Increased Expression of α(1,3)-Fucosyltransferase-VII and P-Selectin Binding of Synovial Fluid T Cells in Juvenile Idiopathic Arthritis

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ABSTRACT. ***Objective***. The mechanisms controlling the recruitment of T helper type 1 (Th1) cells to the inflamed synovium are not fully understood. Here, we focus on α(1,3)-fucosyltransferase-VII (FucT-VII), an enzyme responsible for the generation of functional P- and E-selectin ligands that is upregulated in Th1 cells.

Methods. Expression of transcripts encoding FucT-VII, interferon-γ (IFN-γ), and interleukin 12Rß2 (IL-12Rß2) were analyzed in T cells purified from synovial fluid (SF) and from peripheral blood (PB) of children with juvenile idiopathic arthritis (JIA) using kinetic reverse transcriptase polymerase chain reaction analysis. Binding of SF and PB T cells to P-selectin was determined by flow cytometry using a soluble P-selectin/IgG1 fusion molecule. Recruitment of T cells to synovial tissue *in vivo* was studied by analyzing the migration of FucT-VII transfected Jurkat T cells into human rheumatoid synovial tissue grafted into SCID mice.

Results. In patients with JIA, the mRNAlevels of FucT-VII, as well as of IFN-γ and IL-12Rß2, were up-regulated in SF T cells compared to paired PB T cells. A higher expression of FucT-VII mRNA in SF T cells was associated with increased binding of T cells to P-selectin. Moreover, FucT-VII expression and increased P-selectin binding capacity of T cells were associated with a polyarticular course of oligoarticular JIA. Expression of FucT-VII in Jurkat T cells resulted in an increased accumulation of these cells in human rheumatoid synovial tissue grafted into SCID mice.

Conclusion. Our data indicate an important role of FucT-VII in the enhanced homing of T cells to the inflamed synovium. (J Rheumatol 2003;30:1611–5)

Key Indexing Terms:

T LYMPHOCYTES | Th1/Th2 CELLS | RHEUMATOID ARTHRITIS
ADHESION MOLECULES | CELLTRAFFICKING | INFLAMMATION

In rheumatoid arthritis (RA) and juvenile idiopathic arthritis (JIA) synovium is infiltrated with mononuclear cells, which are predominantly T cells with a proinflammatory type 1 cytokine profile, producing interferon-γ (IFN-γ) and tumor necrosis factor-α (TNF-α)[1-3]. The first step of T lymphocyte extravasation is tethering to, and subsequent rolling on, the vascular endothelium, largely mediated by the interaction of P- and E-selectin expressed on endothelium with functional selectin ligands on T cells. Functional P- and E-selectin ligands are preferentially expressed on Th1 versus Th2 cells and have a critical role in the recruitment of Th1 cells to inflammatory sites[4,5]. Fucosylation is essential for

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Supported by a grant from the EC (TAGAPO 1999-00202).

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Submitted April 1, 2002; revision accepted December 5, 2002.

the function of selectin ligands, and it is controlled by fucosyltransferases, in particular by α(1,3)-fucosyltransferase-VII (FucT-VII). The crucial role of FucT-VII has been demonstrated by impaired lymphocyte homing and leukocyte extravasation to sites of inflammation in FucT-VII$^{-/-}$ mice[6]. In T cells FucT-VII expression is restricted to the Th1 subset and up-regulated by interleukin 12 (IL-12)[7-10].

We evaluated FucT-VII mRNAexpression and P-selectin binding capacity of synovial fluid (SF) T cells of patients with JIA, and the role of ectopic expression of FucT-VII in Jurkat cells in their accumulation into human rheumatoid synovium grafted into SCID mice.

MATERIALS AND METHODS

Twenty-three patients with JIA (age range 1.8-18.3 yrs; disease duration 1.1-10.6), according to the ILAR criteria[11], were studied. Sixteen had antinuclear antibody positive oligoarticular, 3 had polyarticular, and 4 systemic JIA. None were positive for HLA-B27 or rheumatoid factor. Oligoarticular JIA patients were divided in 2 groups according to disease course: (1) persistent oligoarticular (affecting ≤ 4 joints through the disease course) (2) extended oligoarticular (≥ 5 joints through the disease course)[11]. SF and peripheral blood (PB) were collected at the time of intraarticular steroid injection. Permission to draw extra blood during routine venipuncture was obtained from parents.

Mononuclear cells (MC) from SF and PB were isolated by Ficoll-Paque and T cells purified (> 95% CD3+) by anti-CD3 coated magnetic microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). Total RNA was purified with RNeasy columns (Qiagen, Valencia, CA, USA). The relative expression levels of IFN-γ, IL-12Rß2, and FucT-VII were determined using kinetic reverse transcription polymerase chain reaction (RT-PCR) as described[10]. For analysis of P-selectin binding, all incubation and washes were performed in saline, 5% fetal calf serum, 3 mM $CaCl_2$ and 7 mM $MgCl_2$. MC were incubated for 30 min at 4°C with a FITC-conjugated anti-CD3 (Becton Dickinson, Mountain View, CA, USA) in the presence or absence of 40 µg/ml of P-selectin/IgG1 (Pharmingen, San Diego, CA, USA). Binding of P-selectin was revealed with a PE-conjugated $F(ab)_2$ goat anti-human IgG (Jackson Laboratories, West Grove, PA, USA). Cells were analyzed with a FACScan cytometer (Becton-Dickinson). Jurkat cells were transfected with FucT-VII cDNAcloned in pIREShyg (Clontech, Palo Alto, CA, USA) and selected with hygromycin B. P-selectin binding was evaluated in a static adhesion assay[10]. Synovial tissue was transplanted from adult RApatients requiring joint replacement into Beige SCID C.B-17 mice as described[12]. Transplants were extracted, snap-frozen 48 h post intravenous injection into the tail vein of transplanted SCID mice of 5×10^6 PKH26-labeled wild-type and FucT-VII-transfected Jurkat cells (3 animals/condition), and analyzed by fluorescence microscopy.

RESULTS

Consistent with a Th1 skewing in synovium, mRNA levels of IFN-γ and of the IL-12 receptor ß2 chain were significantly up-regulated in SF T cells compared to PB T cells (Figure 1A and B). Analysis of FucT-VII mRNA showed that SF T cells expressed significantly higher levels ($p < 0.001$) of FucT-VII mRNAthan PB T cells (Figure 1C), with no significant difference among the 3 JIA onset types (not shown). The fold-increase in FucT-VII mRNAexpression in SF T cells compared to PB T cells was significantly higher ($p = 0.01$) in patients with extended versus in those with persistent oligoarticular JIA(Figure 1D).

Since FucT-VII is crucial in the generation of functional P-selectin ligands[6], we analyzed whether the increased FucT-VII mRNAexpression in SF T cells was related to P-selectin binding. A typical staining with the P-selectin/IgG fusion construct of PB and SF T cells is shown in Figure 2A. In JIA patients both the mean intensity of staining (not shown) and the percentage of positive cells for the P-selectin reagent were significantly higher for SF than for PB T cells ($p < 0.001$) (Figure 2B). This finding provides a functional correlation between the increased FucT-VII mRNA expression and the ability of T cells to bind P-selectin in SF T cells. Similar to what was found for FucT-VII mRNA, the fold-increase in the percentage of SF T cells binding P-selectin compared to PB was significantly higher ($p = 0.02$) in patients with extended versus those with persistent oligoarticular JIA (Figure 2C).

Overexpression of FucT-VII and the subsequent increased P-selectin binding in SF T cells points to a potential role of FucT-VII in the recruitment of Th1 cells into inflamed joints. To investigate whether FucT-VII expression could directly contribute to T cell migration/retention into synovium *in vivo*, we studied the capacity of Jurkat cells stably transfected with FucT-VII (Jurkat/FucT-VII) to migrate into rheumatoid synovium transplanted into SCID mice. In contrast to wild-type Jurkat cells, which do not express detectable levels of FucT-VII mRNA (not shown), FucT-VII transfected Jurkat cells expressed sialy Lewis^x related epitopes at a high level (Figure 3A) and displayed also high avidity P-selectin ligands capable of mediating cell binding in static adhesion assays (Figure 3B). We injected wild-type and Jurkat/FucT-VII, labeled with the PKH26 dye, intravenously into transplanted SCID mice with human synovium. Jurkat/FucT-VII accumulated in the synovium at least 3-fold more than wild-type Jurkat cells (Figure 3C).

DISCUSSION

Using kinetic RT-PCR that allows precise quantification of the initial amount of mRNA, we found elevated mRNA levels for FucT-VII, IFN-γ, and IL-12Rß2 in SF T cells of JIApatients, compared to paired samples of PB T cells. The Th1 skewing found in SF T cells, shown by the marked increase in IFN-γ mRNA, is in agreement with studies showing that lymphocytes in JIA and in RA synovium display a predominant Th1 pattern of cytokine production[1,3]. Since IL-12 is required for development of Th1 cells and a functional IL-12 receptor is necessary for maintaining IL-12 responsiveness and controlling Th1 commitment[13], the finding of increased IL-12Rß2 mRNAlevel in SF is consistent with a Th1 skewing in the synovium of JIA patients. The finding that FucT-VII mRNA expression is markedly increased in SF T cells of JIA patients, together with our previous observation that FucT-VII mRNA expression is increased in SF T cells of adult RA patients[10], suggests that

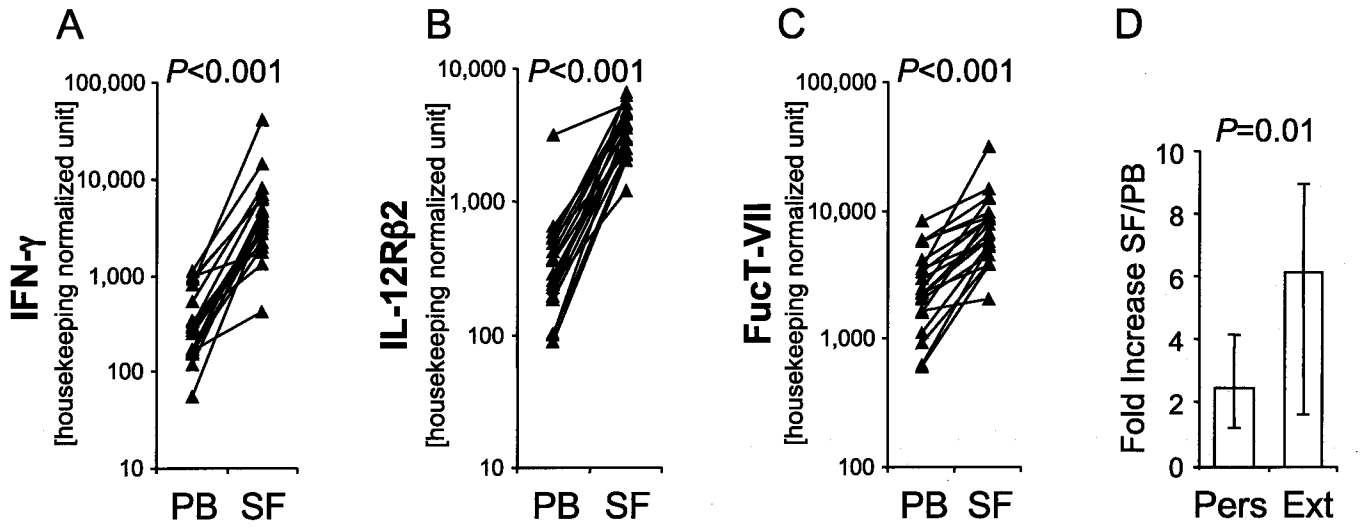

Figure 1. Interferon-γ (IFN-γ), interleukin 12Rß2 (IL-12Rß2), and FucT-VII transcripts were up-regulated in SF T cells from patients with oligoarticular JIA. The relative expression levels of FucT-VII, IFN-γ, and IL-12Rß2 in SF and PB T cells were determined using kinetic RT-PCR as described[10]. The ratio between the levels of the pertinent gene and of translation elongation factor-1 α1 (EEF1) was used to determine the gene expression level (housekeeping normalized unit, HNU) and the fold-change among samples. Shown is the normalized expression level (HNU) of IFN-γ (A), IL-12Rß2 (B), and FucT-VII (C) in T cells from paired samples of PB and SF from patients with oligoarticular JIA. T cells were purified as described in Materials and Methods. The p values were calculated by the Wilcoxon test for paired samples. D. A higher fold increase in FucT-VII mRNAexpression in SF T cells compared to PB T cells is associated with a polyarticular course of oligoarticular JIA. Patients with oligoarticular JIAwere divided according to their disease course into persistent (Pers) or extended (Ext) oligoarticular JIAas described in Materials and Methods. Data are shown as median, minimum, and maximum values. The p value was calculated by Mann-Whitney U test for unpaired samples.

higher FucT-VII mRNA expression is a general phenomenon in chronic arthritides. Moreover, the increased binding to P-selectin of SF T cells is consistent with the increased FucT-VII mRNA expression and provides a functional correlation for the role of FucT-VII in determining the ability of T cells to bind P-selectin.

However, our results may not necessarily reflect only selective recruitment, but may also be the result of selective retention. In addition, since the inflammatory milieu (e.g., IL-12) may affect FucT-VII expression[7-10], they may be the consequence of T cell activation inside inflamed joints. Using the synovial tissue/SCID model, we observed a marked increase in the capacity of FucT-VII transfected Jurkat cells to accumulate into the grafts. Although this experiment does not allow direct extrapolation to the chronic inflammatory condition observed in JIA or RA and the evaluation of the other molecules involved in T cell trafficking, our results suggest that FucT-VII contributes directly to T cell migration/retention *in vivo* into synovial tissue.

We have also found that the preferential accumulation in SF of T cells expressing FucT-VII mRNA and binding P-selectin is associated with a more severe course of oligoarticular JIA. In oligoarticular JIA the presence of the Th2 cytokine IL-4 in the synovium has been reported to be associated with a benign course[3]. In adult RA it has been shown that patients with a low Th1/Th2 ratio are more likely to respond satisfactorily to treatment[14]. Therefore, parameters that evaluate the Th1/Th2 balance may serve as prognostic indicators.

In conclusion, we demonstrate that FucT-VII mRNA expression and the subsequent ability to bind P-selectin are markedly increased in SF T cells from JIA patients and correlate with disease severity. In addition, we show that ectopic expression of FucT-VII in Jurkat cells results in their accumulation into human rheumatoid synovium grafted into SCID mice. These observations suggest a role for FucT-VII in T cell recruitment into inflamed joints, implying that inhibition of the enzyme's activity may represent a valuable therapeutic strategy.

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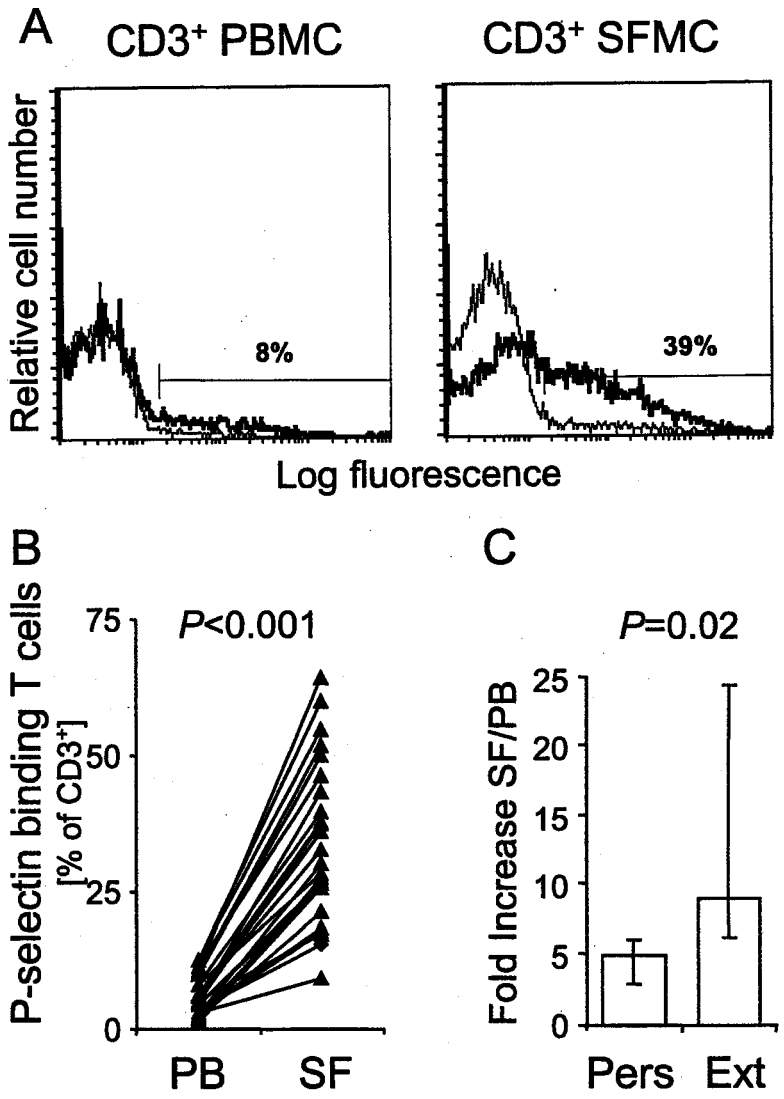

Figure 2. A. Increased binding of P-selectin by SF T cells from patients with JIA. Mononuclear cells from PB or SF were stained with anti-CD3 monoclonal antibody and a P-selectin/IgG fusion protein. The heavy lines represent staining with the P-selectin/IgG fusion construct after gating on CD3+ cells and the light lines show control staining. B. The percentage of the cells stained with the P-selectin/IgG reagent after gating on the CD3+ cells. The p value was calculated by Wilcoxon matched pairs test. C. A higher fold increase in the percentage of T cells binding P-selectin in SF compared to PB is associated with a polyarticular course of oligoarticular JIA. Patients with oligoarticular JIA were divided according to their disease course in persistent (Pers) or extended (Ext) oligoarticular JIAas described in the Materials and Methods. Data are shown as median, minimum, and maximum values. The p value was calculated by the Mann-Whitney U test for unpaired samples.

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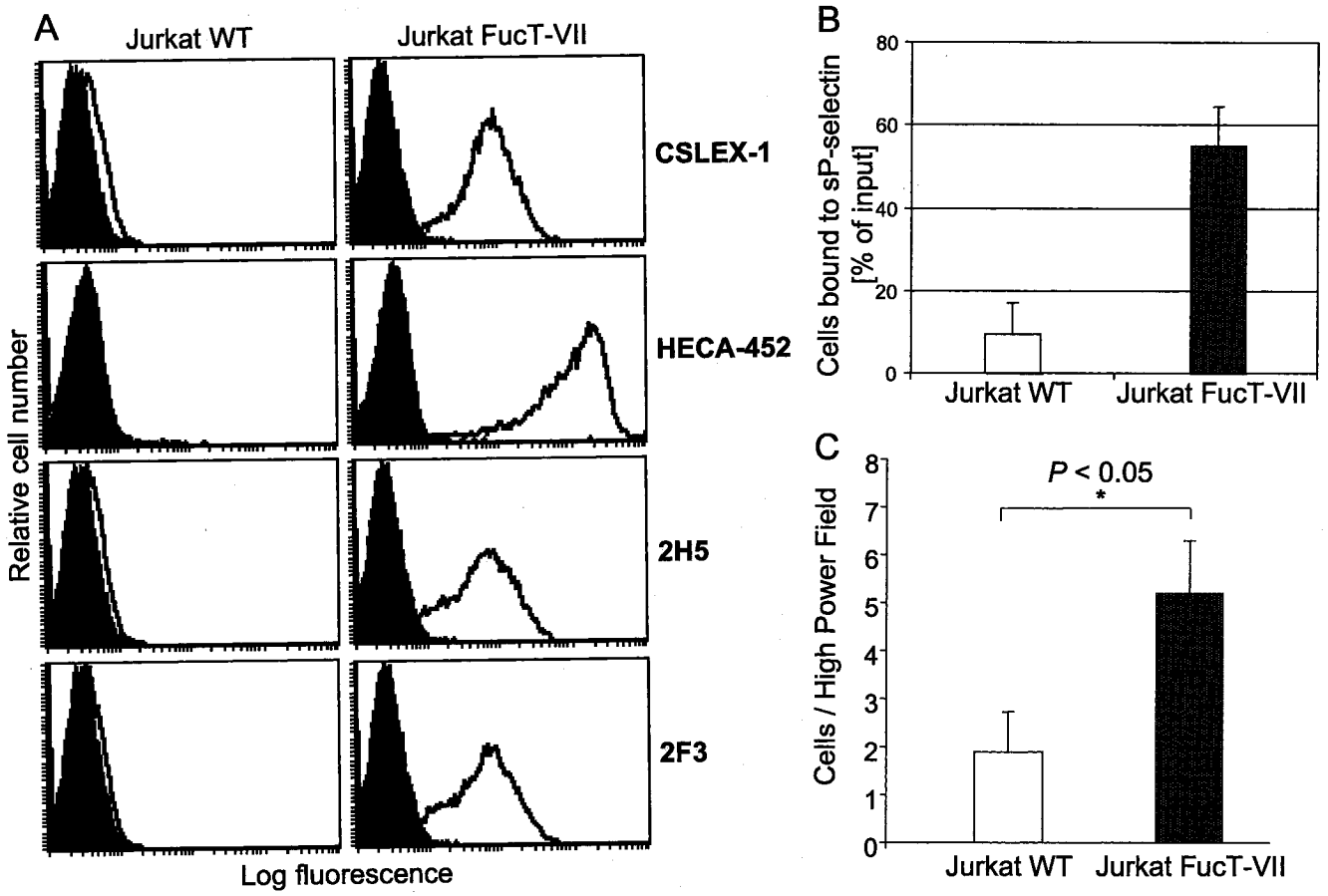

Figure 3. A. Jurkat cells transfected with FucT-VII express the sialy Lewis^x epitope. Jurkat (Jurkat WT) and FucT-VII-transfected Jurkat cells (Jurkat FucT-VII) were stained with monoclonal antibodies CSLEX-1, HECA-452, 2H5, and 2F3 as indicated (solid lines) or isotype-matched control antibodies (filled histograms). Experiments were performed with bulk cultures rather than with a particular clone. B. Jurkat cells transfected with FucT-VII bind to P-selectin. Binding assays were performed using 96-well plates coated with soluble P-selectin (1 µg/well). The amount of bound cells was determined colorimetrically as described[10]. The data represent mean and SEM of 3 independent experiments. C. FucT-VII transfection enhances the capacity of Jurkat cells to migrate into human synovial tissue transplanted into SCID mice. PKH26-labelled wild type (open bar) and FucT-VII-transfected (filled bar) Jurkat cells were injected intravenously into SCID mice transplanted with human synovial tissue as described[12]. Cell migration into the grafts was assessed histologically by immunofluorescence microscopy 48 h post injection. Shown is the mean and SEM of the total number of cells identified in each section in which, on average, more than 100 high power fields were counted. Two way ANOVA on ranks was performed and Tukey's multiple comparison test was used for pairwise comparison of treatment and control groups.

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