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A Comparison of Primary and Secondary Haemolysin Responses to Sheep Erythrocytes in Neonatally Thymectomized, Sham-Thymectomized and Normal Swiss Mice

A TIME-COURSE STUDY OF TOTAL, 19S AND 7S ANTIBODY

N. R. St C. Sinclair*

Chester Beatty Research Institute, Institute of Cancer Research, Royal Cancer Hospital, Fulham Road, London, S.W.3

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Summary. A time-course study of total, 19S and 7S haemolysins showed that Swiss albino mice were able to give a normal secondary response to sheep erythrocytes at an age when the primary response was much reduced and delayed.

INTRODUCTION

In the past few years, some attention has been given to comparisons of the effect of neonatal thymectomy on the primary and secondary antibody responses (Hess, Cottier and Stoner, 1963; Svet-Moldavsky, Zinzar and Spector, 1964). Previous studies have been carried out on one group of thymectomized mice in which the primary response was compared to a secondary response induced later in the life of the animal. Recent studies have shown that even the primary response changes with the increasing age of the animal (Rogister, 1965; Dukor, Dietrich and Rosenthal, 1966; Sinclair and Millican, 1967). Therefore it is advisable to re-investigate this comparison under conditions when the age of the animals is the same when the primary and secondary antibody responses are induced.

Since the distinction between primary and secondary responses to sheep erythrocytes is difficult to make when studying the total haemolysin response, a time-course study (investigation of the serum haemolysin antibody levels as a function of time after injection of antigen) was carried out on the 19S and 7S as well as the total haemolysin.

MATERIALS AND METHODS

Mice

Colony bred male and female Swiss albino mice were used for all the experiments, and were maintained under conventional conditions. The animals were given water and commercial cubed food *ad libitum*. Mice were weaned and separated according to sex at 1 month of age.

* Fellow of the Medical Research Council of Canada.

Thymectomy

Neonatal thymectomy was performed according to the method of Miller (1960, 1964 personal communication), and was controlled with both sham-operated and non-operated mice. Completeness of thymectomy was assessed by autopsy and histological examination of the thymic area. Incomplete thymectomies accounted for less than 10 per cent of all thymectomized animals.

Immunization

Sheep erythrocytes in Alsever's solution (Wellcome Research Laboratories, Beckenham, England) were washed 4 times in 0·9 per cent saline. All mice were injected intraperitoneally with 0·1 ml of a 10 per cent suspension of washed sheep erythrocytes. Thymectomized, sham-thymectomized and normal mice were each divided into two groups, one receiving sheep erythrocytes at 4 weeks of age, and the other receiving sheep erythrocytes at 10 days and 4 weeks of age. The comparison of primary and secondary responses was made between the responses which occurred at 4 weeks of age.

Measurement of serum haemolysin activity

Blood was obtained from an incision in the ventral aspect of the tail, and diluted 2:1 with 0·9 per cent saline to avoid gel formation of the serum. The serum was collected following centrifugation, and endogenous complement inactivated by incubating the serum at 56° for 30 minutes. Serum samples were serially diluted 1:1 with 0·9 per cent saline in Microtiter plates (Cooke Engineering Co., Arlington, Virginia, U.S.A.) (Sever, 1962). A standard amount of guinea-pig complement (Wellcome Research Laboratories) was added, and the serum and complement incubated for 30 minutes at 37°. The pre-incubation of serum and complement lowered the incidence of titrations which were negative at low dilutions but became positive at higher dilutions. Washed sheep erythrocytes (0·05 ml of a 0·5 per cent suspension) were added and the complete mixture incubated for 2 hours at 37° and then read. The plates were stored overnight at room temperature and read again the following morning. Titres were usually one $\log_2$ unit higher on the second reading. All titres are expressed as the $\log_2$ of dilution.

Ultracentrifugation

Pooled serum was layered on a 10–40 per cent linear sucrose gradient and spun in a Spinco Model L ultracentrifuge at 35,000 rev/min (100,000 ***g***) for 18 hours at 4° (Kunkel, 1960). Twenty-four fractions were collected by puncturing the centrifuge tube at the bottom with a number 18 lumbar puncture needle.

Calculations

The determinations of total, 19S and 7S were carried out as previously described (Sinclair, 1967).

RESULTS

Fig. 1 shows the primary responses in neonatally thymectomized, neonatally sham-thymectomized and intact Swiss mice when they were injected with sheep erythrocytes at 10 days (Fig. 1b) or 4 weeks (Fig. 1a) of age, and the secondary response when the mice were injected at 4 weeks of age (Fig. 1b). Any comparison of primary with secondary

responses should be made between responses induced in animals of the same age, that is, between the primary response in Fig. 1(a) and the secondary response in Fig. 1(b). The arrows indicate the times at which the antigen was injected.

In the primary response study (Fig. 1a), the thymectomized mice had a much reduced response compared to the sham-thymectomized and intact mice, but the titres in the thymectomized mice did rise somewhat towards the end of the period of observation.

In the secondary response study (Fig. 1b), the haemolysin titres in the thymectomized mice had dropped to below threshold level at the time of second injection, but nevertheless the secondary response in the neonatally thymectomized mice did not differ significantly from that which occurred in sham-thymectomized or normal animals ($P>0{\cdot}3$). The secondary response began at roughly the same time in all three experimental groups.

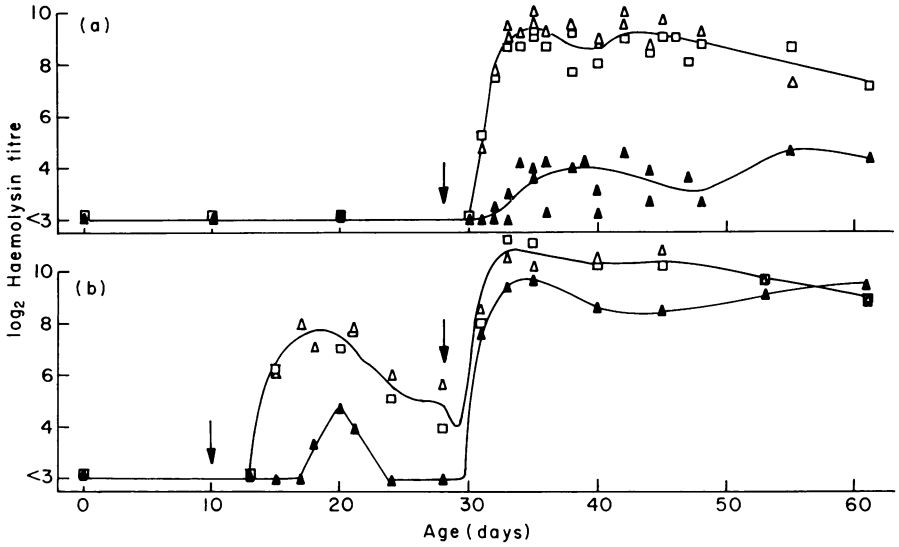

FIG. 1. Haemolysin responses in thymectomized (▲), sham-thymectomized (△) and intact (□) Swiss mice. Average haemolysin titre is plotted against age of animals, and arrows indicate the ages at which animals were injected. (a) Primary response when sheep erythrocytes were injected intraperitoneally at 4 weeks of age. An average of 8·5 mice is represented by each point. *P*-value of the difference between thymectomized and control groups is 0·01. (b) Primary and secondary responses when antigen was injected at 10 days and 4 weeks respectively. Average number of mice represented by each point is 14. In the secondary response, the *P*-value of the difference between thymectomized and control groups was greater than 0·3.

Fig. 2 shows the 19S and 7S primary response in thymectomized, sham-thymectomized and intact mice. In the thymectomized group of mice, the 19S response was much reduced. The 7S haemolysin response in thymectomized mice rose slowly over a long period of time, whereas the 7S response of the controls reached near maximal levels early in the response.

Fig. 3 shows the 19S and 7S secondary responses in thymectomized, sham-thymectomized and intact mice. The behaviour of the 19S and 7S were similar for all three experimental groups except for a few minor differences. In the thymectomized group, the 7S haemolysin might have risen a day later than in the normal, but at the same time as in the sham-thymectomized mice. After an initial fall from the peak titre, the 19S haemolysins remained well above the threshold level in all three experimental groups, and, in the thymectomized group, appeared to increase slightly towards the end of the experiment.

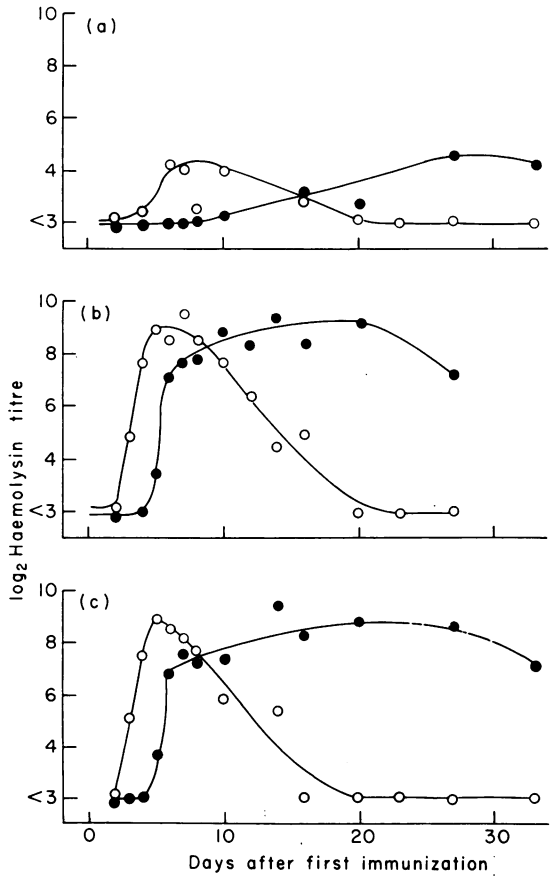

FIG. 2. Primary response—the calculated 19S (○) and 7S (●) haemolysin titres were plotted against days after immunization for thymectomized (a), sham-thymectomized (b) and intact (c) Swiss mice.

DISCUSSION

A study dealing with comparison of the effects of neonatal thymectomy on primary and secondary responses should be carried out on a biological system in which it is possible to distinguish between primary and secondary responses. Since the level of total haemolysin is not much greater in the secondary response to sheep erythrocytes than in the primary response, it is advisable to have some other criteria which distinguish between these two responses.

In sham-thymectomized and intact mice in the present study, the behaviour of 19S and 7S haemolysins in the primary response differed in a number of ways from that in the secondary response. The 19S haemolysin responses remained detectable throughout the whole observation period of the secondary response, whereas the 19S antibody decreased logarithmically to threshold levels in the primary response. The 7S rose at almost the same time as the 19S in the secondary response and reached maximal levels quickly, whereas, in the primary response, the 7S tended to rise later and increase more slowly to maximal titres. The crossover point (the time at which the 7S antibody surpassed the 19S) occurred at 9–10 days in the primary response, but at 3–4 days following immunization in the secondary response.

Which of these differences represents the best criteria for distinguishing between primary and secondary response? When the behaviour of 19S and 7S antibody in the primary and secondary responses in this study is compared with that observed in an inbred strain of Swiss mice and reported in the previous paper (Sinclair, 1967), it can be seen that some of the characteristics which distinguish primary and secondary responses in this study, were not present in the study on inbred Swiss mice. Two such distinguishing

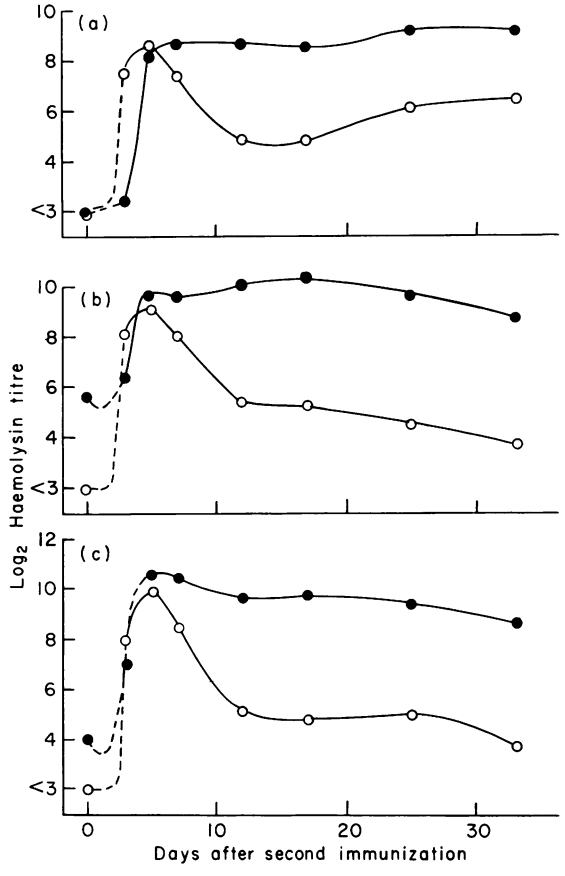

FIG. 3. Secondary response—the calculated 19S (○) and 7S (●) haemolysin titres were plotted against days after immunization for thymectomized (a), sham-thymectomized (b) and intact (c) Swiss mice.

features however are present in both this study and the previous one—the time at which the 7S rose after immunization, and the crossover point. The crossover points in the study on inbred Swiss mice were 10 days for the primary response and 4 days for the secondary response, and these agree very well with the crossover points in the present study. Thus, on this basis alone, one may distinguish between primary and secondary haemolysin responses to sheep erythrocytes in Swiss mice.

The primary response in the thymectomized group after injection at 10 days showed no evidence of being 7S in type (unpublished findings). It is possible that 'immunological memory' was not induced, but that the animals were at least pre-conditioned so that they could respond after the next injection of antigen with a normal primary-type response.

Consequently, the normal behaviour of total haemolysin in the thymectomized animals, injected for the second time at 4 weeks of age, could indicate that either a normal secondary response or a response more closely allied to a primary response had occurred. On comparing the behaviour of 19S and 7S haemolysins in the thymectomized group after second injection of antigen with that which occurred in the primary and secondary responses of sham-thymectomized animals, it can be seen that the response of thymectomized animals more closely approximated the secondary response of sham-thymectomized mice. The 7S haemolysin rose between 3 and 5 days in the thymectomized group, as compared with 5–6 days in the primary response, and 3–5 days in the secondary response of sham-thymectomized mice. In the thymectomized animals, the crossover point occurred at 5–6 days after the second injection, as compared with 10 days after immunization in the primary response and 4 days after immunization in the secondary response of sham-thymectomized mice. Therefore, the haemolysin response in thymectomized mice after second injection of sheep erythrocytes at 4 weeks was not only a normal response but a secondary response with close to normal characteristics.

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