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# A role for the integrin $\alpha_6\beta_1$ in the differential distribution of CD4 and CD8 T-cell subsets within the rheumatoid synovium

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**Objective.** CD4 and CD8 T-cell subsets accumulate in distinct microdomains within the inflamed rheumatoid synovium. The molecular basis for their differential distribution remains unclear. Since chemokines and adhesion molecules play an important role in the positioning of leucocytes at sites of inflammation, we tested the hypothesis that the differential expression and function of chemokine and/or adhesion molecules explains why CD4$^+$ T cells accumulate within perivascular cuffs, whereas CD8$^+$ T cells distribute diffusely within the tissue.
**Methods.** Expression of an extensive panel of chemokine receptors and adhesion molecules on matched CD4$^+$ and CD8$^+$ T cells from peripheral blood (PB) and synovial fluid (SF) was analysed by multicolour flow cytometry. Migration assays and flow-based adhesion assays were used to assess the functional consequences of any differences in the expression of chemokine and adhesion receptors.
**Results.** CD4$^+$ and CD8$^+$ T cells from PB and SF expressed unique yet consistent patterns of chemokine and adhesion receptors. SF CD8$^+$ T cells were much less promiscuous in their expression of chemokine receptors than SF CD4$^+$ T cells. The $\alpha_6\beta_1$ integrin was highly expressed on PB CD4$^+$ T cells, but not on PB CD8$^+$ T cells. Laminin, the ligand for $\alpha_6\beta_1$, retained CD4$^+$ T cells, but less so CD8$^+$ T cells, within inflamed synovial tissue.
**Conclusion.** Infiltrating PB CD4$^+$ T cells, but not CD8$^+$ T cells, express functional levels of the $\alpha_6\beta_1$ integrin. We propose that this leads to their retention within the rheumatoid synovium in perivascular cuffs, which are defined and delineated by the expression of laminin.

Key words: T cells, Alpha 6 integrin, Chemokine receptors, Rheumatoid arthritis.

## Introduction

RA is a chronic persistent inflammatory disease characterized by hyperplasia of the synovial tissue and the infiltration of leucocytes within the inflamed synovium [1]. The predominant population of leucocytes within the inflamed synovium are T lymphocytes [2]. We have previously shown that the production of IFN-$\beta$ by synovial fibroblasts supports the survival of T cells and that infiltrating CD4 T cells up-regulate a number of chemokine receptors such as CXCR4, CXCR3 and CCR5 contributing to their retention within the synovium [3–5]. The infiltrating lymphocytes often form well-defined, organized structures within the synovium. There are three characteristic types of infiltrate: diffuse, where there is a lack of leucocyte organization; perivascular cuffs, where predominantly CD4$^+$ leucocytes surround the endothelium; and ectopic lymphoid structures, where the leucocytes are organized in such a way as to resemble lymphoid tissues [6].

An intriguing feature of the rheumatoid synovium in tissue where there is an organized lymphocyte infiltrate is the difference in distribution of CD4$^+$ and CD8$^+$ T cells within the inflamed synovium. CD4$^+$ T cells are located next to blood vessels, in perivascular cuffs, while CD8$^+$ T cells are distributed more sparsely within the tissue parenchyma [7]. We have previously shown that CD8$^+$ T cells display an increased ability to migrate under synovial fibroblasts *in vitro* compared with CD4$^+$ T cells suggesting that factors intrinsic to the different T-cell subsets contribute to their differential distribution within the rheumatoid synovium [8].

In this study, we examined a wide range of chemokine and adhesion receptors that might be responsible for these intrinsic differences between CD4$^+$ and CD8$^+$ T cells. We show that CD4$^+$ and CD8$^+$ T cells express different levels of the $\alpha_6\beta_1$ integrin. This integrin is the main laminin receptor for leucocytes. Laminin is a major component of the basement membrane and surrounding vascular parenchyma in rheumatoid synovial tissue [9, 10]. Using flow-based detachment assays we demonstrate that CD4$^+$ T cells but not CD8$^+$ T cells selectively bind to laminin using the integrin $\alpha_6\beta_1$. Importantly, the expression of this integrin on CD4$^+$ T cells from the synovial fluid (SF) was decreased compared with the expression found on peripheral blood (PB) and synovial membrane (SM) CD4$^+$ T cells. Our findings suggest that differential expression and function of the $\alpha_6\beta_1$ integrin contributes to the retention and spatial distribution of CD4$^+$ and CD8$^+$ T-cell subsets within the rheumatoid synovium.

## Materials and methods

### Media, cytokines and antibodies

Chemokines CCL5, CCL19, CXCL9 and CXCL12 were purchased from PeproTech, Peterborough, UK. These chemokines were titrated to achieve maximal migration for both PB and SF samples and were used at CXCL12 1 $\mu$g/ml; CCL5 200 ng/ml; CCL19 1 $\mu$g/ml; CXCL9 1 ng/ml; CCL22 10 ng/ml; CCL2 20 ng/ml; CCL20 100 ng/ml; CXCL16 1 $\mu$g/ml. Antibodies to chemokine receptors were the same as used previously [4].

Antibodies to adhesion molecule receptors were: CD49a ($\alpha_1$ integrin) (HP2B6, IgG1) Immunotech (Marseille, France), CD49b ($\alpha_2$ integrin) (AK7, IgG1) Serotec, CD49c ($\alpha_3$ integrin) (P1B5, IgG1) AbD Serotec (Oxford, UK), CD49d ($\alpha_4$ integrin) (HP2/1 IgG1) Serotec, CD49e ($\alpha_5$ integrin) HLDA 8 workshop 8, CD49f ($\alpha_6$ integrin) (GoH3, rat IgG2a) BD Pharmingen (San Jose, CA, USA), CD103 ($\alpha_E$ integrin) (2G5, IgG2a) Immunotech, CD162 (PSGL-1) (5D8.8.12, IgG1) Immunotech, CLA (HECA-452, rat IgM) BD Pharmingen, $\alpha_4\beta_7$ (Act-1, IgG1) a gift from Leukosite Inc., Cambridge, MA, USA, CD29 ($\beta_1$ integrin) 3S3, IgG1 BD Pharmingen, CD18 ($\beta_2$ integrin) (MHM23, IgG1) Dako (Glostrup, Denmark), CD61 ($\beta_3$ integrin) (RUU-PL 7F12, IgG1) BD Pharmingen.

### Patients, PB, synovial tissue and SF preparation

All 29 patients with RA used in this study, fulfilled 1987 ACR classification criteria for RA [11]. The details, including which

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samples (PB, SF and SM) were taken from which patients, are shown in Table 1. Samples from venous PB and SF were collected into preservative-free heparin. PB lymphocytes (PBLs) and SF lymphocytes (SFLs) were isolated as previously described [4]. Synovial tissue was obtained from patients undergoing routine synovectomy. Tissue was finely minced with a scalpel being careful to remove fat. The minced tissue was then incubated with 2 mM EDTA solution for 4 h at 4°C and then passed through several filters (100 $\mu$m size, Falcon, San Jose, CA, USA). Lymphocytes were separated on a Ficoll gradient. This enzyme-free method ensured that no cell surface molecules would be cleaved off the surface of the lymphocytes. Patient consent as well as ethical approval from the Birmingham Research Ethics Committee (REC 2002/088 and LREC 5735) was obtained for the use of these samples.

### *IF microscopy*

Synovial tissue was removed from patients undergoing joint replacement surgery. All samples fulfilled the 1987 criteria for RA [11]. IF was performed as previously described [4].

### *Flow cytometry*

Analysis of cell surface molecules was performed using three-colour IF as previously described [4]. For analysis of chemokine receptor expression PBL and SFL were stained with anti-chemokine receptor antibodies followed by FITC-labelled secondary antibodies (Southern Biotechnology Associates Inc., Birmingham, AL, USA). The samples were analysed on an EPICS XL Flow cytometer (Beckman-Coulter Inc., Fullerton, CA, USA). Cytometer calibration was standardized using fluorospheres (Immunocheck and Standardbrite, Beckman-Coulter Inc., USA). Data was analysed using WinMDI v2.8, Scripps Institute (La Jolla, CA, USA).

### *Transmigration assays for functional chemokine receptor expression*

Viable cells (determined by trypan blue exclusion) were placed in 5 $\mu$m pore Transwell inserts (Corning, NY, USA). Chemokines used for the assay were pre-warmed in 0.5% BSA/RPMI at 37°C before adding them to the bottom chamber of the Transwell. Specific migration was calculated as the percentage of input cells migrated in the presence of the appropriate chemokine minus percentage of input cells migrated in medium alone.

### *Controlled detachment adhesion assay*

Controlled detachment assays were performed as previously described [12]. Glass microslides were coated with either 10 $\mu$g/ml recombinant laminin or fibronectin (Sigma) with 2 $\mu$g/ml of CXCL12 before being blocked with a 1% solution of albumin before use. $CD4^+$ and $CD8^+$ $CD45RA^-$ (primed) T cells were isolated from PB of volunteers using a MoFlo cell sorter and were allowed to adhere to the micro-slides for 4 min under static conditions before the flow (0.1 Pa) was switched on again. The percentage adhesion was calculated from the average number of cells in eight fields of view, counted before and after the flow was switched on using images captured on videotape.

### *Statistical methods*

The unpaired Student's *t*-test was used to compare results obtained by comparing SF with PB samples, or CD4 *vs* CD8 T cells.

## Results

### *Expression of chemokine receptors on $CD4^+$ and $CD8^+$ T cells from PB and SF*

We have previously shown that specific chemokine receptors contribute to the accumulation of $CD4^+$ T cells in the rheumatoid synovium [4, 13]. However, to our knowledge there have not been any studies that have directly compared the pattern of chemokine and adhesion receptor expression on $CD4^+$ *vs* $CD8^+$ T cells in the rheumatoid synovium. Matched $CD4^+$ and $CD8^+$ T cells from PB and SF were compared for their expression of chemokine receptors using flow cytometry. We found significant differences in the expression of chemokine receptors on $CD4^+$ and $CD8^+$ T cells

TABLE 1. Details of patients used in this study

| Patient code | Sex | Date of birth | Duration of treatment | RF latex | Sample site | Use of sample | Treatment |
|---|---|---|---|---|---|---|---|
| T03.143 | Male | 30.07.54 | 2 yrs | – | Left knee | PB, SF | Celecoxib |
| T03.167 | Female | 17.7.35 | 15 yrs | + | Knee | PB, SF | MTX |
| T03.172 | Female | 3.12.24 | | | Left knee | PB, SF | Prednisolone |
| T03.175 | Male | | <1 yr | | Left knee | PB, SM | SSZ, prednisolone |
| T03.176 | Female | 3.11.80 | | | | PB, SF | MTX |
| T03.185 | Female | 25.10.02 | | | Left knee | PB, SF | SSZ, MTX |
| T03.224 | Female | 12.3.56 | | | | PB, SF | Nil |
| T03.223 | Male | 15.5.46 | | | Right knee | PB, SM | MTX |
| T03.226 | Female | 5.6.61 | 13 yrs | + | Knee | PB, SM | Prednisolone |
| T03.229 | Female | 20.10.38 | 35 yrs | + | Knee | PB, SM | Infliximab, MTX, prednisolone |
| T03.241 | Female | 10.05.41 | <5 yrs | | Knees | PB, SF | Celecoxib |
| T03.254 | Female | 12.10.49 | 26 yrs | + | Knee | PB, SM | LEF, prednisolone |
| T03.258 | Female | 27.10.57 | 22 yrs | N/A | Knee | PB, SM | LEF, nabumetone |
| T03.261 | Male | 29.04.42 | <6 months | | Right knee | PB, SF | MTX |
| T03.342 | Female | 26.5.41 | 10 yrs | + | Knee | PB, SF | Meloxicam, gold, prednisolone |
| T03.351 | Male | | 10 yrs | + | Knee | PB, SF | MTX |
| T03.426 | Female | 02.05.76 | | + | Knee | PB, SM | MTX |
| T04.47 | Female | 27.03.43 | 6 yrs | + | Knee | PB, SF | Etanercept |
| T04.54 | Male | 23.11.60 | 5 yrs | N/A | Knee | PB, SM | MTX |
| T04.68 | Female | 06.03.53 | 30 yrs | – | Knee | PB, SF | MTX |
| T04.89 | Female | 27.02.60 | 15 yrs | + | Knee | PB, SF | Naproxen |
| T04.92 | Female | 11.09.79 | 4 yrs | N/A | Knee | PB, SF | Gold |
| T04.143 | Female | 14.09.22 | | N/A | Knee | PB, SF | MTX |
| T04.144 | Male | 27.04.35 | | + | Knee | PB, SM | MTX |
| T04.165 | Female | 18.12.47 | | + | Knee | PB, SM | MTX |
| T04.192 | Female | 02.07.32 | 30 yrs | + | Knee | PB, SM | Meloxicam, AZA |
| T04.228 | Female | 01.07.58 | | N/A | Knee | PB, SF | MTX |
| T04.263 | Female | 17.09.36 | 6 yrs | + | | PB, SF | Meloxicam, adalimumab |
| T04.268 | Male | 10.07.38 | | N/A | Knee | PB, SM | MTX |

The age, sex, duration of treatment (if known), RF, sample site and treatment for all 29 patients used in this study are shown. Details as to which patients provided PB, SF or synovial membrane (SM) are also included (use of sample column).

from PB and SF (Fig. 1A). As we have previously reported [4] the chemokine receptors CCR2, CCR4, CCR5, CXCR3, CXCR4 and CXCR6 were up-regulated on CD4$^+$ T cells in the SF compared with PB. For CD8$^+$ T cells fewer chemokine receptors were up-regulated on SF compared with matched PB namely, CCR5, CXCR4 and CXCR6. Of note, compared with CD4$^+$ SF T cells, CD8$^+$ SF T cells did not express CCR2, CCR4 or CCR6 (Fig. 1A and B). This suggested to us that differences in the function of CCR2, CCR4 or CCR6 on CD4$^+$ *vs* CD8$^+$ SF T cells might contribute to their differential accumulation.

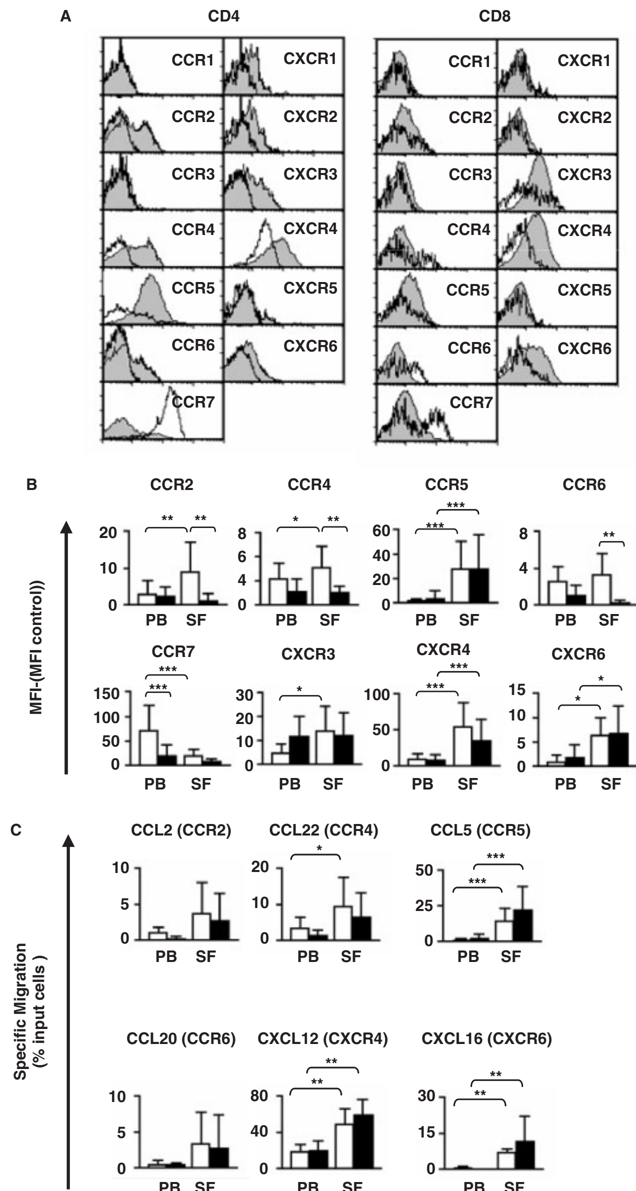


Fig. 1. Expression and function of chemokine receptors on PB and SF CD4$^+$ and CD8$^+$ T cells. (**A**) The expression of chemokine receptors on primed (CD45RA$^-$[RO$^+$]) T cells from PB and SF were compared on CD4$^+$ and CD8$^+$ T cells. Representative histograms are shown with SF (grey) and PB (white). (**B**) Collected results in which the expression of chemokine receptors is indicated as the median fluorescent intensity (MFI) minus that of the isotype control. Clear bars: CD4$^+$; filled bars CD8$^+$: T cells. Error bars show the mean ± s.d. from a minimum of three different experiments. $^*P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$, Student's unpaired *t*-test. (**C**) Migration assays were performed to examine the functional consequences of the differences in chemokine receptor expression on PB and SF CD4$^+$ and CD8$^+$ T cells. Clear bars: CD4$^+$ CD45RA$^-$ T cells; filled bars: CD8$^+$ CD45RA$^-$ T cells. Specific migration (percentage of input cells migrated in the presence of the appropriate chemokine minus spontaneous migration in medium alone) is shown. Error bars show the mean ± s.d. from a minimum of three different experiments $^*P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$, Student's unpaired *t*-test.

## *Migration of PB and SF CD4$^+$ and CD8$^+$ T cells*

In order to examine whether the expression of the differentially expressed chemokine receptors CCR2, CCR4 or CCR6 on CD4$^+$ and CD8$^+$ cells was functionally relevant we performed transmigration assays to the known ligands for these receptors. As we have previously reported, T cells (CD4 and CD8) in SF migrate more robustly in Transwell chambers than PB T cells (CD4 and CD8) in the absence of chemokines [4]. Spontaneous migration to medium alone was about 10% of input cells for both CD4 and CD8 SF T cells compared with <2% for CD4 and CD8 PB cells (data not shown). This spontaneous migration was subtracted from all assays to show chemokine-specific migration. Both SF CD4$^+$ and CD8$^+$ T cells migrated equally well to CCL2 (MCP-1), CCL22 (MDC) and CCL20 (MIP-3$\alpha$), ligands for CCR2, CCR4 and CCR6, respectively (Fig. 1C). This was despite the fact the SF CD8$^+$ T cells expressed much lower levels of CCR2, CCR4 and CCR6 than SF CD4$^+$ T cells (Fig. 1B). As positive controls we found that CCL5, CXCL12, CXCL16, ligands for CCR5, CXCR4 and CXCR6, respectively, all induced higher levels of migration in both SF CD4 and CD8 T cells compared with PB CD4$^+$ and CD8$^+$ T cells (Fig. 1C). As these effects were seen over a wide dose response, these results suggested to us that CCR2, CCR4 and CCR6 were unlikely to play a role in regulating the relative distribution of CD4$^+$ and CD8$^+$ T cells within the rheumatoid synovium.

## *Expression of adhesion molecules on CD4$^+$and CD8$^+$ T cells*

We next examined whether differential adhesion molecule expression on CD4$^+$ and CD8$^+$ T cells from PB and SF might provide an explanation for the differential distribution of CD4$^+$ and CD8$^+$ T cells (Fig. 2). We noted that CD4$^+$ T cells from PB always expressed higher levels of the $\alpha_6$ integrin than PB CD8$^+$ T cells. In comparison, CD4$^+$ T cells from SF expressed lower levels of this integrin compared with matched PB CD4$^+$ T cells (Fig. 2A and B). Both PB and SF CD8$^+$ T cells expressed very low levels of the $\alpha_6$ integrin. The $\alpha_6$ integrin is the main laminin receptor on leucocytes and laminin is a major component of the basement membrane, a structure that surrounds the basolateral surface of blood vessels, where CD4$^+$ T cells accumulate [14–17]. We therefore hypothesized that this receptor–ligand pair contributed to the differential accumulation of CD4$^+$ T cells in perivascular cuffs.

## *PB CD4$^+$ T cells bind to laminin*

To test whether these differences in $\alpha_6$ integrin expression were functionally relevant we performed flow-based detachment assays comparing PB and SF CD4$^+$ and CD8$^+$ T cell adhesion to human laminin. CD4$^+$ and CD8$^+$ T cells were isolated from PB by FACS cell sorting and their adhesion onto glass microslides coated with laminin was assessed. Figure 3A shows that neither CD4$^+$ nor CD8$^+$ T cells can bind efficiently to laminin without the presence of a chemokine to activate their integrins. With the addition of CXCL12 (SDF-1) more PB CD4$^+$ T cells bound to laminin (50–60%) compared with PB CD8$^+$ T cells (20%). The inability of CD8$^+$ T cells to bind laminin was not due to an intrinsic loss of adhesion by CD8$^+$ compared with CD4$^+$ cells as both PB CD4$^+$ and CD8$^+$ T cells bound to fibronectin (Fn) in the presence of CXCL12. Neither SF CD4$^+$ nor SF CD8$^+$ cells were able to bind laminin either in the absence or presence of CXCL12 (Fig. 3B). To confirm that the adhesion of PB CD4$^+$ was specific for the $\alpha_6\beta_1$ integrin, CD4$^+$ T cells were pre-incubated with an isotype control antibody, a blocking antibody to the $\alpha_6$ integrin or a blocking antibody to the $\beta_1$ integrin before adhesion was measured. Figure 3C shows that the addition of blocking antibodies to both $\alpha_6$ and $\beta_1$ integrin but not an isotype control antibody completely inhibited the ability of CD4$^+$ T cells to adhere to laminin confirming that PB CD4$^+$ T cells adhere to laminin using the $\alpha_6\beta_1$ integrin.

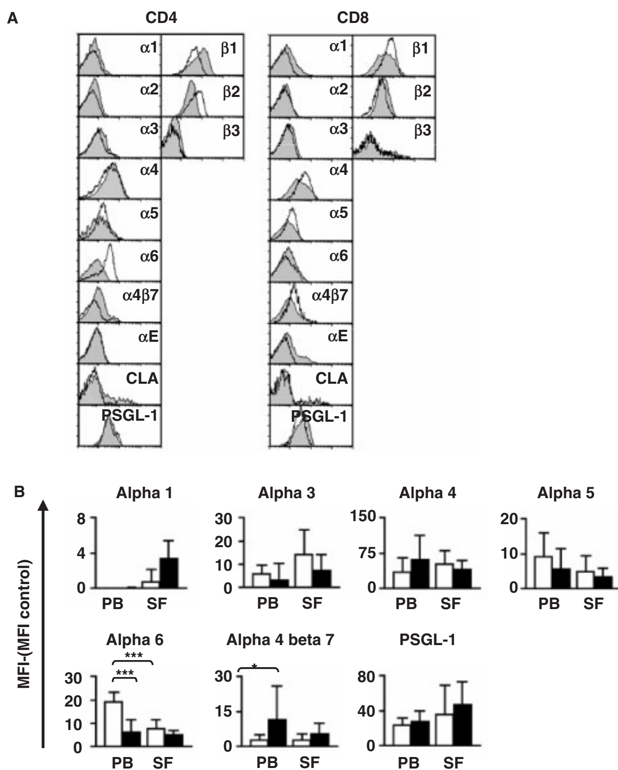


Fig. 2. Expression of adhesion molecule receptors on $CD4^+$ and $CD8^+$ T cells from PB and SF. (**A**) The expression of adhesion molecule receptors on primed ($CD45RA^-[RO^+]$) from PB and SF was compared on $CD4^+$ and $CD8^+$ T cells. Representative histograms are shown with SF (grey) and PB (white). (**B**) Collected results in which the expression of adhesion molecule receptors is indicated as the MFI minus that of the isotype control. Clear bars: $CD4^+$; filled bars: $CD8^+$ T cells. Error bars show the mean ± s.d. from a minimum of three different experiments. $^*P < 0.01$, $^{**}P < 0.001$, $^{***}P < 0.0001$, Student's unpaired *t*-test.

### *Expression of $\alpha_6$ integrin on CD4 T cells in the rheumatoid synovium*

These findings suggested to us that the $\alpha_6$ integrin, expressed on $CD4^+$ T cells that enter the synovium from the PB, might contribute to the accumulation of $CD4^+$ cells within perivascular cuffs in the rheumatoid synovium. In order to examine this possibility we performed flow cytometric analysis of PB, SM and SF $CD4^+$ and $CD8^+$ T cells. SM $CD4^+$ T cells but not $CD8^+$ T cells, expressed high levels of the $\alpha_6$ integrin, comparable with that observed in PB (Fig. 4A). In order to determine the relationship of $CD4^+$ and $CD8^+$ T cells to laminin, the major ligand for $\alpha_6\beta_1$, we performed confocal microscopy on rheumatoid synovial tissue from three different patients (Fig. 4B). $CD4^+$ T cells were confined within a perivascular cuff defined by a clear laminin boundary compared with a much less confined distribution of $CD8^+$ T cells.

## Discussion

In this study, we set out to determine whether differences in the expression of chemokine receptors and adhesion molecules on $CD4^+$ and $CD8^+$ T cells might contribute to their differential distribution in perivascular cuffs within the rheumatoid synovium. We compared $CD4^+$ and $CD8^+$ T cells from matched PB and SF and found that $CD4^+$ T cells from SF expressed high levels of CCR2, CCR4 and CCR6 whereas $CD8^+$ T cells from SF do not (Fig. 1). Both $CD4^+$ and $CD8^+$ T cells up-regulated the chemokine receptors CCR5, CXCR4 and CXCR6 on SF compared with PB suggesting that there was selective up-regulation of chemokine receptors depending upon the T-cell subset examined. However, as these differences in expression did not translate into functional differences in migration, these results suggested to us that CCR2,

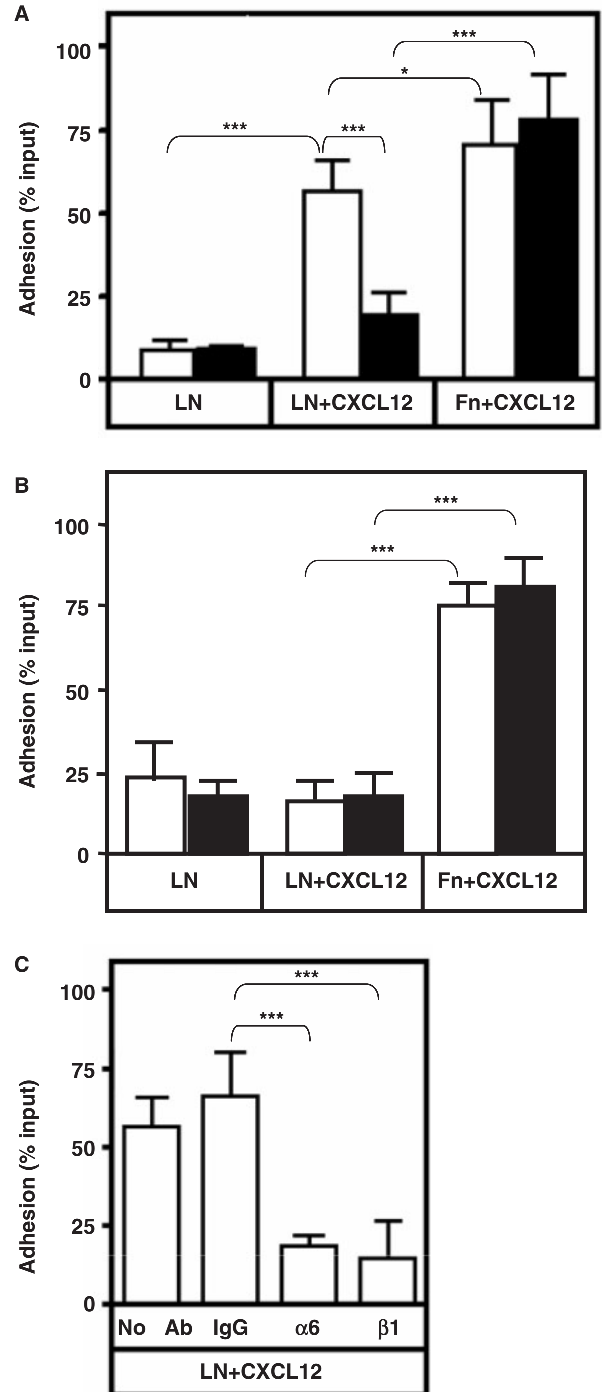


Fig. 3. PB $CD4^+$ but not $CD8^+$ T cells bind to laminin. (**A**) $CD4^+$ and $CD8^+$ T cells ($CD45RA^-$) were isolated from PB by cell sorting (see Materials and methods) and used for flow-based adhesion assays. Only $CD4^+$ T cells bound to laminin (LN) in the presence of CXCL12. Both CD4 and CD8 T cells bound to fibronectin (Fn) in the presence of CXCL12. Clear bars: $CD4^+$ T cells; filled bars: $CD8^+$ T cells. Error bars show the mean ± s.d. of cell adhesion from a minimum of three different experiments. $^*P < 0.01$, $^{***}P < 0.0001$, Student's unpaired *t*-test. (**B**) $CD4^+$ and $CD8^+$ T cells ($CD45RA^-$) were isolated from SF by cell sorting (see Materials and methods) and used for flow-based adhesion assays. Neither $CD4^+$ nor $CD8^+$ T cells bound to laminin (LN) in the absence or presence of CXCL12. Clear bars: $CD4^+$ T cells; filled bars: $CD8^+$ T cells. Error bars show the mean ± s.d. of cell adhesion from a minimum of three different experiments. $^{***}P < 0.0001$, Student's unpaired *t*-test. (**C**) The binding of $CD4^+$ T cells to laminin is specific to the $\alpha_6\beta_1$ integrin as blocking antibodies to either the $\alpha_6$ or $\beta_1$ subunit completely abrogated the ability of $CD4^+$ T cells to bind to laminin. Rat IgG2a was used as an isotype control. Error bars show the mean ± s.d. of cell adhesion from a minimum of three different experiments. $^{***}P < 0.0001$, Student's unpaired *t*-test.

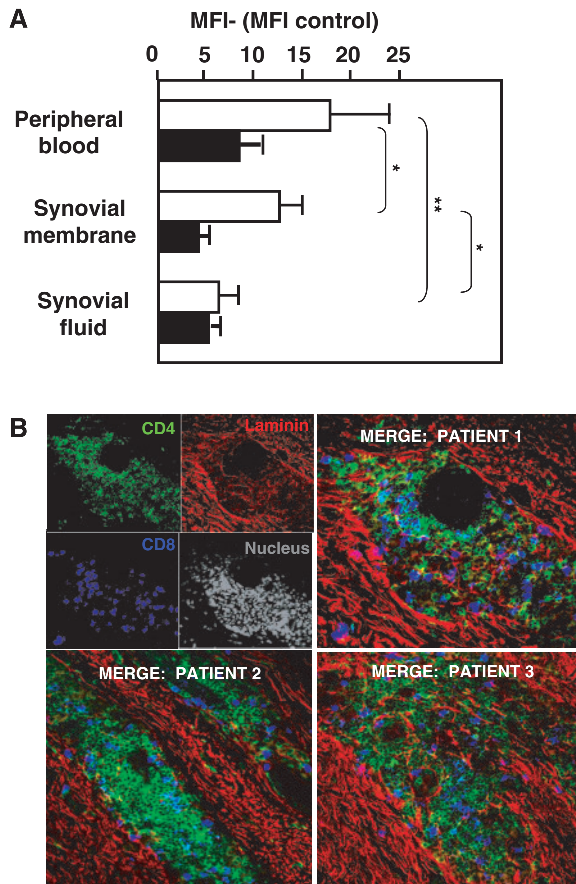


FIG. 4. Expression of $\alpha_6$ integrin on CD4 and CD8 T cells in the rheumatoid synovium. (**A**) Comparison of the expression of the $\alpha_6$ integrin on $CD4^+$ and $CD8^+$ T cells in PB, SM and SF. Clear bars: $CD4^+$ T cells; filled bars: $CD8^+$ T cells. MFI $\pm$ S.D. of three separate experiments from three different patients are given. $^*P < 0.01$, $^{**}P < 0.001$, Student's unpaired *t*-test. (**B**) Immunofluorescent confocal microscopy was performed on synovial tissue from six different patients. Representative pictures from three patients with CD4 in green, CD8 in blue and laminin in red are shown. Nuclear counterstain is shown in grey for the first patient. All images are $\times 25$ magnification 2.

CCR4 and CCR6 were unlikely to play a role in regulating the relative distribution of $CD4^+$ and $CD8^+$ T cells within the rheumatoid synovium.

We found that $\alpha_6$ integrin was highly expressed on PB $CD4^+$ but not $CD8^+$ T cells. PB $CD8^+$ T cells and $CD4^+$ and $CD8^+$ T cells from the SF lacked expression of this integrin. The $\alpha_6$ integrin is the main laminin receptor on leucocytes and has been proposed, in mice, to play a role in leucocyte trans-endothelial (TE) migration [15]. Since laminin is a major component of the tissue parenchyma that surrounds blood vessels [16, 17], this suggests that the $\alpha_6\beta_1$ integrin might play a role in the retention of $CD4^+$ T cells by binding to laminin found within the sub-endothlial tissue and thereby contribute to the formation of $CD4^+$ perivascular cuffs. In contrast, PB $CD8^+$ T cells that do not express $\alpha_6\beta_1$ integrin would not be retained within the parenchymal synovial tissue. In support of this, we have shown that PB $CD4^+$ T cells, but not $CD8^+$ T cells adhere to laminin in the presence of CXCL12 (SDF), and that this was dependent on the $\alpha_6\beta_1$ integrin (Fig. 3).

The $\alpha_6$ integrin has been shown to be up-regulated on PB $CD4^+$ T cells undergoing TE migration [18]. This result presents a paradox in that SF $CD4^+$ T cells that have undergone TE migration, but which have in addition migrated through synovial tissues, express lower levels of the $\alpha_6\beta_1$ integrin compared with PB and SM $CD4^+$ T cells (Fig. 4B). The mechanism(s) responsible for the down-regulation of $\alpha_6\beta_1$ expression in SF is not clear. We cultured PB $CD4^+$ T cells in the presence of SF, but this had no effect on the expression of the $\alpha_6\beta_1$ integrin (data not shown). It has also been shown previously that $PGE_2$ signalling contributes to the ability of Langerhans cells to migrate from the epidermis to the draining lymph node [19]. The process of Langerhans cell migration from the skin was shown to involve the down-regulation of the expression of the $\alpha_6\beta_1$ integrin [20]. It is not clear if a similar mechanism exists for $CD4^+$ T cells. However, when PB $CD4^+$ T cells were cultured in the presence of $PGE_2$, this had no effect on $\alpha_6\beta_1$ integrin expression (data not shown).

Our findings suggest that $CD4^+$ T cells from the PB expressing high levels of the $\alpha_6\beta_1$ integrin enter the inflamed tissue where the $\alpha_6\beta_1$ integrin becomes active, possibly though activation by CXCL12. This leads to their retention through binding to laminin in the subendothelial parenchyma. In contrast, the low expression of $\alpha_6\beta_1$ integrin on $CD8^+$ T cells might allow them to migrate out of synovial tissue into SF as they would be unable to adhere to the laminin within the synovial tissue.

In conclusion, we have found novel differences between $CD4^+$ and $CD8^+$ T cells in their expression of chemokine receptors and adhesion molecules that might contribute to the differential retention of $CD4^+$ T cells in perivascular cuffs found in inflamed rheumatoid synovium. Our findings also offer a potential molecular explanation for the well-known but poorly understood differences in CD4: CD8 ratios between the synovial tissue (more $CD4^+$ than $CD8^+$ T cells) compared with SF (more $CD8^+$ than $CD4^+$ T cells) [7].

## Rheumatology key messages

- Synovial tissue CD4 and CD8 T cells express different functional levels of the $\alpha_6\beta_1$ integrin.
- This difference may account for the differential accumulation of CD4 but not CD8 T cells in perivascular cuffs in the rheumatoid synovium.

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*Disclosure statement*: The authors have declared no conflicts of interest.

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