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ORIGINAL ARTICLE

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Synoviocytes in chronic synovitis in situ and cytokine stimulated synovial cells in vitro neo-express α1, α3 and α5 chains of β1 integrins

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Abstract The expression of the $\beta 1$ integrins was examined immunohistochemically in synoviocytes from normal synovial membrane and from chronic synovitis of different aetiology and intensity. Normal synoviocytes were $\alpha 6\beta 1$-positive but lacked $\alpha 1$ through $\alpha 5$. In mild inflammation type A synoviocytes neo-expressed $\alpha 1$, $\alpha 3$, and $\alpha 5$ chains. In severe inflammation both type A and B synoviocytes expressed $\alpha 3$, $\alpha 4$, $\alpha 5$, and $\alpha 6$ chains. The effects of inflammatory cytokines, as single agents or in combination, on the $\beta 1$ integrin expression in cultured normal synoviocytes was determined by immunocytochemistry and flow cytometry. The $\alpha 1$ chain, while absent in unstimulated synoviocytes, was induced by interleukin-1β (IL-1β), tumour necrosis factor-α (TNF-α), and interferon-γ (IFN-γ). This effect was enhanced by combining IL-1β and TNF-α. Expression of the $\alpha 3$ chain was up-regulated by IL-1β and, more intensely, by IFN-γ. Transforming growth factor β (TGF-β) inhibited the up-regulating effect of IL-1β and antagonized the effect of IFN-γ on $\alpha 3$ chain expression. Expression of the $\alpha 5$ chain was up-regulated significantly by co-stimulation through IL-1β together with TGF-β or TNF-α. Thus, the $\beta 1$ integrin profile of cytokine activated synoviocytes in vitro resembled that of synoviocytes in synovitis in situ. These data suggest that IL-1β, TNF-α, IFN-γ, and TGF-β are likely to be among the effectors regulating $\beta 1$ integrin expression in synoviocytes in vivo.

Key words Interferon-γ · Tumour necrosis factor-α Transforming growth factor-β · Interleukin-1β Inflammation

Introduction

Chronic synovitis is characterized by mononuclear cell infiltration, synoviocyte proliferation, neo-vascularization and deposition of extra-cellular matrix proteins like fibronectin, collagen and laminin. Important mediators of these inflammatory changes are the cytokines which are found abundantly in synovial tissue and synovial fluid [8, 19, 28]. They are produced locally by infiltrating cells such as monocytes and lymphocytes and by resident cells such as endothelial cells [14, 32, 37] and synoviocytes (SC) [22]. The tissue localization of inflammatory cells by adherence to endothelial cells and interaction with extracellular matrix components is mediated through various cell surface receptors including integrins [1].

Integrins are heterodimers consisting of non-covalently associated α and β subunits. On the basis of different β subunits, integrins are subdivided into subfamilies. At present, at least 8 β subunits and up to 14 α subunits may associate to give rise to more than 20 different integrin heterodimers [29]. $\beta 1$ integrins are composed of a minimum of 9 different α subunits, named $\alpha 1$ through $\alpha 8$ and αv which associate with a common β chain, $\beta 1$. $\beta 1$ integrins are adhesion receptors mediating cell-cell and cell-matrix interactions [29]. The $\alpha 3\beta 1$ was shown to react with epiligrin, a compound of epithelial basement membranes [11]. The $\alpha 4\beta 1$ was found to mediate cell-cell as well as cell-matrix interactions (Table 1). $\alpha 2\beta 1$ was shown to recognize the sequence DGEA in type I collagen and to bind to laminin [29]. Recently, $\alpha 2\beta 1$ was identified as the RGD independent tenascin receptor [18, 40]. Furthermore, a recent report suggests the involvement of $\alpha 2\beta 1$ in cell-cell interaction on epithelial cells [10]. The $\alpha 4\beta 1$ heterodimer recognizes the sequence EILDV in fibronectin and mediates the attachment to the vascular cell adhesion molecule (VCAM-1) [16] and to the intercellular adhesion molecule (ICAM-2) [38]. Moreover, this molecule was shown to co-mediate B-T and B-B lymphocyte aggregation [26, 34]. Various cytokines, including transforming growth factor-β (TGF-β), interleukin-1β (IL-1β), tumour necrosis factor α (TNF-α), basic fibroblast growth factor (bFGF), interferon-γ (IFN-γ) have been shown to regulate and modulate the in vitro expression of $\beta 1$ integrins in various cell types [7, 17, 36].

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Table 1 Integrin subunits detected and monoclonal antibodies used in this study

Integrin-subunit	MW (kDa)	Clone	Isotype	CD number[a]	Receptor for/function	References
$\alpha 1$	210	TS2/7	IgG1	CD49a	Collagen/laminin	[25]
$\alpha 2$	170	Gi9	IgG1	CD49b	Collagen/laminin/tenascin	[21]
$\alpha 3$	130	P1B5	IgG1	CD49c	Collagen/laminin/fibronectin/epiligrin	[20]
$\alpha 4$	150	HP2/1	IgG1	CD49d	Fibronectin/VCAM-1 (CD106)/ ICAM-2 (CD102)	[35]
$\alpha 5$	135	SAM-1	IgG2b	CD49e	Fibronectin	[42]
$\alpha 6$	120	GOH3	IgG2a	CD49f	Laminin	[39]
$\beta 1$	130	K20	IgG2a	CD29	Common β chain of $\alpha 1$ through $\alpha 6$ chains	[4]

[a] According to the fixing of the Nomenclature Committee of the 5th International Workshop and Conference on Leucocyte Differentiation Antigens, Boston, November 1993

Cytokines modulate the adhesive properties of cells via regulation of $\beta 1$ integrin expression. Through this mechanism cytokines may target the inflammatory cell traffic indirectly and may contribute to the persistence of the local inflammatory response. The present study aimed at investigating the effect of various cytokines on the expression of $\beta 1$ integrins in SC in vitro by immunocytochemistry and by flow cytometry and is the first report on the functional study of $\beta 1$ integrins in SC in vitro. Furthermore, we describe the distribution of $\beta 1$ integrins in SC of chronically inflamed synovia with different intensities of inflammatory changes in situ in comparison with the $\beta 1$ integrin status of SC in normal synovial membrane by immunohistochemistry. Our data indicate that the $\beta 1$ integrin profile of cytokine-stimulated SC in vitro resembles that of SC in synovitis in situ.

Materials and methods

Synovial tissues were obtained from 5 patients with chronic synovitis of different aetiology and intensity undergoing knee replacement surgery or synovectomy. Specifically, two samples of synovial membranes were taken from patients with rheumatoid arthritis, one of a patient with osteoarthritis (according to the criteria of the 'American College of Rheumatology', formerly, the 'American Association of Rheumatology' [3, 5]), one of a patient with reactive arthritis induced by an osteosarcoma in the inferior extremity and one of a persons with psoriatic arthritis. Normal specimens were obtained at autopsy from 6 patients without arthritis who died of unrelated causes. Autopsy specimens were taken within 6 h after death. Four of these specimens were quick-frozen in liquid nitrogen and stored at −70° C. The other two specimens were dissected under sterile conditions, kept in phosphate-buffered saline (PBS; pH 7.5) and immediately prepared for synoviocyte culture.

Serial frozen sections of about 1 cm^2 in area and 4–6 µm in thickness were air-dried over-night, fixed in acetone for 10 min at room temperature, and immunostained immediately or stored at −20° C for 1–3 weeks.

For synoviocyte isolation the synovial samples were extensively washed with PBS supplemented with penicillin-streptomcyin-fungizone (10 units/ml, 10 mg/ml and 0.25 mg/ml, respectively; Seromed-Biochrom, Berlin, Germany). The synovial membrane was dissected from fibrous tissue and fat and was minced into small pieces with scissors and incubated for 2 h at 37° C with 2 mg/ml collagenase type Ia (Sigma Chemical Co., St. Louis, Mo., USA) in serum-free basal Iscove medium (Seromed-Biochrom). The samples were then filtered through a nylon mesh, extensively washed and suspended in basal Iscove Medium, supplemented with 10% fetal calf serum (FCS, Bio Pro, Karlsruhe, Germany) with the above mentioned mixture of antibiotics. Finally, they were seeded in 25 cm^2 culture flasks (Falcon, Lincoln Park, N.J., USA) and cultured in a humidified 5% carbon dioxide atmosphere. After overnight culture, non-adherent cells were removed. Fresh medium was added and the incubation continued. At confluence, cells were trypsinized, split at a 1:3 ratio and recultured in medium. SC from passages 3 through 9 were used in these experiments. The cultured cells comprised a homogeneous population of SC with respect to morphological and immunocytochemical criteria (<1% $CD11b^+$, <1% $CD11a^+$, <1% $CD11c^+$, <1% $CD53^+$, <1% $CD3^+$).

The source of the cytokines used was as follows: Phorbol 12-myristate 13-acetate (PMA) was obtained from Sigma; human recombinant IL-1β (specific activity 5×10^8 units/mg, purity>95%) was supplied by Genzyme (Cambridge, Mass., USA); human recombinant TGF-$\beta 1$ (purity>95%) was purchased from Boehringer Mannheim (Mannheim, Germany); human recombinant basic fibroblast growth factor bFGF (purity=90%) was obtained from Genzyme; human recombinant IFN-γ (specific activity 2×10^7 units/mg, purity>98%) and human recombinant TNF-α (specific activity 5×10^7 units/mg, purity>95%) were generous gifts from Knoll (Ludwigshafen, Germany). In each preparation the final concentration of lipopolysaccharide (LPS) in culture after dilution of recombinant cytokines was <0.005 ng/ml.

SC were cultured for 72 h in the presence of different cytokines, either alone or in various combinations. The concentrations used were as follows: PMA: 5 ng/ml; IL-1β: 40 U/ml; TGF-$\beta 1$: 10 ng/ml; bFGF: 10 ng/ml; IFN-γ: 100 U/ml; TNF-α: 10 ng/ml. At the end of the incubation, cells were washed with PBS, and harvested by brief trypsinization (which does not affect detection of surface adhesion molecules). The cell viability as measured by the trypan blue exclusion test was >95%. Cells were washed twice in PBS and resuspended with RPMI 1640 (Gibco, Paisley, Scotland, UK) at a concentration of 2×10^4 ml cells. Cells were centrifuged at 1000 r.p.m. for 5 min, air-dried, fixed in acetone for 10 min, and stained immediately or stored at −20° C for up to 2 weeks.

The monoclonal primary antibodies (mAb) against the $\beta 1$ integrin subunits used in this study are listed in Table 1. MAb TS2/7 and GOH3 were supplied by T Cell Sciences (Cambridge, Mass., USA); mAb Gi9, HP2/1, K20 and SAM1 were obtained from Dianova-Immunotech, (Hamburg, Germany); mAb P1B5 was purchased from Telios (San Diego, Calif., USA). A polyclonal biotinylated sheep antibody to mouse Ig (reactive with all mouse isotypes), a polyclonal biotinylated sheep antibody to rat Ig for detection of rat-derived mAb GOH3, and a streptavidin-biotinylated peroxidase complex, were provided by Amersham (High Wycombe, UK) and served as a detection system for the primary antibodies. 3-Amino-9-ethylcarbazole (AEC) and N'N-dimethylformamide (DMF) were obtained from Sigma.

For immunostaining the frozen sections and cytospin preparations were incubated for 1 h with purified mAb at appropriate dilutions (TS2/7, 1:100; Gi9, 1:50; P1B5, 1:3000; HP2/1, 1:50;

Table 2 Expression of $\beta 1$ and $\alpha 1$ through $\alpha 6$ integrin subunits in type A and B synoviocytes in situ. (Scoring of cell reaction : '+++', all cells positive; '–', all cells negative; '*' low level of antigen-expression; reactivity (x/y): number of reactive specimens per number of cases examined)

Antigen	Normal synovium	Mild inflammation		Severe inflammation
	Type A and B	Synoviocytes		Type A and B
		Type A	Type B	
$\alpha 1$ (CD49a)	– (0/4)	+++* (3/3)	– (0/3)	– (0/2)
$\alpha 2$ (CD49b)	– (0/4)	– (0/3)	– (0/3)	– (0/2)
$\alpha 3$ (CD49c)	– (1/4)	+–+* (3/3)	+++* (3/3)	+++* (2/2)
$\alpha 4$ (CD49d)	– (0/4)	– (0/3)	– (0/3)	+++ (2/2)
$\alpha 5$ (CD49e)	– (1/4)	+–+ (3/3)	– (0/3)	+++ (2/2)
$\alpha 6$ (CD49f)	+++ (4/4)	+–+ (3/3)	+++ (3/3)	+++ (2/2)
$\beta 1$ (CD29)	+–+* (4/4)	+++ (3/3)	+–+ (3/3)	++– (2/2)

SAM1, 1:50; GOH3, 1:50; K20, 1:50). The sections and cytospin preparations were then incubated with biotinylated anti-mouse immunoglobulin (1:50) and streptavidin-biotinylated peroxidase complex (1:100) for 30 min, respectively. All incubation steps were carried out in a humid chamber at room temperature, followed by rinsing twice the slides in PBS. Using AEC as a chromogen (0.4 mg/ml in 0.1 M acetate buffer, pH 5.0, with 5% DMF and 0.01 hydrogen peroxide for 10 min), the peroxidase reaction caused an intensive red precipitate. The sections and cytospin preparations were rinsed in tapwater, counterstained with Harris' haematoxylin and mounted with glycerol gelatin.

Negative controls were performed without the primary antibody. No staining was observed except for the reaction of granulocytes in tissue sections whose endogenous peroxidase was not destroyed. In tissue sections, strongly stained endothelial cells and/or lymphocytes and/or histiocytes and/or stromal cells, always present in combinations characteristic of the respective antigen under study, served as intrinsic positive controls. The staining of SC in tissue sections and cytospins was evaluated in a semi-quantitative fashion: '+', indicates strong intensity of the detected antigen; '+*', is regarded as weak intensity of antigen expression and '±‡', indicates that strongly positive and weakly positive cells were found in variable amounts. The absence of antigen was symbolized as '–'. Whenever the staining intensity within SC was heterogeneous, a simple semi-quantitative statement was made: '+++', meaning all cells positive; '++', meaning more positive than negative cells; '+', meaning the minority of cells positive; '–/+', meaning sporadic cells positive.

One million SC were used for flow cytometry. SC, cultured in medium with cytokines as described above, were suspended in fluorescence activated cell sorter (FACS)-medium containing RPMI 1640, 10% fetal calf serum, 0.1% sodium azide (Merck, Darmstadt, Germany) and 2% HEPES buffer (Seromed-Biochrom). The expression of $\alpha 1$, $\alpha 3$, $\alpha 5$ chains was assayed by flow cytometry, performed on a FACScan (Becton Dickinson) using the Lysis II Software. W6/32, a monoclonal antibody which reacts with the HLA-A,B,C/β_2m-complex, served as a positive control. Negative controls were performed by omitting the primary mAb. The cells were incubated with the primary mAb TS2/7, P1B5 and SAM1, diluted 1:20, 1:600, 1:10, respectively, for 1 h at 4° C. Subsequently, the SC were extensively washed and incubated with the polyclonal fluorescein isothiocyanate-coupled goat-anti-mouse antibody (Dianova-Immunotech) diluted 1:50 and placed 45 min on ice. After extensive washing cells were resuspended in 300 µl of FACS-medium containing 1 µg/ml propidium iodide (PI, Sigma). Cells that had taken up PI were regarded as damaged or dying and were excluded from further analysis by gating on PI-negative cells.

In tissue sections two major SC types were discriminated: macrophage-like (type A) SC and fibroblast-like (type B) SC [6]. Type A synovial lining cells are phagocytic cells (probably derived from bone marrow stem cells [9]). In mild synovitis type A SC expressed HLA-DR, -DP, -DQ, CD11a, CD11b, CD11c (unpublished data). Type B synovial lining cells secrete collagen, and are derived from mesenchymal cells [9]. In mild synovitis type B SC did not express any of the molecules listed above (unpublished data). On the basis of these negative characteristics they were regarded as fibroblast-like cells although their relationship to other fibroblasts is unclear.

Fibroblast-like type B SC predominated in SC cultures in vitro. However, it is at present unknown whether the fibroblast-like SC that grow out in culture of synovial tissue samples originate from the intima or from the underlying tissue.

Results

The immunohistochemical data on $\beta 1$ integrin expression in lining cells of normal synovial membrane and in SC from synovitis of different aetiology and intensity are summarized in Table 2. As normal counterparts of synovial tissue, we examined synovial membranes that were macroscopically inconspicuous and histologically devoid of inflammatory changes. Using histopathological criteria such as synovial lining hyperplasia, neo-vascularization and infiltration by mononuclear cells, we operationally subclassified the chronic synovitis into a mild and a severe form intentionally disregarding aetiology. The pattern of $\beta 1$ integrin expression in inflamed synovial tissue was compared to that found in the normal synovial membrane.

Serial immunostained sections of normal synovial membrane showed that in 4 out of 4 cases all SC expressed the $\alpha 6$ subunit strongly and the common $\beta 1$ chain weakly, while being devoid of $\alpha 1$, $\alpha 2$, and $\alpha 4$ chains. The $\alpha 3$ and $\alpha 5$ subunits were negative in 3 out of 4 specimens (Fig. 2, c and e).

In contrast to the normal synovial membrane, SC in mild synovitis showed a heterogeneous $\beta 1$ integrin sub-

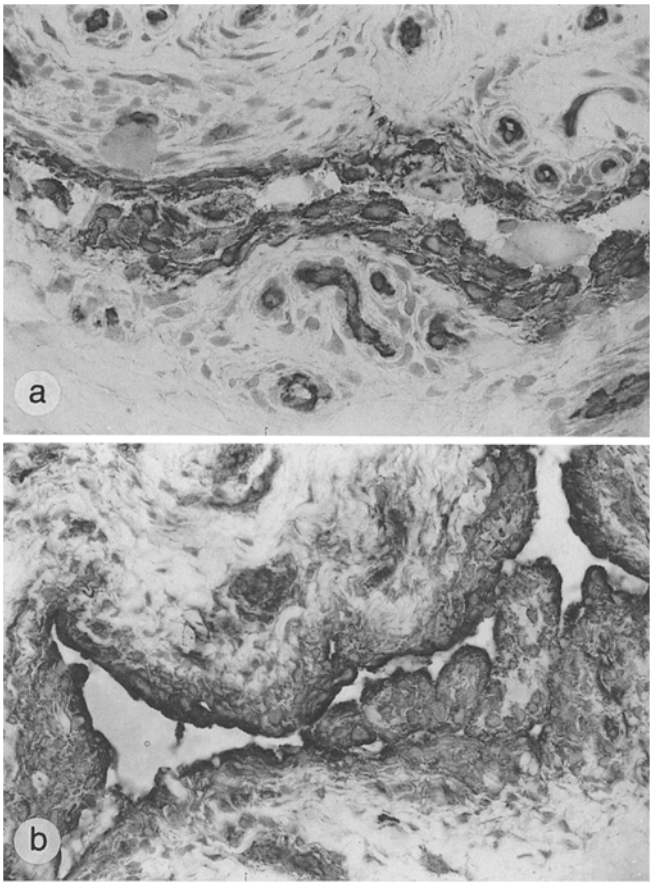

Fig. 1 Expression of $\alpha 6$ (**a**) and $\beta 1$ integrin (**b**) subunits in mild synovitis. All synovial cells (SC) display $\alpha 6$ and $\beta 1$ integrin subunits at high levels; ×340

unit expression. A subset of SC displayed weak expression of the $\alpha 1$ chain in 2 out of 3 cases (Fig. 2b) and strong staining for the $\alpha 5$ chain (Fig. 2f). In order to determine the origin of this subset of SC, additional stainings for HLA-DR, -DP, -DQ, CD11b and CD11c were carried out. Size and distribution of cells expressing these molecules corresponded essentially to those expressing $\alpha 1$ and $\alpha 5$ chains (not shown). As stated in the Methods, this antigenic profile corresponds to that

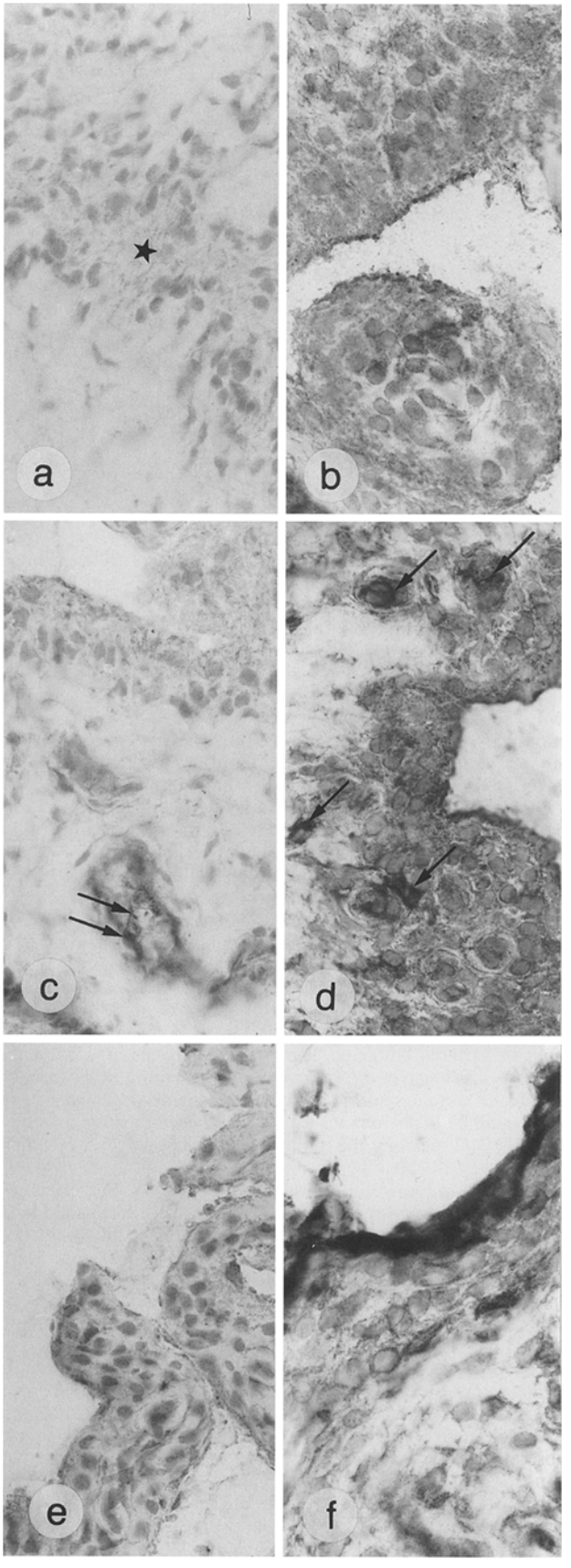

Fig. 2 Expression of $\alpha 1$, $\alpha 3$ and $\alpha 5$ subunits in normal synovial membrane (**a, c** and **e**) and in mild synovitis (**b, d** and **f**). (**a**) Normal, non-inflamed SC are small and lack the $\alpha 1$ chain (joint space marked by *asterisk*); ×340. (**b**) In mild synovitis the SC are clearly larger. A subset of SC, which was defined through additional immunohistological analysis as type A SC (c.f. Methods) faintly express the $\alpha 1$ chain; ×340. (**c**) All SC are small and $\alpha 3$-negative, in contrast to the weakly positive endothelial cells (*arrowheads*); ×540. In (**d**) SC are larger and express the $\alpha 3$ chain at low levels, as compared to the positivity of endothelial cells; ×340. (**e**) SC are small and lack $\alpha 5$ subunits (*arrows*); ×340. (**f**) A subset of SC, which was immunohistochemically characterized as type A SC, strongly express $\alpha 5$ subunits, whereas type B SC are $\alpha 5$-negative; ×340

Table 3 Effect of different cytokines and phorbol ester on the expression of β1 and α1 through α6 integrin subunits in/on synoviocytes 72 hours after stimulation in vitro, as evaluated on cytospins

Antigen	Control	IL-1β	TGF-β	IL-1β +TGF-β	TNF-α	TNF-α +IL-1β	TNF-α +IL-1β +TGF-β	IFN-γ	IFN-γ +TGF-β	bFGF	PMA
α1 (CD49a)	–	+ c/s	–/+ c	++‡ c/s	–/+* c/s	++‡ s	+* s	+* c/s	++* c/s	–	–/+* c/s
α2 (CD49b)	–	–	–	–	–	–	–	–	–	–	–
α3 (CD49c)	+* c	+* c/s	+* c/s	+* c/s	+ c/s	+ s	–/+*	++ s	+ c/s	++ c/s	++ c/s
α4 (CD49d)	–	–	–	–	–	–	–	–	–	–	–
α5 (CD49e)	++‡ c/s	++‡ c/s	++‡ c/s	+++‡ c/s	+++‡ c/s	+++* s	+++* s	+++‡ c/s	+++‡ c/s	+++‡ c/s	+++ c/s
α6 (CD49f)	–	–	–	–	–	–	–	–	–	–	–
β1(CD29)	+++* c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s	+++ c/s

(Scoring of the cell reaction: '*+++*', all cells positive; '*++*', more positive than negative cells; '*+*', the minority of cells positive; '*–/+*', sporadic cells positive; '–', all cells negative; '*' low level of antigen-expression; '‡' weakly and strongly positive cells in various amounts; '*c*', predominantly cytoplasmic staining; '*s*', predominant surface associated staining)

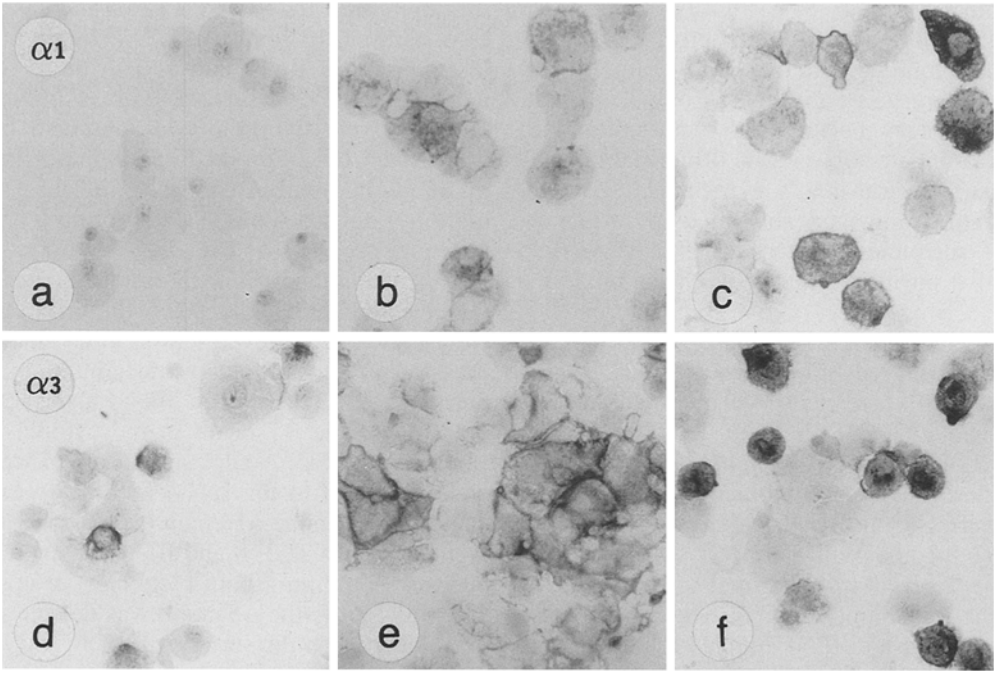

Fig. 3 Expression of the α1 integrin subunit in cytospin preparations of unstimulated SC (**a**); SC treated by co-stimulation through IL-1β together with tumour necrosis factor-α (TNF-α) (**b**) and with transforming growth factor β (TGF-β) (**c**) for 72 h; ×170. (**a**) Unstimulated SC lack the α1 chain. (**b**) Interleukin-1β (IL-1β) and TNF-α induce the expression of the α1 chain, which is predominantly confined to the surface membrane of SC. (**c**) Upon co-stimulation through IL-1β and TGF-β, most SC synthetize (cytoplasmic staining!) and surface express α1 chain in various amounts. Expression of the α3 subunit in cytospin preparations of unstimulated SC (**d**), of SC incubated with interferon-γ (IFN-γ) (**e**) and with pharbol-12-myristate-13-acetate (PMA) (**f**), for 72 h; ×170. (**d**) Few SC are α3-positive, prevalently in the cytoplasm. (**e**) Upon incubation with IFN-γ SC increase in size and show increased α3-positivity which is in parts clearly confined to the surface of most SC. (**f**) PMA induces high levels of both cytoplasmic and surface staining for the α3 chain in the majority of SC

of type A SC. Both type A and B SC showed weak expression of the α3 chain and strong expression of the α6 and the common β1 chains (Fig. 1, a and b; Fig. 2d) while lacking the α2 and α4 chains. In mild synovitis, type B SC were α3-, α6-, and β1-positive. Thus, there was a neo-expression of α1, α3, α5 subunits and an enhancement of the β1 subunit in type A SC. When compared with SC in normal synovial membrane, type B SC in mild synovitis showed a neo-expression of the α3 chain together with an enhanced expression of the β1 chain.

In contrast to the heterogeneous β1 integrin subunit profile in mild inflammation, the distribution of β1 integrins was essentially homogeneous in severe inflammation. Both type A and B SC showed a consistent expres-

Table 4 Effect of cytokines on the expression of $\alpha 1$, $\alpha 3$ and $\alpha 5$ chains on synoviocyte cultures of two individual specimens 72 h after stimulation, as evaluated by flow cytometry (MF, mean fluorescence; % positive, percent of positive cells)

Case No. 1	$\alpha 1$ (CD49a)		$\alpha 3$ (CD49c)		$\alpha 5$ (CD49e)	
	MF	% positive	MF	% positive	MF	% positive
Medium[a]	15	10.9	26	31.2	30	49.7
IL-1β	39	55.3	38	43.7	38	64.3
TGF-β	19	19.2	26	27.7	34	56.6
IL-1β+TGF-β	37	56.3	26	27.6	49	79.7
IL-1β+TNFα	80	82.5	44	42.5	43	77.4
Case No. 2						
Medium[a]	17	17.1	22	42.8	70	99.0
IFN-γ	n.d.	n.d.	47	84.1	93	98.7
TGF-β	21	28.3	17	18.3	75	98.0
IFN-γ+TGF-β	36	65.7	14	16.5	67	97.0

[a] Negative control without cytokine

sion of $\alpha 4$, $\alpha 5$, $\alpha 6$, and $\beta 1$ subunits, while the $\alpha 3$-antigenic density was regularly low. The $\alpha 1$ and $\alpha 2$ subunits were undetectable.

Comparing the $\beta 1$ integrin subunit profile of SC in normal synovial membrane with those of SC in mild and severe synovitis, there was an induction of $\alpha 3$, $\alpha 4$, and $\alpha 5$ chains in parallel to the intensity of inflammation. The $\alpha 1$ chain was undetectable in SC of normal synovial membrane, but was transiently expressed by type A SC in mild inflammation, and abrogated in severe inflammation (the surrounding $\alpha 1$-positive small vessels serving as positive intrinsic controls of the immune reaction).

The data on the expression of $\beta 1$ integrins in untreated and stimulated cultured SC of two different origins are given in Table 3. To investigate whether cytokines influence $\beta 1$ integrin subunit expression, SC were kept in the presence of IL-1β, TGF-β, IFN-γ, bFGF, PMA and in the presence of the following combinations of them: IL-1β and TGF-β; TGF-β and IFN-γ; IL-1β and TNF-α; TNF-α and IL-1β together with TGF-β.

Untreated SC derived from normal synovial membranes lacked the $\alpha 1$ chain (Fig. 3a). The treatment of SC with IL-1β, TNF-α and IFN-γ induced the $\alpha 1$ chain in and on a subset of SC. This effect was increased by combining TGF-β with IL-1β (Fig. 3c): in fact, all SC contained the $\alpha 1$ subunit in the cytoplasm and expressed the $\alpha 1$ chain weakly or strongly on the surface. By combined treatment with TNF-α and IL-1β (Fig. 3b), a large fraction of SC was induced to express the $\alpha 1$ chain weakly on their surface. The addition of TGF-β had no influence on the up-regulating effect of IFN-γ. In contrast, the addition to TGF-β in conjunction with TNF-α decreased the effect of IL-1β on the $\alpha 1$ chain expression. The effect of TGF-β and PMA on the $\alpha 1$ chain expression was marginal. No changes were observed after bFGF treatment. The $\alpha 3$ chain was present at low levels in the cytoplasm of a subset of unstimulated SC (Fig. 3d). In response to PMA and bFGF, the $\alpha 3$ staining was strongly enhanced in the cytoplasm and appeared on the surface of a major fraction of SC (Fig. 3f). This was achieved to a lesser extent by TNF-α. Combining IL-1β and TNF-α increased the $\alpha 3$ surface staining. In contrast, TGF-β inhibited the up-regulating effect of IL-1β and TNF-α on the $\alpha 3$ subunit. A marked increase in surface expression and in the number of $\alpha 3$-positive cells was obtained by stimulation with IFN-γ (Fig. 3e). TGF-β inhibited the IFN-γ effect on SC. A minimal or no effect was observed after stimulation with IL-1β and TGF-β, either alone or in combination.

In unstimulated SC expression of the $\alpha 5$ chain was rather heterogeneous as far as the different experiments were concerned. Nevertheless, bFGF increased the expression of the $\alpha 5$ chain and similar effects were induced by PMA treatment. IFN-γ also increased the expression of the $\alpha 5$ chain in the cytoplasm and on the surface of all SC. A very similar effect was observed by adding TGF-β to the IFN-γ stimulated SC. No significant changes in the $\alpha 5$ chain expression were seen in SC incubated with TGF-β and IL-1β as single agents. However, by combining both cytokines an up-regulating effect was found: the $\alpha 5$ chain was detectable in the cytoplasm and/or on the surface of all SC and was expressed at low or high levels. A similar effect emerged after stimulating SC with TNF-α alone. By adding IL-1β to TNF-α, or combining IL-1β, TGF-β and TNF-α, the $\alpha 5$ subunit was weakly induced and appeared on the surface of all SC.

The $\alpha 2$, $\alpha 4$ and $\alpha 6$ molecules were consistently absent in unstimulated SC and could not be induced by the cytokine stimuli applied. The common $\beta 1$ chain was detectable at high levels in all SC. Upon cytokine treatment no changes in staining intensity or antigen location were observed.

To confirm and to quantify the surface expression of $\beta 1$ integrins, unstimulated and stimulated SC were analysed by flow cytometry. Table 4 gives the values of mean fluorescence and the relative percentage of $\alpha 1$-, $\alpha 3$- and $\alpha 5$-positive SC induced by each cytokine. IL-

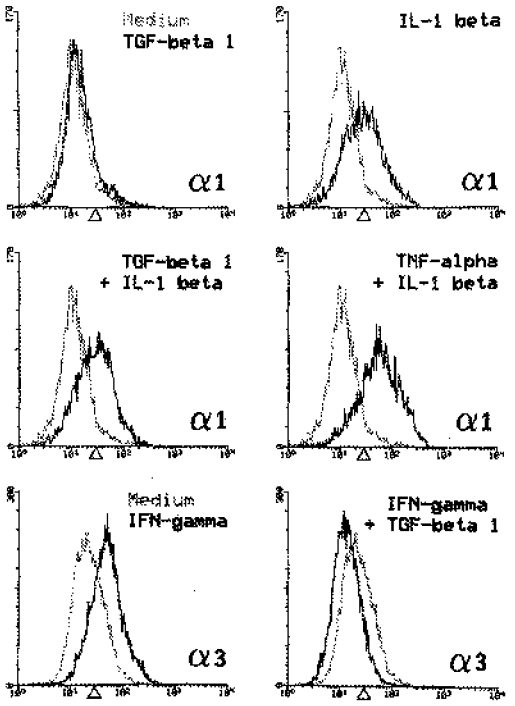

Fig. 4 Histograms of flow cytometric analysis for the $\alpha 1$ and $\alpha 3$ chain expression on untreated (*grey line*) and stimulated SC (*black line*). The threshold of positivity is indicated by a triangle on the abscissa and was determined on the basis of the relative fluorescence intensity of the negative control. Cells of case no. 1 (c.f. Table 4) were stimulated with TGF-β, IL-1β and by combining either TGF-β and IL-1β or IL-1β and TNF-α for 72 h. TGF-β had no effect on the expression of the $\alpha 1$ chain; IL-1β alone or combined with TGF-β induced the expression of the $\alpha 1$ chain. TNF-α enhanced the up-regulating effect of IL-1β. Cells of case no. 2 were treated either with IFN-γ alone or in combination with TGF-β for 72 h. The up-regulating effect of IFN-γ on the expression of the $\alpha 3$ chain was antagonized by TGF-β

1β alone and the addition of either TGF-β or TNF-α to IL-1β induced the expression of the $\alpha 1$ chain which was only marginal in untreated SC (Fig. 4). IL-1β treatment of SC resulted in an increase in the percentage of $\alpha 1$-positive cells as well as in an increase in the amount of the $\alpha 1$ chain per cell (Fig. 4). TGF-β alone and TNF-α alone had no significant influence on the expression of the $\alpha 1$ chain (not shown). The addition of TGF-β to IL-1β did not influence the effect of IL-1β. In contrast, TNF-α clearly enhanced the up-regulating effect of IL-1β on the $\alpha 1$ subunit. Furthermore, SC from case number 2 that were stimulated by combining TGF-β with IFN-γ. TGF-β showed minimal effect on the $\alpha 1$ chain expression when compared with the control, whereas the addition of IFN-γ to TGF-β increased the percentage of $\alpha 1$-positive SC as well the intensity of the $\alpha 1$ staining significantly. The inducing effect of IFN-γ on the $\alpha 1$ subunit as observed immunocytologically was not detected by flow cytometry and, therefore, is likely to consist of intracytoplasmic $\alpha 1$-synthesis without surface expression. Concerning the $\alpha 3$ chain, a minor fraction of unstimulated SC (31.2%) was positive at low levels. IL-1β resulted in a small increase in $\alpha 3$ chain expression. No significant difference in $\alpha 3$ chain staining was observed by the addition of TNF-α to IL-1β stimulated SC. The addition of TGF-β inhibited the IL-1β up-regulating effect on the $\alpha 3$ chain expression, whereas TGF-β alone was ineffective. The expression of the $\alpha 3$ chain was more clearly increased by IFN-γ (84.1% positive SC) (Fig. 4). Nevertheless, the addition of TGF-β to the IFN-γ-stimulated SC led to a strong decrease of $\alpha 3$ expression, the level of which was even lower than that of the untreated SC (Fig. 4).

Immunocytologically, untreated SC showed heterogeneity in $\alpha 5$ expression. TGF-β or IL-1β treatment did not induce any significant changes in the expression of the $\alpha 5$ chain. However, the addition of either TGF-β or TNF-α increased the minor inductive effect of IL-1β. SC of case nomber 2, which were spontaneously $\alpha 5$-positive, showed no increase in $\alpha 5$ expression upon cytokine treatment.

Discussion

In this study we have shown that synovial lining cells in normal, non-inflamed synovial membranes express the $\alpha 6$ integrin subunit together with the $\beta 1$ chain and thus most probably carry functional laminin-receptors [39]. These findings essentially support in situ data of Johnson et al. [30] and Demaziere and Athanasou [13]. However, Demazier's and Athanasou's observation [13] that SC of normal synovial membranes are also $\alpha 5$-positive is at variance with our data. One explanation would be that the specimens these authors used as 'normal' showed inflammatory activation of SC despite the reported lack of significant mononuclear infiltration. In mild synovitis type A SC neoexpressed $\alpha 1$, $\alpha 3$ and $\alpha 5$ chains, while lacking $\alpha 2$ and $\alpha 4$ chains. In this condition type B SC expressed $\alpha 3$ and $\alpha 6$ and $\beta 1$ subunits, but were devoid of $\alpha 1$, $\alpha 4$ and $\alpha 5$ chains. In severe synovitis type A and B SC expressed $\alpha 3$, $\alpha 5$, $\alpha 6$ and $\beta 1$ chains, but still were devoid of the $\alpha 2$ chain. In severe synovitis type A and B SC neo-expressed the $\alpha 4$ chain as compared to SC from mild synovitis, but seem to have abrogated the transiently expressed $\alpha 1$ chain. In this condition type B SC additionally neo-expressed the $\alpha 5$ chain. Our findings suggest that the expression of $\alpha 1$, $\alpha 3$, $\alpha 4$ and $\alpha 5$ chains and the increase of the $\beta 1$ chain expression in SC are changes associated with the intensity of inflammation. Since we intentionally chose a very simple scoring system neglecting histopathological criteria associated with different forms of synovitis, it is still open whether there are changes in the integrin profile which might be typical for a certain disease. SC in rheumatoid arthritis have been reported to display $\alpha 6$ and $\beta 1$ subunits [15, 33, 39] which is in agreement with our results. The available data concerning the presence of $\alpha 1$, $\alpha 3$ and $\alpha 5$ subunits in SC in rheumatoid arthritis are still somewhat conflicting: El-Gabalawy and Wilkins [15] found SC inconsistently expressed $\alpha 1$, $\alpha 3$ and

$\alpha 5$ chains. In another study such effects were not found [33].

Nevertheless, the very few published observations on $\beta 1$ integrin expression in SC in inflamed synovial tissues are essentially in agreement with our data. To further substantiate our in situ findings, in vitro studies on SC cultures were carried out. In particular, we studied the effect of cytokines applied as single agents or in various combinations, on the $\beta 1$-integrin subunit expression. The data is given in Table 4. The cytokines which we chose, i.e., TNF-α, IL-1β, TGF-β, bFGF and IFN-γ, have largely been detected either in situ or in synovial fluid of inflamed joints [8, 19, 22, 28]. The dosages used are standard for in vitro studies. These cytokines are known to be involved in inflammation-induced tissue damage and joint destruction [31]. We show here that unstimulated SC in vitro express the $\beta 1$ chain at high levels, the $\alpha 3$ chain at low levels and the $\alpha 5$ chain at variable levels, while being devoid of $\alpha 1$, $\alpha 2$, $\alpha 4$ and $\alpha 6$ subunits.

The absence of the $\alpha 1$ chain proved not to be a stable feature of cultured SC, since TNF-α, IL-1β and IFN-γ induced the $\alpha 1$ chain expression. These findings parallel reports on $\alpha 1$ induction by TNF-α and IL-1β in MG-63 human osteosarcoma cells [36] and $\alpha 1$ induction by TNF-α in endothelial cells [12]. Santala and Heino [36] reported that IL-1β potentiates the effect of TGF-β on the $\alpha 1$ chain expression in MG-63 osteosarcoma cells. However, in SC we could not find such an additive or synergistic effect by combining these two cytokines. Instead, we found an additive effect on the $\alpha 1$ chain expression when TNF-α was combined with IL-1β while TGF-β alone had but a minimal influence on $\alpha 1$ chain expression. TGF-β was reported to increase the expression of the $\alpha 1$ chain on human skin fibroblasts and on hepatoma cells [23, 24], but not in MG-63 human osteosarcoma cells [36] indicating cell type-related effects of this cytokine. We have shown that only a subset of unstimulated SC weakly express the $\alpha 3$ chain in situ. In vitro, IFN-γ, TNF-α and IL-1β, alone or in combination, bFGF, and PMA lead to some increase of $\alpha 3$ expression. IFN-γ showed the most prominent effect. The effect of TGF-β alone on the expression of the $\alpha 3$ chain was marginal. The published data on $\alpha 3$ chain regulation by TGF-β do not yet yield consistency: TGF-β was shown to decrease $\alpha 3$ chain expression on MG-63 osteosarcoma cells [23], but to enhance the $\alpha 3$ chain expression on WI-38 human lung fibroblasts [24]. In our hands, TGF-β inhibits the up-regulating effect of IL-1β and antagonizes the effect of IFN-γ on $\alpha 3$ chain expression. Whether TGF-β has different effects on different cell types on the expression of $\alpha 1$ and $\alpha 3$ integrin subunits will have to be investigated in a comparative study.

In situ, SC of non-inflamed synovial membranes were devoid of the $\alpha 5$ integrin subunit. In vitro, however, stimulated SC showed a rather heterogeneous pattern of $\alpha 5$ chain expression. A similar "spontaneous" in vitro $\alpha 5$ induction was reported for endothelial cells and melanocytes [2] and might be due to undefined serum factors in the culture medium. There is evidence that the $\alpha 5$ subunit may be induced in resting peritoneal macrophages in vitro by a possible autoregulation pathway related to the secretion of inflammatory mediators [27]. We observed a marginal increase of $\alpha 5$ expression in SC in response to IL-1β and TNF-α. We further found increases in expression of the $\alpha 5$ chain by IFN-γ, alone or in combination with TGF-β, and by bFGF. Accordingly, TGF-β and bFGF have been reported to increase the level of $\alpha 5$ expression in endothelial cells significantly [17], and, as far as TGF-β is concerned, in human lung fibroblasts [24]. When TGF-β or TNF-α were added simultaneously to IL-1β pre-stimulated SC we measured an additive effect on the increased $\alpha 5$ expression. This effect was not found by simultaneous triggering through TNF-α, IL-1β and TGF-β. However, PMA treatment led to a considerable increase in the level of $\alpha 5$ expression. Unstimulated SC lacked $\alpha 2$, $\alpha 4$, $\alpha 6$ chains in vitro and in this state were refractory to the cytokine-stimulations applied. However, TGF-β was shown to increase the expression of the $\alpha 2$ chain in human lung fibroblasts and that of $\alpha 2$, $\alpha 4$, $\alpha 6$ chains in other cell types [24]. Since $\alpha 2\beta 1$ was found to be functionally active on fibroblasts [41] the consistent absence of this molecule in cultured SC might be a cell type specific marker of SC, allowing their discrimination from typical fibroblasts of other tissues.

Our results support the view of an interactive role for cytokines in the regulation of integrin expression. IL-1β and TNF-α exerted an additive effect on the expression of $\alpha 1$, $\alpha 3$, and $\alpha 5$ chains. TGF-β together with both IL-1β and IFN-γ positively influenced the $\alpha 1$ and $\alpha 5$ chain expression but decreased the expression of the $\alpha 3$ chain. Furthermore, we have shown that cytokines may have different effects on the expression of various integrin subunits. In particular, TGF-β enhanced the effect of IFN-γ and IL-1β on the $\alpha 1$ and $\alpha 5$ chains expression while inhibiting the up-regulating effect of IL-1β and antagonizing the effect of IFN-γ on the $\alpha 3$ expression.

In conclusion, we have shown that cytokines lead to changes in the expression of various $\beta 1$ integrin subunits in SC in vitro. In particular, the $\beta 1$ integrin subunit profile of cytokine activated SC in vitro resembles that of type A SC in mild inflammation in situ. This implies that the neo-expression of $\alpha 1$, $\alpha 3$ and $\alpha 5$ chains in SC represents an inflammation-associated change. Therefore, the correspondence between the in situ situation and the in vitro model of inflammation suggests that IL-1β, TGF-β, TNF-α and IFN-γ play an important role in effecting the regulation of $\beta 1$ integrin expression in vivo.

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