Changes in the expression of adhesion molecules as peripheral blood monocytes differentiate into peritoneal macrophages

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Abstract Peritoneal macrophages, derived from peripheral blood monocytes, are the chief cellular defenders against invasion of the peritoneal cavity by infectious organisms. Monocyte migration into the peritoneal cavity depends upon a coordinated series of adhesive events, utilizing cell surface receptors known as adhesion molecules. In order to better understand the mechanisms of leucocyte infiltration of the peritoneum during peritonitis, we studied the relative expression of adhesion molecules on monocytes and peritoneal macrophages from patients on continuous ambulatory peritoneal dialysis (CAPD). Peripheral blood and spent peritoneal dialysis fluid were obtained from patients undergoing CAPD, and the level of expression of various adhesion molecules on the monocytes/macrophages analysed by flow cytometry using receptor-specific monoclonal antibodies. Monocytes were also purified from the peripheral blood of volunteer donors, cultured in vitro for varying periods, and analysed in the same manner. Consistent differences in expression of certain adhesion molecules were found between monocytes and peritoneal macrophages, and similar changes occurred on monocytes cultured in vitro. Concurrent infection had no clear effect. Several receptors (integrins α4β1, α6β1, αLβ2 and αIIbβ3, and platelet endothelial cell adhesion molecule-1) were significantly decreased on peritoneal macrophages, while only the integrin αvβ5 increased. It is concluded that monocyte differentiation into peritoneal macrophages is accompanied by characteristic alterations in the adhesion molecule repertoire on the cell surface, emphasizing the different adhesive requirements of these two cell types.

Key words: adhesion molecules; CAPD; monocytes; peritoneal macrophages

Introduction

Advances in peritoneal dialysis techniques during the last decade have reduced the incidence of peritonitis, but it continues to be a major cause of morbidity in this patient group. The fact that peritonitis is relatively uncommon (~ once every year per patient) is a tribute to the efficiency of the sophisticated cellular and humoral immunological system that protects the peritoneal cavity. This system affords almost fool-proof protection, despite the presence of a permanent foreign body (the catheter) that several times each day connects the peritoneal cavity to the outside world for infusion of an unphysiological, hypertonic sugar solution. The initial cellular defence against bacterial invasion rests primarily with the macrophages that are normally resident within the peritoneal cavity [1]. There are several subpopulations of these cells. Some float free in the peritoneal fluid (normal volume is about 50 ml, but increased to 2-3 l during peritoneal dialysis), and there are also macrophages associated with the peritoneal membrane itself. These lie just beneath the mesothelial cell monolayer that lines the cavity, as well as associated with the capillaries within the membrane [2]. It is also thought, but yet to be demonstrated directly, that a population of macrophages remains adherent in vivo to the intracavity face of the mesothelial cell layer. There is ongoing debate as to which of these macrophage populations most efficiently kills invading microorganisms. During episodes of peritonitis their numbers increase dramatically, and they are also supplemented by a marked influx of mainly polymorphonuclear cells. It is very likely that other body cavities (e.g. urinary tract, pleural and pericardial spaces) possess similar if not fundamentally identical mechanisms of cellular defence.

The peritoneal macrophages derive from peripheral blood monocytes that have emigrated from the intravascular space and undergone differentiation. During this pathway of migration the monocytes traverse a series of barriers, including vascular endothelial cells and their basement membrane, the interstitium within the peritoneal membrane, and the peritoneal mesothelial cells and their associated basement membrane. A low level of trafficking of monocytes occurs continuously into the peritoneal space (and into other tissues and body spaces), but a potent source of leucocyte...
In the presence of appropriate stimuli, the leukocytes adhere more firmly to the endothelial cells, via interactions between their C-type lectin domains and carbohydrate residues on their ligands [5]. This induces the cells to 'roll' along the vessel wall, which is a necessary prelude to the firmer interactions that follow. Selectin-mediated adhesive events are confined to the intravascular space. In order to reach the peritoneal space, cells also need to navigate their way across the mesothelial cell layer. These interactions are mediated by members of the integrin and immunoglobulin superfamilies. The key receptor–ligand adhesive interactions that occur during monocyte migration into the peritoneal space are shown in Fig. 1.

The adhesive events that occur during monocyte (and other cells) migration are regulated in a complex manner. Factors that are involved include changes in the expression of the receptors and/or their ligands, changes in the function of receptors, and changes in the utilization of the receptors [7]. The repertoire of adhesion molecules expressed by cells will primarily dictate their potential for adhesive interactions, and likewise changes in that repertoire presumably reflect fluctuations in adhesive requirements.

In order to better understand the mechanisms whereby peripheral blood monocytes enter the peritoneal space, we have studied the expression of integrins and immunoglobulin superfamily adhesion molecules on blood monocytes and peritoneal macrophages taken from patients having continuous ambulatory peritoneal dialysis (CAPD). The receptors studied, as well as their ligands, are shown in Table 1, and they cover the known major adhesive interactions (after the initial selectin-mediated tethering) that monocytes utilize during the extravasation process. We consistently find a pattern of altered receptor expression on macrophages compared to monocytes, and suggest that these changes reflect the different adhesive requirements for the two cell types. Peripheral blood monocytes cultured in vitro also exhibit similar changes in receptor expression. Until now there has been a paucity of information about the adhesion molecules expressed by tissue macrophages, and we suggest that the changes that we

<table>
<thead>
<tr>
<th>Receptor</th>
<th>Antibody</th>
<th>Ligand(s)</th>
</tr>
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<tbody>
<tr>
<td>Anti-integrin antibodies</td>
<td></td>
<td></td>
</tr>
<tr>
<td>aL/b2</td>
<td>T82/7</td>
<td>Collagens, laminin</td>
</tr>
<tr>
<td>a2/1</td>
<td>RMAC11</td>
<td>Collagens, laminin</td>
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<tr>
<td>a3/b1</td>
<td>P1B5</td>
<td>Laminin, fibronectin(orange), collagens(orange)</td>
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<tr>
<td>a4/b1</td>
<td>P4C2</td>
<td>Fibronectin, VCAM-1</td>
</tr>
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<td>a5/1</td>
<td>PHM2</td>
<td>Fibronectin</td>
</tr>
<tr>
<td>a6/b1</td>
<td>GoH3</td>
<td>Laminin, ferritin</td>
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<td>pan-b1</td>
<td>4B5</td>
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<tr>
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<td>TS1/22</td>
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<td>OKM1</td>
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<td>KB90</td>
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<td>TS1/18</td>
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<td>LM609</td>
<td>Vitronectin, fibrinogen, von Willebrand factor, fibronectin, osteopontin, collagen, thrombospondin</td>
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<tr>
<td>pan-b3</td>
<td>15.4.2</td>
<td></td>
</tr>
<tr>
<td>avf5</td>
<td>15F11</td>
<td></td>
</tr>
</tbody>
</table>

| Antibodies against immunoglobulin superfamily members | |
| ICAM-1 | QE2.1B4 | aL/b2, aM/b2 |
| ICAM-2 | 6D5 | aL/b2 |
| PECAM-1 | 2BD4.D5 | CD31, sulphated glycosaminoglycans |

Abbreviations: VCAM-1, vascular cell adhesion molecule-1; ICAM, intercellular adhesion molecule; C3bi, inactivated complement component C3; PECAM-1, platelet endothelial cell adhesion molecule-1.
have observed play a significant role in monocyte migration into and localization within the peritoneal space.

**Subjects and methods**

**Patients**

Peritoneal cells and peripheral blood monocytes from 10 CAPD patients (seven female, three male) were studied on 13 different occasions. Nine studies were during episodes of peritonitis, and the other four were infection-free. The age range of the patients was 45–77, and duration of dialysis was 1–67 months. Relevant medical data and the organisms that caused peritonitis are summarized in Table 2. One patient (no. 3) was studied three times—once when infection-free, and twice during episodes of peritonitis. Another patient (no. 7) was studied during an episode of peritonitis as well as when infection-free. A third patient (no. 2) was studied during the recovery phase from a prolonged episode of acute renal failure, and so was not actively receiving peritoneal dialysis at the time but still had a Tenckhoff catheter in situ.

**Isolation of cells**

 Resident peritoneal leukocytes were isolated from peritoneal dialysate effluent that was either obtained fresh or had been stored overnight at 4°C. The fluid was centrifuged for 15 min at 400 g at 4°C. The cell pellet was washed twice with phosphate-buffered saline (PBS; pH 7.4) containing 1% fetal calf serum (FCS), and the cells were resuspended in the same buffer at a density of 1 to 5 x 10^6 cells/ml. Receptor expression on peritoneal macrophages was analysed by flow cytometry (see below). Venous blood from volunteer donors. Mononuclear cells were isolated by centrifuging through a Ficoll (Pharmacia Biotech, Sweden) gradient, according to the manufacturer’s instructions, resuspended in RPMI 1640 containing 10% FCS, and incubated in 25 cm^2 tissue culture flasks (Nunc, Denmark). Following 2 h incubation, non-adherent cells were washed off, and the adherent monocytes were incubated for varying times in RPMI 1640 plus 10% FCS at 37°C in a 5% CO2 incubator. The adherent monocytes were detached for analysis by incubating them for 5 min at 37°C in 0.1% EDTA in PBS.

**Monoclonal antibodies**

The following monoclonal antibodies (see Table 1) were used in this study: TS2/7 (murine anti-α2β1 integrin, from the American Tissue Type Collection (ATCC)); RMAC11 (murine anti-α2β1, from Dr G. Russ, The Queen Elizabeth Hospital, Adelaide, South Australia); P1B5 (murine anti-α3β1, from Dako, Denmark); P4C2 1-1 (murine anti-α4β1, from Dr E. Wayner, University of Minnesota, USA); PHM2 (murine anti-α5β1, from Professor R. Atkins, Monash Medical Centre, Melbourne, Australia); GoH3 (rat anti-α6β1, from Dr A. Sonnenberg, Amsterdam, The Netherlands); 4B5 (murine anti-β1 chain, from Dr N. Kovach, University of Washington, Seattle, USA); TSI/22 (murine anti-αLβ2, from the ATCC); OKM1 (murine anti-α2β2, from the ATCC); KB90, murine anti-αXβ2, from Dako, Denmark; TSI/18 (murine anti-β2 chain, from the ATCC); 2G12 (murine anti-αIbβ3, from Dr V. Woods, University of California, San Diego, USA); LM609 (murine anti-αvβ3, from Dr D. Cheresh, The Scripps Research Institute, La Jolla, California); 15.4.2 (murine anti-β3 chain, from Dr M. Ginsberg, The Scripps Research Institute, La Jolla, California); 15F11 (murine anti-αβ5β3, from Dr I. Stuiver, The Scripps Research Institute, La Jolla, California); QE2.1B4 (murine anti-ICAM-1, from the ATCC); RMAC11 (murine anti-ICAM-1, from Professor R. Atkins, Monash Medical Centre, Melbourne, Australia); P1B5 (murine anti-αLβ2, from Dr E. Wayner, University of Minnesota, USA); PHM2 (murine anti-α5β1, from Professor R. Atkins, Monash Medical Centre, Melbourne, Australia); GoH3 (rat anti-α6β1, from Dr A. Sonnenberg, Amsterdam, The Netherlands); 4B5 (murine anti-β1 chain, from Dr N. Kovach, University of Washington, Seattle, USA); TSI/22 (murine anti-αLβ2, from the ATCC). Relevant medical data and the organisms that caused peritonitis are summarized in Table 2. One patient (no. 3) was studied during an episode of peritonitis as well as when infection-free. A third patient (no. 2) was studied during the recovery phase from a prolonged episode of acute renal failure, and so was not actively receiving peritoneal dialysis at the time but still had a Tenckhoff catheter in situ.

**Flow cytometry**

Peritoneal cells (1–5 x 10^6 cells) or anticoagulated blood (containing 0.5–1.0 x 10^9 leukocytes) were incubated for 10 min at 22°C with a saturating amount (1–2 μg) of the appropriate monoclonal antibody (mAb). Irrelevant

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/sex</th>
<th>Primary diagnosis</th>
<th>Dialysis duration (months)</th>
<th>Clinical setting</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>56/F</td>
<td>Scleroderma</td>
<td>9</td>
<td>Peritonitis, culture negative</td>
</tr>
<tr>
<td>2</td>
<td>77/M</td>
<td>Acute renal failure</td>
<td>2</td>
<td>Recovery phase from renal failure, catheter flush only</td>
</tr>
<tr>
<td>3</td>
<td>66/M</td>
<td>IgA nephropathy</td>
<td>28</td>
<td>(i) Peritonitis (Staphylococcus epidermidis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ii) Infection-free — first follow-up</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(iii) Infection-free — second follow-up</td>
</tr>
<tr>
<td>4</td>
<td>77/F</td>
<td>Renovascular disease</td>
<td>2</td>
<td>Peritonitis (Pseudomonas fluorescens)</td>
</tr>
<tr>
<td>5</td>
<td>77/F</td>
<td>Analgesic nephropathy</td>
<td>1</td>
<td>Peritonitis (S. epidermidis)</td>
</tr>
<tr>
<td>6</td>
<td>71/F</td>
<td>Analgesic nephropathy</td>
<td>67</td>
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<tr>
<td>7</td>
<td>45/F</td>
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<td>7</td>
<td>(i) Peritonitis (S. epidermidis)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(ii) Infection-free at follow-up</td>
</tr>
<tr>
<td>8</td>
<td>63/F</td>
<td>Analgesic nephropathy</td>
<td>38</td>
<td>Peritonitis, culture negative</td>
</tr>
<tr>
<td>9</td>
<td>60/M</td>
<td>Diabetic nephropathy</td>
<td>21</td>
<td>Peritonitis, culture negative</td>
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<tr>
<td>10</td>
<td>62/F</td>
<td>Diabetic nephropathy</td>
<td>23</td>
<td>Peritonitis, culture negative</td>
</tr>
</tbody>
</table>
species-specific mAbs were used as negative controls. The cells were then washed twice with PBS containing 1% FCS, and resuspended in 200 μl of the same buffer containing a 1:200 dilution of fluorescein (FITC)-conjugated anti-mouse or anti-rat IgG (according to the species of origin of the primary antibody) (Silenus Laboratories, Victoria, Australia). After incubation for 10 min at 22°C, the erythrocytes in the blood were lysed with 2 ml of lysis buffer (0.826% NH4Cl, 0.1% NaHCO3, 0.0037% EDTA, w/v). Cells were then washed twice with PBS containing 1% FCS, and resuspended in 0.5 ml of 1% PBS-buffered formaldehyde. Fixed cells were stored at 4°C until analysis.

Stained cells were analysed on a FACScan flow cytometer (Becton-Dickinson, Mountain View, California). Monocytes and peritoneal macrophages were specifically studied using selective gating based on their characteristic forward and side scatter parameters. Their identity was also confirmed by staining the cells with phycoerythrin-labelled anti-CD14 monoclonal antibody (Becton-Dickinson). The relative differentiation of the peritoneal macrophages compared to the blood monocytes was confirmed by their selective expression of the differentiation antigen RM3/1 [9] (not shown).

The relative expression of a receptor on the cells was expressed as a mean fluorescence index (MFI) value, which was defined as the ratio between the mean immunofluorescence intensity of the particular antibody and the mean fluorescence intensity of the negative control.

**Statistical analysis**

The non-parametric Wilcoxon signed-ranks test was used to analyse the data. The level of significance applied to all data was \( P < 0.05 \).

**Results**

**Expression of adhesion molecules on peripheral blood monocytes and peritoneal macrophages**

Visual inspection of the forward versus side scatter histograms confirmed that macrophages were the predominant cells in infection-free peritoneal dialysis fluid, whereas neutrophils became much more prominent during episodes of peritonitis. The macrophages alone were studied in both situations using selective gating, with confirmation by staining with the anti-CD14 monoclonal antibody. Peripheral blood monocytes were studied in a similar way.

Preliminary studies of either peripheral blood monocytes or peritoneal macrophages alone were conducted in order to choose the receptors for more detailed study. As a result, certain receptors were not studied further because of their low level of expression (e.g. integrin \( \alpha v \beta 6 \), vascular cell adhesion molecule-1 (VCAM-1)). The studies reported here were only conducted on monocytes and peritoneal macrophages taken at the same time from each patient, and the following general principles emerged:

1. The overall expression of adhesion receptors was higher on monocytes than on macrophages.
2. There was wider variation between patients in expression on monocytes, although monocyte and macrophage expression was stable in the patients studied more than once (nos 3 and 7).
3. Macrophage expression overall was lower and there was less inter-patient variation.
4. Peritonitis had no clear effect on the changes in expression between monocytes and macrophages. This was suggested by our preliminary studies, and supported by comparing the nine studies during episodes of peritonitis with the four (on patients 2, 3 and 7 (twice)) conducted in the absence of peritonitis (see Fig. 2).

The results of these experiments have been grouped according to the type of adhesion molecules involved, and summarized in Fig. 2.

**\( \beta 1 \) integrins (Fig. 2a)**

Integrins \( \alpha 4/\beta 1 \), \( \alpha 5/\beta 1 \) and \( \alpha 6/\beta 1 \) are expressed on peripheral blood monocytes at levels that vary from low to moderate in different patients, but each is significantly downregulated on peritoneal macrophages. The other \( \beta 1 \) integrins studied (\( \alpha 1/\beta 1 \), \( \alpha 2/\beta 1 \), \( \alpha 3/\beta 1 \)) were weakly expressed or virtually absent on monocytes, and their expression was unchanged on macrophages.

**\( \beta 2 \) integrins (Fig. 2b)**

Peripheral blood monocytes from different patients express moderate to high levels of the \( \beta 2 \) integrins \( \alpha L/\beta 2 \) and \( \alpha M/\beta 2 \), whereas the expression of \( \alpha X/\beta 2 \) was consistently low. This receptor was studied on both cell types in only two patients, because the low expression had been observed in preliminary studies. The expression of \( \alpha L/\beta 2 \) was consistently and significantly decreased on peritoneal macrophages. The \( \alpha M/\beta 2 \) on macrophages was clearly lower in a number of patients, but actually rose in two patients, so that the overall change barely reached statistical significance. The decrease for both receptors was most marked in the three patients who had highest initial expression. The expression of \( \alpha X/\beta 2 \) remained low on the macrophages from the two patients studied.

**\( \beta 3 \) integrins (Fig. 2c)**

The initial experiments with an mAb against the common \( \beta 3 \) chain showed moderate to high expression on peripheral blood monocytes, albeit with wide variation between different patients. In all cases expression was markedly lower on the peritoneal macrophages. In order to determine which of the two known \( \beta 3 \) integrins (\( \alpha 11/\beta 3 \) and \( \alpha v/\beta 3 \)) was involved, we then obtained mAbs specific for either receptor (Table 1).
Adhesion molecules on blood monocytes and peritoneal macrophages

(a) Relative fluorescence intensity

(b) Relative fluorescence intensity

(c) Relative fluorescence intensity

- αβ1
- α2β1
- αβ1
- αβ1
- αβ1
- αβ1
- αβ1
- αβ1
- αβ1
- αβ1

- Blood
- Peritoneal

- Patient 1
- Patient 2
- Patient 3
- Patient 4
- Patient 5
- Patient 6
- Patient 7
- Patient 8
- Patient 9
- Patient 10

- p = 0.009
- p = 0.047
- p = 0.005

- αβ2
- αβ2
- αβ2
- αβ2
- αβ2
- αβ2
- αβ2
- αβ2
- αβ2
- αβ2

- ICAM-1
- ICAM-2

- Blood
- Peritoneal

- Patient 1
- Patient 2
- Patient 3
- Patient 4
- Patient 5
- Patient 6
- Patient 7
- Patient 8
- Patient 9
- Patient 10

- p = 0.003
- p = 0.049

- αβ3
- αβ3
- αβ3
- αβ3
- αβ3
- αβ3
- αβ3
- αβ3
- αβ3
- αβ3

- CD31

- Blood
- Peritoneal

- Patient 1
- Patient 2
- Patient 3
- Patient 4
- Patient 5
- Patient 6
- Patient 7
- Patient 8
- Patient 9
- Patient 10

- p = 0.005
- p = 0.005
- p = 0.005
Staining with these antibodies showed that the β3 expression on monocytes was essentially composed of αIIb/β3. It was much decreased on peritoneal macrophages, and the αvβ3 expression was low on both cell types.

Other integrins

The integrin αvβ5 (Fig. 2c) was weakly expressed by peripheral blood monocytes, but was consistently increased on peritoneal macrophages, although the overall level of expression remained quite low.

Several other integrin receptors were also studied in preliminary experiments, but the data are not shown because of low or absent expression. The integrin β4 chain is not expressed by either monocytes or macrophages. Both α4β7 (a receptor for fibronectin, VCAM-1 and mucosal addressing cell adhesion molecule (MAdCAM-1)) and αvβ6 (a fibronectin receptor) were weakly expressed by peripheral blood monocytes, and there was no clear difference in expression on peritoneal macrophages.

ICAM-1 and ICAM-2 (Fig. 2b)

Both of these receptors are members of the immunoglobulin superfamily, and their distribution includes leucocytes and endothelium [4]. In the patients where these were studied, ICAM-1 and ICAM-2 were both expressed on monocytes at relatively low levels, and there was no consistent change on peritoneal macrophages.

PECAM-1 (CD31) (Fig. 2c)

PECAM-1 is an adhesion molecule that is also a member of the immunoglobulin superfamily. It is normally expressed by platelets and leucocytes, and at the intercellular borders between endothelial cells [10]. In the patients we studied it was expressed at moderate to high levels on peripheral blood monocytes, but in all cases was markedly decreased on peritoneal macrophages.

Expression of adhesion molecules on peripheral blood monocytes cultured in vitro

In order to assess the contribution of cell differentiation to the observed changes in expression of these receptors, we examined the effects of varying periods of in vitro culture of peripheral blood monocytes taken from a volunteer normal, healthy donor. Under these conditions the monocytes attach and spread on the tissue culture plastic, and take on certain macrophage-like characteristics. The relative expression (expressed as RFI) of a selection of the adhesion molecules (β2, β3 and β5 integrins, ICAM-1, PECAM-1) at days 0 (before culture), 1, 2, 4 and 7 is shown in Fig. 3.

The initial high expression of both αLβ2 and αMβ2 on these cells fell to and remained at low levels after 2 days in culture. αXβ2 expression rose slightly after 1 day, but then steadily fell back to low levels. αIIbβ3 was highly expressed at day 0, but quickly fell to low levels in culture, while αvβ3 expression remained low throughout. The expression of αvβ5 was initially low but rose steadily during culture (although remaining at relatively low levels). Like αXβ2, the expression of ICAM-1 rose slightly at day 1 and fell steadily thereafter. The expression of PECAM-1 was high before culture, but fell to persistently low levels within 1 day of culture.

We were interested in the time course of change of PECAM-1 expression, and so specifically studied it during the first few hours of culture (Fig. 4). Detectable reduction occurred within 2 h, and it reached nadir by 6 h.
We failed to find any clear effect of concurrent experiments. We make the following conclusions based on these experiments.

1. Adhesion molecule expression on peripheral blood monocytes alters significantly as they become peritoneal macrophages. In particular, there are significantly fewer of a number of these receptors on macrophages compared to monocytes.

2. Monocytes taken from different patients express widely varying levels of adhesion molecules, whereas there is little variability on peritoneal macrophages. It was not possible from this small sample to determine factors (e.g. age, sex, time on dialysis) which affect monocyte expression. However, the lack of variation on macrophages, despite the wide variation in dialysis duration in this patient group (1–67 months), suggests that the increasing immaturity of these cells that has been observed as duration of dialysis increases [11] has no significant effect on their adhesion molecule repertoire.

3. We failed to find any clear effect of concurrent intraperitoneal infection on the differences in expression between monocytes and macrophages, or on the overall expression on macrophages. This suggests that the observed changes are a consequence of monocyte extravasation/differentiation rather than secondary to the effects of inflammatory cytokines. In addition, the changes are mimicked by those that occur on monocytes cultured in vitro, suggesting that the state of cell differentiation plays a key role in determining its repertoire of adhesive receptors.

The consistent differences between monocytes and macrophages give insights into the relative adhesive requirements of these cells. For example, the integrins α4β1, α5β1 and α6β1 are prominent on monocytes, and their ligands (fibronectin and VCAM-1, fibronectin and laminin, respectively [6]) are all present within the peritoneal membrane (Fig. 1) [2]. These interactions facilitate monocyte passage into the peritoneal space [3]. Laminin is only present in basement membranes, and so the diminution of α6β1 on macrophages is understandable. The changes in α4β1 and α5β1 expression are not as easily explained, because peritoneal fluid contains significant amounts of soluble fibronectin (as do other body fluids [12]). Phagocytosis of fibronectin-coated particles by macrophages (e.g. during infections) requires α5β1-dependent high-affinity binding of fibronectin [13]), and the residual α5β1 on the peritoneal macrophages may be sufficient for this purpose. However, α4β1 is not a high-affinity receptor for fibronectin, and this may be the explanation for its more dramatic downregulation on peritoneal macrophages. The α4β1/VCAM-1 interaction is essential for normal monocyte and lymphocyte adhesion to endothelium during inflammation [14], and seems also to be involved in their adhesion to mesothelial cells [15]. The peritoneal macrophages that we studied were floating free in the peritoneal dialysis fluid, and their α4β1 expression may have decreased following their dissociation from the peritoneal membrane. A similar decrease in α4β1 and α6β1 expression has been observed on alveolar macrophages [16], and differentiation of certain monocyctic cell lines reduces their expression of α4β1 and adhesion to VCAM-1 [17].

The reduction of αLβ2 and αMβ2 on peritoneal macrophages and cultured monocytes is similar to results with alveolar macrophages [16], although studies with differentiated monocytic cell lines [16,18,19] yielded conflicting results, and IL-4 stimulation of cultured monocytes increases their expression of αMβ2 and αXβ2 [20]. αLβ2 has an integral role in leucocyte–leucocyte and leucocyte–endothelial cell interactions through its recognition of the immunoglobulin superfamily adhesion molecules ICAM-1, -2 and -3 [3]. Both ICAM-1 and -2 are expressed by endothelial cells, and the former is upregulated on activated endothelium [3]. ICAM-1 is also expressed by peritoneal mesothelial cells [2]. The integrin αMβ2 is also a receptor for ICAM-1, but has additional ligands that include inactivated complement component C3, fibronogen and factor X. It too is involved in leucocyte adhesion to endothelium, but it also has an important role in phagocytosis of opsonized particles [3]. The downregulation of these two receptors on the floating macrophages may occur when there is no further need for interaction with the endothelium or mesothelium, although the decrease in αMβ2 expression is a little surprising if these cells are to retain their full phagocytic potential. The remaining receptors may be sufficient for this purpose, or those macrophages remaining in contact with the membrane may retain expression.

The pattern of expression of the β3 integrins was
surprising, because αIIbβ3 is normally only found on platelets, megakaryocytes and related cell lines, whereas αvβ3 has a more widespread distribution [6]. The αIIbβ3 on monocytes is probably due to platelet fragments [21] that are lost (shed?) as the cells differentiate and/or pass through the interstitium. The expression of αvβ3 remained low throughout, whereas the low expression of αvβ5 on monocytes was consistently increased on peritoneal macrophages and cultured monocytes. This implies a significant role for these cells, but there are conflicting reports about the function of this receptor [22–24].

Of the immunoglobulin superfamily members studied, only PECAM-1 showed significant change in expression. It is involved in homophilic as well as heterophilic interactions, and homophilic adhesion between leucocyte and endothelial PECAM-1 is necessary for leucocyte extravasation to inflammatory sites [10,25–27]. Its marked (and rapid) downregulation on peritoneal macrophages and cultured monocytes suggests that its role is essentially confined to leucocyte extravasation. In support of this hypothesis, we have found that peritoneal mesothelial cells do not express PECAM-1 (unpublished observations).

In summary, there are consistent and measurable changes in the expression of certain adhesion molecules as blood monocytes become peritoneal macrophages, emphasizing the different adhesive requirements of these two cell types. Our future studies will aim to elucidate the timing and mechanisms of these changes, with the intention of gaining better understanding of the defence of the peritoneal cavity.

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References


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