

VLA family in rheumatoid arthritis: evidence for *in vivo* regulated adhesion of synovial fluid T cells to fibronectin through VLA-5 integrin

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SUMMARY

Adhesion of T cells to extracellular matrix (ECM) proteins through VLA integrin receptors is crucial for lymphocyte trafficking, tissue localization and inflammatory function. We have investigated the expression of different VLA integrins (VLA-1–5) on peripheral blood (PB) and synovial fluid (SF) T lymphocytes from patients with rheumatoid arthritis (RA). Their expression on different cell types from synovial membrane (SM) is also reported. The role of VLA-4 fibronectin (FN) receptors in the interaction of activated SF T cells from RA patients with a 38-kD fragment of FN has been previously demonstrated. Here we have focused functional studies on VLA-5 as an alternative FN receptor for RA T cells. A significant higher proportion of SF T cells were able to bind to an 80-kD fragment of FN, containing the Arg–Gly–Asp (RGD) cell binding site, compared with PB T cells. This attachment was almost completely inhibited by anti-VLA-5 MoAbs as well as by RGD peptides. This enhanced capability by SF T cells appears to be independent of the level of the surface expression of the receptor and correlates better with their activation state as determined by the expression of the activation molecule AIM (CD69). The evidence for the expression of VLA heterodimers on both SF and SM cells from RA patients suggests the possible implication of ECM proteins in mediating and perpetuating inflammation *in vivo*.

Keywords rheumatoid arthritis VLA T lymphocytes fibronectin receptors

INTRODUCTION

The integrin family contains numerous cell surface receptors that have been shown to mediate cell–cell interactions as well as binding of cells to extracellular matrix (ECM) proteins [1–3]. A considerable proportion of research has been focused over the past few years on the $\beta 1$ integrin subfamily, also termed VLA (very late activation antigen) family, derived from the diverse capability of its members to function as receptors for ECM proteins [4]. These cell–ECM interactions can influence the trafficking and probably the anchoring of leucocytes into specific tissues or target organs. Several $\beta 1$ integrins recognize fibronectin (FN). VLA-5 is well recognized as the prototype FN receptor [5]. The VLA-4 integrin has been also described as a receptor for FN [6,7], beside its involvement in different cell–cell adhesion functions [8–12]. The binding of VLA-3 to FN is under controversy [13,14]. Recently, integrins $\alpha V\beta 1$ on neuroblastoma cells and $\alpha V\beta 3$ on melanoma cells have also been described to bind FN [15–17]. The interaction of these molecules with FN is

displayed through different domains of this ECM protein. Thus, VLA-3, VLA-5, and $\alpha V\beta 3$ bind to a segment containing Arg–Gly–Asp (RGD), called central cell attachment domain, which is contained in an 80-kD tryptic fragment of plasma FN, whereas VLA-4 interacts with the connecting segment 1 (CS-1) included in a 38-kD proteolytic fragment [6].

Among hematopoietic cells, VLA-5 is constitutively expressed by a subset of T lymphocytes, platelets, monocytes, and different B and T cell lines [4,6]. Adhesion of resting human T cells to intact FN is poor unless they are activated [18]. In this sense, the increase in VLA-5-mediated T cell binding to FN, without any associated change in VLA-5 expression, is observed after activation of CD4⁺ T cells [18].

Blocking experiments using MoAbs suggest that the interaction of T cells with FN through VLA-4 and VLA-5, provides a costimulatory signal in CD3-mediated T cell activation [19–21], and thus could implicate the ECM protein FN in the regulation of T cell responses.

So far, few studies of human pathology evaluating the expression of VLA proteins at inflammatory sites have been reported. Previously, in accordance with data from other

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investigators, we described the presence of VLA-1 on activated T cells from rheumatoid synovial fluid (SF) [22–24]. Furthermore, we have previously demonstrated the VLA-4-mediated interaction of activated SF T cells from patients with rheumatoid arthritis (RA) with a 38-kD fragment of FN [25]. Here we have analysed, as a first step, the expression of different VLA members (VLA-1–5) on peripheral blood (PB) and SF T cells from RA patients, as well as their distribution into inflamed synovial membrane (SM). In addition, we have investigated in detail the functional capability of VLA-5 as a FN receptor for T cells *in vivo*. We have demonstrated an augmented VLA-5-mediated adhesiveness to FN by SF T cells, suggesting the existence of activation-mediated regulatory mechanisms of the VLA-5–FN interactions at inflammatory sites in this disease.

PATIENTS AND METHODS

Patients

Three men and 10 women with RA (median age 48.5 years, range 21–76) were investigated. All of them fulfilled criteria for the diagnosis of RA according to the American College of Rheumatology [26]. The median duration of disease was 7.3 years (range 8 months to 14 years). All patients were taking non-steroidal anti-inflammatory drugs. In addition, four of them were taking gold derivatives, one anti-malarial drugs, one D-penicillamine, one oral methotrexate and only one patient was under low doses of corticosteroid therapy.

Preparation of purified T lymphocytes

PB and SF were collected at the same time into heparinized tubes, and mononuclear cells (MNC) were isolated by Ficoll-Hypaque gradient centrifugation (Pharmacia Fine Chemicals, Uppsala, Sweden). For purification of T cells, adherent cells were removed from MNC by culturing on plastic Petri dishes for 45 min at 37°C. Briefly, non-adherent cells were placed on a 600 mg nylon-wool column pre-incubated for 30 min with RPMI 1640 (Flow, Irvine, UK) supplemented with 10% FCS (Gibco, Grand Island, NY), 2 mM glutamine and 50 µg/ml penicillin/streptomycin. MNC were then incubated in the column for 45 min at 37°C. Cells were eluted with 20 ml of RPMI 1640. The purified T lymphocyte fractions contained ≥90% of CD3, ≤6% monocytes and ≤1% B cells, as determined by expression of the CD3, Mo-2 (CD14) and B1 (CD20) antigens, respectively.

Monoclonal antibodies

The activation marker AIM/CD69 (activation inducer molecule) was studied with the TP1/55 MoAb [27]. MoAbs used to study VLA heterodimers were directed against the β1 common chain (TS2/16 and Lia1/2) [28], and towards the different α subunits: TS2/7 (anti-α1) [28], P1E6 (anti-α2) [29], P1B5 (anti-α3) [29], HP2/1 (anti-α4) [30] and P1D6 (anti-α5) [29].

In order to assess the purity of T cell preparations, cells were stained with SPV-T3b (anti-CD3) [31], Bear-1 (anti-CD11b) [32], and BC-1 (anti-CD20) [33]. Anti Mo-2 (CD14) was kindly provided by J. E. de Vries (Unycet Lab, Dardilly, France). The MoAb D3/9 specific for the leucocyte common antigen (T200/CD45) [34], was used as control. X63 MoAb (IgG1), used as

negative control, is the immunoglobulin secreted by the mouse myeloma cell line P3–X63.

Flow cytometry analysis of T cell surface antigens

Viable cells ($1-5 \times 10^5$) were suspended in 50 µl aliquots of PBS, pH 7.4. Specific mouse MoAb (1 µg in 50 µl) was added and cells were incubated for 30 min at 4°C, then washed twice and incubated with saturating amounts of fluorescein-conjugated F(ab')₂ goat anti-mouse immunoglobulin (Dakopatts, Copenhagen, Denmark). After three washes, fluorescence was measured using an EPICS C flow Cytometer (Coulter, Hialeah, FL). Fluorescence intensity for different MoAbs was determined on a logarithmic scale, and data for mean fluorescence intensity (MFI) were converted to arbitrary linear units. Background fluorescence for irrelevant MoAb P3X63 was determined for each cell population and subtracted. The specific percentages of positive cells for different MoAbs were obtained by subtracting the number of background cells that were non-specifically stained with the MoAb P3X63.

Immunoperoxidase staining of tissue sections

Synovial samples were obtained by surgical synovectomy from three RA patients, frozen in OCT (Ames Miles, Elkhart, IN) and stored at –80°C. Tissue sections were stained by an indirect immunoperoxidase method as described [35]. Briefly, 4 µm acetone-fixed sections were sequentially incubated with MoAb culture supernatants and peroxidase-conjugated rabbit anti-mouse immunoglobulin (Dakopatts); each incubation was followed by three washes. The reaction was developed with Graham–Karnovsky medium containing 0.5 mg/ml of DAB and hydrogen peroxide. Sections were counterstained with Carazzi's haematoxylin followed by dehydration and mounting by routine methods.

Cell attachment analysis

Human FN 80-kD proteolytic fragment (from Dr A. García-Pardo, CIB, CSIC, Madrid) and type I COL (Sigma, St Louis, MO) were applied to 96-well flat-bottomed microtitre plates (Linbro, Flow) (40 µg/ml, 0.1 ml/well) in CO₂/HNa 0.1 M at 4°C overnight. Non-specific binding sites were saturated with RPMI 1640/1% human seroalbumin for 2 h at 37°C. Purified T cells isolated from PB and SF were added (125 000 cells/well) in 0.1 ml of RPMI 1640 and incubated at 37°C and 5% CO₂. After 30 min, plates were washed with RPMI 1640 several times and examined in an inverted microscope by at least two different observers. Each condition was performed in duplicate. In inhibition conditions, cells were incubated for 30 min at 4°C with: (i) 25% final volume of anti-α5 P1D6, anti-α4 HP2/1 and anti-β1 Lia1/2 hybridoma culture supernatants; (ii) RGDS synthetic peptides (Sigma) (500 µg/ml) and added to the wells. As control incubations, cells were incubated with RPMI 1640 1% human seroalbumin, D3/9 (anti-CD45) hybridoma culture supernatant and RGES synthetic peptides (Sigma) (500 µg/ml). Within each well cells from at least three different fields were counted and referred to a non-washed well (100% or maximum binding).

Statistical analysis

Values of PB and SF T cell samples from RA patients were analysed using Student's *t*-test for paired samples.

Table 1. Expression of adhesion and activation antigens on purified T cells from peripheral blood (PB) and synovial fluid (SF) of rheumatoid arthritis (RA) patients

MoAb	Healthy donors (n=6)		RA patients (n=12)					
	PB		%			MFI		
	%	MFI	PB	SF	P	PB	SF	P
Anti-VLA1 α (TS2/17)	4 \pm 1	1 \pm 0.5	8 \pm 3	18 \pm 4	*	1 \pm 1	3 \pm 1	†
Anti-VLA2 α (P1E6)	4 \pm 1	1 \pm 0	6 \pm 1	9 \pm 3	NS	2 \pm 1	3 \pm 1	NS
Anti-VLA3 α (P1B5)	1 \pm 0	0	5 \pm 2	11 \pm 4	NS	2 \pm 1	5 \pm 2	NS
Anti-VLA4 α (HP2/1)	43 \pm 3	14 \pm 3	48 \pm 6	73 \pm 3	‡	15 \pm 3	23 \pm 3	*
Anti-VLA5 α (P1D6)	30 \pm 3	4 \pm 1	38 \pm 9	39 \pm 7	NS	7 \pm 2	7 \pm 1	NS
Anti-VLA β 1 (TS2/16)	60 \pm 9	23 \pm 4	65 \pm 5	88 \pm 2	‡	29 \pm 7	46 \pm 7	‡
AIM (TP1/55)	3 \pm 2	1 \pm 0	6 \pm 2	48 \pm 6	‡	2 \pm 1	14 \pm 2	‡

Values are the percentages of T cells positive for each antigen (mean \pm s.e.m.). Data were obtained by subtracting the number of background cells that were nonspecifically stained with the control MoAb X63. Data for mean fluorescence intensity (MFI) are expressed in arbitrary linear units (mean \pm s.e.m.). Data shown are after subtraction of background fluorescence of cells in the presence of irrelevant MoAb X63. Statistical significance: * $P < 0.01$; † $P < 0.05$; ‡ $P < 0.001$; NS, not significant.

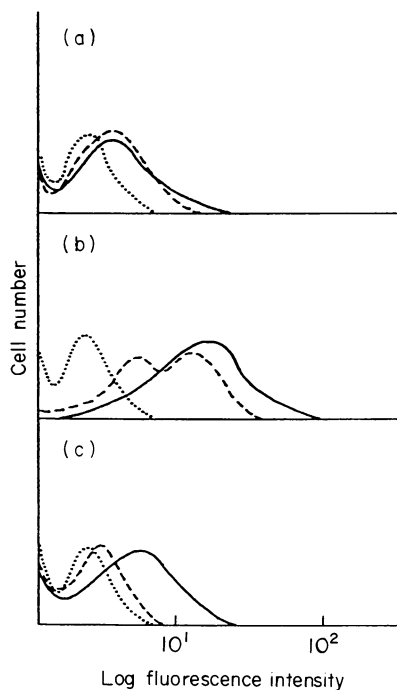


Fig. 1. Immunofluorescence flow cytometry analysis of adhesion (α 5 and β 1) and activation (AIM/CD69) antigens on synovial fluid (solid line), and peripheral blood (dashed line) purified T lymphocytes from a representative patient with rheumatoid arthritis. Cells were labelled with (a) P1D6 MoAb (anti-VLA α 5); (b) TS2/16 MoAb (anti-VLA β 1); (c) TP1/55 MoAb (anti-AIM), and the negative control X63 MoAb (dotted line).

RESULTS

Expression of VLA proteins on PB and SF T cells from RA patients

The distribution of distinct α subunits (VLA-1-5) and β 1 chain was analysed on T cells from PB and SF compartments by flow

cytometry. Results obtained from 12 RA patients are summarized in Table 1.

In agreement with previous reports [22-24], SF T cells expressed low to moderate amounts of α 1 subunit, but in a significant higher level than PB T cells, where there is slight or no detectable expression (Table 1). The expression of α 2 and α 3 on resting PB T cells was actually negligible, and only a slight increase, without significant differences, was found in SF. It is apparent from data in Table 1 that there is a higher expression of α 4 subunit on SF T cells compared with PB compartment from the same patients. By contrast, we have not found significant differences in the expression of α 5 molecules between PB and SF T lymphocytes, in terms of percentage of positive cells and MFI (see profiles of flow cytometry from a representative patient in Fig. 1).

Common β 1 subunit is clearly represented on T cells from PB with a significant increment in its expression on SF T cells (Table 1, Fig. 1).

The levels of surface expression and proportion of positive cells for different VLA-1-5 α and β 1 subunits on PB T cells from both RA patients and healthy donors were generally in the same range (Table 1). Expression levels of α 2 and α 3 subunits were slightly higher on PB T cells from patients but were in all cases very low or undetectable. However, although a subpopulation of PB T lymphocytes obtained from healthy individuals expressed α 5, a higher expression of this antigen was observed in RA patients.

Distribution of VLA proteins in synovial membrane from RA patients

Immunoperoxidase staining of frozen tissue sections from SM of three RA patients was performed for the different α (1-5) and β 1 reactivities (Fig. 2). All synovial biopsy samples contained several lymphocytic aggregates in the subsynovia, usually surrounding blood vessels. Most of the cells in these aggregates exhibited T phenotype, as determined by CD3 MoAb staining (data not shown).

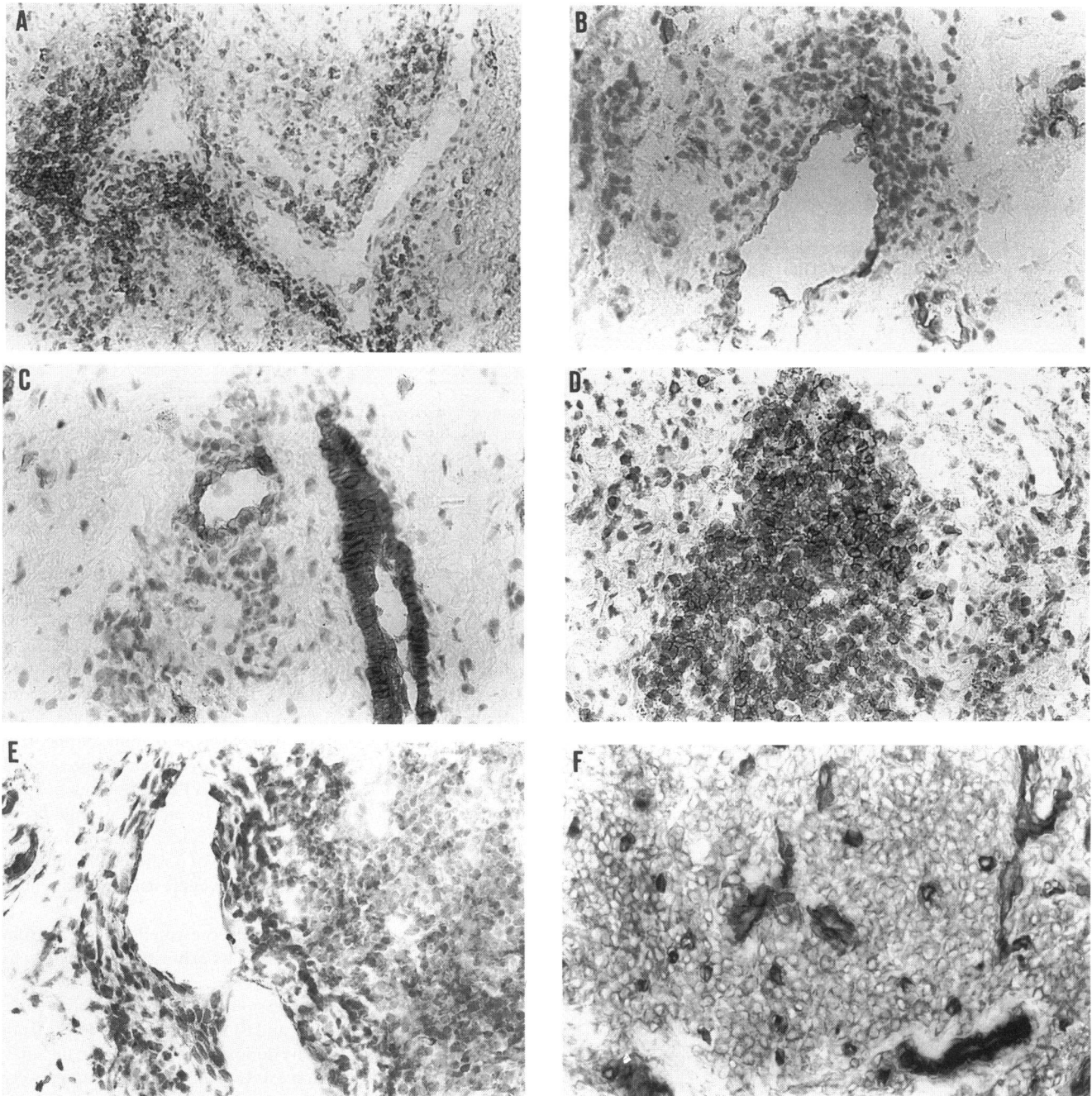


Fig. 2. Immunohistologic identification of the reactivity of MoAbs against VLA α and β molecules within inflamed synovial membrane. Frozen sections of synovial tissue obtained from patients with rheumatoid arthritis were stained with the indicated MoAb by immunoperoxidase technique: (A) TS2/7 (anti- α 1); (B) P1E6 (anti- α 2); (C) P1B5 (anti- α 3); (D) HP2/1 (anti- α 4); (E) P1D6 (anti- α 5); and (F) TS2/16 (anti- β 1). Magnification: (A), $\times 200$; B-F, $\times 400$.

One of the most striking features on tissue staining was the widespread distribution of different VLA α and β chains on synoviocytes within hyperplastic synovial lining, with the exception of α 4. Opposed to this lack of reactivity on synovial cells, anti- α 4 MoAb stained the majority of T lymphocytes in inflammatory infiltrates from the subsynovia (Fig. 2D). In addition, more reduced numbers of T cells stained with anti- α 1 (Fig. 2A) and anti- α 5 MoAb (Fig. 2E). Both anti- α 1, α 2, α 3 and α 5 MoAbs showed positive reaction on endothelial cells from

blood vessels (Fig. 2A, B, C, E). The anti- β 1 MoAb TS2/16 stained strongly synoviocytes and blood vessels, showing a weak diffuse positive reaction on lymphocytes (Fig. 2F).

Expression and function of VLA-5 FN receptor on SF and PB T cells from RA patients

To investigate whether VLA-5 integrin could play a significant role in RA we studied its expression and function on T lymphocytes from PB and SF of RA patients.

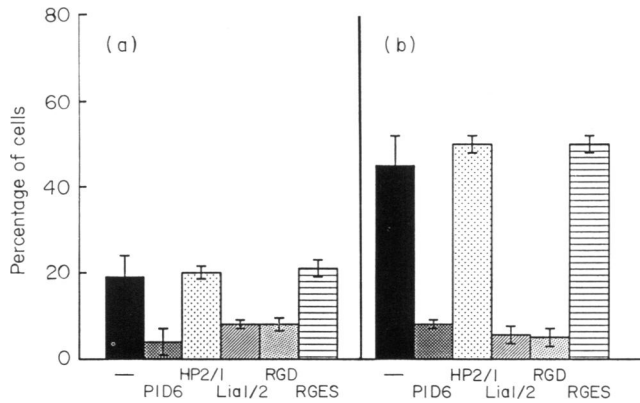


Fig. 3. Comparison of the functional capacity of purified T cells from peripheral blood (a) and synovial fluid (b) compartments, for binding to the 80-kD fibronectin fragment. Adhesion to this FN fragment in the presence of anti- $\alpha 5$ PID6, anti- $\alpha 4$ HP2/1, anti- $\beta 1$ Lia1/2 MoAbs, RGDs and RGES peptides is also represented. Values are mean \pm s.e.m. of data obtained from at least three experiments.

As shown in Table 1, T cell expression levels of $\alpha 5$ subunit in both compartments were quite similar. It is worth emphasizing that weak or no increments in the percentage of positive cells were detected in the SF compartment and no significant differences were observed when paired samples from PB and SF were compared. Those results were in agreement with data obtained from PB and SF T cells of four patients with Reiter's syndrome, psoriatic arthritis, HLA-B27-related oligoarthritis and HLA-B27 reactive arthritis. Little increments in the proportion of $\alpha 5$ -positive cells were observed on SF T cells with minimal changes in fluorescence intensity (data not shown).

We also studied the activation state of T cells from both compartments by measuring the expression of the activation antigen AIM (CD69). Our results showed the presence of elevated amounts of AIM on SF T cells whereas low or undetectable expression of this molecule was observed on PB T cells (Table 1 and Fig. 1). Similarly, a significant proportion of the CD3⁺ lymphocytes infiltrating the SM stained strongly with the anti-AIM MoAb [25].

Next, we examined the adhesive capacity of PB and SF T cells to FN through VLA-5 FN receptors. Adhesion assays to an 80-kD proteolytic fragment of plasma FN containing the RGD sequence were performed with PB and SF T cells from seven patients. We observed that SF T cells displayed a significantly higher capacity to bind to the 80-kD FN fragment than did paired PB T cells from the same patients. Binding of T cells to FN was almost completely inhibited by anti- $\alpha 5$ MoAb in all cases, in PB and SF compartments (Fig. 3). Adhesion was also significantly abrogated when T cells were pre-incubated with either RGDs peptides or the blocking anti- $\beta 1$ Lia.1.2 MoAb (Fig. 3) but it remained unaffected in the presence of RGES peptides (Fig. 3) or anti-CD45 MoAb (data not shown). As expected, pretreatment of T cells with anti- $\alpha 4$ HP2/1 MoAb did not affect their subsequent attachment to the 80-kD FN fragment, lacking the VLA-4 binding site (Fig. 3). Finally, in substrate control conditions, binding of T cells to COL I or serum seroalbumin ranged in all cases between 0 and 10%.

DISCUSSION

Synovial micro-environment can be considered as an intricate scenario where multiple cell-cell and cell-ECM interactions develop influenced by local release of soluble mediators. In the present study, we have shown that VLA heterodimers ($\alpha 1-5$, $\beta 1$) are widely distributed in the SM of RA patients, emerging thus as cell surface structures potentially relevant in the interactions of immune cells with ECM proteins. VLA $\alpha 1$, 2, 3 and 5 subunits are expressed on synoviocytes as well as on endothelial cells, whereas the great majority of infiltrating T cells are $\alpha 4^+$ and only a small percentage stains with anti- $\alpha 1$ or $\alpha 5$ MoAb. T cells in the SF compartment display a very similar pattern of VLA expression. Thus, SF T cells bear moderate expression levels of $\alpha 1$ and $\alpha 3$, raised levels of $\alpha 5$, and $\alpha 4$ was clearly over-represented. A large subset of these T cells both in SF and SM appears to be in an activation state, as shown by the expression of the activation antigen AIM (CD69) [25; this study]. This correlates with the suggested activated phenotype of SF T cells using HLA-DR and other activation markers [22-24]. An important consideration is that PB T lymphocytes, which virtually lack expression of VLA-1, 2 and 3 molecules, clearly display VLA-4 expression, although to a significant lesser extent than SF T cells. By contrast, similar expression values were detected for VLA-5 heterodimers within PB and SF from our patients. These results were consistent with those obtained in patients with other chronic rheumatic diseases.

It is well known that T lymphocytes use $\alpha 5\beta 1$ (VLA-5) as a receptor for FN, through the central cell attachment domain which is contained in the 80-kD FN cell binding fragment [6]. In addition, T cells also use $\alpha 4\beta 1$ (VLA-4) to bind to a totally distinct site in an RGD-independent manner [6,7]. We found that a significant proportion of SF T cells is capable of binding to the 80-kD proteolytic fragment FN, whereas only a small fraction of resting PB T cells displays this capacity. T cell binding to this fragment is specifically mediated by VLA-5 since anti- $\alpha 5$ MoAb and RGDs peptides abrogate T cell attachment in both compartments significantly. Otherwise, the ligand for VLA-4 is not contained in the 80-kD FN fragment and unaltered binding in the presence of anti- $\alpha 4$ MoAb rules out this adhesion pathway. However, the enhanced binding activity in SF (mean 45%) with respect to PB (mean 19%) in RA patients cannot be completely explained by increments in the SF VLA-5 population or by minimal changes in the surface expression of the receptors, indicating that additional mechanisms must be involved in these 80-kD FN-T cell interactions. Function of VLA-5 integrin might be modulated independently of the level of the receptor expression on the cell surface. T lymphocytes in SF and those infiltrating SM constitute an activated cell population as determined by their high expression of the activation antigen AIM. These findings suggest a possible *in vivo* upregulation of the function of VLA-5 FN receptors mediated by activation of T cells. In this context, conformational changes occurring during *in vitro* cell activation have been detected in other integrins such as LFA-1, resulting in active forms that enable receptor-ligand interactions [36]. Recently, the enhanced binding activity of three VLA members to FN (VLA-4 and VLA-5) and LN (VLA-6) has been described on PB T cells upon *in vitro* activation with phorbol esters or anti-CD3 antibodies, without detectable changes in expression [18]. Interestingly, the existence of an additional level of regulation of VLA binding

to ECM components as a result of T cell differentiation from 'naive' (CD45RA⁺RO⁻) to 'memory' T cells (CD45RA⁻RO⁺) has also been reported [18]. The latter subset exhibits a more efficient attachment to its ligand. It seems that these mechanisms act by overlapping in T cell functional behaviour, since *in vitro* studies have shown the acquisition of T cell memory marker CD45RO upon T cell activation [37]. Our phenotypic studies of T cells from different compartments show that 70–80% of SF T cells express the T cell 'memory' marker CD45RO⁺ whereas only 30–35% in PB exhibit this phenotype [25]. Such findings correlate with results from other investigators, obtained with T cells from either patients with RA and other chronic rheumatic diseases [38–40]. Further evidence for modulation of VLA-5 function has been obtained from studies in human epidermal keratinocytes [41]. In this study, a decrease in adhesion of keratinocytes to FN was correlated with a decrease in the ability of the $\alpha 5\beta 1$ receptor to bind to their ligand, preceding by several hours the loss of the integrin from the cell surface [41]. It is conceivable that cells which are capable of adhering better to ECM proteins would tend to be retained in the tissue. We propose that activated SF T cells acquire functional active VLA-5 receptors upon T cell activation, which enable interaction with their ligand FN. This may represent a further mechanism of persistence at inflammatory foci of cells supposed to be previously exposed to antigen.

However, increased levels of FN have been detected in SF from RA patients compared with plasma [42,43]. This may be the consequence of a restricted and localized increase in the production of this protein by stimulated synovial cells [44–45]. The relative contribution of $\alpha 5\beta 1$ to anchoring T cells into synovial tissue remains unclear. The local generation and exposure of different binding sites of FN may influence the preferential migration of cells with specific functional active receptors toward gradients of higher FN fragment concentration.

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REFERENCES

- Hynes RO. Integrins: a family of cell surface receptors. *Cell* 1987; **48**:549–54.
- Springer TA. Adhesion receptors of the immune system. *Nature* 1990; **346**:425–34.
- Ruoslahti E. Integrins. *J Clin Invest* 1991; **87**:1–5.
- Hemler ME. VLA proteins in the integrin family: structures, functions and their role in leukocytes. *Ann Rev Immunol* 1990; **8**:365–400.
- Pytela R, Pierschbacher MD, Ruoslahti E. Identification and isolation of a 140 kd cell surface glycoprotein with properties expected of a fibronectin receptor. *Cell* 1985; **40**:191–8.
- Wayner EA, García-Pardo A, Humphries JA, et al. Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain in plasma fibronectin. *J Cell Biol* 1989; **109**:1321–30.
- Guan JL, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor $\alpha 4\beta 1$. *Cell* 1990; **60**:53–61.
- Campanero MR, Pulido R, Ursa MA, et al. An alternative leukocyte homotypic adhesion mechanism, LFA-1/ICAM-1 independent, through the human VLA-4 integrin. *J Cell Biol* 1990; **110**:2157–65.
- Bednarczyk JL, McIntyre BW. A monoclonal antibody to VLA-4 α chain (CDw49d) induces homotypic lymphocyte aggregation. *J Immunol* 1990; **144**:777–84.
- Clayberger C, Krensky AM, McIntyre BW, et al. Identification and characterization of two novel lymphocyte function-associated antigens, L24 and L25. *J Immunol* 1987; **138**:1510–14.
- Elices MJ, Osborn L, Takada Y, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a distinct site from the VLA-4/fibronectin binding site. *Cell* 1990; **60**:577–84.
- Schwartz BR, Wayner EA, Carlos TM, et al. Identification of surface proteins mediating adherence of CD11/CD18 deficient lymphoblastoid cells to cultured human endothelium. *J Clin Invest* 1990; **85**:2019–22.
- Takada Y, Wayner EA, Carter WG, et al. The extracellular matrix receptors, ECMR II and ECMR I, for collagen and fibronectin correspond to VLA-2 and VLA-3 in the VLA family of heterodimers. *J Cell Biochem* 1988; **37**:385–93.
- Hynes RO, Marcantonio EE, Stepp MA, et al. Integrin heterodimer and receptor complexity in avian and mammalian cells. *J Cell Biol* 1989; **109**:409–20.
- Vogel BE, Tarone G, Giancotti FG, et al. A novel fibronectin receptor with an unexpected subunit composition $\alpha V\beta 1$. *J Biol Chem* 1990; **265**:5934–7.
- Dedhar S, Gray V. Isolation of a novel integrin receptor mediating ARG-GLY-ASP-directed cell adhesion to fibronectin and type I collagen from human neuroblastoma cells. Association of a novel $\beta 1$ -related subunit with $\alpha 5$. *J Cell Biol* 1990; **110**:2185–93.
- Charo IF, Nanizzi L, Smith, et al. The vitronectin receptor $\alpha V\beta 3$ binds fibronectin and acts in concert with $\alpha 5\beta 1$ in promoting cellular attachment and spreading on fibronectin. *J Cell Biol* 1990; **111**:2795–800.
- Shimizu Y, Van Seventer GA, Horgan KJ, et al. Regulated expression and binding of three VLA ($\beta 1$) integrin receptors on T cells. *Nature* 1990; **345**:250–3.
- Matsuyama T, Yamada A, Kay J, et al. Activation of CD4 cells by fibronectin and anti CD3 antibody. *J Exp Med* 1989; **170**:1133–48.
- Shimizu Y, Van Seventer GA, Horgan KJ, et al. Costimulation and proliferative responses of resting CD4 T cells by the interaction of VLA-4 and VLA-5 with fibronectin or VLA-6 with laminin. *J Immunol* 1990; **145**:59–67.
- Davis LS, Oppenheimer-Marks N, Bednarczyk JL, et al. Fibronectin promotes proliferation of naive and memory T cells by signalling through both the VLA-4 and VLA-5 integrin molecules. *J Immunol* 1990; **145**:785–93.
- Laffón A, Sánchez-Madrid F, O de Landázuri M, et al. Very late activation antigens on synovial fluid T cells from patients with rheumatoid arthritis and other rheumatic diseases. *Arthritis Rheum* 1989; **32**:386–92.
- Hemler ME, Glass D, Coblyn JS, et al. Very late activation antigens on Rheumatoid synovial fluid T lymphocytes: association with stages of T cell activation. *J Clin Invest* 1986; **78**:696–702.
- Cush JJ, Lipsky PE. Phenotypic analysis of synovial tissue and peripheral blood lymphocytes isolated from patients with Rheumatoid Arthritis. *Arthritis Rheum* 1988; **10**:1230–8.
- Laffón A, García-Vicuña R, Humbria A, et al. Upregulated expression and function of VLA-4 fibronectin receptors on human activated T cells from Rheumatoid Arthritis. *J Clin Invest* 1991; **88**:546–52.
- Arnett CA, Edworthy SM, Bloch DA, et al. The American Rheumatism Association 1987. Revised criteria for the classification of rheumatoid arthritis. *Arthritis Rheum* 1988; **31**:315–24.

- 27 Cebrián M, Yagüe E, Rincón M, *et al.* Triggering of T cell proliferation through AIM, an activation induced molecule on activated human lymphocytes. *J Exp Med* 1988; **168**:1621–37.
- 28 Hemler ME, Sánchez-Madrid F, Flotte TJ, *et al.* Glycoproteins of 210000 and 130000 m.w. on activated T cells: cell distribution and antigenic relation to components on resting cells and T cell lines. *J Immunol* 1984; **132**:3011–18.
- 29 Wayner EA, Carter WG, Piotrowicz RS, *et al.* The function of multiple Extracellular Matrix Receptors in mediating cell adhesion to extracellular matrix: preparation of monoclonal antibodies to the fibronectin receptor that specifically inhibit cell adhesion to fibronectin and react with platelet glycoproteins Ic-IIa. *J Cell Biol* 1988; **107**:1881–91.
- 30 Sánchez-Madrid FO, de Landázuri M, Morago G, *et al.* VLA-3: a novel polypeptide association within the VLA molecular complex: cell distribution and biochemical characterization. *Eur J Immunol* 1986; **16**:1343–9.
- 31 Spits H, Yssel H, Leewenberg J, *et al.* Antigen-specific cytotoxic T cell and antigen-specific proliferating T cell clones can be induced to cytolytic activity by monoclonal antibodies against T3. *Eur J Immunol* 1985; **15**:88–91.
- 32 Keizer GD, Borst J, Figdor CG, *et al.* Biochemical and functional characteristics of the human leukocyte membrane antigen family LFA-1, Mo1 and p150,95. *Eur J Immunol* 1985; **15**:1142–7.
- 33 Anegón I, Vilella R, Gallart T, *et al.* B-Cl, B-C2, B-C3: monoclonal antibodies against B cell differentiation antigens. In: Reinherz EL, Haynes BF, Nadler L, Berstein ID, eds. *Leukocyte typing II*. New York: Springer-Verlag, 1986:121–4.
- 34 Pulido R, Cebrián M, Acevedo A, *et al.* Comparative biochemical and tissue distribution of four distinct CD45 antigen specificities. *J Immunol* 1988; **140**:3851–7.
- 35 Graham RC, Karnovsky MI. The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney. Ultrastructural cytochemistry by a new technique. *J Histochem Cytochem* 1986; **14**:291–8.
- 36 Dustin ML, Springer TA. T cell receptor cross-linking transiently stimulates adhesiveness through LFA-1. *Nature* 1989; **341**:619–24.
- 37 Akbar AN, Terry L, Timms A, *et al.* Loss of CD45R and gain of UCHL1 reactivity is a feature of primed T cells. *J Immunol* 1988; **140**:2171–8.
- 38 Lasky HP, Bayer K, Pope RM. Increased helper inducer and decreased suppressor inducer phenotypes in the rheumatoid joint. *Arthritis Rheum* 1988; **31**:52–9.
- 39 Emery P, Gentry KC, Mackay IR, *et al.* Deficiency of the suppressor inducer subset of T lymphocytes in Rheumatoid Arthritis. *Arthritis Rheum* 1987; **30**:849–56.
- 40 Pitzalis C, Kingsley G, Murphy J, *et al.* Abnormal distribution of helper-inducer and suppressor-inducer T lymphocyte subsets in the rheumatoid joint. *Clin Immunol Immunopathol* 1987; **45**:252–8.
- 41 Adams JC, Watt FM. Changes in keratinocyte adhesion during terminal differentiation. Reduction in fibronectin binding precedes $\alpha 5 \beta 1$ integrin loss from the cell surface. *Cell* 1990; **63**:425–35.
- 42 Carsons S, Mosesson MW, Diamond HS. Detection and quantitation of fibronectin in synovial fluid from patients with rheumatic diseases. *Arthritis Rheum* 1981; **24**:277–80.
- 43 Clemmensen I, Andersen RD. Different molecular forms of fibronectin in rheumatoid synovial fluid. *Arthritis Rheum* 1982; **25**:25–31.
- 44 Herbert KE, Coppock JS, Griffiths AM, *et al.* Fibronectin and immune complexes in rheumatoid diseases. *Ann Rheum Dis* 1987; **46**:734–40.
- 45 Scott DL, Wainwright AC, Walton KW, *et al.* Significance of fibronectin in rheumatoid arthritis and osteoarthritis. *Ann Rheum Dis* 1981; **40**:142–53.