CAPPING OF COMPLEMENT RECEPTORS ON HUMAN NEUTROPHILS INDUCED BY GROUP A STREPTOCOCCAL CELL WALLS

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Peptidoglycan-polysaccharide polymers derived from group A streptococcal cell walls (PG-PS) were opsonized with either purified C3 or normal human serum and were used as a probe to investigate the mobility of CR1 and CR3, the C3b and iC3b receptors, respectively, on human neutrophils. Incubation of monolayers or cell suspensions of neutrophils with PG-PS opsonized with C3b or serum resulted in capping of PG-PS, as detected by fluorescein-labeled antibody to PS. No binding of PG-PS to neutrophils was observed with heat-inactivated serum. By 30 min the cell walls were internalized and observed in one to three vacuoles. Capping was totally inhibited when PG-PS opsonized with C3b or serum was preincubated with Fab'-anti-C3b. Similar inhibition was observed when C3b-opsonized PG-PS was incubated with neutrophils that were preincubated with anti-CR1 or fluid-phase C3b; only partial inhibition of neutrophil capping was observed by using serum-opsonized PG-PS. Because anti-CR1 blocks only the C3b receptor, the cap formation observed with serum-opsonized PG-PS is probably due to CR3. These results suggest that both CR1 and CR3 on neutrophils cap after stimulation by group A streptococcal cell wall fragments.

Neutrophilic polymorphonuclear leukocytes (neutrophils) and monocytes have been shown to express different two types of membrane complement (C) receptors, which have been designated as C receptor type one (CR1), and C receptor type three (CR3). These receptors have specificities for different sites on the C3 molecule (1–3). The CR1 binds to both C4b and C3b, whereas CR3 binds to iC3b. In addition to these receptors, B lymphocytes express another C receptor, C receptor type two (CR2), which binds to the C3d or d region of iC3b (3, 4). Recently the CR1 and CR2 receptors were isolated and shown to be glycoproteins with m.w. of 205,000 and 72,000, respectively (2).

Activation of the classical or alternative C pathways by target particles or soluble complexes leads to covalent binding of C3b to these components. The bound C3b or its degradation product, iC3b, can mediate several important biologic functions, including enhancement of immune adherence, phagocytosis, and activation of B cells (6–14). It is suggested that a primary role of the C3b receptor is to mediate efficient binding of particles to neutrophils during phagocytosis (7, 10). Both C3b and iC3b receptors can efficiently promote phagocytosis in monocytes (15), and the mobility of these receptors appears to correlate with macrophage activation (15, 16). Frenet et al. (17), using immunofluorescence, have demonstrated clusters of C3b receptors on the neutrophil membrane. Internalization of CR1 can result from cross-linking of the respective receptor by F(ab')2, anti-CR1, with subsequent pinocytosis. Similar evidence was obtained in studies with C3b-fixed sheep erythrocyte membrane proteins (C3b-OR) and F(ab')2, anti-C3. Capping of receptors was not reported (17, 18).

The peptidoglycan from group A streptococci has been reported both in vitro (19) and in vivo (20) as the most active cell wall component of this species for activation of the alternative C pathway. Furthermore, these wall fragments are postulated to play a role in rheumatic-like heart lesions in mice (21, 22) and chronic polyarthritis in rats (23, 24). The opsonic recognition of streptococcal cell wall by neutrophil C receptors offers another approach to study the distribution and endocytosis of CR1 and CR3 receptors and the mechanism of clearance of bacterial debris by neutrophils. In the present study, peptidoglycan-polysaccharide sonicates of polymers derived from group A streptococcal cell walls (PG-PS) were opsonized with either C3 or normal human serum and were used to investigate the mobility of CR1 and CR3 on human neutrophil membranes. The data demonstrate that C3b and iC3b, fixed to PG-PS, induce capping of CR1 and CR3 on the neutrophil membrane.

MATERIALS AND METHODS

Fixation of serum components to streptococcal cell walls. The physical, chemical, and arthropathic properties of a 100P fraction of purified cell walls derived from group A streptococci have been described (24). This preparation has a m.w. average of 5.0 × 107 and is composed of peptidoglycan-polysaccharide polymers. This fraction can be sedimented at 100,000 × G for 1 h in a T-865 rotor in a Sorvall OTD-2 ultracentrifuge. For these studies the pellet was resuspended in phosphate-buffered saline (PBS) to a concentration of 2 mg rhannose/ml. Rhannose content of cell wall fragments was determined by the method of Dische and Shettles (25). These cell wall fragments will be referred to as PG-PS.

PG-PS was incubated with normal human serum or C3 as follows: Four milligrams of PG-PS in a total volume of 2 ml of PBS were incubated for 15 min at 37°C with whole human type AB serum to achieve a final concentration of 10% serum (serum-PG-PS). The pellet was washed three times with cold PBS at 4°C by centrifugation at 100,000 × G and resuspended in PBS to a final concentration of 2 mg rhannose/ml. A control fraction was prepared as above, but incubated in PBS. For C3b fixation to PG-PS (C3b-PG-PS), 4 mg of PG-PS in a total volume of 2 ml were incubated with 5 mg C3 in the
RESULTS

Capping of PG-PS on neutrophils was observed in both monolayers (Fig. 1) and cell suspensions (Fig. 2) incubated with serum-opsonized PG-PS. The cap was distributed on the posterior end (uropod) of polarized cells. No surface binding of PG-PS to neutrophils was detected in the absence of fresh serum or when heat-inactivated serum was used. The kinetics of cap formation is shown in Figure 1A. The PG-PS was observed within 5 min as clusters randomly distributed around the cell periphery and as caps distributed at one pole of the cell. A plateau of about 80% capped cells was reached by 10 min. The distribution of PG-PS during cap formation is shown in Figure 2.

When monolayers were fixed with formalin-acetone and subsequently stained for PG-PS, internalized PG-PS was observed (Fig. 3). Within 20 min, approximately 22% of the cells had endocytic vacuoles; the number of cells with vacuoles increased to 88% by 30 min. Cells were observed that had both caps and endocytic vacuoles. Because serum-opsonized PG-PS probably has both C3b and iC3b on its surface, binding of PG-PS opsonized with purified C3b was investigated. As indicated in Table II, similar observations were noted when neutrophil suspensions were incubated with 30 min at 37°C with C3b(PG-PS) or serum(PG-PS). No binding was observed on neutrophils incubated with heat-inactivated serum(PG-PS) or non-opsonized PG-PS. Inhibition studies were subsequently performed to further identify the neutrophil receptors involved in capping. The results are presented in Table II. Capping was totally abolished when C3b-opsonized or serum-opsonized PG-PS was incubated with Fab anti-C3b before exposure to the cells. Similar inhibition was observed when C3b(PG-PS) was incubated with neutrophils that were preincubated with anti-CR1 or fluid-phase C3b. These observations suggest that C3b(PG-PS) and serum(PG-PS) induce capping of CR1. To determine whether CR2 also caps when cells are incubated with serum(PG-PS), neutrophils were preincubated with antisera to CR1 or fluid-phase C3b and subsequently exposed to serum(PG-PS). As shown in Table III, only partial inhibition of PG-PS binding was observed with serum(PG-PS). Approximately 85% of the neutrophils showed caps or clusters of PG-PS on the cell surface after prior treatment of cells with anti-CR1. In addition, 27% of the neutrophils displayed clusters of PG-PS after preincubation of neutrophils with C3b. Because anti-CR blocks only the C3b receptor, the cap formation observed with serum(PG-PS) must be due to CR3.

DISCUSSION

Group A streptococcal cell wall fragments consisting of peptidoglycan-polysaccharide polymers with m.w. of 5.0 × 10⁶ (24) have been used as a probe to study the mobility of C receptors on human neutrophils. Peptidoglycan activates the alternative pathway both in vitro (19) and in vivo (20), with subsequent fixation of C3b molecules on its surface. The fixation of C by PG-PS is probably favorable because rILH, a cofactor required for cleavage of C3b, is minimally active with molecules deficient in sialic acid residues (32). The involvement of C receptors in neutrophil recognition of PG-PS is supported by the fact that heat-inactivated serum(PG-PS) is not bound by neutrophils. By comparing C3b(PG-PS) with serum(PG-PS), the mobility of receptors to C3b(CR1) and iC3b(CR3) was investigated. Both receptors have the ability to cap. C3b(PG-PS) did not bind to
neutrophils after cells were preincubated with excess C3b or anti-CR1, or after preincubation of C3b-(PG-PS) with anti-C3b. These inhibition studies support the involvement of CR1 in the recognition of this bacterial component by neutrophils. Complete inhibition of capping was also noted when serum-(PG-PS) was preincubated with anti-C3b. However, partial inhibition of capping was observed when serum-(PG-PS) was incubated with cells that were preincubated with fluid-phase C3b or anti-CR1. This suggests that in addition to CR1, CR3 is also involved in the induction of capping by serum-(PG-PS). The greater inhibition by C3b (73%) compared with anti-CR1 (15%) when cells were incubated with serum-(PG-PS) is probably because neutrophil proteolytic enzymes cleave C3b to iC3b, resulting in binding by both C receptors (unpublished observations).

The C3b receptor is localized on neutrophils in clusters (17; also observed in our laboratory) and pinocytosed after cross-linkage of receptors with F(ab')2 anti-CR1. In addition, solubilized C3b-fixed to sheep erythrocytes (C3b-OR) binds to neutrophils and is internalized, as assayed by 125I-C3b-OR (17). Internalization was observed only after cross-linkage with F(ab')2 anti-C3b receptor. Localization studies were not performed with C3b-OR. Our studies indicate that C-opsonized PG-PS is of sufficient size to permit cross-linking of receptors and redistribution of receptors to a cap. The PG-PS is first distributed on the neutrophil as patches or as clusters and is finally distributed to the uropod as a cap, where it is probably internalized. Internalization of the PG-PS appears to be different than that induced by cross-linkage of C receptors by antibody. The antibody C receptor complexes are pinocytosed (17), whereas the PG-PS is concentrated at the uropod. It seems that the distance between receptor clusters cannot be bridged by IgG, but can be connected with PG-PS. This bridging of receptor clusters, which is probably similar to cross-linking and aggregation of IgG on lymphocytes, results in capping and endocytosis of PG-PS by neutrophils.

Hafeman et al. (33) have found that the distribution and motion of the C3b receptor is affected by the nature of the substratum to which the cells are attached. Our data suggest that the distribution and motion of the C3b receptor does not appear to be affected once the receptors are bound by the antigen. Cap-
CAPPING OF COMPLEMENT RECEPTORS ON NEUTROPHILS


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REFERENCES


