Adhesion and locomotion of granulocytes under flow conditions

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Summary

The aim of this study was to compare the strength of the surface adhesion of migrating human granulocytes and the main parameters of their locomotion under the influence of various external forces. The strength of adhesion of granulocytes moving in a quiescent medium was studied by detaching them gravitationally from the plane surface situated above them. In these conditions the force of adhesion is of the same order of magnitude as the gravitational force operating upon the cells. The locomotion characteristics of granulocytes migrating on a surface below them are similar to those observed during their movement on a surface above. When the granulocytes migrate with the medium flowing past them, they are not detached from the surface even by relatively great shearing forces (10 and 100 times greater in comparison with the gravitational force) and the locomotion parameters are only slightly modified. The results show that granulocytes are able to migrate in a similar manner when they are subjected to various external forces.

Key words: cell adhesion, cell locomotion, granulocytes, medium flow.

Introduction

The aim of the present work is to study the adhesion of granulocytes migrating in a quiescent medium and under the influence of the flow of the medium, and to find out whether the locomotion of these cells is modified by the hydrodynamic force generated by movement of the medium. The above problems are strictly interconnected and may be reduced to a fundamental question, the essence of which is the dependence of granulocyte locomotion on their surface adhesion. From the physiological or pathophysiological point of view, however, the action of the medium flow on the motile behaviour of granulocytes is interesting also because they perform an important part of their function, i.e. interaction with the blood vessels' endothelium, while they are subjected to the flow of the blood.

The adhesion of granulocytes and the connection of this phenomenon with their locomotion have been studied especially by Lackie (Brown & Lackie, 1981; Forrester & Lackie, 1984; Lackie, 1982; Lackie & Brown, 1982; Lackie & Wilkinson, 1984; Wilkinson et al. 1982). In our previous studies the influence of external forces on the adhesion and locomotion of malignant as well as normal cells were investigated (Doroszewski et al. 1981, 1986). The present work aims at making the relation between adhesion and locomotion of granulocytes more precise and straightforward.

In the study of cell-substrate adhesion numerous methods have been used, including those based on the operation on the cells of gravitational and hydrodynamic forces (for a review, see Hubbe, 1981); both the latter methods were applied in the present work. The fluid shear stress has also been used (e.g. by Franke et al. 1984) as a factor inducing intracellular structural changes.

Materials and methods

Materials
Granulocytes were obtained from human blood by the method of Harris (1973), which consists of placing a drop of fresh blood on a microscope slide and washing off the coagulated layer containing erythrocytes after a 30-min incubation at 37°C; the granulocytes remained adherent to the glass surface.

In all experiments Hanks' solution supplemented with 10% calf serum was used.

Measurement of cell adhesion and locomotion
The granulocytes were observed in a glass chamber, the
Table 1. Parameters of the medium flow

<table>
<thead>
<tr>
<th></th>
<th>( V_m ) (mm s(^{-1} ))</th>
<th>( S ) (l/s)</th>
<th>( F ) (N)</th>
<th>( T ) (Nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>0.74</td>
<td>3.588</td>
<td>( 4 \times 10^{-12} )</td>
<td>( 1.12 \times 10^{-17} )</td>
</tr>
<tr>
<td>II</td>
<td>0.759</td>
<td>3.684</td>
<td>( 4 \times 10^{-11} )</td>
<td>( 1.15 \times 10^{-16} )</td>
</tr>
</tbody>
</table>

Mean fluid velocity \( (V_m) \), shear rate \( (S) \), shearing force \( (F) \) and torque \( (T) \) were calculated according to Goldman et al. (1967).

Fig. 2. Cell locomotion parameters. A. Length of cell steps (L) and absolute angles (AA), i.e. angles between the direction of the cell steps and that of the medium flow (vertical arrow). B. Relative angles (RA), i.e. angles between consecutive cell steps; L, as in A.

The cells were observed in interference contrast (Biolar microscope, PZO, Poland, magnification \( \times 100 \)) on a TV monitor (total magnification about \( \times 1100 \)) that was connected to the TV camera mounted on the microscope.

The adhesion of the migrating granulocytes under static conditions was studied in the following way. After the preparation of granulocytes on the microscope plate and sealing on the coverslip (see above), the chamber was left at 37°C for about 30 min; during this time the cells were observed microscopically to ascertain that they were migrating normally. Then the chamber was inverted, leaving cells adhering to the ceiling of the chamber. They were left in this position for 1 h at 37°C; during which time they migrated as before. After this time (always at 37°C), the cells that remained on the upper plane as well as those that had fallen onto the lower surface were counted in 50 different, randomly chosen microscope fields. By comparing the number of cells on both planes, the fraction (%) of granulocytes that had fallen off the upper plane was calculated.

In the experiments, the aim of which was to study the behaviour of granulocytes under flow conditions, the chamber was perfused with medium moving with small (no. I) or great (no. II) velocity (Table 1). The estimation of medium velocity was based on the values of the flow rates. During the experiments the fluid velocity was checked in an approximate way by measuring the velocity of red blood cells moving in the chamber, which appeared from time to time above the migrating granulocytes. In each experiment the locomotion of granulocytes was first studied under static conditions and then under flow for a comparable period. The shearing force and torque operating on cells were calculated according to Goldman et al. (1967).

Analysis of cell locomotion parameters

The analysis of the cell tracks was performed using the same method as previously (Doroszewski et al. 1986). The cell trajectories were drawn on a transparent sheet covering the TV monitor screen. The positions of the cell centres (as determined visually) were marked every 30 s and cell positions were connected by straight lines (steps). The lengths of steps and the angles between steps (relative angles) were measured; from these data, the angles between cell steps and the coordinates relating to the plane (absolute angles) were calculated. In the detailed analysis of the migration of granulocytes under flow conditions (Fig. 2), the plane on which the cells moved has been theoretically divided into four 90° sectors (upstream, downstream, lateral right, lateral left).

The same methods of analysis were applied for the assessment of cell locomotion parameters in a quiescent...
medium (either on the lower or on the upper chamber plane) as well as in flow conditions.

Results

Cell adhesion and locomotion in a quiescent medium
During one-hour’s observation about 70% of granulocytes migrating on the ceiling of the experimental chamber (under the action of gravitational force) fall off it and sediment on the lower surface (Table 2).

The locomotion parameters of cells migrating on the floor of the chamber are similar to those observed during their migration on the ceiling before dropping off (see next section, Tables 3 and 4): the differences are statistically insignificant (Kolmogorov-Smirnov test, α ≥ 0.05).

Cell adhesion and locomotion in a flowing medium
When granulocytes are actively moving on the lower surface of the chamber in the medium flowing either with a slower (condition no. I) or with a faster (condition no. II) speed, their number did not change during the whole period of observation, which was always longer than 1 h. In all experiments performed under flow only a few cells (out of about 2000) detached from the surface. Thus, it appears that the adhesive bonds of migrating (as well as non-locomoting) granulocytes are not disconnected under the action of the shearing force generated by the medium flow (velocity no. I and velocity no. II).

When this observation is compared with the results on adhesion of granulocytes migrating under the influence of gravitational force directed downward in relation to the migration plane (see above), the difference is striking.

Global analysis of the parameters of granulocyte locomotion in a quiescent medium and under flow (conditions no. I and no. II) does not reveal any important differences that could be attributed to the action of the shearing force. Some slight but unquestionable evidence indicating that medium flow does exert some influence on the locomotion parameters of the cells under study is provided by the study centred on cells that migrate in a certain direction in relation to the course of the flow.

The length of the cell steps (i.e. the distance covered by the cells in 30-s intervals) is similar in all conditions in which they were observed, i.e. in a quiescent medium and in medium flowing with small (no. I) and great (no. II) velocity (Table 3); the differences are statistically insignificant (Kolmogorov-Smirnov test, α ≥ 0.01).

Concerning the relative angles, there is a statistically significant prevalence of angles in the region near 180° (Table 4). In this way a tendency of cells to persist in a certain direction is expressed. This parameter also does not differ with different flow conditions (Kolmogorov-Smirnov test, α ≥ 0.5). A global estimation of the directions of cell tracks (absolute angles) suggests that they are similar under various flow conditions (Table 5). The test of signs reveals that the distribution of absolute angles is random and its parameters are similar whether the medium is static or flowing fast or slowly.

A more detailed analysis is based on classifying the cell steps according to the relation of their direction to the medium flow (i.e. downstream, upstream or sideway; see Materials and methods); the results are presented in Fig. 3. In control conditions (quiescent medium) the number of steps and their length are evenly distributed in the sectors. When the medium is
set in motion (conditions no. I and no. II), the steps the direction of which corresponds to the downstream sector are more numerous in comparison with the upstream sector and the fraction of longer steps is greater. In the upstream sector, the number of steps is smaller and the proportion of shorter steps is greater. In other words, in a moving medium more cell tracks are directed downstream and in this direction the cells move somewhat faster.

Discussion
The main advantage of the gravitational method of studying cell adhesion lies in the fact that the physical conditions of the experiment are well-defined. The force that operates on the cells is perpendicular to the surface; for granulocytes it is approximately equal to $5 \times 10^{-12} \text{N}$ (Duszyk & Doroszewski, 1982). The cells detach from the upper surface only when they are actually migrating; their non-locomoting, rounded-up forms adhere much more strongly, which makes them insensitive to the gravitational force. The gravitational method makes it possible to estimate directly the adhesion during locomotion, and from this point of view it furnishes more information in comparison with
other methods that have been applied in research on granulocytes' adhesiveness (Lackie, 1982).

The fact that granulocytes are able to migrate when suspended, as it were, on the ceiling of the chamber is well known. For example, Lackie and others studied the locomotion of granulocytes in these conditions (Brown & Lackie, 1981; Forrester & Lackie, 1984; Lackie & Brown, 1982) and found that their velocity increases before falling off (this fact was attributed to a decrease in adhesion); in our experiments the granulocytes' movement parameters were similar when they moved on the lower or upper surface of the chamber.

The use of the shearing force (Goldman et al. 1967) operating on adherent cells is another method of measuring cell adhesion force. For example, in a study on leukaemia L5222 cells (Kowalczyńska et al. 1982) it has been demonstrated that a shearing force of the order of $10^{-12}$ N detaches almost all these cells from a glass surface, endothelial cell layer or collagen sub-stratum.

In our experiments hydrodynamic forces exceed gravitational forces by one or two orders of magnitude (it is of the order of $10^{-13}$ N in the former case, and of the order of $10^{-12}$ N and $10^{-11}$ N in the latter). It is an unexpected finding that a relatively small gravitational force detaches a considerable proportion of migrating granulocytes, while a much greater shearing force is ineffective. There are at least two possible interpretations. (1) The adhesion of migrating granulocytes surrounded by medium flowing with considerable velocity is greater than that of cells migrating in a quiescent medium. (2) The difference in the mechanisms of action of gravitational and shearing forces (perhaps especially the rotational component) is a dominant factor.

The action of the shearing force in the studied range only slightly modifies the characteristics of the granulocytes' locomotion. The most important effect consists of a certain decrease in the number and the speed of cells moving upstream and an increase in the number of cells migrating downstream, accompanied by their slight acceleration; it seems also that the tendency to directional persistence of moving granulocytes is somewhat diminished under flow. Thus, it appears that the shearing force does not substantially change the operation of the cell's motile apparatus and locomotion mechanisms.

The results of the present work may be considered also from the point of view of the physiological role and in vivo behaviour of granulocytes. During their circulation in the blood they are always surrounded by a moving medium and when they enter into contact with the endothelium they are subjected to shearing forces. The emigration of granulocytes from the circulatory system occurs predominantly in the post-capillary venules, in which the blood flow and the interaction of granulocytes with the walls has been investigated and discussed in a number of studies (e.g. see Atherton & Born, 1973, 1972; Lackie, 1982). Unfortunately, it is impossible to compare strictly the results of observations in vivo with the conditions in which the adhesion and locomotion of granulocytes have been studied in the present work. Neglecting geometrical differences, however, it may be supposed that the shearing force operating on granulocytes when the medium flows in the chamber used in our experiments (especially with the greater velocity) is similar to that due to the blood flow in small veins. The hydrodynamic forces operating on migrating cells in our experiments were almost equal to those used by Forrester & Lackie (1984) in their study of adhesion of granulocytes from flowing suspension.

Coming back to the question (raised in the Introduction) concerning the relation of granulocyte locomotion to the force of adhesion, it seems likely that a direct and simple connection between these two phenomena does not exist. This view is based on the fact that similar locomotion parameters of granulocytes are observed when the cells migrate in different conditions that vary considerably depending on the adhesion force, i.e. when on the floor or ceiling of the chamber, in a quiescent medium or surrounded by medium flowing with varying velocity.

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### References


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Table 5. Distribution of the absolute cell step angles (AA) in a quiescent medium (0) and in flowing medium (at velocities I and II)

<table>
<thead>
<tr>
<th>AA (deg.)</th>
<th>0</th>
<th>I</th>
<th>II</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$n/2$ (%)</td>
<td>$n/2$ (%)</td>
<td>$n/2$ (%)</td>
</tr>
<tr>
<td>0–45</td>
<td>13.97</td>
<td>20.05</td>
<td>15.60</td>
</tr>
<tr>
<td>45–90</td>
<td>10.59</td>
<td>11.30</td>
<td>8.61</td>
</tr>
<tr>
<td>90–135</td>
<td>12.65</td>
<td>11.41</td>
<td>12.79</td>
</tr>
<tr>
<td>135–180</td>
<td>12.51</td>
<td>7.72</td>
<td>9.47</td>
</tr>
<tr>
<td>180–225</td>
<td>15.01</td>
<td>10.83</td>
<td>9.25</td>
</tr>
<tr>
<td>225–270</td>
<td>10.70</td>
<td>9.47</td>
<td>8.82</td>
</tr>
<tr>
<td>270–315</td>
<td>13.45</td>
<td>13.93</td>
<td>18.27</td>
</tr>
<tr>
<td>315–360</td>
<td>12.14</td>
<td>15.27</td>
<td>17.10</td>
</tr>
<tr>
<td>Σ</td>
<td>1740</td>
<td>1192</td>
<td>930</td>
</tr>
</tbody>
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