The Thymus and Immunocompetence: The Target Cell of the Thymic Factor[1] (34480)

V. M. Rosenoer, G. Biano, and B. L. Brown
(Introduced by S. Malkiel)

Laboratories of Experimental Therapeutics, The Children's Cancer Research Foundation and Department of Pediatrics, Harvard Medical School, Boston, Massachusetts 02115

The experimental basis for the role of the thymus in the development of the primary immune response is now well established (1–3). The restoration of immunocompetence in neonatally thymectomized mice by thymic grafts implanted intraperitoneally in cell-proof Millipore chambers (4) and by cell-free thymic extracts (5, 6) strongly suggests the existence of a humoral thymic factor required for the differentiation of immunocompetent cells. The mode of action of this thymic factor is at present unknown.

The present study was undertaken to determine more precisely the role of the thymus in the functioning of the immune mechanism. The processing of antigen by macrophages and the transmission of information from these cells to the antibody-producing lymphocytes may be essential for the primary immune response (7, 8). The availability of an *in vitro* system in which the two cell populations can be separated (9, 10) provided an opportunity to study the specific target cell of the thymic factor.

Methods. Spleen cell suspensions were prepared from 4-week-old BDF1 or C57BL/6J mice pretreated with *Bordetella pertussis* phase I vaccine (6×10^9 killed organisms in 0.1 ml ip) 24 hr earlier. This treatment enhances antibody production and under similar conditions neonatal mice, normally considered immunoincompetent, are capable of antibody production (11), but the vaccine is ineffective if added to the spleen cell cultures from untreated mice.

The spleens were teased apart in cold Eagle's minimal essential medium (MEM) prepared with Earle's balanced salt solution, and the fragments were forced through a tantalum wire mesh (0.003 in.). After allowing cell aggregates to sediment by gravity for 3 min, the resulting single cell suspensions were passed through a 25-gauge needle and resuspended at a concentration of $1–5 \times 10^7$ cells/ml in Eagle's MEM supplemented with nonessential amino acids (12) sodium pyruvate (110 mg/liter) and calf serum (100 ml/liter; Microbiological Assoc. Inc.). The cultures were maintained for 4–5 days at 37° in an atmosphere of 5% CO_2 in air on a rocking platform (12 oscillations/min) in paraffin-sealed glass Petri dishes, 30-mm diameter, with a side tube to facilitate daily feeding with 0.15 ml of a nutritional mixture (2 parts Eagle's MEM with 1 part calf serum). The cultural conditions approximated those described by Mishell and Dutton (13).

For the cell-separation studies (Fig. 1) advantage was taken of the ability of macrophages to stick to glass or to plastic surfaces. The method described by Mosier and Coppleson (10) was adopted in which spleen cells ($1–5 \times 10^7$/ml from pooled spleen cell suspensions) were cultured in Petri dishes for 30 min at 37° on the rocking platform. Those cells remaining free in the culture medium were designated the nonadherent population. Morphologically they were lymphocytes. They were aspirated and subjected to two further separation procedures. The cells remaining firmly adherent to the dish after three washings with Eagle's MEM were designated adherent cells. They had the mor-

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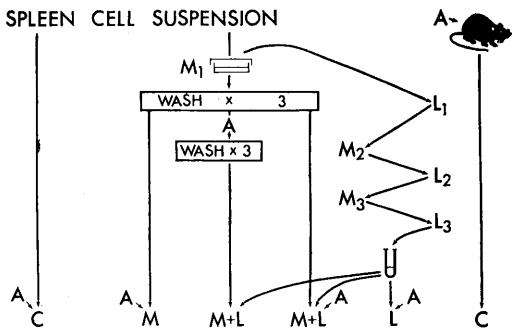

FIG. 1. *In vitro* immunization, normal mice. Experimental design for cell separation and recombination studies. M, adherent macrophage cells, L, nonadherent lymphocytes, A, antigen (sheep red blood cells), C, controls *in vitro* and *in vivo*.

phological and functional attributes of macrophages and could be harvested by scraping the dishes with a plastic "policeman." The separation methods were applied to spleen cell suspensions from neonatally thymectomized mice and from sham-operated and nonoperated littermates.

The sheep erythrocytes (SRBC) were washed three times in cold physiological saline and once in complete medium prior to use. For the *in vitro* studies the spleen cell suspensions were cultured (either separately or after recombination) with SRBC ($1–5 \times 10^7$ spleen cells with $1–10 \times 10^7$ SRBC) for 4–5 days. For studies in which the adherent macrophage population was preincubated with SRBC, the culture fluid and free erythrocytes were aspirated after 30 min of incubation and discarded. The adherent cells were washed three times with Eagle's MEM. After the washing approximately 10^6 adherent cells and fewer than 5×10^5 SRBC remained in the dish.

The antibody-producing cells were assayed either by a slide modification of the Jerne plaque assay (14) or by the more sensitive microchamber technique described by Cunningham (15). In this technique the ghosts of the hemolyzed red cells in the plaques can easily be counted in phase-contrast microscopy, thus affording a quantitation of the hemolysis.

In all studies the experiments were controlled by the simultaneous plaque assay of spleen cell suspensions from mice immunized *in vivo* 4 days previously by the intraperitoneal injection of 4×10^8 washed SRBC and/or of spleen cell suspensions from control animals cultured *in vitro* with SRBC for 4–5 days.

Preliminary studies confirmed the marked immunological deficiency of neonatally thymectomized mice. *In vitro* the immunological defect was readily apparent (2.6 ± 0.7 plaques per 10^6 cells compared with 13.1 ± 1.5 plaques per 10^6 cells in the sham-operated controls; $n=5$). In cell-isolation and recombination experiments nonadherent lymphocytes or adherent macrophages incubated alone with SRBC produced no plaques. The recombined cells produced a definite plaque-forming response although less than that produced by the original spleen cell suspension. Of particular interest was the production of plaques by recombined cells after exposing the adherent macrophages alone to SRBC for only 30 min.

Thymectomies were carried out within the first 24 hr of life using the technique described by Sjodin *et al.* (16). The completeness of thymectomy was in all cases confirmed at autopsy on completion of the studies.

Results and Discussion. An elaboration of the cell-isolation and recombination experiments in which the separated spleen cells from thymectomized and sham-operated animals were cross-combined, enabled an approach to be made to the problem of the target cell of thymic action. The results of this study are shown in Table I. Macrophages from thymectomized animals when incubated with lymphocytes from normal animals produced a number of plaques which did not differ significantly from that produced by macrophages from normal animals incubated with their own lymphocytes. On the other hand, the lymphocytes from thymectomized animals produced fewer plaques when incubated with macrophages from normal animals and no plaques when incubated with their own macrophages. The results were similar, regardless of whether the antigen was present for the duration of the culture or was incubated with the adherent macro-

TABLE I. *In Vitro* Immunization: Cell Recombination Studies Using Adherent Macrophages and Nonadherent Lymphocytes from Normal (N) and Thymectomized (TH-X) Mice.

		Plaques/10^6 cells (Cunningham)[a]	
		Culture of combined cells with antigen	Preincubation of macrophages with antigen
Macrophages (TH-X)	Lymphocytes (TH-X)	0	0
	Lymphocytes (N)	5.4 ± 1.4	5.0 ± 1.2
Macrophages (N)	Lymphocytes (TH-X)	3.0 ± 1.1	1.0 ± 0.5
	Lymphocytes (N)	7.5 ± 2.0	7.0 ± 2.0

[a] Mean ± SE ($n = 5$).

phages alone for only 30 min. Thus, the abnormality in the spleen cells from neonatally thymectomized mice appears to be related to the nonadherent lymphocytic population of cells.

The significance of the discrepancy between the results obtained with lymphocytes from thymectomized animals when recombined with "normal" and "thymectomized" macrophages is not entirely clear. The thymic factor might also exert an influence on the macrophages so that its absence might interfere with their capacity to "process" antigen or transmit information. However, the finding that "thymectomized" macrophages apparently act normally when recombined with "normal" lymphocytes indicates that the thymus is not essential for the role of the macrophage. Further, a small population of thymus-independent lymphocytes present in the spleen might account for the small number of plaques still found with the combination "normal" macrophages–"thymectomized" lymphocytes.

From these considerations, we conclude that thymectomy influences critically the nonadherent population of spleen cells. A small thymus-independent population of nonadherent cells is capable of antibody production, but still requires an intact antigen-processing activity of the macrophages. The slightly diminished capacity of the "thymectomized" macrophage is apparently insufficient for such thymus-independent cells from a thymectomized mouse to produce antibodies.

Taylor and Wortis (17) reported that the efficacy of SRBC as antigen increased up to 100-fold in the presence of the thymus and proposed that the thymus is concerned with the development of a system for trapping antigen. In contrast, the present studies suggest that the thymus acts upon the nonadherent lymphocyte population of spleen cells—and only slightly upon the adherent macrophage population which is involved in the initial "processing" of antigen. It is of interest that recently Roseman, Fitch, and Rowley (18), in a study utilizing recombination techniques, reported that X-irradiation also acts upon the nonadherent lymphocyte cell population.

The clinical counterparts of these animal studies have already been described and fit well the antigen-processing antibody-producing two-cell hypothesis discussed. The thymic aplasia syndrome (Di George syndrome) is equivalent to experimental neonatal thymectomy and leads to marked immunological deficiency in the efferent limb of the immune response, with impairment of delayed hypersensitivity reactions, allograft rejection, and lymphocyte stimulation by phytohemagglutinin *in vitro* (19). These cell-mediated immune responses are restored by fetal thymic transplants (20). In contrast, the defect in the Wiskott—Aldrich syndrome lies presumably in the afferent limb of the immune response—in the recognition or processing of antigen, as both the immunoglobulin production and the lymphocyte behavior is normal (21).

Summary. *In vitro* studies utilizing techniques which separate adherent macrophage and nonadherent lymphocyte populations of cells from the spleens of normal and neonatally thymectomized mice, have permitted

the definition of the target cell of the thymic factor concerned in the development of immunocompetence. The thymic factor is essential for the normal functioning of the antibody-producing lymphocytes and not for the initial "processing" of antigen by macrophages.

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