Weight Reduction of Thymus and Depletion of Lymphocytes of T-Dependent Areas in Peripheral Lymphoid Tissues of Mice Infected with *Francisella tularensis*

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When BALB/c mice (young and adult animals of both sexes) were infected intraperitoneally with $10^3$ viable cells of *Francisella tularensis* (10$^5$ 50% lethal dose), all mice in these groups died on day 4. Reductions in thymus weights and in numbers of thymic cortex lymphocytes were observed in all the groups, but the decline was not so severe in the young females. Increases of plasma corticosterone in the adult males began 1 day after infection, but in the young females, the levels did not increase until day 3, the same days on which the respective thymus weights began to decline. Depletion of the thymus weights in the infected mice was prevented by adrenalectomy. The lymphocytes of the thymus (T)-dependent areas in peripheral lymphoid tissues in all groups were destroyed. By using an electron microscope, we found a large quantity of *F. tularensis* within the macrophages in the T-dependent areas but not in the thymus. The destruction of lymphocytes in the T-dependent areas was not prevented by adrenalectomy. Therefore, it was concluded that the weight reduction of the thymus is due to the stress of the *F. tularensis* infection. However, we think other mechanisms are responsible for the depression of lymphocytes in the T-dependent areas of peripheral lymphoid tissues.

*Francisella tularensis* gives rise to tularemia in rodents and occasionally in humans, who are usually infected by contact with infected animals (3). The disease can be reproduced in many experimental animals. In the case of mice, the disease is fatal. The histological characteristic is a necrotic exudative inflammation in the liver and spleen (10), and the bacteria can replicate within the liver cells and phagocytic cells (6, 9, 13).

Recently, several reports have shown that intracellularly growing microorganisms like *Listeria monocytogenes* can deplete the lymphocytes of the thymus (T)-dependent areas and the thymus (1, 8, 12, 17, 20). In tularemia, however, we do not know of any specific study carried out on the thymus and the peripheral lymph nodes. We thought, therefore, that the morphological interpretation of changes in the spleen, lymph nodes, and thymus in the infected mice should be examined in the light of immunological concepts.

We report here marked decreases in thymus weights and depletion of cortical lymphocytes in the thymus and of lymphocytes in T-dependent areas of spleen and lymph nodules in mice infected with *F. tularensis*.

**MATERIALS AND METHODS**

BALB/c young (4-week-old) and adult (7-week-old) mice of both sexes were used in these experiments. The animals were divided into four groups: young males, young females, adult males, and adult females. *F. tularensis* cells used were of the SCHU S4 strain and were cultured for 18 h in pig liver medium composed of pig liver infusion agar (pH 7.4) containing 10% human blood, 1% glucose, and 0.01% cystine (14). The mice in each group were inoculated intraperitoneally with $10^3$ viable cells of the bacteria (10$^5$ 50% lethal dose) or with 10 mg of heat-killed bacteria (60°C, 30 min). Every day at 10 a.m. the infected mice were sacrificed by bleeding under ether anesthesia. The spleen, liver, and thymus were weighed, small pieces of these organs were cultured with pig liver medium, and the remaining pieces were fixed in Formalin. Histological examinations were made on sections stained with hematoxylin eosin or Mallory.

**Measurement of plasma corticosterone.** The blood from the mice in each group was collected in iced, heparinized plastic centrifuge tubes. The blood was rapidly centrifuged at 4°C, and the plasma was separated and frozen at −20°C for subsequent determination of corticosterone concentrations. Corticosterone levels were measured by radioimmunoassay (7).

**Ultrastructure.** The tissue for electron microscopy was quickly dissected and fixed for 2 h at 4°C in 1% osmium tetroxide in 0.2 M phosphate buffer (pH 7.4) solution. After fixation, the tissue was embedded in Epon 812 and sectioned by glass knives in a Porter-Blum microtome. Sections were stained for 15 min at room temperature with 1% aqueous uranyl acetate–0.2% lead citrate and examined in a Hitachi HS8 electron microscope.

**Adrenalectomy.** Adrenalectomy was performed on adult male BALB/c mice under pentobarbital sodium anesthesia (40 mg/kg of body weight intravenously) by the method of Bolton et al. (2). The mice were given normal saline in lieu of drinking water to alleviate the electrolyte imbalance associated with adrenalectomy.

**RESULTS**

BALB/c mice infected intraperitoneally with *F. tularensis* died on day 4. Marked reductions of the thymus weights were observed (Fig. 1). In the adult male group, the thymus weights were reduced to half 1 day after infection. After 3 days, no thymus or any trace of it was found macroscopically in the mice at the moment of death or thereafter. Two days after infection, the thymus weights of adult female and young male were approximately half of the thymus weights of noninfected mice. The weights decreased markedly after that. On the other hand, in the young female group, thymus reduction did not begin until day 3. Although the young female mice died on day 4, the reduced thymus still re-
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mained. The body weights in all groups decreased about 20% during the 4 days. There were no marked changes in spleen and liver weights in any group. All mice injected with 10 mg of the heat-treated bacteria survived, and no changes in the thymus, spleen, or liver were observed macroscopically or microscopically over the 4 days (data not shown).

**Histological observation. Thymus.** No change was detected in the thymus on day 1 after infection. On day 2 or 3, in all the groups except the young female group, destruction of cortical thymic lymphocytes became obvious with the production of much cell debris, which was phagocytized by macrophages (Fig. 2B). On day 4, lymphocytes had almost completely disappeared from the cortex of the atrophied thymus, but moderate numbers of small lymphocytes were present in the medulla (Fig. 2C). In the young female group, histological change of the thymus was not observed until day 3. On day 4, the cortex lymphocytes were decreased, but the reduction in the young females was slighter than in the other groups (Fig. 2D).

**Spleen.** Histological change in the spleen was almost the same in all groups.

**White pulp.** On day 2, the histological change was visible as pale areas of reticulum cell mesh with a small amount of neutrophil and also as the absence of small lymphocytes in the white pulp of the spleen around the central artery. Little

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**FIG. 1.** Changes of thymus weights during *F. tularensis* infection. Symbols: △, adult male mice; △, adult female mice; ●, young male mice; ○, young female mice. Each point represents the mean figure for five mice; bars represent standard deviation.

**FIG. 2.** Histological observations of a normal thymus and a thymus after *F. tularensis* infection. (A) Thymus of an adult male mouse. Numerous lymphocytes exist in the cortex, whereas a moderate number of the cells appear in the medulla. Magnification, ×120. (B) Thymus of an adult male mouse at 2 days after infection, showing numerous macrophages with cell debris in the cortex. There is no change in the medulla. Magnification, ×120. (C) Thymus of a young male mouse at 4 days after infection, showing almost complete disappearance of lymphocytes from the cortex of the atrophied thymus. There are lymphocytes in the medulla. Magnification ×80. (D) Thymus of a young female mouse at 4 days after infection. Note the moderate numbers of lymphocytes and the numerous macrophages in the cortex. Magnification, ×120.
FIG. 3. Histological observations of normal and *F. tularensis*-infected spleens in adult male mice. (A and B) Normal spleen. There are many lymphocytes in the thymus (T)-dependent area around the central artery (CA). Magnification, $\times 240$ (A) and $\times 480$ (B). (C and D) Spleen of adult male mouse at 3 days after infection, showing destruction and disappearance of lymphocytes in the T-dependent area around the central artery (CA). Magnification, $\times 240$ (C) and $\times 480$ (D).

or no change was seen in the T-independent area. On day 3, the neutrophil disappeared, and only a few scattered small lymphocytes, a lot of cell debris, and occasional macrophages phagocytizing the cell debris could be seen in the T-dependent area (Fig. 3C and D). On day 4, extensive cell debris and fibrinoid degeneration were observed, but reticulum cells were also observed in the area. Destruction of remaining small lymphocytes was also prominent in the T-dependent area. There was no change in germinal center until day 3.

**Red pulp.** No changes were detected in the red pulp 2 days after infection. On day 3, red cells, lymphocytes, and

FIG. 4. Histological observations of a normal mesenteric lymph node and the mesenteric lymph node of an *F. tularensis*-infected mouse. (A) Normal mesenteric lymph node of an adult female mouse. There are small lymphocytes in the T-dependent area (TD). Magnification, $\times 120$. (B) Mesenteric lymph node of the adult female at 3 days after infection, showing disappearance of small lymphocytes in the T-dependent area (TD). Magnification $\times 120$. 

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megakaryocytes were seen in the area, but a striking feature was the accumulation of an eosinophilic material in the extracellular space of the pulp. On day 4, the cells could not be found, and the accumulation of the material increased.

**Mesenteric lymph nodes.** After day 3, slight depressions in the numbers of small lymphocytes and the deposition of eosinophilic materials were observed in the paracortical area and trabecular sinuses, but the changes were not so severe as that in the spleen (Fig. 4B).

**Peyer’s patches.** On day 3, changes in the Peyer’s patches were observed, viz., a depression in the number of small lymphocytes and a proliferation of reticulum cells in the dome area and in the internodular zone (Fig. 5A). On day 4, there were also macrophages phagocytizing the cell debris in the area, but small lymphocytes had not completely disappeared.

**Ultrastructural features. Thymus.** On day 2, the nuclei of many cortex thymocytes in the infected male mice were densely osmiophilic, and the nuclear membranes were not dissolved. Smaller, dense masses, probably lymphocytic nuclear remnants, were also present lying free in an extracellular position. Many macrophages were seen at this stage. These were very large cells containing within their cytoplasm lymphocytes in which either nucleus and cytoplasm or osmiophilic bodies corresponding in size to lymphocytic nuclei (Fig. 6B) could still be identified. In the medulla, however, there were still many lymphocytes without evidence of nuclear degeneration, and there was no phagocytosis of nuclear debris. *F. tularensis* could not be found in the cortex and medulla of the thymus by electron microscope. In adult female and young male groups, the same histological changes were observed on day 3. However, in the young female group, the changes were still not so marked even on day 4.

**Spleen.** Ultrastructural changes of the spleen were almost the same in all groups.

On day 3, in the T-dependent area, very few small lymphocytes were found, as well as cell debris, macrophages with many engulfed lymphocytes in various stages of degeneration with the cytoplasm, and a large number of the bacteria, although the bacteria were not found in any cells of the thymus (Fig. 7B). We often observed that arms of the macrophage, which phagocytized the bacteria, made contact with the adjacent lymphocyte (Fig. 7C and D).

![Fig. 5](image1.png)

**FIG. 5.** Histological observations of a normal Peyer's patch and the Peyer's patch in an *F. tularensis*-infected mouse. (A) Normal Peyer's patch of an adult male mouse. There are small lymphocytes in the T-dependent area between nodules (internodules [IN]) and in the dome area (D). Magnification, ×120. (B) Peyer's patch of an adult male mouse at 3 days after infection. Note depleted T-dependent area in the internodules (IN) and depletion in the dome area (D). Magnification, ×120.

![Fig. 6](image2.png)

**FIG. 6.** Ultrastructures of a normal thymus and a thymus from an *F. tularensis*-infected mouse. (A) Cortical thymic cells in a normal adult male mouse. N, Nucleus of the macrophage. Magnification, ×6,500. (B) Thymocytes and a thymic macrophage in an adult male mouse at 3 days after infection. The macrophage has many engulfed lymphocytes in various stages of degeneration with the cytoplasm. N, Nucleus of the macrophage; magnification, ×5,500.
**FIG. 7.** Ultrastructure of a normal splenic macrophage and the macrophage in an adult male mouse at 3 days after infection. (A) The splenic macrophage in a noninfected male mouse. The macrophage has a few degenerated substances and no *F. tularensis*. N, Nucleus of the macrophage. Magnification, ×8,200. (B) The macrophage has many degenerated substances and *F. tularensis* (arrow). N, Nucleus of the macrophage. Magnification, ×8,000. (C) Part of two adjacent membranes of the macrophage and the lymphocyte (L), in which *F. tularensis* (arrow) existed, became narrow. Magnification, ×18,400. (D) The arm of the macrophage, in which *F. tularensis* (arrow) existed, makes contact with a membrane of a neighboring lymphocyte (L). Magnification, ×23,000.

**Plasma corticosterone response to the *F. tularensis* infection in adult male and young female mice.** As it was known that an increase of plasma levels of corticosteroid hormones had been observed in animals under various applied stresses, we measured the plasma corticosterone levels in adult male and young female mice infected with *F. tularensis*, by using radioimmunoassay. Plasma corticosterone levels in the adult males increased to more than twice those of the controls on day 1, and the levels continued rising until day 4. However, in the young female, the corticosterone did not increase until day 3 (Fig. 8). The beginning of the weight reduction corresponded to the increase of the plasma corticosterone in each group.

**Effect of adrenalectomy on thymus weight depletion.** To test the possibility that the mechanism behind the thymus weight depletion is due to the stress-induced release of corticosteroid hormones from the adrenals, adrenalectomized or sham-adrenalectomized adult male mice were infected 1 day after the operation. The mice died 3 to 4 days postinfection. Thymus weights were determined 3 days after infection. Adrenalectomy prevented the depletion of the thymus weights, but sham adrenalectomy did not prevent it (Table 1). The sham operation itself caused the thymus involution, presumably due to stress, which was prevented by adrenalectomy. The depletion of lymphocytes in the T-dependent area of the spleen was not prevented by adrenalectomy.

**FIG. 8.** Plasma corticosterone levels and thymus weight reductions during *F. tularensis* infection. Symbols: △, corticosterone levels of adult male group; ○, corticosterone levels of young female group; ▲, thymus weight of adult male group; ●, thymus weight of young female group. Each point represents the mean figure for five mice; the bars represent standard deviation.
TABLE 1. Thymus weights of adrenalectomized mice on day 3 of *F. tularensis* infection*\(^a\)

<table>
<thead>
<tr>
<th>Operation</th>
<th><em>F. tularensis</em> infection</th>
<th>Thymus wt (mg)*(^b)</th>
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<tbody>
<tr>
<td>Adrenalectomy</td>
<td>−</td>
<td>55.2 ± 3.0</td>
</tr>
<tr>
<td>Adrenalectomy</td>
<td>+</td>
<td>50.5 ± 8.0</td>
</tr>
<tr>
<td>Sham</td>
<td>−</td>
<td>33.3 ± 5.0</td>
</tr>
<tr>
<td>Sham</td>
<td>+</td>
<td>11.0 ± 8.3</td>
</tr>
</tbody>
</table>

*\(^a\) At day 1 after adrenalectomy, adult male mice were infected with 10^7 *F. tularensis* cells. Thymus weights were determined 3 days after infection.

\(^{b}\) Arithmetic mean of results for three mice ± standard deviation.

DISCUSSION

In this experiment, we observed that the weight of the thymus and the number of its cortex lymphocytes were reduced in adult male, adult female, young male, and young female mice infected with *F. tularensis*.

The thymus is an organ which can be easily reduced by various stresses (19). The stresses induce the release of glucocorticoid hormones which can destroy thymus lymphocytes (18). In steroid- or stress-induced involution, a rapid and widespread lymphocytosis is observed in the cortex of the thymus. The degenerating lymphocytes are rapidly digested by macrophages within a few hours (11).

The lymphoid cells of the medulla have a stronger resistance to the steroids. They may cause an inverted picture of the organ, i.e., a dense medulla and a pale cortex.

In our experiment, changes of the thymus infected with *F. tularensis* were very similar to the changes arising from stress or from corticosteroids (Fig. 2B); that is, the changes were degeneration of the cortex thymocytes and phagocytizing of the cell debris by macrophages. However there was no change in the medulla.

The bacteria were not found in the thymus in any group by the bacteria culture method or by electron microscope.

High numbers of heat-killed bacteria did not cause any depletion of thymus weight in any group. Therefore, it is reasonable to assume that the thymus reduction was due to the stress of the infection and not due to the direct effect of the bacteria. In the young female group, the thymus reduction was moderate. Why are there differences in the thymus reductions among the four groups?

It has been reported that normal levels of plasma corticosterone, which is the principal glucocorticoid secreted by the adrenal cortex in mice, are higher in female mice than in male mice and that the levels in male mice become higher than in female mice when the animals suffer from stress (4). In our experiment, control levels of plasma corticosterone in the adult males became higher 1 day after infection, but in the young females the increase began on day 3 (Fig. 8). The beginning of the weight reduction and the histological changes corresponded to the increase of the plasma corticosterone in each group. Adrenalectomy prevented the depletion of the thymus weights in the adult male mice infected with the bacteria (Table 1).

Therefore, it appears that the difference observed between the two groups is due to differing sensitivity to the stress.

The concept of T-dependent areas of lymphoid tissues has been proposed by Parrott et al., who have found that T-lymphocytes around central arterioles in spleens and postcapillary venules in lymph nodes and in internodes and dome areas of Peyer's patches are diminished when a mouse is neonatally thymectomized (15, 16).

Besides neonatal thymectomy, it has been reported that the lymphocytes in T-dependent areas are diminished when humans or animals suffer from infectious diseases such as lepromatous leprosy, visceral leishmaniasis, *Echinococcus multilocularis* infection, lymphocytic choriomeningitis virus infection, and listeria infection (1, 8, 12, 17, 20). These microbes (except leishmaniasis) can deplete not only the lymphocytes in the thymus-dependent areas but also cortical lymphocytes of the thymus. Those authors have suggested that the thymus reductions in the animals suffering from the various infections described above were due to stress (12, 17). However, they did not explain exactly in their reports why these microbes deplete the lymphocytes in the T-dependent area of peripheral lymphoid organs (1, 8, 12, 17, 20). In our experiment, in the T-dependent area of the spleen, there were degenerated lymphocytes, cell debris, and macrophages which contained a large number of the bacteria and the lymphocytes (Fig. 7B). Degrees of lymphocyte depletion in the T-dependent area were not different from group to group. In addition, *F. tularensis* within a macrophage often existed near the macrophage membrane in the spleen. As the part of the membrane became very narrow or was not found by electron microscopy (Fig. 7D), the two neighboring membranes of the macrophage and the neighboring lymphocyte looked like one membrane; alternatively, the bacteria seemed to lyse the membranes of adjacent lymphocytes. However, we do not have any direct evidence indicating whether the macrophages containing the bacteria destroy the neighboring lymphocytes.

It is not possible that the bacteria directly destroyed the lymphocytes, because we could not find any pictures with the bacteria attached directly to the lymphocytes or the cells engulfing the bacteria. Many reports have shown that the lymphocytes in T-dependent areas of peripheral lymphoid tissues are not degenerated specifically by stress or corticosteroids (5, 21). Here too, just as in the cases of other microbes, the mechanism is difficult to explain. However, *F. tularensis*, *Mycobacterium leprae*emurium, *Leishmania donovani*, and *Listeria monocytogenes* possess a common property, viz., the power to survive within macrophages and degenerate lymphocytes in T-dependent areas of lymphoid tissues.

A large number of heat-killed *F. tularensis* cells did not cause any depletion of the lymphocytes of T-dependent areas. Therefore, it seems to be necessary for the degeneration that the microbes be able to proliferate within the macrophages in the T-dependent areas.

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LITERATURE CITED

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