The CGM1a (CEACAM3/CD66d)-mediated Phagocytic Pathway of Neisseria gonorrhoeae Expressing Opacity Proteins Is Also the Pathway to Cell Death*

Received for publication, November 26, 2000
Published, JBC Papers in Press, February 5, 2001, DOI 10.1074/jbc.M010609200

Tie Chen‡‡, Silvia Bolland‡, Ines Chen, James Parker, Milica Pantelic‡, Fritz Grunert**, and Wolfgang Zimmermann**

From the ‡Department of Microbiology, Immunology and Medicine, Walther Oncology Center, Indiana University School of Medicine, Indianapolis, Indiana 46202, the †Laboratory of Bacterial Pathogenesis and Immunology, the **Laboratory of Molecular Genetics and Immunology, The Rockefeller University, New York, New York 10021, and the ‡‡Institute of Molecular Medicine and Cell Research, University of Freiburg, D-79104 Freiburg, Germany

Phagocytosis of Opa+ Neisseria gonorrhoeae (gonococcus, GC) by neutrophils is in part dependent on the interaction of Opa proteins with CGM1a (CEACAMS/CD66d) antigens, a neutrophil-specific receptor. However, the signaling pathways leading to phagocytosis have not been characterized. Here we show that interaction of OpaI bacteria with neutrophils or CGM1a-transfected DT40 cells induces calcium flux, which correlates with phagocytosis of bacteria. We identified an immunoreceptor tyrosine-based activation motif (ITAM) in CGM1a, and showed that the ability of CGM1a to transduce signals and mediate phagocytosis was abolished by mutation of the ITAM tyrosines. We also demonstrated that CGM1a-ITAM-mediated bacterial phagocytosis is dependent on Syk and phospholipase C activity in DT40 cells. Unexpectedly, the activation of the CGM1a-ITAM phagocytic pathway by Opa+ GC results in induction of cell death.

Gonorrhea is one of the most frequently reported sexually transmitted diseases (1). Neisseria gonorrhoeae (gonococcus, GC), the etiologic agent of gonorrhea, can adhere to and penetrate mucosal cells (2, 3) and attain access to submucosal sites. Among the GC surface proteins that mediate this process is the opacity (Opa) protein family. This family consists of 11 unlinked opa genes, whose sequences are known (4). In addition, each Opa protein is able to switch its expression on and off, resulting in the unavoidable tendency of GC to alter their phenotypes by antigenic variation. Opa-expressing GC and Escherichia coli (Opa+ GC or E. coli) can attach to and invade human fallopian tube epithelium (5), indicating that Opa proteins alone are sufficient to promote adhesion to and invasion of human cells. Furthermore human challenge experiments (6, 7) strongly suggest that in vivo expression of Opa proteins plays an important role in gonococcal pathogenesis on the mucosal surface.

It is well recognized that Opa proteins have the ability to stimulate adherence and phagocytosis of the Opa+ bacteria by polymorphonuclear leukocytes (PMN). This interaction with PMN occurs in an opsonin-independent manner (8–12). In addition, Opa+ bacteria stimulate a chemiluminescent response in human neutrophils as a result of oxidative burst activity. Furthermore, recent studies demonstrated that members of the carinoembryonic antigen (CEA) or CD66 family serve as receptors mediating adherence and possibly phagocytosis of Opa+ bacteria (13–16). These findings were mainly obtained with HeLa transfectants expressing individual CEA family members. However, neither the nature of the signals nor the pathways that are involved in phagocytosis of the bacteria are clear.

The CEA gene is a member of a family of 18 expressible closely related genes (17) that belong to the immunoglobulin (Ig) gene superfamily (18). Recently, the nomenclature of the CEA family has been revised to be CEACAM (CEA-related cell adhesion molecules) family (19). The human CEA family includes BGP (biliary glycoprotein), CGM6 (CEA gene family member 6), NCA (nonspecific cross-reacting antigens), CGM1, CEA and PSG (pregnancy-specific glycoproteins), which are also designated CD66a (CEACAM1), CD66b (CEACAM8), CD66c (CEACAM6), CD66d (CEACAM5), CD66e (CEACAM5), and CD66f, respectively. CEACAM (CEA-related cell adhesion molecules) was renamed from the CEA family (19) recently. It is noteworthy that CGM1a (CD66d), which promotes the strongest phagocytosis of Opa+ bacteria, is only expressed in human neutrophils (20).

Various components of the B cell receptors (BCR), T cell receptors (TCR), and Fc receptors (FcR) contain common sequence motifs in their cytoplasmic tails, called the immunoreceptor tyrosine-based activation motif (ITAM; Ref. 21). The phosphorylated tyrosine residues within ITAMs can recruit protein-tyrosine kinases (PTK), e.g. Syk, whereupon these become activated. Recruitment of substrates such as phospholipase C-γ (PLC-γ) by these kinases leads to stimulation of calcium flux from intracellular stores (22–25). This calcium flux reflects an early event after activation of PTK.

In this study, we demonstrate that CGM1a bears a func-
tional ITAM in its cytoplasmic tail, which mediates phagocytosis of Opa” bacteria following by cell death.

EXPERIMENTAL PROCEDURES

Bacterial Strains, Cell Culture, and Antibodies—GC strain MS11 was cultured and maintained as previously described (26). Only pilus GC and LOS, (lacto-N-neotetraose) phenotype were used (27). Recombinant opa genes from GC MS11 were constructed and expressed in E. coli HB101 as described previously (28). The designation of Opa proteins of both GC and E. coli are based on Swanson et al. (6) and Belland et al. (28). The Opa” bacteria used in this study are Opa”, OpaC, OpaD, OpaF, and OpaG, and Opa” and E. coli.

The wild-type chicken B cells DT40, their mutants DT40-Syk (Syk5) and DT40-PLC-γ1 (PLC-γ1) (29, 30) and transfectants were maintained in RPMI 1640 medium supplemented with 10% fetal bovine serum, 1% chicken serum, 50 μM 2-mercaptoethanol and 2 mM l-glutamine.

COL-I monoclonal antibody (MAB), specific for CGM1 and CEA, was purchased from Zymed Laboratories Inc. Laboratories INC., California. B1.1 antibody, which reacts with BGP, NCA, CGM1, and CEA, was generously provided by Dr. Jeffrey Schlam, NCI, National Institutes of Health. The YTH7I.3 antibody, which recognizes BGP, NCA, and CGM1, was purchased from Harlan Bioproducts (Indianapolis, IN). Anti-Fc antibody 2.4G2 was purchased from PharMingen (San Diego, CA).

Preparation of Neutrophils—Neutrophils from human blood were purified by centrifugation through Polymorphprep (Life Technologies, Inc.). The purified PMN were suspended either in RPMI or Hanks’ balanced salt solution (HBSS; Cellgro, Herndon, VA) at a concentration of 2 × 10⁶/ml.

Transfections—CGM1a cDNA and its mutants were cloned into pBEH expression vector, and NCA and BGPα were cloned into pRC/CMV vector. 10 μg of expression plasmids were co-transfected with 1 μg of pBabe-puro-γ vector (31) into 5 × 10⁶ DT40 cells by electroporation at 250 V and 960 μF in 0.5 ml. Stable transfectants were selected in 0.5 μg/ml puromycin 24 h after electroporation. The presence of CD86 antigens in individual clones was confirmed by flow cytometry analysis using FACScan (Becton Dickinson, Mountain View, CA) analysis with COL-I, YTH7I.3, and secondary antibodies conjugated with FITC.

Generation of CGM1a Mutants—The CGM1a cDNA cloned in pBEH has been previously described (20). The tyrosine residues Tyr-196 and Tyr-207 (position 1 corresponds to the first amino acid of the mature protein after removal of the leader peptide) in the wild-type cytoplasmic tail of Tyr-207 (position 1 corresponds to the first amino acid of the mature protein after removal of the leader peptide) in the wild-type cytoplasmic tail of CGM1a, was purchased from Harlan Bioproducts (Indianapolis, IN). COL-1 monoclonal antibody (MAB), specific for CGM1 and CEA, was purchased from Zymed Laboratories Inc. Laboratories INC., California.

Calcium Measurements—DT40 cells or neutrophils (4 × 10⁶) were suspended in 3 ml of RPMI medium. 6 fl of 1 μM Fura-2AM (Molecular Probe, Eugene, OR) dissolved in Me2SO were added to the suspension, and TyrI-II3 9 ised sequences of CGM1a were confirmed by DNA sequencing using the GTTTGTGTCATGTTTTAGCAATTCCTCAAAGATGGAAGC. The mutated Tyr-207 of DT40-CGM1a, even when cross-linked with secondary antibodies.

Phagocytosis Assays—DT40 cells transfected with CGM1a (DT40-CGM1a) and control DT40 cells were suspended in RPMI with 2% fetal calf serum at the concentration of 2–4 × 10⁷/ml. 0.5 ml of each of these cell suspensions was added to 24-well plates and after addition of 50 μl of bacterial suspensions in RPMI at the concentration of 8 × 10⁶/ml, the cells were allowed to incubate for 3 h at 37°C in the presence of 5% CO₂. Then gentamicin was added into each well to the final concentration of 100 μg/ml and incubated for another 90 min. The cells were washed three times with RPMI and lysed in phosphate-buffered saline containing 0.5% saponin (CalBiochem Corp.,), and dilutions were plated on LB-agar or CG-agar. The level of internalization of bacteria into cells was calculated by determining the colony-forming units (cfu) recovered from the DT40 cell lysates.

Detection of Cell Death—Annexin V-FITC binding to phosphatidylserine (PS) was used as the cell death assay according to the manufacturer’s recommendations (PharMingen, San Diego, CA). Annexin V-FITC was used in conjunction with propidium iodide (PI) to distinguish apoptotic cells (annexin V-FITC positive, PI negative) from necrotic cells (annexin V-FITC positive, PI positive). Cells exhibited apoptotic cell-specific phosphatidylserine on their surface and, therefore, bound annexin V-FITC or took up PI, which cannot penetrate live cells. However, in our experiment, the cells that were either annexin V-FITC positive or PI positive were classified as dead cells (see Fig. 6). Neutrophils or DT40 cells (1 × 10⁶) were suspended in 0.1 ml of 2% fetal calf serum in RPMI containing 1.5 × 10⁶ bacteria (GC) and incubated at 37°C for 30 min with occasional shaking. 50 μl of binding buffer (10 mM Hepes/NaOH, pH 7.4, 140 mM NaCl, and 2.5 mM CaCl₂) 7.5 μl of Annexin V-FITC and 15 μl of PI were added in each vial. The cell suspensions were gently mixed and incubated for 15 min at room temperature in the dark. Finally, after addition of 550 μl of binding buffer, samples were analyzed immediately by flow cytometry.

RESULTS

Opa” GC Stimulates Calcium Flux in Neutrophils and DT40-CGM1a but Not in DT40-BGPa or DT40-NCA—Studies have indicated that Opa protein-mediated binding and phagocytosis of GC by PMN occurs through the CEA family members on PMN (13, 14, 16). Both BGPα and CGM1a can contain possible ITAM-like motifs (Fig. 1A). NCA, which is membrane anchored by a glycosylphosphatidyl inositol (GPI) moiety, is expressed on granulocytes and has also been shown to bind to Opa proteins. To examine whether the interaction between GC and neutrophils results in any signaling in the PMNs, measurement of calcium flux was performed. Opa” GC were used to stimulate neutrophils. As shown in Fig. 2A, Opa” GC but not Opa” GC induced calcium flux in neutrophils, suggesting that at least one of the three CEA family members in question conveys these signals, possibly by an ITAM.

To determine which of the three CEA-related receptors is responsible for the calcium flux in neutrophils induced by Opa” GC (Fig. 2A), the DT40 B cell line was chosen to perform functional studies. There are several reasons why we chose this cell line. First, the ITAM, which could be responsible for the signaling through the CEA-related molecules, was originally identified in the Igα/Igβ heterodimer of the BCR (32, 33), and DT40 B cells have proven to be suitable for studying the functionality of presumed ITAMs (34, 35) e.g. by induction of calcium flux as observed after BCR activation. Furthermore, human neutrophils are terminally differentiated cells and cannot be genetically manipulated. Manipulation of established myeloid lines of human origin such as HL60 cells cannot be performed readily either because endogenous CD66 antigen expression changes during induction of maturation by retinoic acid. Finally, no Opa” GC were found to interact exclusively with individual CEA antigens like CGM1a (36, 37). In short, a cellular system that lacks endogenous human CEA antigens and can be manipulated, like the DT40 cell line, is needed to understand the behavior of CEA-related molecules in neutrophils. We believe that the DT40 cell line is the best model available.

CGM1a, BGPα (both represent splice variants that code for...
tyrosine-containing cytoplasmic tails) and NCA cDNAs were cloned into pBEH or pRc/CMV expression vectors, and the resulting plasmids were stably transfected into the DT40 B cell line, generating the DT40-CGM1a, DT40-BGPa, and DT40-NCA transfectants. All three cell lines expressed similar levels of the respective CEA-related antigens (Figs. 2B and 3). When OpaI GC were added to these three cell lines, calcium flux was only observed in the DT40-CGM1a transfectant (Fig. 2B). In contrast, Opa2 GC could not stimulate any calcium flux. Like-wise, no calcium flux was detected in untransfected DT40 cells treated with OpaI GC. These results show that only CGM1a is able to convey the calcium flux and suggest that it contains a functional ITAM.

Mutation of Tyrosine Residue Tyr-196 in CGM1a Abolishes Its Signaling Capability—Phosphorylation of tyrosine residues within an ITAM is a key step for its activation. The cytoplasmic domain of CGM1a contains two tyrosine residues in its potential ITAM. To test whether these tyrosine residues were required for activation of the calcium flux, we substituted them with phenylalanine. Three different tyrosine mutants, CGM1a-Y196F, CGM1a-Y207F, and CGM1a-Y196F/Y207F (Fig. 1B), were constructed and the resulting expression plasmids were transfected into DT40 cells. Transfectants that expressed similar levels of the mutated molecules were selected for the calcium flux experiments (Fig. 3). As depicted in Fig. 3, mutation of tyrosine residue 196 (Y196F) or mutation of both tyrosines in the cytoplasmic tail of CGM1a (Y196F/Y207F) completely abolished its signaling ability as measured by calcium flux. On the other hand, OpaI GC were able to stimulate a low level of calcium flux in the Y207F CGM1a mutant. These results indicate that both tyrosines are necessary to constitute a fully functional ITAM with Tyr-196 being the essential one. This mutation analysis of the cytoplasmic tyrosines of CGM1a confirms that the motif surrounding these tyrosines (YX₇LX₇YX₇M) is a functional ITAM.

The ITAM in CGM1a Mediates Phagocytosis of Opa⁺ Bacteria—CGM1a promotes strong phagocytosis of Opa⁺ bacteria. We wanted to know whether signaling through the ITAM in the cytoplasmic domain of CGM1a is involved in this process. First, using a colony-forming assay to quantify the number of internalized bacteria, we demonstrated that DT40-CGM1a cells are also capable of phagocytosing OpaI GC or E. coli (Fig. 4A). This result was confirmed by electron microscopy analysis (Fig. 4, B and C). To elucidate the importance of the tyrosines in this process, the tyrosine mutants...
were tested in the phagocytosis assay. As in the calcium flux experiments, mutation of Tyr-196 or mutation of Tyr-196 and Tyr-207 in the ITAM severely impaired or abolished, respectively, the ability of CGM1a to confer phagocytosis of OpaI E. coli (Fig. 4D). However, some capacity to phagocytose OpaI E. coli was still preserved in DT40 cells expressing CGM1a-Y207F. From these results, we conclude that the ITAM in CGM1a mediates both calcium flux and the phagocytosis of OpaI + bacteria.

---

**Signaling Controls Phagocytosis of Opa + GC**

**FIG. 3.** Mutation of Tyr-196 from CGM1a abolished the calcium flux in DT-CGM1a. The calcium flux response was measured as described in the legend to Fig. 2 in DT40 B cells. DT40-CGM1a, DT40-CGM1a-Y196F, DT40-CGM1a-Y207F, and DT40-CGM1a-Y196F/Y207F cells upon addition of OpaI GC. The expression level of the different chimeric molecules in stable DT40 B cell transfectants was determined by flow cytometry (right panel) using anti-CGM1a COL-1 antibody. Untransfected DT40 B cells were used as a negative control.

---

**DISCUSSION**

Internalization of microorganisms into either professional or non-professional phagocytic cells requires the interaction of phagocytosis-promoting receptors on the cell surface with ligands on the surface of the microorganisms. In the present study, we demonstrated that the neutrophil-specific receptor, CGM1a contains an ITAM, which is essential for CGM1a-mediated phagocytosis of Opa + bacteria. This conclusion is further validated by showing that CGM1a-transfected, Syk-
FIG. 4. DT40-CGM1a-mediated phagocytosis of Opa GC and Opa\textsuperscript{+} E. coli is dependent on CGM1a ITAM. A, DT40-CGM1a and control DT40 were plated onto 24-well plates. Opa and Opa\textsuperscript{+} bacteria were added to the cultures and incubated for 3 h. The extracellular bacteria were killed by addition of 100 \( \mu \)g/ml (final concentration) of gentamicin. The number of phagocytosed bacteria was determined by counting CFUs recovered following gentamicin treatment. Transmission electron micrograph shows internalization of Opa GC (B) and Opa\textsuperscript{+} E. coli (C) by DT40-CGM1a cells. The large numbers of internalized Opa\textsuperscript{+} GC and Opa E. coli enclosed within vesicles are clearly visible as indicated with arrows. D, mutation of either of the tyrosine residues on ITAM impaired the ability of CGM1a to phagocytose Opa GC.

FIG. 5. Syk and PLC-\( \gamma \) participate in CGM1a-mediated calcium flux, phagocytosis, and cell death. DT40, DT40-CGM1a, DT40-Syk-CGM1a and DT40-PLC-\( \gamma \)-CGM1a cells were tested for their ability to generate calcium flux (A), phagocytosis (B), and to be induced cell death (C) by interaction with Opa. The same procedures as for calcium flux in Fig. 2, bacterial phagocytosis in Fig. 4 and cell death in Fig. 6 were followed, respectively. DT40-Syk-CGM1a lost all these abilities, whereas DT40-PLC-\( \gamma \)-CGM1a preserved some capacity to phagocytose bacteria.
deleted DT40 cells are no longer able to promote effectively either calcium ion influx or phagocytosis of OpaI bacteria. Finally, we showed that CGM1a-mediated phagocytosis of opacity (Opa) proteins-expressing Neisseria gonorrhoeae leads to cell death.

Because CGM1a is only expressed in PMNs, it is a concern whether CGM1a-mediated signaling data obtained from the B cell line DT40 reflects its biological properties in neutrophils. ITAM-containing receptors have nearly exclusively been identified in cells from immune system. Despite the differences in the structures they recognize and the effector functions they carry out, BCRs, TCRs, and several FcRs utilize remarkably similar signal transduction components to initiate and propagate their signaling responses. They use components that contain distinct recognition (ITAM) and signal transduction subunits such as Syk, with which the ITAM in CGM1a may be also associated (Fig. 5). This type of receptors is crucial for transmission of activation signals in immune cells. Here, we show that the human CGM1a, although ectopically expressed in a chicken B cell line, exhibits the ITAM characteristics of BCRs, supporting the notion that ITAMs are highly conserved motifs, and that the CGM1a ITAM might play a similar role in neutrophils.

Opa+ Neisseria interact with BGP and CGM1 on neutrophils during in vitro infection, and both antigens promote phagocytosis of Opa+ bacteria in HeLa transfectants (13, 14, 16). Do CGM1a and BGPa work independently or do they collaborate during phagocytosis of GC by neutrophils? What kind of collaboration could be envisaged? BGPa can deliver inhibitory signals through its ITIM in the cytoplasmic tail to counteract ITAM-mediated positive signals, which requires SHP-1 and SHP-2. The protein-tyrosine phosphatases SHP-1 and SHP-2, which are typically involved in negative signaling, bind to the ITIM-like motif in the cytoplasmic tail of BGPa (40-41). Indeed, it has recently been shown that interaction of Opa+ GC with BGPa antigens down-regulates the activity of SHP-1 and SHP-2 (42). Therefore, BGPa may be co-ligated with CGM1a on the surface of neutrophils when they interact with Opa+ bacteria. Cross-linkage of the two molecules might regulate the phagocytosis process. Opa+ GC-stimulated signal transduction events in neutrophils might consequently be analogous to the positive (ITAM) versus negative (ITIM) signaling seen with lymphocyte receptors such as BCR and FeRIB, respectively (43).

In addition, this study raises an interesting issue. This is the first report of phagocytosis of bacteria by B cells. Antigen-immunoreceptor interaction is an important step for the establishment of the immune response, because there is signaling from the receptor to activate different cellular functions. Internalization of antigen-bound receptors by B cells is critical for antigen processing and presentation to T lymphocytes. Here we show that DT40-CGM1a B cells were able to phagocytose Opa+ bacteria (either GC or E. coli) in a very efficient manner. Although the cell line we used was manipulated to express CGM1a antigen, our data, nevertheless, suggest that B cells possess the machinery to phagocytose whole bacteria. Demonstration of the ability of B cells to phagocytose bacteria is a new information with a potential.

What is the biological fate of Opa+ GC after phagocytosis by PMN? It is generally thought that the phagocytosed gonococci are killed. However, neutrophils are often packed with intact, seemingly viable, intracellular GC (44). Thus, the possibility exists that these Opa+ GC may be protected from bactericidal attack by the granulocytes and instead use these cells as an intracellular niche or as vehicles to reach the next host. Alternatively, they could simply kill the PMNs. In fact, it was recently found that the ITAM activation pathway also induces cell apoptosis in immune cells (23, 38, 45-49). For example, activation of the ITAM in the BCR of DT40 B cells results in apoptosis (38, 48). In this context it is interesting to note that OpaI and OpaF GC but not OpaD and OpaF GC stimulate the death of neutrophils (Fig. 7) although the level of interaction of Opa+ GC by neutrophils with Opa antigens down-regulates the activity of SHP-1 and SHP-2 (42). Therefore, BGPa may be co-ligated with CGM1a on the surface of neutrophils when they interact with Opa+ bacteria. Cross-linkage of the two molecules might regulate the phagocytosis process. Opa+ GC-stimulated signal transduction events in neutrophils might consequently be analogous to the positive (ITAM) versus negative (ITIM) signaling seen with lymphocyte receptors such as BCR and FeRIB, respectively (43).

In addition, this study raises an interesting issue. This is the first report of phagocytosis of bacteria by B cells. Antigen-immunoreceptor interaction is an important step for the establishment of the immune response, because there is signaling from the receptor to activate different cellular functions. Internalization of antigen-bound receptors by B cells is critical for antigen processing and presentation to T lymphocytes. Here we show that DT40-CGM1a B cells were able to phagocytose Opa+ bacteria (either GC or E. coli) in a very efficient manner. Although the cell line we used was manipulated to express CGM1a antigen, our data, nevertheless, suggest that B cells possess the machinery to phagocytose whole bacteria. Demonstration of the ability of B cells to phagocytose bacteria is a new information with a potential.

What is the biological fate of Opa+ GC after phagocytosis by PMN? It is generally thought that the phagocytosed gonococci are killed. However, neutrophils are often packed with intact, seemingly viable, intracellular GC (44). Thus, the possibility exists that these Opa+ GC may be protected from bactericidal attack by the granulocytes and instead use these cells as an intracellular niche or as vehicles to reach the next host. Alternatively, they could simply kill the PMNs. In fact, it was recently found that the ITAM activation pathway also induces cell apoptosis in immune cells (23, 38, 45-49). For example, activation of the ITAM in the BCR of DT40 B cells results in apoptosis (38, 48). In this context it is interesting to note that OpaI and OpaF GC but not OpaD and OpaF GC stimulate the death of neutrophils (Fig. 7) although the level of interaction of Opa+ GC by neutrophils with Opa antigens down-regulates the activity of SHP-1 and SHP-2 (42). Therefore, BGPa may be co-ligated with CGM1a on the surface of neutrophils when they interact with Opa+ bacteria. Cross-linkage of the two molecules might regulate the phagocytosis process. Opa+ GC-stimulated signal transduction events in neutrophils might consequently be analogous to the positive (ITAM) versus negative (ITIM) signaling seen with lymphocyte receptors such as BCR and FeRIB, respectively (43).

In addition, this study raises an interesting issue. This is the first report of phagocytosis of bacteria by B cells. Antigen-immunoreceptor interaction is an important step for the establishment of the immune response, because there is signaling from the receptor to activate different cellular functions. Internalization of antigen-bound receptors by B cells is critical for antigen processing and presentation to T lymphocytes. Here we show that DT40-CGM1a B cells were able to phagocytose Opa+ bacteria (either GC or E. coli) in a very efficient manner. Although the cell line we used was manipulated to express CGM1a antigen, our data, nevertheless, suggest that B cells possess the machinery to phagocytose whole bacteria. Demonstration of the ability of B cells to phagocytose bacteria is a new information with a potential.
death in B-cell and possibly in neutrophils is very significant. Whether BGPa mediated internalization of Opa+ GC in neutrophils could reduce apoptosis of neutrophils remains to be determined, because BGPa contains an ITIM to counteract the activated ITAM.2

It may seem to a paradox that host cells phagocytosing microorganisms will kill them and also may be killed by microorganisms. However, the two aspects might in reality represent two separate battlefields in the interaction between bacteria (GC) and host cells (PMNs). This phenomenon also occurs in interaction between other pathogenic bacteria and host cells. For example, whether the outcome of macrophages phagocytosing Shigella or Salmonella is the killing of the microorganisms or the death of the host cell (50, 51) remains to be defined in vivo. The study of the interaction of Opa+ bacteria with host cells through CEA (CEACAM/CD66) antigens addresses two facets of phagocytosis of GC by PMNs, which are critical for the understanding of the pathogenesis of gonococcal infection.

Acknowledgments—We thank Dr. M. Kuroki for generously providing the cDNA of BGPa. We are indebted to John Swanson and Bob Belland for supplying the Opa+ bacteria. We also thank Dr. Stanley Spinola for useful suggestions and editorial comments. We always thank Emil Gotschlich for insightful scientific advice.

REFERENCES
1. Centers for Disease Control (1993) Morbid. Mortal. Weekly Rep. 42, 55–63
2. Evans, B. A. (1977) J. Infect. Dis. 136, 248–255
3. Ward, M. E., and Watt, P. J. (1972) J. Infect. Dis. 126, 601–605
4. Bhat, K. S., Gibbs, C. P., Barrera, O., Morrison, S. G., Jahnig, P., Stern, A., Kupsch, E. M., Mayer, T. F., and Swanson, J. (1991) Mol. Microbiol. 5, 1889–1901
5. Gorby, G., Simon, D., and Rest, R. (1994) Ann. New York Acad. Sci. 730, 286–289
6. Swanson, J., Barrera, O., Sola, J., and Boslego, J. (1988) J. Exp. Med. 168, 2121–2129
7. Jerse, A. E., Cohen, M. S., Drown, P. M., Whicker, L. G., Isbey, S. F., Seifert, H. S., and Cannon, J. G. (1994) J. Exp. Med. 179, 911–920
8. Swanson, J., Sparks, E., Young, D., and King, G. (1975) Infect. Immun. 11, 1352–1361
9. Swanson, J., King, G., and Zeligs, B. (1975) Infect. Immun. 11, 65–68
10. Virji, M., and Everson, J. S. (1981) Infect. Immun. 31, 965–970
11. Virji, M., and Heckels, J. E. (1986) J. Gen. Microbiol. 132, 503–512
12. Fischer, S. H., and Rest, R. F. (1988) Infect. Immun. 56, 1574–1579
13. Virji, M., Makepeace, K., Ferguson, D. J. P., and Watt, M. (1996) Mol. Microbiol. 22, 941–950
14. Chen, T., and Gotschlich, E. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 14851–14856
15. Chen, T., Grunert, F., Medina-Marino, A., and Gotschlich, E. (1997) J. Exp. Med. 186, 1557–1564
16. Gray-Owen, S., Dehio, C., Haude, A., Grunert, F., and Meyer, T. (1997) EMBO J. 16, 3435–3445
17. Hammerstrom, S. (1999) Semin. Cancer Biol. 9, 67–81
18. Paxton, R., Mooser, G., Pande, H., Lee, T. D., and Shively, J. E. (1987) Proc. Natl. Acad. Sci. U. S. A. 84, 920–924
19. Beauchemin, N., others, and Zimmermann, W. (1999) Exp. Cell Res. 252, 243–249
20. Nagel, G., Grunert, F., Kuijpers, T., Watt, S., Thompson, J., and Zimmermann, W. (1993) Eur. J. Biochem. 214, 27–35
21. Cambier, J. (1995) J. Immunol. 155, 3281–3285
22. Bijsterbosch, M., Meade, C., Turner, G., and Klaus, G. (1985) Cell 41, 999–1006
23. Takata, M., Homma, Y., and Kurosaki, T. (1995) J. Exp. Med. 182, 97–104
24. Tuveson, D. A., Carter, R. H., Soltoff, S. P., and Fearon, D. T. (1993) Science 260, 886–889
25. Buhl, A. M., Pleiman, C. M., Rickert, R. C., and Cambier, J. C. (1997) J. Exp. Med. 186, 1897–1910
26. Swanson, J., and Barrera, O. (1983) J. Exp. Med. 157, 1405–1420
27. Swanson, J. (1991) in Proceedings of the Seventh International Pathogenic Neisseria Conference (Achtman, M., Kohl, P., Marchal, C., Morelli, G., Seiler, A., and Thiesen, B., eds), pp. 391–396, Walter de Gruyter & Co., Berlin
28. Belland, R. J., Chen, T., Swanson, J., and Fischer, S. H. (1992) Mol. Microbiol. 6, 1729–1737
29. Takata, M., Sahe, H., Hata, A., Inazu, T., Homma, Y., Nukada, T., Yamauma, H., and Kurosaki, T. (1994) EMBO J. 13, 1341–1349
30. Takata, M., and Kurosaki, T. (1996) J. Exp. Med. 184, 31–40
31. Morgenstern, J., and Land, H. (1999) Nucleic Acids Res. 18, 3587–3596
32. Reth, M. (1989) Nature 338, 383–384
33. Reth, M. (1985) Seminars in Immunol. 7, 21–27
34. Cox, D., Chang, P., Kurosaki, T., and Greenberg, S. (1996) J. Biol. Chem. 271, 16597–16602
35. Maeda, A., Kurosaki, M., and Kurosaki, T. (1998) J. Exp. Med. 188, 991–995
36. Bos, M., Grunert, F., and Belland, R. (1997) Infect. Immun. 65, 2353–2361
37. Gray-Owen, S., Lorenzen, D., Haude, A., Meyer, T., and Dehio, C. (1997) Mol. Microbiol. 26, 971–980
38. Ono, M., Okada, H., Bolland, S., Yanagi, S., Kurosaki, T., and Ravetch, J. (1997) Cell 90, 293–301
39. Kupsch, E. M., Knepper, B., Kuroki, T., Heuer, I., and Meyer, T. F. (1993) EMBO J. 12, 641–650
40. Beauchemin, N., Kunath, T., Robitaille, J., Chow, B., Turbide, C., Daniels, E., and Veillette, A. (1997) Oncogene 14, 783–790
41. Huber, M., Izzii, L., Grodinsk, P., Haupe, C., Kunath, T., Veillette, A., and Beauchemin, N. (1999) J. Biol. Chem. 274, 335–344
42. Hauck, C., Gulbins, E., Lang, F., and Meyer, T. (1999) Infect. Immun. 67, 5490–5494
43. Healy, J. I., and Goodnow, C. C. (1998) Annu. Rev. Immunol. 16, 645–670
44. Shafer, W. M., and Rest, R. F. (1989) Ann. Rev. Microbiol. 43, 121–145
45. Combadiere, B., Freedman, M., Chen, L., Shores, E. W., Love, P., and Lenardo, M. J. (1996) J. Exp. Med. 183, 2109–2117
46. Yao, X. R., Flavwinkel, H., Reth, M., and Scott, D. W. (1995) J. Immunol. 155, 652–661
47. Tseng, J., Eifelder, B. J., and Clark, M. R. (1997) Blood 90, 1513–1520
48. Sugawara, H., Kurosaki, M., Takata, M., and Kurosaki, T. (1997) EMBO J. 16, 3078–3088
49. de Aos, I., Metzger, M. H., Exley, M., Dahl, C. E., Misra, S., Zheng, D., Varticovski, L., Terhorst, C., and Sancho, J. (1997) J. Biol. Chem. 272, 25310–25318
50. Zychlinsky, A., Prevost, M., and Sansonetti, P. (1992) Nature 358, 167–169
51. Monack, D., Raupach, B., Hromsckyj, A., and Falkow, S. (1996) Proc. Natl. Acad. Sci. U. S. A. 93, 9833–9838
The CGM1a (CEACAM3/CD66d)-mediated Phagocytic Pathway of Neisseria gonorrhoeae Expressing Opacity Proteins Is Also the Pathway to Cell Death
Tie Chen, Silvia Bolland, Ines Chen, James Parker, Milica Pantelic, Fritz Grunert and Wolfgang Zimmermann

J. Biol. Chem. 2001, 276:17413-17419.
doi: 10.1074/jbc.M010609200 originally published online February 5, 2001

Access the most updated version of this article at doi: 10.1074/jbc.M010609200

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 50 references, 26 of which can be accessed free at http://www.jbc.org/content/276/20/17413.full.html#ref-list-1