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Kinetics of IgM and IgG Responses in Neonatally Thymectomized Swiss Mice Under Persistent Immunization with Sheep Red Blood Cells

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The kinetics of IgM and IgG responses (direct and indirect PCF) in the Swiss mice, neonatally thymectomized, injected three times a week with 5×10^8 sheep erythrocytes at the age of two months, were exactly the same as the controls from the same litters. The thymectomized animals showed a statistically significant decrease in both responses (18.6 and 30.2 %, respectively). The kinetics of the switch from IgM to IgG and of the latter response inhibition, is the same in both the control and the thymectomized animals.

There is a rather general agreement in considering the IgM response as thymus independent and the IgG as thymus-dependent, or with a higher degree of thymus dependency (2-4, 8, 11). However, it has also been observed that the 19S *in vitro* response is inhibited by treatment with anti-serum and complement (6). Some authors (9) have pointed out that an increase in the dose of sheep red blood cells is necessary to induce a primary response when the Swiss mice are thymectomized neonatally. The same increase is needed to produce a secondary response. We have observed (1) a clear response both of IgM and IgG in this strain in two-month-old neonatally thymectomized mice. Nevertheless the responses were clearly lower than the ones obtained in the non-thymectomized control mice. These control mice came from the same litter as the experimental group. The decrease in the response affected both IgM and IgG to the same degree. It has also been observed that the response of the thymectomized mice does not differ from the response of the controls if, instead of only one dose, a set of them was given (10). Therefore we consider it interesting to study the kinetics of both IgM and IgG responses during a persistent immunization in the

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neonatally thymectomized mice in order to analyze if there was some difference either in the kinetics of the switch or in the intensity of the responses. The data obtained in this work confirm that there is no difference in the kinetics of the switch. The sole difference observed was a decrease in the intensity of IgM and IgG responses affecting both of them to the same degree.

Materials and Methods

Animals. Swiss mice bred in our Institute have been used. Thymectomy was carried out under cryanaesthesia with the suction method during the first 24 hours after their birth. Mothers were also anaesthetized on returning the thymectomized mice in order to avoid cannibalism (8). Each litter was divided in two groups, one experimental and one sham-thymectomized control group.

Antigens. Sheep red blood cells from a sole donor were used throughout the experiment. A dose of 5×10^8 cells (0.1 ml of 20 % suspension) was given three times a week.

Evaluation of PFC. Biozzi's modification of Jerne's plaque forming cell (PFC) technique was used to evaluate the immune responses. Both types of plaques, direct and indirect were evaluated. For the latter an anti-mouse IgG from rabbit was used (Behring); number of indirect plaques were estimated by subtracting number of direct plaques from the number of total plaques.

Results

A dose of 5×10^8 sheep red blood cells was given three times a week to a group of fifty, two-month-old neonatally thymectomized mice. The same dose was injected in the control group of fifty animals of the same litter. The animals were sacrificed on days 4, 7, 10, 20 and 30 from the beginning of the immunization, i.e., they got a set of 2, 5, 10 and 15 doses of red blood cells. Spleen suspensions were used to analyse the direct and indirect PFC on the day of the killing. Figure 1 shows that the kinetics of response in both the thymectomized and the control groups are identical. Otherwise the intensity of the response was rather different in both the direct (IgM) and the indirect (IgG) PFC response. The IgM and IgG responses

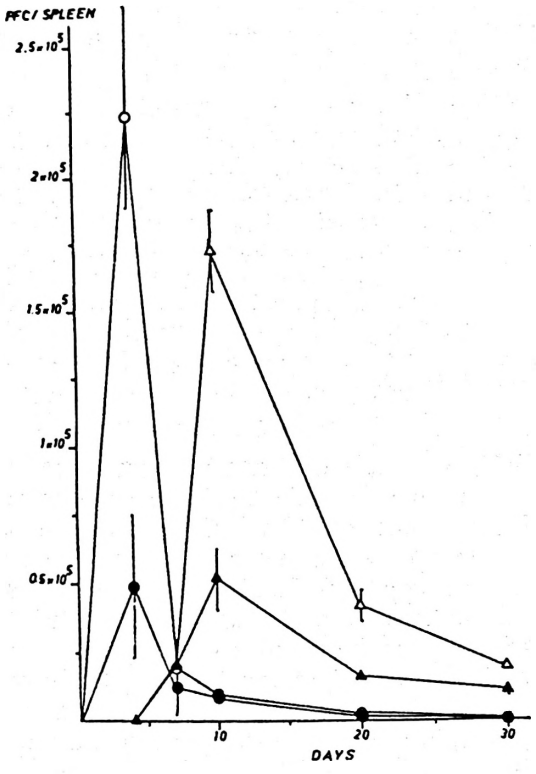

Fig. 1. *IgM (direct PFC) and IgG (indirect PFC) responses mean curves ± SE in neonatally thymectomized Swiss mice (● = IgM, ▲ = IgG) and in control animals of the same litters (○ = IgM, △ = IgG), persistently injected, i.p., with 5×10^8 SRBC, 3 times/week.* Each point corresponds to 10 animals.

of the experimental group are 18.6 % and 30.2 % respectively of the control responses. These differences are statistically significant ($0.02 > P > 0.01$ for IgM and $0.05 > P > 0.02$ for IgG).

Discussion

SINCLAIR and ELLIOTT (9) have observed that a higher dose of sheep red blood cells was required to induce either a primary response or to sensitize for a secondary response in the neonatally thymectomized mice. The results show that when these animals are immunized persistently with sheep red blood cells, a clear response in both IgM and IgG is obtained, exactly with the same kinetics in the control and experimental groups. However, the level of both IgM and IgG responses is clearly lower in the thymectomized animals, with very similar differences for both Ig, and statistically significant. The data presented in this work show clearly that the kinetics of IgM to IgG switch are exactly the same in neonatally thymectomized than in normal mice, which seems to imply that the switch is not T-dependent, or at least it requires a very small number of T cells. The use of sheep erythrocytes in non-irradiated neonatally thymectomized mice is particularly suitable, because partial T cell depletion still allows sheep red blood cells to amount a suboptimal (although measurable) response; on the other hand, total T cell depletion would not allow any response to take place. Although convenient ,this experimental design does not allow to rigorously exclude T cell involvement in the above mentioned experiments. Nevertheless, while thymectomy decreases clearly the IgM and IgG responses, it does not affect the kinetics of IgM to IgG switch (fig. 1). It implies that the switch took place in the absence of the bulk of T cells, and, at least, that, if it is T cell-dependent, it requires a small number of them.

The probable T-independence of the switch from IgM to IgG observed in our results agrees with the results obtained by SCHRADER (7). This author using DNP conjugated to different protein carriers has found that although T cells are required during the proliferative phase of the IgG response, they are not necessary for the switch to IgG. Some authors have pointed out a stronger T dependency of the IgG responses. These results are probably due to the fact that they gave a sole dose of antigen (2, 5), not the ideal conditions to get an optimal IgG response.

The kinetics of IgM and IgG responses are identical in both the control groups and the neonatally thymectomized mice (fig. 1); i. e., the curves follow a parallel course. This fact suggests the lack of suppressor T cell participation in the decline of both IgM and IgG responses; otherwise, the decline would be presumably slower in thymectomized.

Resumen

La cinética de las respuestas en IgM (CFP directas) e IgG (CFP indirectas) en el ratón suizo, neonatalmente timectomizado, inmunizado persistentemente con 5×10^8 hematíes de carnero, tres veces por semana, es la misma que la de los animales control de la misma camada sham-timectomizados; la única diferencia es una disminución neta en la intensidad de ambas respuestas (18,6 y 30,2 %, respectivamente) en los animales timectomizados. diferencia estadísticamente significativa. La cinética del cambio de IgM a IgG y de la inhibición de la respuesta en esta última es la misma en los animales control que en los timectomizados.

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