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Stimulation of Complement Component C3 Synthesis in Macrophagelike Cell Lines by Group B Streptococci

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Complement levels and complement activation are key determinants in streptococcus-induced inflammatory responses. Activation of macrophage functions, such as complement synthesis, by group B streptococci (GBS) was examined as a possible component of GBS-induced chronic inflammation. Using an enzyme-linked immunosorbent assay, secreted C3 from mouse macrophagelike cell lines (PU5-1.8 and J774A.1) was monitored after cultivation with GBS. Whole, heat-killed GBS (1 to 10 CFU per macrophage) of both type Ia and III strains induced 25 to 300% increases in secreted C3 in both cell lines after a 24-h cultivation. GBS-treated cell lines exhibited increases in secreted lysozyme (10%) and in cellular protein (25 to 50%). Inhibition of macrophage phagocytosis by cytochalasin B inhibited GBS stimulation of C3. Purified cell walls of GBS type III strain 603-79 (1 to 10 μg/ml) also enhanced C3 synthesis. Local enhancement of macrophage C3 production by ingested streptococci or by persistent cell wall antigens may serve to promote chronic inflammatory responses.

The severity and duration of streptococcus-induced inflammation depends upon the capacity of streptococci or their cell walls to activate the alternative complement pathway (ACP) (34) and to resist degradation by tissue enzymes (7, 31, 34). Macrophages laden with streptococcal antigens persist in lesions of both group A streptococcus- and group B streptococcus (GBS)-induced inflammation. The limited resistance of GBS peptidoglycan to lysozyme digestion appears to be a critical pathogenic factor in GBS-induced arthritis (33, 34). Lysozyme digestion of GBS enhances their ability to activate the ACP, but digestion is insufficient to reduce persistence of the GBS antigens (33). Complement plays an important role in the pathogenesis of inflammatory arthritis induced by group A streptococci or by GBS (18, 34), and cell wall-complement complexes have been suggested to play a role in rat models of streptococcus-induced arthritis (11).

Common characteristics of highly inflammatory substances such as streptococci include the ability to activate the ACP and the ability to activate macrophage secretory functions (8, 34). These characteristics are directly related, since complement activation and generation of free or bound C3b activates macrophage secretion of lysosomal hydrolases (29, 30). As a correlate, local opsonization of ACP activators is mediated by macrophage-derived complement proteins (12).

In sites of chronic inflammation, enhanced local production of C3 and other complement components by macrophages is hypothesized to promote inflammation (4, 20, 38). Autoregulation of complement synthesis by recognition of free or bound complement peptides, perhaps via macrophage complement receptors, may also operate (13). Enhanced C3 synthesis is characteristic of lymphokine-activated, lipopolysaccharide (LPS)-treated, or gamma interferon-activated macrophages (17, 20).

To further assess the role of macrophages and complement in the pathogenesis of GBS-induced inflammation, I investigated the ability of whole heat-killed GBS and their antigens to activate C3 synthesis in mouse macrophagelike cell lines. These cell lines secrete C3 and respond to immunologic (lymphokine) and nonimmunologic (LPS) activation with enhanced C3 synthesis and secretion as do normal macrophages (25; K. J. Goodrum, J. Leukocyte Biol., in press).

Whole GBS and their purified cell walls enhanced C3 synthesis by the macrophage cell lines, and this enhancement was inhibited by cytochalasin B.

MATERIALS AND METHODS

Streptococci. Streptococci grown on sheep erythrocyte agar were inoculated into 10 ml of glucose- and phosphate-supplemented Todd-Hewitt broth (Difco Laboratories, Detroit, Mich.) (3), incubated for 24 h at 37°C, and used as the inoculum for 200 ml of broth. These cultures were grown for 4 to 6 h at 37°C, harvested by centrifugation (10,000 × *g*, 15 min, Beckman J2-21 centrifuge), and washed three times in phosphate-buffered saline (PBS). Streptococci were suspended in PBS, and the number of CFU per milliliter was determined by counting viable colonies on sheep erythrocyte agar. Streptococci were heat killed by incubation at 60°C for 30 min or Formalin killed in 0.3% (vol/vol) Formalin in PBS. GBS type Ia strains 090 and 509-80, GBS type III strains D136C and 603-79, and group D streptococcal strain F24 were obtained from J. K. Spitznagel (Emory University, Atlanta, Ga.) GBS DS1204 (type Ia) and M732 (type III) were provided by Toby Eisenstein (Temple University, Philadelphia, Pa.). GBS strains 542 and 372 (type Ia) and 512 (type III) were provided by David Scholl (Diagnostic Hybrids, Athens, Ohio). Group A streptococci (A-2, A-3) were fresh clinical isolates provided by M. C. Modrzakowski (Ohio University, Athens).

PCW preparations. Streptococcal cell walls are prepared from GBS (strain 603-79) as described by Spitznagel et al. (34). Purified cell walls (PCW) contain a complex of peptidoglycan and streptococcal polysaccharides and are free of protein and membrane components (34).

Reagents. Cytochalasin B and LPS from *Escherichia coli* O26:B6, trichloroacetic acid extract) were all from Sigma Chemical Co., St. Louis, Mo.

TABLE 1. C3 levels in mouse macrophagelike cell lines stimulated with GBS

Stimulus[a]	C3 (U/culture)[b] in the following cell lines:		
	PU5-1.8[c]		J774A.1
	Expt 1	Expt 2	
Untreated	102 ± 14 (6)	234 ± 33 (4)	2,752 ± 253 (8)
GBS, type Ia			
090	189 ± 22 (4)[d]	NT[e]	3,276 ± 112 (4)[d]
DS1204	219 ± 30 (4)[d]	441 ± 134 (2)	3,575 ± 239 (4)[d]
542	NT	373 ± 23 (2)	NT
372	222 ± 18 (4)[d]	366 ± 13 (2)	NT
509-80	191 ± 20 (4)[d]	NT	NT
GBS, type III			
603-79	173 ± 15 (4)[d]	359 ± 52 (4)[d]	NT
M743	NT	416 ± 9 (2)	NT
512	205 ± 7 (4)[d]	418 ± 4 (2)	3,295 ± 175 (4)[d]
D136C	179 ± 23 (4)[d]	NT	3,172 ± 161 (4)
Group A streptococci			
A-1	117 ± 16 (2)	219 ± 54 (2)	NT
A-2	NT	326 ± 40 (4)[d]	NT
A-3	NT	266 ± 14 (4)	NT
Group D streptococci			
F24	184 ± 29 (2)	388 ± 28 (4)[d]	NT

[a] Heat-killed GBS and Formalin-killed group A and D streptococci were added to PU5-1.8 cells at doses of 5 to 10 CFU per cell (experiment 1) and 5 CFU per cell (experiment 2). The dose in J774A.1 cultures was 5 to 10 CFU/cell.

[b] Antigenic C3 (mouse serum standard defined as 10^7 U/ml) assayed 24 h after the addition of streptococci. Values represent means ± Standard deviation, with the numbers of samples per group shown in parentheses.

[c] PU5-1.8 cultures in experiments 1 and 2 contained 1×10^6 cells and 2×10^6 cells, respectively.

[d] Significantly different from untreated control ($P \leq 0.05$).

[e] NT, Not tested.

Cell lines. The mouse macrophagelike cell lines J774A.1 (26, 27; BALB/c) and PU5-1.8 (26; monocytic tumor, BALB/c) were obtained from American Type Culture Collection, Rockville, Md. Frozen stocks were stored in liquid nitrogen.

Cell culture. Macrophages were maintained in Dulbecco modified Eagle medium (high-glucose formula; GIBCO Laboratories, Grand Island, N.Y.) with 10% (vol/vol) defined fetal bovine serum (Hyclone Labs, Utah) and antibiotic-antimycotic (GIBCO) at 37°C in a humidified atmosphere with 5% CO_2. Cells grew to confluence as attached monolayers and were subcultured by gently detaching cells by rinsing and dilution (1:20 to 1:50) into fresh medium. Cells were subcultured for experiments into 24-well cluster dishes (Corning Glass Works, Corning, N.Y.) at 1×10^5 to 2.5×10^5 cells per well in 1 ml of medium. When wells exhibited confluent growth (~2×10^6 cells per well), fresh medium (1 to 2 ml per well) with experimental treatments was added. Cultures were incubated for 24 h, followed by collection of conditioned medium for assays of secreted C3 and lysozyme. Monolayers were washed twice with PBS and either suspended in 1 ml of PBS for counting cell numbers per well or lysed in 0.5 ml of 0.2% Triton X-100 in PBS for protein determination.

Protein Assay. A modified Lowry protein assay (9) for samples containing Triton X-100 was used.

Lysozyme assay. Lysozyme was monitored in all samples by the lysoplate method of Osserman and Lawlor (21) with a hen egg white lysozyme (Sigma) as a standard.

C3 determinations. An enzyme-linked immunosorbent assay for antigenic mouse C3 was used for quantitation of secreted C3 in macrophage cultures. Specific goat anti-mouse C3 (Cooper Biomedical, Inc., Malvern, Pa.) is nonspecifically bound to 96-well flexible polyvinyl chloride enzyme immunoassay plates (Falcon; Becton Dickinson Labware, Oxnard, Calif.). Each well received 0.1 μg of antibody protein (representing a 1:4,000 dilution of antiserum) in 0.05 ml of 0.015 M Na_2CO_3–0.035 M $NaHCO_3$ (pH 9). Binding occurred over 4 h at room temperature or overnight at 4°C. Antibody was decanted, and other nonspecific binding sites on wells were blocked by filling wells with 1% bovine serum albumin in PBS for 1 h at room temperature. Bovine serum albumin was decanted, and 0.05-ml samples were added and incubated for 4 h at room temperature or overnight at 4°C. Samples were decanted, and wells were washed vigorously three times with 0.25% bovine serum albumin–0.05% Tween 20 in PBS. Bound C3 was detected directly with horseradish peroxidase-conjugated goat anti-mouse C3 (Cooper Biomedical; 0.1 μg per well in 1% bovine serum albumin for 2 h at room temperature). Secondary antibody was decanted, and wells were washed three times as above. Colorimetric detection of secondary antibody followed the addition of peroxidase substrate *o*-phenylenediamine (0.2%, wt/vol; in 0.017 M citrate–0.035 M phosphate [pH 6]–0.015% hydrogen peroxide). The reaction was stopped after incubation (15 min at room temperature in the dark) by the addition of 0.05 ml of 12.5% sulfuric acid per well. The optical density at 490 nm was determined in sample wells versus control wells with a Biotek EIA Reader (Biotek Instruments, Inc., Vt.). A standard curve relating optical density at 490 nm to C3 concentration was constructed by using pooled mouse serum as a standard. A unit of C3 antigen is defined as that amount of C3 in a $1{:}10^7$ dilution of the standard. Antibody concentrations used in the assays give the lowest backgrounds while retaining linearity over a range of C3 (10 to 500 U/ml) similar to that found in macrophage cultures.

Statistics. Significant differences between treatment groups was analyzed by the Wilcoxon rank sum test (28).

RESULTS

Stimulation of C3 levels in macrophagelike cell lines by GBS. Whole heat-killed GBS stimulated C3 levels in both PU5-1.8 and J774A.1 cell lines (Table 1). At doses of 5 to 15 CFU per cell all strains of GBS tested induced significant increases in secreted antigenically detectable C3. Formalin-killed whole group D and group A strains were also stimulatory; however only one of three group A isolates (at 5 CFU per cell) induced significant change in C3. The J774A.1 cell line secreted much higher basal levels of C3 than did PU5-1.8 cells but was less stimulated by GBS relative to changes seen in PU5-1.8.

Some strain differences in the degree of macrophage activation were evident. With recently isolated clinical strains of GBS (372, 512) as little as 1 CFU per cell would induce a 50% increase in secreted C3 in PU5-1.8 cells. The standard laboratory strains of GBS (090, D136c) were less stimulatory to both PU5-1.8 and J774A.1 cells, requiring 5 CFU per cell to induce a 50% increase in C3 in PU5-1.8 (data not shown). Phagocytosis of all strains was evident in stained smears of 24-h macrophage cultures. At least a 2-h pulse treatment of macrophages with GBS was required to

stimulate C3 levels in 24-h cultures, indicating that stimulation of C3 required some prolonged interaction of macrophage with GBS. Routinely, macrophages were kept in the continuous presence of GBS for the 24-h incubation period of experiments.

Significant increases in C3 levels in GBS-treated macrophages above the level secreted by untreated macrophages were not seen until 24 h of culture (data not shown). This timing indicates a synthetic stimulation and subsequent secretion of C3 rather than an early release of preformed C3.

GBS stimulation of cultural protein levels. GBS-treated cells also exhibited increased total cellular protein (25 to 50% above untreated control; Table 2) and increased secreted lysozyme activity (10% above control; Table 2). GBS-induced C3 levels paralleled increases in cellular protein with little increase in C3 levels per milligram of cellular protein. Compared with the constitutive synthesis and secretion of lysozyme by macrophages (15), which showed no significant change upon macrophage stimulation (at 10 CFU/ml; Table 3), secreted C3 was substantially increased by GBS treatment of PU5-1.8 cells (46%, Table 3). Significant increases in secreted C3 levels in GBS-treated macrophages required 1 to 10 CFU per macrophage (Table 3). Treatment of macrophage cultures with an inert phagocytosable particle (latex beads) did not induce cultural protein, lysozyme, or C3 levels (210 ± 3 U of C3 per culture in controls versus 164 ± 18 U of C3 per culture in latex-treated PU5-1.8; five beads per cell, four samples per group). No significant response to latex particles was seen over a range of 1 to 100 particles per macrophage.

Stimulation of C3 levels in PU5-1.8 cells by PCW. PCW stimulated signficant increases in C3 secretion above levels in untreated macrophages at doses of 1 to 10 μg/ml (Table 3) as previously reported (Goodrum, in press). Some toxicity was evident at doses of 100 μg/ml as shown as decreases in the secretion of lysozyme. The proportional levels of C3 (units per milligram of cultural protein, or units per microgram of secreted lysozyme) in PCW-treated cultures increased significantly, indicating a differential stimulation of C3 synthesis rather than an overall increase in protein synthesis (Table 3). PCW from group D streptococcus strain F24 were also effective stimulants of macrophage C3 secretion at doses as low as 1 μg/ml (data not shown).

TABLE 2. Stimulated levels of C3, lysozyme, and cellular protein in the PU5-1.8 macrophagelike cell line stimulated with killed streptococci

Stimulus[a]	Total cultural levels of [b]:		
	Secreted C3[c] (U)	Cellular protein (μg)	Secreted lysozyme (μg)
Untreated	161 ± 12	259 ± 14	10.3 ± 0.7
Streptococci			
603-79	224 ± 15[d]	382 ± 27[d]	11.6 ± 0.3[d]
DS1204	222 ± 13[d]	368 ± 15[d]	11.4 ± 0.6[e]
M732	188 ± 20	345 ± 6[d]	11.6 ± 0.2[d]
A-2	200 ± 10[d]	334 ± 5[d]	12 ± 0.5[e]
F-24	206 ± 10[d]	386 ± 26[d]	13.2 ± 1.3[e]

[a] Heat-killed GBS (603-79, DS1204, M732) and Formalin-killed group A (A-2) and group D (F24) streptococci were added at 5 CFU per cell to PU5-1.8 cultures.
[b] Values represent means of six samples ± standard deviations.
[c] Antigenic C3 (mouse serum standard defined as 10^7 U/ml) assayed 24 h after the addition of streptococci.
[d] Significantly different from untreated control ($P \leq 0.01$).
[e] Significantly different from untreated control ($P \leq 0.05$).

TABLE 3. C3, Lysozyme, and cellular protein levels in the PU5-1.8 macrophagelike cell line treated with whole heat-killed cells and PCW of GBS strain 603-79

Treatment[a]	Total cultural levels of[b]		
	Secreted C3[c] (U)	Cellular protein (μg)	Secreted lysozyme (μg)
Untreated	474 ± 68	161 ± 22	9.8 ± 0.2
Killed 603-79 (CFU/cell)			
0.1	464 ± 60	Not tested	9.6 ± 1.4
1.0	550 ± 48	Not tested	10.4 ± 1.0
10.0	692 ± 14[d]	224 ± 18[d]	9.2 ± 0.6
100.0	680 ± 20[d]	232 ± 17[d]	5.4 ± 0.6
PCW (μg/ml)			
0.1	450 ± 20	Not tested	8.4 ± 1.2
1.0	580 ± 40[d]	225 ± 21[d]	10.8 ± 1.6
10.0	852 ± 46[d]	244 ± 24[d]	11.8 ± 1.4[d]
100.0	802 ± 50[d]	210 ± 12[d]	5.0 ± 0.8

[a] Heat-killed 603-79 cells or PCW were added to cultures of PU5-1.8 cells and incubated for 24 h.
[b] Values represent means of four samples ± standard deviations
[c] Antigenic C3 (mouse serum standard defined as 10^7 units/ml).
[d] Significantly different from untreated control ($P \leq 0.05$).

Modulation of GBS-stimulated C3 responses by cytochalasin B treatment of macrophage cultures. The role of phagocytosis in GBS- and PCW-induced C3 synthesis in PU5-1.8 cells was tested by comparing phagocytosis and C3 levels at 24 h in macrophages treated with or without cytochalasin B. A dose of cytochalasin that inhibited phagocytosis of GBS (determined by direct microscopic count of ingested GBS in stained smears) completely eliminated any increases in C3 levels in GBS-treated or PCW-treated macrophages (Table 4). Cytochalasin B alone did not affect C3 levels in control cultures. LPS stimulation of C3 levels, which should not be phagocytosis dependent, was unaffected by cytochalasin.

DISCUSSION

Whole heat-killed GBS and their PCW were shown to stimulate secreted C3 levels in the mouse macrophagelike cell lines PU5-1.8 and J774A.1. Significant increases in antigenic C3 were detectable in culture medium of GBS- or PCW-treated PU5-1.8 cells as compared with the constitutive levels in untreated cultures. Inhibition of phagocytosis with cytochalasin B inhibited GBS or PCW stimulation of C3 in macrophage cultures. GBS induction of C3 synthesis in

TABLE 4. C3 secretion by the mouse macrophagelike cell line (PU5-1.8): inhibition of cell activation by cytochalasin B

Macrophage activator[a]	Total C3 (U/culture)[b]	
	Untreated	Cytochalasin treated[c]
None	212 ± 13	209 ± 12
LPS	444 ± 15[d]	394 ± 19[d]
PCW	291 ± 30[d]	220 ± 15
GBS strain (090)	304 ± 13[d]	198 ± 8

[a] LPS (10 μg/ml); PCW (10 μg from GBS strain 603-79 per ml); GBS (5 CFU of heat-killed GBS strain 090 per cell of PU5-1.8 cultures).
[b] Antigenic C3 (mouse serum standard defined as 10^7 U/ml). Values represent means of six samples ± standard deviations, assayed 24 h after the addition of activators.
[c] Cytochalasin B (5 μg/ml) was added 1 h before the other additions.
[d] Significantly different from untreated control receiving no activators ($P \leq 0.01$).

macrophages shows that these bacteria possess potent modulators of macrophage function. Stimulation of local C3 levels by GBS or other persistent bacterial antigens at sites of inflammation may potentiate the inflammatory reaction.

C3 is synthesized and secreted by monocytes and macrophages constitutively (5, 6, 23), and enhanced C3 synthesis has been reported in macrophages "activated" by various particulate and soluble stimuli including lymphokine-rich lymphocyte culture supernatant fluids (17), gamma interferon (23), LPS (20, 35), and *Corynebacterium parvum* (38). Koestler et al. (17) demonstrated that enhanced C3 synthesis in macrophages correlated with their activation to tumoricidal function. Enhanced extracellular C3 in activated macrophages, including PCW-stimulated macrophages (Goodrum, in press), is due to increased synthesis (23, 35) as shown by cycloheximide-inhibitable changes in C3 levels. C3 synthesis is evidently induced, as is the synthesis of many proteins during macrophage activation (1, 22). The relative changes in total cellular protein vs. extracellular C3 and extracellular lysozyme levels in GBS-or PCW-treated macrophages indicates at least a differential increase in synthesis or secretion (or both) of C3 and lysozyme. Since intracellular pools of C3 and lysozyme were not assayed, direct comparisons cannot be made; however intracellular pools of C3 similar to lysosomal pools of lysozyme have not been reported.

Differences in C3 levels between GBS- PCW-treated and untreated cultures were not detectable until 24 h after GBS treatment and do not parallel early release reactions of lysosomal enzymes by PCW-treated macrophages (8). This probably reflects the time required for synthesis induction as well as the time required for accumulation of detectable levels of C3 in the medium.

Doses of cytochalasin B which inhibited GBS uptake by macrophages but did not influence secreted levels of C3 in control cultures completely blocked GBS or PCW stimulation of C3 levels. The lack of effect of cytochalasin B on LPS induction of macrophage C3 indicates that the signal for C3 synthesis induction is different for LPS than for GBS, which appears to require the phagocytic process. Phagocytosis alone, however, is not a sufficient stimulus to induce C3 synthesis in these cell lines, since the uptake of latex beads has no effect on C3 levels. The anti-inflammatory agent hydrocortisone was also tested (data not shown) for antagonism of macrophage C3 responses to GBS, but the reduced basal C3 in hydrocortisone-treated macrophages made interpretation difficult.

All GBS strains tested stimulated C3 in the PU5-1.8 cell line. Limited dose-response studies indicated that strain differences exist in GBS-induced C3 synthesis. All strains of GBS appeared to be phagocytosed, but strict comparisons of phagocytosis rates of GBS versus C3 induction were not made. Low doses of a group D streptococcus and two of three strains of group A streptococcus however were not as stimulatory as were GBS isolates. These differences could relate to rates of phagocytosis or to the presence of stimulatory agents. The use of Formalin-killed group A and D organisms versus heat-killed GBS may contribute to the differences noted; however, Formalin-killed and heat-killed GBS were equally active. Group D PCW were highly active inducers of C3, and group A PCW are known to activate various macrophage functions (32).

The significance of C3 synthesis and secretion by macrophages is unknown, since the bulk of plasma C3 is synthesized by parenchymal cells of the liver (2). Interestingly, C3 functions as an acute-phase protein, being induced early during infection or inflammation in response to interleukin 1 (24). Many investigators have hypothesized that local C3 secretion by macrophages at sites of inflammation may regulate continued inflammation (4, 38), act to promote local opsonization and phagocytosis (12), or influence primary immune reactions (19).

The significance of GBS-induced C3 synthesis can be found in the fact that a common characteristic of many particulate activators of macrophages is their ability to activate the alternative complement pathway (29, 30), a characteristic of GBS and their PCW (34). It is known that ACP activators induce lysosomal enzyme release from macrophages (30), probably via C3b that is generated by ACP activation (29), but no similar relationship has been established between ACP activators and induction of macrophage synthetic functions. An autoregulatory circuit for C3 synthesis might operate in which ACP activators opsonized with C3b interact with CR3 (membrane receptor for C3bi), resulting in phagocytosis as well as signaling the macrophage synthetic machinery to make additional C3 (4, 13). Evidence for autoregulation of C2, C4, and C5 has been reported (4). Serotype Ia GBS can directly activate the classical complement pathway via binding and activating C1 (10), and their interaction with macrophage cultures may depend on macrophage-derived C1 and C1 inhibitor (23).

In addition to chronic inflammatory responses, whole heat-killed GBS have been reported to exhibit an acute toxicity in animals (36). Early-onset infection with GBS has symptoms similar to those of adults suffering from endotoxemia (14). A cell-associated toxin of GBS has been reported by Hellerqvist et al. (16). The activation of macrophage C3 secretory activity by GBS antigens as shown here is similar to macrophage activation by endotoxin (LPS). Interaction of GBS antigens with macrophages, complement proteins (10), and perhaps with other cells and humoral effector systems may activate acute toxic mechanisms similar to those known to occur with endotoxemia. The use of cell walls, which contain both type and group antigens of GBS as well as peptidoglycan, in these studies prevents any conclusion on which GBS antigens play a role in such toxic or inflammatory activities. However, the virulence of GBS strains has been related to their biosynthetic capacity for synthesis of the type-specific antigen (37). Induction of macrophage functions, including C3 synthesis, may be a target for such toxic molecules.

Stimulation of C3 synthesis by GBS or their PCW is likely a very significant factor in GBS-induced rat models of inflammatory disease (7, 33). Whereas low levels of antibody may be important in promoting ACP activation by streptococcal cell walls (11), the important pathology of streptococcus-induced arthritis seems to involve complement and macrophages (18, 32, 34). ACP activators are potent stimulants of macrophage secretory activity, and GBS antigens are known to activate the ACP as well as the classical pathway and to persist within macrophages. GBS induction of local complement synthesis may well promote further opsonophagocytosis, macrophage activation, and enzyme release as well as generation of inflammatory complement peptides. Chronic GBS-induced inflammation then may reflect both antigen persistence and positive feedback of macrophage activation by their own secretions.

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