# Response of the Thymus and Immunologically Induced Lymphopenia.* (32294)

A. MICHAEL DENMAN,† EUGENE P. FRENKEL, AND MORRIS ZIFF
*Department of Internal Medicine, University of Texas Southwestern Medical School, Dallas*

The thymus has a higher mitotic rate than any other lymphoid tissue(1,2) and produces small lymphocytes in great numbers. These cells appear to arise from reticulum cells through a sequence of maturation of reticular lymphoblasts and large lymphocytes(3,4) and are released into the peripheral circulation as small lymphocytes(5). Following cellular destruction induced by x-irradiation(4) or non-specific stress, such as partial hepatectomy(6), deoxyribonucleic acid (DNA) synthesis in the thymus is increased as demonstrated by biochemical and autoradiographic studies. The functional importance of this regenerative capacity has been indicated by the poor recovery of immunological capacity after whole body irradiation in thymectomized as compared to intact animals (7). However, irradiation and partial hepatectomy leading to increased thymic cell proliferation represent relatively non-specific stimuli. In this report, evidence is presented that the thymus is capable of responding to a specifically induced lymphopenia with an increased production of lymphocytes.

The purpose of the present study was to evaluate the kinetics of lymphocyte production in rats subjected to chronic immune destruction of lymphocytes by specific anti-lymphocyte antibody.

*Materials and methods.* Rabbits were immunized with purified preparations of small lymphocytes obtained from inbred female Fischer rats‡ by filtration of lymph node and spleen cell suspensions through glass-wool columns by the method of Hildemann(8). The rabbit antiserum was repeatedly absorbed with rat erythrocytes; the gamma globulin fraction was then precipitated with ammonium sulfate and submitted to DEAE cellulose chromatography(9). *In vivo* activity against lymphocytes was found to be associated with the immunoglobulin G (IgG) component. To eliminate the immune response to rabbit globulin, the recipient rats were rendered immunologically tolerant to normal rabbit IgG by repeated injections during the first 10 weeks of life. This also permitted a more prolonged effect of the anti-lymphocyte antibody and more specific interpretation of the changes in the lymphoid mass(10).

Twenty tolerant female Fischer rats received daily intraperitoneal injections of this anti-lymphocyte IgG preparation (A.L. IgG) which resulted in the induction of a specific persistent lymphopenia. The absolute lymphocyte counts fell from 15-20,000/mm$^3$ to less than 500/mm$^3$ after the 6th day of injection of the anti-lymphocyte IgG. No effect on the circulating level or type of polymorphonuclear leukocytes resulted. Sixteen tolerant controls received daily injections of normal rabbit IgG without significant effect on the peripheral white cell count or differential count. These experiments were not continued beyond the 15th day because continued administration of A.L. IgG resulted in "wasting disease" with severe hemolysis and splenomegaly(10).

At intervals between the 8th and 15th days, groups of 4 rats each were labeled by intravenous injection of 1.0 $\mu$c of tritiated thymidine (H3-Tdr) (spec. act. 6.4 c/m mole) per gram of body weight. They were then sacrificed 1, 8, 24 or 72 hours later. The thymus was weighed and a portion taken for determination of the specific activity of the isolated DNA(11). Imprints and sections of all the lymphoid tissues were made for autoradiography as previously described(4). On each of the imprints, differential counts were made on a minimum of 4000 consecutive cells and expressed as the number of cells in each maturation compartment. The degree of labeling was expressed as the number and

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† Trainee in Arthritis, NIAMD.

‡ From Charles River Labs., Brookline, Mass.

TABLE I. Effect of Anti-Lymphocyte IgG on Mean Grain Count Per Precursor Cell in Thymus at Various Times After Injection of H3Tdr.*

| | 1 hr | | | 8 hr | | | 24 hr | | | 72 hr | | | 100 hr | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | R | ML | LL | R | ML | LL | R | ML | LL | R | ML | LL | R | ML | LL |
| Experimental | 30.9 | 30.7 | 19.5 | 13.2 | 21.2 | 11.8 | 10.7 | 11.2 | 7.2 | 5.3 | 4.6 | 5.1 | 5.1 | 5.5 | 4.9 |
| Control | 8.0 | 10.7 | 6.3 | 5.3 | 7.2 | 5.5 | 5.0 | 4.2 | 4.0 | 4.0 | 4.4 | 4.1 | — | — | — |

* Expressed as grains above background (3/cell) per each type of cell. Mean value for each group of 4 rats.

R = Reticulum cell; ML = Monocytoid lymphoblast; LL = Large lymphocyte.

grain count of each type of labeled cell. The tissue sections were processed for routine histologic examination and autoradiographic study to determine the histologic distribution of the labeled cells.

*Results and comments.* The thymus of the lymphopenic rats became progressively smaller during the course of the A.L. IgG injections. Histologically there was an accompanying loss of small lymphocytes, but there was no disturbance of the normal thymic architecture. A good correlation was observed between the fall in thymic weight and the decline in small lymphocyte population (Fig. 1). Serial light microscopic evaluation of the imprints and sections failed to demonstrate evidence of structurally altered or destroyed lymphocytes as a mechanism of the depletion of small lymphocytes and the associated decline in the weight of the thymus. The selective loss of small lymphocytes, apparent from imprints and histologic preparations, may have resulted from their release into the peripheral blood. There is experimental evidence for the migration of thymocytes into the blood stream(12,13). It is possible that the number of thymocytes released was increased in response to the peripheral lymphocytopenia.

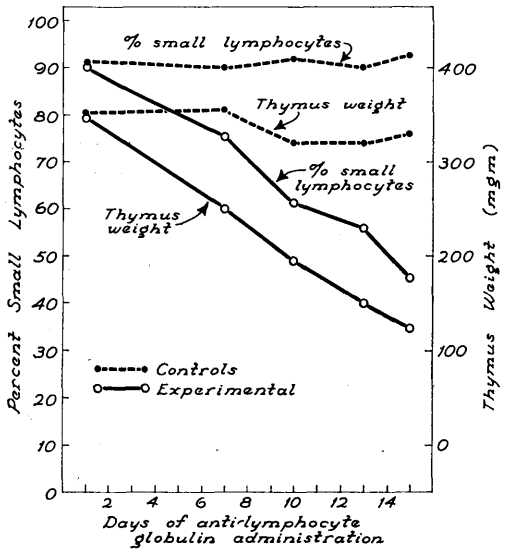


FIG. 1. Effect of serial administration of Anti-Lymphocyte globulin on percentage of small lymphocytes and weight of thymus.

TABLE II. Per Cent Small Lymphocytes of Thymus Labeled at Various Times After Injection of H3Tdr in Animals Injected with Anti-Lymphocyte IgG.

| | 1 hr | 8 hr | 24 hr | 72 hr | 100 hr |
|---|---|---|---|---|---|
| Experimental | 0 | 23.3 | 53.5 | 52.3 | 40.0 |
| | 0 | 13.1 | 44.5 | 7.8 | 32.3 |
| | 0 | 7.0 | 44.5 | 6.0 | 9.6 |
| | | 0 | 17.6 | 1.0 | 1.9 |
| Control | 0 | 0 | 6.0 | 3.0 | |
| | 0 | 3.5 | 4.5 | 1.0 | |
| | 0 | 3.0 | 3.1 | 1.0 | |
| | 0 | 1.5 | 1.2 | 1.0 | |

As indicated in Table I the mean grain counts per precursor cell were significantly increased in the lymphopenic animals during the 24 hour period following the injection of H3-Tdr. The percentage of each cell type labeled as well as the total number of labeled cells were also increased during this period. The progressive fall in mean given counts which was observed over the 100 hours following the initial labeling and the accompanying increase in per cent of small lymphocytes labeled (Table II), reaching a maximum at 24 hours, suggest that division of precursor cells and maturation of newly formed cells was proceeding normally. Although the increase in labeling observed might represent merely a decrease in pool size of thymidine leading to an apparent increase in labeling, this is considered unlikely. First, because a true thy-

TABLE III. Specific Activity (cpm/$\mu$g DNA) in Thymus of Lymphopenic and Control Fischer Rats at Various Times After Injection of $H^3$-Tdr.*

| | 1 hr | 8 hr | 24 hr | 72 hr | 100 hr |
|---|---|---|---|---|---|
| Experimental | 40.2 | 46.8 | 126.8 | 10.3 | 4.0 |
| Control | 8.0 | 5.6 | 1.3 | 4.3 | |

* Mean values for each group of 4 rats.

midine pool is not known to exist as such(14) and secondly since *in vivo* lympholysis was concurrent one might anticipate increased quantities of breakdown products available for reutilization.

The morphologic and autoradiographic findings presented above were confirmed by the high specific activity of the DNA isolated from the thymus (Table III). Although there was considerable variation from animal to animal, the specific activity over the first 24 hours was significantly higher than in the controls ($P<0.001$). These findings furnish additional evidence that increased DNA synthesis is present in the thymus of rats subjected to chronic, immunologically induced lymphopenia. Extensive studies of the peripheral lymphoid tissues also demonstrated a proliferative pattern. These will be reported later.

Although the thymus is normally impermeable to antigenic agents(20), experiments were carried out to determine whether A.L.IgG entered the gland. This was considered necessary in order to differentiate between a direct effect of A.L.IgG on the thymus and an indirect effort resulting from changes in the peripheral lymphatic tissue. This was especially important because of recent evidence that exposure of human buffy coat cells to rabbit anti-serum in tissue culture results in a blast cell-like response(18,19). Evidence from 3 types of experiments suggested that little, if any, anti-lymphocyte IgG entered the thymus. Since light microscopy of the thymus failed to demonstrate evidence of lymphocyte destruction, electron microscopic studies were performed in an attempt to identify subtle alterations in the cell membrane, cytoplasm or nucleus. Electron microscopic examination of the thymus glands of the lymphopenic rats showed no significant evidence of local destruction of lymphocytes. By contrast, damaged cells and cell debris were readily evident in the peripheral lymphoid tissues of the same animals. Second, rabbit IgG could not be detected in the thymus of the lymphopenic rats by immunofluorescent staining, utilizing fluoresceinated sheep anti-rabbit gamma globulin serum. Finally, in a third experiment, rabbit A.L.IgG was conjugated with $I^{131}$(21), and then injected intravenously into rats previously treated for 8 days with the A.L.IgG as well as into controls. The radioactivity of the thymus, spleen and lymph nodes was measured, and autoradiography performed. In contrast to the other tissues, relatively low levels of activity were detected in the thymus, and this was virtually limited to the blood vessels of the medulla. From this evidence it was concluded that the A.L.IgG exerted its effect on the thymus by an indirect mechanism.

*Discussion.* The present experiments have demonstrated increased proliferation of precursor cells in the thymus in apparent response to chronic immunologic depletion of the presumed end stage cell of the proliferative response, the small lymphocyte. A similar pattern of increased synthesis(4,11) has been observed following irradiation. Irradiation, however, is followed by a number of local metabolic changes in addition to lymphocytopenia(11), which may not occur in the A.L.IgG treated animal.

There are several possible mechanisms by which immunologically induced lymphopenia might stimulate the increased proliferative activity observed in the precursor cells of the thymus. It is possible that the simple removal of mature lymphocytes may stimulate the multiplication of precursor cells. It is also possible that, just as antibody formation can be enhanced by the injection of DNA(22), the increased blast cell proliferation observed in the thymus during the immune destruction of lymphocytes may be activated by DNA or its metabolic intermediates arising from the cells undergoing destruction. It is also conceivable that the breakdown of lymphocytes peripherally liberates other active agents which stimulate increased precursor cell proliferation. Finally, experiments demonstrating reconstitution of neonatally thymec-

tomized mice with allogenic thymus grafts in cell-tight millipore chambers(23) offer evidence that a humoral factor may mediate the influence of the thymus on the peripheral lymphoid system, and evidence for such a humoral factor has recently been adduced in adult animals(24). From this point of view, the state of the peripheral lymphatic tissue may exert a controlling influence on thymic cell production. Experiments are currently in progress to evaluate the relative importance of the several mechanisms presented above in the regeneration of the thymus and the state of the remainder of the lymphoid system.

*Summary.* Administration of specific rabbit anti-rat lymphocyte gamma globulin (IgG) produced prolonged severe lymphopenia in rats made tolerant to normal rabbit IgG. A decrease in thymic weight due to loss of small lymphocytes occurred. Combined morphologic and biochemical studies demonstrated marked proliferative activity of the precursor cells. The anti-lymphocyte IgG did not appear to gain entrance into the thymus.

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## Radioimmunoassay for Human Luteinizing Hormone.* (32295)

CHARLES FAIMAN†‖ AND ROBERT J. RYAN‖
*Department of Medicine, University of Illinois, College of Medicine, Chicago*

During the past year radioimmunoassays for human luteinizing hormone (LH) have been developed in several laboratories(1-4). In all of these, the basis of the assay rests upon the development of antisera to human chorionic gonadotropin (HCG) and the cross-reactivity of LH with these HCG antisera(5). The labeled antigen used has varied from highly purified pituitary LH(1) to purified

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† Research fellow, Medical Research Council of Canada.

‖ Present address: Mayo Clinic, Rochester, Minn.