Assessment of neutrophil chemotaxis and random migration in childhood

Comparison between leading-front and lower surface count methods

SARAB AL-NAKEEB AND EILEEN N THOMPSON

Department of Child Health, Welsh National School of Medicine, Llandough Hospital, Penarth, South Glamorgan

SUMMARY Neutrophil chemotaxis and random migration were studied in 65 healthy children and 18 normal adults. The method used, the leading-front technique, was more accurate and reproducible than the lower surface count method. Chemotaxis in children under 15 years differed from that in adults. This age effect was most pronounced in those less than 6 years, and particularly in those less than 2 years. When investigating chemotaxis in childhood, comparisons with age-matched controls should be made.

Many workers¹⁻⁴ have drawn attention to immaturity of specific and nonspecific immune mechanisms in early childhood. Impaired neutrophil chemotaxis has been demonstrated in early infancy,³ but studies at other ages show conflicting results (P C Wilkinson, 1975, personal communication).⁵⁻⁷ Furthermore the duration of the dysfunction found in the young infant has not been established. Chemotaxis, the directed migration of cells towards different attractants, is an early and fundamental step in the process of inflammation, while random migration may be another mechanism of cell movement; yet a selective defect of one mechanism, or both, has been reported in different conditions of repeated infection.⁸ Abnormalities in this function can be due to humoral factors (abnormality in the serum) but are most often the result of an intrinsic abnormality of leucocytes,⁸ which may be congenital, acquired, or developmental. The cellular aspect of chemotaxis was investigated and the efficiency of neutrophil polymorphonuclear neutrophil leucocyte migration in infants and young children was compared with that of adults.

Materials and methods

83 normal subjects were studied. 18 were adults aged between 20 and 62 years (9 men and 9 women) and 65

were children aged between 1 and 16 years. The children (except for 10) had either been seen in outpatients for noninfective conditions or had been admitted for minor elective surgery (all tests done before surgery). No child was bled specifically for the test. The other 10 children aged between 9 and 16 years were asymptomatic children of staff members; consent was obtained from parent and child. Great care was taken in selecting the control individuals to avoid any factor that might affect chemotaxis—such as infection (however trivial), drugs, or pregnancy. These precautions were taken because certain drugs are known to have a suppressive effect on chemotaxis, and infections produce antigen-antibody reactions which activate the complement system which in turn stimulates migration.⁹ Furthermore, phagocytosis of infecting organisms is known to release inhibitory factors which prevent migration of these cells away from the site of infection.¹⁰

Chemotaxis. This was assayed by using Wilkinson's modification⁹ of the Boyden technique. Ficol/Triosil separated neutrophils, obtained from 10 ml venous heparinised blood, were washed once in Hanks's solution. Subsequently 2×10^5 were suspended in Hanks's solution and added to the upper chamber. The attractants used were: casein (Hammarstern from Merck AG) at a concentration of 5 mg/ml, and pooled normal human sera (5 donors per pool), activated by adding 2 µg/ml *Escherichia coli* endotoxin (Difco), a final concentration of 20% in Hanks's solution was used. Neutrophil migration

Department of Child Health, Welsh National School of Medicine

SARAB AL-NAKEEB, research fellow
EILEEN N THOMPSON, senior lecturer

was quantified by two methods: the lower surface count method after 3 hours of incubation⁶ and the leading-front technique after 50 minutes of incubation.¹¹ After the appropriate incubation, the membrane was separated, fixed in alcohol, stained with haematoxylin, and cleared with xylene. The lower surface count method was a ratio of total number of cells in 10 rectangles (11.5×7 mm/rectangle; Leitz Ortholux microscope) on the lower surface, to number of cells in 10 circles (1.5 mm diameter/circle) on the upper surface after 3 hours of incubation. The leading-front technique measured the distance travelled by the leading front of neutrophils using the

micrometer on the fine adjustment of the microscope ($\times 40$ objective).

Random migration. This was assessed in the absence of a concentration gradient. 20% serum was added to both the upper and lower compartments of the chemotactic chamber.

Results

Consistent results were impossible to achieve using the lower surface count method, due to the large but variable migration of cells through the membrane into the fluid compartment below, which has been noted previously.¹² With the leading-front technique consistent results were obtained in the same individual (chamber/chamber, filter/filter, and day/day variation $<10\%$). However a wide person-to-person variation was found in the 18 adults (11, 15, and 21% of the mean in casein, serum, and random migration respectively).

This interperson variation was much greater in children, but when the values were looked at in relation to age a steady increase was obtained (Figure). There was a significant difference between the 0- to 6- and the 6- to 15-year-old groups ($P < 0.01$, $P < 0.001$, and $P < 0.01$) for casein, serum, and random migration, and between the 6- to 15-year-old and adult groups in casein and serum ($P < 0.001$) and in random migration ($P < 0.01$).

Discussion

The leading-front technique for measuring chemotaxis proved reproducible. The wide range of values between normal individuals has already been reported,^{5, 13-14} and shows the need to compare a single result with a normal range, rather than a single control run concurrently. This is particularly important in children where the interperson variation is so much greater. Although delayed chemotaxis in the very young (<2 years) is well documented, no normal data for older children were available.

Our data clearly show an age-dependent effect. It is of interest that Klein *et al.*¹⁴ using a different method for measuring chemotaxis noted a similar age-dependency. The failure of other workers^{2, 6-7} to show this age-dependent effect is probably due to the fact that they did not study a wide age range, and also used the lower surface technique. A recent study by Farhoudi *et al.*¹⁵ of chemotaxis in children with an increased susceptibility to infection, compared the results with adult controls, using a method identical with ours, and giving a similar adult control range. These workers did not cite a normal range for

Figure Neutrophil chemotaxis (casein and serum) and random migration in relation to age. Chemotaxis, measured by the leading-front technique, is the distance travelled (in microns) during 50 minutes of incubation.

children, although they suggested that normal values for children probably are in the lower normal adult range. Our findings however, show a considerable range of normality in childhood which is age-dependent. Thus when investigating children in whom a chemotactic defect is suspected, comparison with age-matched controls is essential.

The reason for the relative inefficiency of neutrophil chemotaxis in young children is unknown, but as adult levels were ultimately achieved a maturation defect seems likely. It is interesting that other age-dependent mechanisms such as immunoglobulin production¹ and lymphocyte transformation to phytohaemagglutinin² reach adult levels during adolescence. The practical importance of delayed maturation, both of the specific immune mechanism and neutrophil function, is obvious in relation to the well-known observation of increased susceptibility to infection in childhood.

References

- ¹ Stiehm E R, Fudenberg H H. Serum levels of immune globulins in health and disease. *Pediatrics* 1966; **37**: 715–27.
- ² Pentycross C R. Lymphocyte transformation in young people. *Clin Exp Immunol* 1969; **5**: 213–6.
- ³ Miller M E. Chemotactic function in the human neonate: humoral and cellular aspects. *Pediatr Res* 1971; **5**: 487–92.
- ⁴ Miller M E, Stiehm E R. Phagocytic, opsonic, and immunoglobulin studies in newborns. *California Med* 1973; **119**: 43–63.
- ⁵ Baum J, Mowat A G, Kirk J A. A simplified method for the measurement of chemotaxis of polymorphonuclear leucocytes from human blood. *J Lab Clin Med* 1971; **77**: 501–9.
- ⁶ Hill H R, Warwick W J, Dettloff J, Quie P G. Neutrophil granulocyte function in patients with pulmonary infection. *J Pediatr* 1974; **84**: 55–8.
- ⁷ Anderson R, Rabson A R, Sher R, Koornhoff H J. Defective neutrophil motility in children with measles. *J Pediatr* 1976; **89**: 27–32.
- ⁸ Miller M E. Pathology of chemotaxis and random mobility. *Semin Hematol* 1975; **12**: 59–82.
- ⁹ Wilkinson P C. *Chemotaxis and inflammation*. Edinburgh: Churchill Livingstone, 1974.
- ¹⁰ Mowat A G, Baum J. Polymorphonuclear leucocyte chemotaxis in patients with bacterial infections. *Br Med J* 1971; **iii**: 617–9.
- ¹¹ Zigmond S H, Hirsch J G. Leukocyte locomotion and chemotaxis. New methods for evaluation and demonstration of a cell derived chemotactic factor. *J Exp Med* 1973; **137**: 387–410.
- ¹² Keller H U, Borel J F, Wilkinson P C, Hess M W, Cottier H. Reassessment of Boyden's technique of measuring chemotaxis. *J Immunol Methods* 1972; **1**: 165–8.
- ¹³ Ketchel M M, Favour C B. The acceleration and inhibition of migration in human leucocytes *in vitro* by plasma protein fractions. *J Exp Med* 1955; **101**: 647–63.
- ¹⁴ Klein R B, Fischer T J, Gard S E, Biberstein M, Rich K C, Stiehm E R. Decreased mononuclear and polymorphonuclear chemotaxis in human newborns, infants, and young children. *Pediatrics* 1977; **60**: 467–72.
- ¹⁵ Farhoudi A, Harvey B A M, Soothill J F. Clinicopathological findings in patients with primary and secondary defects of neutrophil motility. *Arch Dis Child* 1978; **53**: 625–30.

Correspondence to Dr E N Thompson, Department of Child Health, Welsh National School of Medicine, Llandough Hospital, Penarth, South Glamorgan CF6 1WX.

Received 17 May 1979