Neutrophil chemotaxis in ankylosing spondylitis, Reiter's disease, and polymyalgia rheumatica

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SUMMARY Neutrophil chemotaxis was found to be normal in 14 patients with ankylosing spondylitis, in 10 patients with Reiter's disease, and in 8 patients with polymyalgia rheumatica.

The main function of the polymorphonuclear leucocyte (neutrophil) is the phagocytosis and digestion of foreign and dead material. Chemotaxis is an essential component of the directed movement of a neutrophil from the blood vessel towards such material in the extravascular space or synovial joint cavity. Abnormalities in neutrophil chemotaxis and phagocytosis have been shown in patients with rheumatoid arthritis and systemic lupus erythematosus (Mowat and Baum, 1971; Zivkovic and Baum, 1972; Turner *et al.*, 1973; Clark *et al.*, 1974). The impairment in cell function in both diseases has been attributed, at least in part, to the prior ingestion of immune complexes.

Recent work suggests that immunological abnormalities, often with the formation of immune complexes, occur in patients with ankylosing spondylitis, Reiter's disease, and polymyalgia rheumatica. This study was undertaken to determine whether such abnormalities led to changes in neutrophil chemotaxis.

Materials and methods

Peripheral blood neutrophils from 14 patients with ankylosing spondylitis, 10 patients with Reiter's disease, and 8 patients with polymyalgia rheumatica were used. The patients with ankylosing spondylitis (12 male, 2 female) with a mean age of 33 years (range 21–44 years) fulfilled the New York criteria (Bennett and Wood, 1968), were being treated with a wide range of nonsteroidal anti-inflammatory agents which in earlier studies have not been shown to alter neutrophil chemotaxis (Mowat and Baum, 1971), and had widespread active disease at the time of testing. The mean erythrocyte sedimentation rate (Westergren) was 31 mm/h (range 5–81 mm/h) and

the mean duration of disease 7.8 years (range 2–20 years).

The patients with Reiter's disease (8 male, 2 female) with a mean age of 34 years (range 22–55 years) all had urethritis and arthritis although only 5 patients had conjunctivitis and 3 patients subsequently developed keratoderma blennorrhagica. Only one patient, with 30 years' duration of disease, had sacroiliac joint involvement either clinically or radiographically. All had active disease which had been treated with a nonsteroidal, anti-inflammatory agent. The mean ESR was 57 mm/h (range 5–100 mm/h), and the mean duration of disease (excluding the patient who had had intermittent symptoms and signs for 30 years) was 3 weeks (range 1–4 weeks).

The diagnosis in the patients with polymyalgia rheumatica (4 male, 4 female) had been made on the typical presentation of severe pain and stiffness of the muscles of the shoulder and pelvic girdles, accompanied by general malaise and a modest hypochromic, normocytic anaemia. A raised ESR was not considered essential for the diagnosis (Mowat and Hazleman, 1974). No patient had overt evidence of temporal ('giant cell' or cranial) arteritis and arterial biopsy was not undertaken. The patients' prompt response to corticosteroid therapy and subsequent follow-up did not suggest that an incorrect diagnosis had been made. At the time of testing all had active disease and none had been treated with corticosteroids. The mean age of the patients was 68 years (range 55–81). The mean ESR was 56 mm/h (range 4–130 mm/h) and the mean duration of disease was 5–6 months (range 1–9 months).

The controls (40 men, 42 women) with a mean age of 53 years (range 22–80 years) were medical and laboratory staff and patients with no intercurrent disease and on no drug therapy who were awaiting minor orthopaedic surgery. Since earlier studies have shown that neutrophil chemotaxis is not dependent

upon the age and sex of the subject, exact matching of the controls was not done (Mowat and Baum, 1971; Mowat, 1976).

Neutrophil chemotaxis was measured by the method of Baum *et al.* (1971). The neutrophils from 10 ml heparinised peripheral blood were sedimented and concentrated by the addition of 2% methyl cellulose. Aliquots of the cell suspension were diluted with Hanks's solution and deposited on a 3 μ m millipore filter using a cytocentrifuge. The filter was enclosed in a Sykes-Moore tissue culture chamber, the upper compartment was filled with Hanks's solution and the lower attractant compartment was filled with a mixture of casein (5 mg/ml) and standard AB serum, stored in aliquots at -70°C , as a source of complement. After 3 hours' incubation at 37°C the filter was removed, stained, and mounted. The number of cells in the upper and lower layers of 10 random areas of the filter were counted using standard areas in the microscope eyepiece reticule. The chemotactic index equals (number of cells (attractant side))/(number of cells (starting side)) $\times$ 1000. Duplicate chambers were used and showed a mean variability of 7% in the index.

Results

The mean $\pm$ 1SD neutrophil chemotactic index for the controls was 544 ± 78 . The neutrophil chemotactic index was normal in all 3 patient groups and no patient had an index outside the normal range. The mean index $\pm$ 1SD for the patients with ankylosing spondylitis was 562 ± 36 , for the patients with Reiter's disease 536 ± 32 , and for the patients with polymyalgia rheumatica 548 ± 26 .

Discussion

Defective neutrophil chemotaxis *in vivo* may arise due to intrinsic defects in the leucocytes, either familial or acquired, and these have been shown in a number of conditions including rheumatoid arthritis and systemic lupus erythematosus (Mowat and Baum, 1971; Clark *et al.*, 1974), while a number of drugs have been implicated in the causation of such defects. Defects may also arise due to familial or acquired abnormalities in the complement system, while inhibitors against both the leucocytes or chemotactic factors have been described (Ward, 1972; Keller *et al.*, 1975; Miller, 1975). Thus it is possible to find either similar defects in several functions of one cell type or similar cell lines similarly affected, for instance by cell poison, or a specific defect in one cellular function with other cellular activities being unaffected. The standardised *in vitro* method used here eliminates a number of

possible mechanisms, in particular many of those relating to the complement system and its inhibitors as standard AB serum rather than the patient's serum is used. *In vivo* a wide range of chemoattractants including complement components and immune complexes, may be present. The present study has used the standardised generation of complement components by casein as the chemoattractant, but comparable results have been found using *E. coli* endotoxin and immune complexes (Baum, 1975).

No abnormality in neutrophil chemotaxis in patients with ankylosing spondylitis, Reiter's disease or polymyalgia rheumatica was demonstrated. Baum (1975), who used a similar method, reported that neutrophil chemotaxis was normal in 5 patients with ankylosing spondylitis although no clinical details were given.

Ankylosing spondylitis and Reiter's disease have until recently been considered to be immunologically 'silent'. However, the association of both diseases with a high incidence of HLA B27 positivity (Brewerton, 1975) has led to a series of reports which suggest that immunological abnormalities are frequently present. Bluestone *et al.* (1975) found abnormalities in the serum concentrations of one or more immunoglobulins in half the patients and diminished concentrations of serum complement (C3) in 20% of patients with Reiter's disease, while specific cellular immune responsiveness to IgG was present in the majority of patients with ankylosing spondylitis. Nikbin *et al.* (1975) found raised values for IgG and IgM and complement components in patients with ankylosing spondylitis, while Eghtedari *et al.* (1976a) confirmed the raised value for serum immunoglobulins and found increased numbers of circulating immunoblasts in 11 of 39 patients with ankylosing spondylitis. Yates *et al.* (1975) found evidence of complement activation and synovial tissue immunoglobulin complexes in patients with Reiter's disease.

It has been suggested that the impairment in neutrophil chemotaxis in patients with rheumatoid arthritis and systemic lupus erythematosus can be attributed, at least in part, to the prior ingestion of immune complexes (Mowat and Baum, 1971; Clarke *et al.*, 1974), and in patients with rheumatoid arthritis a correlation has been shown between neutrophil chemotaxis and the amount of soluble immune complexes in the serum of patients with both seropositive and seronegative disease (Roberts, Thomson *et al.*, 1976). Cats *et al.* (1975) found phagocytosis of IgG and IgM by granulocytes from the synovial fluid in patients with ankylosing spondylitis, while Corrigan *et al.* (1978) found evidence of circulating immune complexes, detected by the inhibition of antibody-mediated lymphocyte induced cytotoxicity in 11 of 18 patients with

ankylosing spondylitis. Thus, an impairment of neutrophil chemotaxis in either ankylosing spondylitis or Reiter's disease might have been expected.

There is less evidence of immunological abnormalities in patients with polymyalgia rheumatica or the closely related condition temporal arteritis, and thus the failure to show an impairment in neutrophil chemotaxis attributable to an immunological mechanism is not unexpected. However, lymphocytes of patients with polymyalgia rheumatica show an increased transformation response to arterial antigen (Hazleman *et al.*, 1975); immunoglobulin has been found deposited in the media of involved vessels (Liang and Simkin, 1973), raised levels of immunoglobulins, especially IgM, are found in some patients (Bacon *et al.*, 1975), and Eghtedari *et al.* (1976b) found an increased number of circulating immunoblasts in the majority of patients.

Although in some patients ESR levels were only modestly raised, this did not invalidate the diagnosis or suggest that their disease was inactive (Mowat and Hazleman, 1974). Finally, although corticosteroid therapy can improve the neutrophil chemotaxis in patients with severe rheumatoid arthritis and other connective tissue diseases (Mowat and Baum, 1971), no patient had been treated with these drugs at the time of testing.

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