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Estimation of PFC and Serum Haemolysin Response to SRBC in 'Nude' Mice

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Summary. The time course of the primary haemolysin response of thymusless 'nude' mice to sheep red blood cells differs from that of intact animals in one main respect. After the initial peak, which is reached 4–6 days from injection of the cells, haemolysin level falls to zero at about Day 10; and there is a concurrent decline of the counts of plaque-forming cells.

The prolonged phase of slow decline of haemolysin level, reflecting the production of 7S antibodies and typical of the response of normal animals, is absent. It is concluded that production of 7S haemolysins is thymus-dependent, whilst that of 19S haemolysins is thymus-independent.

INTRODUCTION

The production by mice of haemolysins in response to the injection of sheep red cells is studied widely as a system that depends on the synergism of both bone marrow-derived and thymus-derived cells. The experiments reported here are a contribution to the elucidation of the degree and nature of this thymus dependence, and were carried out on congenitally thymusless mice of the genotype, *nu nu* ('nude').

MATERIALS AND METHODS

The mutation, *nude* (*nu*) is maintained in this laboratory in a closed colony. Homozygous *nu nu* ('nude') mice are obtained by crossing heterozygous individuals. Mice used for immunization were all around 6-week-old. Unfortunately, about 2 months is the age of maximum mortality of 'nude' mice, which are also rather difficult to raise in the first place. The size of the experimental groups was often reduced by deaths (see Table 2).

Sheep red blood cells were sedimented from blood freshly collected at the abattoir and were washed three times in saline. An injection of 4×10^8 such cells was given to each mouse intraperitoneally.

At intervals of from 5–19 days (Table 1) after injection, the mice were exsanguinated under ether anaesthesia and the serum was obtained by centrifugation of the blood. Spleens were removed immediately, teased into ice-cold medium '199' and filtered through a stainless steel gauze of 200 mesh. The suspension was then passed through a 22-gauge needle to break up the few remaining cell clumps. Aliquots were taken for cell counts, carried out with a model D1 Coulter counter. The rest of the suspension was kept

in an ice-water bath and was used, within 2·5 hours from harvesting, for the counts of plaque-forming cells.

Haemolysin titres were measured in the standard way, following the procedure described by Sever (1962). The haemolytic plaque-forming cell (PFC) assay was carried out as described by Cunningham and Szenberg (1968), in slide chambers made from two slides separated by two strips of double-sided Sellotape. Chambers prepared in this way could accommodate 150 μl of the cell suspension. Plaques were counted after incubation at 37° for 1 hour. This method is about three times more sensitive than the classical Jerne technique.

RESULTS

The counts of plaque-forming cells (PFC) are set out in Table 1 and Fig. 1. It should be noted that the technique used detects only 19S antibody producing cells.

TABLE 1
PFC AFTER IMMUNIZATION WITH 4×10^8 SHEEP RBC

	PFC/10^6		PPC/Spleen	
Days from immunization	+/?	nu/nu	+/?	nu/nu
5	3 780	94	213 192	4 606
	3 670	221	189 372	1 657
	4 160	762	592 384	24 993
	690	115	90 390	6 854
8	254	11·5	23 978	107
	94	12·2	8 873	732
	107	5·1	31 886	481
	379	11·2	87 928	258
	377	14·6	150 800	613
	210		172 200	
10	102	4·1	17 646	344
	146	31	18 425	794
12	214	5·8	23 968	534
	99	4·6	17 127	561
	62	8·0	21 700	1 040
	140	4·6	65 100	552
	63		17 892	
	26		15 964	
15	108	16·5	22 032	1 254
	88	1·3	14 256	156
	56		7 280	
19	25	4	3 750	440
	19·5	2·8	3 354	179
	13	1·3	2 158	130

The number of plaques is, throughout the period examined, about ten times smaller in 'nude' animals than in their normal sibs. It declines steadily from 5 days and by Day 12 it reaches the very low value of about 10 per 10^6 spleen cells.

The haemolysin titres are given in Table 2 and Fig. 2. The 'nude' mice consistently fail to achieve the same titres as the normal controls. The most important feature of the results, however, is that in 'nude' mice the haemolysin level drops to zero after Day 10. Control animals on the other hand exhibit a titre of 4–8 for the whole period to Day 18.

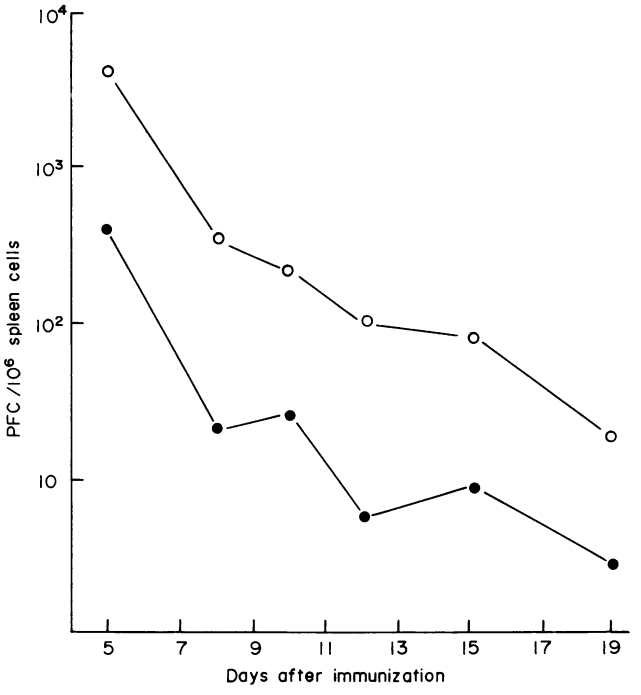

FIG. 1. The appearance of plaque-forming cells in (●) nu/nu and (○) +/? spleens following immunization with 4×10^8 sheep RBC.

DISCUSSION

The time course of the primary response of normal mice to the injection of sheep red cells is well-established (Dietrich, 1966). After a period of about 3 days, haemolysins become detectable and their level rises to a peak towards the end of the first week. Following this, there is a slow and gradual decline for about 3 weeks. Our control mice gave a response that fits into this pattern.

Many workers have studied the haemolysin responses of mice subjected to thymectomy soon after birth. It has been found that titres are depressed but by no means eliminated (Sinclair, 1967a; Davies, 1969). In fact, there is a certain degree of recovery with time (Sinclair, 1967a, b; Taylor and Wortis, 1968). Furthermore, the level of haemolysins produced by thymectomized mice can be raised to near normal by the injection of a large enough number of sheep red cells (Sinclair and Elliott, 1968).

Investigations on thymectomized mice, although very valuable, are of necessity associated with a certain residual uncertainty. This is why Miller and Mitchell (1969) remarked: 'The increased response of neonatally thymectomized Swiss mice, brought about by increasing the dose of SRBC (Sinclair and Elliott, 1968) may be explained, as mentioned above, by an antigen-induced expansion of a very limited pool of SRBC-reactive cells, initially derived from the thymus before thymectomy.'

In fact, Miller and Mitchell (1969) state in their review of this field: 'It is clear that thymus-derived cells, on their own, do not produce haemolysin-forming cells. Likewise,

bone marrow-derived cells, on their own, do not appear to be able to respond to SRBC by differentiating to PFC.'

The residual risk referred to above does not apply to experiments with 'nude' mice. These are born without a thymus; the foetal thymic rudiment is at no stage equipped with lymphoid cells (Pantelouris and Hair, 1970). It is already known, however, that these

TABLE 2
LOG_2 HAEMOLYSIN TITRE AFTER IMMUNIZATION WITH 4×10^8 SRBC

Days from injection	'Nude' injected	'Nude' surviving	'Nude' titres	+/? titres
3	3	2	2	0
			1	0
				1
5	10	10	2	5
			2	4
			4	2
			3	4
			4	2
			3	5
			0	7
			3	6
			4	5
			3	6
7	4	2	5	7
			2	3
8	6	5	2	9
			1	6
			0	9
			2	8
			4	9
				9
10	4	2	0	7
			0	6
11	6	3	0	6
			0	7
			0	7
12	6	4	2	7
			1	8
			2	8
			0	8
				5
				4
15	6	2	0	7
			0	8
				6
18	6	2	0	6
			0	5
				6

mice are able to produce haemolysins and plaque-forming cells, albeit at a low level (Pantelouris, 1971; Wortis, 1971). It cannot be said therefore that no haemolysins can be produced in the absence of thymus-derived cells.

The primary haemolysin response involves the production of both 19S and 7S antibodies. There is no doubt that 19S predominate in the first week but then decline steadily. The 7S antibodies begin to be synthesized about 2 days or so later than 19S, and gradually

take over. Thus, 7S antibodies are almost solely responsible for the prolonged phase of response from Days 10–12 onwards (see Sinclair, 1967a).

Taylor, Wortis and Dresser (1967) and Taylor and Wortis (1968) found that thymectomy and irradiation depress the production of 7S (γG) haemolysins more severely than of 19S (γM) haemolysins. Similarly, Carter *et al.* (1968) showed that, whenever the influence of the thymus is reduced, it is the level of 7S antibodies that is particularly depressed. The concept has been extended to the response to BSA linked to the hapten, NIP (Aird, 1971).

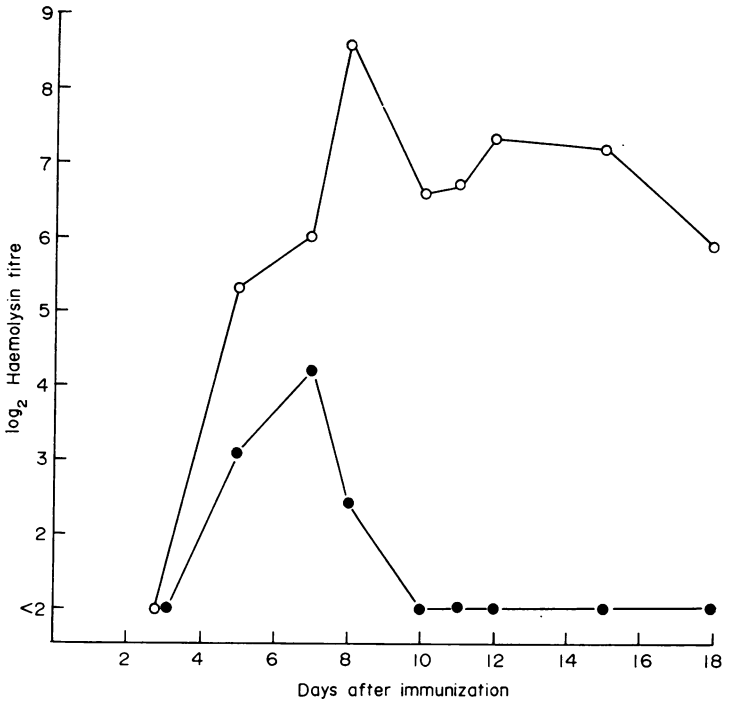

FIG. 2. Haemolysin response in (●) nu/nu and (○) +/? mice after immunization with 4×10^8 sheep RBC.

In our 'nude' mice, haemolysin titres dropped to zero level after Day 10, and the number of plaque-forming cells producing 19S antibodies declined concurrently. Of course, the persistence of traces of 19S after Day 10 cannot be rigorously excluded. Any negligible background readings could be attributed to such traces, or could be due to cross-reactions.

It thus appears almost certain that 'nude' mice do not exhibit that part of the primary haemolytic response that is generally attributed to 7S antibodies. It is reasonable to conclude that production of 7S haemolysins is thymus-dependent, whilst production of 19S haemolysins is independent, or at least largely independent, of the thymus.

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