British Journal of Dermatology 1997; **136:** 730–733.

IgG subclasses specific to *Staphylococcus epidermidis* and *Propionibacterium acnes* in patients with acne vulgaris

H.R.ASHBEE, S.R.MUIR, W.J.CUNLIFFE* AND E.INGHAM

*Skin Research Centre, Departments of Microbiology and *Dermatology, University of Leeds, Leeds, U.K.*

Accepted for publication 18 December 1996

Summary IgG subclasses specific *to Staphylococcus epidermidis* and *Propionibacterium acnes* were determined in sera from patients with mild, moderate or severe acne and from a control group. Titres specific to *S. epidermidis* were all within the same range and did not differ between groups. The titres of IgG subclasses specific to *P. acnes* did vary between groups. IgG_1 and IgG_3 were significantly higher in severe acne patients compared with moderate acne patients, while IgG_2 was significantly higher in moderate and severe patients compared with controls. Titres of IgG_4 did not differ between groups. The pattern of titres observed suggests that, while the antibody response to *S. epidermidis* is relatively harmless, antibodies to *P. acnes* may be involved in the pathogenesis of acne vulgaris.

The pathogenesis of inflammatory acne is not fully understood. The available evidence favours the concept that inflammatory lesions arise from non-inflamed comedones, which are the clinical manifestation of abnormal ductal hypercornification.[1] Several lines of evidence have implicated skin bacteria, in particular *Propionibacterium acnes*, in the pathology of inflammatory lesions. Previous investigators have shown that, in inflammatory acne, there is an immune response, particularly to the antigens of *P. acnes*.[2,3] Studies in acne patients have suggested that this is a typical response to antigenic stimulation and that there is no regulatory defect of the host's immune system.

An investigation carried out by Holland *et al.*[4] demonstrated that patients with severe acne had significantly higher titres of total IgG than controls. This was due to higher IgG_2 and IgG_3 titres in the patient group, and it was suggested that this was due to antigenic stimulation from Gram-positive bacterial cell wall components, in particular *P. acnes*. However, acne lesions are colonized by two genera of Gram-positive bacteria, *Propionibacterium* and staphylococci. The relative contributions of these bacteria to antigenic stimulation of potentially pathological antibodies has not been studied previously.

The aim of this present study was to examine the titres of the IgG subclasses specific to *Staphylococcus epidermidis* and *P. acnes* in patients with varying degrees of acne and control subjects. In this way, the contribution to the humoral immune response evoked by each organism could be assessed.

Correspondence: Dr H.R.Ashbee, Immunology Research Lab, Department of Microbiology, University of Leeds, Leeds, LS2 9JT, U.K.

Materials and methods

Reagents

Rabbit antihuman IgG_{1-4} fluorescein isothiocyanate (FITC) conjugates, sheep antihuman IgG_{1-4} and donkey antisheep FITC conjugate were obtained from The Binding Site, Birmingham, U.K. Multispot microscope slides were obtained from C.A.Hendley, Loughton, U.K. and phosphate-buffered saline (PBS) from Oxoid, Basingstoke, U.K.

Patient and control subjects

Sera were obtained from four groups, each consisting of eight males and two females. The groups were designated on the basis of their acne severity, according to the grading scheme of Burke and Cunliffe.[5] The mild acne group had acne grades <2 (mean = 1·355) and their ages ranged from 14 to 26 years (mean = 19·6). The moderate acne group had acne grades from 2·1 to 5·0 (mean = 3·665) and their ages ranged from 17 to 24 years (mean = 20·5). Severe acne patients had acne grades >5·0 (mean = 8) and their ages ranged from 14 to 34 years (mean = 21·3). The group used as controls only had physiological acne and had acne grades <1, and their ages ranged from 18 to 28 years (mean = 22). The duration of disease was not recorded in the patient

groups, but the mean age and range of ages in the groups were found not to be significantly different (data not shown).

Determination of IgG subclasses specific to S. epidermidis

A formalized, mid-exponential phase, whole cell suspension of *S. epidermidis* NTCC 11047 was adjusted to a concentration of $2{\cdot}5\times10^7$ cells/ml. To each of the wells of the multispot slides, 10 μl volumes of washed *S. epidermidis* suspension were added, air dried and heat fixed. The test sera were then placed in duplicate 10 μl volumes at various dilutions on duplicate wells of the slides. Control wells had 10 μl of PBS in place of the test sera. The slides were incubated in a moist box at room temperature for 30 min, after which they were washed and soaked in PBS for 3×10 min. Surplus moisture was removed from the slides using a tissue, taking care to leave the wells untouched. The optimal dilution of each of the subclass conjugates were determined (data not shown) and Evans blue was used as a counterstain. To each of the wells, 10 μl of the appropriate subclass conjugate was added and incubated in a moist box at room temperature for 30 min. The conjugate was washed off and the slides washed in PBS for 3×10 min. Surplus moisture was again removed from the slides and a drop of glycerol/PBS mountant placed on to each well. Slides were observed under coverslips at $\times500$ magnification using a Leitz fluorescent microscope. Titres were defined as the highest dilution of the sera which gave positive fluorescence.

Determination of IgG subclasses specific to P. acnes

A formalized, whole cell suspension of *P. acnes*[6] (strain P37) was diluted to a concentration of 3×10^7 cells/ml. The method used was the same as that for *S. epidermidis*, with the exception that sheep antihuman IgG_{1-4} (1 : 80) and donkey antisheep IgG FITC (1 : 50) were used to detect the *P. acnes* specific IgG. Titres were defined as the highest dilution of serum giving positive fluorescence.

Statistical analysis

Antibody titres were normalized by conversion to the reciprocal $\log_2$ titres, analysed by analysis of variance (ANOVA[7]) and the results expressed as the mean reciprocal $\log_2$ titres $\pm$ 95% confidence limits. Differences between individual means were tested by minimum significant difference using Tukey's T-procedure.

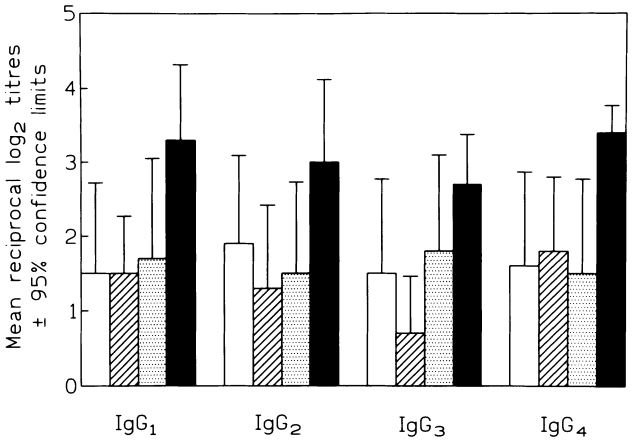

Figure 1. Mean reciprocal $\log_2$ titres of IgG_{1-4} specific to *Staphylococcus epidermidis* in patients with mild ▨, moderate ▩ and severe acne ■, and in controls □. Results are shown as the mean reciprocal $\log_2$ titre $\pm$ 95% confidence limits.

Results

Determination of IgG subclasses specific to S. epidermidis

The mean reciprocal $\log_2$ titres $\pm$ 95% confidence limits for the IgG subclasses specific to *S. epidermidis* for the four groups are shown in Fig. 1. The reciprocal $\log_2$ titres ranged from 0 to 4 in the sera from mild and moderate acne patients; and 0–5 in sera from severe acne patients and controls. Titres of IgG_1, IgG_2 and IgG_4 ranged from 0 to 5; while those of IgG_3 ranged from 0 to 4. Analyses of these results demonstrated that there were no significant differences in the titres of any of the IgG subclasses between the groups ($P > 0{\cdot}05$).

Determination of IgG subclasses specific to P. acnes

The mean reciprocal $\log_2$ titres $\pm$ 95% confidence limits for the IgG subclasses specific to *P. acnes* for the four groups are shown in Table 1. The reciprocal $\log_2$ titres ranged from 4 to >10 in the sera from mild acne patients; from 5 to >10 in sera from moderate acne patients; from 6 to >10 in sera from severe acne patients and from 5 to 9·5 in sera from controls. Titres of IgG_1 ranged from 5 to >10; for IgG_2 from 7·5 to >10; for IgG_3 from 4 to 10·5 and for IgG_4 from 4·5 to 10.

Analysis of the reciprocal $\log_2$ titres of IgG_1 revealed a significant difference between the severe and moderate groups ($P < 0{\cdot}05$). Both severe and moderate acne patients differed significantly in their IgG_2 titres from the control group ($P < 0{\cdot}001$). Titres of IgG_3 demonstrated a significant difference between moderate and severe acne patients ($P < 0{\cdot}05$). There were no

Table 1. Mean reciprocal $\log_2$ titres ± 95 % confidence limits of IgG_{1-4} to *P. acnes* for patient and control sera

	Control	Mild	Moderate	Severe
IgG_1	7·30 ± 0·84	7·75 ± 0·90	6·75 ± 0·77	8·60 ± 1·18
			$P < 0·05$	
IgG_2	8·75 ± 0·54	9·40 ± 0·50	9·80 ± 0·25	9·85 ± 0·17
	$P < 0·001$			
	$P < 0·001$			
IgG_3	6·70 ± 0·59	6·35 ± 0·94	6·1 ± 0·41	7·50 ± 1·08
			$P < 0·05$	
IgG_4	6·25 ± 0·85	7·40 ± 1·33	6·70 ± 0·91	7·65 ± 0·89

significant differences between the groups in the titres of IgG_4 ($P > 0·05$).

Discussion

The aim of this study was to measure the titres of the IgG subclasses specific to *S. epidermidis* and *P. acnes* in patients with varying degrees of acne and control subjects in order to assess the nature of the humoral immune response to the organisms. For *S. epidermidis*, the titres for all the subclasses were within the same range and did not differ significantly between the groups. This contrasts with the proportions of IgG_{1-4} in normal human serum, which are 65, 25, 6 and 4%, respectively.[8] The relative increase in the levels of IgG_3 and IgG_4 specific to *S. epidermidis* suggest the following: as IgG_3 is normally produced in response to protein, the protein components of *S. epidermidis* appear to be important antigens. Secondly, as IgG_4 is the last IgG subclass produced during class switching, it indicates that chronic antigen exposure is occurring in both acne patients and controls. However, IgG_4 has also been found to have a protective effect, for example in patients undergoing hyposensitization treatment. This, together with the lack of significant difference between the IgG subclass titres in acne patients and controls, indicates that there is no evidence for *S. epidermidis*-specific IgG in the pathogenesis of acne.

The pattern of responses to *P. acnes* in the same groups was very different. Titres of IgG_1 to *P. acnes* were significantly higher in patients with severe acne when compared with those in moderate acne patients. This may simply be a reflection of the normal predominance of IgG_1 in human serum, or result from the greater opportunity for sensitization in patients with more severe disease. IgG_1 may bind to *P. acnes* and activate the complement cascade and contribute to the pathology of inflammatory acne.

Patients with both moderate and severe acne had significantly higher titres of IgG_2 when compared with controls. IgG_2 tends to be elicited in response to carbohydrate antigens, suggesting that the polysaccharide present in *P. acnes*, probably in the cell wall, are important immunogens in acne vulgaris patients. This finding is in accord with that of Holland *et al.*[4] who found significantly higher titres of total IgG_2 in patients with severe acne.

Titres of IgG_3 were also higher in severe acne patients compared with moderate acne patients. When class switching occurs from IgM to IgG, IgG_3 is the first subclass formed and hence may be indicative of a recent class switch. However, as appreciable amounts of IgG_4 (the last IgG subclass formed after class switching) were also present, it is likely that the high titres of IgG_3, in association with IgG_1, demonstrate the importance of the protein antigens of *P. acnes* in stimulating an immune response during acne vulgaris. IgG_3 is the most effective subclass at activating the complement cascade and the high titres in severe acne patients may be responsible for considerable complement activation. This result also supports the previous studies of Holland *et al.*[4] who found higher titres of total IgG_3 in patients with severe acne. Titres of IgG_4 were comparable in all the patient and controls groups. High titres of IgG_4 may have protective effects by inhibiting the binding of IgG_1 to complement. If this was an important protective mechanism in acne, patients with mild disease would be expected to have higher titres of IgG_4 than patients with severe disease, but this was not observed.

In conclusion, the pattern of IgG subclass titres specific to *S. epidermidis* and *P. acnes* in patients with acne are very different. While the response to *S. epidermidis* is relatively harmless, the IgG antibody response to *P. acnes* supports the hypothesis that this organism may be involved in the pathogenesis of inflammatory acne vulgaris.

References

1 Norris JFB, Cunliffe WJ. A histological and immunohistochemical study of early acne lesions. *Br J Dermatol* 1988; **118:** 651–9.
2 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:** 43–7.
3 Knop J, Ollefs K, Frosch PJ. Anti-*P. acnes* antibody in comedonal extracts. *J Invest Dermatol* 1983; **80:** 9–12.
4 Holland DB, Ingham E, Gowland G, Cunliffe WJ. IgG subclasses in acne vulgaris. *Br J Dermatol* 1986; **114**: 349–51.

5 Burke B, Cunliffe WJ. The assessment of acne vulgaris: the Leeds technique. *Br J Dermatol* 1984; **111:** 83–92.
6 Ingham E, Holland KT, Gowland G, Cunliffe WJ. Purification and partial characterization of hylauronate lyase (EC 4.2.2.1) from *Propionibacterium acnes. J Gen Microbiol* 1979; **115:** 411–18.
7 Sokal RR, Rohlf FJ. In: *Biometry. The Principles and Practice of Statistics in Biological Research*, 2nd edn. San Francisco: W H Freeman and Company, 1981; 208–70.
8 Papadea C, Check IJ. Human immunoglobulin G and immunoglobulin G subclasses: biochemical, genetic and clinical aspects. *Crit Rev Lab Sci* 1989; **27:** 27–58.