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Complement Activation in Acne Vulgaris: Consumption of Complement by Comedones

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Comedones, the contents of acne lesions, were shown to consume complement hemolytic activity in normal serum. This consumption was stimulated by the addition of serum from patients with inflammatory acne. Absorption of acne serum with *Propionibacterium acnes* cells removed all stimulating activity. Immunoelectrophoretic analysis of serum incubated with comedones revealed the conversion of C3 and factor B in normal serum. The addition of acne serum resulted in cleavage of C4. In serum treated with ethylene glycol-bis(β-aminoethyl ether)-*N*,*N*′-tetraacetic acid, only C3 and factor B were converted. This indicates that comedones may activate complement by either the classical or the alternative pathway. It is suggested that *P. acnes* cells in comedonal material are responsible for the complement activation.

Acne vulgaris is the most common disease of humans (1). The clinical expression of this disorder ranges from noninflamed open and closed comedones ("blackheads" and "whiteheads") to inflammatory papules, pustules, and nodules. The site of the disease is the pilosebaceous follicle, which becomes distended and impacted with abnormally desquamated follicular epithelial cells. This process can progress to a completely impacted follicle which is clinically visible (comedones) or may evolve into inflammatory papules, pustules, or nodules (6).

The possible factors involved in the development of inflammation recently have begun to be systematically investigated. The weight of current evidence incriminates the anaerobic diphtheroid *Propionibacterium acnes* to be central in the development of inflammation. The evidence includes the following observations. (i) *P. acnes* is seen to accumulate in follicles which are just beginning to show the earliest histological changes of acne (6). (ii) Acne patients have significantly greater numbers of *P. acnes* than do age-matched controls (6). (iii) *P. acnes* produces lipases which release free fatty acids from sebaceous gland triglycerides; these free fatty acids have been shown to be capable of inducing abnormal desquamation or comedo formation (6) and may also enhance inflammation (11). (iv) Topical and systemic antibiotics which significantly suppress *P. acnes* produce clinical benefit in acne, whereas those which do not lower *P. acnes* levels have been judged to be ineffective (2). (v) Intradermal injections of *P. acnes* produce more inflammation in acne patients than they do in normal controls (11). (vi) Antibody titers to *P. acnes* tend to parallel the severity of inflammation (10). More recent work has centered on possible mechanisms for *P. acnes* participation in the pathogenesis of inflammatory acne lesions. Puhvel and Sakamoto (12) have shown that *P. acnes* produces chemotactic factors and that chemotactic activity can be retrieved from comedones. Recently, we and others have shown that *P. acnes* cells will activate complement (8, 14) and in doing so generate neutrophil chemotactic factors (14). Limited immunofluorescent studies of early inflammatory acne lesions have shown the presence of C3 in the lesion and in surrounding tissues, implying a role for the complement system in vivo (3).

In this study we investigated the ability of materials retrieved from acne lesions (comedones) to activate complement and the effect of serum from acne patients on this reaction.

MATERIALS AND METHODS

Collection of comedones. Open and closed comedones were extracted from the faces of acne patients, pooled, and frozen at −20°C.

Sera. Sera from 10 healthy, acne-free adults were collected, pooled, and frozen at −80°C. This pool served as a source of complement and had an agglutination titer against *P. acnes* 6919 of 1:128. Sera from four inflammatory acne patients were pooled and heat inactivated at 56°C for 30 min. The anti-*P. acnes* titer of this pool was 1:1,024. A 5-ml volume of this pool was absorbed with 100 mg of lyophilized *P. acnes* 6919 for 3 h at 37°C and overnight at 4°C on a rotator. Bacteria were removed by centrifugation and filtration. After this absorption the *P. acnes* titer was 1:4.

Activation of complement. Comedones were suspended at various concentrations in gelatin-Veronal buffer (9). Equal volumes of suspension, serum, and acne serum, absorbed acne serum, or gelatin-Veronal buffer were reacted for 30 min at 37°C. As a control, all reagents were tested for complement-activating activity in the absence of comedones. Incubation of comedones with ethylenediaminetetraacetic acid (EDTA)-treated serum served as a control for non-complement-mediated consumption of activity. After incubation, residual hemolytic activity was assayed by established methods (9).

Immunoelectrophoresis was performed as previously described (10). Comedones at 20 mg/ml were suspended in gelatin-Veronal buffer and reacted with serum treated with ethelene glycol-bis(β-aminoethyl ether)-*N,N'*-tetraacetic acid (EGTA) supplemented with 0.05 M $MgSO_4$, 0.01 M EDTA, or untreated normal human serum. After 30 min of incubation at 37°C, the reaction mixture was clarified by centrifugation, and the supernatant was electrophoresed for 90 min at 40 V in 1% agarose with 0.01 M EDTA. Gels were developed with anti-C3, anti-factor B, and anti-C4. Rabbit anti-human C3 and C4 were described previously (7). Factor B was demonstrated by immunoelectrophoresis with a rabbit antiserum with specificity for the Ba and Bb fragments (14).

RESULTS

Comedones were capable of consuming 94.5% of the total hemolytic activity at a final concentration of 13.3 mg/ml and 12.5% at 6.65 mg/ml. The addition of acne serum stimulated this consumption to 72% at 6.65 mg/ml. The absorption of acne serum removed the stimulating factor(s) and reduced consumption to near that with gelatin-Veronal buffer alone (Fig. 1). In the absence of comedones no hemolytic activity was consumed by acne serum or absorbed acne serum.

The ability of comedones to initiate alternative pathway activation was tested immunoelectrophoretically. As shown in Fig. 2, C3 and factor B were partially cleaved by comedones in normal serum. C4 was cleaved only when acne serum was added to the incubation mixture. Mg^{2+}-EGTA chelation of Ca^{2+} inhibited the cleavage of C4, but not factor B or C3. EDTA completely blocked the cleavage of all three components. This indicates that comedones may activate complement by either the alternative or the classical pathway, since paralysis of Clr and Cls with Mg^{2+}-EGTA did not inhibit C3 and factor B cleavage.

DISCUSSION

Activation of the complement system results in the generation of factors which cause mast cell degranulation, leukocyte chemotaxis, and release of lysosomal enzymes (5). In this study we have shown that the contents of acne lesions are capable of activating complement systems in normal serum. This activation was increased by a serum reagent which had a high anti-*P. acnes* titer. The effects of this reagent could be removed by preabsorption with *P. acnes* cells, indicating that antibody may be involved in this reaction. Immunoelectrophoresis of serum incubated with comedones revealed cleavage of C3, C4, and factor B when both Mg^{2+} and Ca^{2+} were present. When the classical pathway was inhibited by chelation of Ca^{2+}, only C3 and factor B were cleaved. The stimulation by acne serum was also reflected in the conversion of C4 to C4d. Thus, comedones may activate complement by either the classical or the alternative pathway.

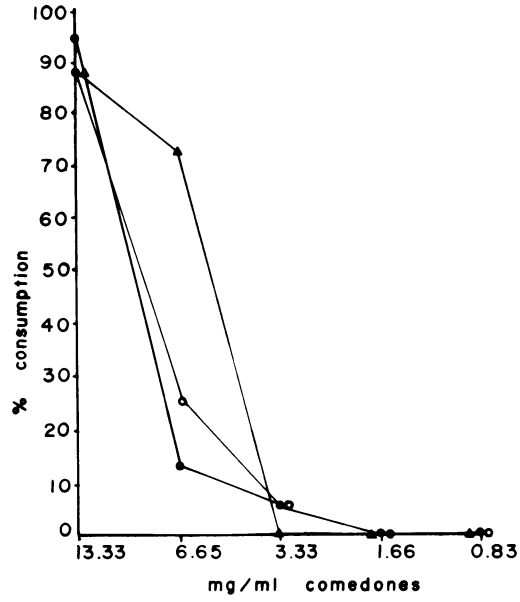

FIG. 1. *Consumption of hemolytic complement activity by comedones. Doses of comedones plus gelatin-Veronal buffer and serum (●), plus acne serum and serum (△), or plus absorbed acne serum and serum (○) were incubated at 37°C for 30 min, and residual hemolytic activity was assayed. The results of one experiment are shown. Similar results were obtained in other experiments, with variation being less than 10%.*

Histological studies indicate that large visible comedones only rarely become inflamed. Rather, it is the much smaller "microcomedo" which gives rise to inflammatory lesions (6). In this study we used comedones instead of microcomedones for reasons of practicality, as it would be extremely difficult to obtain sufficient microcomedones to perform these investigations.

We found that between 6.65 and 13.3 mg of comedones per ml is required to produce significant hemolytic consumption in normal serum. The necessity of this large dose implies that the

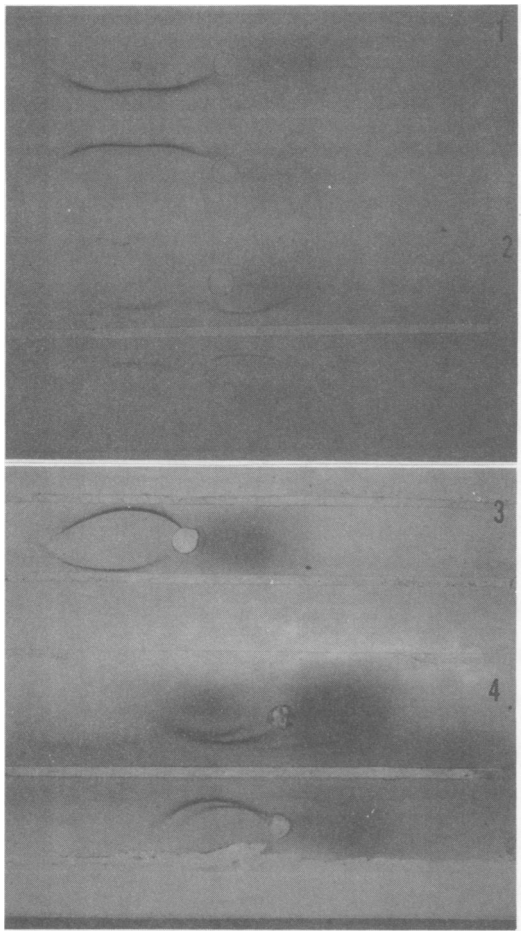

FIG. 2. *Immunoelectrophoresis of comedones incubated with serum. Upper wells on (1) and (2) contained normal serum which had been incubated with comedones. Lower wells contained Mg^{2+}-EGTA-treated serum which had been incubated with comedones. (1) was developed with anti-C3 serum, and (2) was developed with anti-factor B serum. (3) is immunoelectrophoresis of EDTA-treated serum which had been incubated with comedones. The upper trough contained anti-C3 serum, and the lower trough contained anti-factor B serum. (4) was developed with anti-C4 serum. The upper well contained serum incubated with comedones, and the lower well contained serum treated with EDTA and incubated with comedones. The C4 conversion is identical to that reported elsewhere (13). The anti-C4 serum also contained antibody reacting with an euglobulin contaminant and immunoglobulin A.*

activator in comedones is a small proportion of the total mass. *P. acnes* cells are such a comedonal component. Hernandez and Puhvel (4) fractionated a pool of comedones and found that the insoluble keratinous microbial fraction was 18.75% of the total mass. In our hands, microscopic examination of similar preparations revealed that the amount of comedonal keratinous material far outnumbers that of the bacteria. Furthermore, in a previous study we found that *P. acnes* cells will activate complement in normal serum at concentrations as low as 75 μg/ml (14). Possibly, the most direct argument for the role of *P. acnes* in comedonal complement activation is the stimulation of consumption by sera from acne patients, and its ablation by preabsorption with *P. acnes* cells.

It has long been known that antibody titers to *P. acnes* correspond to the severity of inflammation in acne (10) and that acne patients respond more vigorously to an injection of comedonal material (11); yet evidence for a direct connection between antibody titer and the mechanisms of inflammation has not been provided.

Our results indicate that factors in the serum of acne patients which bind *P. acnes*, surely antibodies, enhance complement consumption by comedonal constituents. Once disruption of the follicular epithelium occurs and follicular contents are extruded into the viable dermis, *P. acnes* activation of the complement system undoubtedly contributes to the degree of inflammation which ensues.

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