Increment of CD8S6F1 cells in synovial fluid from patients with rheumatoid arthritis

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Abstract

Objective—To investigate the role of CD8 cell subsets in the pathogenesis of rheumatoid arthritis (RA) and the phenotypes of T cells adherent or non-adherent to the target cells (endothelial cells and synovial cells) pre-treated with IL-1 β .

Methods—The expression of S6F1 on CD8 cells and that of an activation marker on CD8 cells and CD8 cell subsets was evaluated in specimens of peripheral blood and synovial fluid obtained from 15 patients with RA and 10 with osteoarthritis (OA) using a two- or three-colour immunofluorescence method for analysis.

Results—The percentage of CD8S6F1 cells among CD8 cells in synovial fluid was significantly greater than that of peripheral blood. Synovial fluid from RA patients had a greater percentage of CD8S6F1 cells compared with either peripheral blood of matched patients or synovial fluid of OA patients. The percentage of CD8HLA-DR cells in synovial fluid was markedly greater than that in paired samples of peripheral blood in patients with RA. In the CD8S6F1 cells from both groups of patients, synovial fluid showed an increased percentage of HLA-DR cells compared with peripheral blood. Similar results were observed in CD8 cells lacking S6F1 expression (CD8S6F1⁻) from both groups of patients. There was no significant difference in the percentage of HLA-DR cells between CD8S6F1 and CD8S6F1⁻ cell populations in peripheral blood. In contrast with peripheral blood, in synovial fluid of RA patients the percentage of HLA-DR cells in the CD8S6F1 cell population was markedly greater than that in the CD8S6F1⁻ population. However, the percentage of HLA-DR cells in both cell populations was similar in synovial fluid of OA patients. In both the endothelial and the synovial cell adhesion assays, the percentage of CD8S6F1 among CD8 cells and the mean fluorescence intensity of S6F1 antigen on CD8S6F1 cells were significantly greater in the adherent T cell population than that in the non-adherent T cell population.

Conclusion—These results suggest that increased expression of S6F1 antigen and the increased percentage of HLA-DR cells on CD8 cells in synovial fluid may be responsible for the migration of these cells into inflamed synovial tissues, and for cellular interactions between these cells

and synovial cells or the extracellular matrix.

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There is increasing evidence that autoimmune processes are important in the pathogenesis of rheumatoid arthritis (RA).¹⁻⁴ The synovial membrane in RA is characterised by a marked infiltration of mononuclear cells, and extensive proliferation of synovial cells and small blood vessels. Leucocytes migrate from the latter vessels to synovial tissue,⁵ where they may become involved in cellular interactions, leading to proliferation of synovial tissue, production of autoantibodies, and secretion of biochemical mediators such as cytokines. It has been established that most synovial inflammatory cells in RA are T cells.⁶ These cells are highly activated and differ from circulating T cells in many aspects in patients with RA and healthy subjects.⁷⁻¹⁷ Development of newer monoclonal antibodies and flow cytometry has allowed better identification of functional subsets of CD4 cells and CD8 cells. The CD8 population contains precytotoxic, cytotoxic, natural killer, presuppressor and suppressor effector cells.¹⁸ Several studies have investigated the heterogeneity of CD4 cells with regard to function, cell surface antigens and lymphokine secretion profile,¹⁹⁻²⁵ but little is known of the exact phenotypic identities of the CD8 population. Morimoto *et al*²⁶ have developed a monoclonal antibody, anti-S6F1, which can distinguish killer effector and suppressor effector cells in CD8 lymphocyte populations. Hale *et al*²⁷ showed that the number of T cells that bind to human umbilical endothelial cells increased significantly when the endothelial cells were pretreated with IL-1 β , and we obtained similar results from IL-1 β stimulated synovial cells.²⁸

The aim of our present study was to characterise further the CD8 cell subsets in peripheral blood and synovial fluid in RA patients using two- or three-colour immunofluorescence techniques, and to compare them with those of normal healthy subjects and patients with osteoarthritis (OA). We also conducted a flow cytometry analysis to characterise CD8 cells that bind to IL-1 β -stimulated endothelial and synovial cells.

Materials and methods

PATIENTS AND NORMAL SUBJECTS

We studied 15 patients (two male, 13 female; mean (SD) age 52 (13) years (range 23-71))

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who met the American College of Rheumatology criteria for RA.²⁹ Patients were randomly selected from those attending the outpatient clinic of the Department of Internal Medicine, Sasebo Chuo Hospital. All patients were taking non-steroidal anti-inflammatory drugs (NSAIDs) and disease-modifying antirheumatic drugs (DMARDs) during the study. We also studied 10 patients with OA (two male, eight female; mean (SD) age 54 (13) years (range 36–71)), who were taking NSAIDs. Ten age- and sex-matched normal healthy subjects served as controls (two male, eight female; mean (SD) age 53 (13) years (range 31–70)).

SEPARATION OF MONONUCLEAR CELLS FROM PERIPHERAL BLOOD AND SYNOVIAL FLUID

A total of 25 samples of synovial fluid was obtained (15 from RA patients and 10 from OA patients) during therapeutic joint aspiration. In some cases, a paired sample of venous blood was obtained at the same time. Separation of peripheral blood mononuclear cells (PB-MNC) in a 10 ml heparinised venous blood sample was performed using Ficoll-Conray density-gradient centrifugation (Daiichi Pharmaceutical Co., Tokyo, Japan), according to the method described by Burmester *et al.*⁸ Mononuclear cells from synovial fluid (SF-MNC) were isolated using the same method. PB-MNC and SF-MNC were washed three times with phosphate buffered saline solution (PBS) containing 2% fetal bovine serum (FBS) (Gibco, Grand Island, NY), and then adjusted to a concentration of 1×10^6 cells/ml for immunofluorescence staining. In each experiment, cell viability, assessed by the trypan blue dye (Gibco, Grand Island, NY) exclusion test, was greater than 95% in both PB-MNC and SF-MNC.

PREPARATION OF T CELLS FROM PERIPHERAL BLOOD

Isolated PB-MNC were depleted of adherent cells by incubation in cell suspensions in Petri dishes (Falcon 3003, Becton Dickinson, Oxnard, CA) for 2 hours at 37°C. A T cell enriched population was prepared from non-adherent cells by rosetting with 5% sheep red blood cells (Nippon Biotest Laboratory, Tokyo, Japan). In each experiment, the purity of T cells was determined using monoclonal antibodies to CD2, CD20 and MY4 (Coulter Immunology, Hialeah, FL) that react with mature T cells, pan B cells and monocytes, respectively. T cell enriched populations containing more than 95% T cells, less than 1% B cells, and less than 1% monocytes were designated as T cells.

PREPARATION OF ENDOTHELIAL CELLS

Endothelial cells were prepared from samples of human umbilical cord vein and a modified enzymatic digestion method described by Kawakami *et al.*³⁰ Briefly, umbilical cord veins

were infused with 100 µg/ml of collagenase (Sigma Chemical Co., St Louis, MO) in Hanks's balanced salt solution (HBSS). After 10 minutes of incubation at room temperature, the solution containing detached endothelial cells was flushed out with PBS. Cells were suspended in RPMI 1640 supplemented with 20% heat-inactivated FBS (Gibco, Grand Island, NY), 25 µg/ml of endothelial cell growth supplement (Sigma), 5 U/ml of heparin, and antibiotics (100 U/ml of penicillin and 100 µg/ml of streptomycin). Cells were then cultured in Petri dishes (Falcon 3003) pre-coated with fibronectin (Sigma). The culture medium was changed every two to three days and endothelial cells were briefly exposed to trypsin-EDTA-HBSS. The identity of the cells was established by their characteristic cobblestone appearance under phase-contrast microscopy, and by an enzyme-labelled antibody technique using a mouse monoclonal anti-human Von Willebrand factor (Immunotech, Marseille, France) to which the preparation of endothelial cells was more than 99% reactive as shown by the avidin-biotin-immunoperoxidase technique. The cells grew well under these culture conditions and were used at the third or fourth passage for our experiments.

PREPARATION OF SYNOVIAL CELLS

A sample of synovial tissue was trimmed of fat, and the residue cut up finely with scissors and added to a mixture of collagenase (Sigma) and dispase (Godo Shusei Co., Tokyo, Japan), according to the method described by Wernick *et al.*³¹ The tissue mixture was digested for 15 minutes, with gentle stirring at 37°C, and the resulting suspension passed through a nylon mesh. The harvested cells were allowed to adhere to Petri dishes (Falcon 3003) by culturing for 18 hours at 37°C in RPMI 1640 supplemented with 10% FBS. The plated cells were thoroughly washed with PBS to eliminate non-adherent cells, and then cultured with RPMI 1640 supplemented with 10% FBS at 37°C in a humidified atmosphere of 5% carbon dioxide in air. The resultant synovial cells were used at the third or fourth passage in subsequent experiments.

PHENOTYPIC CHARACTERISATION OF ADHERENT AND NON-ADHERENT CELLS

We also characterised the T cells phenotypically, by assaying adhesion to endothelial or synovial target cells. The target cells (2.4×10^6) were cultured for 8 hours in Petri dishes with rIL-1β (10 IU/ml). After thorough washing, T cells (4.8×10^7) from peripheral blood healthy subjects were added to the cell monolayer, and incubated for two hours. Non-adherent T cells were collected by aspiration and washed gently five times with warm culture medium. The adherent T cells were collected by a rubber policeman.³² To investigate the phenotypes of both adherent and non-adherent T cells, we determined the percentage of S6F1 cells among CD8 cells and the mean

fluorescence intensity of S6F1 antigen on CD8S6F1 cells using monoclonal antibodies and a flow cytometer (FACScan, Becton Dickinson Immunocytometry Systems, Inc., San Jose, CA).

MONOCLONAL ANTIBODIES

Murine monoclonal antibodies, fluorescein isothiocyanate (FITC)-conjugated antibodies to CD8, phycoerythrin (PE)-conjugated antibodies to S6F1, and Peridinin Chlorophyll Protein (PerCP)-conjugated antibodies to HLA-DR were purchased from Coulter Immunology, Hialeah, FL. Their production and characterisation have been described elsewhere.³³⁻³⁵

TWO- AND THREE-COLOUR

IMMUNOFLUORESCENCE ANALYSES

Two-colour immunofluorescence analysis was as described elsewhere.³⁶ Briefly, 100 µl of mononuclear cells (1×10^6) was incubated with 2% normal mouse serum for 45 minutes at 37°C and then with 5 µl of FITC-conjugated CD8 monoclonal antibodies and 5 µl of PE-conjugated S6F1 monoclonal antibodies for 30 minutes at 4°C, to prevent non-specific binding of Fc fragments to the Fc receptor on mononuclear cells. The cells were washed twice after incubation using PBS supplemented with 2% FBS. Two-colour immunofluorescence was quantified using an FACScan.

Multi-colour immunofluorescence analysis of cell surface molecule expression by flow cytometry is widely used to identify distinct sub-populations of cells. We used PerCP, which is a superior energy transfer fluorochrome,³⁷ has a strong absorption at 488 nm and maximal fluorescent emission at 677 nm, and is suitable for use in three-colour immunofluorescence analysis on flow cytometers equipped with a single low power argon ion laser. Triple labelling experiments were performed by simultaneous addition of 5 µl of FITC-conjugated CD8 antibody, PE-labelled S6F1 antibody, and PerCP-conjugated HLA-DR antibody to cell pellets. After 30 minutes of incubation at 4°C, the cells were washed three times with PBS containing 2% FBS and suspended in 1 ml of PBS with 2% FBS and kept at 4°C until required for three-colour immunofluorescence analysis by FACScan software.

DATA ANALYSIS

Unpaired *t* tests were used to compare differences between groups. *P* < 0.05 denoted statistical significance.

Results

EXPRESSION OF S6F1 AND HLA-DR ANTIGENS ON CD8 CELLS

We studied initially the expression of S6F1 on CD8 cells in peripheral blood and synovial fluid in patients with RA and OA and in peripheral blood of healthy subjects. Table 1 depicts the proportions of CD8 cell subsets. There was no significant difference between subject groups in the percentage of CD8 cells in peripheral blood, or in the percentage of CD8S6F1 and CD8S6F1⁻ cells, but expression of S6F1 in synovial fluid differed substantially from that in peripheral blood. Synovial fluid from RA and OA patients had significantly greater percentages of CD8S6F1 cells compared with peripheral blood from the same patient, and synovial fluid of RA patients had a greater percentage of CD8S6F1 cells than that of OA patients (table 1). In contrast, the percentage of CD8S6F1⁻ cells in synovial fluid of RA patients was significantly smaller than that in peripheral blood in the same patient or in healthy subjects. In addition, analysis of the expression of HLA-DR antigen on CD8 cells revealed that the percentage of CD8HLA-DR cells in synovial fluid also was markedly greater than that in paired samples of peripheral blood from patients with RA. Similar data were obtained in synovial fluid of OA patients, while peripheral blood of RA patients had greater percentage of CD8HLA-DR cells compared with that of OA patients and healthy subjects.

PERCENTAGE OF HLA-DR CELLS ON CD8 CELL SUBSETS

Figure 1 shows results of a three-colour profile of the expression of HLA-DR, S6F1, and CD8 in peripheral blood and synovial fluid of a representative patient with RA. The percentage of HLA-DR antigen among CD8S6F1 cells in synovial fluid was greater than that in peripheral blood (80.7% *v* 36.2%). Table 2 summarises the percentages of HLA-DR cells among CD8 cell subsets. In CD8S6F1 cells from both groups of patients, synovial fluid showed a greater percentage of HLA-DR cells than did peripheral blood. Similar results were observed in CD8S6F1⁻ cells from both groups of patients.

COMPARISON OF HLA-DR EXPRESSION BETWEEN CD8S6F1 AND CD8S6F1⁻ CELLS

Because synovial fluid from patients contained a greater percentage of CD8S6F1 cells in the CD8 population, we studied the expression of HLA-DR antigen on CD8S6F1 and CD8S6F1⁻ cells in peripheral blood and synovial fluid of our population sample. As shown in table 2, there was no significant difference in the percentage of HLA-DR cells

Table 1 Percentage of S6F1 and HLA-DR cells among CD8 cells in peripheral blood (PB) and synovial fluid (SF) from patients with rheumatoid arthritis (RA) and osteoarthritis (OA), and in peripheral blood from healthy subjects (HS)

		CD8 (%)	CD8S6F1 (%)	CD8S6F1 ⁻ (%)	CD8HLA-DR (%)
SF	RA (n = 15)	45.4 (12.9)*	39.5 (14.5)*	5.9 (2.9)*†	34.1 (9.5)*†
	OA (n = 10)	31.7 (6.0)*	21.8 (4.8)	9.9 (2.8)*	17.4 (6.9)*
PB	RA (n = 15)	30.3 (5.9)	17.2 (6.1)	13.1 (4.2)	8.7 (4.6)‡
	OA (n = 10)	27.5 (3.7)	13.7 (1.6)	13.8 (2.6)	2.7 (0.4)
	HS (n = 10)	27.4 (3.9)	14.7 (3.6)	12.7 (3.0)	2.2 (0.9)

Data expressed as mean (SD). Significant differences (unpaired *t* tests): *p* < 0.01 compared with *PB from paired patients, †SF from OA patients, and ‡PB from patients with OA and from healthy subjects.

Figure 1 Comparison of expression of HLA-DR antigen on CD8S6F1 cells in peripheral blood (top) and synovial fluid (bottom) in a patient with RA, by triple labelling experiments. Each panel illustrates intensity of staining by one antibody versus a second antibody, each dot representing one cell. In the middle histogram, relative cell numbers are plotted against the logarithmically increasing green fluorescence profiles of CD8S6F1-gated cells from peripheral blood (a), synovial fluid (b), and control cells (c).

between CD8S6F1 and CD8S6F1⁻ cell populations in peripheral blood, from patients with either RA or OA. In contrast with this, the percentage of HLA-DR cells among CD8S6F1 cell populations in the synovial fluid of patients with RA was markedly greater than that among CD8S6F1⁻ cell populations; however, synovial fluid of patients with OA showed no significant difference in the percentage of HLA-DR cells between these cell populations.

PHENOTYPIC CHARACTERISATION OF ADHERENT AND NON-ADHERENT T CELLS

Table 3 shows the results of the adhesion assay in the adherent, non-adherent, and unfractionated T cell populations. The percentage of CD8S6F1 cells among CD8 cells was

Figure 2 Comparison of S6F1 antigen expression on CD8S6F1 cells in unfractionated T cells (A) and in non-adherent (B) and adherent (C) T cell populations pre-incubated with IL-1 β stimulated endothelial cells. Mean fluorescence intensity of the S6F1 antigen detected by two-colour flow cytometry.

significantly greater in the adherent T cell population than in the non-adherent population in both the endothelial and that synovial cell assays. Our data indicate that CD8S6F1 cells bind preferentially to IL-1 β treated endothelial and synovial cells. As shown in figure 2 and table 3, the mean fluorescence intensity of S6F1 antigen on CD8S6F1 cells in the adherent T cell population was significantly greater than that of the non-adherent population. Similar results were obtained in the synovial cell adhesion assay.

Discussion

The present study confirmed our previous findings^{38, 39} and those of other investigators^{40, 41} that synovial fluid from RA patients contains a significantly greater percentage of cytotoxic T cells and a smaller percentage of suppressor T cells compared with peripheral blood of the same patient or normal subjects. We demonstrated previously³⁸ that the percentage of CD8CD11b⁻ cells was significantly greater in synovial fluid and synovial tissue compared with peripheral blood in RA patients and healthy subjects.

Using monoclonal antibodies different from those used in the present study. Goto *et al*⁴⁰ reported a low percentage of CD8Leu15 T cells in both peripheral blood and synovial fluid of RA patients. However, these phenotypic findings do not correlate with functional studies. For example, CD8CD11b cells are thought to be heterogeneous and include both HLA class I-restricted CD8 cytotoxic effector and suppressor cells, while CD8CD11b⁻ cells include natural-killer-like cells.⁴² Morimoto *et al*²⁶ have developed a monoclonal antibody, anti-S6F1, which can distinguish killer effector and suppressor effector cells in CD8 lymphocyte populations. The cell surface structure defined by this antibody comprises two glycoproteins of molecular weights 180 and 95 kDa. Sequential immunoprecipitation studies and two dimensional gel electrophoresis indicate that anti-S6F1 recognised the α (CD11a) and β (CD118) subunits of the

Table 2 Percentage of HLA-DR cells among CD8S6F1 and CD8S6F1⁻ cells from patients with rheumatoid arthritis (RA) and osteoarthritis (OA)

		CD8S6F1 HLA-DR/CD8S6F1 (%)	CD8S6F1 ⁻ HLA-DR/CD8S6F1 ⁻ (%)
SF	RA (n = 15)	77.5 (10.8)#	60.9 (13.2)#
	OA (n = 10)	48.1 (16.9)¶	40.6 (11.3)¶
PB	RA (n = 15)	30.1 (14.5)§	26.9 (11.5)§
	OA (n = 10)	11.7 (2.4)†*	8.3 (3.1)†*

Data expressed as mean (SD). Significant differences (unpaired *t* test): p < 0.01 compared with *SF of OA, †PB of RA, #CD8S6F1⁻ HLA-DR cells among CD8S6F1⁻ cells in SF of RA, §SF of RA; ¶p < 0.001 compared with SF of RA.

Table 3 Phenotypic characterisation of adherent and non-adherent T cells

Target	T cell fractions	CD8S6F1/CD8 (%)	S6F1 antigen on CD8S6F1 (Mean fluorescence intensity)
Endothelial cells	Adherent	86.5 (6.5)	152.3 (36.5)
	Non-adherent	34.2 (7.5)	16.3 (4.8)
	Unfractionated	52.2 (8.6)	56.3 (24.5)
Synovial cells	Adherent	75.3 (6.9)	120.4 (40.3)
	Non-adherent	41.3 (6.8)	23.4 (8.9)
	Unfractionated	53.5 (7.6)	49.8 (21.7)

Data expressed as mean (SD) of three experiments. Significant differences (unpaired *t* test): *p < 0.01, compared with non-adherent T cells.

lymphocyte function associated antigen 1 (LFA-1). Moreover, although the LFA-1 antigen is expressed on a broad spectrum of cell types,⁴³ the S6F1 antigen is expressed primarily on a subset of T cells, null cells, and a small fraction of macrophages and B cells. In the present study, synovial fluid of RA and OA patients contained significantly greater percentages of CD8S6F1 cells compared with peripheral blood in the same patient. In addition, our results demonstrated that synovial fluid of RA patients had a greater percentage of CD8S6F1 cells compared with that of OA patients. Abnormalities of T cell subsets in synovial fluid of RA patients support many of the reported findings on synovial fluid lymphocyte function.^{17 44–50} There were similarities in phenotypic and functional abnormalities in synovial fluid mononuclear cells in patients with RA. However, it is not known if the phenotypically defined cell subset, CD8S6F1 cells, in synovial fluid of RA patients show exactly the same cytotoxic T lymphocyte function as do normal cells. It is necessary, therefore, to investigate further the precise roles of CD8S6F1 cells in synovial fluid.

The induction of T cells by specific antigens or mitogens lead to the orderly appearance of a number of surface molecules that are important in cellular growth and function. In the present study, we analysed T cell activation, using a monoclonal anti-HLA-DR antibody to non-T-cell-restricted antigen with a known function.⁵¹ More CD8 cells than CD4 cells in rheumatoid synovial fluid have been reported to express HLA class II antigens,^{8 52} more CD8 cells than CD4 cells are reported to incorporate [³H] thymidine in sections of rheumatoid synovial tissue,⁵³ and most T cell lines and clones of CD8 cells from RA synovial fluid are more easily established than are CD4 cells.^{54–56} However, activated T cells in CD8 cell subsets have not been analysed previously. Our study demonstrated that the percentage of HLA-DR cells among CD8S6F1 cells in synovial fluid was significantly greater than that in peripheral blood in RA patients. There was no significant difference in the percentage of HLA-DR cells between CD8S6F1 and CD8S6F1[−] cell populations in peripheral blood. The percentage of HLA-DR cells in the CD8S6F1 cell population in synovial fluid of patients with RA was markedly greater than that in the CD8S6F1[−] cell population.

It has been demonstrated that induction of CD8 cytotoxic cells has a preferential requirement for CD4CD29 cells,⁵⁷ and that CD8CD29S6F1 cells exert class I-restricted killer effector activity.⁵⁸ Reciprocal CD4CD45RA cells were previously reported to induce CD8 cells to suppress antibody production.¹⁹ Thus it appears that activation of CD8 cells for suppression of cytotoxicity requires different subsets of CD4 cells. This implies that suppression and cytotoxicity have different activation requirements and represent distinct CD8 cell functions. In a previous study, we found that synovial CD4 cells carried mostly CD29 antigen, while only a few carried CD45RA.³⁹ On the basis of the observations

presented in the present and previous studies, we suggest that the combined increments of CD8S6F1 cells and CD4CD29 cells may lead to excessive cytotoxic activities in rheumatoid joints.

Adhesion molecules may mediate the interaction between lymphocytes and various accessory cells or target cells.^{59 60} These molecules are involved not only in extensive cell adhesion per se, but also in signal transduction and mediation of cellular events such as activation or proliferation.⁶¹ A wide variety of adhesion dependent lymphocyte interactions, in both antigen-dependent and -independent processes, are mediated through interaction of integrin LFA-1, with the Ig supergene family intercellular adhesion molecule-1 (ICAM-1), and interaction of the Ig supergene family LFA-2 (CD2) with LFA-3. Those interactions include T cell interaction with antigen presenting cells, cytotoxic T lymphocyte mediated killing, and lymphocyte binding to the endothelium.⁶² S6F1 recognise an epitope of LFA-1, which is a CD11a/CD18 integrin adhesion protein.²⁶ This antigen appears to facilitate endothelial adherence in association with specific vascular homing molecules. In the present study, the percentage of CD8S6F1 among CD8 cells and the mean fluorescence intensity of S6F1 antigen on CD8S6F1 cells were significantly greater in the adherent T cell population compared with the non-adherent T cell population. We have previously reported that T cells expressing LFA-1 and CD2 were more adherent to IL-1-stimulated synovial fibroblasts, and that adherent T cells had greater densities of LFA-1 α , LFA-1 β and CD2 than non-adherent T cells.²⁸ A recent immunofluorescence study showed that ICAM-1 was strongly expressed on vascular endothelial cells in the sublining layer of the synovium in patients with RA and, to a lesser extent, in that of patients with OA.^{63 64} Those results demonstrated that CD8S6F1 cells are activated by antigen stimulation in the synovial tissues and express a greater density of LFA-1. The present results suggest that the preferential capture of circulating CD8S6F1 cells by the endothelium, with a greater retention of these cells through their adhesion to extravascular cells (e.g. synovial cells), is important in the perpetuation of RA synovitis.

In summary, our results provide strong support for the hypothesis that CD8S6F1 cells may preferentially localise to inflammatory lesions of joints, where they consequently contribute to subsequent tissue destruction.

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