ABSTRACT To investigate the role of complement protein factor B (Bf) and alternative pathway activity in vivo, and to test the hypothesized potential genetic lethal effect of Bf deficiency, the murine Bf gene was interrupted by exchange of exon 3 through exon 7 (including the factor D cleaving site) with the neo" gene. Mice heterozygous for the targeted Bf allele were interbred, yielding Bf-deficient offspring after the F1 generation at a frequency suggesting that Bf deficiency alone has no major effect on fertility or fetal development. However, in the context of one or more genes derived from the 129 mouse strain, offspring homozygous for Bf deficiency were generated at less than expected numbers (P = 0.012). Bf-deficient mice showed no gross phenotypic difference from wild-type littermates. Sera from Bf-deficient mice lacked detectable alternative complement pathway activity; purified mouse Bf overcame the deficit. Classical pathway-dependent total hemolytic activity was lower in Bf-deficient than wild-type mice, possibly reflecting loss of the alternative pathway amplification loop. Lymphoid organ structure and IgG1 antibody response to a T-dependent antigen appeared normal in Bf-deficient mice. Sensitivity to lethal endotoxic shock was not significantly altered in Bf-deficient mice. Thus, deficiency of Bf and alternative complement activation pathway activity led to a less dramatic phenotype than expected. Nevertheless, these mice provide an excellent model for the assessment of the role of Bf and the alternative pathway in host defense and other functions in vivo.

The alternative pathway of complement activation provides a mechanism for defense against infectious agents. Because the alternative pathway is activated in the absence of specific antibody, it provides an immediately available line of defense that does not require prior exposure to a specific microorganism, though specific antibody may also contribute to alternative pathway-mediated defenses (1, 2).

Factor B (Bf), a 93-kDa single-chain glycoprotein, is required for the initiation and propagation of alternative pathway activation. When Bf associates with a cleavage product of complement protein C3 (C3b) it is cleaved by factor D, releasing a 33-kDa amino-terminal fragment (Ba), and the bound 63-kDa carboxyl-terminal serine proteinase (Bb). Because C3 is a component of the C3bBb enzyme as well as its substrate, this pathway serves as a positive amplification loop for both pathways of complement activation (3). Bf is synthesized in liver (4) and at extrahaepatic sites in endothelial, epithelial, and mesenchymal cells (5).

In addition to its role in activation of the alternative pathway, Bf is a cofactor in antibody-independent monocyte-mediated cytotoxicity (6), macrophage spreading (7), activation of plasminogen (8), and proliferation of B lymphocytes (9–11). All of these Bf-dependent functions were demonstrated in vitro. Inferences regarding the essential actions of the alternative pathway and Bf in vivo have been based on evaluation of patients and experimental animals in which several of the alternative pathway proteins are reduced in concentration, including C3, the target of both classical and alternative complement activation systems. Hence, definitive information about the importance of alternative pathway functions has not been available. Genetic deficiencies of classical pathway complement proteins, on the other hand, provided important insights into the role of this pathway in immunopathology (reviewed in ref. 12).

One of the most common of the human genetic deficiencies of complement is deficiency of C2, a functional and structural homologue of Bf that is encoded by a gene ~400 bp upstream of Bf within the class III region of the major histocompatibility complex. Homozygous C2 deficiency occurs at a high frequency among populations of Western European origin (13), but until a recent preliminary report (14), no homozygous Bf-deficient individuals had been recognized. On the basis of this and the apparent importance of the alternative pathway in host defenses, many suggested that Bf deficiency might be a genetic lethal. To test this hypothesis and to provide a model for evaluating the importance of the alternative pathway in vivo, we generated a Bf-null mouse by gene targeting.

We found that mice rendered deficient in Bf were viable and showed no gross phenotypic abnormalities in spite of the complete absence of alternative pathway function. However, Bf deficiency in the context of one or more other genes imposed a reproductive disadvantage. Lymphoid organ structure was overtly normal in Bf-deficient mice, and the T-dependent antigen response was indistinguishable from that of wild-type mice. Sensitivity to endotoxic shock was also unaltered. Thus, Bf-deficient mice showed less dramatic phenotypic changes than had been anticipated. Nevertheless, this Bf-deficient mouse strain provides an excellent model in which to study the role of Bf and the alternative pathway in systemic host responses.

MATERIALS AND METHODS

**Bf Targeting Vector.** The Bf targeting vector was assembled by using fragments of the BALB/Ç cosmid clone D-9 (15) and

Abbreviations: Bf, factor B; LPS, lipopolysaccharide; SRBC, sheep red blood cells; CR1, complement receptor 1; NP, (4-hydroxy-3-nitrophenyl)acetyl.

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the pBluescript KS (+) vector (Stratagene). A gel-purified 5.3-kb BamHI–EcoRI 5' Bf fragment, a 5.5-kb HindIII–SacII 3' Bf fragment, and a 1.8-kb EcoRI–HindIII fragment encoding neomycin resistance (neo') under control of the pgk promoter (16) were ligated together with BamHI–SacII-digested pBluescript KS (+). Subsequently, its 12.6-kb insert was excised from the vector and transferred into pBluescript KS (+) containing the gene encoding herpes simplex virus-thymidine kinase (HSV-tk) under the control of a polyoma-virus promoter and enhancer (17). Plasmid DNA for electroporation was linearized by digestion with SalI.

**Generation of Bf-Deficient Mice.** D3 (provided by T. Doetschman, University of Cincinnati, Cincinnati, OH) and J1 (provided by R. Jaenisch, Whitehead Institute for Biomedical Research, Cambridge, MA) embryonic stem (ES) cells were electroporated with the linearized Bf targeting vector as previously described (18). Homologous recombinants were selected using 250 \( \mu \)g/ml G418 (GIBCO/BRL) and 2 \( \mu \)M ganciclovir (Cytovene; Syntex Laboratories, Palo Alto, CA) and screened by Southern blotting using a 3' Bf external probe (Fig. 1A, probe A). After targeted cells had been injected into C57BL/6J blastocysts, resulting chimeric male mice were mated to C57BL/6J females (The Jackson Laboratory), and germ-line transmission was established. Mice were maintained under specific pathogen-free (SPF) conditions. Eight- to 12-week-old mice were used.

**Northern Blotting.** Mice were injected i.p. with 10 \( \mu \)g of lipopolysaccharide (LPS; *Escherichia coli* O111:B4; Sigma) and sacrificed 24 h later. Total RNA was extracted from liver and kidney using guanidinium thiocyanate/\( \text{CsCl} \) density gradient ultracentrifugation as previously described (19). Poly(A)+ RNA was prepared using oligo(dT)-cellulose spin columns (CLONTECH). Hybridization was with \( ^{32} \text{P} \)-labeled nearly full-length fragments of the mouse Bf or C2 cDNAs (20).

**Metabolic Labeling and Immunoprecipitation.** Resident peritoneal macrophages were cultured overnight in RPMI medium 1640 (Life Technologies), containing 10% heat-inactivated fetal bovine serum (FBS) (Life Technologies) as previously described (21). The cells were washed, then incubated in methionine-free medium with \( ^{35} \text{S} \)-methionine (ICN; specific activity 1,100 Ci/mmol; 1 Ci = 37 GBq) at 350 \( \mu \)Ci/ml and dialyzed FBS for 6 h. Bf protein was detected in the cell lysates and culture medium by immunoprecipitation (21) with goat anti-human Bf polyclonal Ab (Quidel, San Diego).

**Alternative Pathway Activation on Zymosan Particles.** C3 deposition on zymosan particles was assessed by using flow cytometry as described (22). Zymosan (Sigma) suspension was boiled and washed twice in gelatin/Veronal-buffered saline (GVBS) (23). Particles (1 × 10^6) and mouse sera (10 \( \mu \)l) in 100 \( \mu \)l of GVBS containing either 2 mM MgCl\(_2\) and 10 mM EGTA or 10 mM EDTA were incubated at 37°C for 15 min. The particles were washed three times in phosphate-buffered saline, pH 7.4 (PBS) with 1% bovine serum albumin (BSA), cooled to 4°C, and incubated with fluorescein-conjugated IgG goat anti-mouse C3 fraction (Cappel Laboratories) at 4°C for 30 min. After three more washes, deposition of C3 was analyzed by FACScan (Becton Dickinson) with CELLQuest software.

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**Fig. 1.** Targeted disruption of the gene encoding Bf in mice. (A) Black boxes = exons of the Bf and C2 genes. Factor D cleavage site in Bf exon 6 is shown by an open triangle. The locations of the 3' external probe (probe A) and internal probe (probe B) are indicated. Restriction sites: B, BamHI; E, EcoRI; H, HindIII; S, SacII; R, EcoRV. For HindIII, only the site relevant to the construction of the targeting vector is shown. (B) Southern blot of genomic DNA from offspring of Bf+/− intercrosses. Tail DNA was digested with either EcoRI and EcoRV or BamHI and was hybridized with probe A or probe B. (C) Detection of Bf exon 6 by PCR. Oligonucleotide primers: GAGAACAGCAGAAGAGGAAGATTGTCCTAG and CTTCCTCAATCAAAGTTGGTGAGGACCCTT. Exon 6 sequences were not amplified in tail DNA of Bf−/− mice.
**Hemolytic Activity.** *Alternative pathway.* Serial dilutions of serum in Veronal/Mg/EGTA buffer with 0.1% gelatin were added to rabbit erythrocytes in microtiter plates and incubated 1 h at 37°C, and the extent of lysis was measured in an automated enzyme-linked immunosorbent assay (ELISA) reader at 405 nm, and AP50 units (50% lysis) were calculated (24).

*Classical pathway.* Sheep red blood cells (SRBC) were sensitized with polyclonal mouse antisera. Twenty microliters of mouse serum diluted in GVBs with 0.15 mM CaCl2 and 0.5 mM MgCl2 (GVBSS (25)) was incubated with 70 µl of sensitized SRBC suspension at 37°C for 90 min (25). Percent lysis and calculation of CH50 was performed according to standard methods (23).

**Purification of Murine Bf.** Bf was purified from pooled normal mouse serum (Rockland, Gilbertsville, PA) according to a modification of the method of Williams and Sim (26). The supernatant, after precipitation with saturated ammonium sulfate (45%) and after addition of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 2 mM iodoacetamide) in 25 mM Tris HCl buffer, pH 7.4, with 0.5 mM Mg2+, 0.5 mM Ca2+, and 25 mM sodium caproate was applied to a 5-ml HiTrap Blue column (Pharmacia). After elution with a linear 0–2 M KCl gradient, fractions containing Bf were pooled and dialyzed overnight at 4°C against 10 mM potassium phosphate/5 mM NaEDTA buffer (pH 7.0) and applied to a 5-ml HiTrap SP ion-exchange column (Pharmacia), equilibrated with the same buffer. Bf-containing fractions were eluted with a linear 0–1 M NaCl gradient and dialyzed against Veronal-buffered saline (pH 7.3) overnight, at 4°C. The Bf yield was about 22% of the original serum. The final product had a protein content of 35 mg/ml. On Western blots bands corresponding to 108 kDa (Bf), 68 kDa (Bb), and 35 kDa (Ba fragment of Bf) were seen. Approximately 30% of Bf was converted to Bb and Ba. Only one other protein band (with the approximate mobility of albumin in the starting serum) was observed in the final preparation.

**Mouse Factor B ELISA.** Micro ELISA plates (Nunc Maxi-Sorp) were coated with 10 µg/ml antiserum to human Bf (Incastor) overnight at 4°C, blocked by PBS (pH 7.4, containing 2% nonfat dry milk and 15 mM NaN3). Samples were diluted 7.4, with 0.5 mM Mg2+, 0.5 mM Ca2+, and 25 mM sodium caproate, after precipitation with saturated ammonium sulfate (45%) and after addition of protease inhibitors (1 mM phenylmethylsulfonyl fluoride, 2 mM iodoacetamide) in 25 mM Tris HCl buffer, pH 7.4, with 0.5 mM Mg2+, 0.5 mM Ca2+, and 25 mM sodium caproate was applied to a 5-ml HiTrap Blue column (Pharmacia). After elution with a linear 0–2 M KCl gradient, fractions containing Bf were pooled and dialyzed overnight at 4°C against 10 mM potassium phosphate/5 mM NaEDTA buffer (pH 7.0) and applied to a 5-ml HiTrap SP ion-exchange column (Pharmacia), equilibrated with the same buffer. Bf-containing fractions were eluted with a linear 0–1 M NaCl gradient and dialyzed against Veronal-buffered saline (pH 7.3) overnight, at 4°C. The Bf yield was about 22% of the original serum. The final product had a protein content of 35 mg/ml.

**Flow Cytometric Analysis.** Flow cytometric analysis of spleen cells, thymocytes, and peritoneal exudate cells was performed as previously described (27), using anti-CD3ε (clone 145–2C11), anti-CD45R (clone 145–2C11), anti-CD4 (L3T4), and anti-CD8, all from PharMingen.

**Immunohistochemistry.** Ten days after i.p. injection of 100 µl of a 10% SRBC suspension in PBS, spleens were harvested and frozen sections were stained with anti-complement receptor 1 (CR1) mAb (8C12) (28) or peanut agglutinin (PNA) (Vector Laboratories) and polyclonal rat anti-mouse IgD (Southern Biotechnology Associates, Birmingham, AL) as previously described (29).

**Antigen-Specific Antibody Response.** Mice were immunized i.p. with 5 µg of (4-hydroxy-3-nitrophenyl)acetyl (NP)-ovalbumin (30) admixed with 0.1 ml of SRBC and 0.1 ml of Freund’s adjuvant. Twenty-one days later, the mice were boosted using the same dose of antigen in alum. Sera were harvested at days 10 and 28 after the initial immunization. Anti-NP IgG1s of low and high affinity were assessed by ELISA with NP13-BSA- and NP25-BSA-coated plates, respectively (29). Mice were immunized i.v. with 1 × 106 SRBC per mouse and the response was analyzed by hemolytic plaque assay on days 5 and 13. Cobra venum factor was given 24 h before immunization in selected experiments.

**Endotoxic Shock.** Shock was induced by i.p. injection of 0.75 or 1.0 mg of LPS (Salmonella typhosa 0901; Difco) in 200 µl of sterile PBS. Lethality was assessed 3 days later. Mice that survived at day 3 were observed for 1 week and appeared to recover completely.

### RESULTS AND DISCUSSION

**Generation of Bf-Deficient Mice.** The Bf gene was disrupted by replacing a segment from the EcoRI site in exon 3 through exon 7 by the neo" gene (Fig. 1A). This site for targeting was chosen to avoid compromising C2 expression (the 3' end of the C2 gene overlaps Bf promoter elements and is only ∼100 bp upstream of the most 5' transcriptional Bf start site) and the deletion encompasses exon 6, which encodes the factor D cleavage site necessary for Bf activation. Homologous recombiantons were obtained at a frequency of 1 of 30 doubly drug-resistant colonies for both D3 and J1 cell lines. Germ-line transmission was obtained with one D3 and one J1 targeted line. Heterozygous deficient mice were crossed to obtain homozygous Bf-deficient offspring. The Bf targeted allele was demonstrated by the presence of a 7.7-kb EcoRV–EcoRI fragment and a 6.6-kb BamHI fragment detected by 3' Bf and C2 cDNA probes, respectively (Fig. 1B). Hybridization with a neo" probe confirmed a single integration event (data not shown). Exon 6 sequences were not amplified from homozygous deficient mice (Fig. 1C).

**BfExpression.** Targeted disruption of the Bf gene was confirmed by Northern blotting and by studies of Bf synthesis in cell culture. Poly(A)+ RNA from liver of wild-type mice showed the normal Bf transcript (Fig. 2A). Bf-deficient liver showed no detectable intact Bf transcript, but did show a 1.6-kb band. This truncated transcript was detected with Bf cDNA probes encompassing sequences either 5' or 3' of the deleted segment. No neo" signal was detected in the truncated transcript (data not shown), suggesting that neo" is spliced out of the targeted transcript. Using oligonucleotides for exon 2 (5'-GATTTGTCAGAGCCGG-3') and 10 (5'-TTCAGGGAGTTCCACACGG-3'), reverse transcription–PCR amplification and sequencing of the truncated Bf mRNA from Bf--/-- mouse liver revealed a deletion of exons 3–7 with in-frame splicing of exon 2 and 8. This mRNA in the Bf--/-- mice is therefore capable of generating the truncated Bf protein observed in cell culture (see Fig. 3). Ala-97 is replaced by Asp because the codon GCA at the exon 2–3 junction is replaced by GAC at the new exon 2–8 junction in the truncated Bf. When the same oligonucleotides were used, amplification of...
Finally, alternative pathway-dependent hemolytic activity was in vitro cleavage of C3 by cobra venom factor (data not shown). 2
pathway activity in wild-type serum. Bf
Mixing Bf
Bf
ited C3 on zymosan in Mg
no detectable Bf activity. Serum from wild-type mice depos-
evidence established that the sera of the Bf-deficient mice had transcript from the LPS-responsive Bf promoter.
increased by treatment of the Bf-deficient mice with LPS (data not shown), consistent with transcription of the truncated transcript from the LPS-responsive Bf promoter.
Alternative Pathway Activity. Three independent lines of evidence established that the sera of the Bf-deficient mice had no detectable Bf activity. Serum from wild-type mice deposited C3 on zymosan in Mg/EGTA buffer, whereas serum from Bf−/− mice showed no detectable C3 deposition (Fig. 3). In Bf-deficient mice, no intact Bf protein was detected. However, small amounts of a truncated Bf protein (≈50 kDa) were found in cell lysate and media from Bf−/− macrophages. Bf+/− macrophages showed both intact and truncated Bf protein. Levels of the truncated transcript and proteins were increased by treatment of the Bf-deficient mice with LPS (data not shown), consistent with transcription of the truncated transcript from the LPS-responsive Bf promoter.
Growth and Development of Bf-Deficient Mice. Bf−/− mice grew normally and showed no gross phenotypic differences from their wild-type littermates. Although heterozygous matings in one mouse line, J24, produced homozygous deficient mice at a frequency of 1 in 4, matings of heterozygous animals from mouse line D68 produced fewer homozygous deficient mice than expected (P = 0.030) (Table 1). F1 heterozygous matings of the D68 strain showed even greater deviation from the predicted number of Bf−/− offspring (P = 0.012). These data suggest an effect on reproduction of Bf, together with one or more genes derived from the 129 strain which is/are lost by breeding of the Bf− locus into C57BL/6. Variable numbers of genes from 129 are contributed apparently randomly to each of the founders, so the difference between the D68 and J24 strains is not surprising. That this effect is specific for Bf and certain 129 gene(s) is also suggested by analysis of two other targeted deletions in D3 embryonic stem cells that showed no significant bias in genotype of the F1 heterozygous matings. Matings of homozygous males and heterozygous females or matings of homozygous females and heterozygous males produced appropriate numbers of offspring. Furthermore, live pups were obtained from crosses between male and female homozygous Bf-deficient mice for both the D68 and J24 mouse lines. These results suggest that Bf deficiency alone has no major effect on fertility or fetal development.
Taken together, these data indicate that under unstressed conditions in specific pathogen-free animal quarters, homozygous deficiency of Bf alone and absence of alternative pathway function has no significant adverse effect on reproductive performance.
Table 1. Genotype frequency: Offspring from heterozygous matings of Bf-deficient mice

<table>
<thead>
<tr>
<th>Strain/generation</th>
<th>n</th>
<th>−/−</th>
<th>+/−</th>
<th>+/+</th>
</tr>
</thead>
<tbody>
<tr>
<td>J24</td>
<td>45</td>
<td>11 (0.244)</td>
<td>23 (0.512)</td>
<td>11 (0.244)</td>
</tr>
<tr>
<td>D68</td>
<td>260</td>
<td>47 (0.181)*</td>
<td>138 (0.531)</td>
<td>75 (0.288)</td>
</tr>
<tr>
<td>F1</td>
<td>125</td>
<td>17 (0.136)*</td>
<td>70 (0.560)</td>
<td>38 (0.304)</td>
</tr>
<tr>
<td>≥F2</td>
<td>160</td>
<td>38 (0.238)*</td>
<td>82 (0.513)</td>
<td>40 (0.250)</td>
</tr>
</tbody>
</table>

J24 is from J1 embryonic stem cells, and D68 is from D3 embryonic stem cells. Difference from expected analyzed by 1 × 3 χ²: *, P = 0.030; †, P = 0.012; ‡, not significant.
capacity and postnatal growth. This was surprising because of past predictions that homozygous Bf deficiency was likely to be a genetic lethal or at least impose a major selective disadvantage. That prediction was based on several considerations: (i) The alternative pathway provides innate host defense against certain microorganisms. Relative decreases in alternative pathway proteins, due to either developmental delay or hypercatabolism, result in increased susceptibility to infection (31, 32). Clearly the importance of complement and other innate host defenses could not be assessed from these earlier studies because the “relative deficiency” was not selective for Bf or the alternative complement activation pathway. The development of this Bf-deficient mouse strain will make it possible to directly test this question in the future. (ii) Studies of Bf expression suggested that Bf might be necessary for several nonimmune functions (33) that affect reproductive efficiency and/or optimum postnatal survival—i.e., that Bf has a major role in diverse functions of fat cells, uterine and renal epithelium, etc. At least under unstressed conditions, our data indicated that Bf is not absolutely required for these functions. However, thus far the results suggest a negative effect of Bf deficiency on reproduction in the context of gene(s) found in the 129 mouse strain. (iii) Genetic deficiency of C2 (C2D), the highly homologous Bf counterpart in the classical component pathway, is the most common deficiency of complement in populations of European origin (gene frequency of C2-null = 1–1.5%) (12, 13), but no instances of homozygous Bf deficiency in humans or other animal species had been reported.‡‡ More than 93% of cases of C2 deficiency (type I) are due to a single mutation (a 28-bp deletion) found in the context of a specific major histocompatibility complex extended haplotype (34). Only three other C2 deficiency alleles in two kindreds have been recognized (ref. 35; X.W., A.C., R.A.W., M.L., and H.R.C., unpublished data). Hence, the high frequency of C2 deficiency results from a founder effect for C2D type I in which a single mutation occurs within an HLA haplotype (that itself may confer selective advantage). A comparison of the frequencies of type II C2 deficiency with Bf deficiency would show that they do not differ significantly.

Total Hemolytic Activity. Activation of the classical complement pathway generates C3b, which can serve either in the amplification of further C3 cleavage (by means of components of the alternative pathway) or as a constituent of the C5 cleaving enzyme, which in turn leads to assembly of the membrane attack complex (C5b–9) (5). To test the importance of this amplification loop in cytolysis, we measured total hemolytic complement activation (CH50) in wild-type and Bf−/− mouse sera from littermates. CH50 in Bf-deficient sera was 50% that in wild-type sera (Fig. 5). This reduction in CH50 was significant (P = 0.006) and not likely due to relative deficiencies of classical pathway constituents (C3 and C4 protein and C2 mRNA levels were similar in wild-type and Bf-deficient mice).

Lymphoid Organ Development and Antibody Response. Both Ba and Bb fragments have been implicated as modulators of the proliferation of B lymphocytes in vitro (9, 10, 36). To evaluate these activities of Bf in vivo, we examined the lymphoid compartment and humoral immune response in Bf-deficient mice. In Bf-deficient and wild-type mice numbers of peripheral blood lymphocytes and total serum IgM, IgG, and IgA were equivalent. Flow cytometric analysis showed similar expression of B220, CD3, CD4, and CD8 in spleen and thymus of Bf−/− and wild-type mice. Peritoneal B-1 cell population detected by CD5 mAb showed no phenotypic differences between wild-type and Bf−/− mice (data not shown). Histology of spleen, organization of follicular den...

‡‡An individual with genetic deficiency of Bf was described in an abstract (14), but important molecular biological details are lacking.
fore, it is possible that relative B cell growth support in Bf−/− is the result of absence of both Ba and Bb. Alternatively, this subtle change could be due to potential differences between the genetic background of the wild-type (C57BL/6) and homozygous deficient (Sw129 × C57BL/6) partially backcrossed to C57BL/6 mice. Immunization of Bf-deficient and wild-type mice with SRBC (5 × 10^5 i.v.) showed similar numbers of IgM on day 5 and IgG anti-SRBC plaque-forming cells (PFC) on day 13 (direct PFC/10^5: Bf+/+ = 723 ± 328; Bf−/− = 810 ± 325; indirect PFC/10^5: Bf+/+ = 2230 ± 1272; Bf−/− = 2312 ± 1047) although in cobra venom factor-treated animals the IgM and IgG responses were decreased nearly to background levels. Taken together, these data showed no major effect of Bf on the humoral immune response.

**Endotoxic Shock.** It has been suggested that activation of the complement system by endotoxin contributes to the pathophysiology of endotoxic shock (37). Although some data suggest that endotoxin-induced complement activation primarily involves the alternative pathway (38), the role of the alternative pathway in the development of endotoxic shock in vivo remains unclear. To evaluate this question, Bf-deficient mice were injected i.p. with either 0.75 or 1 mg of *S. typhosa* LPS. Wild-type and Bf-deficient mice showed similar endotoxin sensitivities in this shock model. [Mortality for 0.75 and 1.0 mg challenge: Bf+/+ = 3/8 (37.5%) and 31/38 (81.6%); Bf−/− = 7/13 (53.8%) and 19/30 (63.3%).] An apparent increased susceptibility to the lethal effects of LPS in C3- and C4-null mice has been reported by Carroll and co-workers (39). Our data, therefore, support the conclusions of Carroll that the *classical* pathway of complement is a major mechanism for LPS clearance and that the complement system is protective in endotoxic shock.

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