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DELAYED DEVELOPMENT OF IMMUNOLOGICAL RESPONSIVENESS IN NEONATALLY THYMECTOMIZED MICE: A TIME-COURSE STUDY

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SUMMARY

Haemolysin responses to first injection of sheep erythrocytes in neonatally thymectomized, neonatally sham-thymectomized and intact Swiss albino mice were tested when the mice were 10 days, 4 weeks, 6–7 weeks and 6 months of age. The serum haemolysin activity was assessed at a number of times after injection of antigen (time-course study). Neonatal thymectomy of Swiss mice was followed by a decreased and delayed haemolysin response. These abnormalities in antibody response following neonatal thymectomy became less obvious when the age at which the mice were injected was increased, indicating that delayed development of immunological responsiveness had occurred in neonatally thymectomized Swiss mice.

INTRODUCTION

A number of studies dealing with the delayed development of the immune response in thymectomized mice with age have been carried out recently (Rogister, 1965; Dukor, Dietrich & Rosenthal, 1966; Grant, 1966, personal communication). None of these studies were time-course studies (investigation of serum antibody levels as a function of time after injection of antigen), so that the antibody response to an antigen, injected at any one particular age, has not been investigated sufficiently enough to demonstrate clearly the change in the response characteristics with age.

The present report deals with the time-course of the haemolysin response to the first injection of sheep erythrocytes in neonatally thymectomized, neonatally sham-thymectomized and non-operated Swiss mice when the sheep erythrocytes were injected at various times after birth.

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MATERIALS AND METHODS

Animals

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.

Thymectomy

Neonatal thymectomy was performed according to the method of Miller (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% of all thymectomized animals.

Immunization

Sheep erythrocytes in Alsever's solution (Wellcome Research Laboratories, Beckenham, England) were washed four times in 0·9% saline. All mice were given 0·1 ml of a 10% suspension of sheep erythrocytes by the intraperitoneal route. Mice were immunized at 10 days, 4 weeks, 6–7 weeks and 6 months of age.

Measurement of serum haemolysin activity

The blood was obtained from an incision in the ventral aspect of the tail, and diluted 2:1 with 0·9% saline to avoid gel formation of the serum. The serum was collected following centrifugation, and endogenous complement inactivated by incubating the serum at 56°C for 30 min. Serum samples were serially diluted 1:1 with 0·9% 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 min at 37°C. 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% suspension) were added and the complete mixture incubated for 2 hr at 37°C 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 logarithm to the base 2 ($\log_2$) of the dilution.

RESULTS

Immunization at 10 *days of age* (Fig. 1)

When antigen was injected at 10 days of age, neonatally thymectomized mice gave a much lower response than the control group. In the thymectomized group, the commencement of the detectable haemolysin response, and the time of attainment of near-maximal levels was delayed by roughly 3 days.

Immunization at 4 *weeks of age* (Fig. 2)

Normal and sham-thymectomized mice, immunized at 4 weeks of age, gave a response similar to that of older mice, but the neonatally thymectomized mice gave a much lower

response. At most times after immunization the average antibody titres of the thymectomized mice were 3–6% of the controls, and this difference had the statistical significance of $P = 0{\cdot}01$). Normal and sham-operated mice have been separated to show that the trauma of the operation itself is not a very significant factor in lowering the haemolysin response.

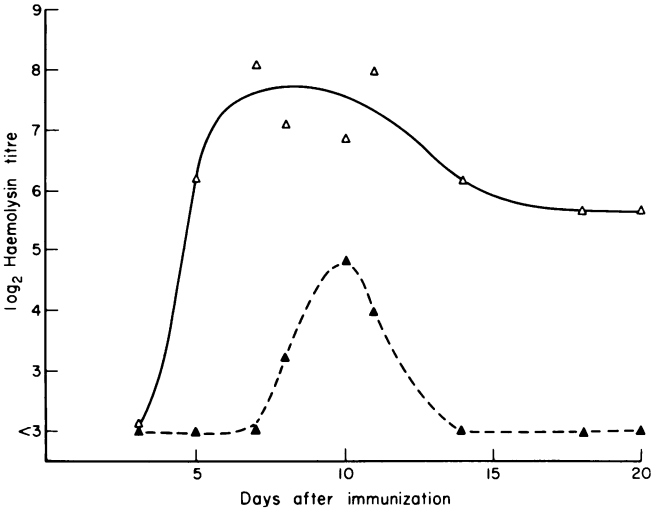

FIG. 1. Haemolysin response in thymectomized (▲) and sham-thymectomized plus intact (△ Swiss mice when the sheep erythrocytes were injected at 10 days of age. An average of 11·5 thymectomized mice and 13·5 control mice are represented by each point.

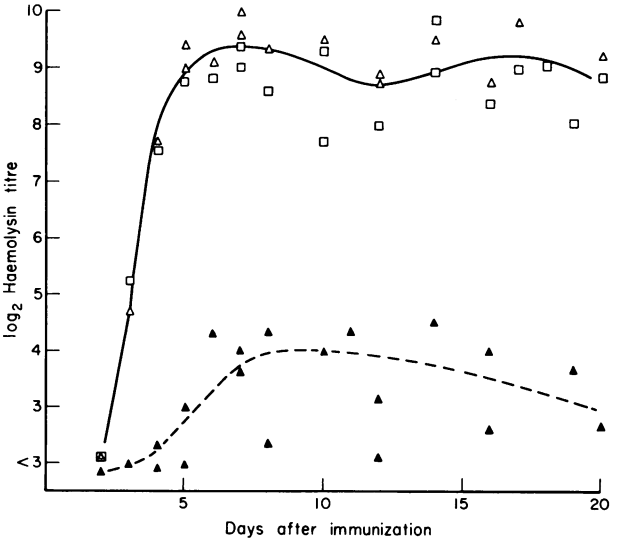

FIG. 2. Haemolysin response in thymectomized (▲), sham-thymectomized (△) and intact (□) Swiss mice when the sheep erythrocytes were injected at 4 weeks of age. An average of 8·5 mice is represented by each point.

Immunization at 6–7 weeks of age (Fig. 3)

Neonatally thymectomized mice developed serum haemolysin activities which were near to the control levels, but the time required for the response in thymectomized mice to reach maximal levels was longer than that in normal and sham-operated animals. The difference

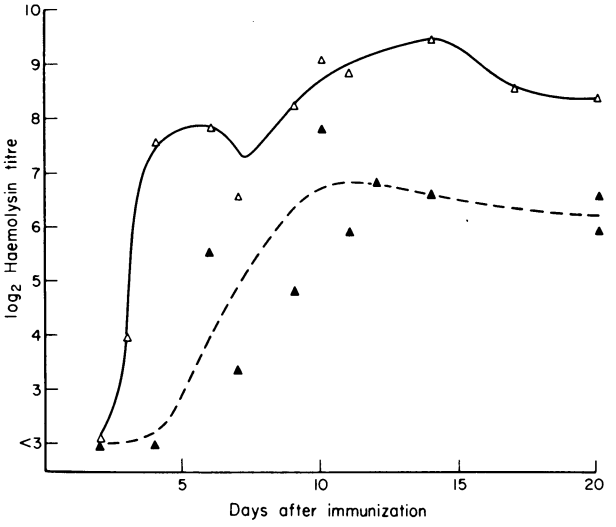

FIG. 3. Haemolysin response in thymectomized (▲) and sham-thymectomized plus intact (△) Swiss mice when the sheep erythrocytes were injected at 6–7 weeks of age. An average of eleven mice is represented by each point.

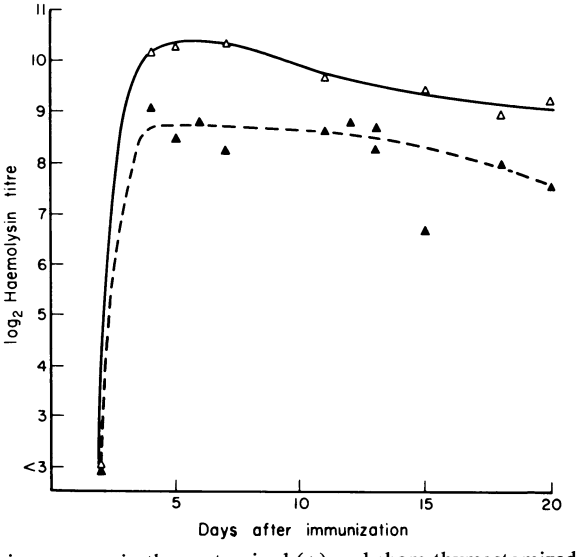

FIG. 4. Haemolysin response in thymectomized (▲) and sham-thymectomized plus intact (△) Swiss mice when the sheep erythrocytes were injected at 6 months of age. An average of twelve mice is represented by each point.

between thymectomized and control groups had the statistical significance of $P > 0{\cdot}05$ at the later times, but $P = 0{\cdot}01$ at 4 days after immunization.

Immunization at 6 months of age (Fig. 4)

When neonatally thymectomized mice were immunized at 6 months of age, their response was only slightly lower than that of the non-operated animals, being about one-half the controls. Statistical analysis showed the difference between the thymectomized and control groups to have a *P*-value of 0·1–0·05 at 7 days after immunization, 0·02 at 15 days after immunization and 0·05 at 20 days after immunization. Differences between the thymectomized and control groups at the other times tested were not statistically significant.

DISCUSSION

Neonatal thymectomy was followed by a delayed as well as a decreased haemolysin response to intraperitoneal injection of sheep erythrocytes. A similar finding was reported previously when an inbred strain of Swiss mice was used (Sinclair, 1965). Both the delay and the decrease in antibody levels became less obvious when the age of the mice at the time of immunization was increased. The normal Swiss mouse attains the normal adult-type of haemolysin response by about 2 weeks of age, but the neonatally thymectomized Swiss mouse did not reach a near normal response until some time after 6–7 weeks in the present study, and at some time between 9 and 13 weeks in the study made by Dukor *et al.* (1966). Therefore, thymectomized Swiss mice are able to develop a near normal haemolysin antibody response to sheep erythrocytes, but this development is delayed.

Preliminary results of experiments carried out in this laboratory indicate that even normal Swiss mice proceed through a period of life when the haemolysin response to sheep erythrocytes is decreased and delayed. This type of haemolysin response is most marked between 5 and 6 days of age. Therefore, the reduced and delayed type of haemolysin response described in this report as occurring in thymectomized mice is also present in normal mice during an earlier period of their life.

Thymectomized Swiss mice seem to be more prone to infections than intact animals, and the role played by the increased incidence of infections in thymectomized Swiss mice in producing the delayed and even the decreased haemolysin response is not yet known. Studies on 'pathogen-free' and 'germ-free' Swiss mice will help in deciding to what extent the observations reported in this paper may be attributable to an increased rate of bacterial infections.

Two previous reports have used the word 'recovery' when referring to the delayed development of the immunological responsiveness in thymectomized mice. The word 'recovery' is ambiguous when applied to the conditions present in experiments employing neonatal thymectomy, since it would infer that a measurable immunological responsiveness was present, then lost, and then regained. It is suggested that the words 'delayed development' be used to describe the condition present in neonatally thymectomized animals, and the words 'delayed recovery' or 'delayed re-development' be used to describe the condition in adult thymectomized and irradiated animals. Both of these phenomena must be distinguished from the 'delayed response' to sheep erythrocyte injection described previously and in this paper.

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