treatment as those which were 100 days old at the termination of therapy. During the coasting period the grafts appeared, at least grossly, to be perfectly healthy, and when rejection finally occurred it was invariably precipitous. Usually no more than 1 day

intervened between the appearance of the first outward signs of epithelial breakdown and reduction of the graft to a dry scab.

Advantage was taken of the "coasting phenomenon" to try to elucidate the causes of xenograft immunity. Rat and mouse skin xenografts placed contralaterally on the same ALS-treated recipient both generally enjoyed a coasting period of about 20 days ($\pm 1-2$ days) (Table 2). If, however, hosts bearing such grafts were injected intravenously with 400×10^6 lymph node cells from isologous donors (the lymphoid cell donors being in the final throes of actively rejecting "second-set" rat skin xenografts), the rat skin xenografts on the animals receiving the cell inocula underwent precocious rejection (median coasting period < 11 days) while the control contralateral mouse skin grafts remained in a healthy condition. The latter observation indicates at least a species specificity for this transferred immune state.

Rejection of rat xenografts by hamsters whose immune machinery had been reconstituted through the transfer of sensitized lymph node cells was invariably accompanied by the "walling off" of the graft by a large accumulation of mononuclear cells interspersed with a few eosinophils. Death of the graft, in these circumstances, may therefore have been chiefly caused by ischaemic necrosis. The existence of a moderate degree of mononuclear cell infiltration in the graft stroma, however, may indicate that the cellular factors responsible for the rejection of solid tissue allografts⁹ were also involved.

Table 2. A COMPARISON OF THE ABILITY OF TRANSFERRED PUTATIVELY IMMUNE ISOLOGOUS LYMPH NODE CELLS AND SERUM TO PRECIPITATE THE REJECTION OF LONG ESTABLISHED RAT SKIN XENOGRAFTS ON ALS-TREATED HAMSTERS WHICH ALSO BEAR LONG-STANDING CONTRALATERAL MOUSE TRUNK SKIN XENOGRAFTS

Number of animals tested	Treatment of the host	Approximate median coasting period (and range) for rat skin xenografts (days)	Approximate median coasting period (and range) for mouse skin xenografts (days)
12	ALS days -7 to +50	20.0 (18-21)	21.0 (19-22)
7	ALS days -7 to +50; then 400×10^6 viable hamster anti-rat lymph node cells intraperitoneally on day +52	10.5 (9-11)	20.5 (19-21)
6	ALS days -7 to +50; then 4 ml. of hamster anti-rat antiserum intraperitoneally on day +52	20.0 (20-21)	21.0 (18-22)

All attempts to procure rejection of well established rat skin grafts on hamsters by transferring as much as 4 ml. of putatively immune serum were unsuccessful (Table 2). There are three possible explanations for this success of lymphoid cell inocula in transferring immunity to xenografts and the failure of serum. (a) Rejection of skin xenografts (at least in the present species combination) is mediated primarily by a cellular immunity. (b) The notorious inability of Syrian hamsters to produce circulating isoantibodies¹⁰ extends to their ability to produce heteroantibodies, precluding the participation of humoral factors in the process of xenograft rejection in this species. Or (c) antibodies against xenografts are produced in such small quantities that at any given time the amounts present in the blood are insufficient to transfer immunity by passaging serum, whereas viable antibody producing cells, when transferred, continue to make antibodies the cumulative effects of which are sufficient to bring about the rejection of a xenograft.

Antilymphocyte serum has already been used clinically for the abrogation of allograft immunity^{11,12}. The present studies extend to a new species combination an earlier observation¹ that mammalian tissues of very distant genetic relationship can grow and flourish in close proximity *in vivo* over periods of several months and perhaps indefinitely. Furthermore, the results suggest that the unique capacity of ALS to sustain xenograft survival may be used as a tool to determine mechanisms

