Influence of Hyperimmune Immunoglobulin G on the Physicochemical Properties of the Surface of *Salmonella typhimurium* 395 MS in Relation to Interaction with Phagocytic Cells

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Partition in an aqueous, two-polymer phase system containing dextran and polyethylene glycol was employed to investigate the physicochemical changes inflicted by the presence of immunoglobulin G (IgG) antibodies on the cell surface of a smooth strain of *Salmonella typhimurium*. Adding increasing amounts of anti-*Salmonella* IgG to the bacteria decreased the affinity for the polyethylene glycol-rich top phase, with a concomitant increase in vivo clearance and in vitro phagocytosis by rabbit polymorphonuclear cells. Similarly, S → R mutations in the same *S. typhimurium* strain decrease the affinity for the top phase and increase the liability to phagocytosis. The limiting antibody concentration to demonstrate increase of in vitro phagocytosis was approximately the same as that to produce a significant effect in the phase system, whereas lower concentrations were needed to increase in vivo clearance. The results show that adsorption of IgG antibodies to bacteria brings about physicochemical changes of the cell surface which seem to promote the phagocytosis by polymorphonuclear cells and uptake in the reticuloendothelial system.

It is well known that immunoglobulins function as opsonins for a great number of otherwise phagocytosis-resistant bacteria, e.g., virulent streptococci, pneumococci (5), and *Salmonellae* (14). The phagocytosis of *Salmonella typhimurium* S and R bacteria by rabbit polymorphonuclear (PMN) cells in the absence of serum components is dependent on their physicochemical surface properties as demonstrated by the partition of the bacteria in a dextran-polyethylene glycol (PEG) aqueous two-phase system (15, 16). Such systems are becoming more widely employed, analytically and preparatively, to discriminate and separate particles different in surface characteristics such as charge and hydrophobicity (1). This study was undertaken to investigate whether opsonization of *Salmonella* S bacteria inflicts a change in the physicochemical properties of the bacterial surface which might increase the adherence of the bacteria to phagocytes.

**MATERIALS AND METHODS**

**Bacterial strain and cultivation.** The smooth, virulent *S. typhimurium* 395 MS was kept at 4 C on agar slants before use. The mean lethal dose of the strain was 50 bacteria after intraperitoneal injection of 0.2-ml suspensions in saline into albino mice (Anticimex, Stockholm, Sweden). The bacteria were grown for 16 h in nutrient broth (Difco) at 37 C, harvested by centrifugation (6,000 x g) and washed once in phosphate-buffered saline solution (PBS), pH 7.2 to 7.4. The cells were then heat killed at 56 C for 1 h, washed twice in PBS, and labeled with 35Cr as previously described (14).

**Immunization and preparation of IgG.** The production of rabbit hyperimmune sera against *S. typhimurium* 395 MS was performed as described earlier (2). The blood was collected aseptically, clotted, and centrifuged, and the sera were stored at −70 C. The fractionation of rabbit serum was carried out at 4 C on diethylaminoethyl-cellulose. The serum, dialyzed against 0.01 M phosphate buffer, pH 7.6, was loaded on a column (0.9 x 25 cm) filled with diethylaminoethyl-cellulose (Whatman) and eluted with the buffer. The immunoglobulin G (IgG) material was collected and concentrated to 5 mg of protein per ml. Protein was quantitated after nitrogen determination (24). Only one precipitation line was observed by immunoelectrophoresis with goat anti-rabbit serum. This gave reaction of identity with goat anti-rabbit IgG.

**Opsonization.** To 1 ml of PBS containing 3 x 10⁴ labeled bacteria was added 1 ml of IgG in different concentrations. The mixtures were incubated at 4 C for 30 min and then washed twice in PBS and once in 0.03 M tris(hydroxymethyl)aminomethane (Tris)
buffer, pH 7.0. Finally, the cells used for phagocytosis were suspended in Krebs-Ringer glucose (10 mM) solution, and those used for two-phase analysis were suspended in 0.03 M Tris. No agglutination was observed microscopically at serum concentrations less than 1:32.

Phagocytosis experiments. The procedure for phagocytosis in vitro has been described in detail (14). Briefly, PMN leukocytes were collected from the peritoneal cavity of rabbits after injection of 0.1% glycosen solution. The leukocytes were allowed to adhere to cellulose acetate filters (Millipore Corp.), on the bottom of petri dishes, and bacteria were added. The petri dishes were incubated at 37 C on a rocking table, the filters were removed after indicated times, and the radioactivity of each filter was measured. Relative phagocytosis was determined as the percentage of added bacteria adhering to the filters in 60 min.

Phagocytosis in vivo was studied as clearance from blood in mice. One-half milliliter of a bacterial suspension (5 x 10^8 bacteria) was injected into the tail vein of each mouse. Within 30 s and at regular intervals, 0.05 ml of blood was withdrawn from the retro-orbital venous plexus and the radioactivity was determined. The clearance rate (phagocytic index = K) was estimated by determining the slope of the best straight line drawn between the plots of the logarithms of the radioactivity against time. Each value represents the mean of three individual mice. Clearance of killed and radioactively labeled bacteria is more rapid than that of living cells (K = 0.012).

Phase system preparation and single-step partition. The two-phase system was prepared from stock solutions of 20% PEG 6000 (Carbowax 6000, Union Caribide, New York, N.Y.) and 20% dextran T 500 (Pharmacia Fine Chemicals, Uppsala, Sweden) in 0.1 M Tris, and was allowed to equilibrate at 4 C overnight (1). The final system contained 4.4% (wt/wt) PEG and 6.2% (wt/wt) dextran in 0.03 M Tris buffer. The partition procedure was carried out by pipetting 2 ml of bottom phase (dextran-rich) and 2 ml of top phase (PEG-rich) into test tubes. To each tube, 0.1 ml of bacteria (approximately 2 x 10^9) suspended in 0.03 M Tris was added. The tubes were inverted repeatedly for mixing, and the phases were allowed to settle for 20 min at 4 C. Then 0.5-ml samples were carefully withdrawn from each phase, and the radioactivity was measured in each sample, and the percentages of cells in top and bottom phase, and of those adhering to the interface, were calculated.

RESULTS

Earlier experiments (15, 16) have shown that the smooth strain S. typhimurium 395 MS and other phagocytosis-resistant bacteria collected mainly in the PEG-rich top phase, and rough bacteria mainly in the dextran-rich bottom phase. Thus, 82% of S. typhimurium 395 MS is collected in the top phase and 13% at the interface, whereas its R mutants R0-R10 accumulate in the bottom phase (34 to 80%) and interface (16 to 40%). The mutants most sensi-
phase (not shown), which is similar to the behavior of rough bacteria in the absence of antibodies (15, 16). Still, at a dilution 1:256, a change in partition was observable. In contrast, addition of preimmune serum or bovine serum albumin to the S bacteria did not affect their partition.

To assess whether these changes in surface characteristics, revealed by decreased affinity for the PEG-rich top phase, influence interaction with phagocytic cells, part of the opsonized bacteria was exposed to rabbit PMN leukocytes, and another part of them was injected intravenously into mice. A concomitant increase in uptake by rabbit leukocytes was noticed (Fig. 1B). IgG diluted to 1:256 still had opsonizing effect, whereas at the dilution of 1:512, where most cells collected to the top phase, the phagocytic uptake approached the control value (absence of IgG). The clearance rate was more sensitive to the presence of IgG on the cell (Fig. 1C, Table 1); IgG diluted 1:1,024 still had an opsonizing effect on reticuloendothelial phagocytosis. The increased clearance rate, due to IgG on the cell surface, caused more bacteria to collect in the liver, whereas in the absence of IgG, more cells collected in the spleen (Table 1).

**DISCUSSION**

Two mechanisms for the attachment of particles to phagocytic cells have been proposed: (i) specific recognition by phagocyte membrane receptors for the Fc part of IgG (4, 6, 8) and for activated C3 (6, 7, 13), and (ii) aspecific adhesion based on physicochemical characteristics of the particle and phagocyte surfaces such as shape, charge, and hydrophobicity (9–11, 17–20). Arguments for the first mechanism are

(i) the small quantity of IgG required for opsonization, for S. adelaide less than 2,200 molecules (less than eight molecules of IgM) (13); (ii) inhibition of attachment in the presence of IgG and Fc fragments (6, 8); and (iii) a certain species specificity in the immunoglobulin-phagocyte combinations (4). Arguments for the second mechanism are (i) certain particles are phagocytosed in the absence of serum and complement (14); (ii) antibody to S. london increases the uptake of the bacteria by a primitive phagocyte such as the slime mold Dictyostelium discoideum (3), and antibodies to sheep erythrocytes increase the uptake of the erythrocytes by Acanthamoeba (12); (iii) addition of antiserum to otherwise phagocytosis-resistant bacteria such as Diplodoccus pneumoniae, Escherichia coli O55, and Klebsiella pneumoniae increases the interfacial tension of the bacteria to a level higher than that of phagocytes, which makes phagocytosis possible; complement increases the interfacial tension and the phagocytosis of the antibody-treated bacteria even further (19); and (iv) old erythrocytes that are approaching elimination from the blood stream are changed with respect to charge and partition in a two-phase system (21, 22).

Our results add arguments to the second mechanism. Addition of antibody IgG to S. typhimurium S bacteria increased the affinity of the bacteria to rabbit PMN cells in vitro and to the dextran-rich phase. Furthermore, the quantity of antibodies required was the same to produce the increased affinity in the cellular and the two-phase system, respectively, implying that possible receptors on the rabbit PMN cells are no more sensitive for opsonized rabbit IgG than the physicochemical system. Such opsonized IgG presumably presents structures that are recognized as well by the PMN cells as the two-phase system, indicating a low degree of specificity. In contrast, clearance of bacteria from blood stream after intravenous injection required smaller quantities of antibodies. This could be due to the antibodies eliciting a cascade effect, e.g., via the complement system, to more favorable conditions for phagocytosis, and to a sensitive recognition mechanism in the reticuloendothelial system.

Little is known about the chemical bonds operating in phagocyte-prey interactions. It has recently been demonstrated that lightly heat-aggregated human IgG and Fc fragments of certain subclasses adhere to and cause perturbation in phospholipid liposomal membranes, presumably by hydrophobic association between the Fc regions and membrane phospho-

### Table 1. Influence of different amounts of anti-MS IgG on the clearance rate of S. typhimurium 395 MS in mice and the accumulation of the bacteria in the liver and the spleen

<table>
<thead>
<tr>
<th>Dilution</th>
<th>Clearance rate (K)</th>
<th>Liver</th>
<th>Spleen</th>
</tr>
</thead>
<tbody>
<tr>
<td>1:8</td>
<td>0.20 ± 0.03</td>
<td>81</td>
<td>4</td>
</tr>
<tr>
<td>1:16</td>
<td>0.20 ± 0.04</td>
<td>69</td>
<td>4</td>
</tr>
<tr>
<td>1:32</td>
<td>0.20 ± 0.02</td>
<td>64</td>
<td>6</td>
</tr>
<tr>
<td>1:64</td>
<td>0.19 ± 0.03</td>
<td>84</td>
<td>6</td>
</tr>
<tr>
<td>1:128</td>
<td>0.22 ± 0.02</td>
<td>64</td>
<td>4</td>
</tr>
<tr>
<td>1:256</td>
<td>0.23 ± 0.04</td>
<td>80</td>
<td>5</td>
</tr>
<tr>
<td>1:1,024</td>
<td>0.12 ± 0.03</td>
<td>65</td>
<td>10</td>
</tr>
<tr>
<td>0</td>
<td>0.045 ± 0.005</td>
<td>45</td>
<td>22</td>
</tr>
</tbody>
</table>

*a Mean of three mice (± SD).

*b Sixty minutes after injection of bacteria.

*c Initial protein concentration = 5 mg/ml.

*d Percent of injected bacteria.
lipid (23). We hypothesize that in the adherence of one opsonized bacterium to a PMN cell, several presumably weak bonds between the Fc regions of a number of sterically ordered IgG molecules, and components of the PMN cell surface, cooperate to promote the adherence. The adherence may or may not be promoted by conformational changes in the Fc regions by the antigen-antibody reaction. Since all particles investigated so far that have shown a great affinity for the dextran phase have also been liable to phagocytosis, we postulate that particles with such characteristics do not normally circulate in the body with a fair life-span.

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LITERATURE CITED