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Classic diseases revisited

Acne: a review of immunologic and microbiologic factors

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Summary
Acne vulgaris is a self-limiting skin disorder seen primarily in adolescents, whose aetiology appears to be multifactorial. The four main aetiological factors are hypercornification of the pilosebaceous duct, increased sebum production, colonization with *Propionibacterium acnes*, and subsequently the production of inflammation. Considerable investigation has addressed the immunologic reaction to extracellular products produced by the acne-causing organism, *P acnes*. The immunologic response involves both humoral and cell-mediated pathways. Further research should clarify the role of complement, cytotoxins, and neutrophils in this acne-forming response.

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Acne vulgaris, the most common cutaneous disorder, is manifested by comedones, papules, pustules, and cysts. The aetiology of acne appears to be multifactorial. The exact mechanism triggering the development of the comedone and the stimuli causing the non-inflamed lesion to become inflamed are poorly understood. The microbiology of acne vulgaris and its immunologic ramifications constitute the major thrust of present research in the elucidation of the pathogenesis of the inflammatory acne lesion.

The microbiology of the pilosebaceous unit involves three coexisting groups of microorganisms: Gram-positive, coagulase-negative cocci (staphylococci and micrococci); anaerobic diphtheroids (*Propionibacterium acnes* and *Propionibacterium granulosum*); and lipophilic yeasts (*Pityrosporum* species). The microflora of comedones is qualitatively identical to that of the normal sebaceous follicle.

The staphylococci and micrococci are aerobes; therefore, their site of growth within the sebaceous unit is superficial, and these organisms are unable to reside in the anaerobic conditions of the infra-infundibulum where the inflammatory reaction occurs in acne. Antibiotics that selectively eliminate these organisms do not affect the clinical response of patients,[1] and their role in acne pathogenesis is negligible.

The lipophilic yeasts that reside in hair follicles have been divided morphologically into *Pityrosporum ovale* and *Pityrosporum orbiculare*. These represent a complex of organisms and have been reclassified as *Malassezia furfur* as well as other *Malassezia* species.[2] The filamentous forms that are usually associated with a pathologic condition are not seen in acne lesions and, save for tinea versicolor, these organisms appear not to play a significant aetiologic role in any disease state.

If the microbial flora is significant in the pathogenesis of acne, the most likely organism to blame is *P acnes*, a strict anaerobe that has been shown serologically and biochemically to be identical to *Corynebacterium parvum*, a potent stimulator of the reticuloendothelial system.[3] This organism has been used as an immunostimulatory adjunct in chemotherapy of numerous tumours.[3–6] *P acnes* is overwhelmingly the predominant microorganism in the normal pilosebaceous follicle, as well as in the acne state, and has been divided into two serotypes and five biotypes. Up to 10^7 viable *P acnes* have been isolated from a single sebaceous unit. *P acnes* is not pathogenic by normal standards because there is no correlation between the number of bacteria and the severity and type of acne. Nevertheless, *P acnes* appears to be the target of oral and topical antibiotic usage, and the reduction in numbers of *P acnes* is a just parameter of therapeutic effectiveness of antibiotics.

P acnes secretes several extracellular products that may be significant in the aetiology of acne. These include hyaluronidase, proteases, lipases, and chemotactic factors for neutrophils, lymphocytes, and macrophages. The microenvironment of the pilosebaceous unit is likely to play a major role in the amount of exoenzymes that are produced by the organism because *in vitro* studies demonstrate that their production is altered by factors such as pH and oxygen tension.

The current interest in *P acnes* revolves around whether its immunopotentiating properties are pertinent in the pathogenesis of acne. Specifically, there is reason to believe that *P acnes* may be a direct instigator of inflammation in acne via its interaction with antibody and complement, its chemotactic properties, and via cell-mediated immunity.

Humoral immunity

Patients with inflammatory acne develop an immune response to *P acnes*. Circulating immune complexes have been reported to be elevated in some acne patients. The degree of elevation has been correlated with the severity of acne inflammation.[7 8] Additionally, complement-fixing antibody titres to *P acnes* are

elevated, and the titres parallel the severity of the inflammation.[9] In minimal acne, these antibody titres are rarely greater than the levels found for most adults. The antibodies to *P acnes* have not been characterised fully, although they are reported to be largely of the IgG class. Total IgG levels are slightly increased in some patients with severe acne, which may reflect an enhanced B-cell activity,[10] although they may be lower than normal in others.[11] Titres of IgG3 have been demonstrated to be higher in severe cases.[12 13] We have shown the dominant antigen to be in the soluble extract of *P acnes* and to have a carbohydrate component.[14]

The elevated antibody response to *P acnes* appears to be specific because antibody titres for *Staphylococcus epidermidis* are not raised in acne. At least four major antigenic components have been detected on analysis of the extracellular supernatant fluid from dialysed *P acne*s cultures.[15] Variations in the antigenic composition of *P acnes* may account for some of the differences in antibody patterns that are seen in individuals.

Immunofluorescence studies have revealed *P acnes* antigens in the dermis surrounding the pilosebaceous units in acne patients. In contrast, the antigens are confined totally within the follicular walls in normal skin.[16]

Cell-mediated immunity

Cell-mediated immunity could contribute to the development of inflammation in acne; however, this role is far from having been proven. Skin tests, made with common recall antigens such as trichophytin, mumps, or purified protein derivative, have demonstrated that patients with severe acne may have a depressed or absent reactivity.[17] In addition, sensitisation to dinitrochlorobenzene may not occur.[17] Such deficiencies are not matched by defects in mitogen-induced lymphocyte blastogenesis *in vitro* where the responses of patients' lymphocytes to phytohaemagglutinin occur at normal levels.[17–19] However, acne patients have a depressed number of E-rosette-forming cells, indicating that some form of T-cell deficiency may be present.[11]

Specific responses to antigens prepared from *P acnes* have been studied. Various types of skin test responses are reported, including both the classic immediate-type and delayed-type hypersensitivity reactions, as well as ill-defined erythematous reaction that disappears before 48 hours postinjection.[20] The latter may be caused by the inflammation brought about from the activation of complement by antibody–antigen complexes or by materials in the antigen preparation.

The type of skin test response shown by acne patients appears to depend, at least in part, on the nature of the antigen preparation, the dosage used for the skin test, and possibly on the isolate of *P acnes* from which the antigen is prepared. Thus, Puhvel *et al*[20] reported immediate hypersensitivity reactions as being characteristic of acne patients who had been skin-tested with *P acnes* antigen prepared from disrupted cells or from a dialyzed culture filtrate. Less than half of their patients developed a delayed reaction that remained visible at 48 hours. In contrast, Kersey *et al*[21] used heat-killed *P acnes* as antigen and reported strong delayed responses in the patients, the strongest response being found for the most severe acne cases. It is a distinct possibility that the strong immediate skin test response reported by Puhvel *et al*[20]prevented subsequent development of a delayed response, a phenomenon that was described over 40 years ago for the trichophytin skin test.

Although the results from specific skin tests are still somewhat confusing, it is generally accepted that the *in vitro* tests for lymphocyte transformation and for production of the lymphokine leukocyte migration inhibitory factor show that acne patients' lymphocytes develop a hyperreactivity to *P acnes* antigens.[19 22] Thus, the stage is set for a contribution by cell-mediated immune responses to the inflammation in acne.

Complement activation

The activation of complement leads to the release of inflammatory mediators, causing mast cell degranulation, leukocyte chemotaxis, and lysosomal enzyme release. Both comedonal contents and *P acnes* have been shown to activate complement via both the classic and the alternate pathways.[23]

Immunofluorescence of skin specimens from acne patients have revealed the presence of C3 deposits in the dermal vessel walls.[24] On occasion, immunoglobulins are seen in addition to complement, and this provides suggestive evidence of the formation of immune complexes around the acne lesion. Thus, complement fixation may play a major role in inducing the inflammation seen in the acne lesion.

Cytotoxins and neutrophil function

Early acne lesions reveal polymorphonuclear leukocytes accumulating at the periphery of pilosebaceous units and later migrating within the hair follicle. The production of cytotoxins, which stimulate chemotactic activity independently of complement, has been investigated in acne. Materials produced by *P acnes* and by other comedonal bacteria grown *in vitro* can stimulate neutrophil chemotaxis.[25] The fraction of *P acnes* with chemotactic activity appears to consist of predominantly low molecular weight material.[26] The lipid-containing fraction extracted from comedones can induce neutrophil chemotaxis.[27] However, crude comedonal extracts are reported to be toxic for neutrophils, with the free fatty acids appearing to be responsible for the cytotoxicity.[28] The crude comedonal extract is, however, a chemoattractant for monocytes, even though toxicity is reported.[28] Thus, the importance of cytotoxin production for acne lesion formation is unclear at present. Indeed, the activation of complement by both comedonal material and *P acnes* may be of greater importance for inducing neutrophil chemotaxis in acne.

There have been relatively few studies on neutrophil function in acne patients. Enhanced chemotactic and random migratory activities have been reported by some,[29] but others find activity at normal levels.[30] In general, phagocytic activities appear normal for bacteria such as *Staphylococcus aureus*.[30] Lee and Shalita[10] have indicated that some patients with severe acne have a marked depression in their neutrophil chemotaxis, while in other patients chemotaxis is increased. In this latter group, they reported finding a defect in phagocytosis that was specific for *P acnes*. Further research should clarify the role of abnormal neutrophil functions in the pathogenesis of acne.

Treatment considerations

Acne therapy must address the aetiological factors involved in acne pathogenesis. These treatment considerations include correcting the altered pattern of follicular keratinization, reducing sebaceous gland production, diminishing the *P acnes* population in the follicle and inhibiting its production of extracellular inflammatory products, and producing an anti-inflammatory effect.

Coexistent with the immune theories of the pathogenesis of acne, there have been several modes of therapy applied to patients with severe acne. Possibly because of the presence of isotretinoin or the venerable microcomedonal theory of acne, few clinical trials have materialised since the early 1980s.

Intralesional and oral steroids are occasionally used to reduce inflammation in severe cases of acne. Cimetidine, which affects cell-mediated immunity as well as being anti-androgenic, has had conflicting results as to its therapeutic efficacy.[31] Levamisole restored the impaired T-cell function in acne patients as well as bringing about some clinical improvement.[11] A polyvalent *P acnes* vaccine has been reported to have had modest success,[32] as has transfer factor.[33] Finally, it has been suggested that tetracycline, which becomes concentrated in inflamed lesions and has been the mainstay of acne treatment for two decades, could act by inhibiting neutrophil chemotaxis rather than its antibacterial actions.[29]

P acnes plays a central role in acne pathogenesis. Not only does this anaerobic bacterium produce lipases, proteases, and other extracellular enzymes, it also secretes chemotactic factors attracting polymorphonuclear leukocytes, lymphocytes, and macrophages. The inflammatory response initiated by these extracellular products stimulates the classical and alternative complement pathways and other immune respones. Thus, *P acnes* directly contributes to the existence of acne via its effects on humoral and cell-medicated immunity, complement activation, and cytotoxin production. Further studies on the immunological factors involved in acne pathogenesis are warranted.

1 Plewig G, Kligman AM. *Acne*. New York, Springer-Verlag, 1975; pp 38–41.
2 Gueho E, Boekhout T, Ashbee HR, Guillot J, Van Belkum A, Faergemann J. The role of *Malassezia* species in the ecology of human skin and as pathogens. *Med Mycol* 1998;**36**(suppl 1):220–9.
3 Adlam C, Scott MT. Lymphoreticular stimulatory properties of *Corynebacterium parvum* and related bacteria. *J Med Microbiol* 1973;**6**:261–5.
4 Senaldi G, Yin S, Shaklee CL, Piguet P, Mak TW, Ulich TR. *Cornyebacterium parvum* and *Mycobacterium bovis* Bacillus Calmette-Guerin-induced granuloma formation is inhibited in TNF Receptor I Knockout Mice and by treatment with soluble TNF-RI. *J Immunol* 1996;**157**:5022–6.
5 Herberman RB. Natural killer cells and their possible roles in resistance against disease. *Clin Immunol Rev* 1981;**1**:1–14.
6 Cantrell JL, Wheat RW. Antitumor activity and lymphoreticular stimulation properties of fractions isolated from *Corynebacterium parvum*. *Cancer Res* 1979;**39**:3554–5.
7 Woolfson H. Acne fulminans with circulating immune complexes and leukaemoid reaction treated with steroids and azathioprine. *Clin Exp Dermatol* 1987;**12**:463–6.
8 Kellett JK, Beck MH, Chalmers RJ. Erythema nodosum and circulating immune complexes in acne fulminans after treatment with isotretinoin [letter]. *BMJ* 1985;**290**:820.
9 Puhvel SM, Hoffman K, Sternberg TH. *Corynebacterium acnes*. Presence of complement fixing antibodies to *Corynebacterium acnes* in the sera of patients with acne vulgaris. *Arch Dermatol* 1966;**93**:364–8.
10 Lee WI, Shalita AR. Leukocyte abnormalities in acne conglobata. *J Invest Dermatol* 1980;**74**: 258–62.
11 DeCree J, DeCock W, Verhaegen H. Levamisole treatment of inflammatory acne. Restoration of impaired T-cell function accompanied by clearance of the lesions. *Biomedicine* 1979;**31**:95–8.
12 Holland DB, Ingham E, Gowland G, Cunliffe WJ. IgG subclasses in acne vulgaris. *Br J Dermatol* 1986;**114**:349–51.
13 Ashbee HR, Muir SR, Cunliffe WJ, Ingham E. IgG subclasses specific to *Staphylococcus epidermidis* and *Propionibacterium acnes* in patients with acne vulgaris. *Br J Dermatol* 1997;**136**;730–3.

14 Lehmann PF, Burkhart CG, Cantrell JL, Butcher CL. *Propionibacterium acnes* : interaction with complement and development of an enzyme-linked immunassay for the detection of antibody. *Int J Dermatol* 1999; in press.

15 Puhvel SM. Acne from an immunological perspective. *Cutis* 1976;**17**:502–4.

16 Imamura S, Pochi PE, Strauss JS, McCabes WR. The localization and distribution of *Corynebacterium acnes* and its antigens in normal skin and in lesions of acne vulgaris. *J Invest Dermatol* 1969;53:143–8.

17 Palatsi R. Delayed hypersensitivity and febrile acne conglobata. *Acta Dermatovener* 1977;**57**: 51–2.

18 Rajka G, Froland S. Lecture at the XI Scand Congr Allerology, Helsinki, May 25, 1973, quoted in Fajka G: On cell-mediated immunity in acne conglobata. *Acta Dermatovener* 1977;**57**; 141.

19 Puhvel SM, Amirian D, Weintraub J, Reisner RM. Lymphocyte transformation in subjects with nodulocystic acne. *Br J Dermatol* 1977;**97**: 205–7.

20 Puhvel SM, Hoffman IK, Reisner RM, Sternberg TH. Delayed hypersensitivity of patients with acne vulgaris to *Corynebacterium acnes*. *J Invest Dermatol* 1967;**49**:154–8.

21 Kersey P, Sussman M, Dahl M. Delayed skin test reactivity to *Propionibacterium acnes* correlates with severity of inflammation in acne vulgaris. *Br J Dermatol* 1980;**103**:651–6.

22 Gowland G, Ward RM, Holland KT, Cunliffe WJ. Cellular immunity to *P acnes* in the normal population and patients with acne vulgaris. *Br J Dermatol* 1978;**99**:43–5

23 Webster GF, Leyden JJ, Norman ME, Nilsson UR. Complement activation in acne vulgaris: in vitro studies with *Propionibacterium acnes* and *Propionibacterium granulosum*. *Infect Immun* 1978;**22**:523–6.

24 Dahl MGC, McGibbon DH. Complement in inflammatory acne vulgaris. *BMJ* 1976;**2**:1383–4.

25 Puhvel S, Sakamoto MA. Cytotoxin production by comedonal bacteria (*Propionibacterium acne*, *Propionibacterium granulosum*, and *Staphylococcus epidermidis*). *J Invest Dermatol* 1980;**74**:36–40.

26 Webster GF, Leyden JJ. Characterization of *Propionibacterium acnes* chemotactic factor. *J Invest Dermatol* 1980;**74**:254–6.

27 Puhvel S, Sakamoto M. The chemoattractant properties of comedonal components. *J Invest Dermatol* 1978;**71**:324–6.

28 Tucker SB, Rogers RS III, Windelmann RK. Inflammation in acne vulgaris: leukocyte attraction and cytotoxicity by comedonal materials. *J Invest Dermatol* 1980;**74**:21–6.

29 Gould DJ, Gowland G, Cunliffe WJ. An investigation of leukocyte function in acne vulgaris. *Br J Dermatol* 1978;**99**(suppl 16):17–21.

30 Rebora A, Dallegri F, Patrone F. Neutrophil functions in acne conglobata. *Dermatologica* 1979;**159**:217–20.

31 Lyons F, Cook J, Shuster S. Inhibition of sebum excretion by a H_2 blocker. *Lancet* 1979;**1**:1376.

32 Goldman L, Michael JG, Riebel S. The immunology of acne: a polyvalent *Propionibacteria* vaccine. *Cutis* 1979;**23**:181–3.

33 Grohn P, Kuokkanen K, Krohn K. The effect of transfer factor on cystic acne. *Acta Dermatovener* 1978;**58**:153–6.