165. Effects of Neonatal Thymectomy on Immunity against *Listeria monocytogenes* Infection

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The thymus has recently been the subject of intensive investigation and is now considered as an organ responsible for the maturation of various immunological capacities. Neonatal thymectomy in rodents is found to be closely associated with a diminished capacity to produce serum antibodies,¹⁾⁻⁶⁾ a depressed capacity to develop delayed hypersensitivity^{5),7)} and a failure of the capacity for homograft rejection.^{1)-3),8)-13)} On the other hand, the role of the thymus in the development of the protective immunity against bacterial and viral infections has not yet been elucidated. In this paper, the effects of neonatal thymectomy on immunity against *Listeria monocytogenes*, the acquired resistance to which is known to be associated with cellular immunity,¹⁴⁾ will be reported.

Experimentals. *Organisms.* *Listeria monocytogenes* (strain #2318) of serotype 4b was used. Although this strain is apt to produce R-type colonies, only the cells remaining in a smooth phase were used throughout the experiment. The bacteria were grown in brain heart infusion broth (Difco Laboratories, Inc., Detroit). The LD₅₀ for the organism by the intravenous route of injection was found to be $0.9-1.3 \times 10^5$.

Animals. Mice of the colony-bred CF1 strain were used. Thymectomy was performed 6 through 12 hours after birth by the method reported by Sjodin *et al.*¹⁵⁾ (TE group). As a control group, sham-operated mice (SH group) were handled in an identical fashion, except that the thymus was left *in situ*. Both groups of mice were used for the immunization experiment at 4 weeks of age.

Immunization. The *Listeria* cells were grown in 5 ml of brain heart infusion broth for 24 hours at 37°C and harvested by centrifugation. After washing by centrifugation, the cells were suspended in a suitable amount of saline. A dose of 1/30 LD₅₀ in 0.1 ml was intravenously injected for immunization.

Challenge. Mice immunized with a dose of 1/30 LD₅₀ and mice of the nonimmunized control group were challenged with a dose of 10 LD₅₀ ten days after the primary infection, because evidence has been reported by Mackaness and Ackerman¹⁶⁾ that the challenging *L. monocytogenes* was inactivated without a preliminary growth phase

during 2 weeks following immunization.

The results are shown in Fig. 1. Immunized mice both in SH (Fig. 1a) and TE (Fig. 1b) groups are more or less resistant to the challenge infection. All the mice of the nonimmunized control group (Figs. 1c and 1d) died within 7 days after challenge, while in the immunized group 11 of 15 SH group mice (Fig. 1a) and 4 of 15 TE group mice (Fig. 1b) survived within the observation period of 2 weeks. The degree of acquired resistance to lethal infection, however, was markedly depressed in the TE group as compared with the SH group.

In nonimmunized control group mice, the sensitivity to *Listeria* infection seems to be lower in the TE group than in the SH group. All the mice in the latter group died within 2 days after lethal infection, while in the former group, 7 days were required to obtain 100% mortality. On the other hand, the LD₅₀ dose of *Listeria* for the TE group proved to be identical to that of the SH group.

Discussion. Acquired resistance to *Listeria* has been proved to be based not upon the serum antibodies, but upon cellular immunity.¹⁴⁾ The present experiment was undertaken to elucidate the role of the thymus in the development of cellular immunity, and has disclosed that the capacity to produce cellular immunity to *Listeria* infection was depressed by neonatal thymectomy.

The mechanism of cellular immunity has not been clarified yet, but it seems to resemble that of a delayed hypersensitivity and of homograft rejection, considering that passive transfer of the immunity is not enabled by serum but by immune cells. Several experiments already published have disclosed that in the rodents thymectomized at birth, the capacity to develop delayed hypersensitivity was depress-

Fig. 1. The effects of neonatal thymectomy on immunity against *L. monocytogenes* infection.

No. of Mice: Number of mice surviving after challenge with a dose of 10 LD₅₀.

- 1a: Immunized sham-thymectomized mice.
- 1b: Immunized neonatally thymectomized mice.
- 1c: Nonimmunized sham-thymectomized mice.
- 1d: Nonimmunized neonatally thymectomized mice.

ed, and the capacity for homograft rejection was abolished. In this experiment, cellular immunity against *Listeria* infection was also found to be markedly impaired by neonatal thymectomy. The present result is consistent with that obtained in tuberculosis, the acquired resistance to which is also known to be mainly based upon cellular immunity and is markedly impaired by neonatal thymectomy.¹⁷⁾

Another interesting fact found in this experiment is that all the nonimmunized mice in the SH group succumbed within 48 hours after lethal infection, but in the TE group a significant prolongation of survival time was recognized. This may be correlated with the finding obtained in a preliminary experiment that the number of viable bacteria in the spleen at 24 hours after sublethal infection was smaller in the TE group than in the SH group. Further investigations will be required to clarify the mechanism of pathogenesis of *Listeria* infection. Indeed, the present findings observed in the *L. monocytogenes*-neonatally thymectomized mice relationship would contribute to these studies.

Summary. In mice thymectomized at birth, the protective immunity against *Listeria monocytogenes* infection was markedly impaired. The time required for the death caused by lethal *Listeria* infection was considerably longer in neonatally thymectomized mice than in sham-thymectomized mice.

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