

Increased Macrophage Activity in Psoriasis

M. S. KOH, B. B. J. MAJEWSKI and E. L. RHODES

Department of Dermatology, St Helier Hospital, Wrythe Lane, Carshalton, Surrey, England

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Macrophages derived from circulating blood monocytes of psoriatic patients demonstrated an enhanced release of β -glucuronidase and lysozyme compared with macrophages from healthy subjects. The relationship of cell activation to the pathogenesis of psoriasis is discussed. (Received November 17, 1984.)

MS Koh, Department of Dermatology, St Helier Hospital, Wrythe Lane, Carshalton, Surrey, SM5 1AA, England.

A number of reports have been published on the functional changes in bone-marrow derived cells in psoriatic patient. In some cases, the extent of the change is related to activity of the disease, and may simply be a consequence of the cutaneous lesions. However, several groups of workers have found alterations in polymorphonuclear and mononuclear cell functions which seem to be characteristic of the psoriatic individual, being independent of the extent (or even the presence) of cutaneous pathology. Wahba et al. (1) and Michaelsson (2) showed that leucocytes derived from the peripheral blood of psoriatic patients have enhanced chemotactic responses compared with leucocytes from healthy subjects. Similarly Kruger et al. (3) observed increased chemotaxis of psoriatic blood monocytes to lymphokine and bacterial factor while Bar Eli et al. (4) observed enhanced phagocytosis of labelled bacteria by psoriatic monocytes. They suggested that this increase is an inherent disorder probably mediated via a decrease in the cAMP/cGMP ratio.

Mononuclear cells that are present in the tissue component are either lymphocytes or transformed blood monocytes, i.e. macrophages. Macrophages present in tissues have characteristics which differ from circulating monocytes (5, 6) and these changes in function and biochemical characteristics are associated with a maturation or differentiation process. Current evidence that macrophages are altered in psoriatic subjects is indirect. Molin & Reizenstein (7) reported enhanced uptake of radioactively labelled red blood cells by the reticuloendothelial system. We have attempted to study macrophages grown from circulating psoriatic monocytes to see if the hyperactivity of psoriatic neutrophils and blood monocytes also occurs in these cells.

MATERIALS AND METHODS

Eighteen psoriatic patients and 23 healthy control subjects (hospital personnel closely matched for age) were studied. Eight of the patients were female and 10 were male while 10 of the controls were female and 13 were male. Nine of the patients had minimal skin involvement, e.g. a few small plaques on the elbows or palmar psoriasis with nail pitting. Eight other patients had widespread skin lesions while one patient had very severe generalized pustular psoriasis. None of the patients studied had been receiving oral steroids or immunosuppressive drugs for the last 6 months.

Peripheral blood cell separation and macrophage culture

Fifty ml of blood was collected by venesection and divided into two portions in sterile 30 ml containers. One tube containing 20 ml of blood was allowed to clot for 2 hours and then centrifuged at

800×g for 20 min to obtain the serum. The other tube containing 30 ml of blood plus heparin 10 µ/ml was allowed to stand for 2 hours at room temperature. The leucocyte rich plasma was decanted and differential cell count made. Cell suspension containing 6×10^6 mononuclear cells was pipetted into Leighton tubes containing plastic coverslips and incubated at 37°C in 5% CO₂ for 30 min. The cells were then washed with sterile warm phosphate buffered saline (PBS) to remove non-adherent cells. Two ml of medium 199 (Wellcome) containing 50% autologous serum plus penicillin 100 µ/ml and streptomycin 100 µg/ml were put into the Leighton tubes and incubated for a further 48 hours. By this time most of the PMN became detached from the coverslip while the monocytes adhered firmly to the plastic. After washing twice with sterile PBS, 2 ml of medium 199 (plus 50% autologous serum and antibiotics) were put in the tube and the cells were cultured for a further 4 days by which time most of the monocytes had spread and transformed into macrophages (morphological definition). At the end of 6 days culture, the supernatant was collected and macrophages washed thoroughly before being removed with 2 ml of Triton X-100 (0.2%). The cells were disrupted by freezing and thawing and the cell lysate collected by centrifugation (1200×g). Three Leighton tubes were used for each test.

B-glucuronidase

The amount of β-glucuronidase was determined by incubating 0.1 ml of the Triton X-100 lysate or the culture supernatant with 0.1 ml substrate (4 methyl umbelliferyl B D glucuronide) at 37°C for 3 hours. The reaction was terminated by adding 3.8 ml of glycine buffer pH 10.4 and the product measured fluorometrically at 446 nm. One unit of enzyme activity is defined as 1 nmole of 4 methyl umbelliferone liberated per hour and specific enzyme activity was expressed as units/µg of cellular protein.

Lysozyme

Lysozyme in culture supernatant or cell lysates was quantified by measuring the lysis of *Micrococcus lysodieticus* as determined by a reduction in optical density at 450 nm (8). Specific enzyme activity is expressed as units per µg cellular protein where one unit is the amount of enzyme which causes a change in optical density of 0.001 per min.

Lactate dehydrogenase

This was measured spectrophotometrically as described by Bergmeyer et al. (9).

Protein determination

The protein content of 0.1 ml of Triton X-100 cell lysate or culture supernatant was determined by the method of Lowry et al. (10) using bovine serum albumin as the standard.

Statistical analysis

The results were expressed as mean ± one standard deviation and the significance was analysed using the Student's *t*-test.

RESULTS

When cultured for 6 days blood monocytes showed features characteristic of macrophages. As shown in Fig. 1 most of the cells have spread extensively with pseudopod protrusions. About 10%–20% of the cells have 2 nuclei suggesting mitosis has occurred. Giant cells, both of foreign body and Langhans types, could be seen scattered throughout the coverslip.

B-glucuronidase release

Intracellular and extracellular levels of β-glucuronidase were measured. Both the release and cellular content of the lysosomal enzyme were significantly higher in psoriasis than in controls. As shown in Fig. 2 the mean intracellular and extracellular enzyme levels were 3.47 ± 0.5 and 1.03 ± 0.12 for psoriasis and 3.04 ± 0.5 and 0.86 ± 0.1 for controls. These differences are statistically significant ($p < 0.005$).

Lysozyme production

As shown in Fig. 3 extracellular but not intracellular levels of lysozyme were elevated in psoriasis culture. The extracellular enzyme level for psoriasis was 17.33 ± 1.8 and for

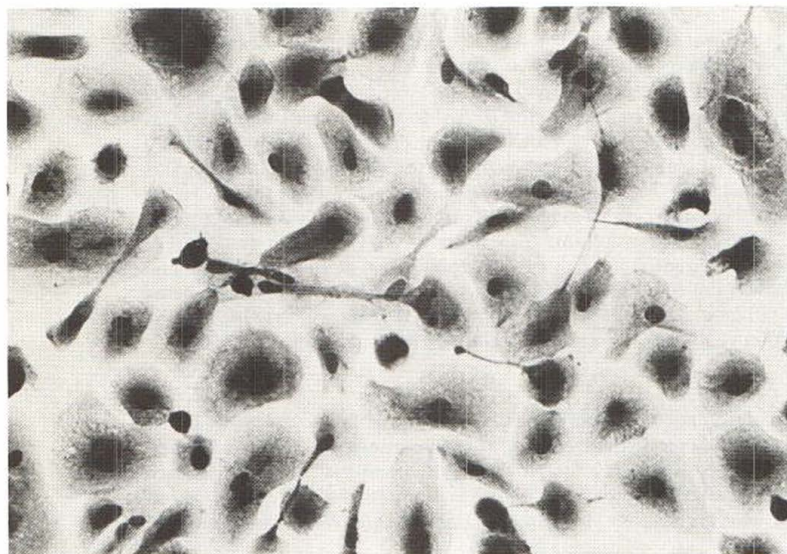


Fig. 1. Monolayer of macrophages after 5 days of culture.

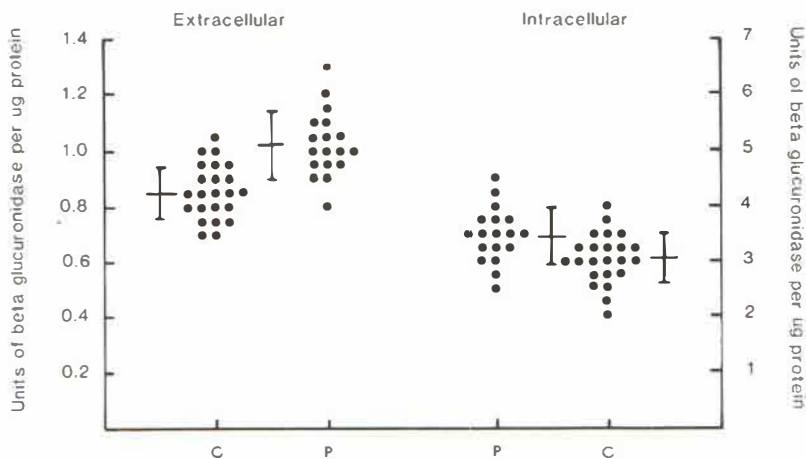


Fig. 2. Production of β -glucuronidase by psoriatic (P) and control (C) macrophages after 6 days of culture.

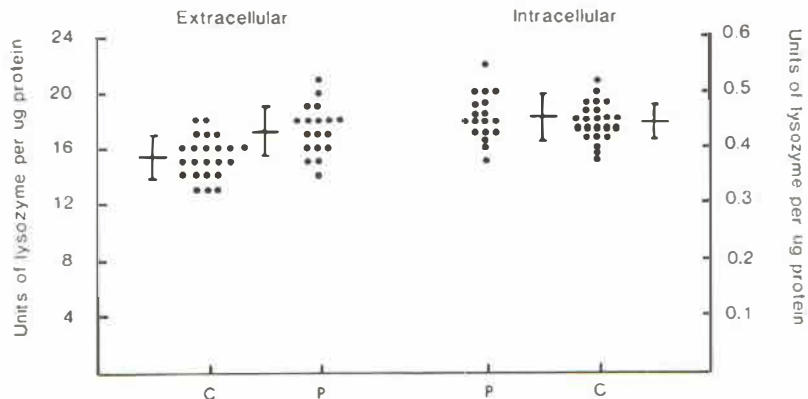


Fig. 3. Production of lysozyme by psoriatic (P) and control (C) macrophages after 6 days of culture.

control it was 15.35 ± 1.51 and this difference is statistically significant ($p < 0.01$). No significant difference was observed in the intracellular level between psoriatic and control cultures.

Lactate dehydrogenase (cytoplasmic marker)

There was no significant difference in lactate dehydrogenase level between psoriatic and control cultures. Cell viability remained high (90%) for the first 7 days of culture, but decreased sharply after 8 days.

There was no significant difference in macrophage activity between limited and widespread psoriasis, but a particularly high level was found in the one case of generalized pustular psoriasis.

DISCUSSION

We have demonstrated that macrophages derived from psoriatic monocytes are more active than those from control monocytes. Psoriatic macrophages produce more β -glucuronidase and lysozyme than control macrophages. This hyperactivity of psoriatic macrophages is analogous to the enhanced chemotactic and phagocytic activity of psoriatic neutrophils (1, 2). It seems that in psoriasis there is an intrinsic abnormality which is expressed in the bone marrow. This has led to the concept that the primary expression of the 'psoriatic' gene is in the monoblast; this is perpetuated in the promonocyte, the monocyte and finally the macrophage. The precise nature of this defect, although unknown, is probably manifested by a decrease in cyclic AMP level. Cyclic AMP modulates polymorphonuclear and mononuclear cell activities and possibly the differentiation of keratinocytes. It has been shown experimentally that increases in the intracellular level of cyclic GMP enhance chemotaxis (11) while increases in the level of cyclic AMP inhibit chemotaxis and phagocytosis (12). Similarly it has been found that increased intracellular cyclic AMP reduces lysosomal enzyme release during phagocytosis while increased intracellular cyclic GMP enhances their release (13, 14). Although we did not measure the levels of cyclic AMP and cGMP in psoriatic macrophages, it seems logical to assume that a decreased cyclic AMP/cyclic GMP ratio does occur in this cell, similar to the imbalance between these two cyclic nucleotides found in psoriatic monocytes (15). Thus it is possible that the hyperactivity of psoriatic macrophages reported here, and the enhanced neutrophil and monocyte activities observed by others, together with the excessive epidermal proliferation found in psoriasis, are caused by a common underlying mechanism.

Finally, although it is apparent that neutrophils, monocytes and macrophages in psoriasis behave abnormally, the relationship this bears to the proliferative changes in the epidermis has yet to be elucidated. The possibility that the macrophage produces substances which enhance epidermal cell proliferation as it does for fibroblasts (16) and vascular tissue (17) cannot be discarded.

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REFERENCES

1. Wahba A, Cohen HA, Bar-Eli M, Gallily R. Enhanced chemotactic and phagocytic activities of leucocytes in psoriasis vulgaris. *J Invest Dermatol* 1978; 71: 186-188.
2. Michaëlsson G. Increased chemotactic activity of neutrophil leucocytes in psoriasis. *Br J Dermatol* 1980; 103: 351-356.

3. Krueger GG, Jederberg WW, Ogden BE, Reese DL. Inflammatory and immune cell function in psoriasis. II. Monocyte function, lymphokine production. *J Invest Dermatol* 1978; 71: 195-201.
4. Bar-Eli M, Gallily R, Cohen HA, Wahba A. Monocyte function in psoriasis. *J Invest Dermatol* 1979; 73: 147-149.
5. Elias PM, Epstein WL. Ultrastructural observations on experimentally induced foreign body and organised epitheloid-cell granulomas in man. *Am J Pathol* 1968; 52: 1207-1223.
6. Van der Rhee JJ, van der Burgh-deWinter CPM, Daems WTh. The differentiation of monocytes into macrophages. I. Fine structure. *Cell Tissue Res* 1979; 197: 355-378.
7. Molin L, Reizenstein P. Haematological changes in psoriasis. *Acta Derm Venereol (Stockh)* 1974; 54: 465-469.
8. Shugar D. Measurement of lysozyme activity and ultra-violet inactivation of lysozyme. *Biochim Biophys Acta* 1952; 8: 302-309.
9. Bergmeyer HU, Bernt E, Hess B. Lactate dehydrogenase. In: Bergmeyer HU, ed. *Methods of enzymic analysis*. New York: Verlag Chemie, Academic Press, 1963; 736.
10. Lowry OH, Rosebrough NJ, Farr AC, Randall RJ. Protein measurement with the protein phenol reagent. *J Biol Chem* 1951; 193: 265-275.
11. Rivkin T, Becker EL. Possible implication of cyclic AMP in the chemotaxis of rabbit peritoneal polymorphonuclear leucocytes. *Fed Proc* 1972; 31: 657 abs.
12. Bourne HR, Lehrer RI, Cline MJ, Melman KL. Cyclic AMP in human leucocyte: Synthesis, degradation and effect of neutrophil candidacidal activity. *J Clin Invest* 1971; 50: 920-929.
13. Weissman G, Lurier GB, Spieler PJS, Goldstein JM. Mechanisms of lysosomal enzyme release from leucocytes exposed to immune complexes and other particles. *J Exp Med* 1971; 134: 149s-165s.
14. Colombo C, Ignarro LJ, Chart JJ. Enzyme release from polymorphonuclear leucocyte lysosomes, inhibition by catecholamines and cyclic AMP, acceleration by cholinergic agents and cyclic GMP. *Fed Proc* 1973; 32: 291 abs.
15. Geerdink JPM, Bergers M, Van Erp PEJ, Gommans JM, Mier PD, Roelfzema H. Cyclic AMP is decreased in mononuclear leucocytes from psoriasis patients. *Br J Dermatol* 1980; 103: 107-108.
16. Leibovich SJ, Ross R. A macrophage dependent factor that stimulates the proliferation of fibroblasts in vitro. *Am J Pathol* 1976; 84: 501-514.
17. Polverini PJ, Cotran RS, Gimbrone MA, Unanue ER. Activated macrophages induced vascular proliferation. *Nature* 1977; 269: 804-806.