This might be useful for photopatch testing (photoallergy), and testing of patients with porphyria cutanea tarda, solar urticaria and polymorphic light eruptions (2).

We obtained the BSP 580, the KG3 and the WG 340 from the Optisk Laboratorium, the Technical University of Denmark, Lyngby, and the Wratten gelatin filters from Kodak.

REFERENCES

1. Berger, D. S.: Specification and design of solar ultraviolet simulators. J Invest Dermatol *53*: 192, 1969.
2. Magnus, I. A.: Dermatological Photobiology: Clinical and Experimental Aspects. Blackwell Scientific Publications, Oxford, 1976.
3. Wiskemann, A.: Abnormal action spectra. *In* The Biologic Effects of Ultraviolet Radiation (ed. F. Urbach), 197 pp. Pergamon Press, Oxford, 1969.

Leukocyte Inhibitory Factor (LIF) in Granuloma annulare: A Comparative Study between the Generalized and the Localized Types

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Abstract. Leukocyte inhibitory factor (LIF) was investigated in 9 patients with localized granuloma annulare and 8 patients with the generalized form of the disease. The control group consisted of 10 matched, apparently healthy subjects. LIF values were significantly higher in patients with granuloma annulare of both groups than in controls ($p<0.05$ and 0.01). However, no significant difference was revealed between the two groups of patients. Though the results seem to speak for a cell-mediated immune response in granuloma annulare, they do not add a further support to the previously demonstrated differences between the two forms of granuloma annulare.

Granuloma annulare (GA) is a chronic skin disease, characterized histologically by necrobiotic epithelioid granulomas surrounded by a palisade of histocytes, lymphocytes, epithelioid and giant cells (3). Two main forms of the disease are recognized, the localized (LGA) and the generalized (GGA) (7).

In previous studies we pointed out the differences between the two forms of GA regarding age distribution, their relationship to diabetes (8), frequency of microangiopathy (9) and frequency of HLA-Bw 35 (7).

There are conflicting reports concerning the possibility of a defective cell-mediated immune response in patients with GA (3, 12). We found it of interest, therefore, to investigate the leukocyte inhibitory factor (LIF) in patients with GA and, furthermore, to search for a possible difference in this regard between the two forms of the disease.

MATERIAL AND METHOD

Nine patients with LGA and 8 with GGA were included in this study. The LGA group consisted of 7 females and 2 males aged 31 to 60 years. In the GGA group there were 8 females aged 50 to 65 years. The diagnosis of GA was confirmed in each case by histological examination. Ten apparently healthy matched controls were simultaneously and similarly tested. The test of leukocyte migration inhibition was adopted from Erard (6) with a slight modification. 5 µl of 1×10^7 leukocyte-containing lymphocytes with and without PPD were placed in Petri dishes containing 2% agarose, medium M 199 and fetal calf serum. The Petri dish preparations were incubated overnight, fixed with methanol and stained with Giemsa. Images of migration area were projected with an overhead view projector. The areas of migration were measured and the inhibition index was calculated as follows:

$$100 - \frac{\text{Area migration} + \text{Ag}}{\text{Area migration} - \text{Ag}} 100$$

RESULTS

The index of inhibition for each of the three tested groups is shown in Table I. Our data reveal that the index of inhibition in controls was significantly lower than in patients with LGA and GGA as well ($p<0.05$ and 0.01 respectively). No difference was observed between the two types of the disease.

DISCUSSION

Epithelioid granulomas have been considered to be an expression of delayed hypersensitivity reaction to an obscure antigen (2). In support of a similar mechanism in GA we found reports on the development of GA-like histological changes after intradermal injection of tuberculin (1) and lymphogranu-

Table I. *Summary of results*

Material	Mean	Significance
LGA ($n=9$)	27.1±9.7	NS
GGA ($n=8$)	36.7±18.7	
LGA	27.1±9.7	$p<0.05$
Control ($n=10$)	14.7±9	
GGA	36.7±18.9	$p<0.01$
Control	14.7±9	
GA Total ($n=17$)	31.9±14.3	$p<0.01$
Control	14.7±9	

loma venereum antigen (10). An additional point in favour of this hypothesis in GA is the constant finding of fibrin deposition in the necrobiotic areas (11, 13). The presence of fibrin has been demonstrated by immunofluorescence in biopsies of delayed hypersensitivity reaction (5). As further evidence of a cell-mediated immune reaction in GA, Umbert & Winkelmann (14) have found blastogenic lymphocytes in tissue adjacent to necrobiotic areas.

Antigen-induced migration inhibition of human peripheral blood leukocytes (LIF) is extensively used as an in vitro parameter of cell-mediated immunity. It has many similarities to the macrophage inhibitory factor (MIF) which is among the lymphokines postulated to be related to delayed hypersensitivity reaction in vivo (4). Umbert, Belcher & Winkelmann (12) have demonstrated MIF-like activity in sera of patients with GA. However, neither MIF nor LIF were detected in plasma of patients with GA by Cherney et al. (3).

In our study, LIF values were significantly higher in LGA and in GGA as well than in controls ($p<0.05$ and 0.01 respectively). This may constitute further evidence implicating a cell-mediated immune mechanism in the pathogenesis of GA. However, in spite of a lower mean value of LIF obtained in patients with LGA than in those with GGA (27.1+9.7% and 36.7+18.9) the difference between these two values was not statistically significant. Thus these data fail to add any further support in favour of the previously demonstrated differences between the two forms of GA.

REFERENCES

1. Beer, W. E. & Wilson Jones, E.: Granuloma annulare following tuberculin Heaf tests. Trans J Hosp Dermatol Soc *52:* 68. 1966.
2. Broom, B. C. & McLaurin, B. P.: Sarcoidosis: correlation of delayed hypersensitivity, MLC reactivity and lymphocytotoxicity with disease activity. Clinic Exp Immunol *15:* 355, 1973.
3. Cherney, K. J., Lindros, W. E., Goltz, R. W. & Dahl, M. V.: Leukocyte function in granuloma annulare. Br J Dermatol *101:* 23, 1979.
4. Clausen, J. E.: Leukocyte migration inhibitory factor. *In* Manual of Clinical Immunology (ed. Rose & Friedman), p. 56. Am Soc Microbiol, Washington, 1976.
5. Colvin, R. B. & Dvorak, H. F.: Role of the clotting system in cell-mediated hypersensitivity. II. Kinetics of the fibrinogen/fibrin accumulation and vascular permeability changes in tuberculin and cutaneous basophil hypersensitivity reaction. J Immunol *114:* 377, 1975.
6. Erard, P.: Technical study of the leukocyte migration inhibition agarose. Clin Exp Immunol *18:* 439, 1974.
7. Friedman-Birnbaum, R., Haim, S., Gideone, O. & Barzilai, A.: Histocompatibility antigens in granuloma annulare. Comparative study of the generalized and localized types. Br J Dermatol *98:* 425, 1978.
8. Haim, S., Friedman-Birnbaum, R., Haim, N., Shafrir, A. & Ravina, A.: Carbohydrate tolerance in patients with granuloma annulare. Study of fifty-two cases. Br J Dermatol *88:* 447, 1973.
9. Haim, S., Shafrir, A., Haim, N. & Lichtig, C.: Microangiopathy in cases of granuloma annulare. Dermatologica *147:* 261, 1973.
10. Schaumann, J. & Seeberg, G.: On cutaneous reactions in cases of lymphogranulomatosis benigna. Acta Dermatovener (Stockholm) *28:* 158, 1948.
11. Thyresson, H. N., Doyle, J. A. & Winkelmann, R. K.: Granuloma annulare: histopathologic and direct immunofluorescent study. Acta Dermatovener (Stockholm) *60:* 261, 1980.
12. Umbert, P., Belcher, R. W. & Winkelmann, R. K.: Lymphokines (MIF) in the serum of patients with sarcoidosis and cutaneous granuloma annulare. Br J Dermatol *95:* 481, 1976.
13. Umbert, P. & Winkelmann, R. K.: Granuloma annulare, direct immunofluorescence study. Br J Dermatol *95:* 487, 1976.
14. — Histologic, ultrastructural and histochemical studies of granuloma annulare. Arch Dermatol *113:* 1681, 1977.