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Role of p150,95 in adhesion, migration, chemotaxis and phagocytosis of human monocytes*

The leukocyte function-associated antigen-1 (LFA-1), the C3bi receptor (CR3) and the p150,95 antigen belong to a family of leukocyte surface molecules consisting of bimolecular complexes with α chains of 170 kDa, 165 kDa and 150 kDa, respectively, and a common β subunit with a mol. mass of 95 kDa. In order to determine the function of the p150,95 antigen on human monocytes and U937 cells, and to study the functional relationship between this antigen and LFA-1 or CR3, we investigated the influence of monoclonal antibodies (mAb) directed against these cell surface molecules on the adhesive properties of these cells. The observation that anti-β chain mAb strongly inhibited migration, chemotaxis, adhesion and phagocytosis of monocyctic cells indicates a major role for LFA-1 family antigens in monocyte functions. Detailed analysis with a panel of anti-α chain antibodies demonstrated that both p150,95 and LFA-1 mediate random migration whereas in contrast, p150,95 and CR3 were shown to be involved in the directed migration of monocytes to f-Met-Leu-Phe. Furthermore, adhesion of monocytes to plastic surfaces or monolayers of endothelial cells as well as phagocytosis of latex particles was mediated by p150,95. The results demonstrate that, in spite of its relative low expression, the p150,95 glycoprotein is a major adhesion-associated molecule expressed by human monocytes.

1 Introduction

The p150,95 antigen belongs to a family of cellular adhesion molecules consisting of p150,95, the leukocyte function-associated antigen-1 (LFA-1) and the C3bi receptor (CR3, Mo1, Mac-1) [1]. All three antigens consist of an α chain with a mol. mass of 150, 170 and 165 kDa, respectively, and share a common β subunit with a mol. mass of 95 kDa [1-3].

CR3 is present on null cells, granulocytes and monocytes [4]. Monoclonal antibodies (mAb) directed against CR3 have been shown to inhibit granulocyte adherence [5, 6], migration [7] and phagocytosis of C3bi opsonized particles [8-10]. In addition, Dana et al. described two functional domains in CR3, one involved in C3bi binding and the other in stimulation of granulocyte adhesion-dependent functions [11].

LFA-1 is present on virtually all leukocytes [12, 13], and acts as an adhesion molecule involved in cell-cell interactions [14, 15]. Anti-LFA-1 mAb have been demonstrated to interfere with the lytic activity of cytoxic T lymphocytes (CTL) [13, 15, 16] and natural killer (NK) cells [13, 17-19] and are able to block T helper cell-monocyte-associated functions [2] by preventing cell-cell contact.

In addition to the structural relationship between p150,95, CR3 and LFA-1, p150,95 has other characteristics in common with either CR3 or LFA-1. Similar to CR3, p150,95 is mainly expressed on monocytes, granulocytes and null cells [20-22]. Furthermore, a rapid increase in expression of both p150,95 and CR3 antigens is obtained after stimulation of granulocytes with chemoattractant, probably by mobilization of an internal pool of these antigens [5, 6]. In addition, mAb directed against p150,95 have been shown to inhibit granulocyte adherence to serum-coated glass [6, 7] but not the phagocytosis of C3bi opsonized particles [7]. However, since Malhotra et al. showed that p150,95 bound to C3bi [23], it cannot be excluded that, similar to CR3, p150,95 also has two functional domains [23]. Recently, it has been demonstrated that p150,95 can be expressed on CTL clones [24, 25] and that anti-p150,95 mAb like anti-LFA-1 antibodies are able to inhibit lysis of target cells [25]. The inhibition occurred at the level of conjugate formation and in addition additive effects were observed when a mixture of anti-LFA-1 and anti-p150,95 mAb were used [25]. Since the function of p150,95 on human monocytes is still largely unknown, we investigated its role in various adhesion associated processes in comparison to LFA-1 and CR3. Our data indicate that p150,95 is an important cellular adhesion molecule, contributing to all functions tested (adherence, chemokinesis, chemotaxis and phagocytosis).

2 Materials and methods

2.1 Cells

The histiocytic cell line U937 was cultured in RPMI 1640 medium supplemented with L-glutamine (2 x 10^{-3} M), sodium pyruvate (50 mg/ml), HEPES (10^{-2} M), penicillin (100 IU), streptomycin (100 μg/ml) and 10% fetal calf serum (FCS). U937 cells could be induced to differentiate into adherent macrophage-like cells by 12-O-tetradecanoylphorbol 13-acetate (TPA; 10 ng/ml) [26]. TPA was dissolved in ethanol (10 mg/ml), diluted in medium to a concentration of 100 μg/ml and stored frozen at -20°C.
Monocytes were isolated from buffy coats of healthy donors by centrifugal elutriation, as described previously [27]. Monocytes obtained in this way were >95% pure, as judged by staining for nonspecific esterase and by staining with monocyte-specific antibodies.

### 2.2 Antibodies

The mAb SPV-L7 (IgG1) and SPV-L11 (IgG1), both reacting with the α chain of LFA-1, the mAb Bear-1 (IgG1) which detects the α chain of CR3 (Mo1, Mac-1), and the mAb Gepi-1 (IgM) which reacts with sugar moieties on bone marrow cells, monocytes, granulocytes and monocytoid cell lines, were raised in our laboratory [2, 18]. CLB54 (IgG1) (CLB-LFA-1/1) [18] and MHM-23 [19], which are directed against the β chain of LFA-1 [1, 2, 19] were kindly provided by Dr. F. Miedema (Central Laboratory of the Netherlands Red Cross Blood Transfusion Service, Amsterdam, The Netherlands) and by Dr. A. McMichael (Nuffield Department, John Radcliffe Hospital, Oxford, GB), respectively. S-HC13 (IgG2b) reacts with the p150,95 antigen [20, 21]. The chain specificity of SPV-L7, SPV-L11, Bear-1, CLB54 and S-HC13 was determined by immunoprecipitation studies ([2]; results not shown). Antibodies were purified on a Baker-Bond mAb HPLC column (J. T. Baker, Philipsburg, NJ).

In all experiments the mAb Rupi-1 (IgG1) which detects a determinant on monocytes with a molecular mass of 35 kDa and the mAb SAM-1 (IgG2b) which is monocyte specific and detects a determinant of 70 kDa, were used as controls. These mAbs did not interfere with all functions tested.

### 2.3 ELISA to measure adhesion

Five x 10⁶ monocytes or U937 cells were labeled with 100 μl of Gepi-1 antibody, conjugated with horseradish peroxidase [28], by incubation at 4°C for 30 min. Subsequently the cells were washed twice with phosphate-buffered saline (PBS) containing 0.2% bovine serum albumin (BSA) and resuspended in RPMI 1640 (Flow Laboratories, Rockville, MD). The capacity of the monocytoid cells to adhere to plastic surfaces or to dense monolayers of vascular endothelial cells was determined as described [29]. Briefly, 5 x 10⁶ labeled monocytes or U937 cells were incubated in flat-bottom microtiter wells (Costar no. 3596, Cambridge, MA) in the presence or absence of mAb (dilution of ascites 1:250). After an incubation period of 60 min at 37°C the plates were carefully washed twice with 100 μl warm (37°C) PBS/BSA. Labeling of monocytes with mAb Gepi-1-p.o. did not affect the adhesive properties of the monocytes [29].

To quantify the number of adherent cells 100 μl TMB (3,3′,5,5′-tetramethylbenzidine; Crystalline Sigma T2885; 100 mg TMB/10 ml dimethyl sulfoxide 1:100 diluted in 0.1 M sodium acetate, 0.006% H₂O₂, pH 6.0) were added. The reaction was stopped after 5–60 min depending on the intensity of the colour of the reaction product, by addition of 100 μl 0.8 M H₂SO₄. The staining intensity correlated directly with the number of adherent cells and was determined by a calibration curve. Measurements were carried out with a multiscan photocolorimeter (Titertek, Flow Labs.) at a wavelength of 450 nm.

### 2.4 Determination of migration, chemokinesis and chemotaxis

The migration of U937 cells and monocytes was measured as described by Wilkinson [30]. Briefly, 2 x 10⁶ cells/well were seeded in microtiter plates (Costar 3596) and allowed to migrate out of an agar droplet for 24 h, in the presence or absence of mAb. The areas of migration were determined under a microscope which was coupled to a microprocessor as described by Thurman [31]. Chemokinesis and chemotaxis were tested in a microchemotaxis chamber (Neuroprobe, Bethesda, MD). The chamber is divided in an upper and lower compartment by a 10 μm thick polycarbonate membrane (Nuclepore Corp., Pleasanton, CA) with 8 μm pores. Two x 10⁶ cells were added in RPMI medium containing 2% autologous heat-inactivated serum to the upper compartment in the presence or absence of mAb. Chemotaxis was measured by adding f-Met-Leu-Phe (fMLP, 10⁻⁷ m) dissolved in RPMI 1640 supplemented with 2% heat-inactivated autologous human serum to the lower compartment. Chemokinesis was measured by adding fMLP both to the lower and upper compartment. In both situations the chambers were incubated 90 min at 37°C and 5% CO₂. After this incubation the membrane filter was washed and cells which migrated through the pores were counted microscopically. The percentage of migrating cells was calculated.

### 2.5 Phagocytosis

Two x 10⁶ monocytes were resuspended in 100 μl RPMI 1640 medium supplemented with 10% heat-inactivated normal mouse serum and cultured in teflon bags [32], followed by addition of 10² fluorescent monodisperse carboxylated microspheres (latex beads, Polysciences Inc., Warrington, PA). The monocytes were incubated in the presence or absence of mAb for 2 h at 37°C, 5% CO₂. The number of beads phagocytized per monocyte were counted under the microscope. In total 200 monocytes were counted.

### 2.6 Immunofluorescence

Cells were sequentially incubated (PBS containing 1% BSA and 0.01% sodium azide) with appropriate dilutions of the different mAb and fluorescein isothiocyanate (FITC)-labeled goat F(ab')₂ anti-mouse IgG antibody (Nordic, Tilburg, The Netherlands) for 30 min at 0°C. The relative fluorescence intensity was measured by FACS IV analysis and was enumerated according to the formula:

\[
\text{Relative fluorescence} = \frac{\text{Fluorescence intensity of cells labeled with a reactive antibody (indirect FITC-labeled)}}{\text{Fluorescence intensity of unlabeled cells treated with a FITC-labeled goat anti-mouse immunoglobulin antibody}}
\]

### 3 Results

#### 3.1 Expression of p150,95, LFA-1, CR3 on monocytoid cells

The expression of p150,95, LFA-1 and CR3 on the myeloid cell line U937 was low. However, when these cells were induced to differentiate into mature monocytes or macrophage-like cells by incubation with TPA, a 3–5 times
Table 1. Relative fluorescence intensities of monocytes and U937 cells labeled with mAb by indirect immunofluorescence as measured by FACS IV analysis:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>mAb</th>
<th>Relative immunofluorescence intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 control (p35)</td>
<td>Rupi-1</td>
<td>14.7</td>
</tr>
<tr>
<td>IgG2b control (p70)</td>
<td>SAM-1</td>
<td>28.6</td>
</tr>
<tr>
<td>LFA-1 α</td>
<td>SPV-L7</td>
<td>23.5</td>
</tr>
<tr>
<td>LFA-1 α</td>
<td>SPV-L11</td>
<td>22.1</td>
</tr>
<tr>
<td>CR3 α</td>
<td>Bear-1</td>
<td>31.8</td>
</tr>
<tr>
<td>pl50,95 α</td>
<td>S-HC13</td>
<td>18.9</td>
</tr>
<tr>
<td>Common β</td>
<td>MMH-23</td>
<td>32.6</td>
</tr>
<tr>
<td>Common β</td>
<td>CLB54</td>
<td>36.4</td>
</tr>
</tbody>
</table>

Table 2. Effects of mAb on the random migration of monocytes and U937 cells:

<table>
<thead>
<tr>
<th>Antigen</th>
<th>mAb</th>
<th>U937 Monocytes % Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgG1 control (p35)</td>
<td>Rupi-1</td>
<td>2 ± 3</td>
</tr>
<tr>
<td>IgG2b control (p70)</td>
<td>SAM-1</td>
<td>1 ± 4</td>
</tr>
<tr>
<td>LFA-1 α</td>
<td>SPV-L7</td>
<td>52 ± 10 (a)</td>
</tr>
<tr>
<td>LFA-1 α</td>
<td>SPV-L11</td>
<td>46 ± 29 (a)</td>
</tr>
<tr>
<td>CR3 α</td>
<td>Bear-1</td>
<td>3 ± 3</td>
</tr>
<tr>
<td>pl50,95 α</td>
<td>S-HC13</td>
<td>41 ± 10 (a)</td>
</tr>
<tr>
<td>Common β</td>
<td>MMH-23</td>
<td>81 ± 4 (b)</td>
</tr>
<tr>
<td>Common β</td>
<td>CLB54</td>
<td>89 ± 9 (b)</td>
</tr>
</tbody>
</table>

a) Cells were resuspended in agar and were allowed to migrate in the presence of mAb (10 ng/ml) for 24 h, followed by the measurement of migration as described [30]. Percentages of inhibition are given as mean ± SD of 4 experiments. The average migration of control cells was 300 µm.
b) p < 0.001.

3.2 Migration of monocytoid cells

U937 cells and monocytes have been shown to migrate spontaneously [33]. To determine whether pl50,95, LFA-1 and CR3 played a role in the migration of these cells we investigated whether mAb directed against these antigens blocked migration. The anti-pl50,95 α, the anti-LFA-1 α and anti-β chain mAb inhibited migration of both cell types (Table 2). Particularly the anti-β chain mAb showed strong inhibitory effects. These results indicate that pl50,95, LFA-1 and probably also CR3 are associated with the motility of monocytes and U937 cells. Furthermore, the results demonstrate that the anti-CR3 α chain mAb did not affect the migration of monocytes and U937 cells, probably due to the mAb used, since other investigators defined a role for CR3 in this process using different mAb [6, 7].

3.3 Adhesion of monocytoid cells to plastic surfaces

Since adherence is required for migration [34] we investigated the effects of the various anti-LFA-1 family mAb on the adhesion of monocytes and U937 cells. To induce the capacity to adhere, U937 cells were stimulated with TPA. It is shown in Table 3 that mAb directed against the pl50,95 α and against the common β chain had a strong inhibitory effect on the adhesion of U937 cells (up to 100%) and monocytes (up to 60%) to plastic surfaces, whereas the anti-CR3 α and the anti-LFA-1 α mAb were not effective. These data indicate that the various anti-LFA-1 family mAb had differential inhibitory effects on the adhesive properties of monocytoid cells. Furthermore, it is concluded from these data that the pl50,95 antigen is strongly associated with adhesion despite its relatively low expression on human monocytes (Table 1).

3.4 Adhesion of monocytoid cells to endothelial cells

LFA-1 has been described to act as a cellular adhesion molecule with an important role in lymphoid cell-cell interactions [13-15]. The anti-LFA-1 mAb used here did not block the adhesion of monocytoid cells to artificial substrates such as plastic surfaces (Table 3). Since monocytes extravasate to migrate into tissues it may be possible that LFA-1 is involved in adhesion of these cells to vascular endothelial cells. Therefore, we tested the effects of the various anti-LFA-1 family mAb on the adhesion of monocytes and TPA-stimulated U937 cells to a confluent monolayer of vascular endothelial cells obtained from umbilical veins. In the absence of mAb, monocytes but not U937 cells adhered to the monolayers of vascular endothelial cells. Adhesion of monocytes could be blocked by mAb recognizing the pl50,95 α chain or the common β chain but not by anti-LFA-1 α chain or anti-CR3 α chain mAb (Table 4).

In addition we excluded that the Fc receptor was involved in the adhesion of the monocytes to plastic or the endothelial cells, since ELISA and immunofluorescence experiments demonstrated that the mAb did not bind to endothelial cells or adhered nonspecifically to plastic [35]. These results provide evidence that the pl50,95 antigen plays a physiological role in the adhesion of monocytes to natural substrates.
3.5 Chemotaxis and chemokinesis of monocytoid cells

To study the effects of these mAb on directed migration, the capacity of the mAb to inhibit chemokinesis and chemotaxis of monocytes and U937 cells in response to fMLP was tested. Monocytes showed strong chemotactic and chemokinetic responses to fMLP whereas U937 cells treated with or without TPA did not respond [36]. Maximal inhibition was observed after addition of anti-β chain antibodies, whereas the anti-α chain antibodies p150,95 mAb were most effective (Table 5). Furthermore, anti-CR3 mAb showed similar inhibitory effects in contrast to anti-LFA-1 mAb which did not block directed or random migration. These results indicate that p150,95 and CR3 are involved in chemokinesis and chemotaxis of human monocytes.

3.6 Phagocytosis of latex beads by monocytoid cells

Another important function of monocytes is their capability to phagocytose. To investigate the antigens involved in this process, phagocytosis blocking studies were performed with the LFA-1 family mAb. Table 6 shows the number of carboxylated latex beads ingested by each monocyte in the presence of different mAb. Anti-p150,95 α chain and anti-CR3 α chain antibodies blocked the uptake of latex beads equally well. Both anti-LFA-1 α chain and anti-CR3 α chain antibodies were ineffective. These results, together with the results from Sects. 3.3 and 3.4, indicate that p150,95 is an important general adhesion molecule, since it exhibits low substrate specificity.
4 Discussion

mAb directed against the common β chain of the LFA-1 family inhibit adhesion, chemotaxis and phagocytosis of human monocytes [3, 18, 19, 37]. These data do not allow identification of the function of each member of this family, since binding of an anti-β chain mAb can affect the function of LFA-1, CR3 and p150,95. Although the function of CR3 and p150,95 has been investigated on human granulocytes [6, 7, 9, 11], less is known on the role of these molecules on human monocytes. In the present report we show that p150,95 plays an important role in adhesion-associated functions of human monocytes. mAb directed against the α chain of p150,95 and the common β chain were potent inhibitors of adhesion, migration, chemotaxis and phagocytosis of human monocytes and inhibited the adhesion of TPA-stimulated U937 cells. In contrast, antibodies directed against the α chains of LFA-1 and CR3 were less efficient in their blocking properties and only affected random migration (LFA-1) and chemotaxis (CR3). Previous studies, however, clearly demonstrated that both the anti-α chain LFA-1 and the anti-α chain CR3 antibodies used in this study, recognize functional epitopes since they inhibit conjugate formation between effector and target cells [25, 35].

The differences observed in the capacity of the anti-p150,95 and anti-common β chain antibodies to block adhesion of TPA-treated U937 cells or monocytes were not related to the level of expression of LFA-1, CR3 or p150,95, since both cell types expressed equal amounts of these antigens (Table 1). This finding may indicate the involvement of additional molecules in adhesion-related processes of human monocytes or granulocytes and is in line with data of Wallis et al. and Harlan et al. who observed that mAb directed against the common β chain never completely abrogated the adhesion of monocytes or neutrophils to endothelial cells [38, 39]. Similar findings were made by Anderson et al. [7] who applied mixtures of mAb directed against all peptides of the LFA-1 family to study granulocyte adhesion.

The role of each of the LFA-1 family antigens in random migration is difficult to define since cell migration is a complex process in which both adhesion and motility play an important role [34, 40]. It is even more difficult to determine the function of these molecules in migration upon stimulation with chemotactic compounds such as fMLP, because rapid increases in the expression of CR3 and p150,95 on monocytes [6, 41, 42] are observed. Our data demonstrate that p150,95 plays a major role in cell motility, cell adhesion and chemotaxis. In addition, from our data and from results presented by others, it seems fair to conclude that both LFA-1 and CR3 also can participate in these processes. This notion is derived from the results of inhibition studies. These studies show that anti-β chain mAb are more potent inhibitors of adhesion and chemotaxis than anti-α chain antibodies alone [37, 39, 41-43], whereas an increased inhibition was observed when a selected mixture of mAb directed against all three α chains and against the common β chain of the LFA-1 family were applied [39]. Similarly, we demonstrated that p150,95 is involved in phagocytosis whereas other investigators showed that LFA-1 [44] or CR3 [6, 9, 44] mediate phagocytosis applying different targets.

As discussed above, anti-β chain mAb interfere with all functions associated with LFA-1, CR3 or p150,95. Therefore, it cannot be excluded that the β chain by itself contributes to the adhesion reaction. Despite a possible role in adhesion, the function of the common β chain is not clearly understood. It has been suggested that it acts as a transport molecule for the various α chains [1, 41], comparable to β2-microglobulin which has been shown to act as a vehicle to insert major histocompatibility complex antigens into the cell membrane [45]. This notion is supported by studies in patients deficient for LFA-1, p150,95 and CR3 [46-48]. These patients have a very low level of β chain precursor in contrast to LFA-1 α chain and CR3 α chain precursors [41, 44]. Until now it was not possible to relate the functional deficiencies of monocytes and granulocytes observed in these patients to one or more of the LFA-1 family antigens.

The observations presented in this study indicate that the LFA-1, CR3 and p150,95 antigens all contribute to many adhesion-related reactions each with its own specificity. A detailed analysis of the binding sites of these molecules by antibodies reacting with different epitopes of the same antigen and elucidation of their respective counterstructures will be helpful to further define the function of these adhesion-associated glycoproteins.

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5 References
