Immunology 1995 **85** 461–466

Synovial fibroblasts as accessory cells for staphylococcal enterotoxin-mediated T-cell activation

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SUMMARY

Rheumatoid arthritis (RA) is thought to be the result of T-cell-mediated autoimmune phenomena. So far, a critical autoantigen has not been identified. Recently, superantigens have been implied in the pathogenesis of RA. In the present study it was tested whether major histocompatibility complex (MHC) class II-positive synovial fibroblast cells (SFC) function as superantigen-presenting cells. SFC were stimulated with interferon-γ (IFN-γ) to express class II antigens; then they were cultivated in the presence of T cells with or without staphylococcal enterotoxins (SE). T-cell activation was measured as proliferation and interleukin-2 (IL-2) production. Depending on the dose and type of SE, activation of T-cell clones and also of peripheral T cells was seen. T-cell activation was inhibited by antibodies to MHC class II antigens and also by antibodies to intracellular adhesion molecule type-1 (ICAM-1). The data suggest that class II-positive SFC have the capacity to serve as accessory cells for superantigen-mediated T-cell activation. Thus SFC may participate in the propagation of a T-cell dependent immune response.

INTRODUCTION

The pathogenesis of rheumatoid arthritis (RA), one of the most common autoimmune diseases, is still unclear. There is increasing evidence that T cells play an important role in the pathology of the disease. T cells expressing activation markers such as interleukin-2 (IL-2) receptor and human leucocyte antigen (HLA)-DR are found in the inflamed synovia.[1,2] In addition, susceptibility to RA is associated with particular alleles of the HLA type, suggesting major histocompatibility complex (MHC)-restricted T-cell activation.[3,4] However, so far no critical antigen or autoantigen has been identified. Analyses of the heterogeneity of T cells in the synovium have been controversial.[5–7]

Several different arthritogenic stimuli may be involved in the development of RA. Apart from endogenous substances such as connective tissue proteins (e.g. collagen), exogenous infectious agents have been discussed (reviewed in Refs 8 and 9). In adjuvant arthritis, a model of autoimmune arthritis in rats, and in RA as well, the reactivity of T cells to mycobacterial proteins, particularly the 65 000 MW heat-shock proteins, has been implicated in the pathogenesis of joint inflammation due to cross-reactivity with human heat-shock proteins on synovial cells.[8] It has also been speculated that RA might be a slow bacterial infection, for example of unusual forms of mycobacteria with extremely slow growth characteristics.[9] In addition, there is increasing evidence that bacteria or bacterial products of the gut flora may play a role in the pathogenesis of RA.[8]

Speculating about bacterial infections as primary cause of RA, bacterial superantigens are of special interest since they activate a high frequency of T cells. Superantigens bypass antigen-specific T-cell activation by linking HLA class II molecules to the Vβ region of the T-cell receptor.[10] The best examined superantigens are the staphylococcal enterotoxins (SE). SE selectively activate T cells bearing particular Vβ elements.[10]

Recently, T cells bearing specific β-chains were reported to be preferentially enriched in the inflamed synovia of RA patients, pointing to T-cell activation by superantigens.[11,12] In the inflamed synovia of RA patients HLA class II-positive synovial fibroblasts have been found;[13] so far it is not known whether these cells can function as antigen-presenting cells.

In the present study we have examined whether class II-positive synovial fibroblasts may serve as antigen-presenting cells for superantigen-mediated T-cell activation.

MATERIALS AND METHODS

Preparation and culture of synovial fibroblasts

Synovial material was obtained from patients with degenerative joint disorder (20) or RA (5). Adherent synovial cells were isolated according to the protocol of Dayer *et al.*[14] with modifications. The lining cell layers were separated from the synovial material, minced and digested with collagenase

Received 30 June 1994; revised 6 February 1995; accepted 7 March 1995.

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(850 U/ml, type Ia; Sigma, Deisenhofen, Germany) and hyaluronidase (3000 U/ml; Serva, Heidelberg, Germany) in Dulbecco's minimum essential medium (DMEM; Gibco, Berlin, Germany) containing 1% antibiotic/animycotic (Gibco), for 2–4 hr. The cells were then sieved through sterile gauze, washed and seeded on 24-well plates (Greiner, Nürtingen, Germany) at a density of 10^5 cells/well. After 7–14 days in culture, when the cells reached confluence, they were subcultivated. After removal with trypsin–EDTA (Gibco) they were passaged onto new 24-well or 96-well plates. Synovial fibroblast cell (SFC) cultures were usually used for experiments between passages 3 and 7. At this time-point all cells had the appearance of fibroblasts. They were all positive for vimentin and stained negative with anti-Leu-M3 and anti-HLA-DR.

Cytochemical characterization

SFC were characterized as described previously.[15] Briefly, cells were fixed to coverslips with ice-cold methanol. For characterization by indirect immunofluorescence, the following antibodies were used: anti-HLA-DR (Dianova, Hamburg, Germany), anti-vimentin (Dakopatts, Hamburg, Germany), anti-Leu-M3 (Becton Dickinson, Heidelberg, Germany) and anti-intracellular adhesion molecule-1 (ICAM-1; Dianova). Expression of class II by SFC after treatment with interferon-γ (IFN-γ; 200 U/ml) was determined by staining cells with an anti-HLA-class II monoclonal antibody (mAb) (Dianova) and fluorescein isothiocyanate (FITC)-conjugated anti-mouse IgG (Dianova). The samples were then analysed on a FACScan cytofluorumeter (Becton-Dickinson).

T-cell clones

The $CD4^+$ $CD8^-$, T-cell receptor (TCR) $\alpha\beta^+$ T-cell clones D894 and D798 were established and propagated as described elsewhere.[16] Briefly, purified T cells from human peripheral blood were cultured at 0·3 cells/well in 96-well round-bottomed culture plates (Nunc, Wiesbaden, Germany) in the presence of 10^5 irradiated (3000 rads) peripheral blood mononuclear cells (PBMC) and 1 μg/ml phytohaemagglutinin (PHA; Wellcome, Burgwedel, Germany). Growing clones were expanded in RPMI-1640 (Biochrom KG, Berlin, Germany) supplemented with 2 mM L-glutamine, 10 mM HEPES, antibiotics, 10% heat-inactivated fetal calf serum (complete medium) and 30 U/ml recombinant (r)IL-2 (EuroCetus, Amsterdam, the Netherlands). The clones were restimulated every 2–3 weeks with a feeder cell mixture of irradiated PBMC and irradiated Epstein–Barr virusEBV-transformed lymphoblastoid cell lines and PHA.[16]

Purification of T cells from the peripheral blood

Peripheral T cells were obtained from adult peripheral blood. Briefly, PBMC were separated into E^+ and E^- cells by rosette formation with sheep erythrocytes (E) and subsequent centrifugation on Ficoll–Hypaque density gradients.

Experimental procedures

SFC grown in 96-well plates were stimulated with IFN-γ (200 U/ml) for 5 days, then washed with RPMI complete medium. T cells were added at $2–10 \times 10^4$ cells/well; staphylococcal enterotoxin A (SEA), SEB, SEC, SED or SEE (Serva, Heidelberg, Germany) were used at concentrations of 0·1–30 ng/ml and added in a final volume of 200 μl.

Proliferative responses were measured in replicates (as indicated in the figure legends) of $2–10 \times 10^4$ cloned T cells or purified peripheral T cells at the peak days. Assays using cloned T cells as responders were pulsed with [^{3}H]thymidine (TdR; 1 μCi; specific activity 6·7 Ci/mmol) after 2 days for 6–16 hr; assays using purified T cells were pulsed after 3–5 days for 6–16 hr.

Proliferation was quantified as the amount of radiolabel incorporated into the DNA, as determined by liquid scintillation counting.

Monoclonal antibodies anti-HLA class II (Dianova), anti-ICAM-I (Dianova), anti-IL-2 receptor (Dianova), mouse IgG or rat IgG (Dianova) were preincubated with SFC for 20 min before T cells were added.

Determination of IL-2 production

Ten to 14 days after the addition of feeder cells and PHA, T cells were washed and cultured at $5–10 \times 10^4$ cells/well with SFC in 96-well plates in the absence or presence of SE. After 24 hr, cell-free supernatants were tested for IL-2 content using IL-2-dependent murine CTLL cells. Serial dilutions of the test supernatants were added to 3×10^4 CTLL cells for 24 hr. The viability of the CTLL cells was measured in a colorimetric assay (cleavage of tetrazolium salt MTT) as described elsewhere.[17]

RESULTS

Class II-positive SFC as accessory cells for SEE-mediated proliferation of T-cell clones

SFC cultivated *in vitro* for two to three passages did not express class II antigens, as also shown previously elsewhere.[15] To induce class II expression, SFC were cultured in the presence of IFN-γ (200 U/ml). Consistent with previous findings, optimal class II expression was seen after 3–5 days;[18] at that time IFN-γ-treated SFC also expressed ICAM-1 (data not shown).[19]

To test whether class II-positive SFC could function as accessory cells for superantigen-mediated T-cell activation, SFC were cultured with a SEE-reactive T-cell clone in the presence of 1 ng/ml SEE. After 2 days, T-cell proliferation was

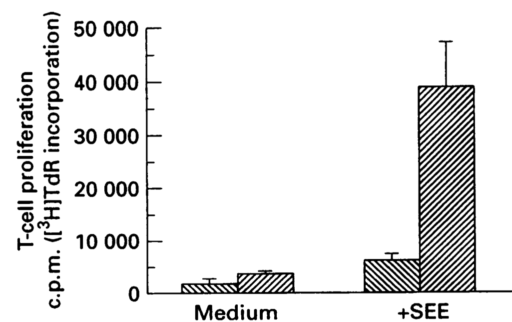

Figure 1. IFN-γ-treated SFC as accessory cells for SEE-mediated T-cell activation. T cells (5×10^4, T-cell clone D894) were cultured on SFC (▧) or SFC pretreated with IFN-γ (200 U/ml) (▨) in the absence or presence of SEE (1 ng/ml). After 2 days T-cell proliferation was measured as incorporation of [^{3}H]TdR. One of four independent experiments is shown. Data are shown as mean ± SD of five replicates. (T cells + SEE 1 ng/ml without SFC: 6600 ± 1779 c.p.m.).

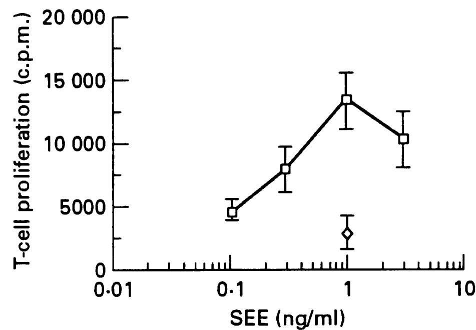

Figure 2. Dose-dependent increase of SEE-mediated T-cell proliferation. T cells (5×10^4, T-cell clone D894) were cultured on SFC pretreated with IFN-γ in the presence of increasing amounts of SEE (□). After 2 days T-cell proliferation was measured. No proliferation was seen when T cells were cultured on class II-negative SFC in the presence of SEE (1 ng/ml) (◇) [T cells cultured on SFC without SEE: 3806 ± 578 c.p.m.; T cells + SEE (1 ng/ml) without SFC: 1273 ± 223 c.p.m.]. One of three independent experiments is shown. Data are presented as mean ± SD of three replicates.

measured (Fig. 1 shows one of four independent experiments with the T-cell clone D894). In the presence of SEE a significant increase of T-cell proliferation was seen, whereas in the absence of SEE no proliferation was observed. When class II-negative SFC were used, no or only a small increase of T-cell proliferation was measured in the presence of SEE (Fig. 1). However, throughout all experiments, the SE-mediated proliferation was always considerably higher in the presence of class II-positive SFC. A minimum 3·7- to a maximum 11-fold increase was seen, indicating that SFC can function as accessory cells for T-cell activation. With increasing amounts of SEE an increase of proliferation was seen (Fig. 2.).

SE-induced T-cell proliferation was not limited to SEE. T-cell proliferation was also seen with SED and SEA, although higher concentrations of these SE were necessary to induce significant T-cell proliferation (Table 1). No significant increase was measured with 1 ng SED, and with 1 ng SEA only a 2·2-fold increase was seen, which was less effective than 1 ng SEE (11·2-fold increase). Accordingly, less proliferation was measured in response to 30 ng SED compared to 30 ng SEA. No proliferation was seen with SEB or SEC (data not shown).

To test whether SFC were equally efficient in presenting enterotoxins as professional antigen-presenting cells, class II-positive SFC were compared with EBV-transformed B cells with respect to their accessory cell function for SE-mediated T-cell proliferation. In the presence of different doses of SEA, SED and SEE, T-cell proliferation was the same for both cell types, suggesting that SFC were as efficient as EBV-transformed B cells in mediating SE-induced proliferation of T-cell clones (Table 1).

Table 1. Comparison of SFC and EBV-transformed B cells to function as APC for SE-mediated T-cell activation

T cells incubated with	SFC as APC	B cells as APC
	Proliferation (c.p.m.)	
No SE	1 808 ± 269	1 064 ± 118
SEA 1 ng/ml	3 980 ± 332	1 209 ± 372
SEA 30 ng/ml	23 189 ± 2333	20 919 ± 2454
SED 1 ng/ml	2 420 ± 419	1 651 ± 257
SED 30 ng/ml	10 267 ± 1090	20 830 ± 2864
SEE 1 ng/ml	20 231 ± 1421	24 739 ± 3975

T cells (clone D894, 10^5 cells/well) were cultured with class II-positive SFC (5×10^4–10^5 cells/well) or B cells (10^5 cells/well) as antigen-presenting cells (APC) in the presence of various SE. T-cell proliferation was measured after 48 hr. Data are shown as mean ± SD of five replicates.

SFC as accessory cells for SE-mediated activation of peripheral T cells

Next it was tested whether class II-positive SFC might also function as accessory cells for SE-mediated activation of freshly purified peripheral T cells. Again T-cell proliferation was observed in the presence of SEE and class II-positive SFC (Fig. 3). Proliferation did not occur when class II-negative SFC were used or in the absence of SEE. These data rule out the possibility that T-cell proliferation was due to a T-cell allo-response. No or only weak proliferation was seen when T cells were cultivated in the presence of SEE but without SFC (1–1·6-fold increase), whereas in the presence of class II-positive SFC a minimum 3·8 to a maximum 13·3-fold increase was seen. These results indicate that the proliferation was not due to contaminating mononuclear cells (MNC). Moreover, SE-mediated autoactivation of T cells appeared to play a minor role in this experimental set up. Activation of peripheral T cells was not limited to SEE, but was also seen with SED. With increasing amounts of SE a dose-dependent increase in T-cell proliferation was seen (Fig. 3).

The accessory cell function of class II-positive SFC for peripheral T cells was independent of the source of SFC and T

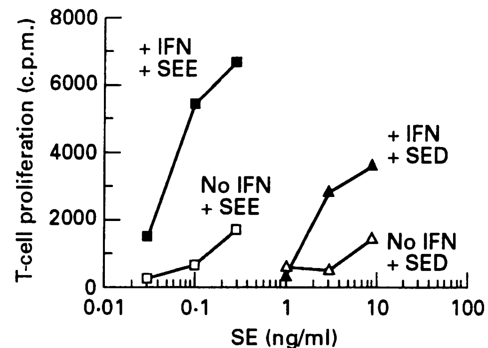

Figure 3. SFC as accessory cells for SE-mediated activation of peripheral T cells. Purified peripheral T cells (3×10^4/well) were cultured on class II-negative or class II-positive SFC in the presence of increasing concentrations of SEE and SED. Proliferation of T cells was measured after 3 days (T cells + class II-positive SFC: 1039 c.p.m.; T cells + SFC: 650 c.p.m.; T cells + SEE 0·3 ng/ml without SFC: 271 c.p.m.; T cells + SED 9 ng/ml without SFC: 225 c.p.m.). One of three independent experiments is shown. Results are shown as mean of duplicates.

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Table 2. Class II-positive SFC as accessory cells for activation of freshly purified T cells

	Class II-positive SFC incubated with	
	T cells	T cells + SEE
	Proliferation (c.p.m.)	
SFC (A) T cells (MB)	1734 ± 427	20 041 ± 3086
SFC (B) T cells (MB)	999 ± 169	3 775 ± 579
SFC (C) T cells (SF)	1756 ± 390	12 024 ± 4019
SFC (C) T cells (SF)*	860 ± 186	11 438 ± 1169
SFC (D) T cells (FH)	5985 ± 1134	60 064 ± 6202
SFC (E) T cells (MK)*	623†	7 107†

Class II-positive SFC from various donors (A–E) were treated with IFN-γ to express class II antigens. Then freshly purified T cells from different donors were added in the absence or presence of 0·3 ng/ml SEE. After 3–5 days [^{3}H]TdR was added and proliferation was measured. Data are presented as mean ± SD of three replicates.

* In these experiments T cells were harvested after 3 days and cultured independently of SFC in the presence of [^{3}H]TdR.

† Data are presented as mean of duplicates.

cells. Similar results were seen with T cells from four different donors cultured on SFC obtained from five different patients (Table 2).

To exclude the possibility that the incorporation of [^{3}H]TdR measured was due to proliferation of SFC, in a few experiments T cells were harvested 3 days after stimulation and were cultured independently of SFC in the presence of [^{3}H]TdR. Following this experimental protocol the same results were seen as with continuous SFC/T-cell cultures (Table 2).

Inhibition of SE-mediated T-cell proliferation by anti-class II and anti-ICAM-1

So far we had only demonstrated that IFN-γ-stimulated SFC could function as accessory cells for SE-induced T-cell proliferation, suggesting that MHC class II expression was necessary for SE presentation. This assumption was confirmed by experiments using a mAb directed against class II antigens. Class II-positive SFC were preincubated for 20 min with the anti-class II mAb or a control antibody before T cells and SEE were added. In the presence of anti-class II (5 μg/ml), SE-mediated T-cell proliferation was inhibited (Fig. 4). At suboptimal SE concentrations (0·1 ng/ml), T-cell proliferation was inhibited up to 93%. With increasing SE concentrations (0·3 ng/ml), T-cell proliferation was reduced by only 23%. Antibodies to ICAM-1 also inhibited T-cell proliferation, indicating that this adhesion molecule also participates in the SFC–T-cell interaction. With suboptimal SE concentrations T-cell proliferation was inhibited by 95%; with increasing concentrations inhibition was only reduced by 52%. However, when the two antibodies, anti-class II and anti-ICAM-1, were combined the inhibitory effect was enhanced (97%). No inhibition was seen with a mouse IgG control serum.

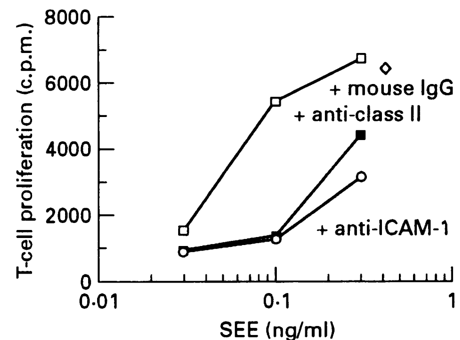

Figure 4. Inhibition of SEE-mediated activation of peripheral T cells by anti-class II mAb and anti-ICAM-1 mAb. Class II-positive SFC were pretreated for 15 min without (□) or with anti-class II antibody (5 μg/ml) (■), anti-ICAM-1 antibody (5 μg/ml); (○) or mouse IgG (5 μg/ml); (◇) before 3×10^4 peripheral T cells and SEE at the indicated concentrations were added. Proliferation was measured after 3 days. One of three independent experiments is shown. Data are presented as mean of duplicates.

Similar results were observed when cloned T cells were cultured with SEE on class II-positive SFC preincubated with anti-class II mAb. Even at relatively high concentrations of SEE (10 ng/ml) T-cell proliferation was inhibited by 43% (Table 3). In the presence of the anti-ICAM-1 mAb inhibition was 65%, further supporting the hypothesis that ICAM-1 plays an important role in the SFC–T-cell interaction.

IL-2 production by T cells cultured with SE and SFC

To test whether T-cell proliferation was paralleled by IL-2 production, T-cell clones were cultured for 1 day with SEA, SED or SEE in the presence of SFC treated with or without IFN. Supernatants were tested for IL-2 production. Only T cells cultured with SE in the presence of class II-positive SFC produced significant amounts of IL-2 (Table 4). IL-2 production was only seen at SE concentrations that also stimulated T-cell proliferation. When peripheral T cells were cultured with class II-positive SFC and SE, no IL-2 release could be demonstrated although T-cell proliferation occurred. In the

Table 3. Inhibition of SEE-mediated T-cell activation by anti-class II mAb and anti-ICAM I mAb

T cells incubated with	SFC with IFN	SFC without IFN
	Proliferation (c.p.m.)	
No SEE	2423	1922
SEE (10 ng/ml)	8352	2266
SEE + anti-class II	4828	2041
SEE + anti-ICAM I	2898	2510
SEE + mouse IgG	7587	2203

T cells (10^5, clone D798) were cultured on SFC treated with or without IFN-γ in the presence of SEE (10 ng/ml) and anti-class II mAb (5 μg/ml) or anti-ICAM I mAb (5 μg/ml). Data are shown as mean of duplicates. One of two independent experiments is shown.

Table 4. Class II-positive SFC as accessory cells for SE-mediated IL-2 production by cloned T cells

T cells incubated with	IL-2 production (U/ml)
No SE	ND
SEA 1 ng/ml	ND
30 ng/ml	28
SED 1 ng/ml	ND
30 ng/ml	4·8
SEE 1 ng/ml	72

T cells (clone D894, 10^5 cells/well) were cultured with class II-positive SFC and SE. After 24 hr culture, supernatants were harvested and tested for IL-2. One of three independent experiments is shown.

ND, not detectable.

presence of an antibody to IL-2 receptor, T-cell proliferation was inhibited. A control IgG or anti-IL-4 antibody had no effect, indicating that the SE-stimulated proliferation of peripheral T cells was mediated by IL-2 (Table 5).

DISCUSSION

In the present study, we demonstrated that class II-positive SFC in culture function as accessory cells for SE-mediated T-cell activation. T-cell activation measured as T-cell proliferation and IL-2 production was dependent on class II-positive SFC. Although cloned T cells also express class II molecules and thus might bind SE, no or only very weak T-cell proliferation was seen when T cells were cultivated with SE, but in the absence of SFC. Our data are in line with a recent paper by Koning & Rust[20] who reported weak T-cell autoactivation in the presence of SE. Our data indicate that in addition to class II, a second signal was required, which might be provided by the SFC.

One such second signal might be provided by the interaction of ICAM-1 molecules on SFC and lymphocyte function-associated antigen-1 (LFA-1) molecules on T cells. As IFN-γ-stimulated SFC also express ICAM-1 molecules, and as anti-ICAM-1 mAb inhibited the SE-mediated T-cell activation markedly, our data support the idea that the interaction between LFA-1 and ICAM-1 molecules participates critically in a successful T-cell activation. This observation is in line with earlier studies by others[21] demonstrating that optimal T-cell activation with SEA was dependent on the expression of ICAM-1 molecules on accessory cells, and was absolutely required when antigen-presenting cells expressed only low levels of MHC class II. The stimulatory effect of ICAM-1 may be due to the prolonged increase of adhesion and to cellular signalling as well.

Table 5. Inhibition of SE-mediated proliferation of peripheral T cells by anti-IL-2 receptor

	Proliferation (c.p.m.)
No mAb	3775 ± 579
Anti-IL-2R (5 μg/ml)	847 ± 362
Anti-IL-2R (1 μg/ml)	2794 ± 297
Rat IgG (5 μg/ml)	3865 ± 591

Peripheral T cells (2×10^4/well) were cultured with SEE (1 ng/ml) on class II-positive SFC in the presence of different concentrations of a rat anti-IL-2 receptor mAb (anti-IL-2R) or a rat IgG antibody as control. After 3 days, proliferation was measured. One of two independent experiments is shown. Data are presented as mean ± SD of three replicates.

SE-mediated T-cell activation requires the presentation of SE by class II molecules.[12] There are, however, reports suggesting that SE-mediated T-cell activation may also occur in the absence of class II,[22] probably in association with another as yet unknown molecule. With respect to SFC as accessory cells, class II expression seemed to be required because (1) only class II-positive SFC could function as accessory cells, and (2) anti-class II antibody inhibited T-cell activation.

The extent of T-cell proliferation was variable when comparing different experiments. This might be due to the different sources of T cells, but also to the origin of SFC. The percentage of SE-reactive T cells may vary from donor to donor, but also individual T-cell clones may respond differently to SE (compare Figs 1 and 2; Table 3). On the other hand, the cell cycle or passage number of SFC might influence the outcome of T-cell proliferation. The underlying clinical disease, degenerative disorder versus RA, did not affect the reactivity of SFC. This is not too surprising, as in previous studies it was shown that after multiple passages SFC obtained from RA patients did not differ morphologically or functionally from SFC obtained from patients with osteoarthritic joint disorder in the non-inflammatory state.[15]

SFC are not the only tissue cells that may function as accessory cells. Only recently, it has been reported that after stimulation with IFN-γ human umbilical vascular endothelial cells, as well as keratinocytes, gained the capacity to bind SE and to participate in T-cell activation.[23,24] We have extended these observations by studying the accessory cell function of SFC for SE-mediated proliferation and IL-2 production of cloned and freshly purified T cells. It has been shown that IFN-γ-treated skin fibroblasts do not stimulate allogeneic T-cell proliferation and are insufficient antigen-presenting cells, particularly for resting T cells.[25] This seemed to be related to a diminished capacity to accomplish accessory cell functions unrelated to class II expression. However, our results show that class II-positive synovial fibroblasts are effective accessory cells for superantigen-mediated T-cell activation. These results suggest that fibroblasts do not function as true antigen-presenting cells but can provide costimulatory signals for superantigen stimulation or may reflect different accessory cell capacities of skin versus synovial fibroblasts.

With regard to the development of RA, the fact that IFN-γ-stimulated SFC served as accessory cells for freshly purified peripheral T cells as well suggests a role of SFC as enterotoxin-presenting cells inasmuch as recently superantigens have been implied in RA.[11,12] Howell *et al.*[11] demonstrated a clonal dominance among Vβ3, Vβ14 and Vβ17 T cells with a significant homology in the fourth complementary determining

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region (CDR4), which suggests Vβ-specific T-cell activation by superantigens. Uematsu *et al.*[12] reported that T cell receptors (TCR) expressing Vβ2.1 and Vβ3.1 are enriched in the synovial fluid.[12] T cells expressing Vβ2.1 or Vβ3.1 can be stimulated by certain bacterial toxins. The enrichment of specific Vβ sequences of synovial T cells may reflect a previous bacterial infection, leading to stimulation and expansion of T cells expressing these Vβ segments.

Several infectious agents have been discussed to play a role in triggering and perpetuating RA, e.g. mycobacterial antigen with its homology with mammalian heat-shock proteins[8] or peptidoglycan peptides from intestinal bacteria also present in mammalian tissue.[26] With respect to the role of superantigens in RA, one might speculate that bacterial infection results in T-cell activation with subsequent homing of activated cells to the joints. Homing of T cells to the joints after bacterial infection has been demonstrated in animal models, e.g. adjuvant arthritis in rats induced by *Mycobacterium tuberculosis*.[27] Release of IFN by the invaded T cells may lead to class II expression on SFC. Subsequent binding of superantigens such as bacterial products or presentation of endogenous superantigens will activate or reactivate more T cells, which in turn may stimulate collagenase induction in SFC (our observation), leading to subsequent collagen degradation and release of autoantigens. Whether SFC can also function as antigen-presenting cells for antigens such as collagen is not clear at the moment.

The co-culture system of SFC and T cells shown in this paper can be used as a model to study further the potential role of SFC as accessory cells for T-cell activation and as effector cells for collagenase release in the pathogenesis of RA.

REFERENCES

1. Burmester G., Jahn B., Gramatzki M., Zacher J. & Kalden J.R. (1984) Activated T cells *in vivo* and *in vitro*: divergence in expression of Tac and Ia antigens in the non-blastoid small T cells of inflammation and normal T cells activated *in vitro*. *J Immunol* **133,** 1230.
2. Hovdenes I., Gandernack G., Kvien T.K. & Egeland T. (1989) Expression of activation markers on $CD4^+$ and $CD8^+$ cells from synovial fluid, synovial tissue and peripheral blood of patients with inflammatory arthritis. *Scand J Immunol* **29,** 631.
3. Gregersen P.K., Seilver J. & Winchester R.J. (1987) The shared epitope hypothesis. An approach to understanding the molecular genetics of susceptibility to rheumatoid arthritis. *Arthritis Rheum* **30,** 1205.
4. Todd J.A., Acha-Orbea H., Bell J. *et al.* (1988) A molecular basis for MHC class II-associated autoimmunity. *Science* **240,** 1003.
5. Keystone E.C., Minden M., Klock R. *et al.* (1988) Structure of T cell antigen receptor β chain in synovial fluid cells from patients with rheumatoid arthritis. *Arthritis Rheum* **31,** 1555.
6. Stamenkovic I., Stegagno M., Wright K.A. *et al.* (1988) Clonal dominance among T-lymphocyte infiltrates in arthritis. *Proc Natl Acad Sci USA* **85,** 1179.
7. Miltenburg A.M.M., van Laar J.M., Daha M.R., de Vries R.R.P., van den Elsen P.J. & Breedveld F.C. (1990) Dominant T-cell receptor β-chain gene rearrangements indicate clonal expansion in the rheumatoid joint. *Scand J Immunol* **31,** 121.
8. Epstein F.H. (1990) Rheumatoid arthritis: pathophysiology and implications for therapy. *New Engl J Med* **322,** 1277.
9. McCulloch J., Lydyard P.M. & Rook G.A.W. (1993) Rheumatoid arthritis: how well do the theories fit the evidence. *Clin Exp Immunol* **92,** 1.
10. Herman A., Kappler J.W., Marrack P. & Pullen A.M. (1991) Superantigens: mechanism of T-cell stimulation and role in immune responses. *Annu Rev Immunol* **9,** 745.
11. Howell M.D., Diveley J.P., Lundeen U.A. *et al.* (1991) Limited T-cell receptor β-chain heterogeneity among interleukin 2 receptor-positive synovial T cells suggests a role for superantigen in rheumatoid arthritis. *Proc Natl Acad Sci USA* **88,** 10921.
12. Uematsu Y., Wege H., Straus A. *et al.* (1991) The T-cell receptor repertoire in the synovial fluid of a patient with rheumatoid arthritis is polyclonal. *Proc Natl Acad Sci USA* **88,** 8534.
13. Lindbald S., Klareskog L., Medfors E., Forsum U. & Sundstrom C. (1983) Phenotypic characterization of synovial tissue cells in situ in different types of synthesis. *Arthritis Rheum* **26,** 1321.
14. Dayer J.M., Krane S.M., Russell G.G. & Robinson D.R. (1976) Production of collagenase and prostaglandins by isolated adherent rheumatoid synovial cells. *Proc Natl Acad Sci USA* **73,** 945.
15. von Kempis J., Torbohm I., Schönermark M., Jahn B., Seitz M. & Hänsch G.M. (1989) Effect of the late complement components C5b-9 and of platelet-derived growth factor on the prostaglandin release of human synovial fibroblast-like cells. *Int Archs Allergy Appl Immun* **90,** 248.
16. Kabelitz D. & Wesselborg S. (1992) Life and death of a superantigen-reactive human $CD4^+$ T cell clone: staphylococcal enterotoxins induce death by apoptosis by simultaneously trigger a proliferative response in the presence of HLA-DR^+ antigen-presenting cells. *Intern Immunol* **4,** 1381.
17. Joos J., Zanker B., Wagner H. & Kabelitz D. (1988) Quantitative assessment of interleukin-2-producing alloreactive human T cells by limiting dilution analysis. *J Immunol Meth* **112,** 85.
18. Burmester F.R., Jahn B., Rohwer P., Zacher J., Winchester R.J. & Kalden R. (1987) Differential expression of Ia antigens by rheumatoid synovial lining cells. *J Clin Invest* **80,** 595.
19. Chin J.E., Winterrowd G.E., Krzesicki R.F. & Sanders M.E. (1990) Role of cytokines in inflammatory synovitis. The coordinate regulation of intercellular adhesion molecule 1 and HLA class I and class II antigens in rheumatoid synovial fibroblasts. *Arthritis Rheum* **33,** 1776.
20. Koning F. & Rust C. (1992) Staphylococcal enterotoxin-mediated human T–T cell interactions. *J Immunol* **149,** 317.
21. Fischer H., Gjörloff A., Hedlund G. *et al.* (1992) Stimulation of human naive and memory T helper cells with bacterial superantigen. Naive $CD4^+$ $45RA^+$ T cells require a costimulatory signal mediated through the LFA-1/ICAM-1 pathway. *J Immunol* **148,** 1993.
22. Herrmann T., Romero P., Sartoris S. *et al.* (1991) Staphylococcal enterotoxin-dependent lysis of MHC class II negative target cells by cytolytic T lymphocytes. *J Immunol* **146,** 2504.
23. Uchiyama T., Araake M., Yan X.J., Miyanaga Y. & Igarashi H. (1992) Involvement of HLA class II molecules in acquisition of staphylococcal enterotoxin A-binding activity and accessory cell activity in activation of human T cells by related toxins in vascular endothelial cells. *Clin Exp Immunol* **87,** 322.
24. Nickoloff B.J., Mitra R.S., Freen J. *et al.* (1993) Accessory cell function of keratinocytes for superantigens. Dependence on lymphocyte function-associated antigen-1/intercellular adhesion molecule-1 interaction. *J Immunol* **150,** 2148.
25. Geppert T. & Lipsky P.E. (1985) Antigen presentation by interferon-γ-treated endothelial cells and fibroblasts: differential ability to function as antigen-presenting cells despite comparable Ia expression. *J Immunol* **135,** 3750.
26. Hazenberg M.P., Klasen I.S., Kool J., Ruseler-van Embden J.G.H. & Severijnen A.J. (1992) Are intestinal bacteria involved in the etiology of rheumatoid arthritis? *APMIS* **100,** 1.
27. Holoshitz J., Matitiau A. & Cohen I.R. (1984) Arthritis induced in rats by cloned T lymphocytes responsive to mycobacteria but not to collagen type II. *J Clin Invest* **73,** 211.