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REPORT #1

"Studies of Immunological Abnormalities Following Burn Trauma"

ANNUAL SUMMARY REPORT

Ann B. Bjornson, Ph.D.
William A. Altemeier, M.D.
H. Stephen Bjornson, M.D., Ph.D.

JUNE, 1976

Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND

Washington, D.C. 20314

Contract No. DAMD17-76-C-6023

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Studies were performed to determine the temporal sequence of humoral abnormalities which occur following burn trauma, their relationship to the severity and type of burn injury and to the occurrence of sepsis. Serum opsonic activity for Escherichia coli 075, conversion of C3 by inulin, total hemolytic complement (CH_{50}), levels of native C3, factor B, C3 inactivator (KAF), properdin (P), and immunoglobulins (Ig) were determined in 14 patients with burns involving 13% to 91% body surface during 6 to 8 weeks postburn. In the 12 uninfected patients, levels of IgG and IgA were reduced during the first 10 days postburn (continued)

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and decreased concentrations of P and IgM were demonstrated from 3 to 6 weeks postburn. C3 conversion was reduced from 10 days to 6 weeks postburn. Levels of C3, factor B, and KAF were normal or elevated for the entire study period. No difference in the occurrence of humoral abnormalities was noted in patients with burns caused by flame, immersion scald, or acid contact. Reduction in C3 conversion by inulin and P concentration were the only abnormalities which correlated with increasing burn size. Septicemia was documented in the other two patients. In one of these patients, reduction in CH_{50} occurred during septicemia due to Staphylococcus aureus, and in the other, reduction in all measurements of complement was associated with candidemia and Pseudomonas septicemia and occurred prior to the development of shock. Serum opsonic activity was only reduced significantly during sepsis, suggesting that this abnormality occurred as a result rather than a cause of infection. These results indicated that consumption of components of the classical and/or alternative pathways of complement activation may compromise host defense and perpetuate infection in the burn patient.

Methodology was developed for measuring the in vitro interaction of normal human serum and peripheral leukocytes with Bacteroides fragilis and Fusobacterium mortiferum. In aerobic and anaerobic environments, clinical isolates of Bacteroides fragilis were killed only by leukocytes in the presence of serum; Fusobacterium mortiferum was killed by leukocytes or serum alone or in combination. These observations provide a foundation for investigations into host defense mechanisms against anaerobic microorganisms.

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Abstract

The primary objectives of the present study were to establish the relationship between reduced serum opsonic activity for Escherichia coli 075 and other humoral abnormalities which occur following burn trauma and to determine the association between these abnormalities, the severity of the injury, and the occurrence of septicemia. In addition, because of the lack of fundamental knowledge concerning the definition of normal serum proteins involved in opsonization of gram-negative aerobic bacilli, an additional objective was to define the normal serum opsonic requirements for E. coli 075 and a clinical isolate of Pseudomonas aeruginosa. Also, due to the increased prevalence of gram-negative non-sporulating anaerobic microorganisms in surgical infections, studies of the interaction of human serum and leukocytes in the killing of Fusobacterium mortiferum and Bacteroides fragilis were undertaken.

Serum opsonic activity for E. coli 075, conversion of C3 by inulin, total hemolytic complement (CH_{50}), levels of native C3, factor B, C3 inactivator (KAF), properdin (P), and immunoglobulins (Ig) were determined in 14 patients with burns involving 13% to 91% body surface during 6 to 8 weeks postburn. In the 12 uninfected patients, levels of IgG and IgA were reduced during the first 10 days postburn, and decreased concentrations of P and IgM were demonstrated from 3 to 6 weeks postburn. C3 conversion was reduced from 10 days to 6 weeks postburn. Levels of C3, factor B, and KAF were normal or elevated for the entire study period. No difference in the occurrence of humoral abnormalities was noted in patients with burns caused by flame, immersion scald, or acid contact. Reduction in C3 conversion by inulin and P concentration were the only abnormalities which correlated with increasing burn size. Addition of normal human serum to the burn sera did not normalize C3 conversion, suggesting that reduced C3 conversion might be caused by a circulating inhibitor. Similar results were

obtained in an additional five patients whose level of C3 and C3 conversion by inulin were also determined over a 3-week postburn period.

Septicemia as documented by positive blood cultures and clinical findings was observed in two of the fourteen patients. In one of these patients, reduction in CH_{50} occurred during septicemia due to S. aureus, and in the other, reduction in all measurements of complement was associated with candidemia and Pseudomonas bacteremia and occurred prior to the development of shock. Serum opsonic activity was only reduced significantly during sepsis, suggesting that this abnormality occurred as a result rather than a cause of infection. These results indicate that consumption of components of the classical and/or alternative pathways of complement activation may compromise host defense and perpetuate infection in the burn patient.

The ability of partially purified preparations of human C3, factor B, properdin, and normal IgG to promote phagocytosis and intracellular killing of E. coli 075 and P. aeruginosa by normal human peripheral leukocytes was determined. When the preparations were tested individually or in combination in physiological concentration, no phagocytosis and intracellular killing by leukocytes was demonstrable. These findings are preliminary and only begin to approach the complex problem of determining which components of the alternative complement pathway participate in opsonization of the microorganisms and the requirement for normal IgG.

Methodology was developed for measuring the effects of human serum and peripheral leukocytes on the killing of gram-negative non-sporulating anaerobic microorganisms in vitro under aerobic and anaerobic conditions. F. mortiferum was killed directly by leukocytes or serum alone or in the presence of serum and leukocytes; similar results were obtained in aerobic and anaerobic environments. In both environments, B. fragilis subspecies fragilis was killed by leukocytes in the presence of serum but not by serum alone. Killing of this

microorganism by leukocytes in the absence of serum occurred in the aerobic environment, but not under anaerobic conditions. In both environments, B. fragilis subspecies thetaiotaomicron was only killed by leukocytes in the presence of serum and not by either leukocytes or serum alone. In the anaerobic environment, total counts in the reaction mixtures containing leukocytes, bacteria, and serum, or leukocytes and bacteria alone were compared to bacterial counts in the supernatants of these reaction mixtures. Similar counts for all three microorganisms were obtained, suggesting that phagocytosis of the microorganisms was rapidly followed by intracellular killing. These observations provide a foundation for further investigations into host defense mechanisms against anaerobes.

FOREWORD

In conducting the research described in this report, the investigators adhered to the "Guide for Laboratory Animal Facilities and Care," as promulgated by the Committee on the Guide for Laboratory Animal, Resources, National Academy of Sciences - National Research Council.

ACKNOWLEDGEMENT

The investigators express their gratitude to Dr. Clark D. West, Children's Hospital Research Foundation, Cincinnati, Ohio, for the gift of reference antisera to human properdin, KAF, factor B, and B antigen of C3, and to Mrs. Marcia Bacon for the excellent technical preparation of this report.

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I. INTRODUCTION

Antibiotic therapy, used widely for over a quarter of a century, has not reduced the overall incidence of microbial infections in surgical patients (1,2). General use of antibiotics has been associated with a decrease in the number of surgical infections caused by Staphylococcus aureus, beta streptococci, and Streptococcus pneumoniae. However, there has been a disturbing increase in the incidence of surgical infections caused by microorganisms previously considered to be of relatively low virulence. The bacteria most commonly associated with this increased incidence of infection are the gram-negative aerobic bacilli including Escherichia coli, Enterobacter, Proteus, Pseudomonas aeruginosa, and Serratia marcescens. In addition, there has been a growing awareness of the importance of gram-negative non-sporulating anaerobic infections produced principally by members of the Bacteroides.

Management of surgical infections has classically involved antibiotic therapy and the meticulous care of the surgical wound. However, in addition to these therapeutic modalities, attention has recently been focused upon defining immunological abnormalities of the host which may predispose him to microbial infection. Studies of alterations of host defense mechanisms in surgical patients have predominantly been carried out in patients with severe thermal injury. The data obtained from these investigations have provided preliminary evidence to suggest that neutrophil anti-staphylococcal activity (3), phagocytic function of the reticuloendothelial system (4), cell-mediated immunity (5), and levels of immunoglobulins (6-10) and classical complement components (11) are reduced following burn trauma. These altered parameters of host defense have not, however, been clearly linked to increased susceptibility to microbial infections.

Recently, the important role of serum opsonins in protection against infection with gram-negative aerobic bacilli has been demonstrated in patients with

severe thermal injury (12-14). Reduced serum opsonic activity for E. coli and P. aeruginosa in these patients was associated with the onset of septic episodes with gram-negative aerobic bacilli. The primary objectives of our research were to study the relationship between reduced serum opsonic activity and other humoral abnormalities which occur following burn trauma and to determine the relationship between the abnormalities, the severity of the injury, and the occurrence of septicemia. In addition, because of the lack of fundamental knowledge concerning the definition of normal serum proteins involved in opsonization of gram-negative aerobic bacilli, an additional objective was to study concurrently the normal serum opsonic requirements for these microorganisms. Also, due to the increased prevalence of gram-negative non-sporulating anaerobic microorganisms in surgical infections, studies of their opsonic requirements were undertaken as a means of providing a foundation for investigations into host defense mechanisms against anaerobes.

II. BACKGROUND

In 1903, Wright and Douglas established that phagocytosis of various pathogenic microorganisms by human leukocytes could not occur in the absence of normal human serum (15,16). The phagocytosis promoting activity of normal serum was found to be heat-labile and appeared to be due to adsorption of serum components onto the bacteria which rendered the organisms susceptible to the phagocytes. The serum components responsible for promoting phagocytosis were termed "opsonins."

Until recently, no agreement existed concerning the nature of serum opsonins and their relationship to complement and antibody. Although it is known that immune IgG antibodies and the first four components of the classical complement pathway can promote opsonization of bacteria (17), the discovery of an alternative[1] (alternate) or properdin pathway of complement activation has greatly broadened this field of investigation.

The properdin system was originally described by Pillemer et al. in 1954 as a group of serum factors which interacted with zymosan and a variety of other polysaccharides and lipopolysaccharides to inactivate C3 (18). Studies by several investigators showed that the properdin system consisted of properdin itself and two other serum components, hydrazine-sensitive factor A (19) and heat-labile factor B (50°C, 30 min.) (20). The properdin system was implicated in opsonic (21), bactericidal (22), and viricidal reactions (23), and in the lysis of erythrocytes from patients with paroxysmal nocturnal hemoglobinuria (24), and it was proposed that the properdin system was a unique and nonspecific serologic mechanism.

Since that time, an activated form of properdin ($\overline{P}$) has been isolated and shown to be a protein distinct from known immunoglobulins and complement components (25). More recently, Gotze and Muller-Eberhard have established that an alternative pathway of complement activation exists which preferentially activates

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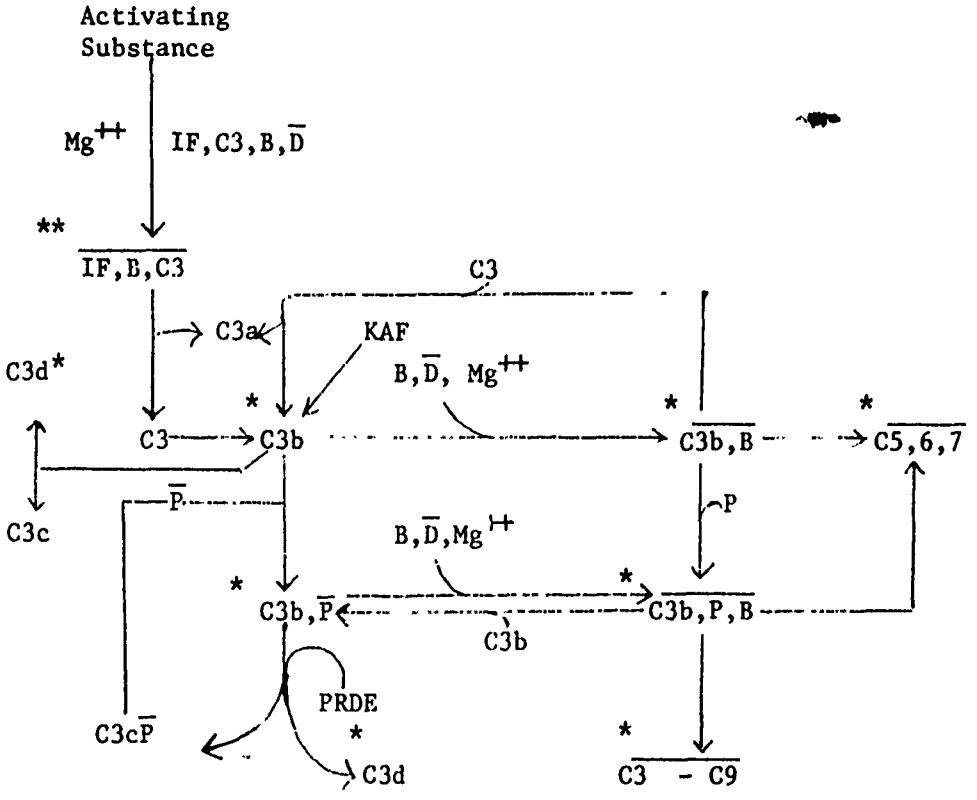
[1]Nomenclature used for the alternative pathway components were those recommended at the Second International Congress of Immunology, Brighton, England, 1974; the terminology used in the original contract is in parentheses.

C3 and bypasses C1, C4, and C2 (26). These investigators originally proposed that unactivated properdin (P) functioned in the early stages leading to activation of C3-C9 via this pathway (27,28). It was postulated that the activation of properdin ($P \longrightarrow \overline{P}$) did not occur by direct interaction with activating substances but was mediated by additional serum factors such as properdin convertase (29), or initiating factor (28). In the fluid phase, it was shown that $\overline{P}$ could interact with C3 to generate an enzymatic activity which in the presence of factors B (C3 proactivator) and $\overline{\overline{D}}$ (C3 proactivator convertase) cleaved C3 to C3a and C3b (28,30). The latter fragment was shown to initiate a positive feedback mechanism by modulating the conformation of factor B so that this molecule could be activated by factor $\overline{D}$ to cleave C3 (28,31,32). This activation of factor B has been shown to involve cleavage to two fragments, a gamma globulin, $\overline{B}$, and an alpha globulin; $\overline{\overline{B}}$ is the active C3 convertase. This feedback mechanism is independent of $\overline{P}$ and can be inhibited by C3b inactivator (KAF) which cleaves C3b to C3c and C3d (33). It has also been recently shown that, in the fluid phase, native C3 and factors B and $\overline{D}$ can act together to convert C3 (34), however the significance of this finding has not been established.

Recently, unactivated or precursor properdin (P) has been obtained from normal human serum in purified form, and its function has been established (35). P has been shown to stabilize the enzyme $\overline{C3b,B}$ which is formed by the feedback mechanism involving C3b and factors B and $\overline{D}$. Both $\overline{C3b,B}$ and stabilized $\overline{\overline{C3b,P},B}$ serve as C3 or C5 convertases, suggesting that the function of P in the alternative complement pathway may be merely to stabilize an enzyme. The C3 and C5 convertase functions of $\overline{C3b,P,B}$ are inhibited by fluid phase C3b which causes dissociation of factor B. The resultant C3b, $\overline{P}$ enzyme can be dissociated by an enzyme referred to as properdin receptor destroying enzyme (PRDE) which cleaves $C3b,\overline{P}$ to $C3c\overline{P}$ and C3d.

The initial stage in the conversion of C3 by zymosan has recently been shown to require native C3, factors B and $\overline{D}$, and initiating factor (IF), a globulin which reacts immunochemically with C3 nephritic factor (36). The tentative reaction sequence of the alternative pathway may be visualized below. The single asterisk indicates that the protein was bound to sensitized erythrocytes (EA), and the double asterisk indicates that the protein was bound to zymosan; these were the particulate activating substances which were used to demonstrate the various steps in the pathway.



The participation of an alternative complement pathway in opsonization of S. pneumoniae, S. aureus, P. mirabilis, E. coli, and P. aeruginosa has been suggested by several studies (37-42), although the necessary components have not been identified. Factor B has been shown to be a heat-labile component in normal human serum which participates in opsonization of E. coli (40) and P. aeruginosa (42).

No general conclusions have been formulated concerning the requirement for immunoglobulin in "natural" opsonic processes; however, it is clear that S. pneumoniae and P. aeruginosa utilize IgG antibodies in normal human serum as opsonins (37,41, 42). Unfortunately, there have been no reports concerning requirements for serum factors for opsonization or direct killing of anaerobic gram-negative microorganisms due to the lack of development of experimental methods.

Increased susceptibility of patients with severe thermal injury to gram-negative bacteremia has been associated with reduced serum opsonic activity by Bjornson and Alexander (12-14). The opsonic activity for P. aeruginosa and E. coli of sera obtained within 12 days following injury from nine patients with burns involving 36% to 85% total body surface was found to be reduced. Two of three sera obtained from patients with burns involving 14% to 25% body surface during the first week postburn had normal opsonic activity for both bacterial strains. In four of five patients with burns involving greater than 25% body surface, opsonic activity for P. aeruginosa returned to normal within 15 days postburn, and remained normal thereafter for up to 49 days postburn. Fluctuations in opsonic activity for E. coli were observed in three of these patients after the first week postburn. These three patients also had positive blood cultures during their clinical course. One of the patients had a series of positive blood cultures with P. vulgaris and S. marcescens during times in which opsonic activity of his serum for P. aeruginosa and E. coli was markedly reduced. Blood cultures became negative in this patient when serum opsonic activity returned to normal. In another patient, bacteremia with Herellea, alpha-streptococci, and diphtheroids occurred after opsonic activity of the serum for both bacterial strains had returned to normal; however, this patient had clinical evidence of burn wound sepsis with P. aeruginosa on the 4th postburn day associated with reduced serum opsonic activity for both bacterial strains. In the fifth patient, opsonic activity for

P. aeruginosa was not restored to normal until the 23rd postburn day, and reduced opsonic activity for E. coli was observed in all sera examined during 28 days postburn. This patient had two positive blood cultures and died on day 28 of sepsis with E. coli and yeast.

Bjornson and Altemeier subsequently measured serum opsonic activity for E. coli in an additional five patients with burns involving 45% to 80% total body surface during a 3-week postburn period (Figure 1). In all patients, opsonic activity was reduced immediately following the injury. In patients 1 and 2, opsonic activity was reduced during the entire 3-week postburn period. Patient 1 had a positive blood culture with P. mirabilis on the 11th postburn day, and patient 2 had a series of positive blood cultures with P. aeruginosa during the first 11 days postburn. In the other three patients, opsonic activity was fully restored to normal within 1 week postburn. Patients 3 and 4 had positive blood cultures with Herellea, Flavobacterium, diphtheroids, or yeast or mixtures of these organisms at times during the second and third postburn weeks when their serum opsonic activity for E. coli was normal. In patient 5, opsonic activity became reduced again during the third week postburn. This patient had no positive blood cultures, but developed pneumonia on the fourth postburn day which persisted during the period of study. Sputum cultures during this time were positive for S. aureus, P. morganii, and C. albicans. Addition of normal human serum to all of the burn sera from patients 1 and 2 and to the initial serum samples from patients 3, 4, and 5 fully restored their opsonic activity suggesting that reduction in opsonic activity of the burn sera was related to a deficiency of one or more critical normal serum proteins required for opsonization.

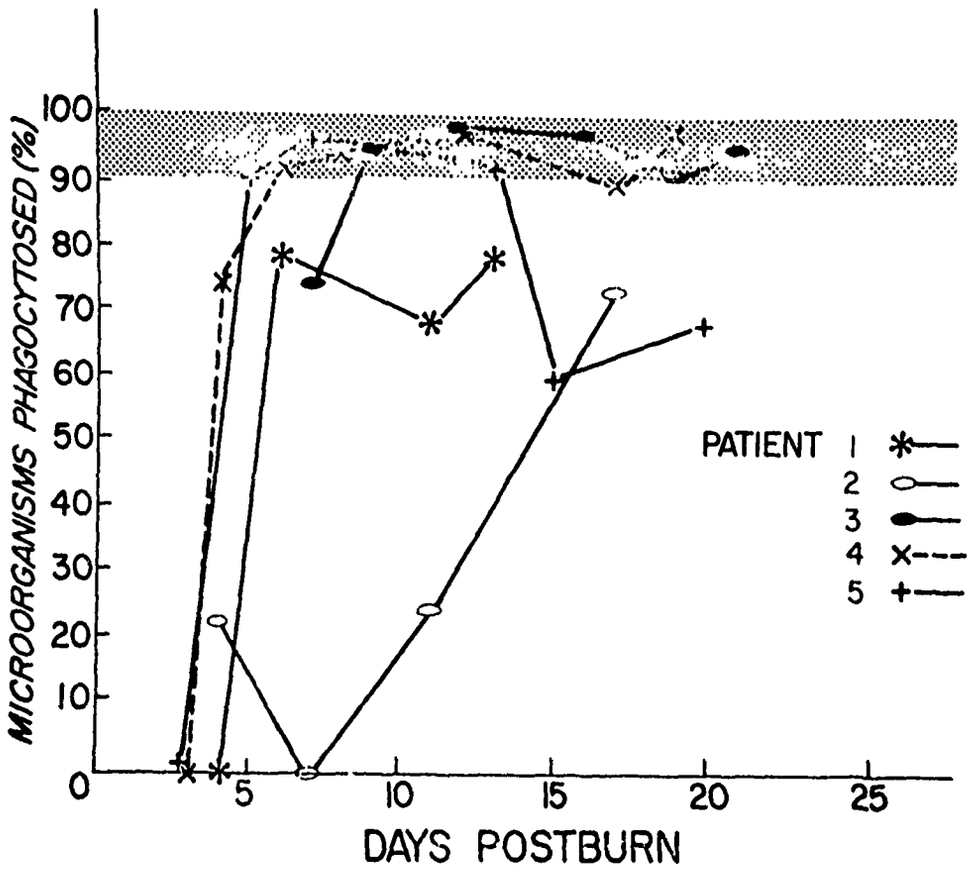


Fig. 1. Opsonic activity of the burn sera for E. coli 075. The shaded area represents the opsonic activity of 10 normal sera (± 2 SD). The total burn sizes were as follows: patient 1, 80%; patient 2, 71%; patient 3, 65%; patient 4, 55%; patient 5, 45%.

III. EXPERIMENTAL APPROACH

The proposed study was divided into three integrated parts. In the first part of the study, various parameters were to be measured in the sera of 10 to 20 severely burned patients during 4 weeks postburn as follows:

a. opsonic activity for *E. coli* 075

b. concentrations of C3, factor B, properdin, and KAF

c. conversion of C3 by inulin

d. total hemolytic complement

e. immunoglobulin levels (IgG, IgA, IgM)

If a deficiency of complement components or immunoglobulin was demonstrated in a burn serum with reduced opsonic activity, then restoration of opsonic activity was to be attempted using the respective purified protein or proteins. Blood cultures were to be drawn from the patients concurrently with serum samples and microorganisms, if present in the cultures, were to be isolated and identified.

In the second part of the study, components of the alternative complement pathway and immunoglobulin G (IgG) were to be obtained in purified form from normal human serum and the ability of combinations of these proteins to promote phagocytosis of *E. coli* 075 (40) and a strain of *P. aeruginosa* (41,42) by normal human peripheral leukocytes was to be determined. These were to be the strains of bacteria selected for these experiments for several reasons: (a) Both genera of gram-negative aerobic bacilli are frequent pathogens in surgical infections; (b) neither strain was susceptible to the bacteriolytic action of normal human serum; (c) both strains were rapidly killed by human peripheral leukocytes in the presence of normal human serum; (d) the strain of *E. coli* 075 was to be used as the test strain for measuring opsonic activity of the burn sera; definition of the normal

serum opsonins for this bacterium would provide essential information for delineating alterations of these factors in the burn sera.

In the third part of the investigation, methodology was to be developed for measuring opsonic requirements for F. mortiferum (formerly referred to as F. ridiculosum) and various clinical isolates of B. fragilis using modifications of presently available techniques.

IV. PROGRESS

A. Changes in Humoral Factors of Host Defense Following Burn Injury

1. Results

Our initial efforts were directed toward obtaining components of the alternative complement pathway from normal human serum in purified form suitable for preparation of antisera. The antisera were necessary for developing methodology for measuring serum levels of factor B, KAF, properdin, and B antigen of C3. Partially purified preparations of factor B and properdin were obtained, and goats were immunized for preparation of antisera to these proteins; goat antisera to commercially purified C3 and KAF were also prepared. Aged normal human serum was prepared for adsorption of antibodies to the A and D antigenic determinants in the anti-C3 antiserum. In addition, normal human sera depleted of factor B, properdin, or KAF were prepared for removal of contaminating proteins in each respective antiserum. The antisera were adsorbed with the respective depleted sera. Each of the adsorbed antisera in agarose yielded single lines against normal human serum in immunoelectrophoresis and double immunodiffusion and complete identity in double immunodiffusion with reference goat antiserum to each respective protein. The reference antisera to factor B, KAF, properdin, and B antigen of C3 were generously provided by Dr. C. D. West, Childrens Hospital Research Foundation, Cincinnati, Ohio.

While antisera were being prepared and methodology was being developed as described above, we decided to begin our investigation by determining the concentration of native C3 and C3 conversion by inulin in the sera of the five patients with burns involving 45% to 80% total body surface in which we previously measured opsonic activity for *E. coli* 075 (see Background). Several reasons prompted us to initiate this preliminary study as follows: a) To determine if C3 conversion

via the alternative pathway was reduced following burn trauma; b) to determine if a correlation between opsonic and C3 converting functions could be demonstrated; and c) antisera to the B antigenic determinant of C3 had been prepared in my laboratory prior to the initiation of this contract which could be used for measuring C3 concentration and conversion.

During the first week postburn, the concentration of native C3 was abnormal in two of the five patients and was at a low normal level in the other patients (Figure 2). In all of the patients, C3 levels remained normal or elevated thereafter for the duration of the study. C3 conversion by inulin was reduced in all patients to varying degrees during the 3 week postburn period (Figure 3). In patients 1 and 2, C3 conversion was markedly reduced during 16 days postburn. In patients 3 and 4, C3 conversion was markedly reduced initially and then ranged from an abnormal to low normal level after the sixth postburn day. In patient 5, C3 conversion was at a low normal level initially and became maximally reduced by the 20th postburn day.

Addition of 50% pooled normal serum to sera obtained from patients 1 and 2 did not restore C3 conversion to normal (Figure 4). In patients 3 and 4, C3 conversion was initially restored to an abnormal or low normal level by addition of normal serum. During the second and third postburn weeks when C3 conversion was reduced in patient 5, addition of normal serum to this patient's sera restored C3 conversion to a low normal level. Addition of 50% pooled normal serum to serum depleted of properdin or factor B restored C3 conversion by inulin to normal (55% and 68% respectively); C3 conversion by inulin in the depleted sera ranged from 0% to 20%. Since normal serum was capable of fully restoring C3 conversion of sera depleted of components of the alternative complement pathway, these findings suggested that reduction of C3 conversion in the burn sera was not related to a deficiency of serum proteins but possibly to the presence of an inhibitor.

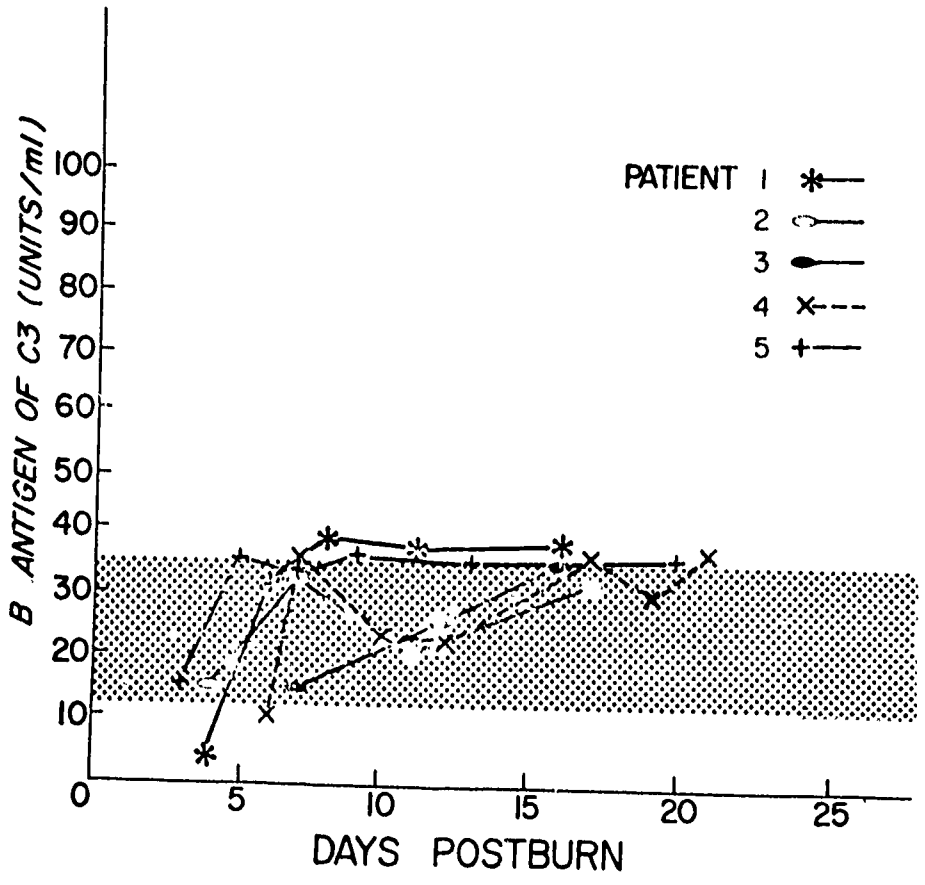


Fig. 2. Concentration of native C3 in the burn sera. The shaded area represents the B antigen concentration in 10 normal sera (± 2 SD).

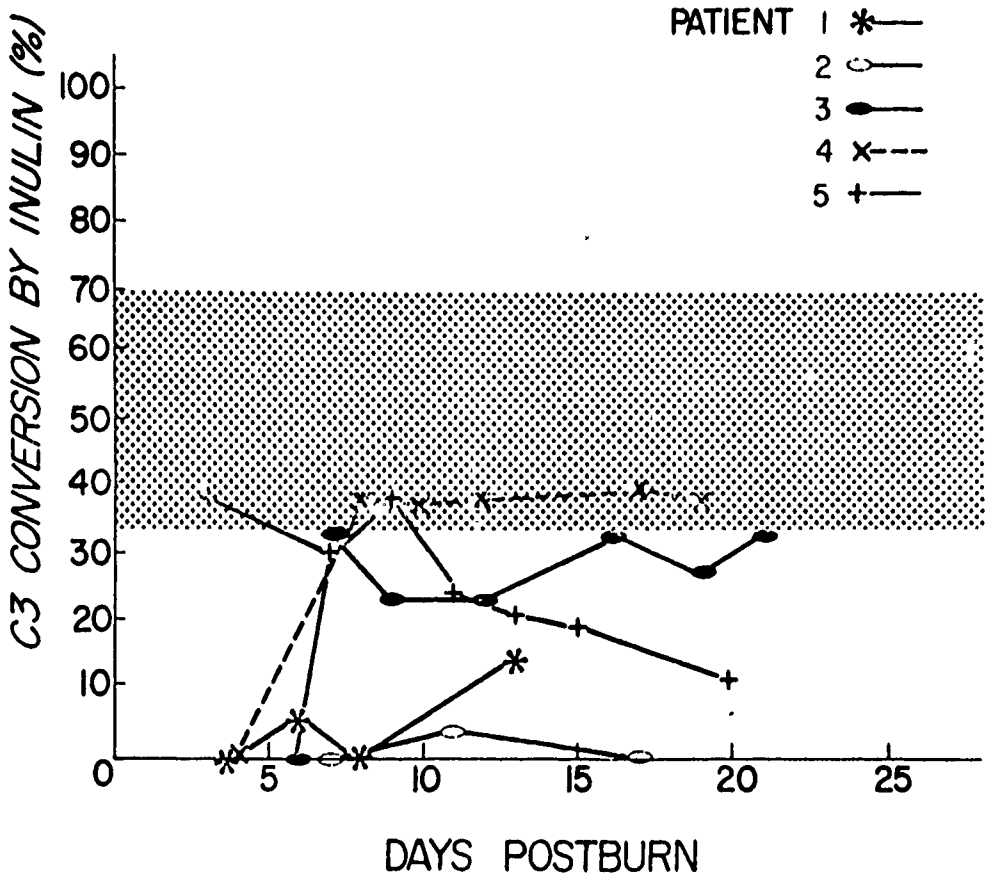


Fig. 3. C3 conversion by inulin in the burn sera. The shaded area represents the percent of C3 conversion in 10 normal sera (± 2 SD).

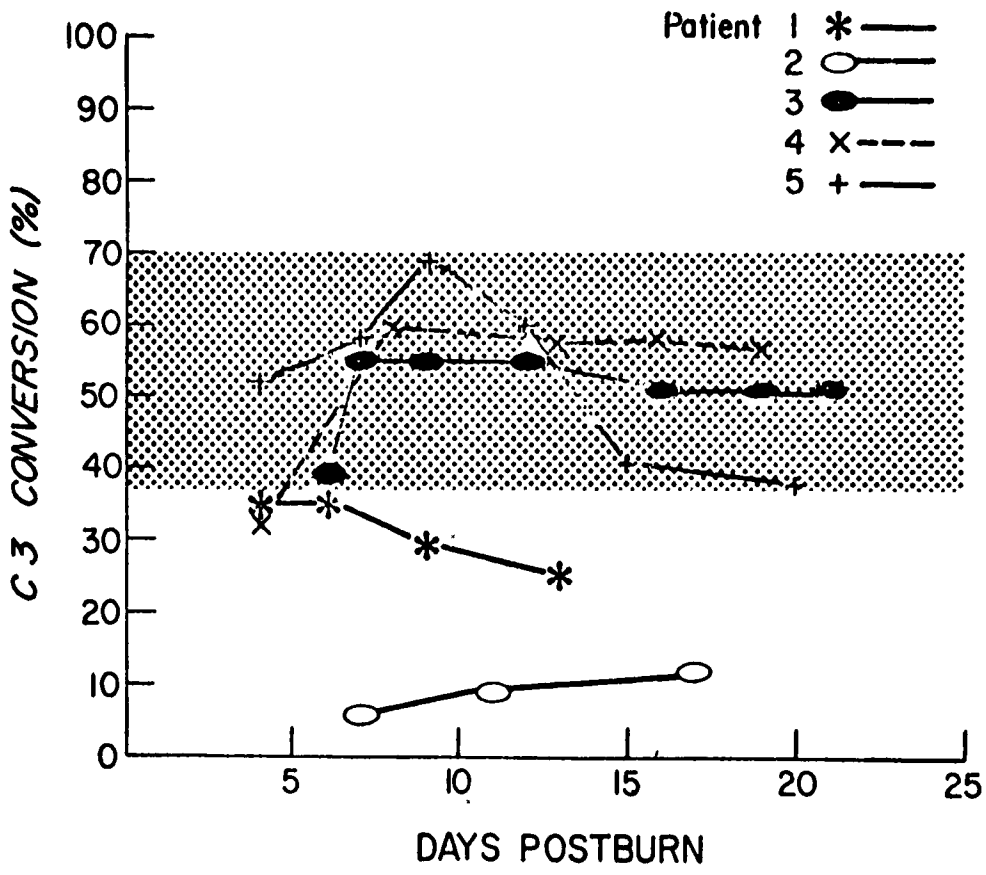


Fig. 4. C3 conversion by inulin in the burn sera supplemented with pooled normal human serum. The shaded area represents the percent of C3 conversion in five normal sera supplemented with pooled normal human serum (± 2 SD).

Conversion of C3 by inulin occurs via the alternative pathway and reduction in this function may compromise host defense by reducing the opsonic capacity of a serum for a potential infecting microorganism. Our preliminary data, however, suggested that a cause and effect relationship probably did not exist between reduction in serum opsonic activity for E. coli 075 and C3 conversion by inulin, primarily because of the finding that in three of the five patients, C3 conversion and opsonization were not always reduced concurrently. These findings suggested that the opsonic capacity of the burn serum for E. coli 075 may not be dependent upon the function of the alternative pathway or that only minimal C3 conversion is required for opsonization of this microorganism. Either possibility would explain why C3 conversion by inulin in a burn serum was reduced while opsonic activity for E. coli 075 was normal. We hoped that further studies of changes in components of the alternative and classical pathways of complement activation and of immunoglobulins in the sera of a larger group of patients would provide information regarding the significance of these observations.

Levels and activities of complement were next determined in the sera of an additional ten patients with injuries caused by flame burns, and the data were grouped according to burn size. Clinical information on the patients within the groups is shown in Table 1. In the patients with the largest burn sizes (group A), C3 conversion by inulin became significantly reduced after the first 10 days postburn and remained markedly reduced through the 41st to 50th day postburn period (Figure 5). C3 conversion in the patients with intermediate burn sizes (group B) was also reduced during 11 to 40 days postburn; however, the reduction was significant only during the 21st to 30th postburn day period. Conversion of C3 by inulin in patients with the smallest burn sizes (group C) was normal for the entire study period. Properdin levels were significantly reduced in all three groups of patients during the first 10 days postburn. Properdin levels remained significantly

Table 1. Clinical Features of 14 Patients with Burn Injury

Group	Patient No.	Age*	Sex‡	Cause of Burn	Body Surface Injured	
					Total %	Third Degree %
A	1	5	F	flame	75	70
	2	21	M	flame	73	56
	3	3	F	flame	60	42
B	1	59	M	flame	45	28
	2	9	F	flame	49	15
	3	9	M	flame	48	14
	4	2	M	flame	45	15
C	1	43	M	flame	40	7
	2	49	M	flame	30	6
	3	11	M	flame	13	10
	I	30	M	flame	91	2
	II	2	F	immersion scald	37	30
	III	16	F	sulfuric acid contact	60	50
	IV	39	F	flame	70	37

*Patients ranging in age from 2 to 11 years were hospitalized at the Shriners Burn Institute and patients over 15 years of age were hospitalized at the Cincinnati General Hospital.

‡M = male, F = female.

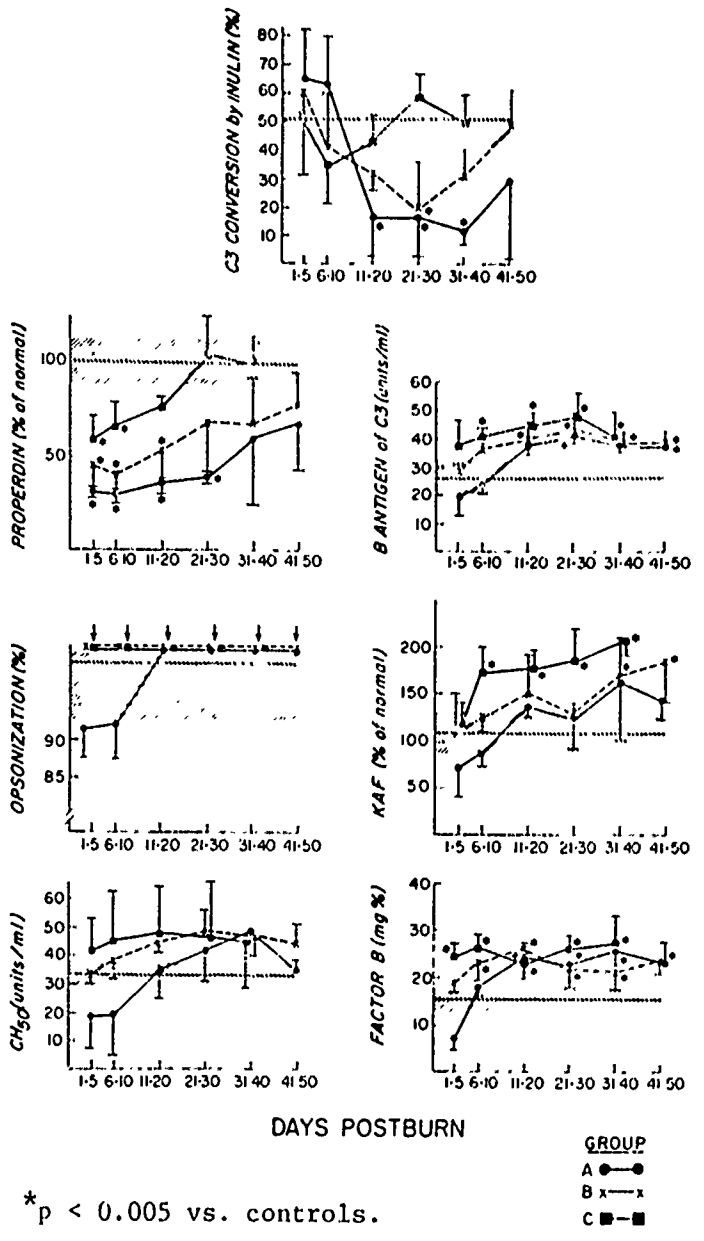


Fig. 5. Levels and activities of components of the classical and alternative pathways of complement in the sera of 10 patients with increasing burn sizes. The average burn ratio {total burn size (%)/area of third degree burn (%)} was 69/56 for group A, 47/18 for group B, and 27/8 for group C. Refer to Table 1 for data on individual patients in each group. The shaded areas represent the variation for 15 normal sera (mean ± 1 SD), and the vertical bars represent 1 SD. The arrows refer to 99.9% opsonization.

reduced during 30 days postburn in group A and during 20 days postburn in group B; properdin levels were subsequently restored to normal in both groups. Properdin levels were restored to normal in group C during the 11th to 20th postburn day period. Serum opsonic and hemolytic activities were reduced concurrently in group A during the first 10 days postburn; the reduction was not, however, statistically significant. In groups B and C, opsonic and hemolytic activities were normal for the entire duration of the study. Levels of native C3, KAF, and factor B were normal or elevated in groups B and C for the entire study period. In group A, C3, factor B, and KAF levels were reduced during the first 5 days postburn, however the reduction was not significant.

Complement levels and activities following extensive second degree thermal injury (patient I), and burn injuries caused by immersion scald (patient II), or by contact with sulfuric acid (patient III) are shown in Figure 6. In patients I and II, C3 conversion was normal during the first 10 days postburn, was reduced during 11 to 40 days postburn, and was restored to normal during the 41st to 50th postburn day period. In contrast, in patient III with acid burns, C3 conversion by inulin was not reduced until the 21st to 30th postburn day period and remained reduced for the entire study period of 60 days postburn. Properdin levels in patients I and II were initially reduced, but were restored to normal by the 31st to 40th postburn day period. In patient III, properdin levels remained depressed through the 60th postburn day. Serum opsonic and hemolytic activities were only reduced in the serum of patient II. Reductions in these activities occurred concurrently during the first 10 days postburn, and thereafter returned to normal or elevated levels. Levels of C3, KAF, and factor B were normal or elevated in all of the three patients for the duration of the study.

During the period of observation, two of the fourteen patients had sepsis as documented by chills, fever, hypotensive episodes, and positive blood cultures.

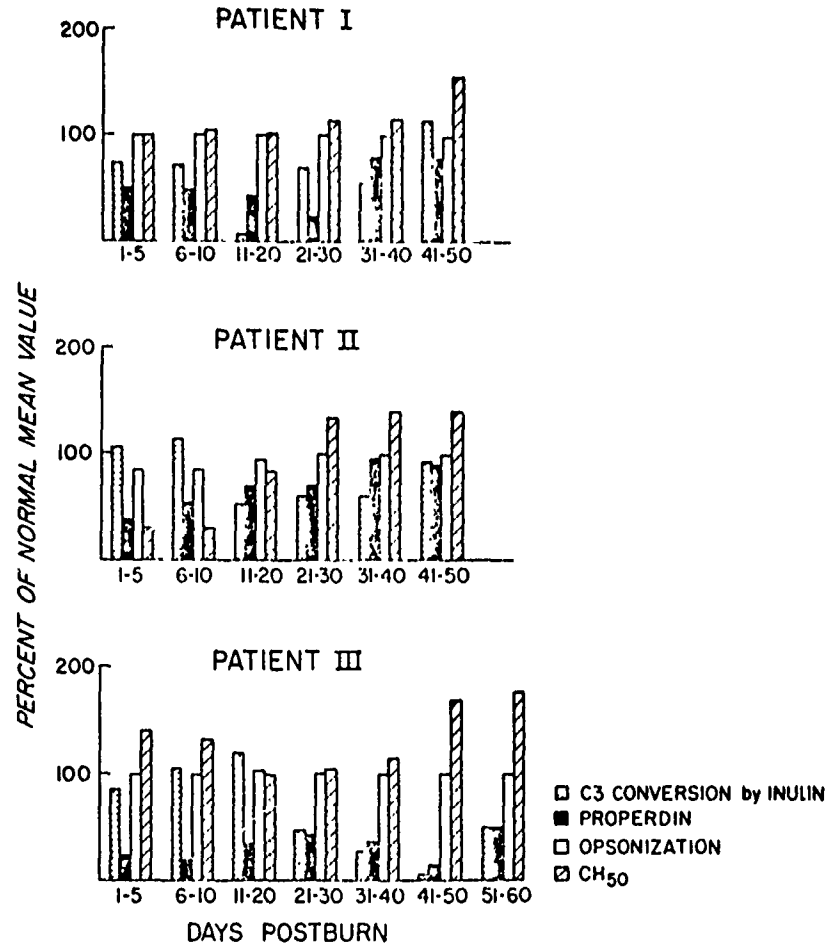


Fig. 6. Levels and activities of components of the classical and alternative pathways of complement following extensive second degree thermal injury (Patient I) and burn injuries caused by immersion scald (Patient II) or by sulfuric acid contact (Patient III).

Patient II developed sepsis with S. aureus on the third postburn day associated with subclavian catheterization. Positive blood cultures for S. aureus were obtained on days 3 and 6; blood cultures were negative thereafter in this patient. Patient IV had multiple septic episodes during her clinical course and died of septic shock on the 42nd postburn day. In this patient, positive blood cultures were obtained on day 5 with P. aeruginosa, on day 19 with P. aeruginosa and C. albicans, and on days 33 and 41 with C. albicans.

The complement profile in patient IV who had severe thermal injury and sepsis was different from that seen in uncomplicated burn trauma (Figure 7). Levels of native C3, factor B, and KAF were low initially, were restored to normal during the sixth to tenth postburn day period, and then became reduced during the 31st to 40th postburn day period prior to the development of shock. Concentration of properdin and conversion of C3 by inulin were markedly reduced during the entire 40-day period. Serum opsonic and hemolytic activities were reduced concurrently during the first 10 days postburn and then again during the 21st to 40th postburn days. These results indicated that consumption of the classical and alternative complement pathways can occur in association with the development of septic shock in the thermally injured patient.

Because of the differences in immunoglobulin levels during various phases of human development, the data were grouped according to two age categories, 0 to 3 years and 9 to 59 years (Figure 8). In both groups, levels of IgA and IgG were significantly reduced during the first 5 days postburn. In the older group, levels of IgG and IgA were restored to normal during the sixth to tenth postburn day period, and remained normal for the duration of the study. In the younger group, levels of IgA were restored to normal during the sixth to tenth postburn day period, although IgG levels were not fully restored to normal until the 21st to 30th postburn day period. IgM levels in both groups were significantly reduced during the entire

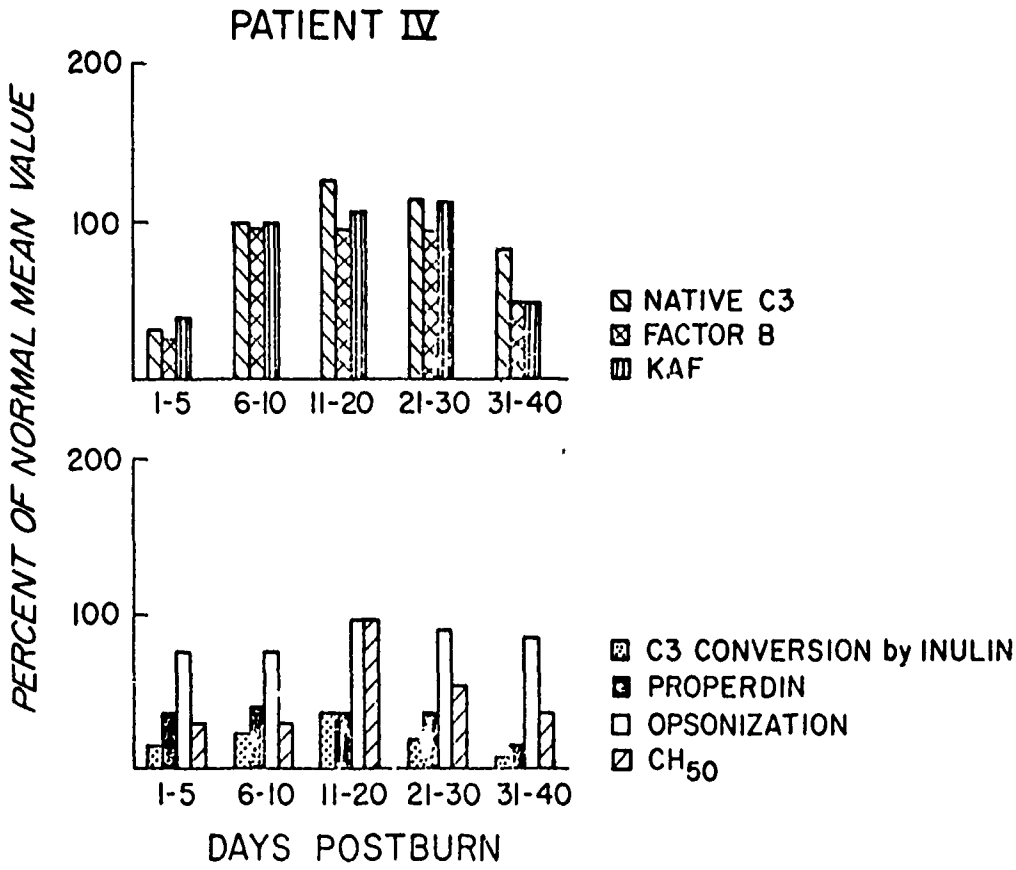
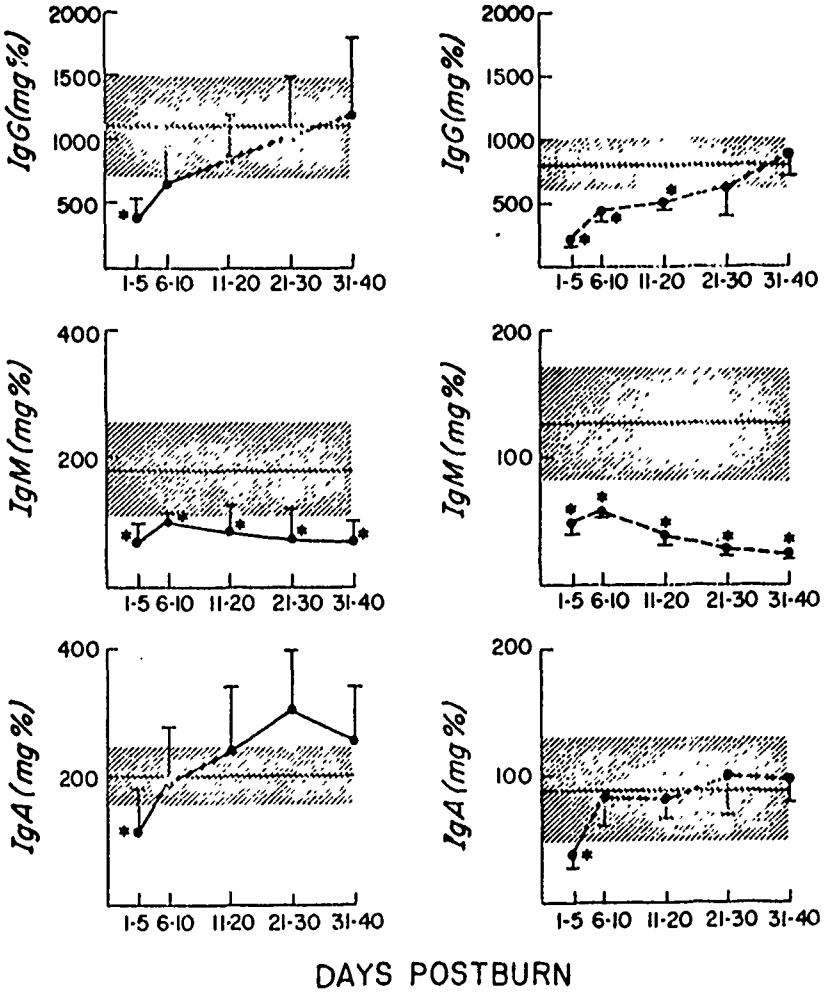


Fig. 7. Levels and activities of components of the classical and alternative pathways of complement in a thermally injured patient with septic shock (Patient IV).



*p < 0.005 vs. controls.

Fig. 8. Levels of immunoglobulins following burn trauma. The solid line represents the mean immunoglobulin levels in 14 patients ranging in age from 5 to 59 years (mean age of 31), and the dotted line represents the mean immunoglobulin levels in three patients under the age of 4. The shaded areas represent the variation for 10 age-matched normal sera (mean ± 1 SD), and the vertical bars represent 1 SD.

40-day period. Differences in immunoglobulin pattern were not found to be related to the type or severity of the burn injuries or to the presence of sepsis.

In an attempt to elucidate the reasons for reduction in opsonization and C3 conversion in the burn sera, 50% of pooled normal human serum was added to 50% of the burn sera, and opsonization and C3 conversion were determined. The volume of pooled normal serum used in these opsonic assays (1%) did not promote phagocytosis of the test strain of E. coli by itself. However, it fully restored the ability of patients' sera to promote phagocytosis (98% to 99.9%), suggesting that reduction in opsonization was related to a deficiency of serum proteins. For these experiments, sera with reduced opsonic activity obtained from Patients II and IV were used. Conversely, C3 conversion by inulin in sera from Patients I to IV and from the patients in groups A and B was not always fully restored to normal by 50% normal serum. In most of the burn sera, C3 conversion ranged from 0% to 42% after addition of normal serum. These findings were in agreement with our previous preliminary observations which suggested that reduction in C3 conversion in the burn sera was probably related to the presence of an inhibitor.

2. Discussion and Recommendations

One of the most dramatic and rapidly fatal complications which occurs as a result of thermal injury is burn wound sepsis. Following thermal injury, staphylococci and streptococci rapidly proliferate and colonize the superficial layers of the burn wound. By the fifth postburn day, gram-negative bacilli are found in significant numbers, and by the end of the first postburn week, predominate in the burn wound. These microorganisms rapidly proliferate and actively invade the adjacent subcutaneous tissue in the untreated burn wound. Blood stream invasion usually occurs via the lymphatics with the onset of sepsis being rare before the third or after the 21st postburn day. A primary purpose of the present investigation was to assess humoral host defense mechanisms in the burn patients during this

critical time period and to determine if alterations in specific serum factors predisposed the patient to infection, or if they were caused by infection.

A decrease in serum IgG was observed in the burn patients during the early postburn period with children under the age of 4 showing the most severe and prolonged deficiency, lasting up to 30 days postburn. In both young and older burn patients, levels of IgA were only reduced during the first 5 days postburn. In all patients, levels of IgM were reduced during the entire 40-day postburn period. Our results confirm those of other investigators who have determined immunoglobulin patterns following burn trauma (4-10), although a more prolonged decrease in IgM level was demonstrated in our study. The absence of a significant difference in immunoglobulin levels in patients with large and small burns and the marked depression in IgM level indicate that exudative loss of protein from the burn surface is probably only partially responsible for the observed changes. Increased catabolism and/or decreased synthesis of immunoglobulins are probably responsible for the observed abnormalities.

Burn injury was also shown to cause changes in the alternative pathway of complement activation. Properdin was shown to be reduced in the burn sera during the first 30 days postburn, and reduction in the C3 converting activity of the sera was demonstrated after the first 10 days postburn and was normalized by the sixth week. In several of our patients, however, C3 conversion was also reduced during the initial postburn period. Reduction in C3 converting activity and properdin level occurred with the same frequency in young and older patients and correlated with increasing burn size. In addition, levels of native C3, KAF, and factor B were found to be increased following burn injury. Patients with the smallest burn sizes had the most striking increase in the levels of these components, probably because they were not losing as much protein through their burn wounds as the patients with the larger burns. C3, KAF, and factor B may be acute phase reactants in the thermally injured patient.

Reduction in the C3 converting activity of the serum appeared to be related to the presence of an inhibitor, since it could not be fully corrected by addition of normal human serum. However, it will be necessary to demonstrate that addition of a purified factor from burn serum reduces C3 conversion in normal serum before any conclusions regarding the nature of the reduction in C3 conversion can be drawn. It is, however, interesting to speculate that a factor produced by tissue injury is responsible for this abnormality of the alternative complement pathway. The significance in the reduction in properdin following burn injury is difficult to evaluate since properdin has recently been shown to play an ancillary role in the formation of C3 and C5 alternative pathway convertases (35,36).

No differences in the occurrence of abnormalities of immunoglobulins or complement were noted in patients with burns caused by flame, immersion scald, or contact with sulfuric acid. However, in the patient with acid burns, the onset of reduction in C3 conversion occurred during the third to fourth postburn week rather than after 10 days postburn. In addition, properdin in this patient's serum was not restored to normal during the entire study period of 60 days postburn. The difference in the temporal sequence of these abnormalities in relation to burns caused by flame or immersion scald may be related to the type of burn injury. Sulfuric acid does not burn tissue by hyperthermic activity; rather, it coagulates protein by desiccation (43).

A cause and effect relationship could not be established between any of the observed abnormalities of immunoglobulins or of the alternative complement pathway which were demonstrated in the burn patients and the development of septicemia. The humoral abnormalities were demonstrated prior to the onset of infection in only one patient; they were also demonstrated in patients who did not develop septicemia. These findings do not, however, rule out the possibility that the humoral abnormalities together with other factors may predispose the patients to infection.

Preliminary evidence indicated that consumption of components of the classical and alternative complement pathways occurred during septicemia, suggesting that these abnormalities of complement were caused by infection. Reduction in the classical complement pathway occurred during the first 10 days postburn in an infant who developed sepsis with S. aureus on the third postburn day, which persisted through the sixth day. Reduction in the classical and alternative pathways of complement activation was also observed in a 39-year-old female who had multiple episodes of Pseudomonas bacteremia and candidemia during her clinical course and who died of septic shock. In this regard, Fearon et al. have demonstrated reduction in components of the alternative complement pathway in cases of gram-negative bacteremia associated with shock, and have suggested that biologically active products released during activation of C3 through C9 by the bacteria contributed to development of shock (44). Our findings, although preliminary, suggest that treatment of thermally injured patients with fresh frozen plasma during periods of septicemia would be of considerable therapeutic value. The potential advantage of this therapy would be the replacement of unactivated complement components which would serve to replace essential opsonins for the infecting microorganisms.

In our study, the classical complement pathway was shown to be utilized preferentially during opsonization of E. coli 075. In the presence of an intact alternative pathway C3 convertase, reduction in the opsonic activity of the burn sera for E. coli 075 was only observed when total hemolytic complement which requires C1 through C9 was also reduced. Jasin previously demonstrated a requirement for factor B of the alternative complement pathway for opsonization of this microorganism (40). It is therefore possible that properdin is also required for opsonization of this microorganism even though it does not appear to be required for formation of a C3 convertase using inulin as the activating substance. Reduction in the properdin level of the burn sera might divert activation from the alternative to the

classical complement pathway. Another explanation for the contradictory results is that antibodies to E. coli 075 are present in high concentration in the burn sera which would promote activation of C1. From the findings presented in our study, it does appear essential to reexamine whether E. coli 075 can activate both the classical and alternative pathways of complement.

One of the most important findings of this investigation was that multiple abnormalities of humoral components of host defense were demonstrated in patients who did not develop systemic infection. This finding emphasizes the importance of the clinical management of the patient in preventing the development of septic complications. The importance of early and appropriate intensive local treatment of the burn wound should be emphasized. If the patient does become septic, consumption of components of the classical and/or alternative pathways of complement activation may compromise host defense by perpetuating infection. Of considerable importance in our future studies will be to address the question of whether reduction in C3 conversion via the alternative complement pathway may also compromise host defense by reducing the opsonic capacity of the serum for a potential infecting organism which normally utilizes components of this pathway during opsonization. This abnormality occurs during the time when the burn patient is the most susceptible to infection, suggesting that it may be potentially important in predisposition to infection. In addition, it would be important to know if changes in humoral components of host defense are unique to patients with burn injury or are also observed in patients with other types of trauma and in septic patients without trauma. Of future interest will also be studies concerning the mechanisms by which abnormalities of the humoral factors occur and their relationship to abnormalities of cellular components of host defense.

B. Normal Human Serum Opsonins for P. aeruginosa and E. coli

1. Results

Partially purified preparations of properdin and factor B were obtained from normal human serum during the contract period. By alkaline polyacrylamide disc

gel electrophoresis, both preparations were found to be contaminated only with gamma globulin; functional activity of the preparations has not yet been tested.

Considerable effort was also expended on purifying human factor $\overline{D}$. This component is present in very low concentration in human serum and its purification is extremely time consuming. The reason for this is because the purification procedures are difficult and because fractions must be tested functionally rather than immunochemically, since antiserum to this protein is presently unavailable commercially. We have had encouraging results in preparing factor $\overline{D}$ depleted serum for the functional testing of fractions potentially containing factor $\overline{D}$. We are planning to test two different methods for purification of factor $\overline{D}$ and to compare the activities of the purified factor $\overline{D}$ preparations.

Normal IgG has been successfully purified by ammonium sulfate fractionation and chromatography on DEAE cellulose. After a second fractionation on TEAE cellulose (Cellex T), further purification was obtained. By immunoelectrophoretic analysis using antiserum to whole human serum, normal IgG preparations contained a single line migrating in the gamma region. By radial immunodiffusion, the preparations contained no IgM or properdin, and only trace amounts of IgA.

The opsonic activities for E. coli 075 and P. aeruginosa of the partially purified preparations of C3, factor B, properdin, and normal IgG have been tested. The results have demonstrated no reduction in bacterial counts by normal human leukocytes in the presence of physiological concentrations of the proteins when tested either individually or in combination. These findings are preliminary and only begin to approach the complex problem of determining which components of the alternative pathway participate in opsonization of the microorganisms and the requirement for normal IgG.

2. Discussion and Recommendations

In the studies which have been reported in the literature regarding participation of the alternative complement pathway in opsonic functions required

for in vitro phagocytosis and intracellular killing of bacteria, a requirement for a specific serum protein in opsonization has been demonstrated by its ability to restore opsonic activity to serum depleted of that respective protein. This approach has the capability of determining whether or not a serum protein is required for phagocytosis and intracellular killing of a bacterial strain, but is limited by its inability to determine all of the proteins required for these events. This determination can only be made by adding combinations of purified proteins to bacteria and determining whether or not phagocytosis and intracellular killing of the bacteria by leukocytes will occur; this is the experimental approach which we are utilizing in the present study.

The success of the experiments depends on the purity of the various proteins and their functional integrity. Although our approach requires a considerable amount of technical time, we believe it will result in new information regarding basic humoral host defense mechanisms against E. coli and P. aeruginosa. We also believe that because of the contradictory results obtained with E. coli 075, which were discussed in the previous section of this report, we should determine if this microorganism and the test strain of P. aeruginosa can activate the alternative pathway; this has not been previously tested directly.

C. In Vitro Interaction of Bacteroides fragilis and Fusobacterium mortiferum with Human Serum and Leukocytes

1. Results

Methodology was developed for measuring the effects of human serum and peripheral leukocytes on the killing of F. mortiferum #9817, B. fragilis subspecies fragilis #1249, and B. fragilis subspecies thetaiotaomicron #1309. Diluent used in the experiments was Hank's Balanced Salt Solution (Microbiological Associates Inc., Bethesda, Md.) containing 0.1% gelatin which was deoxygenated by boiling immediately prior to the experiments. Preliminary experiments indicated

that survival of each of the bacterial strains in diluent in plastic capped tubes was excellent over a 3-hour incubation period at 37° C in room air. Bacteria were maintained in thioglycollate medium at -70° C; prior to each experiment, a tube of thioglycollate medium was inoculated from a frozen culture. The tube was incubated for 4 days at 37° C, and then transferred to a tube of deoxygenated medium containing equal parts of trypticase soy broth and brain heart infusion broth which was incubated at 37° C overnight. The bacteria were washed once and diluted in deoxygenated diluent immediately prior to the experiment. Leukocytes were prepared from the plasma of dextran-sedimented heparinized blood obtained from healthy adult volunteers. The leukocyte-rich plasma was divided, centrifuged at 200 g for 10 minutes and the supernatants were discarded. The leukocytes were washed twice and resuspended in deoxygenated or untreated diluent depending on whether the experiments were to be conducted in an aerobic or anaerobic environment.

Various combinations of pooled normal human serum (10% concentration), leukocytes (5×10^6), bacteria (1.0×10^6), and diluent in a final volume of 1 ml were added together in plastic capped tubes. For the experiments conducted in the anaerobic environment, the diluent added to the reaction mixtures was deoxygenated; for the experiments conducted in room air, untreated diluent was used. For the experiments performed in the anaerobic environment, the reagents were added to the tubes in an anaerobic glove box (Coy Manufacturing Co., Ann Arbor, Mich.). In addition, the tubes to be used were equilibrated overnight in the glove box. The internal atmosphere of the glove box contained 85% nitrogen, 10% hydrogen, 5% carbon dioxide, and not over 25 ppm of oxygen. The next steps in the procedure were carried out entirely in or out of the glove box. The tubes were incubated for 3 hours at 37° C on a rotating platform, and samples were removed at 0 time, 30, 90, and 180 minutes. The samples were diluted in deoxygenated distilled water to disrupt the leukocytes, and the dilutions were plated in thioglycollate agar by the pour plate method. The plates from all experiments were incubated anaerobically in GasPak jars for 4 days, and the colonies were enumerated to determine

the total number of bacteria surviving in each reaction mixture. In some experiments, the number of surviving extracellular bacteria was determined by plating the supernatants of the reaction mixtures after centrifugation at 100 g.

F. mortiferum 9817 was killed directly by serum alone or in the presence of serum and leukocytes; similar results were obtained when the experiments were carried out in or out of the anaerobic glove box (Figure 9). A three log reduction in bacterial counts by leukocytes in the absence of serum was demonstrated in the aerobic environment in comparison to a one log reduction in bacterial counts when this reaction was carried out in the glove box. In both environments, B. fragilis 1249 was killed by leukocytes in the presence of serum, but not by serum alone (Figure 10). Killing of this microorganism by leukocytes in the absence of serum occurred in the aerobic environment, but not under anaerobic conditions. In both environments, B. fragilis 1309 was only killed by leukocytes in the presence of serum and not by either leukocytes or serum alone (Figure 11). In the anaerobic environment, total counts in the reaction mixtures containing leukocytes, bacteria, and serum or leukocytes and bacteria alone were compared to bacterial counts in the supernatants of these reaction mixtures. Similar counts for all three bacterial strains were obtained, suggesting that phagocytosis of the microorganisms was rapidly followed by intracellular killing.

2. Discussion and Recommendations

Prior to this study, no information was available regarding specific humoral and cellular host defense mechanisms against gram-negative anaerobic microorganisms. Phagocytosis and killing by polymorphonuclear leukocytes (PMNS) has been shown to be of primary importance in host defense against extracellular and facultative aerobic microorganisms. Serum bactericidal activity against gram-negative aerobic enteric bacilli is also well recognized. The purpose of the present study was to determine if these bactericidal mechanisms were operative against strains of B. fragilis and F. mortiferum under aerobic or anaerobic conditions.

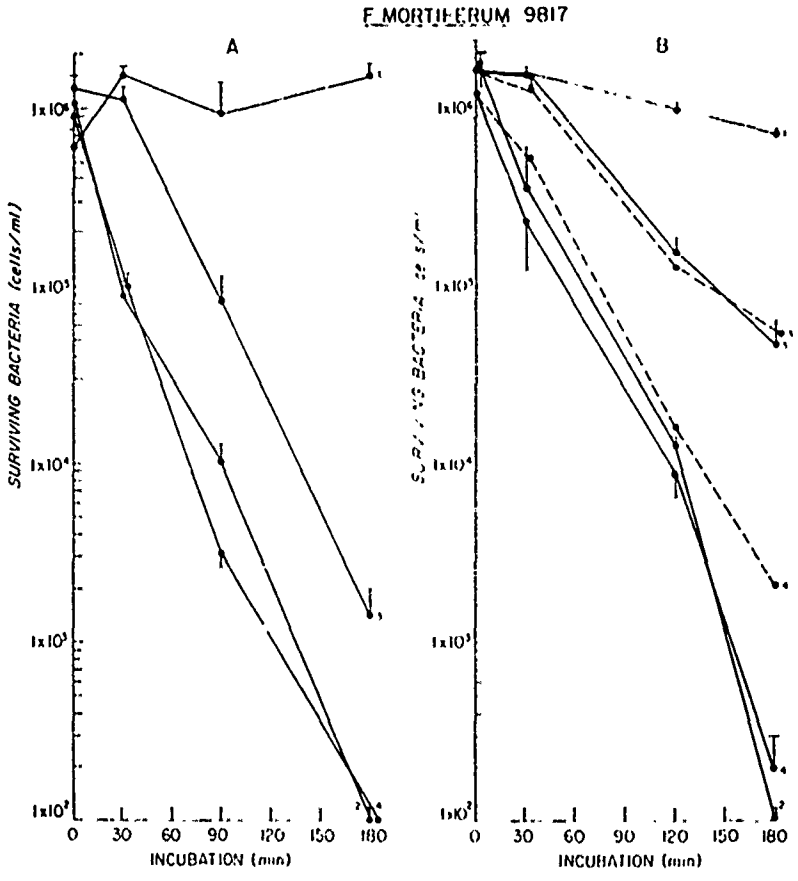


Fig. 9. Effect of human serum and leukocytes on the killing of F. mortiferum 9817. In figure A, experiments were performed in room air; in figure B, experiments were performed in the anaerobic glove box. The following reaction mixtures were tested: 1, diluent and bacteria; 2, serum and bacteria; 3, leukocytes and bacteria; 4, serum, leukocytes and bacteria. The solid lines represent total surviving bacteria, and the dotted lines represent surviving extracellular bacteria. The vertical bars represent the standard error of the mean of duplicate determinations.

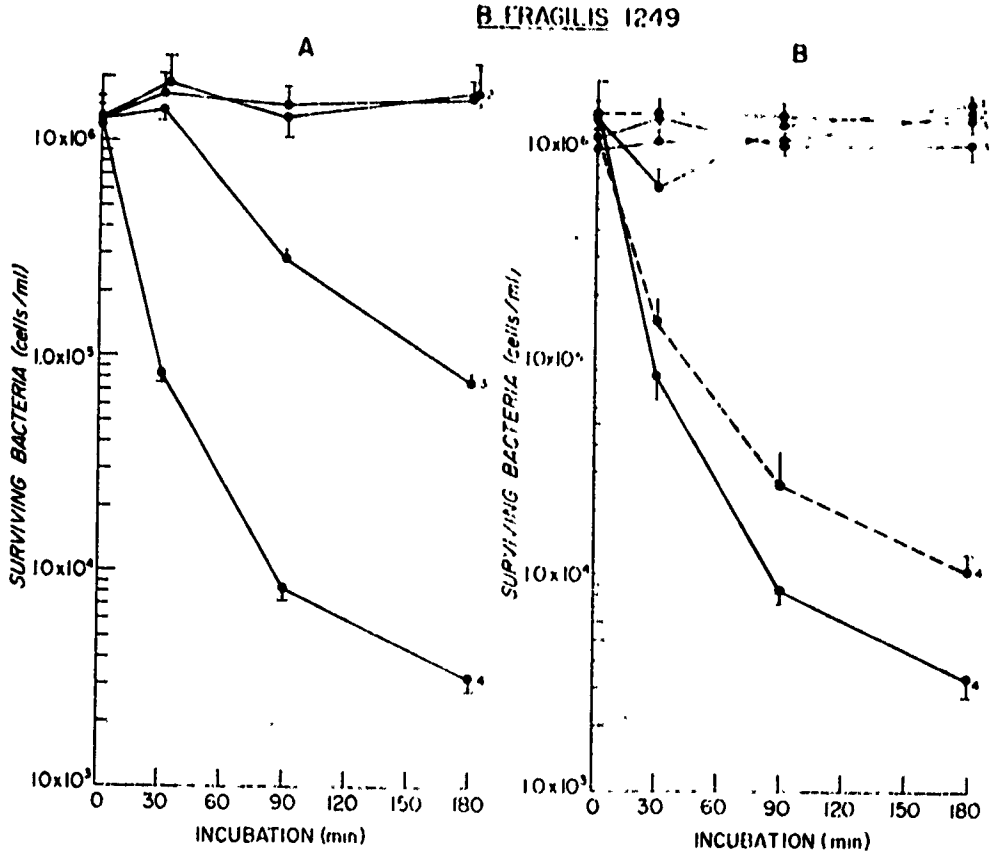


Fig. 10. Effect of human serum and leukocytes on the killing of B. fragilis 1249. In figure A, experiments were performed in room air; in figure B, experiments were performed in the anaerobic glove box. The following reaction mixtures were tested: 1, diluent and bacteria; 2, serum and bacteria; 3, leukocytes and bacteria; 4, serum, leukocytes and bacteria. The solid lines represent total surviving bacteria, and the dotted lines represent surviving extracellular bacteria. The vertical bars represent the standard error of the mean of duplicate determinations.

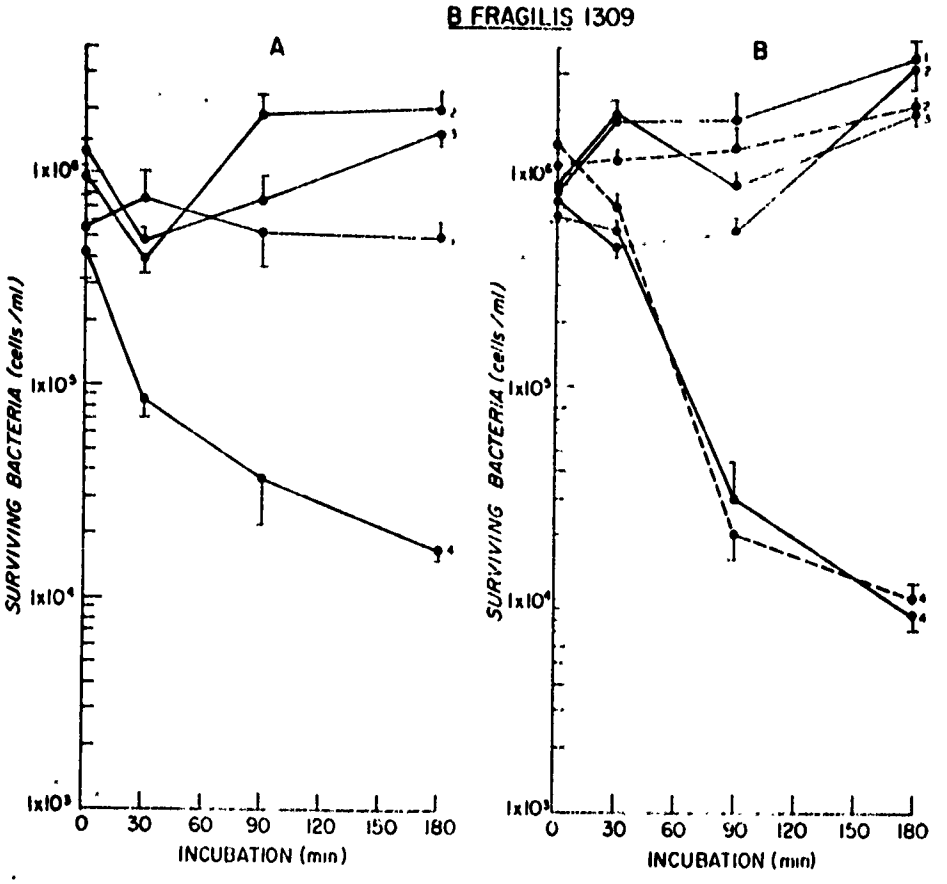


Fig. 11. Effect of human serum and leukocytes on the killing of B. fragilis 1309. In figure A, experiments were performed in room air; in figure B, experiments were performed in the anaerobic glove box. The following reaction mixtures were tested: 1, diluent and bacteria; 2, serum and bacteria; 3, leukocytes and bacteria; 4, serum, leukocytes and bacteria. The solid lines represent total surviving bacteria, and the dotted lines represent surviving extracellular bacteria. The vertical bars represent the standard error of the mean of duplicate determinations.

The strains of B. fragilis subspecies fragilis and thetaiotaomicron used in our study are subspecies which are common clinical isolates. These microorganisms were not susceptible to the bactericidal activity of normal human serum, nor were they phagocytosed and killed intracellularly by peripheral human leukocytes in the absence of serum under anaerobic conditions. Both strains of B. fragilis were, however, phagocytosed and promptly killed intracellularly by leukocytes in the presence of normal serum. These findings suggest that these microorganisms are handled by the host in the same manner as most of the opportunist pathogens such as P. aeruginosa and E. coli. In this regard, infections caused by B. fragilis have been seen with increased frequency in the compromised host (45,46), and the invasiveness of B. fragilis probably depends to a large extent on the functional integrity of the host's phagocytic cells and serum opsonins as well as on the virulence factors of this microorganism.

The humoral and cellular requirements for killing the strain of F. mortiferum used in our study were markedly different from the requirements for killing of the B. fragilis isolates. F. mortiferum was killed directly by normal human serum in the absence of leukocytes and was also phagocytosed and rapidly killed intracellularly by leukocytes alone or by leukocytes and serum. These observations suggest that this strain of F. mortiferum is probably not invasive unless the host's humoral and cellular functions are markedly abnormal. This strain of F. mortiferum was obtained from the American Type Culture Collection and to our knowledge, was not a clinical isolate.

Our observation that gram-negative anaerobic microorganisms were killed by leukocytes in an anaerobic environment supports the findings of Mandell (47). His investigation showed that a variety of aerobic and anaerobic microorganisms were killed efficiently by human PMNS made anaerobic by nitrogen washout, suggesting that mechanisms other than those dependent on hydrogen pyroxide may be important

in intracellular killing by leukocytes. The finding that B. fragilis subspecies fragilis was killed to some extent by leukocytes in the absence of serum in the aerobic but not anaerobic environment remains to be explained but appears to be dependent upon an ingestion mechanism which occurs independently of serum opsonins.

Gram-negative non-sporulating anaerobic microorganisms constitute a major proportion of the normal flora of the human mouth, respiratory, genitourinary, and intestinal tracts. Infections caused by these microorganisms are increasingly being recognized as major problems in surgical practice (48). Gram-negative anaerobes, particularly B. fragilis, are commonly isolated in cases of secondary peritonitis in which perforation of the abdominal wall and intra-abdominal viscus permits the escape of the microorganisms into the peritoneal cavity, adjacent organs, retroperitoneal space, and circulation. Secondary peritonitis is often complicated by intra-abdominal abscesses and is most often caused by gunshot wound of the abdomen or by other penetrating abdominal injury. This type of trauma occurs during combat in the military service and its frequency is increasing in the modern civilian population. Our studies have provided a foundation for future studies of host defense mechanisms against the microorganisms most frequently associated with trauma involving this type of contaminated wound.

Several important areas for future investigation should be pursued. These include a) studies of the role of complement and antibody in opsonization of B. fragilis, and b) studies of host defense mechanisms against clinical isolates of Fusobacterium and of a strain of F. necrophorum which has been demonstrated experimentally to cause liver abscesses in animals.

V. CONCLUSIONS

A. Conversion of C3 by inulin was reduced after 10 days postburn and was restored to normal by 60 days postburn.

B. Properdin levels were reduced immediately following the burn injury and during the first 60 days postburn.

C. Levels of native C3, KAF, and factor B were normal or increased during 60 days following the burn injury.

D. Reduction in C3 conversion and decrease in properdin concentration were related to the extent of the burn injury.

E. Changes in the complement following burn injury were not found to be related to the patient's age.

F. Serum levels of IgG and IgA were reduced during the first 10 days postburn; a more prolonged decrease in IgG was observed in children under 4 years of age.

G. Serum levels of IgM were reduced during 60 days postburn in young and older burn patients.

H. Reduction in the levels of IgG, IgA, IgM were not found to be related to the extent of the burn injury or to the presence of septicemia.

I. Reduction in serum opsonic activity for *E. coli* 075 and in the classical complement pathway only occurred during septicemia in the burned patients.

J. Consumption of components of the alternative pathway of complement activation also occurred during septicemia in a burn patient.

K. No difference in the occurrence of changes in levels of immunoglobulins or complement activation were found in burns caused by flame, immersion scald, or contact with sulfuric acid.

L. Physiological concentrations of purified human C3, properdin, IgG, and factor B either alone or in combination did not promote phagocytosis and intracellular killing of E. coli 075 or P. aeruginosa by normal human peripheral leukocytes.

M. In anaerobic and aerobic environments, a strain of F. mortiferum was killed by normal human serum in the presence or absence of human peripheral leukocytes or to a lesser extent, in the presence of leukocytes alone.

N. In aerobic and anaerobic environments, a strain of B. fragilis subspecies thetaiotaomicron was killed in the presence of normal human serum and human peripheral leukocytes but not by leukocytes or serum alone.

O. In an anaerobic environment, a strain of B. fragilis subspecies fragilis was killed in the presence of normal human serum and human peripheral leukocytes but not by serum or leukocytes alone; in an aerobic environment, killing also occurred by leukocytes in the absence of serum.

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