Expression of adhesion molecules on synovial fluid and peripheral blood monocytes in patients with inflammatory joint disease and osteoarthritis

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Abstract

***Objective*—To determine the presence of adhesion molecules on monocytes/ macrophages (Mϕ) from peripheral blood (PB) and synovial fluid (SF) in patients with osteoarthritis (OA) and inflammatory joint diseases (rheumatoid (RA) and reactive arthritis (ReA)) in order to improve our understanding of the possible mechanisms underlying the inflammatory process.**

***Methods*—Whole blood and SF cells were stained with monoclonal antibodies against CD11a (LFA-1), CD15 s (sialyl-Lewis X), CD44, CD54, VLA-4, and HLA-DR counterstained with anti-CD14 antibodies as a Mϕ marker for dual fluorescence analysis by flowcytometry.**

***Results*—On PB-Mϕ, CD15s was markedly increased in both RA as well as ReA compared with OA. Furthermore, in the PB LFA-1, CD44, and HLA-DR showed a higher surface density on Mϕ in ReA than in OA. Comparison between SF and PB showed significantly higher CD44 and CD54 expression on SF-Mϕ. These molecules play an important part in lymphocyte-Mϕ interaction.**

***Conclusion*—In PB from patients with inflammatory joint diseases, Mϕ are activated, allowing recruitment into the synovial compartment. These disorders, in contrast with OA seem to be "systemic" in nature. Within the SF, different adhesion molecules are expressed on $CD14^+$ Mϕ as compared with PB.**

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A hallmark of arthritic conditions is the accumulation of leucocytes within the synovial fluid (SF). Among those, cells of the monocyte/ macrophage (Mϕ) lineage play an important part[1] and are thought to be involved in the pathogenesis of inflammatory joint diseases.[2]

Mechanisms of cell migration via endothelial cells (EC) into areas of inflammation are increasingly understood. A variety of adhesion molecules and their functions have been partly elucidated in recent years. In respect to Mϕ, differences in certain function associated and differentiation associated molecules have been shown to exist between SF-Mϕ and peripheral blood (PB)-Mϕ from patients with rheumatoid arthritis (RA), reactive arthritis (ReA) and osteoarthritis (OA).[3 4]

In this investigation we intended to elucidate the type and distribution of adhesion molecules on PB-Mϕ and SF-Mϕ as well as possible differences between patients with different diseases.

Methods

PATIENTS

Arthrocentesis of knee joint effusions was performed for therapeutic or diagnostic purposes. No intra-articular injection of hyaluronate or corticosteroids was evident within the past six months. SF samples were obtained in heparinised tubes from 20 patients with inflammatory arthritis (IA), namely 10 RA (diagnosed according to the ACR criteria[5]) and 10 ReA (diagnosed as previously described[6]), as well as 10 patients with OA.[7] All SF were analysed microscopically and crystal induced arthritides ruled out; in parallel PB was collected in heparinised tubes on the occasion of routine laboratory testing. Informed consent was obtained from all patients. Table 1 shows the patient characteristics.

IMMUNOFLUORESCENCE AND FLOW CYTOMETRY

Staining of PB-Mϕ was performed in whole blood for 30 minutes at room temperature.[8] SF cells were pelleted by centrifugation and resuspended in phosphate buffered saline (PBS) containing 3% bovine serum albumin (BSA). To avoid cell activation of these samples, 0.1% sodium azide (Na_3N) was added and probes were kept at room temperature. To remove clots, cell suspensions were filtered through a Cell Strainer 2350 (Falcon, Lincoln Park, NJ). In all experiments 10^7 cells were stained in 100 µl PBS/BSA/Na_3N, containing murine monoclonal antibodies (mab) in predetermined optimal concentrations. Cells were stained with mabs against leucocyte function antigen-1 (LFA-1; CD11a), sialyl Lewis X (sLeX; CD15s), hyaluronic acid receptor (CD44), intercellular adhesion molecule-1 (ICAM-1; CD54), very late activation antigen-4 (VLA-4; CD49d), and MHC-class II (HLA-DR). All mabs were purchased from Becton Dickinson, San Jose, CA, USA, except anti-CD54 (Immunotech, Marseille, France). Mabs were labelled with fluoro-iso-thiocyanate (FITC), except anti-CD15s and anti-VLA-4 (both CAMFolio, Becton Dickinson). Binding of unconjugated mabs was visualised with a rabbit-antimouse IgG mab (FITC labelled, Dako, Glostrup, Denmark). For direct immunofluorescence

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Table 1 Patient characteristics

Sex	*Age*	*ESR*	*CRP*	*RF*	*SF-WBC*	*PB-MO abs/rel*				
Osteoarthritis										
f	59	13	0.5	neg	2000	425/µl; 3.0%				
m	78	10	0.5	neg	1600	302/µl; 2.9%				
m	70	16	0.2	neg	100	239/µl; 2.7%				
f	72	19	0.5	neg	3000	498/µl; 3.9%				
m	68	14	0.2	neg	1500	240/µl; 3.7%				
m	70	18	0.3	neg	500	375/µl; 5.5%				
m	65	8	0.4	neg	1200	296/µl; 2.4%				
m	58	12	0.3	neg	2500	371/µl; 3.1%				
f	73	14	0.6	neg	2800	441/µl; 3.5%				
m	49	4	0.2	neg	100	601/µl; 6.1%				
Rheumatoid arthritis							*Duration*	*DMARD*	*NSAID*	*Corticosteroids*
f	26	54	5.7	pos	14900	321/µl; 3.0%	8	MTX 12.5 mg/wk	none	none
f	48	72	10.1	neg	12000	595/µl; 6.3%	1	MTX 10 mg/wk	diclofenac 150 mg/d	none
f	85	90	12.4	neg	52000	870/µl; 7.5%	19	MTX 12.5 mg/wk	ketoprofen 200 mg/d	prednisolon 5 mg/d
m	55	80	5.1	pos	25000	490/µl; 6.3%	3	SSZ 2000 mg/d	ibuprofen 1800 mg/d	prednosolon 7.5 mg/d
f	77	20	3	pos	10600	298/µl; 3.1%	2	CQ 150 mg/d	diclofenac 150 mg/d	none
f	72	74	6.2	neg	18500	430/µl; 5.2%	10	MTX 15 mg/d	naproxen 1000 mg/d	prednosolon 10 mg/d
m	59	86	7	pos	8000	370/µl; 4.9%	7	SSZ 3000 mg/d	diclofenac 100 mg/d	none
f	79	54	3.3	pos	18100	435/µl; 3.1%	1	SSZ 2000 mg/d	indomethacin 100 mg/d	prednisolon 10 mg/d
m	71	42	5.8	pos	8200	910/µl; 10%	1	none	diclofenac 150 mg/d	none
f	54	38	1.6	neg	7500	700/µl; 11%	2	MTX 15 mg/d	indomethacin 100 mg/d	none
Reactive arthritis							*Infection*	*DMARD*	*NSAID*	*Corticosteroids*
m	26	20	2.3	neg	5000	540/µl; 5.2%	n.id.	none	none	none
m	46	46	1.1	neg	5500	398/µl; 5.4%	n.id.	none	diclofenac 100 mg/d	none
f	33	70	2	neg	19900	422/µl; 6.2%	Chlamydia	none	piroxicam 20 mg/d	none
m	32	40	2.2	neg	2200	500/µl; 8%	n.id.	MTX 12.5 mg/wk	ketoprofen 300 mg/d	none
f	24	112	7.8	neg	29000	400/µl; 6%	Chlamydia	none	diclofenac 100 mg/d	none
m	40	79	4.3	neg	3300	1410/µl; 14%	Yersenia	SSZ 3000 mg/d	none	prednisolone 5 mg/d
m	47	34	2.2	neg	9000	250/µl; 4.5%	n.id.	none	diclofenac 100 mg/d	none
f	28	48	2.1	neg	30000	560/µl; 7.5%	Chlamydia	none	none	none
m	46	34	2.4	neg	4000	880/µl; 10%	n.id.	none	diclofenac 100 mg/d	methyl-pred. 8 mg/d
m	55	48	7.7	neg	19400	625/µl; 5.5%	n.id.	none	ibuprofen 1200 mg/d	none

Sex (m, male; f, female); age (y); ESR, erythrocyte sedimentation rate (mm 1st h; normal <15); CRP, C reactive protein (mg/dl, normal <0.5); RF, rheumatoid factor; SF-WBC, synovial fluid white blood cell count (leucocytes/µl); peripheral blood monocytes (PB-Mo); disease duration (y); infection (n.id.; not identified); disease modifying antirheumatic drug (DMARD: CQ, chloroquine; MTX, methotrexate; SSZ, sulfasalazine); non-steroidal anti-rheumatic drug (NSAID).

staining IgG_1 and IgG_{2a} (FITC and phycoerythrin (PE) labelled, Simultest, Becton-Dickinson) were used as respective isotype controls. Unconjugated murine IgG_{2b} (Dako) was the negative control in the indirect immunofluorescence assay. After two washing steps all samples were counterstained with an anti-CD14 mab (PE labelled, Leu-M3, Becton-Dickinson). To remove erythrocytes all cells were incubated with lysing solution (Becton-Dickinson) for 15 minutes at room temperature, which also leads to fixation of stained cells. Leucocytes were then pelleted and resuspended in 200 µl PBS before analysis was performed with a flow cytometer (FACScan, Becton Dickinson). Five thousand Mϕ were acquired by gating for CD14 positive ($^+$) cells. All results were expressed as mean fluorescence intensity (MFI). To ensure comparable flourescence data, alignment and instrument control were done daily by running Immunocheck beads (Coulter, Hileah, FL, USA) on the cytometer before data acquisition.

STATISTICS

Group data were obtained by calculating mean (SEM) MFI. Statistical analysis of differences was performed by Wilcoxon test. Data were analysed using StatWorks software running on an Apple Macintosh IIvx computer.

Results

PERIPHERAL BLOOD MONOCYTE COUNTS

Statistically significant higher absolute (Mϕ/µl (SEM)) and relative (% of PB leucocytes (SEM)) monocyte counts in the PB have been found in patients with RA (542 (70); 6.0% (0.9%)) and ReA (599 (105); 7.2% (0.9%)) than patients with OA (379 (37); 3.7% (0.4%)) (fig 1).

FLOW CYTOMETRY RESULTS

Table 2 shows the results of mean (SEM) MFI.

CARBOHYDRATE LIGAND RECEPTOR

sLeX (CD15s) expression was more than threefold higher on PB-Mϕ in both, RA ($p<0.05$) and ReA ($p<0.05$) than in OA but comparable on SF-Mϕ.

INTEGRINS

The surface density of LFA-1 (CD11a) tended to be higher on PB-Mϕ in RA (NS) patients than those with OA but only reached statistical significance in ReA patients ($p<0.05$). LFA-1 expression on SF-Mϕ was similar in all groups.

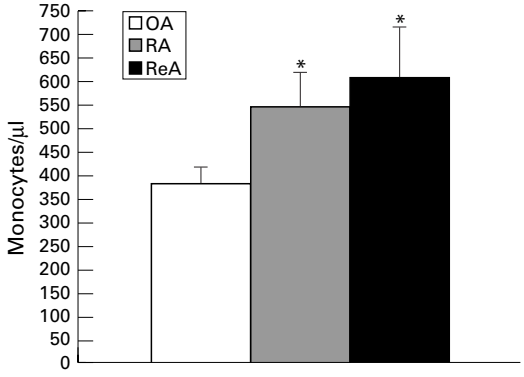

Figure 1 Absolute monocyte counts in the peripheral blood of patients with osteoarthritis (OA), rheumatoid (RA) and reactive arthritis (ReA) are shown. Columns represent mean (SD) values of monocytes/µl.

Table 2 Expression of adhesion molecules on peripheral blood (PB) and synovial fluid (SF) CD14⁺ monocytes of patients with osteoarthritis, rheumatoid and reactive arthritis. Values show mean (SEM) fluorescence intensity

	Osteoarthritis	*Rheumatoid arthritis*	*Reactive arthritis*
sLeX (CD15s)			
PB	109.0 (22.6)	431.0 (60.3)*	402.9 (61.1)*
SF	138.5 (13.8)	169.1 (24.3)	186.4 (43.0)
LFA-1 (CD11a)			
PB	210.9 (7.5)	250.5 (21.7)†	340.2 (12.3)*
SF	189.7 (9.4)	213.6 (21.3)	194.5 (7.8)
VLA-4 (CD49d)			
PB	83.8 (5.2)	76.2 (9.6)	90.7 (5.0)
SF	122.6 (5.3)	93.2 (14.1)†	127.0 (7.3)
ICAM-1 (CD54)			
PB	51.8 (6.8)	47.6 (9.4)†	99.2 (24.0)
SF	99.0 (20.7)	120.0 (24.1)†	217.1 (31.3)*
CD44			
PB	111.1 (13.2)	152.9 (39.3)	234.6 (29.3)*
SF	270.9 (19.6)	382.7 (123.0)	470.1 (26.3)*
HLA-DR			
PB	343.8 (80.1)	568.0 (130.5)	649.1 (85.3)*
SF	1079.5 (187.0)	2003.8 (389.8)*	2233.0 (136.5)*

*Indicates statistically significant difference compared with OA patients. †Indicates statistically significant difference between RA and REA ($p<0.05$).

In general, VLA-4 (CD49d), which binds to endothelial VCAM-1, was found in similar amounts in all disease groups. It is only weakly expressed, on both, PB-Mϕ and SF-Mϕ.

IMMUNOGLOBULIN FAMILY

No significant difference was seen regarding PB-Mϕ ICAM-1 (CD54) expression. The higher surface density on SF-Mϕ in inflammatory joint diseases was only significant in ReA patients ($p<0.05$) when compared with OA.

HOMING RECEPTOR

CD44 expression in ReA patients was higher than in OA on both, PB-Mϕ and SF-Mϕ ($p<0.05$), but no significant difference was seen between OA and RA.

MHC-CLASS II

PB-Mϕ from ReA patients had higher HLA-DR expression than those from OA patients ($p<0.05$), but for RA the difference failed to be statistically significant. Among SF-Mϕ, both, RA and ReA showed significantly higher surface levels than from OA ($p<0.05$).

COMPARISON OF RA AND REA

Adhesion molecule surface densities on Mϕ in ReA patients were comparable to or higher than those in RA patients. A significant difference was found in the SF concerning VLA-4 and ICAM-1. In the PB LFA-1 and ICAM-1 expressions were significantly higher in ReA patients. There were no differences in absolute and relative PB-Mϕ counts in patients with RA and ReA (fig 1).

COMPARISON OF PB-MΦ AND SF-MΦ

Because of different technical isolation and staining procedures, a comparison of MFI values of Mϕ from the SF and the PB is of limited reliability. In all patients a surface density increase of the most adhesion molecules was detected in the SF compared with PB-Mϕ (CD44, ICAM-1, HLA-DR and VLA-4; $p<0.05$). Only CD15s on Mϕ from the SF is expressed to a lower extent than from the PB ($p<0.05$). Moreover, this was only seen in patients with IA.

Discussion

The results obtained show differences in adhesion molecule expression on Mϕ in inflammatory, mainly ReA, as compared with degenerative joint diseases, but also on SF-Mϕ compared with PB-Mϕ.

The different molecules are known to serve different functions. Thus, sLeX (CD15s) and its ligand ELAM-1 on endothelial cells (EC) are engaged in the initial phase of Mϕ adhesion.[9] Patients with IA (both RA and ReA) had more than threefold higher levels of CD15s expression on circulating Mϕ than patients with OA. In SF, however, such an increase was not seen. Downregulation of sLeX after the migration into and through the synovial membrane is one hypothesis to explain the lower expression in the SF. Alternatively, soluble ELAM-1 released by activated EC might have masked the antigen on SF-Mϕ.[10]

LFA-1 (CD11a) interacts with endothelial ICAM-1 on EC, induces "stretching" of endothelium bound Mϕ and leads to transendothelial migration.[9] Similar to CD15s, LFA-1 was higher on PB-Mϕ of patients with ReA than OA and showed such a tendency in RA. On SF-Mϕ comparable levels exist in all disease groups. Cytokines such as interferon (IFN)γ have been demonstrated to downregulate surface LFA-1. The data on sLeX and LFA-1 suggest a systemic activation of PB-Mϕ in IA with possibly increased recruitment via binding to EC.

In contrast, VLA-4 (CD49d), which mediates "rolling" of leucocytes and is required by Mϕ for migration into the joint,[9 11] was expressed to similar extents among Mϕ from patients with IA and OA, although there was a difference between RA and ReA patients, but was slightly higher on SF-Mϕ compared with PB-Mϕ. The activated form of VLA-4 binds to the matrix component fibronectin. Thus, CD49d does not seem to exert its role in arthritis by changes in cell surface density. It is conceivable that conformational changes are more important for adhesive function.

CD44 was found in higher levels on SF-Mϕ compared with PB-Mϕ in all groups. Hyaluronate, which is found abundantly in the SF and binds to its receptor (CD44), can activate monocytes, induces monokine production, and regulates interleukin (IL) 1 and tumour necrosis factor (TNF) α production by Mϕ.[12] No significant differences on PB-Mϕ and SF-Mϕ were found between patients with RA and OA, although CD44 tended to be higher in RA. In contrast, this difference reached statistical significance higher in patients with ReA when compared with the OA.

Interaction between Mϕ and lymphocytes is partly mediated by ICAM-1 (CD54). This molecule was expressed to higher degrees on Mϕ in the SF of patients with arthritis. Therefore SF-Mϕ may intimately interact with T cells, which are known to express high levels of LFA-1.[13] This circumstance is further substantiated by the increase in HLA-DR expression on both SF-Mϕ in arthritis patients. All these changes can be induced by cytokines, such as interleukin IL4, IFNγ or TNFα.[9 14]

Patients with RA showed lower expressions of some adhesion (LFA-1, ICAM-1, VLA-4) molecules as compared with patients with ReA. RA patients had a longer intake and higher dose of anti-rheumatic drugs than ReA patients. Non-steroidal anti-inflammatory drugs have been shown to influence adhesion molecule expression, which probably contributes to these differences.[15] This may also cause the lack of some significant differences in the RA group when compared with OA. Interestingly, there have been comparable absolute and relative PB monocyte counts in RA and ReA patients.

Taken together, the data indicate that $CD14^+$ Mϕ from the PB of patients with inflammatory joint disease are activated to a state that enables binding to EC and transendothelial migration (CD11a and CD15s). Furthermore, Mϕ from the SF seem to express highly some of the adhesion molecules that are needed for interaction with T cells (ICAM-1, HLA-DR) reflecting their propensity for intensive interaction with cognate immunocompetent cells. Interestingly, under both, degenerative and inflammatory conditions SF-Mϕ show higher surface density of receptors for connective tissue constituents (CD44) than PB cells.

All these observations substantiate the importance of the upregulated adhesion molecules on Mϕ in the pathogenesis of IA, but they only provide data on surface expression of these function associated molecules. Further analysis of migratory capacity of Mϕ will be needed to prove the above assumptions; however these assumptions are based on known effects of these molecules. Our data also indicate that therapeutic approaches leading to downregulation of these molecules may be of significant interest.

Finally, it should be borne in mind that our results relate to $CD14^+$ cells. CD14 is expressed by only a part of the Mϕ lineage.[16] Mϕ may also be activated in other compartments—that is, the bone marrow or the synovial membrane before they enter the sites investigated here (PB, SF). Thus, additional studies will also have to focus also on other functional populations.

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