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EVALUATION OF DELAYED TYPE IMMUNE REACTIVITY IN PATIENTS WITH ACNE VULGARIS

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Abstract. The delayed-type immune reactivities of 39 patients suffering from moderate to severe papulopustular or nodulo-cystic forms of Acne vulgaris were compared with healthy acne-free controls. *In vivo* reactivity was assessed by skin testing with four common recall antigens. *In vitro* the afferent arm of the immune system was assayed by antigen and mitogen stimulated lymphocyte transformation and the efferent arm by granulocyte random migration and by granulocyte responsiveness to a standard migration inhibitory lymphokine preparation. Spontaneous lymphocyte transformations were also evaluated. In acne patients the skin test reactivities were in general only slightly decreased. Their lymphocytes showed relatively normal responses to mitogen stimulation, but antigen responsiveness was significantly diminished. Furthermore the patients' lymphocytes showed increased spontaneous transformation. All these changes correlated, to some extent, with the severity of the disease. The granulocytes of acne patients showed increased random migration, of borderline significance, but the granulocyte responses to the lymphokine preparation were completely normal.

The results suggest that immunologic abnormalities are mild and probably secondary to the inflammatory process.

Key words: Acne vulgaris; Skin tests; Lymphocyte stimulation; Leukocyte migration inhibitory factor; Granulocyte random migration

Cellular immunity to *Propionibacterium acnes* (synonymous with *Corynebacterium acnes* and *Corynebacterium parvum*), the predominant organism both in acne lesions and normal skin, is believed to play a role in determining the intensity of the inflammatory response around the lesions (4, 9). This theory is supported by the fact that immediate-type skin reactivity is elevated in patients with acne vulgaris. Elevated titres of serum antibodies to *P. acnes* have also been detected in acne patients (6, 7). However, delayed-type hypersensitivity (DHT) skin reactions to *P. acnes* have been shown to be negative in acne patients as well as in acne-free subjects (8). In vitro, on the other hand, patients with severe acne showed elevated lymphocyte transformation and LIF production when stimulated by *P. acnes*, the lymphocyte transformation to PHA being normal (4, 9).

Patients with severe, conglobatic acne have been shown to have an overall decrease in their DTH skin reactivity to common recall antigens (5, 10), and a significant positive correlation between severity of acne and granulocyte chemotaxis as well as random mobility has been discovered. This did not apply to monocytes, however.

The present study was undertaken to further evaluate the role of cell-mediated immunity (CMI) in acne vulgaris and its correlation to the severity of the disease. To evaluate the afferent arm of immune reactivity, antigen- and mitogen-induced lymphocyte transformation assays were performed, while, in the belief that acne lesions are strongly infiltrated by polymorphonuclear cells, the efferent target cell reactivity was tested by assaying granulocyte random mobility and responsiveness to leukocyte migration inhibitory factor (LIF). In vivo DTH was tested by reactivity to common recall antigens.

MATERIALS AND METHODS

Patients. 39 ambulatory patients, with moderate to severe acne, were selected for the study. Of these, 22 were females and 17 males, with ages ranging from 14 to 29 (mean 21.5) years. The papulopustular form of acne, grades II–IV (5), was seen in 15 patients, and 24 had nodulocystic lesions. The patients were divided into two groups according to the clinical severity of the disease. Group I consisted of 23 patients with grade II papulopustular form or milder nodulo-cystic form of acne. Group II comprised 16 patients with grades III–IV papulopustular acne or severe nodulocystic disease.

Controls. In order to evaluate *in vivo* and *in vitro* the level of immune reactivity to the test antigens in a normal healthy population, a control group consisting of 18 normal, healthy, acne-free people between 15 and 41 years of age (mean 22 years) was formed. They were skin tested with the four test antigens, and lymphocyte stimulations and standard LIF assays were performed.

Table I. *Grading of skin reactions*

Antigen concentration	0–4 mm[a]	5–9 mm	10–14 mm	>15 mm
Highest	1	2	3	4
2nd highest	5	6	7	8
3rd highest	9	10	11	12
4th highest	13	14	15	16

[a] Mean diameter of induration or erythema.

Skin test. The skin tests were performed by injecting intracutaneously 0.1 ml of tuberculin (PPD, State Serum Institute, Copenhagen, Denmark) and oidiomycin (OM, Dermatophyton "O", Hollister Stier, Spokane, Wa., USA), SK-SD (Varidase, Lederle, USA) and parotitis vaccine (kindly provided by Professor Kari Cantell, Central Public Health Laboratory, Helsinki, Finland). The following concentrations or dilutions of the antigens were used: PPD, 0.1 TU, 1 TU, 10 TU and 100 TU; OM, 1 : 500, 1 : 50 and 1 : 5; SK-SD, 5 IU and 50 IU; parotitis, 1 : 10 and 1 : 100. The skin tests were started with the lowest concentrations and repeated at weekly intervals until a positive skin reaction developed, or the highest concentration of the antigen was reached. The skin tests were read for erythema and induration at 24, 49 and 72 hours after injection. The skin reactions, induration and erythema, were graded as shown in Table I. Thus as parotitis and SK-SD were used only in two concentrations these reactions were graded from 0 to 7, OM reactions to three antigen concentrations were graded 0–11, and as PPD was used in four concentrations the reactions were graded from 0 to 15.

Lymphocyte stimulation assay. 25 ml of peripheral venous blood was drawn into a heparinized plastic syringe and the mononuclear cells were separated by Ficoll-Isopaque gradient centrifugation (1). The cultures were set up on flat-bottomed microplates (Sterilin, Middlesex, UK) containing in each well 10^5 lymphocytes per 0.2 ml of minimal essential medium for suspension cultures (MEM-S), supplemented with 15% autologous plasma. Four replicate wells were stimulated with leukoagglutinin (LA, Pharmacia Fine Chemicals, Uppsala, Sweden) at concentrations of 12.5 mg/l and 25 mg/l, and with tuberculin PPD (State Serum Institute, Copenhagen, Denmark) at concentrations of 12.5 mg/l and 50 mg/l. Appropriate controls contained no stimulant. The cultures were incubated for 3 and 4 days (LA) or for 5 and 6 days (PPD) at 37°C in a humidified 5 %CO_2 atmosphere. The cultures were pulsed for 18 h with ^{125}IUdR (0.125 μCi, specific activity 5 000 Ci/mol, Radiochemical Centre, Amersham, UK) before harvesting with an automatic harvester (Skatron, Flow Laboratories, Norway). The filters were counted in a well-type gamma counter (Wallac, Turku, Finland), and the stimulation indices (SI) were calculated followingly:

$$SI = \frac{\text{cpm} \pm \text{S.D.}/10^5 \text{ stimulated lymphocytes}}{\text{cpm}/10^5 \text{ non-stimulated lymphocytes}}$$

The stimulation indices obtained with the highest antigen or mitogen concentrations, and with the culture period yielding highest stimulation indices, were used in the analysis of the results.

Preparation of standard lymphokine. Mononuclear cells, separated by Ficoll-Isopaque gradient centrifugation, were pulse treated with 40 mg/ml of LA for 30 min, after which they were washed three times with Hanks' balanced salt solution (HBSS). The cells were cultured in serum-free MEM-S in polystyrene tubes at a concentration of 2×10^6 cells/ml for 2 days. After culturing the tubes were centrifuged at 1 000 *g* for 10 min and the supernatants pooled. The supernantants were then concentrated to 1 : 50 of the original volume by ultrafiltration at 4°C. This concentrated preparation was stored at −80°C and used as a standard lymphokine.

Leukocyte migration inhibitory assay. LIF responsiveness of the patient's granulocytes was tested by the agarose migration method (2) with minor modifications. Briefly, heparinized peripheral blood was sedimented with dextran and the leukocyte-rich plasma was centrifuged on Ficoll-Isopaque. Leukocytes at the bottom of each tube, containing an average of 98% granulocytes and 2% mononuclear cells, were washed three times before being used as indicator cells in the assay. Dilutions of 1 : 4, 1 : 32 and 1 : 256 in MEM-S of the LIF preparation or plain MEM-S (control) were preincubated at 37°C for 30 min with the isolated granulocytes, after which 5 μl of the suspension, containing 5×10^5 granulocytes, was pipetted into each 2.3 mm diameter well punched in the agarose. The supernantants were tested in four replicate determinations. The plates were incubated at 37°C in a humidified 5% CO_2 atmosphere. The area of the well was excluded from the final area of migration. Means and standard deviations were calculated. Migration indices were calculated as follows:

$$MI = \frac{\text{mean migration area} \pm \text{S.D., LIF supernatant}}{\text{mean migration area, control supernatant}}$$

The results are expressed as dilutions of the LIF preparation corresponding to 50% migration inhibition, calculated by linear regression analysis.

Granulocyte random migration assay. The random migrations of granulocytes were calculated from the controls of the LIF assay.

RESULTS

In vivo. The results are summarized in Table II. The skin reactions of the acne patients were generally decreased, and this change was clearer in the

Table II. *Skin test reactivities of acne patients and healthy controls*

	OM[a]		SK-SD[a]		Parot.[a]		PPD[a]	
	Erythema	Induration	Erythema	Induration	Erythema	Induration	Erythema	Induration
Controls	8.6±0.6	6.4±1.0	6.1±0.5	4.0±0.8	9.3±0.8	6.8±1.1	11.0±0.5	7.6±1.1
Mild acne	7.5±0.7	4.5±0.7	5.9±0.5	3.3±0.7	9.7±0.2	6.9±0.9	9.7±0.8	4.3±1.2
Severe acne	6.1±0.9	2.4±0.9**	4.5±0.7	2.6±0.8	8.7±0.5	5.7±1.2	9.8±0.9	5.6±1.5
Mild+severe acne	6.9±0.6*	3.6±0.7**	5.3±0.4	3.0±0.5	9.2±0.3	6.5±0.7	9.7±0.6	4.9±0.9

[a] Mean grade ± S.E.M. of skin reactions.
* Differs almost significantly from control group ($p<0.05$; independent, 2-dimensional Student's *t*-test).
** Differs significantly from control group ($p<0.01$; independent, 2-dimensional Student's *t*-test).

clinically more severely affected group. However, only the OM induration reaction of the patients differed significantly from the control group ($p<0.01$). Furthermore this reaction differed with a $p<0.05$ value between the clinically milder and more severe groups.

In vitro. The results are summarized in Table III. The lymphocyte transformation indices, both with LA and PPD stimulations, were significantly reduced in acne patients ($p<0.01$–0.001). Again, the stimulation indices in the more severe acne group were lower than in the milder acne group ($p<0.05$). The spontaneous lymphocyte transformation was higher in the acne group than in the healthy control group and again this difference was more profound in the more severe acne group. The cultures serving as controls for the LA stimulations of acne patients differed more from the corresponding spontaneous proliferation of healthy subjects' lymphocytes, than did those serving as control cultures for the PPD stimulations. However, none of the differences was statistically significant.

Granulocyte responses to the standard LIF preparation revealed no differences between the test groups, but the random migration of the acne group's granulocytes was almost significantly ($p<0.02$) increased compared with the control group. This, however, did not correlate with the severity of the clinical status.

DISCUSSION

The present study indicates that only minor *in vivo* abnormalities occur in patients with *Acne vulgaris*. The delayed-type skin reactivities toward all four tested common recall antigens were only slightly decreased, this change correlating to some extent with the severity of the disease. None of the pa-

Table III. *In vitro immune status of acne patients and healthy controls*

	Lymphocyte transformation					
	LA		PPD			
	Spontaneous[c]	Stimulated[d]	Spontaneous[c]	Stimulated[d]	LIF[a]	Granulocyte migration[b]
Controls	17.2±1.2	282±36	19.9±2.0	128±17	370± 90	50.1±2.5
Mild acne	20.6±1.5	230±19**	20.3±1.9	66±11**	230± 76	62.9±4.2*
Severe acne	27.6±5.8	190±27**	25.0±4.5	35± 7***	360±100	61.7±5.6*
Mild + severe acne	23.6±2.7	216±16**	22.2±2.1	54± 8***	340± 60	62.4±3.3*

[a] Means ± S.E.M. of dilutions of Standard LIF preparation corresponding to 50% migration inhibition of test subject's granulocytes.
[b] Mean random migration area ± S.E.M.
[c] Cpm per 10^5 unstimulated cells cultured for the same period as corresponding stimulated cells.
[d] Stimulation index ± S.E.M.
* Differs almost significantly from control group ($p<0.02$; independent, 2-dimensional Student's *t*-test).
** Differs significantly from control group ($p<0.01$; independent, 2-dimensional Student's *t*-test).
*** Differs highly significantly from control group ($p<0.001$; independent, 2-dimensional Student's *t*-test).

tients was anergic. These findings are in agreement with previous work, indicating that only patients with severe, conglobatic acne have significantly decreased skin reactivities (5, 10).

In the lymphocyte transformation assay, acne patients had significantly lower stimulation indices than had healthy controls. However, their lymphocytes showed stronger spontaneous proliferation than those of healthy controls; this probably reflects stimulation by continuous inflammation. The cpm of the unstimulated cultures were used in calculating the stimulation indices. Hence higher spontaneous proliferation leads to a lower index. This difference in spontaneous proliferation between the acne and normal groups largely explains the depressed mitogen (LA) stimulation indices, our results paralelling thus the normal PHA responses in acne patients reported by Puhvel et al. (9). However, the spontaneous proliferation of the patients lymphocytes was not profound enough to account for the depressed antigen (PPD) stimulation indices. Hence it is suggested that there is a slight abnormality in the afferent arm of the immune system of acne patients, manifested by reactivity to antigen but not to mitogen. The fact that PPD skin reactions were relatively normal in acne patients; would appear to contradict this suggestion, though the diameter of skin reactivity may not be a relevant method of quantifying DTH. Also the non-specific inflammatory responsiveness of acne patients may be diminished.

The patients' granulocytes showed increased random mobility, the results thus agreeing with those of Gould et al. (3), whereas the patients' granulocyte reactivities to a standard lymphokine preparation (LIF) seemed to be completely normal, in contrast to the findings of Gould et al. (3), which indicated increased granulocyte responsiveness in a chemotaxis assay. Our results confirm that Gowland et al. (4), in their LIF assay against *Propionibacterium acnes*, really measured increased LIF secretion and not increased granulocyte responsiveness.

Altogether only mild *in vivo* and *in vitro* immunological abnormalities were found in patients with Acne vulgaris, the degree correlated to some extent with the severity of the disease. It is highly unlikely that such minor abnormalities would lead to the development of Acne—hence these are probably secondary to the inflammatory disease.

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