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Expression and functional role of 1F7 (CD26) antigen on peripheral blood and synovial fluid T cells in rheumatoid arthritis patients

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SUMMARY

The expression and the functional role of the CD26 (1F7) T cell surface molecule, an ectoenzyme which seems to represent a functional collagen receptor of T lymphocytes and to have a role in T cell activation, were analysed in both peripheral blood (PB) and synovial fluid (SF) T cell samples from patients with active and inactive rheumatoid arthritis (RA). Although patients with active disease displayed higher percentages of PB $CD26^+$ $CD4^+$ T cells than inactive RA and control subjects, CD26 antigen expression on RA SF T lymphocytes was low. The anti-1F7 binding to the T cell surface, that led to CD26 antigen modulation and enhancement of both IL-2 synthesis by, and ^{3}H-TdR incorporation of, anti-CD3- or anti-CD2-triggered PB T cells in RA and control subjects, was unable to affect significantly both expression and functional activity of RA SF T lymphocytes. Since the 1F7 antigen spontaneously reappeared on the surface of unstimulated SF T cells after 2–5 days of culturing, the low 1F7 antigen expression of anti-1F7 in the SF T cell compartment may be the result of *in vivo* molecule modulation exerted by the natural ligand in the joint, with important implications for T cell activation and lymphokine synthesis.

Keywords T cells CD26 rheumatoid arthritis synovial fluid

INTRODUCTION

Rheumatoid arthritis (RA) is a chronic inflammatory disease histologically characterized by a marked cellular infiltration and accumulation of T lymphocytes in the sublining layer of the synovium, often with a perivascular distribution [1]. Despite an increasing understanding of the immunopathogenesis of RA, the precise role of T cells in the development of rheumatoid synovitis and the mechanisms involved in their activation at the site of inflammation are not completely understood. However, because adhesion-related molecules and a number of activation markers are more densely expressed on synovial fluid (SF) T cells than on peripheral blood (PB) T cells [2,3], the intrinsic migratory capacity and the activation status of circulating T lymphocytes is thought to play a key role in the development of the T cell infiltrate within synovial tissue and fluid [4,5].

CD26 is a membrane-associated intrinsic ectoenzyme with a binding affinity for collagen expressed on the surface of a subset of human resting T lymphocytes [6–8]. This molecule is known as a T cell activation antigen, as its expression on T lymphocytes rapidly increases with short-term mitogenic or antigenic stimulation [9,10]. Furthermore, T cell CD26 appears to be involved in CD3- and CD2-induced T cell activation, since binding of the anti-CD26 MoAb to the 1F7 T cell surface epitope enhances the T cell proliferation induced by submitogenic concentrations of anti-CD3 or anti-CD2 MoAb [11,12]. We therefore attempted to evaluate the role of 1F7 T cell membrane antigen in the T cell activation processes at the site of inflammation during a chronic immune-mediated disorder by analysing the expression of this molecule on, and the functional effects of anti-1F7 binding to, the T cell surface in both PB and SF compartments in patients with active and inactive RA.

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PATIENTS AND METHODS

Patients

PB and/or knee SF samples were obtained from 22 patients who met the American College of Rheumatology (formerly the American Rheumatism Association) 1987 revised criteria for RA [13]. Five were men and 17 women, mean age was 47·5 years (range 15–71 years), and disease duration was 6·3 years (range 1–19 years). Sixteen patients were seropositive for IgM rheumatoid factor, 19 were receiving non-steroidal anti-inflammatory drugs (NSAID), six low-dose prednisone (PDN), and only four disease-modifying anti-rheumatic drugs (DMARD;

auranofin or methotrexate) at the time of sampling. Ten patients were in disease remission, and the other 12 manifested signs of disease activity, as defined by the presence of six or more tender joints, and two of the following conditions: nine or more swollen joints, morning stiffness lasting more than 45 min, or a Westergren erythrocyte sedimentation rate of 28 mm/h or more. Twenty-eight age- and sex-matched healthy volunteers acted as normal controls (NC). SF control samples were also collected from the knee of seven subjects with osteoarthritis (OA). Written informed consent was obtained from all the patients included in the study.

Cell purification

Mononuclear cells (MNC) and T cells were isolated from heparinized PB and SF samples as described previously [14]. Briefly, MNC were depleted of monocytes by adherence to plastic Petri dishes, and the lymphocytes were separated into E-rosette-positive (T cells) and -negative subsets with sheep erythrocytes. T cells were then passed over a nylon wool column to deplete residual contaminating monocytes and B cells.

Monoclonal antibodies

Anti-CD3 (IgG2a), anti-CD4 (IgG2b) and anti-CD8 (IgG2a) were purified from supernatants of hybridoma cells obtained from the American Type Cell Culture Collection (ATCC; Rockville, MD). Anti-1F7 (CD26, IgGl) and mitogenic anti-CD2 ($Tl1_2$ and $Tl1_3$) were kindly supplied by Dr S. F. Schlossman (Dana-Farber Cancer Institute, Boston, MA).

Cell phenotype

Surface phenotyping was carried out by a previously described two-colour immunofluorescence staining technique [15], employing isotype-specific goat anti-mouse antibody (Southern Biotechnology Assoc., Brimingham, AL) conjugated with either fluorescein or PE as developing reagents for each MoAb. The stained cells were fixed in 1% paraformaldehyde in PBS and analysed by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA). Lymphocyte subsets were identified by gating analysis and fluorescence profiles obtained for 10 000 cells of each sample. Negative controls for each experiment were performed with mouse MoAb unreactive with human determinants, followed by the corresponding fluorochrome-conjugated goat anti-mouse antiserum or the latter reagent alone. The specificity of the isotype-specific goat anti-mouse fluorochrome-conjugated antisera was proved by the fact that they never cross-reacted with non-corresponding MoAb.

Cell proliferation

In vitro T cell proliferation (5×10^4/well) was carried out in triplicate in complete medium in 96-well round-bottomed microwell plates (Nunc, Roskilde, Denmark). Anti-CD3 and the combination of anti-$Tl1_2$ and anti-$Tl1_3$ were used at the indicated concentrations. The plates were incubated in a 5% CO_2 humidified atmosphere at 37°C. Cell proliferation was assessed by measuring ^{3}H-TdR incorporation (0·5 μCi; specific activity 25 Ci/mmol; Amersham, Aylesbury, UK) during the last 6 h of culture, and counting in a liquid scintillation counter. For purified T cell anti-CD3-stimulated proliferation assays, microtitre wells were coated with the MoAb as previously described [16]. Briefly, protein-A purified anti-CD3 was diluted in complete medium at the indicated concentrations and 100 μl were placed in each well of a 96-well flat-bottomed microtitre plate (no. 3596; Costar Data Packaging Corp., Cambridge, MA) and incubated at room temperature for 1 h. The wells were then washed twice in medium to remove non-adherent MoAb before T cells were added.

IL-2 assay

IL-2 levels were determined in filtered cell culture supernatants after 48 h stimulation by incubating mouse spleen cells in complete medium supplemented with $2{\cdot}5 \times 10^{-5}$ M 2-mercaptoethanol (2-ME; Sigma, St Louis, MO) and concanavalin A (Con A; 2 μg/ml; Miles Labs, Naperville, IL) for 72 h and then culturing them in 96-well flat-bottomed plates (Nunc; 2×10^4 cells/well) at a final volume of 0·2 ml in the presence or absence of the supernatants (25%) under test. Cells were pulsed with ^{3}H-TdR after 18 h of culture, harvested 6 h later and counted as above. All assays were done in triplicate. The concentration of IL-2, expressed as U/ml, in each culture supernatant was calculated against a standard curve constructed with serial dilutions of an rIL-2 of known activity. Some supernatants were simultaneously assayed on the IL-2-sensitive mouse cell line CTLL. As both assays yielded similar results in terms of U/ml, only the data for murine splenocytes are given.

Modulation studies

For anti-CD26-induced modulation studies, cells were incubated overnight at 37°C in medium containing optimal amounts of anti-1F7 (1 : 400 ascites dilutions). Cell samples were collected for immunofluorescence studies before and after anti-1F7 incubation.

Statistical analysis

Owing to the non-normal distribution of samples, the Mann–Whitney *U*-test was adopted for statistical evaluation. Values of $P < 0{\cdot}05$ were chosen for rejection of the null hypothesis.

RESULTS

Expression of 1F7 antigen on PB and SF T lymphocytes

As shown in Table 1, the percentage of $CD3^+$ T cells coexpressing the 1F7 antigen was significantly higher in RA patients than in NC subjects ($P < 0{\cdot}001$). The increase was mainly evident on the $CD4^+$ subset ($P < 0{\cdot}001$). Despite the fact that there were no differences between patients treated with PDN and/or DMARD and those who received NSAID alone (data not shown), when the RA patients were subdivided into two groups according to disease activity (Table 1), only patients with active disease displayed a significantly higher number of T cells that coexpressed the 1F7 antigen (active RA *versus* NC and *versus* inactive RA: $P < 0{\cdot}001$). These data were confirmed when the absolute number of PB circulating T cells bearing the 1F7 surface antigen was analysed (data not shown). Instead, the expression of 1F7 molecule was strongly reduced on T lymphocytes from the SF of both active and inactive RA ($P < 0{\cdot}001$), but only partially decreased on SF T cells from OA subjects, with respect to NC PB. The decrease in 1F7 surface expression on SF T cells was evident on the $CD4^+$ subset.

Table 1. Percentages and mean fluorescence intensity (MFI) (mean ± s.d.) of 1F7 antigen on $CD3^+$, $CD4^+$ and $CD8^+$ cells in normal controls (NC) and rheumatoid arthritis (RA) peripheral blood (PB) and in RA and osteoarthritis (OA) synovial fluid (SF)

	$CD3^+$		$CD4^+$		$CD8^+$	
	Per cent	MFI	Per cent	MFI	Per cent	MFI
NC PB (28)	58·8 ± 9	89·6 ± 12	64·1 ± 8	99·3 ± 11	48·1 ± 6	73·7 ± 10
RA PB (22)	68·9 ± 11*	119·4 ± 14*	74·3 ± 11*	121·6 ± 15*	52·7 ± 7	81·0 ± 9
Inactive RA PB (10)	60·6 ± 4	92·9 ± 9	65·2 ± 5	100·2 ± 12	49·1 ± 6	77·8 ± 10
Active RA PB (12)	75·9 ± 10*	139·2 ± 15*	81·8 ± 8*	144·8 ± 16*	55·4 ± 7†	94·1 ± 10
RA SF (10)	36·3 ± 10*	39·5 ± 9*	36·2 ± 13*	48·7 ± 10*	44·7 ± 8	59·5 ± 9
OA SF (7)	49·0 ± 11	61·0 ± 10	48·4 ± 10†	56·3 ± 11†	47·1 ± 8	60·1 ± 8

* $P < 0.001$ *versus* NC PB.
† $P < 0.005$ *versus* NC PB.

Effects of surface modulation of the 1F7 antigen

It has been demonstrated that the binding of anti-1F7 to its specific molecule leads to internalization of the antigen/antibody complex and disappearance of the 1F7 antigen from the T cell surface [12]. The modulation of 1F7 antigen results in enhanced proliferative activity of human T cells via either the TCR/CD3 or CD2 pathway [11,12]. The present experiments show that optimal anti-1F7 concentrations significantly reduce 1F7 expression on PB T cells of both RA and NC subjects after 3 h incubation, and that minimum expression occurs after 8 h (Fig. 1). 1F7 antigen could also be modulated on the SF T lymphocyte surface, but mainly in OA and only when the basal percentage of SF $CD3^+$ cells coexpressing the 1F7 molecule was higher than about 35%.

We subsequently analysed the functional effect of anti-1F7 binding to the T cell surface in both PB and SF of RA patients and control subjects (Table 2). 1F7 antigen modulation enhanced the ^{3}H-TdR uptake of PB T cells induced by

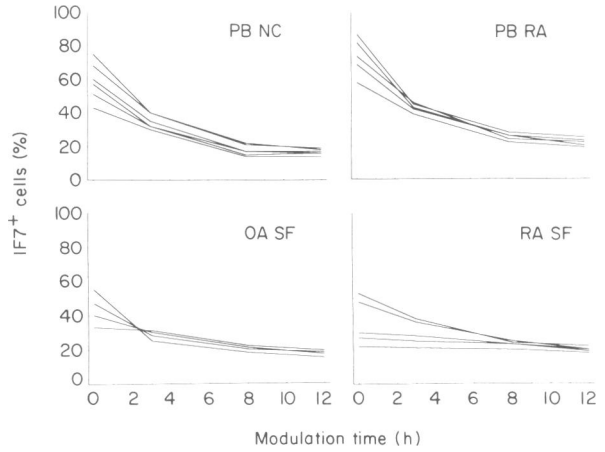

Fig. 1. Anti-1F7-induced modulation of the CD26 antigen on the surface of T cells from the peripheral blood (PB) of both normal controls (NC) and rheumatoid arthritis (RA) subjects and from the synovial fluid (SF) of osteoarthritis (OA) and RA patients. Each line indicates the percentage of $CD3^+$ cells coexpressing the 1F7 antigen before (time 0) and during the incubation with the anti-1F7 MoAb.

Table 2. Effect of anti-1F7 modulation on anti-CD3- or anti-CD2-induced ^{3}H-TdR incorporation (ct/min × 10^{-3}) of rheumatoid arthritis (RA) peripheral blood (PB) and synovial fluid (SF), normal control (NC) PB, and osteoarthritis (OA) SF T cell samples

	Modulation					
	None		Anti-CD3		Anti-CD2	
Stimulus*	None	1F7	None	1F7	None	1F7
NC PB	0·3	0·4	1·0	9·4	2·2	14·2
RA PB	0·4	0·6	0·9	14·2	2·3	20·1
RA SF	0·9	0·8	1·3	2·2	1·5	2·3
OA SF	0·7	0·8	1·1	4·9	1·9	5·7

* Data are representative of eight separate experiments. Immobilized anti-CD3 was used at the submitogenic concentration of 500 ng/ml and anti-$Tl1_2$ and anti-$Tl1_3$ at the 1:3200 ascites submitogenic dilution.

Table 3. Effect of anti-1F7 modulation on anti-CD3- or anti-CD2-induced IL-2 synthesis (U/ml) by rheumatoid arthritis (RA) peripheral blood (PB) and synovial fluid (SF), normal control (NC) PB, and osteoarthritis (OA) SF T cell samples

	Modulation					
	None		Anti-CD3		Anti-CD2	
Stimulus*	None	1F7	None	1F7	None	1F7
NC PB	< 1	< 1	< 1	5·0	1·2	6·8
RA PB	< 1	< 1	< 1	6·6	1·1	8·9
RA SF	< 1	< 1	< 1	1·0	1·0	1·1
OA SF	< 1	< 1	< 1	2·4	1·1	2·5

* Data are representative of four separate experiments. Immobilized anti-CD3 and soluble anti-$Tl1_2$ and anti-$Tl1_3$ were used as descibed in Table 2.

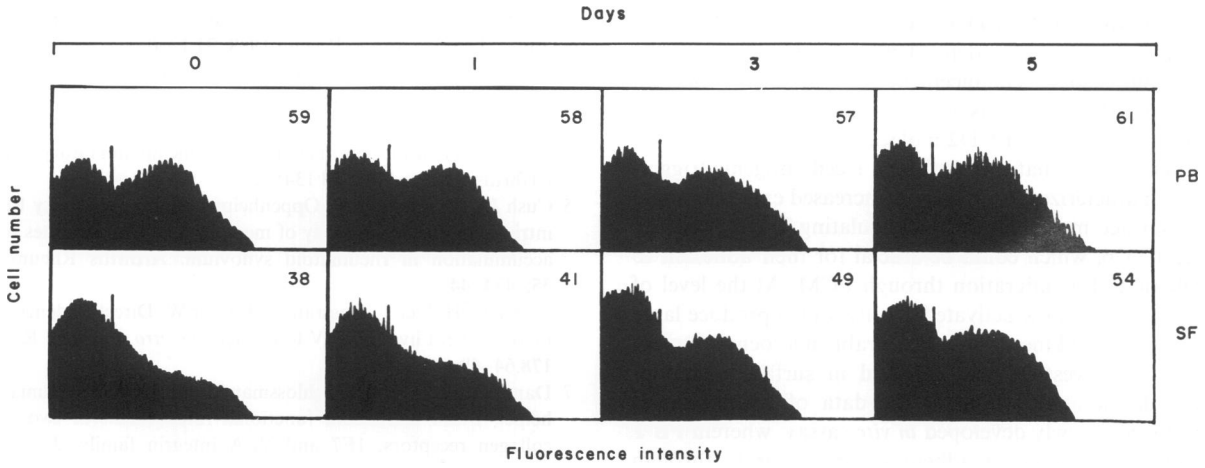

Fig. 2. Spontaneous reexpression of 1F7 antigen on the surface of both normal control (NC) peripheral blood (PB) and rheumatoid arthritis (RA) synovial fluid (SF) $CD3^+$ T cells. Data are representative of six separate experiments.

suboptimal concentrations of anti-CD3 or anti-CD2. The increase in the proliferative response of PB samples was due to enhanced IL-2-dependent T cell activation, as suggested by increased IL-2 levels in the supernatants of anti-1F7-modulated PB T cell cultures (Table 3).

In contrast, binding of anti-1F7 to the surface of RA SF T lymphocytes failed to increase significantly either CD3- or CD2-induced ^{3}H-TdR uptake or IL-2 synthesis (Tables 2 and 3). A partial increase in proliferation and IL-2 production was, however, documented in OA SF T cell cultures.

Re-expression of 1F7 surface antigen after its modulation

Our experiments showed a low 1F7 expression on SF $CD3^+$ T cells of RA patients. Because it has been demonstrated that the *in vitro* modulation of the 1F7 surface antigen is followed by slow, but spontaneous, re-expression of the molecule [12], we designed experiments aimed at establishing whether the reduced number of $1F7^+/CD3^+$ T lymphocytes was a stable or variable feature of RA SF samples. T cells from RA SF and NC PB were incubated at 37°C without activatory stimuli for a period ranging from 1 to 5 days, and then counted and phenotypically analysed. Interestingly, the $1F7^+$ T cell percentage of RA, but not of NC, slowly but spontaneously increased with a peak, which almost reached the range of 1F7 antigen expression on normal PB T cells, on day 5 (Fig. 2). The fact that the viability of the residual T cells after 5 days of culture was higher than 90% by trypan blue dye exclusion, ruled out the presence of a selectively surviving population of $1F7^+$ cells.

DISCUSSION

The CD26 surface molecule is considered a marker for T cells that have undergone triggering by antigen *in vivo* [9,10]. The expression of CD26 on T cells has been found to be associated with the capacity of these cells to produce high amounts of IL-2 and to proliferate strongly in response to recall antigens such as tetanus toxoid [17]. The results of the present experiments demonstrate increased expression of this molecule, as recognized by the anti-1F7 MoAb, on the surface of PB T lymphocytes of RA patients. Although phenotypic data on the activation status of T cells circulating in the PB of RA are conflicting [1,18], our findings appear to support the presence of a previously *in vivo* activated PB T cell subpopulation in these subjects. Discrepancies among these phenotypic studies could arise from differences in selection of patients with regard to treatment and disease activity. It seems unlikely that the increase in 1F7 T cells in the present investigation was attributable to the therapy, since there were no differences in the $1F7^+$ T cell proportion of the few patients treated with PDN and/or DMARD and those who received only NSAID. On the other hand, our results clearly demonstrate that the high expression of the 1F7 molecule, mainly present on the PB $CD4^+$ T cell subset, was associated with clinical signs of disease activity. It is well known that activation of $CD4^+$ T lymphocytes is one of the most important steps in the development of RA synovitis [1,4]. The present finding may, therefore, be a reflection in the PB of the inflammatory processes which occur at the joint level. In other words, the high number of $1F7^+$ $CD4^+$ T lymphocytes in patients with active RA may be an expression of the recirculation of activated helper T cells from the blood stream to the inflamed tissues, and *vice versa*. This hypothesis, however, does not appear to be sustained by our SF phenotypic data, which documented a marked decrease in 1F7 expression on synovial T lymphocytes. The intriguing phenotypic discrepancies in 1F7 expression between PB and SF T cells in a chronic immune-mediated disorder such as RA, where the pathological immune response takes place mainly at the level of inflamed synovium, suggest that our findings may have a more complex explanation.

Immunochemical and immunocytochemical techniques have identified the CD26 molecule as the enzyme dipeptidyl peptidase IV (DPPIV) [19]. Found on a number of tissue types, DPPIV is a surface-associated ectopeptidase which specifically catalyses the hydrolysis of the peptide bond between N-terminal X-Pro and adjacent peptide amino acids [20]. Although CD26 is distinct from the VLA family in molecular size [7], it represents a receptor for collagen involved in the T cell migration processes into the extracellular matrix (ECM) [1]. CD26 has also recently been demonstrated to be involved in T cell activation induced via either CD3 or CD2 pathways [7,11].

Anti-1F7 binding to the CD26 antigen on the T cell surface leads not only to energy-dependent internalization of the molecule, but also to enhancement of cytoplasmic calcium mobilization, IL-2 synthesis and proliferative activity of T cells triggered via CD3 or CD2 molecules [12]. These observations would suggest that the persistent T cell antigenic triggering which characterizes RA results in increased expression of a series of surface molecules on PB circulating T lymphocytes, including CD26, which could be crucial for their adhesion to endothelium and/or migration through ECM. At the level of the inflamed joint, these activated T cells, which produce large amounts of lymphokines, find a favourable microenvironment for adhesion to vessels, also enriched in surface adhesion-related molecules. According to the data of recent studies that employed a newly developed *in vitro* assay, wherein PB T cells with the capacity to adhere to and migrate through endothelial cell (EC) monolayers can be retrieved and assessed, most migrating T cells, unlike EC-adherent T cells, are cells which strongly express CD26 [21]. This indicates that the CD26 molecule is not crucial for adhesion, but probably by means of its proteolytic activity plays a key role in regulating T cell transmigration across EC monolayers. On the basis of our *in vitro* functional data showing that anti-1F7 exerts a comitogenic effect on CD3- or CD2-triggered PB T cell proliferation, it could be hypothesized that *in vivo* activation of migrating T cells, which strongly express CD26 and maximally respond to antigen stimulation, is synergically enhanced by the binding of CD26, and probably other surface receptors, to their ECM natural ligand at the joint level. On the other hand, although a different distribution of $1F7^+$ cells in the SF and membrane cannot be ruled out, the low CD26 surface expression and the low comitogenic capacity of anti-1F7 on CD3- or CD2-induced activation of SF T lymphocytes could well be the result of *in vivo* modulation of the molecule. The spontaneous CD26 resynthesis on the SF T cell surface observed in our *in vitro* experiments seems to sustain this concept, since it rules out that low expression of the molecule is an intrinsic and stable characteristic of SF T lymphocytes.

The present study showed that T cells from the SF of control subjects with degenerative joint disease only partially exhibited the CD26 phenotypic and functional characteristics of SF T cells observed in RA patients. Our preliminary data in other synovitic disorders, however, appear to indicate that such features are not specific to RA, but are shared with other inflammatory joint diseases. Whether the functional role of the CD26 surface molecule is also to influence the pattern of lymphokine secretion by T cells is a question we are now trying to answer.

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