137. Cellular Basis of Immunity. VIII

Cell-bound Antibody in Mononuclear Phagocytes Immunized with Live Vaccine of Salmonella enteritidis

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In the previous papers,¹⁻³⁾ it was reported that the mice super-immunized with live vaccine of *Salmonella enteritidis* acquired high resistance to the infection with the same organism and survived the intravenous infection with 1,000 MLD of a virulent strain 116-54. And the mononuclear phagocytes, monocytes, obtained from the abdominal cavity, subcutaneous tissue, and liver of these mice, inhibited the intracellular growth of a virulent strain 116-54 and resisted the cell degeneration caused by phagocytosis, in the absence of antibody in tissue culture medium. We referred to this cell resistance as cellular immunity.

In contrast, the mice immunized with killed vaccines of *S. enteritidis* were incapable of surviving the infection with the same organism, whereas high titer of antibodies (O, H) against *S. enteritidis* was found in the serum. And the mononuclear phagocytes could not resist infection and were destroyed by engulfment of bacteria.

Employing the method of immune transfer and immune adherence hemagglutination,⁴⁾ the cell-bound antibody was detected in the monocytes of mice immunized with live vaccine of *S. enteritidis*. In the present communication, the outline of this cell-bound antibody is described.

Experimental methods. Experimental animal, bacterial strains, and method of immunization were described previously.¹⁻³⁾ The cell-bound antibody was detected according to the modified method⁷⁾ of immune transfer and immune adherence hemagglutination.⁶⁾ Briefly, the monocytes of normal or immunized mice were obtained from the peritoneal cavity of mice according to the method of previous paper²⁾ and used for the determination of cell-bound antibody (Ab). Erythrocytes and complement (C') were collected by venipuncture from healthy persons in this laboratory. A fully virulent strain 116-54 was used to prepare the antigens (Ag) of immune transfer. For the diluents of immune transfer and immune adherence hemagglutination, GVB⁺⁺ and TVB⁺⁺ buffers were used.⁷⁾

The determination of cell-bound antibody was carried out by the immune transfer and immune adherence hemagglutination method. For the dilution of antigen (Ag) and antibody (Ab), a monocyte (1×10^8 /ml) and bacteria (1×10^8 /ml) suspensions were diluted with GVB⁺⁺ to make a series of 2 fold dilution, respectively. Eight tenth ml each of monocytes suspension (1×10^8 /ml) and dilution series of bacterial suspension, or eight tenth ml each of bacterial suspension (1×10^8 /ml) and dilution series of monocytes suspension, was incubated at 30 C with occasional manual shaking. After 60 min incubation, the sensitized bacteria (Ag-Ab) was obtained by centrifugation, washed twice with TVB⁺⁺ and suspended in 0.8 ml TVB⁺⁺. Two tenth ml of a 30 times diluted human complement (C') was added to 0.8 ml of sensitized bacteria (Ag-Ab), and the mixture was incubated in a 37 C water bath attached to a shaker. After incubation for 15 min, 0.1 ml of the standardized human erythrocytes (E) was added to the mixture of Ag-Ab-C' and incubated in a water bath attached to a shaker. After 10 min incubation, the mixture of Ag-Ab-C'-E was allowed to stand at 37 C for 60 min. The pattern of settled erythrocytes was read according to the method described by Nishioka.⁷⁾ The grade of positive reaction was recorded, varying from 1+ to 3+.

For the absorption of antibodies against killed bacteria, equal volume each of monocytes suspension (1×10^8 /ml) and formalinized bacterial suspension (1×10^8 /ml) was mixed and incubated at 30 C. After standing for 60 min, the monocytes were separated by centrifugation at 2,600 g for 5 min, washed twice by centrifugation with GVB⁺⁺ and suspended in GVB⁺⁺ to a final concentration of 1×10^8 cells/ml.

Results. The monocytes of mice immunized with live or killed vaccine were used for the source of cell-bound antibody. As shown in Table I, maximal reactivity with the monocytes of mice superimmunized with live vaccine was obtained by employing 1.5×10^7 bacteria which were sensitized with monocytes. This was followed in the order of reactivity by monocytes of mice immunized with formalin vaccine.

The antibodies present in monocytes were absorbed with formalinized bacteria as described under the section of Materials and Methods. Thereafter the monocytes were used for immune transfer and immune adherence hemagglutination test.

After the absorption of monocytes with formalinized bacteria, the monocytes of mice hyperimmunized with live vaccine still showed maximal reactivity by employing 3×10^7 live organisms of *S. enteritidis*, whereas the cells of mice immunized with formalin vaccine

turned negative against live organisms of *S. enteritidis*. The results are shown in Table I.

Table I. Hemagglutination patterns after the reaction with *S. enteritidis* sensitized with monocytes absorbed with formalinized *S. enteritidis*

Monocytes obtained from the mice	Absorption with formalinized <i>S. enteritidis</i>	Antigen for hemagglutination	Number of <i>S. enteritidis</i> antigen particles per reaction mixture ($\times 10^7$)							
			24	12	6	3	1.5	0.75	0.38	None
Hyperimmunized with live vaccine Immunized with formalin vaccine	before absorption	formalinized <i>S. enteritidis</i> 116-54	++	+	+	+	+	-	-	-
			++	++	+	-	-	-	-	-
Hyperimmunized with live vaccine Immunized with formalin vaccine	after absorption	live <i>S. enteritidis</i> 116-54	++	++	+	+	-	-	-	-
			-	-	-	-	-	-	-	-

When the series of dilution of monocytes (Ab) were used, maximal reactivity with the monocytes of mice hyperimmunized with live vaccine and immunized with formalin vaccine was obtained by employing 1×10^7 and 2×10^7 monocytes, respectively. After the absorption of monocytes with formalinized bacteria, the monocytes of mice hyperimmunized with live vaccine still showed positive reaction against live organisms of *S. enteritidis*, whereas the cells of mice immunized with formalin vaccine turned negative (Table II).

Table II. Hemagglutination patterns after the reaction with *S. enteritidis* sensitized with monocytes absorbed with formalinized *S. enteritidis*

Monocytes obtained from the mice	Absorption with formalinized <i>S. enteritidis</i>	Antigen for hemagglutination	Number of monocytes per reaction mixture ($\times 10^7$)							
			8	4	2	1	0.5	0.25	0.125	None
Hyperimmunized with live vaccine Immunized with formalin vaccine	before absorption	formalinized <i>S. enteritidis</i> 116-54	++	++	+	+	-	-	-	-
			++	+	+	-	-	-	-	-
Hyperimmunized with live vaccine Immunized with formalin vaccine	after absorption	live <i>S. enteritidis</i> 116-54	++	+	+	-	-	-	-	-
			-	-	-	-	-	-	-	-

These results indicate that the monocytes of mice hyperimmunized with live vaccine of *S. enteritidis* contained antibody other than those reacting with formalinized bacteria, *i.e.*, O, H antigens.

Discussion. The role of plasma cells in antibody synthesis was suggested by many research workers,⁸⁻¹⁰⁾ and it was firmly established by the immunofluorescent studies of Coons¹¹⁾ and by the single cell culture of Nossal.¹²⁾ According to the cytological study made on the role of lymphoreticular cells in macroglobulinemia Waldenstroem,¹³⁾ Amano¹⁴⁾ proposed the hypothesis that there are two functional allotments in immune system, *i.e.*, plasma cellular and lymphoreticular system. It was suggested¹⁵⁾ that the heterogeneity of antibody molecules produced against a single antigen may depend on the participation of different types of cells in the synthesis of these antibodies. It is known facts that neonatal infants synthesize 19s antibody. This fact suggests that the cells other than plasma cells synthesize this antibody, since plasma cells are not found in the tissues of the neonatal infants.

On the other hand, it was reported that antibody was not detected in the peritoneal macrophages of rabbits immunized with *Shigella* and *Salmonella*.^{16,17)}

It was suggested by Fishman¹⁸⁾ that the sequential action of at least two different types of cells was responsible for the antibody formation in tissue culture, *i.e.* phagocytic cells (macrophages) and non-phagocytic cells belonging to the lymphocytic cells. And it was found that the initial step necessary was the phagocytosis of antigen by the macrophages, and thereafter to yield a material which initiated the formation of antibody in the cultures of lymph node cells.¹⁹⁾

In the present article, it was demonstrated that macrophages, obtained from the abdominal cavity of mice hyperimmunized with live vaccine of *S. enteritidis*, contained specific antibody which reacted with live organisms even after absorption with formalinized bacteria. This cell-bound antibody inhibited the growth of *S. enteritidis* with the aid of complement and lysozyme, either on the nutrient agar plate or in the macrophages of normal mice. And this antibody was not detected in the serum of mice hyperimmunized with live vaccine of *S. enteritidis*.

According to the reports described previously, there are two possibilities for the formation of this antibody, in the mice immunized with live vaccine, not with killed vaccine. These are 1) plasmacellular formation and adsorption onto monocytes, and 2) formation by monocytes belonging to reticuloendothelial cell line. These problems still remain to be solved.

Summary. According to the immune transfer and immune adherence hemagglutination technique, the cell-bound antibody was detectable in the monocytes of mice hyperimmunized with live vaccine of *S. enteritidis*. This antibody was not found in the cells of mice immunized with killed vaccine or in the serum of mice immunized with live vaccine.

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