64. Cellular Basis of Immunity. IV

Cross Immunity of the Mouse Mononuclear Phagocytes Immunized with Live Vaccine of *Salmonella choleraesuis* and *S. typhimurium* against the Infection with *S. enteritidis*

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In the preceding paper¹⁾ cross immunity of the mouse mononuclear phagocytes immunized with live vaccine of *Salmonella enteritidis* was described.

Mononuclear phagocytes obtained from the mouse immunized with live vaccine of *S. enteritidis*, inhibited intracellular growth of *S. typhimurium*, *S. choleraesuis*, *S. enteritidis*, and *E. coli* 0-55, regardless of the presence of immune sera. But the cells of normal mice as well as immunized mice with live or dead vaccine of *S. enteritidis* did not inhibit the intracellular multiplication of *M. tuberculosis H37Rv*.

This paper deals with the cross immunity of mononuclear phagocytes of the mouse immunized with live vaccine of *S. typhimurium* or *S. choleraesuis* against the infection with *S. enteritidis*.

Materials and Methods. Experimental animals, immunization method, and antigenic structure of bacterial strains used were described in previous papers.¹⁾⁻⁴⁾ The tissue-culture medium and phagocytic system were reported in the preceding report.³⁾

Results. Cross-immunity of the mouse super-immunized with live vaccine of *S. choleraesuis* or *S. typhimurium* against the infection with *S. enteritidis*. As shown in Table I, mouse super-immunized with live vaccine of *S. choleraesuis*, *S. enteritidis* or *S. typhimurium* resisted completely the intravenous infection of 10 MLD virulent strain of the same organism used for immunization. But the mouse super-immunized with live vaccine of *S. choleraesuis* or *S. typhimurium* showed cross-immunity toward intravenous infection with the virulent strain of *S. enteritidis*.

Cellular resistance of mononuclear phagocytes derived from the mouse super-immunized with live vaccine. Mononuclear phagocytes derived from the mouse super-immunized with live vaccine of *S. choleraesuis* or *S. typhimurium* showed cross resistance against the infection of *S. enteritidis*. The results are shown in Fig. 1.

The normal mononuclear phagocytes were destroyed within 3 days

of incubation by intracellular multiplication of the virulent strain 116-54 of *S. enteritidis*. Number of mononuclear phagocytes which were derived from normal or immunized mouse were calculated after

Table I. Cross immunity of the mouse super-immunized with live vaccine

Organism used for immunization*	Organism used for challenge**	Number of mice	Survival rate (%)
<i>S. choleraesuis</i>	<i>S. enteritidis</i>	20	85
<i>S. choleraesuis</i>	<i>S. choleraesuis</i>	10	100
<i>S. enteritidis</i>	<i>S. choleraesuis</i>	20	75
<i>S. enteritidis</i>	<i>S. enteritidis</i>	10	100
<i>S. typhimurium</i>	<i>S. enteritidis</i>	10	70
<i>S. typhimurium</i>	<i>S. typhimurium</i>	10	100
Non-immunized control	<i>S. enteritidis</i>	10	0
	<i>S. choleraesuis</i>	10	0
	<i>S. typhimurium</i>	10	0

* *S. enteritidis*: Mice were super-immunized intravenously with 10^{-5} mg of attenuated strain and 10^{-7} mg of virulent strain of *S. enteritidis* at 21 days interval.

S. choleraesuis and *S. typhimurium*: Mice were super-immunized intravenously with 10^{-2} mg of attenuated strain and 10^{-7} mg of virulent strain of both strains at 21 days interval.

** Mice were challenged intravenously with 10^{-7} mg of virulent strain.

Fig. 1. Intracellular multiplication of the virulent strain 116-54 of *S. enteritidis* in normal monocytes and cross resistance of immune monocytes.

○—○, cells derived from normal mice; ●---●, cells derived from mice super-immunized with live vaccine of *S. choleraesuis*; ●---●, cells derived from mice super-immunized with live vaccine of *S. typhimurium*; △—△, cells derived from mice super-immunized with live vaccine of *S. enteritidis*.

in vitro infection with the virulent strain 116-54 of *S. enteritidis*.

Monocytes derived from the mouse super-immunized with live vaccine of *S. choleraesuis* or *S. typhimurium* showed cellular resistance and did not show any decrease in cell number after infection, whereas normal monocytes showed about 80 per cent degeneration after 5 days of incubation. The results are shown in Fig. 2.

Fig. 2. Number of monocytes after infection with the virulent strain 116-54 of *S. enteritidis*, which were derived from normal or immunized mouse.

See foot-note of Fig. 1. The number of monocytes is expressed in percent of initial number. The total number of monocytes (ca. 500) in 5 microscopic fields ($20 \times 7 \times 1.5$) were calculated in each experiment. Each point indicates the mean of three experiments.

Discussion. The degeneration of normal mononuclear phagocytes caused by infection with *M. tuberculosis* H37Rv decreased by antiserum from the rabbits immunized with BCG, *Salmonella rutgers*, or crystalline egg albumin.^{5),6)} Immune monocytes obtained from the rabbit vaccinated with BCG resisted the degeneration caused by infection with H37Rv when maintained in immune serum (anti-BCG, anti-*Salmonella*, anti-ovalbumin).

But immune monocytes derived from the rabbit vaccinated with BCG were degenerated by H37Rv infection when cultivated in normal rabbit serum. Immune monocytes derived from animals immunized with *S. rutgers* showed early and marked degeneration by H37Rv infection even when cultivated in anti-BCG or anti-*Salmonella* serum.⁵⁾⁻⁸⁾

In earlier reports^{2),4),8)} by the present authors it was described that mononuclear phagocytes obtained from the abdominal cavity, liver or subcutaneous tissue of mouse super-immunized with live vaccine of *S. enteritidis* inhibited intracellular multiplication of a

virulent strain 116-54 of the same organism regardless of the presence of antibody in cell culture medium. In a previous paper, monocytes super-immunized with live vaccine of *S. enteritidis* inhibited intracellular growth of *S. typhimurium*, *S. choleraesuis*, and *E. coli* 0-55, regardless of the presence of immune sera, whereas the cells were degenerated by infection with *M. tuberculosis* H37Rv. In the course of the study of infection of mouse with Salmonellae group the following results were obtained.

Summary. Mouse super-immunized with live vaccine of *S. choleraesuis* or *S. typhimurium* survived the intravenous infection with 10 MLD virulent strain of *S. enteritidis*. In the course of the study of *in vitro* infection with *S. enteritidis*, it was found that mononuclear phagocytes derived from the abdominal cavity of mouse immunized with live vaccine of *S. choleraesuis* or *S. typhimurium* inhibited intracellular multiplication of bacteria and resisted the degeneration caused by infection, regardless of the presence of immune sera.

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