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PRELIMINARY COMMUNICATIONS

Polymorphonuclear Leucocyte Chemotaxis in Patients with Bacterial Infections

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Summary

A new in vitro method of measuring the chemotaxis of polymorphonuclear leucocytes from peripheral blood has been used to calculate a chemotactic index. The mean chemotactic index in 15 patients with bacterial infection (434) was significantly less ($P < 0.0005$) than in 15 normal controls (553) matched for age and sex. The reduction in chemotaxis could be correlated with the duration of the infection, with the greatest impairment being found in those patients with the shortest duration of infection. In five patients studied before and after appropriate therapy the chemotactic index returned to normal values with clearing of the infection. It is suggested that the impairment in chemotaxis may be due to prior phagocytosis of antibody-antigen complexes by the polymorphonuclear leucocytes.

Introduction

Chemotaxis is an important function of the polymorphonuclear leucocyte in the protection of the individual against invasive agents. Various methods have been used to study chemotaxis, but the technique devised by Boyden (1962) has proved to be the most used because of its simplicity and to some extent its reproducibility. A number of modifications of this technique have been made (Ward *et al.*, 1965; Ward, 1968), but though these simplify the method they do not alter the requirement for large numbers of cells. Consequently few studies of chemotaxis have been carried out on human cells, and investigations of polymorphonuclear leucocyte chemotaxis in patients with various disease states in which infection plays a part have been almost totally ignored. We have recently developed a simple reproducible method for studying chemotaxis of polymorphonuclear leucocytes permitting the use of small volumes of human peripheral blood (Baum *et al.*, 1971) and have compared the results in patients with bacterial infection and in normal controls.

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Material and Methods

The 15 patients with bacterial infection were inpatients in Strong Memorial Hospital who had had a variety of infections lasting from 8 months to 3 days without any other overt disease. Most of the patients were already receiving appropriate antibiotic therapy, but in five (Cases 5, 6, 7, 8, and 13) it was possible to study the polymorphonuclear leucocyte chemotaxis before and after treatment with antibiotics. The age of the patients ranged from 18 to 85 years. The normal subjects, who were matched for age and sex with the patients, were members of the laboratory staff and healthy residents of a home for the aged. The mean age of the patients was 53·2 years and of the normal controls 53·8 years (see Table).

The method for studying the chemotaxis of polymorphonuclear leucocytes, which has been described in greater detail elsewhere (Baum *et al.*, 1971), utilized a Sykes-Moore tissue culture chamber (Bellco Glass Company, Vineland, New Jersey) which was modified by the design of a new gasket. The chamber was assembled with a 25 mm round cover slip, the gasket, a 25 mm 3 μm Millipore filter (Millipore Filter Corporation, Bedford, Massachusetts), another gasket, a 25 mm cover slip again, and then closed with the screw top. Ports in the side wall of the chamber enabled the compartments above and below the Millipore filter to be filled with various solutions.

Polymorphonuclear leucocytes were obtained from peripheral blood. Ten millilitres of blood was drawn into a heparinized tube, 0·75 ml of a 2% solution of methyl cellulose in normal saline was added, and the tube was gently inverted. The methyl cellulose caused the rapid sedimentation of red blood cells and mononuclear leucocytes (Böyum, 1968). After standing for 30-45 minutes the plasma layer, which then contained a concentration of polymorphonuclear leucocytes 20-40% higher than in the peripheral blood, was removed and diluted with approximately one-half its volume of Hank's solution. The cells from four or five drops of this cell suspension were deposited on a circumscribed area of a 3 μm Millipore filter by means of the Shandon cytocentrifuge (Shandon Scientific Company, Sewickley, Pennsylvania). The filter was placed in the Sykes-Moore chamber, and after filling both compartments of the chamber (approximately 0·5 ml each) this was incubated for three hours at 37°C. After incubation the filters were stained by Boyden's (1962) method, trimmed, and mounted.

Cell viability was studied by using the trypan blue dye exclusion method after centrifugation and after incubation, and better than 99% of the cells from both normal controls and patients with bacterial infection were found to be viable at both times.

Since in other techniques some of the cells entering the filter may have done so owing to inherent motility of the polymorphonuclear leucocytes as well as by chemotactic direction, we eliminated as much of the effects of motility as possible by counting only those cells which had completely penetrated the filter and were present on the bottom or attraction surface of the membrane. This was done by focusing beyond any cells seen and then counting only those cells which appeared first in focus as the membrane was approached. The same technique

Chemotactic Indices in Patients with Bacterial Infection and in Normal Controls

Case No.	Age	Sex	Type of Infection	Organism	Duration of Symptoms	Chemotactic Index		Normal Controls	
						Before and During Therapy	After Therapy	Age	Chemotactic Index
1	18	F.	Genital and arthritis	Gonococcus	3 weeks	515		20	536
2	21	F.	Pyelonephritis	*E. coli*	3 days	330		21	525
3	29	M.	Peritonitis	Tuberculosis	6 weeks	551		30	510
4	39	M.	Osteomyelitis	*Staph. aureus* (positive)	9 months	493		42	618
5	46	F.	Pneumonia	Pneumococcus	2 weeks	368	584	48	586
6	54	F.	Pneumonia	Pneumococcus	2 weeks	351	561	54	565
7	54	M.	Pyelonephritis	*E. coli*	1 day	411/422	614	53	476
8	56	M.	Pneumonia	Pneumococcus	4 weeks	454	606	56	577
9	58	M.	Osteomyelitis	*Pseudomonas*	34 months	529		58	510
10	61	M.	Osteomyelitis	*Klebsiella*	4 weeks	541		61	529
11	65	M.	Osteomyelitis	*Pseudomonas, Klebsiella*	38 months	479		64	586
12	68	M.	Septicaemia	*E. coli*	4 days	438		68	540
13	71	M.	Pneumonia	Pneumococcus	5 days	372	540	71	445
14	73	M.	Pneumonia	Pneumococcus	4 weeks	377		76	702
15	85	M.	Pneumonia	Pneumococcus	10 days	310		85	599
Mean values (± 1 S.D.)	53·2					434 ± 81		53·8	553 ± 62

was used for counting those cells on the upper or starting side. The number after counting 10 random fields on each side was expressed as a ratio and called the chemotactic index. In an average experiment this method of cell counting resulted in a figure of 90-100 cells on the starting side of the filter. In this way a precise number of cells did not have to be delivered on to the filter surface and this obviated procedural delays and the necessity for frequent handling and counting of the cells with possible reductions in cell viability. On the starting side the cells were counted within the outer circle (1·5 mm diameter) of the photographic reticule of a Leitz orthomatic microscope. On the opposite (attraction) side of the Millipore filter the cells were counted within the rectangular photographic reticule (11·5 × 7 mm). The cells were counted with a ×10 ocular and a ×25 objective.

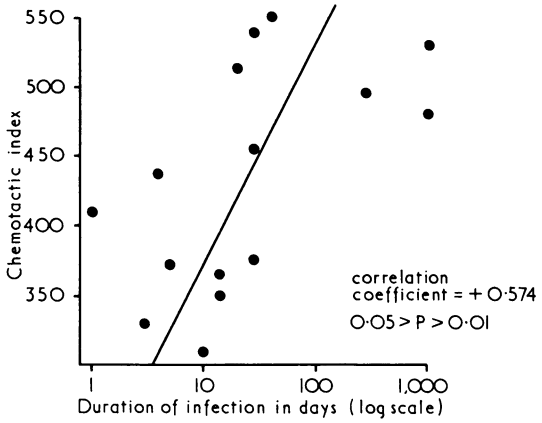
$$\text{Chemotactic index} = \frac{\text{number of cells (attractant side)}}{\text{number of cells (starting side)}} \times 1{,}000$$

Three solutions were used in the study. (1) Hank's solution (Difco Laboratories, Detroit, Michigan) was prepared without the addition of bicarbonate (pH 6·8). (2) Casein was made up in a concentration of 5 mg/ml in the Hank solution. (3) Human complement was provided by human serum, type AB, from one of several healthy donors, whose serum was used throughout the study. The serum was separated immediately after being drawn, kept in small aliquots at −70°C, and thawed just before use. Complement levels were checked in each donor and found to be well within the normal range.

Previous studies (Baum *et al.*, 1971) have shown that a mixture containing 2 parts of the casein solution and 1 part of the human serum produced a reliable standard chemotactic attraction with an accuracy based on triplicate chamber studies of about 7%. This mixture was used to fill the lower, attractant compartments of the chamber and Hank's solution alone was used to fill the upper compartments. Duplicate chambers were used throughout this study.

Results

As can be seen in the Table the mean chemotactic index ±1 S.D. for the 15 patients with bacterial infection before or during treatment was 434 ± 81 compared with 553 ± 62 for the 15 normal controls matched for age and sex ($P < 0.0005$). There was no correlation between the chemotactic index and age or sex in either group. In the patients with bacterial infection the chemotactic index correlated with the duration of the infection (coefficient of correlation +0·574, $0.05 > P > 0.01$; see Chart., Thus patients with the shortest history of infection had the lowest chemotactic indices.



Chemotactic index and duration of the infection in days.

In five patients (Cases 5, 6, 7, 8, and 13) the chemotactic index was measured on blood drawn before and after treatment with an appropriate antibiotic. Clearing of the infection was accompanied by a return of the chemotactic index to a normal value. In Case 7, a patient with pyelonephritis, the chemotactic index was measured before therapy (411) and when he became free of urinary tract symptoms seven days later (422). Pyuria persisted, however, and the chemotactic index did not return to normal values (614) until after the removal of a renal calculus, the underlying cause of the pyelonephritis. We were not able to correlate the chemotactic index with the number of polymorphonuclear leucocytes in the peripheral blood.

Discussion

In the system used in this study chemotaxis of the polymorphonuclear leucocytes occurred in response to the interaction of various components of complement and endotoxin contaminating the casein (Ward, 1968; Ward and Newman, 1969; Baum *et al.*, 1971). We have previously shown the method to be accurate and reproducible in 48 normal controls (Baum *et al.*, 1971). The results of the present study, therefore, indicate a relative deficiency in the chemotaxis of the polymorphonuclear leucocyte in some subjects with bacterial infection compared with normal controls. The deficiency was more striking in those patients with acute infection, with a return to normal values in the five cases studied after the infection had cleared with appropriate antibiotic therapy. Our finding that the chemotaxis of the polymorphonuclear leucocytes was not related to the number of polymorphonuclear leucocytes in the peripheral

blood is in agreement with the results of others (Fruhman, 1964; Hollingsworth and Atkins, 1965).

So far as we are aware there have been no previous full reports of the finding of a reduction in chemotaxis in patients with bacterial infection. A recent abstract (McCall and Caves, 1970) mentioned impairment in many leucocyte functions in these patients including chemotaxis and phagocytosis. Chemotaxis and phagocytosis by the polymorphonuclear leucocyte are energy-dependent and the metabolic activities of these cells are complicated (Karnovsky, 1968). It is possible that the reduction in chemotaxis in the present patients reflects a metabolic abnormality which is corrected when the infection is cleared by appropriate therapy. We have found such a mechanism in diabetes mellitus, where abnormal chemotaxis of cells from patients with this disease are returned towards normal after in-vitro treatment with insulin and glucose (Mowat and Baum, 1971a).

Alternatively the impairment in chemotaxis may reflect previous ingestion of antibody-antigen complexes, with consequent utilization of the limited energy sources of the cells (Sbarra and Karnovsky, 1959; Strauss and Stetson, 1960). Keller and Sorkin (1968) showed impairment of the chemotaxis of rabbit neutrophils after phagocytosis of antibody-antigen complexes. We have reproduced this impairment by feeding normal cells purified rheumatoid factor complexes (Mowat and Baum, 1971b).

The finding in this study that the impairment in polymorphonuclear leucocyte chemotaxis was most pronounced in those patients with the shortest duration of infection could be interpreted to support the concept that the impairment results from the previous ingestion of antibody-antigen complexes. The initial antibody response to bacterial antigens is the production of 19S immunoglobulin, with a gradual change over a period of weeks to the production of 7S immunoglobulin (Baum and Ziff, 1969).

Since these 19S antibodies are more active in agglutination reactions (Pike, 1967) we might expect earlier in the disease the formation of larger aggregates of antigen-antibody complexes which are more readily picked up in the peripheral circulation by the polymorphonuclear leucocytes. This would then have a greater depressive effect on chemotaxis. The deficiency in response to chemotaxis could reflect an increased number of less-responsive immature cells. In rheumatoid arthritis, however, where the impairment appears to be as great or greater, there is an excess of older (hypersegmented) polymorphonuclear leucocytes (Edwin, 1969).

Chemotaxis is a measure of one of the body's defence mechanisms against foreign agents or complexes circulating in the peripheral blood. The importance of the finding of a relative defect in chemotaxis in patients with bacterial infection is difficult to determine, since it is unknown how great the reduction has to be before this defence mechanism becomes incapable of handling invasive agents. Nevertheless, since the results were significantly different from normal they probably represent a genuine reduction in function. This may partly explain the occasional susceptibility of patients with bacterial infection to secondary invasive agents, though other features of the defence mechanism such as impaired phagocytosis (McCall and Caves, 1970) and an already committed antibody-producing system may play a part.

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