

Granulocyte migration *in vivo* and *in vitro* in healthy children of parents with atopic asthma

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Healthy children of atopically asthmatic parents received *in vivo* and *in vitro* granulocyte migration tests. Eleven children whose parents were both asthmatic showed statistically significant inhibition of granulocyte migration compared with controls.

Key words: atopic asthma; granulocyte migration.

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Since 1978 several publications have demonstrated a defective granulocyte migration in patients with atopic asthma (2, 3, 7). We have also demonstrated defective peripheral-blood granulocyte migration in our patients with atopic asthma (4, 6).

According to Ito et al. (3), granulocyte migration defect in atopic asthma is due to IgE hyperglobulinaemia; Rubin et al. (7) suggested the defect to be connected with histamine release during allergic reactions. Our previous studies suggested a disturbed function of the phagocytic cell itself (5). It is also possible that defective granulocyte migration in atopic patients is genetically determined.

Thus, it is the aim of the present study to test peripheral-blood granulocyte migration in healthy children who have at least one parent with atopic asthma.

MATERIAL AND METHODS

The study was carried out on 41 healthy children: 30 aged 5-12 years, mean 7 years, (one parent atopic asthmatic) and 11 aged 6-14 years, mean 8 years, (both parents atopic asthmatic). The asthmatic parents were aged 23-39 years, mean 30 years. The control group comprised 20 healthy subjects aged 25-37 years

(mean 32 years) and 20 healthy children aged 6-14 years (mean 9 years) with no family history of atopic diseases.

The diagnosis of atopic asthma was based on history data, positive skin tests and raised serum IgE level. In the group of atopic patients 42 were allergic to pollens of trees, shrubs and grass, eight to house dust, and two to animal danders.

The tests used were: blind random migration and chemotaxis of granulocytes on agar medium (1) and the skin window test (8). At the time of testing the patients with atopic asthma were in an asymptomatic period.

The migration test *in vitro* was done according to Clausen's method (1). The medium with Parker's solution was supplemented with antibiotics, and a 1% NaHCO₃ solution was added to obtain pH value 7.3. The medium was mixed with an equal amount of properly prepared agarose, and equine serum from the Warsaw Sera and Vaccine Laboratory was added. The dissolved medium at 48°C was placed on Petri dishes and kept for 30 min in a refrigerator at 4°C. Four wells, 2.3 mm in diameter, were cut in the solidified medium and filled with appropriately prepared peripheral blood leucocytes of the studied patients (random migration test).

For chemotaxis investigation, four identical wells were cut at a distance of 4 mm from the first four, and filled with the chemotactic substance (purified transferrin, produced by Bio-med Poland). The dishes were then placed in a thermostat with inflow of air and CO₂. The size of the granulocyte migration area was calculated using the ηr^2 formula after 18 h, after removing agarose with methanol and fixation with 40 % formalin.

The *in vivo* granulocyte migration test was done by the method of Southam (8). The skin on the right forearm was scarified over an area of 0.5 cm², avoiding bleeding. The scari-fied area was covered with a chamber of 1 cm³ capacity filled with autologous serum. Samples were taken after 3, 6, 12 and 24 h of observation assessing the number of gran-ulocytes in 1 µl of exudate in a cell-counting chamber.

In all patients pathological changes in the peripheral blood had been excluded pre-

viously. The obtained results were subjected to statistical analysis using Student's t test.

RESULTS

As seen in Table 1, the parents with atopic asthma showed a statistically significant inhibi-tion of random migration compared with the control goup (30.03 versus 45.91, *P* < 0.01). A similar difference was also found in the chem-otaxis test (48.08 versus 68.13, *P* < 0.01). On the other hand, no significant differences were noted in the random migration test and the chemotaxis test between the children with one atopic parent and the control group (46.01 ver-sus 45.13, *P* > 0.01 and 60.03 versus 67.04, *P* > 0.01 respectively).

Similarly, the ability of the granulocytes to migrate into the skin window was statistically significantly lower in the parents with atopic asthma than in the control group (42 × 10⁶ versus 68 × 10⁶, *P* < 0.01). The ability of the

Table 1
Leucocyte migration *in vitro* in children with one atopic asthmatic parent and in the control group

Investigated group (<i>n</i> = 100)	Children of parents with atopic asthma (<i>n</i> = 30) × mm ² ± SD	Children in control group (<i>n</i> = 20) × mm ² ± SD	Parents with atopic asthma (<i>n</i> = 30) × mm ² ± SD	Adults in control group (<i>n</i> = 20) × mm ² ± SD
Random migration	46.01 (14.32)	45.13 (16.01)	30.03 (16.09)	45.91 (14.32)
Chemotaxis test	60.03 (13.04)	67.04 (13.03)	48.08 (17.13)	68.13 (12.03)
Difference between 1st and 2nd groups and 3rd and 4th groups	<i>P</i> > 0.01		<i>P</i> < 0.01	

Table 2
Number of leucocytes in µl of exudate in children whose one parent had atopic asthma and in the control group

Observation (hours)	Children of parents with atopic asthma (<i>n</i> = 30) × % ± SD	Children in control group (<i>n</i> = 20) × % ± SD	<i>P</i>	Parents with atopic asthma (<i>n</i> = 30) × % ± SD	Adults in control group (<i>n</i> = 20) × % ± SD	<i>P</i>
3	1500 (600)	1700 (500)	> 0.01	1200 (150)	1600 (300)	> 0.01
6	8700 (800)	7800 (700)	> 0.01	5600 (300)	8500 (300)	> 0.01
12	24000 (1700)	22300 (1100)	> 0.01	14800 (1200)	23000 (1500)	< 0.01
24	37000 (2500)	35800 (2000)	> 0.01	20400 (1900)	36000 (1500)	< 0.01
Total in 1 µl	70 × 10 ⁶	67 × 10 ⁶	> 0.01	42 × 10 ⁶	68 × 10 ⁶	< 0.01

granulocytes to migrate *in vivo* in children with one atopic parent was similar to that in the control group (67×10^6 versus 70×10^6 , $P > 0.01$).

Table 3 shows that the random migration of granulocytes in agarose medium was statistically significantly lower in children with both parents having atopic asthma than in the control group (28.06 versus 45.13, $P < 0.01$). The chemotaxis ability in these children was also statistically significantly lower than in controls (36.12 versus 67.28, $P < 0.01$).

As shown in Table 4 the ability of the granulocytes to migrate into the skin window was statistically significantly lower in children with both parents having atopic asthma than in controls (40×10^6 versus 67×10^6 , $P < 0.01$).

DISCUSSION

The defective granulocyte migration in patients with atopic asthma found in our study has been demonstrated in earlier publications (2, 3, 6).

Ito et al. (3) considered granulocyte migration defect in patients with atopy to be connected with IgE hyperglobulinaemia. Immunoglobulin E binding of basophil leucocytes to the cell membrane causes histamine release, and histamine is a powerful blocker of granulocyte migration. This was confirmed by other authors, who observed increased inhibition of granulocyte migration only in patients during attacks of asthma (7).

In our earlier published findings the ability of granulocytes to migrate *in vivo* and *in vitro* in patients with atopic asthma was inversely proportional to the level of immunoglobulin E in their sera (6). Nevertheless we also observed inhibition of granulocyte migration in patients with normal IgE levels.

Our other studies (5) may contest the decisive role of histamine in granulocyte migration in atopic patients. We performed the *in vitro* migration test using the granulocytes of healthy subjects suspended in antigenically compatible

Table 3

Leucocyte migration *in vitro* in children whose parents were both atopic asthmatics and in the control group

Investigated group (<i>n</i> = 73)	Children of parents with atopic asthma (<i>n</i> = 11) × mm ² ± SD	Children in control group (<i>n</i> = 20) × mm ² ± SD	Parents with atopic asthma (<i>n</i> = 22) × mm ² ± SD	Adults in control group (<i>n</i> = 20) × mm ² ± SD
Random migration	28.06 (15.13)	45.13 (16.01)	31.17 (18.31)	45.91 (14.32)
Chemotaxis test	36.12 (16.03)	67.28 (20.03)	43.23 (16.08)	68.13 (12.03)
Difference between 1st and 2nd groups and 3rd and 4th groups	<i>P</i> < 0.01		<i>P</i> < 0.01	

Table 4

Number of leucocytes in µl of exudate in children whose parents were both atopic asthmatics and in the control group

Observation (hours)	Children of parents with atopic asthma (<i>n</i> = 11) × % ± SD	Children in control group (<i>n</i> = 20) × % ± SD	<i>P</i>	Parents with atopic asthma (<i>n</i> = 22) × % ± SD	Adults in control group (<i>n</i> = 20) × % ± SD	<i>P</i>
3	1100 (300)	1700 (500)	> 0.01	1400 (200)	1600 (300)	> 0.01
6	3900 (500)	7800 (700)	< 0.01	6300 (600)	8500 (300)	= 0.05
12	13800 (900)	22300 (1100)	< 0.01	15400 (1000)	23000 (1500)	< 0.01
24	21700 (2600)	35800 (2000)	< 0.01	22000 (1200)	36000 (1500)	< 0.01
Total in 1 µl	40 × 10 ⁶	67 × 10 ⁶	< 0.01	44 × 10 ⁶	68 × 10 ⁶	< 0.01

sera of atopic patients, and, conversely, with the granulocytes of atopic patients suspended in the sera of healthy subjects. It was shown that the sera of atopic patients failed to inhibit the migration of granulocytes from healthy subjects. The granulocytes of these patients suspended in compatible sera of healthy subjects showed the same defect as those suspended in autologous sera. Because of this, we do not consider that "serum factors" are decisive for the presence of granulocyte migration defect in atopic patients. The defect affects the phagocytic cell itself, its morphology or function. It may be genetically determined. This possibility is supported by the results of the present study.

Conclusions

Defective granulocyte migration occurs in healthy children whose parents both suffer from atopic asthma.

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