In vitro Primary Immune Response Resulting from the Interaction Between Bone Marrow-Derived and Thymus Cells[1]

In vivo experiments demonstrated that the primary response to sheep red blood cells (RBC) depends on the interaction between bone marrow-derived and thymus-derived cells[2-4]. Anti-sheep RBC antibodies resulting from this interaction, as it occurs in immunized mice which have been thymectomized in adult life, irradiated, and reconstituted by thymus graft and bone marrow cell injection, were shown to be produced by bone marrow-derived cells but not by thymus-derived cells[5]. This finding was confirmed in thymectomized bone marrow chimeras and extended to neonatally thymectomized mice, both reconstituted by the injection of thymus cells[6,7].

The observations described below demonstrate that the interaction between bone marrow-derived and thymus cells can take place in vitro. Primary response can, indeed, be induced in vitro only if both cell types are cultured with sheep RBC.

Materials and methods. The interaction between thymus cells from normal donors and spleen cells from either thymectomized bone marrow radiation chimeras or neonatally thymectomized mice was investigated by the MISHELL and DUTTON technique[8] whereby normal mouse spleen cells can be stimulated in vitro with sheep RBC to give rise to hemolytic plaque forming cells (PFC). Suspensions of thymus, spleen, or bone marrow cells, and of sheep RBC were prepared as previously described[9,10].

In one series of experiments, male C3HeB/FeJ mice were used to study the interaction between thymus cells from normal donors and spleen cells from thymectomized bone marrow radiation chimeras, which had been prepared as previously described[9]. For each cell culture experiment, groups of 5 mice were sacrificed and used as thymus or spleen donors. Thymuses were removed from 4-week-old normal mice, pooled, and dissociated in cell suspension. Spleens were removed from mice of each of the following 3 groups: 12-week-old normal mice, thymectomized or sham-operated chimeras 6 weeks after irradiation and bone marrow transplantation. Spleens were pooled within each group and dissociated in cell suspensions. After washing, the thymus and spleen cell suspensions were adjusted to the desired cell concentrations and distributed in 6 series of culture dishes. The nucleated cell content per dish of a series was one of the following: 1.5×10^7 normal spleen cells; 1.5×10^7 spleen cells from sham-operated chimeras; 1.5×10^7 spleen cells from thymectomized chimeras; 1×10^7 normal thymus cells and 1.5×10^7 spleen cells from thymectomized chimeras; 1×10^7 normal thymus cells; 1×10^7 normal thymus cells and 1.5×10^7 spleen cells from sham-operated chimeras. Antigen (1×10^8 sheep RBC) was added to a half of the culture dishes of each series. The volume of the final cell suspension per dish was 1 ml. From day 3 on, cells cultured with or without antigen were harvested daily from 2 dishes of the same series, pooled, washed and assayed for the number of PFC by the JERNE technique[11].

In another series of experiments, male DBA/2J mice were used to investigate the interaction between thymus cells from normal donors and spleen cells from neonatally thymectomized donors. The cell suspensions used in each culture experiment were the following: thymus cells from 4-week-old normal donors, spleen cells from 12-week-old normal donors, and spleen cells from 6-week-old donors which had been thymectomized or sham-operated at birth. Cells were distributed in culture dishes in the same numbers according to the same design as for the experiments with radiation chimeras.

Results and discussion. Relevant data from a typical experiment are reported in Figure 1, which illustrates the effect of the interaction between thymus cells from normal donors and spleen cells from thymectomized chimeras on the primary response in vitro. The peak mean number of PFC per dish resulting from the antigenic stimulation of this cell mixture was slightly higher and appeared one day later than that produced by the response of normal spleen cells. Neither spleen cells from thymectomized chimeras nor normal thymus cells ever produced more than 100 PFC per dish when stimulated in separate cultures. Results of the control cultures not shown in Figure 1 indicated that: (1) In vitro stimulation of spleen cells from sham-operated chimeras cultured alone or with normal thymus cells gave rise to numbers of PFC comparable to those resulting from the stimulation of normal spleen cells. (2) When no antigen was added to cultures less than 100 PFC per dish were observed in all series. As shown by the mean number of PFC on day 5 after antigenic stimulation, the immune response of spleen cells from thymectomized chimeras cultured with normal thymus cells was found to be at least 36-fold greater than the response of

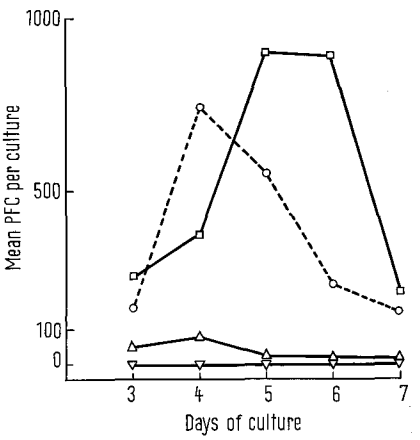

Fig. 1. In vitro primary response to sheep RBC by thymus cells from normal donors and spleen cells from thymectomized bone marrow chimeras. ▽—▽, thymus cells; △—△, spleen cells from thymectomized chimeras; □—□, thymus cells and spleen cells from thymectomized chimeras; ○—○, spleen cells from normal donors.

[1] Supported by CNEN-Euratom Association Contract. Publication No. 512 of the Euratom Biology Division.

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either cell type cultured separately. This enhancement cannot be accounted for by the higher cell concentration of the mixed cell culture, as shown by the results of the following experiment. Either 1.5×10^7 or 2.5×10^7 nucleated spleen cells from sham-operated chimeras were cultured with sheep RBC in 1 ml of medium. The peak response of the 2.5×10^7 cells was never more than 2-fold greater than that of the 1.5×10^7 cells.

The interaction between thymus cells from normal donors and spleen cells from neonatally thymectomized donors is illustrated in Figure 2. As in the radiation chimera experiments, the peak mean number of PFC per dish resulting from the stimulation of normal thymus cells mixed with spleen cells from thymectomized donors was higher and appeared one day later than that produced by the response of normal spleen cells. The response of the mixed cells was at least 70-fold greater than that of either cell type stimulated in separate cell cultures. Results of the control cultures not shown in Figure 2 were very similar to those described in the previous experiment with radiation chimeras.

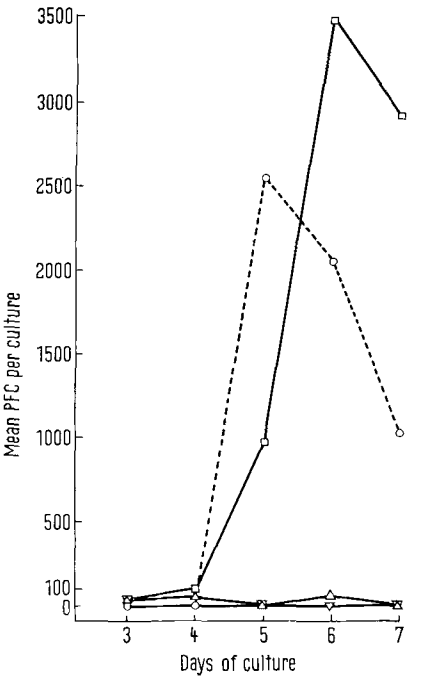

Fig. 2. In vitro primary response to sheep RBC by thymus cells from normal donors and spleen cells from neonatally thymectomized donors. ▽—▽, thymus cells; △—△, spleen cells from thymectomized donors; □—□, thymus cells and spleen cells from thymectomized donors; ○---○, spleen cells from normal donors.

It is worth mentioning that using male DBA/2J or C3HeB/FeJ normal mice thymus cells from 4-week-old donors and bone marrow cells from 12-week-old donors repeatedly failed to produce PFC when stimulated with sheep RBC in mixed or separate cultures, even when as many as 3×10^7 nucleated bone marrow cells (the most that could be obtained from 4 femurs) were cultured with 1×10^7 nucleated thymus cells. The unresponsiveness of this cell mixture suggests that the femural marrow of an adult mouse has too few cells, if any, able to interact with thymus cells in vitro. The interaction observed when spleen cells from thymectomized bone marrow chimeras were used, indicates that bone marrow cells able to interact in vitro or their precursors had proliferated or differentiated to such an extent that enough differentiated cells could be found in the spleen of the irradiated recipients 45 days after bone marrow transplantation. These proliferative and differentiative events can occur in the absence of thymus, as demonstrated by the results with spleen cells from either thymectomized chimeras or neonatally thymectomized mice. In the latter case, the bone marrow origin of the spleen cells able to interact with thymus cells in vitro remains to be determined.

Riassunto. Globuli rossi di pecora inducono in vitro, in colture miste di cellule spleniche di topi timectomizzati e timociti di topi normali, una risposta immune di tipo emolitico paragonabile a quella di cellule spleniche di topi normali. I dati indicano che la risposta immune delle colture miste risulta dall'interazione dell'antigene con timociti e cellule spleniche di origine midollare.

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Molecular Hybridization of RNA from Chicken Spleen Cells after Immunization

Recent studies have shown that new RNA species appear in mouse spleen or peritoneal exudate cells after in vivo[1] or in vitro[2] exposure to erythrocyte antigens. Direct hybridization of labelled RNA and competition experiments indicated that different RNA species appeared with different antigens. The aim of the present work was to confirm these results using chicken spleen cells at various times after immunization with a soluble antigen.

Adult White Leghorn chickens were injected i.v. with 200 mg of human serum albumin (HSA) per 1 kg of body weight. The spleens were excised between the 1st and 5th day after immunization. Spleens were minced by scissors and both washed cells and fragments were suspenedd in Eagle's MEM medium without phosphate. The mixture of fragments and cells from 5–10 chickens was incubated in a total volume of 100–200 ml of medium. P^{32}-labelled phosphate (Na_2HPO_4, carrier free, GmbH Isocomerz, Berlin-Buch) was added to a final concentration of 100 μc per ml. Incubation was stopped after 1 h by cooling in an ice-bath, the cells were collected by centrifugation and frozen.

RNA was isolated from the cells in a mixture of equal volumes of water saturated phenol and 0.05*M* sodium acetate (pH 5.5) containing 0.005*M* EDTA and 0.5% bentonite. Successive extractions were carried out at 25

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