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NEUTROPHIL, MONOCYTE AND LYMPHOCYTE LOCOMOTION IN RHEUMATIC FEVER AND RHEUMATOID ARTHRITIS*

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Neutrophil, monocyte and lymphocyte chemotaxis was investigated in 19 patients with active rheumatic fever (10 with carditis), in 15 patients with rheumatoid arthritis and in 20 healthy, age-matched controls. Chemotaxis assays were repeated in the rheumatic fever patients on the fifth day of therapy and two weeks after remission. Neutrophil and monocyte chemotaxis was found to be significantly decreased in the rheumatoid arthritis patients when compared with the controls and rheumatic fever patients. In contrast, neutrophil chemotactic activity was significantly higher in the rheumatic fever patients when compared with the healthy controls. Monocyte and lymphocyte chemotaxis in patients with rheumatic fever was not significantly different when compared with the controls. Neutrophil, monocyte and lymphocyte locomotion was found to be significantly decreased on the fifth day of salicylic acid or prednisolone treatment. *Key words: rheumatoid arthritis, rheumatic fever, chemotaxis, neutrophil, monocyte, lymphocyte.*

Both rheumatoid arthritis (RA) and acute rheumatic fever (RF) are known as inflammatory connective tissue disorders. Various abnormalities in inflammatory response have been postulated in both diseases. Although the pathogenesis seems to be different in these two diseases, their exact pathogenetic mechanisms are still obscure[1,2].

Immune response and inflammatory reaction develop concomitantly, and the elimination of antigen can be achieved without a clinically obvious inflammatory reaction. But in certain conditions, such as high antigen load, abnormal location of antigen, and antigens difficult to eliminate, an uncontrolled and long lasting inflammatory reaction may develop.

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Since chemotaxis (accumulation of inflammatory cells at the site of the inflammatory reaction) is an important step in the inflammatory response, we investigated neutrophil, monocyte and lymphocyte chemotaxis in 19 RF patients. Studies were repeated at different stages of the disease. The same parameters were investigated in 15 adult RA patients and 20 healthy controls using the same method.

Material and Methods

Fifteen RA patients (identified according to the criteria of ARA)[3], 19 RF patients (diagnosed by the modified Jones criteria)[4] and 20 healthy, age-matched controls were studied. Ten of the 19 RF patients also had carditis. Chemotaxis studies were performed in all patients prior to treatment. Therapy was initiated which consisted of 80-100 mg/kg salicylic acid (for patients without carditis), and 1-2 mg/kg (maximum 60 mg) prednisolone (for patients with active carditis). Chemotaxis studies were repeated on the fifth day of therapy, and again 14 days after cessation of therapy when clinical and laboratory examinations (fever, sedimentation, CRP, etc) showed remission.

Chemotaxis method

Lymphocytes, neutrophils and monocytes were obtained from the peripheral blood by using the two-step method described by Boyum[5]. Chemotaxis was evaluated by the modified[6] Boyden chamber method[7] using millipore filters (a pore size of 3 microns for neutrophils and 5 microns for monocytes and lymphocytes). 2×10^6 neutrophils or monocytes were placed in the upper compartment and chemotaxis was measured by the leading front method, as described by Zigmond and Hirsch[8].

Lymphocyte locomotion was determined by the previously described method without prior overnight incubation[9].

Zymosan activated serum prepared from ten healthy controls was used as the standard chemotactic agent.

Since some patients were lost to follow-up, and a sufficient quantity of cells could not be obtained from some of the other patients, an equal number of patients could not be studied.

The Wilcoxon and Mann-Whitney –*U* tests were used for statistical comparisons.

Results

The results of the neutrophil chemotaxis studies are shown in Table I. Neutrophil chemotaxis in the RF patients before therapy was found to be significantly higher in comparison with the healthy controls ($p<0.05$), RA patients, and the RF patients on the fifth day of therapy ($p<0.01$). Neutrophil chemotaxis was found to

be significantly decreased in the RA patients in comparison with the healthy controls ($p<0.05$). Neutrophil chemotaxis in the RF patients was also found to be significantly depressed during the administration of therapy when compared with this group before given therapy ($p<0.05$) and the controls ($p<0.05$). After therapy, the values showed no significant difference when this group was compared with the controls and before they were administered therapy.

TABLE I: Results of Neutrophil Chemotaxis *(μm)

SUBJECTS	PRIOR TO THERAPY	n	DURING THERAPY	n	AFTER THERAPY	n
RA	71±14	10	N.T.		N.T.	
RF (TOTAL)	86±26	19	71±15	18	76±10	11
RF (PAIRED)	88±17	11	71±17	11	77±11	11
CONTROLS	83±14	20	N.T.		N.T.	

* Results were given as the mean ± 1 standard deviation.
RA=rheumatoid arthritis, RF=acute rheumatic fever, NT=not tested.

The results of monocyte chemotaxis studies are given in Table II. Monocyte chemotaxis was found to be significantly depressed ($p<0.05$) in the RA patients when compared with the controls and the RF patients prior to therapy. Monocyte chemotaxis in this group was found to be significantly depressed ($p<0.01$) on the fifth day of therapy when compared with before and after the administration of therapy and with the controls. There was no difference in monocyte chemotaxis of the RF patients before and after therapy and the controls.

TABLE II: Results of Monocyte Chemotaxis Studies (μm)

SUBJECTS	PRIOR TO THERAPY	n	DURING THERAPY	n	AFTER THERAPY	n
RA	42±9	9	N.T.		N.T.	
RF (TOTAL)	47±9	19	43±10	16	48±6	11
RF (PAIRED)	47±11	8	35±11	8	46±6	8
CONTROLS	51±8	20	N.T.		N.T.	

Results of lymphocyte locomotion are given in Table III. Although there was no significant difference found in lymphocyte locomotion in any of the groups, a difference was demonstrable in the paired-test of the RF patients before and after the administration of therapy. Lymphocyte locomotion was found to be significantly decreased in RF patients on the fifth day of therapy compared with this group before and after therapy ($p<0.05$).

TABLE III: Lymphocyte Locomotion Results (μm)

SUBJECTS	PRIOR TO THERAPY	n	DURING THERAPY	n	AFTER THERAPY	n
RA	38±3	8	N.T.		N.T.	
RF (TOTAL)	31±10	16	30±9	16	34±10	11
RF (PAIRED)	32±11	8	26±9	8	35±11	8
CONTROLS	35±8	20				

The RF patients were further divided into two subgroups according to the presence or absence of carditis. No statistically significant difference in chemotaxis was found in any of the cell types (Table IV).

TABLE IV: Results of Chemotaxis in Rheumatic Fever Patients With and Without Carditis (μm)

CELL TYPE	WITH CARDITIS	n	WITHOUT CARDITIS	n
NEUTROPHIL	89+12	10	83+17	9
MONOCYTE	46+9	10	48+10	9
LYMPHOCYTE	31+9	9	32+12	7

Discussion

Uncontrolled accumulation of inflammatory cells at any site may cause an excessive inflammatory reaction due to active molecules (lysosomal and, superoxide enzymes causing complement activation, etc) which are released from these cells. Conversely, the delayed arrival of inflammatory cells at the infection site may lead to a prolonged inflammatory reaction because the infective agent would have time to proliferate[10-12].

Neutrophil and monocyte chemotaxis was found to be decreased in RA patients in our study, confirming previous studies[13-16]. The obvious difference in neutrophil chemotactic response in RA and RF patients suggests that the effects of these two diseases on neutrophil chemotaxis is different, or different etiopathogenetic mechanisms are responsible for these two inflammatory diseases. The depression in neutrophil chemotaxis in RA patients was not very significant; 60 percent of their chemotaxis values were within one standard deviation of the healthy controls. Of the numerous studies reported on neutrophil chemotaxis in RA patients, we were able to find only one article describing this activity in RF patients (Naik et al)[17].

These investigators studied 30 patients with active rheumatic fever, 30 with active rheumatic heart disease, and 28 rheumatic fever patients in remission. In contrast to our results, neutrophil chemotaxis was found to be normal in all their groups. In our study neutrophil chemotaxis was found to be elevated during active RF. This controversy could be due to patient selection. We excluded all patients who had taken drugs 48 hours prior to the study.

We observed that neutrophil, monocyte and lymphocyte locomotion was significantly decreased on the fifth day of therapy. It has been reported that both salicylic acid and prednisolone can depress chemotaxis[18-21]. Since we could not find any difference between the RF patients with (given salicylic acid) and without carditis (given prednisolone) on the fifth day of therapy, both drugs seem to be equally effective in depressing chemotaxis (data not shown). There was no difference in neutrophil chemotaxis between the controls and the RF patients in remission (after therapy). This finding indicates that the increase in neutrophil chemotactic response is secondary to active RF disease, and not an inherited abnormality.

Since we found obvious differences in neutrophil chemotaxis between the RA patients, RF patients and healthy controls, using the same method and technical conditions, this observed depression in the RA patients and the increase in the RF patients are most likely not due to technical factors.

An increase in chemotaxis during active RF can be a part of the active inflammatory reaction but the exact mechanism remains to be elucidated. Both prednisolone and salicylic acid are used for the treatment of RA and RF because of their well-known antinflammatory effects. The therapy is followed up by drug monitoring and/or nonspecific laboratory tests such as CRP, and erythrocyte sedimentation rate. Since chemotaxis is an important phase of the inflammatory reaction, monitoring chemotactic activity during drug therapy and correlating these findings with the clinical response may provide valuable information for the researcher.

REFERENCES

1. Silver RM, Zvaifler NJ. Pathogenesis. In Utsinger PD, Zvaifler NJ. Ehrlich GE (eds). *Rheumatoid Arthritis: Etiology, Diagnosis, Management.* Philadelphia: J.B. Lippincott, 1985, pp. 71-89.
2. Wannamaker LW, Kaplan EL. Acute rheumatic fever. In Moss AJ, Adams FH (eds). *Heart Disease in Infants, Children and Adolescents.* Baltimore: Williams and Wilkins, 1983, p. 584.
3. Arnett FC, Edworthy SM, Bloch DA, et al: The American Rheumatism Association 1987 revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* **31**:315, 1988.
4. Jones Criteria revised for guidance in the diagnosis of rheumatic fever. *Circulation* 69:204 A, 1984.
5. Boyum A. Isolation of mononuclear cells and granulocytes from humon blood. Isolation of mononuclear cells by one centrifugation, and of granulocytes by combining centrifugation and sedimentation at 1 g. *Scand J Clin Lab Invest* **21**:Suppl 970:77, 1968.

6. Yeğin O. Chemotaxis in childhood. *Pediatr Res* 17:133, 1983.
7. Boyden S. The chemotactic effect of mixtures of antibody and antigen on polymorphonuclear leucocytes. *J Exp Med* 115:453, 1962.
8. Zigmond SH, Hirsch JG. Leukocyte locomotion and chemotaxis. New methods for evaluation, and demonstration of a cell-derived chemotactic factor. *J Exp Med* 137:387, 1973.
9. Yeğin O, Sanal Ö, Yeralan O, et al. Defective lymphocyte locomotion in Chédiak-Higashi syndrome. *Am J Dis Child* 137:771, 1983.
10. Snyderman R. Mechanisms of inflammation and leukocyte chemotaxis in the rheumatic diseases. *Med Clin North Am* 70:217, 1986.
11. Malech HL, Gallin JI. Current concepts: immunology. Neutrophils in human diseases. *N Engl J Med* 317:687, 1987.
12. Henson PM. The immunologic release of constituents from neutrophil leukocytes. I. The role of antibody and complement on nonphagocytosable surfaces or phagocytosable particles. *J Immunol* 107:1535, 1971.
13. Walker JR, James DW, Smith MJ. Directed migration of circulating polymorphonuclear leucocytes in patients with rheumatoid arthritis: a defect in the plasma. *Ann Rheum Dis* 38:215, 1979.
14. Udén AM, Trang L, Venizelos N, Palmblad J. Neutrophil functions and clinical performance after total fasting in patients with rheumatoid arthritis. *Ann Rheum Dis* 42:45, 1983.
15. Wandall JH. Leucocyte function in patients with rheumatoid arthritis: quantitative in-vivo leucocyte mobilisation and in-vitro functions of blood and exudate leucocytes. *Ann Rheum Dis* 44:694, 1985.
16. Corberand J, Amigues H. de Larrard B, Pradere J. Neutrophil function in rheumatoid arthritis. *Scand J Rheumatol* 8:49, 1977.
17. Naik B, Jambotkar B, Kemet JR, et al. Polymorphonuclear leukocyte functions in patients with acute rheumatic fever and rheumatic heart disease. *J Clin Lab Immunol* 24:189, 1987.
18. Spagnuolo PJ, Ellner JJ. Salicylate blockade of granulocyte adherence and the inflammatory response to experimental peritonitis. *Blood* 53:1018, 1979.
19. Dahl MV. Chemotaxis: death march of the phagocyte [editorial]. *Arch Dermatol* 115:1407, 1979.
20. Losito A, Williams DG, Cooke G, Harris L. The effects on polymorphonuclear leucocyte function of prednisolone and azathioprine in vivo and prednisolone, azathioprine and 6-mercaptopurine in vitro. *Clin Exp Immunol* 32:423, 1978.
21. Dale DC, Fauci AS, Wolff SM. Alternate-day prednisone: leukocyte kinetics and infection susceptibility. *N Engl J Med* 291:1154, 1974.