INFECTION AND IMMUNITY, Sept. 1984, p. 795–797
0019-9567/84/090795-03$02.00/0

Vol. 45, No. 3

Electron Microscopic Study Showing Antibody-Independent Binding of C1q, a Subcomponent of the First Component of Complement, to Serum-Sensitive Salmonellae

FELICITAS CLAS,[1] J. R. GOLECKI,[2] AND M. LOOS[1]*

Institut für Medizinische Mikrobiologie, Johannes Gutenberg-Universität, 6500 Mainz,[1] and Institut für Biologie II, Mikrobiologie, Albert-Ludwigs-Universität, 7800 Freiburg,[2] Federal Republic of Germany

Received 30 April 1984/Accepted 21 June 1984

Effective serum-mediated killing of sensitive gram-negative bacteria requires all the complement components. In the preimmune phase the antibody-independent interaction of the first component of complement, C1, with the bacteria might be especially important. Electron microscopic studies showed that the C1 subcomponent C1q binds only to the serum-sensitive R form of *Salmonella minnesota* and not to the serum-resistant S form.

Effective killing of serum-sensitive, gram-negative bacteria is dependent on an intact classical complement pathway. In the absence of C1, Ca^{2+}, C2, or C4, either the bacteria were not killed or the killing was delayed compared with the effect of normal serum (3). Hemolytic tests and fluorescent-serological studies revealed that serum-sensitive bacteria take up high amounts of C1 from serum (ca. 1,000 to 2,000 C1 molecules per bacterium) (4). Antibodies were not essential for C1 uptake and for the activation of C1. Therefore, the classical complement pathway seems to be important for the killing of some serum-sensitive, gram-negative bacteria in the preimmune phase. Direct binding of C1 and C1q to serum-sensitive bacteria has been confirmed in several other laboratories (1, 4, 8, 12). In the present study electron microscopic techniques were utilized to demonstrate that the Re and S forms of *Salmonella minnesota* interact differently with C1.

The *Salmonella* strains SF1111 and SF1167 were kindly provided by G. Schmidt, Borstel, Federal Republic of Germany. The cultures grew for ca. 16 h at 37°C in complex medium. After being washed twice with 0.01 M phosphate-buffered saline (PBS) (pH 7.5), the suspensions were adjusted to 10^9 bacteria per ml. This solution (100 μl) was incubated with 50 μl of purified human C1q (ca. 2,000 effective molecules per bacterium) for 20 min at 30°C. After this period the bacteria were washed twice with 0.01 M PBS (pH 7.5), and finally the sediments were suspended in 100 μl of PBS and incubated together with 1:10 diluted anti-C1q immunoglobulin G (IgG) (IgG fraction from rabbit serum; Behring) for another 30 min at 30°C. After two further washings in PBS, both strains were treated with 100 μl of ferritin-labeled anti-rabbit IgG from goat (Miles, Frankfurt, Federal Republic of Germany) for 1 h at 30°C, followed by two washings in PBS. As controls, strains which had not been preincubated with C1q were treated in the same way. Afterwards the samples were prepared for freeze etching and for epoxy-resin embedding.

For freeze fracture preparation, the cells were sedimented and prepared on copper disks (5). During this procedure the cells were frozen in liquid propane and then plunged into liquid nitrogen. Freeze fracture preparation was performed in Balzers apparatus 360 MA at a chamber pressure of less than 2×10^{-6} Torr (132.2 μPa) and a temperature of −110°C with etching. Platinum carbon was used for shadowing. After being cleaned in 40% chromic acid and water, the replicas were examined in a Philips EM 400 at 80 kV. The different fracture faces were labeled by the nomenclature of Branton et al. (2).

The samples for ultrathin section preparations were fixed by replacing the buffer with 2% glutaraldehyde in 0.05 M sodium-cacodylate buffer for 2 h at 37°C. Afterwards, the cultures were postfixed in 1% OsO_4 in 0.15 M cacodylate buffer for 30 min at 37°C. The samples were dehydrated in a graded series of ethanol and embedded in Epon (6, 9). The ultrathin sections were contrasted with methanolic uranylacetate solution (13) and with lead citrate according to Reynolds (11). The sections were put on Formvar-coated grids.

Figure 1 shows the ultrathin sections of the S and Re forms of *S. minnesota*. A conspicuous ferritin layer surrounds the Re form, indicating that a lot of cell-bound C1q could be detected by the antibodies on the bacterial surface (Fig. 1b). The S form did not bind C1q, since no ferritin grains were detectable on the outer membrane of the S form (Fig. 1a). Similar observations could be made with freeze-etched preparations (Fig. 2). The outer surface of Re form cells are covered with a intensive ferritin border, as indicated by the thick layer around the cell (Fig. 2b). It is only the outer surface which is able to bind C1q. In contrast, the outer membrane of the S form is smooth, without any ferritin grains (Fig. 2a). In controls it was proven that ferritin labeling was only detected on C1q-pretreated bacteria.

A direct interaction of purified C1q and C1 with purified lipopolysaccharide and lipid A preparations was demonstrated by Loos et al. (7). In further experiments lipid A was shown to be the part of the lipopolysaccharide molecule which is responsible for an antibody-independent activation of C1 by lipopolysaccharide (10).

Direct binding of the first complement component C1 and its subcomponent C1q to the Ra, Rb, Rc, Rd, and Re forms of *S. minnesota* and *Salmonella typhimurium* had also been shown by fluorescent serological studies and several hemolytical test methods (4). The S form did not bind C1 or C1q. Therefore, we assume that the O antigenic sugar chains protect the bacterial surface of the S form. In contrast, the

* Corresponding author.

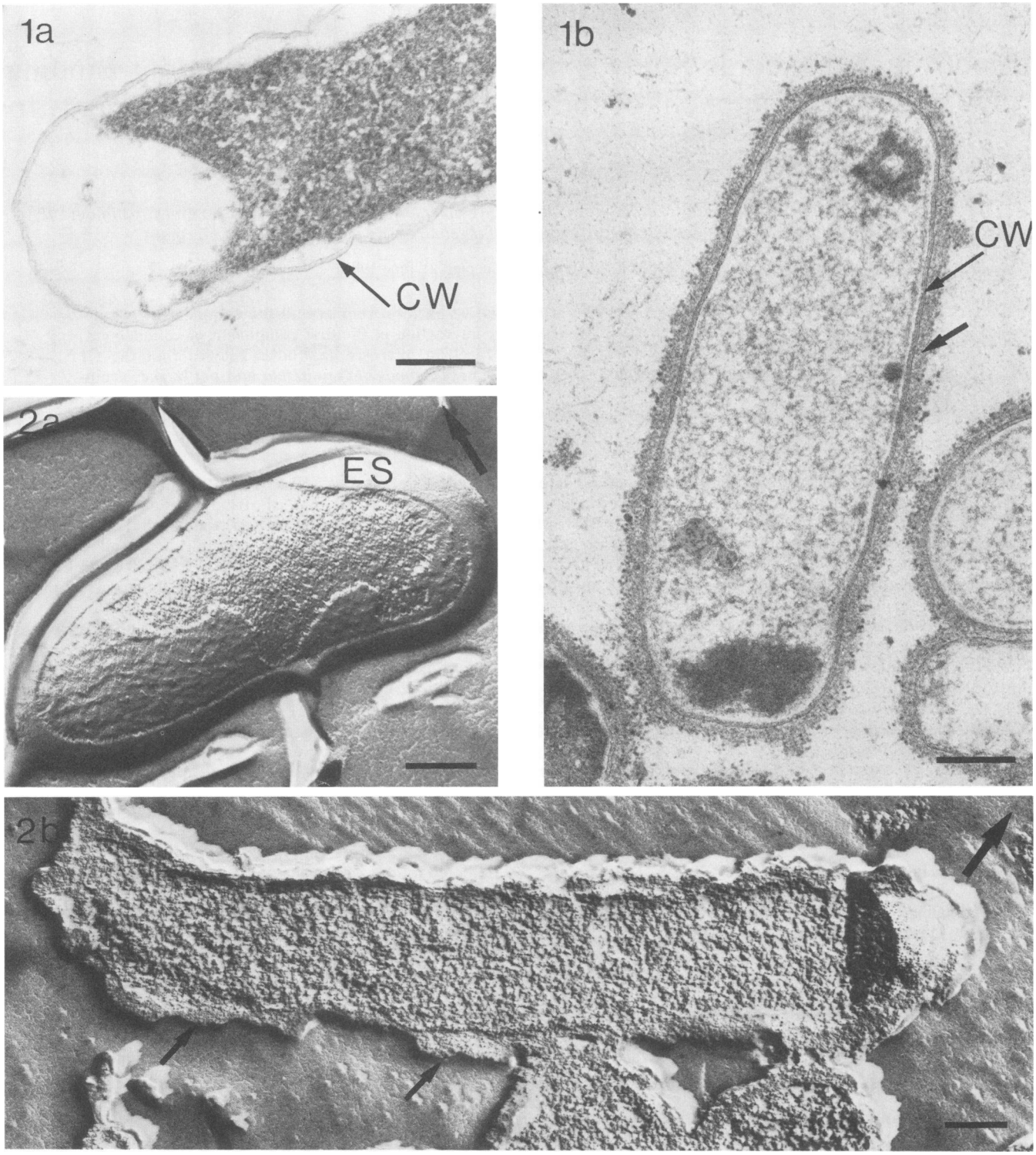

FIG. 1. Ultrathin sections of the S and Re forms of *S. minnesota*. The bacteria were treated with purified C1q, anti-C1q IgG, and anti-IgG labeled with ferritin. The S form (a) presents no ferritin outside the cell wall (CW), whereas the cell wall of the Re form (b) is surrounded by an intensive layer of ferritin (←), indicating the presence of C1q antigen. The shrinkage of the bacteria visible in (a) is caused by osmic conditions during the serum incubation. The bar represents 200 nm.

FIG. 2. Freeze fracture micrographs of the S and Re forms of *S. minnesota*. The bacteria were pretreated with C1q, anti-C1q IgG, and ferritin-labeled IgG. Whereas the exoplasmic surface (ES) of the outer membrane of the S form is smooth and lacks ferritin (a), the Re form is covered by a thick layer of ferritin (←). The arrow in the upper right corner indicates the direction of the shadowing. The bar represents 200 nm.

membrane components, such as lipid A and outer membrane proteins, are accessible on R forms, providing the tight C1 binding to these bacteria (4).

The presented electron microscopic photographs confirm our earlier observations that C1q is bound only by the R form and not by the serum resistant S form of *S. minnesota*. This antibody-independent interaction might be responsible for the killing of some gram-negative bacteria in the preimmune phase.

LITERATURE CITED

1. **Betz, S. J., and H. Isliker.** 1981. Antibody-independent interactions between *E. coli* J5 and human complement components. J. Immunol. **127**:1748–1754.
2. **Branton, D., S. Bullivant, N. B. Gilula, M. J. Karnovsky, H. Moor, K. Mühlethaler, D. H. Northcote, L. Packer, B. Satir, P. Satir, V. Speth, L. A. Staehelin, R. L. Steere, and R. S. Weinstein.** 1975. Freeze-etching nomenclature. Science **190**:54–56.
3. **Clas, F., and M. Loos.** 1980. Killing of the S- and Re-forms of *Salmonella minnesota* via the classical pathway of complement activation in guinea-pig and human sera. Immunology **40**:547–556.
4. **Clas, F., and M. Loos.** 1981. Antibody-independent binding of the first component of complement (C1) and its subcomponent C1q to the S and R forms of *Salmonella minnesota*. Infect. Immun. **31**:1138–1144.
5. **Golecki, J. R., and J. Oelze.** 1980. Differences in the architecture of cytoplasmic and intracytoplasmic membranes of three chemotrophically and phototrophically grown species of the *Rhodosspirillaceae*. J. Bacteriol. **144**:781–788.
6. **Kellenberger, E., A. Ryter, and J. Sechaud.** 1958. Electron-microscope study of DNA-containing plasma. J. Biophys. Biochem. Cytol. **4**:671–683.
7. **Loos, M., D. Bitter-Suermann, and M. Dierich.** 1974. Interaction of the first (C1), the second (C2) and the fourth (C4) component of complement with different preparations of bacterial lipopolysaccharides and with lipid A. J. Immunol. **112**:935–940.
8. **Loos, M., B. Wellek, R. Thesen, and W. Opferkuch.** 1978. Antibody-independent interaction of the first component of complement with gram-negative bacteria. Infect. Immun. **22**:5–9.
9. **Luft, J. H.** 1961. Improvements in epoxy resin embedding methods. J. Biophys. Biochem. Cytol. **9**:409–414.
10. **Morrison, D. C., and F. L. Kline.** 1977. Activation of the classical and properdin pathway of complement by bacterial lipopolysaccharides. J. Immunol. **118**:362–368.
11. **Reynolds, E. S.** 1963. The use of lead citrate at high pH as an electron-opaque stain in electron microscope. J. Cell Biol. **17**:208–212.
12. **Tenner, A. J., R. J. Ziccardi, and N. R. Cooper.** 1983. Antibody-independent C1 activation: *E. coli* strains demonstrate differences in the kinetics and control of C1 activation and in the fate of the activated C1. Immunobiology **164**:306.
13. **Watson, M. L.** 1958. Staining of tissue sections for electron microscopy with heavy metals. J. Biophys. Biochem. Cytol. **4**:475–478.