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# RESPONSIVENESS OF HUMAN T LYMPHOCYTES TO BACTERIAL SUPERANTIGENS PRESENTED BY CULTURED RHEUMATOID ARTHRITIS SYNOVIOCYTES

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*Objective.* **Type B fibroblastic synoviocytes are abundant in inflamed joints of patients with rheumatoid arthritis (RA), and can secrete cytokines and other mediators of inflammation. The aim of this study was to determine whether cell lines derived from RA type B synoviocytes could also serve as accessory cells for T lymphocyte activation.**

*Methods.* **Cells from RA synoviocyte lines, with or without preculture in interferon-$\gamma$ (IFN$\gamma$), were cultured with purified peripheral blood T cells, in the presence or absence of superantigens or other accessory cell–dependent T cell mitogens. T cell proliferation was measured by thymidine incorporation, and synoviocyte surface markers were analyzed by flow cytometry.**

*Results.* **RA type B synoviocyte lines were potent accessory cells for T cell responses to bacterial superantigens or lectins, and direct cell–cell contact was required. Preculture in IFN$\gamma$ augmented synoviocyte expression of major histocompatibility complex (MHC) class II molecules and of ligands for some T cell costimulatory receptors, but synoviocyte accessory cell function was evident even in the absence of IFN$\gamma$. Blocking studies using monoclonal antibodies supported the notion of a role for CD2, CD11a/CD18 and MHC class II molecules in synoviocyte-dependent T cell activation. Monoclonal antibodies against IFN$\gamma$, interleukin-1$\beta$ (IL-1$\beta$), IL-6, IL-8, and tumor necrosis factor $\alpha$ failed to block the T cell proliferative responses, but anti–IL-2 was strongly inhibitory.**

*Conclusion.* **Cultured RA type B synoviocytes can perform some of the functions of professional antigen-presenting cells. If such cells have similar properties in vivo, they may be important participants in activation of immune responses, in addition to their previously described synthetic and proinflammatory roles. If RA synovial tissue T cells, like normal peripheral blood T cells, can respond to superantigens presented by synoviocytes, this interaction could be important in the pathogenesis of RA.**

Rheumatoid arthritis (RA) is an autoimmune disease characterized by proliferation of synoviocytes, persistent inflammation of the synovium, and tissue destruction. Synovial tissue contains at least 2 distinct populations of cells, which have been termed macrophage-like (type A) and fibroblast-like (type B) synoviocytes (1). Local proliferation of type B synoviocytes contributes substantially to the synovial hyperplasia that is characteristic of RA (2). Synthetic functions of type B synoviocytes, including secretion of hyaluronic acid and collagen, have been well documented (3). These cells also contribute to inflammatory responses, by releasing substances such as prostaglandins, metalloproteinases, and cytokines, which regulate cellular function within the synovial compartment and cause tissue damage. These processes are regulated by a variety of cytokines and cytokine inhibitors, acting individually, synergistically, or antagonistically (4–11), as well as by mediators of inflammation, such as kinins (12).

Although some functions of synoviocytes are mediated by secreted products, other functions may require direct interaction with cells of the immune

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system that migrate to the synovium during inflammation. In order to support antigen presentation to CD4+ T cells, accessory cells must express major histocompatibility complex (MHC) class II molecules as well as other, costimulatory molecules. In several reports, type B synoviocytes from patients with RA have been shown to express MHC class II, intercellular adhesion molecule 1 (ICAM-1) (CD54), vascular cell adhesion molecule 1 (VCAM-1) (CD106), integrins, and other molecules involved in cell–cell interaction within the inflamed joint or ex vivo (4,13–21). MHC class II determinants are induced on cultured type B synoviocytes from RA patients in the presence of interferon-$\gamma$ (IFN$\gamma$) (4). In addition, IFN$\gamma$ and other cytokines have been reported to enhance ICAM-1 and VCAM-1 expression (16,18,19). Anti–VCAM-1 antibodies have been found to block the binding of the Jurkat T cell line to type B synoviocytes (16).

Despite this finding that synoviocytes express ligands for T lymphocyte surface glycoproteins, there is as yet little direct evidence for a role of these cells in the afferent arm of the immune responses as antigen-presenting cells. Recent studies demonstrated that the superantigen (SAg) staphylococcal enterotoxin A (SEA) induces MHC class II–positive type B synoviocytes to secrete interleukin-6 (IL-6) and IL-8 (22). Since it has been suggested that SAg may be involved in the pathogenesis of RA (23,24), we investigated the interaction of cultured type B synoviocytes derived from RA patients and peripheral T lymphocytes in the presence of staphylococcal SAg. We demonstrated that synoviocytes can act as accessory cells in SAg and lectin responses, and provide the necessary costimulatory signals to support the activation and proliferation of peripheral blood T lymphocytes. Furthermore, interaction between synoviocytes and T lymphocytes was shown to require direct cell–cell contact and to involve certain costimulatory structures in addition to MHC class II proteins. These data suggest that type B synoviocytes not only possess synthetic, secretory, and proinflammatory capabilities, but can also support T cell activation. Such interactions between synoviocytes, SAg, and T lymphocytes could contribute to the pathophysiology of RA.

## MATERIALS AND METHODS

**Monoclonal antibodies.** Monoclonal antibodies (MAb), MHC class II, CD2, CD4, CD11a (lymphocyte function–associated antigen 1 [LFA-1]), CD11b (MO-1), CD14 (MO-2), CD50 (ICAM-3), CD54 (ICAM-1), CD58 (LFA-3), CD60, CD80 (B7-1/BB1), CD86, (B7-2, B70), CD98, CD102 (ICAM-2), CD106 (VCAM-1), J4-81 (ALCAM) IFN$\gamma$, IL-1$\beta$, IL-2, IL-6, IL-8, and tumor necrosis factor $\alpha$ (TNF$\alpha$), as well as isotype-matched control reagents, were used. The anti-CD14 and anti-CD16 MAb were generously provided by Dr. Rob Todd (University of Michigan). Antibodies to ICAM-1 and VCAM-1 were gifts from Dr. Yoji Shimizu (University of Michigan). Antibodies to ICAM-2 and ICAM-3, and the J4-81 antibody were obtained from the Fifth Leukocyte Typing Workshop Blind Panel (25). Several antibodies were produced as previously described, i.e., anti-CD2 antibody UMCD2 (26), isotype control anti–von Willebrand factor (27), and anti-CD98 antibody UM7F8 (28). Antibody-producing hybridomas, anti-CD58 (TS 2/9.1.4.3.; $IgG_1$), anti–LFA-1$\alpha$ (TS 1/22.1.1.13), and OKT3 (CRL 8001) were purchased from American Type Culture Collection (Rockville, MD). Anti–CD80/B7-1 (Ancell, Bayport, MN), anti–CD86/B7-2/B70 (Pharmigen, San Diego, CA), and neutralizing antibodies to human cytokines IL-1$\beta$, IL-6 (Gibco BRL, Grand Island, NY), IL-2 (R & D Systems, Minneapolis, MN), IL-8, and TNF$\alpha$ (Biosource International, Camarillo, CA) were purchased. A second anti-TNF$\alpha$ MAb termed cA2 (29) and an antibody to IFN$\gamma$ (B133.3.1) were generous gifts from Dr. James Woody (Centocor, Malvern, PA). All reagents were murine monoclonal antibodies except cA2 and B133.3.1 (chimeric mouse–human monoclonals) and antibodies to IL-1$\beta$ and IL-6 (rabbit polyclonals). These reagents were used at a final concentration of 10 $\mu$g/ml.

For flow cytometric analysis, saturating dilutions of ascites fluid or 10 $\mu$g/ml of purified MAb were used. For inhibition of proliferative responses, antibodies were purified by passage over protein A or protein G Sepharose beads and used at a final concentration of 10 $\mu$g/ml.

**Synoviocyte culture.** Synovial specimens obtained at the time of total joint replacement from patients with RA were treated with 4 mg/ml collagenase (type I; Worthington Biochemical, Freehold, NJ) for 4 hours in Dulbecco's modified Eagle's medium (DMEM) at 37°C in 5% $CO_2$. The dissociated cells were then centrifuged at 500$g$, resuspended in DMEM supplemented with 10% fetal calf serum (FCS; Gibco BRL), 10% human AB serum (Biowhitaker, Walkersville, MD), penicillin (100 units/ml), and streptomycin (100 $\mu$g/ml), and plated in 75-cm$^2$ flasks. When the cells reached 95% confluence, they were passaged at a dilution of 1:3 into 175-mm$^2$ flasks. The confluent adherent cells were split by gentle trypsinization (trypsin/EDTA; Irvine Scientific, Walkersville, MD). Cells were used between the third and the twelfth passages. IFN$\gamma$ (Genentech, South San Francisco, CA)–treated synoviocytes were precultured in 1,000 units/ml IFN$\gamma$ for 48–72 hours before assay.

**T lymphocyte purification.** Human peripheral blood mononuclear cells (PBMC) from healthy normal donors were separated by Ficoll-Hypaque (Sigma, St. Louis, MO) density gradient centrifugation. T lymphocytes were isolated from the PBMC by separating E+ rosette cells (T cells) and $E^-$ rosette cells, using sheep red blood cells. Macrophages were coated with MAb to CD14 and MHC class II molecules, and were depleted from the E+ rosette cells by 2 rounds of panning with microselector flasks (Applied Immune Sciences, Santa Clara, CA). Depletion of accessory

**Table 1.** Flow cytometric analysis of non–IFNγ-treated and IFNγ-treated synoviocytes*

| Antibody | Untreated synoviocytes | | | IFNγ-treated synoviocytes | | |
|---|---|---|---|---|---|---|
| | Passages 4–6 | Passages 7–9 | Passages 10–12 | Passages 4–6 | Passages 7–9 | Passages 10–12 |
| CD54 (ICAM-1) | + | – | +++ | +++ | +++ | +++ |
| CD58 (LFA-3) | + | ++ | +++ | +++ | +++ | +++ |
| CD80 (B7-BB1) | +/– | – | + | – | – | – |
| CD86 (B7-2) | – | + | – | – | – | – |
| CD98 | +++ | +++ | ND | +++ | +++ | ND |
| CD106 (VCAM-1) | +++ | +++ | +++ | ++ | +++ | +++ |
| MHC class II | – | – | – | +++ | +++ | +++ |
| J4-81† | +++ | +++ | ND | +++ | ND | ND |

* Cells were cultured in medium alone or supplemented with interferon-γ (IFNγ) as described in Materials and Methods. Cells were stained with antibodies to the surface markers indicated and analyzed by flow cytometry. Although late-passage non–IFNγ-treated synoviocytes expressed intercellular adhesion molecule 1 (ICAM-1) and vascular cell adhesion molecule 1 (VCAM-1) at >50% fluorescence, expression of these molecules was up-regulated significantly by IFNγ. Results shown are pooled data from synoviocyte lines derived from 3 different individuals. – = <5% staining; + = >5–20% staining; ++ = >20–50% staining; +++ = >50% staining. LFA-3 = lymphocyte function–associated antigen 3; ND = not determined; MHC = major histocompatibility complex.
† Recognizes a CD6 ligand termed ALCAM.

cells from T lymphocyte preparations was monitored, and confirmed by lack of T cell proliferation with SEA, SEB, and anti-CD3.

**Proliferation assays.** SEA and SEB were purchased from Sigma, and phytohemagglutinin (PHA) from Munex Diagnostics (Atlanta, GA). Proliferation assays were performed in triplicate. T cells ($5 \times 10^4$) purified as described above, were cultured in 96-well round-bottomed culture plates (Costar, Cambridge, MA). Synoviocytes ($10^3$–$4 \times 10^4$) were added to the wells with OKT3, SEA, SEB, and PHA as stated in figure legends, in a final volume of 0.2 ml RPMI 1640 with 10% FCS. Antibodies recognizing synoviocytes and T cells were used at the concentrations indicated. Plates were incubated for 4 days at 37°C with 5% $CO_2$, pulsed for the final 18 hours of culture with 1 μCi/well of $^3$H-thymidine (ICN Biomedicals, Irvine, CA), harvested, and counted in a liquid scintillation counter (Pharmacia 1205/1204 BS Beta Counter). For proliferation assays, the results shown (mean ± SD) are from single experiments representative of at least 3 similar experiments, using synoviocytes from different patients and T cells from different normal donors.

**Fluorescence-activated cell sorter (FACS) analysis.** Flow cytometric analysis was performed using an Elite cell sorter (Coulter Electronics, Hialeah, FL). Positive cells were defined as those with fluorescence intensity beyond a threshold defined by the negative control (cells stained with isotype-matched nonspecific mouse Ig). Dead cells were excluded from analysis using forward and right-angle light scatter gating. Cell staining was measured in arbitrary units as the log fluorescence intensity and was recorded on a 3-log scale. For indirect immunofluorescence staining, phycoerythrin-conjugated second antibody was used to minimize background autofluorescence of synoviocytes (Molecular Probes, Eugene, OR).

**Effects of synoviocyte supernatants.** Seventh-passage synoviocytes ($5 \times 10^5$/well), both IFNγ-treated and non–IFNγ-treated, were placed in a 24-well plate and stimulated with SEA for 12, 18, or 24 hours. The cells were placed at a final volume of 1 ml RPMI and 10% FCS. Aliquots of the supernatants (150 μl) were placed in 96-well round-bottomed plates in triplicate, and $5 \times 10^4$ T cells in 50 μl were added to each well. Assays were performed in triplicate, and proliferation was measured as described above.

## RESULTS

**Synoviocyte expression of molecules necessary for T cell costimulation.** FACS analysis was used to determine which molecules important for T cell co-

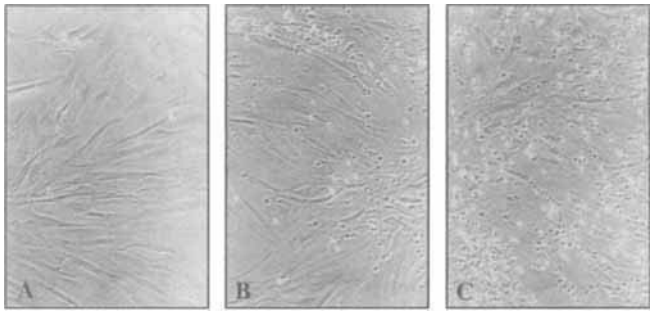


**Figure 1.** Staphylococcal enterotoxin A (SEA) promotes T lymphocyte binding to interferon-γ–treated seventh-passage synoviocytes. **A**, Synoviocytes ($5 \times 10^5$) were placed in 24-well plates and incubated for 4 days. **B**, Synoviocytes ($5.0 \times 10^5$) were cocultured with $2.5 \times 10^6$ T lymphocytes for 2 days in 24-well plates. **C**, Synoviocytes ($5.0 \times 10^5$) and T lymphocytes ($2.5 \times 10^6$) were cultured with SEA (100 ng/ml) for 2 days. Wells were washed twice with RPMI 1640–10% fetal calf serum pior to photographing. (Original magnification ×200.)

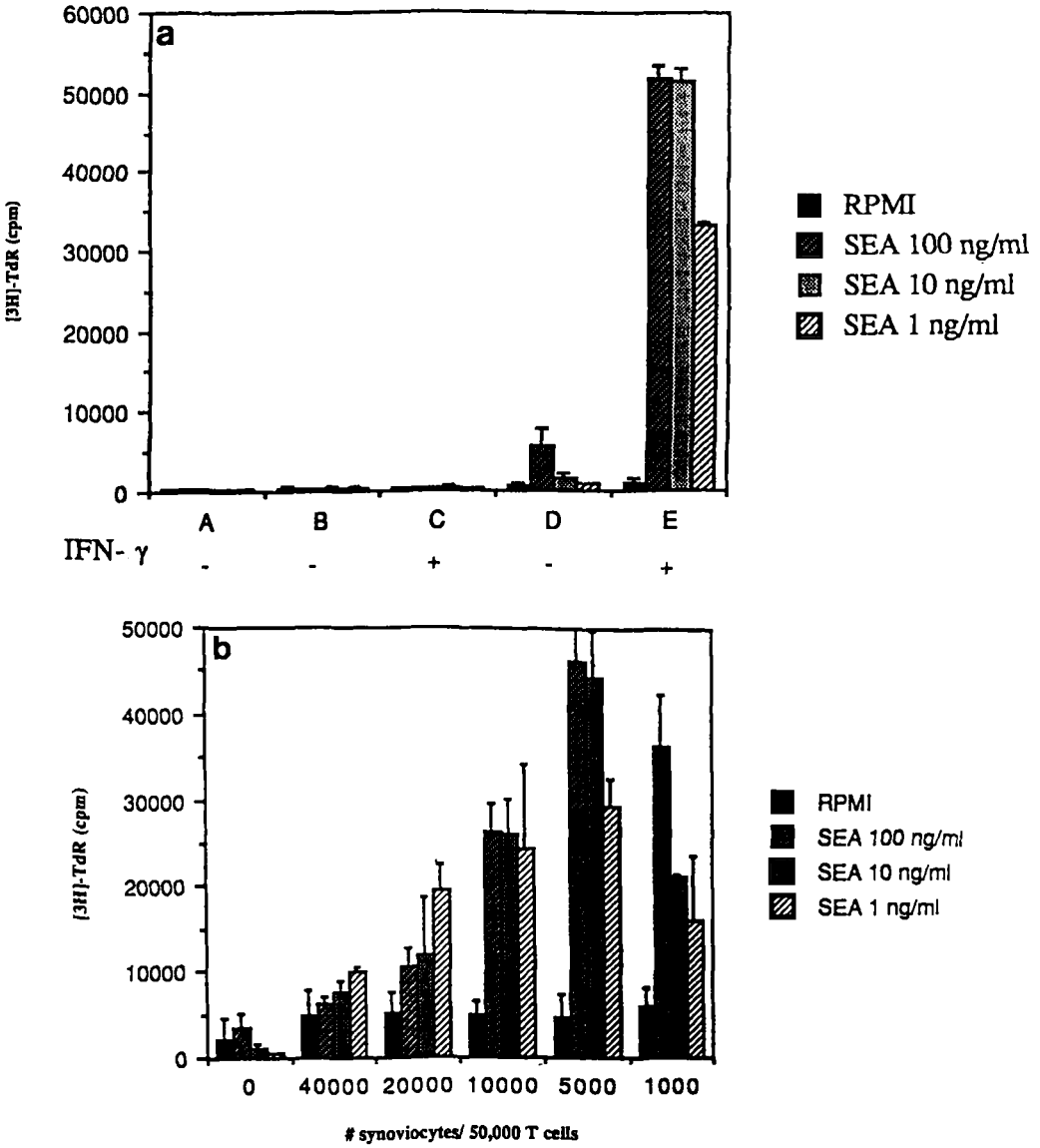


**Figure 2. a,** Function of synoviocytes as accessory cells for T cell responses to staphylococcal enteroxin A (SEA). Synoviocytes ($1.0 \times 10^4$; either interferon-γ [IFN-γ-treated or non–IFN-γ-treated) and purified T cells ($5 \times 10^4$) were added to the wells at culture initiation. Proliferation was determined as described in Materials and Methods, and is expressed as $^3$H-thymidine ([3H]-TdR) incorporation. In the presence of SEA (100 ng/ml, 10 ng/ml, or 1 ng/ml), T lymphocytes alone (A), non–IFN-γ-treated synoviocytes alone (B), and IFN-γ-treated synoviocytes alone (C) did not proliferate, as compared with non–IFN-γ-treated synoviocytes and T cells with SEA (D), or the maximal T lymphocyte response observed with IFN-γ-treated synoviocytes and SEA (E). **b,** Effect of the ratio of T lymphocytes to synoviocytes on the SEA response. Synoviocytes at $4.0 \times 10^4$, $2.0 \times 10^4$, $1.0 \times 10^4$, $0.5 \times 10^4$, and $0.1 \times 10^4$ were incubated with $5.0 \times 10^4$ purified T lymphocytes. Optimal T lymphocyte response was seen with a ratio of $0.5 \times 10^4$ synoviocytes to $5 \times 10^4$ T lymphocytes. Values are the mean and SD.

stimulation were found on the surface of synoviocytes in culture. CD106 (VCAM-1), CD98, and J4-81/ALCAM (a putative ligand for CD6), were found at high densities (Table 1), while CD11b/CD18 (MO-1) and CD14 (MO-2) were found at low levels (results not shown). In contrast, CD80 (B7-BB1), CD86 (B7-2), and the MHC class II molecule were generally not detectable (Table 1). The level of expression of CD54

(ICAM-1) and CD58 (LFA-3) varied according to the extent of passage in vitro.

Synoviocytes were treated with 1,000 units/ml of IFNγ for 48–72 hours to induce MHC class II expression as described by Burmester et al (17). IFNγ-treated synoviocytes were screened by FACS to examine whether any additional surface molecules were positively or negatively modulated on these cells. The expression of CD80 (B7-1), CD86 (B7-2), and CD98 did not change with IFNγ treatment. The expression of CD54 (ICAM-1), CD58 (LFA-3), CD106 (VCAM-1), and MHC class II was significantly upregulated (Table 1). CD14 (MO2) and CD11b/CD18 (MO1) were expressed on a subset of cells at passages 4–6, but were undetectable or present on a much smaller number of cells in later passages (data not shown). CD50 (ICAM-3) and CD102 (ICAM-2) were absent or expressed at very low levels, with or without IFNγ treatment (data not shown).

**SAg promotion of T lymphocyte binding to synoviocytes.** We examined whether SAg affects the ability of peripheral blood T lymphocytes to adhere to type B synoviocytes (Figure 1). RA type B IFNγ-treated synoviocytes bound small numbers of peripheral blood T lymphocytes without SEA (Figure 1). However, when SEA was added, the synoviocytes bound T cells at a much higher density, as measured by phase-contrast microscopy (Figure 1). Enumeration of T cells bound per high power field revealed that IFNγ-treated synoviocytes bound 3.5 times the number of T cells in the presence of SEA compared with binding in the absence of SEA.

**Synoviocyte support of the SEA response.** To investigate the ability of synoviocytes to support the SEA response, IFNγ-treated and non–IFNγ-treated synoviocytes were cocultured with highly purified allogeneic resting T cells. Proliferation experiments showed that, in the presence of SEA, synoviocytes induced T cell activation and proliferation (Figure 2a). The T cell response was SEA dose–dependent, with 10 ng/ml or 100 ng/ml SEA stimulating a greater T cell response than 1 ng/ml. The optimal response was found using 5,000 synoviocytes and 50,000 T cells (Figure 2b). Highly purified T cells did not proliferate in the presence of SEA, confirming the absence of antigen-presenting cells and the purity of the T cells (Figure 2a). In addition, synoviocytes alone, with or without SEA, and synoviocytes with T cells but no SEA, demonstrated negligible proliferation (Figure 2a). Thus, direct mitogenic effects on synoviocytes

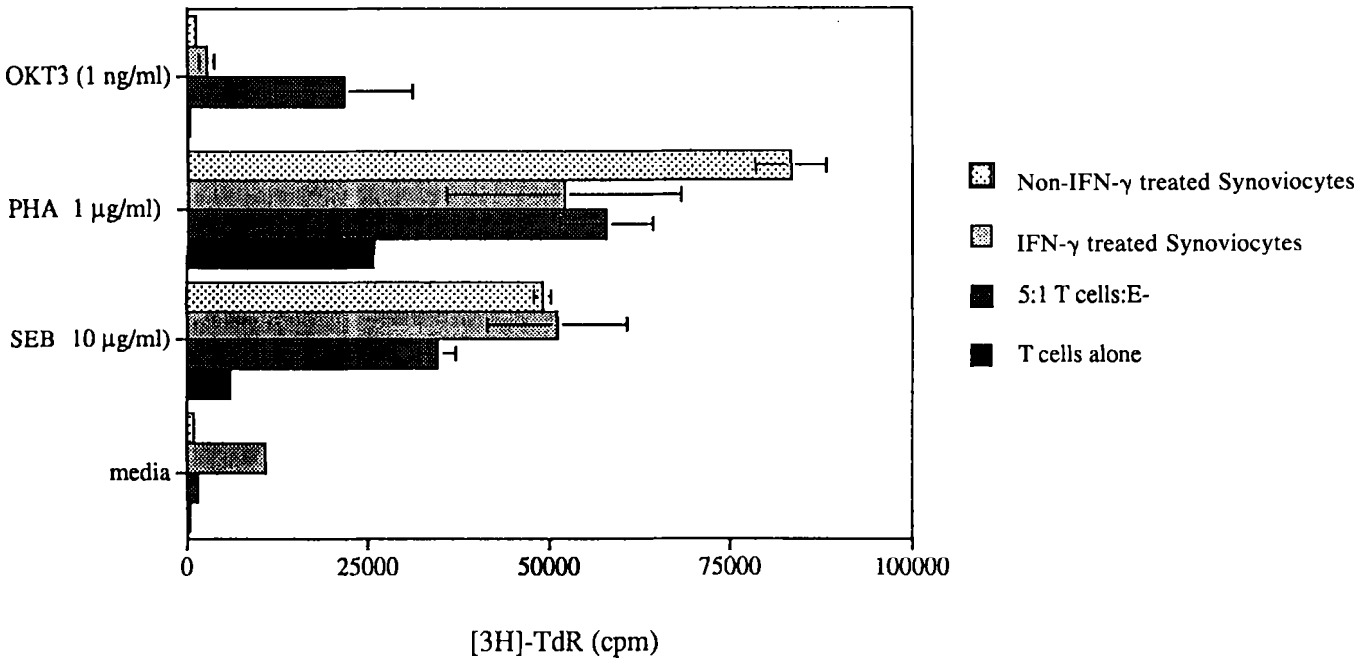


**Figure 3.** Synoviocyte support of both superantigen- and lectin-induced T lymphocyte proliferation. Purified T lymphocytes ($5.0 \times 10^4$) were stimulated with SEB (10 μg/ml), phytohemagglutinin (PHA; 1 μg/ml), or OKT3 (1:500) in the presence of synoviocytes ($1.0 \times 10^4$) (either non–IFN-γ–treated or IFN-γ–treated) and [3H]-TdR incorporation was measured. T lymphocytes ($5.0 \times 10^4$) mixed with peripheral blood E− ($1.0 \times 10^4$) cells as antigen-presenting cells were used as a positive control. Values are the mean ±SD. See Figure 2 for other definitions.

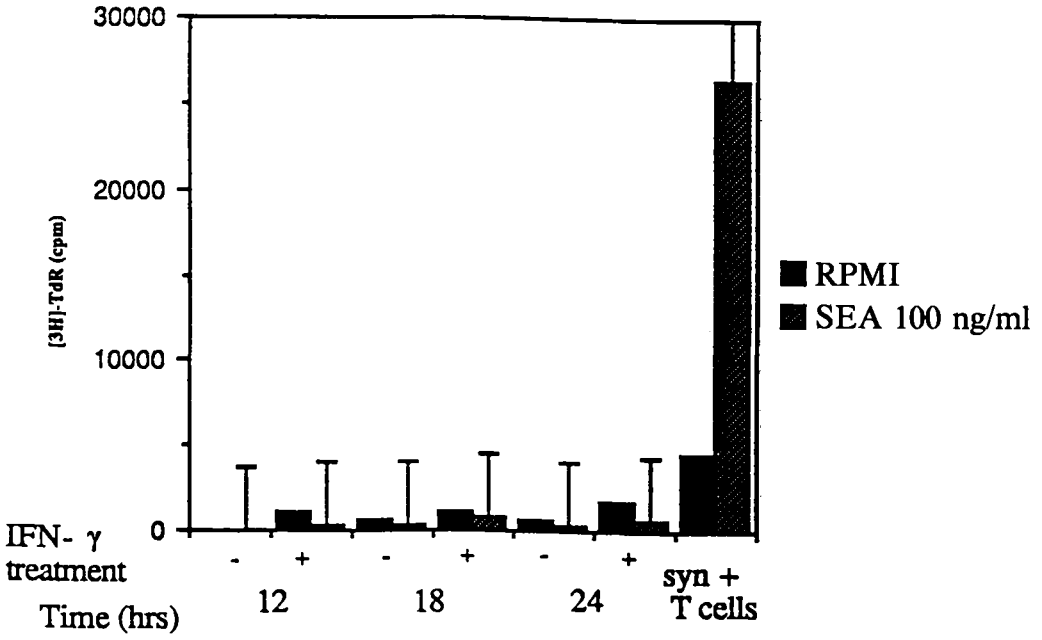


**Figure 4.** Failure of supernatants from rheumatoid arthritis synoviocytes incubated with SEA to induce T cell proliferation. IFN-γ-treated and non–IFN-γ-treated synoviocytes were preincubated with SEA (100 ng/ml) for 12 hours, 18 hours, or 24 hours before the addition of the supernatants to T lymphocytes. Synoviocytes ($5.0 \times 10^5$) were plated in 24-well plates and incubated with 1 ml of media alone or supplemented with 100 ng/ml SEA. Supernatants at 150 μl were added to $5.0 \times 10^4$ T lymphocytes, which were placed in 96-well round-bottomed plates. Cells were harvested 4 days later, after an 18-hour pulse with [3H]-TdR. Proliferation was measured by [3H]-TdR incorporation. The 2 bars on the right show the results from cultures that contained both T cells and synoviocytes (syn), and demonstrate responsiveness of the T cell population to SEA presented directly by the synoviocytes. Values are the mean and SD. See Figure 2 for other definitions.

and allogeneic responses of the T cells could not explain the proliferative responses.

**Synoviocyte support of other SAg and lectin responses.** Studies were performed to test whether synoviocytes, acting as accessory cells, could support additional T cell responses. SEB (10 μg/ml), PHA (1 μg/ml), and MAb OKT3 (1:500) in combination with synoviocytes and T cells were used in proliferation assays. SEB and PHA stimulated T cells to proliferate with both the IFNγ-treated and non–IFNγ-treated synoviocytes. In contrast, soluble OKT3 did not induce a response (Figure 3), except when peripheral blood antigen-presenting cells were cocultured with T cells.

**Necessity of cell contact for synoviocyte support of SAg responses by T cells.** To test whether cytokines secreted by synoviocytes in the presence of SEA could independently induce T cell proliferation, both IFNγ-treated and the non—IFNγ-treated synoviocytes were incubated with SEA for 12, 18, or 24 hours. The supernatants from these cultures were added to highly purified T cells. After 72 hours, T cells in culture with the supernatants demonstrated no proliferative response (Figure 4).

**Ligand–receptor interactions involved in synoviocyte-mediated SAg response.** To identify the accessory molecules necessary for the observed T cell responses, cells were incubated with SEA and MAb against CD2, CD11a/CD18 (LFA-1), MHC class II, CD58, or CD98. When high concentrations of purified MAb (10 μg/ml) were used, marked inhibition of proliferative responses was observed (Figure 5). Less complete inhibition was obtained at lower antibody concentrations (data not shown). Inhibition of T cell proliferation by MAb to CD98 and CD58 was negligible (data not shown).

**Failure of IL-1β, IL-6, TNFα, and IL-8 to independently mediate the T cell–synoviocyte response to SAg.** We tested whether neutralizing monoclonal antibodies to the cytokines IFNγ, IL-1β, IL-2, IL-6,

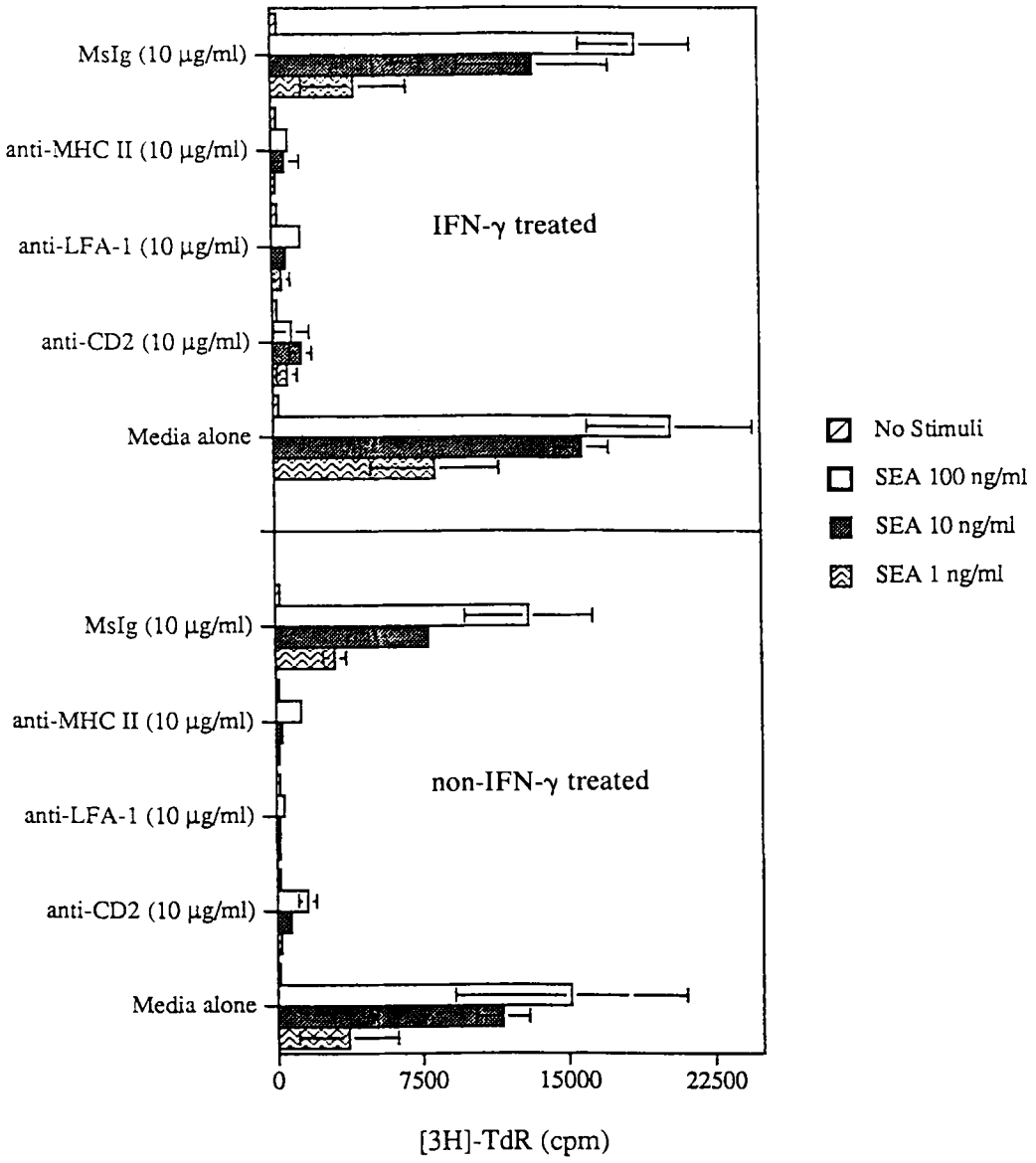


**Figure 5.** Effects of antibodies to cell surface molecules on T lymphocyte responses. Antibodies against major histocompatibility complex (MHC) class II, lymphocyte function–associated antigen 1 (LFA-1), or CD2 (10 μg/ml) were used to block accessory cell function of synoviocytes for T cell responses to SEA (100 ng/ml, 10 ng/ml, or 1 ng/ml). Purified T lymphocytes ($5.0 \times 10^4$) were incubated with synoviocytes ($1.0 \times 10^4$) in 96-well round-bottomed plates at a final volume of 200 μl. Isotype-matched control reagents, for which no blocking of T cell responses was observed, included monoclonal antibodies directed against CD6 and CD98 (data not shown). Values are the mean ±SD. MsIg = nonspecific mouse Ig. See Figure 2 for other definitions.

TNFα, and IL-8 could interrupt the signaling between synoviocytes and T cells. Both IFNγ-treated and non–IFNγ-treated cells were incubated with SEA and purified T cells, along with MAb to these cytokines. Except for anti–IL-2, the anticytokine MAb did not have any inhibitory effect on T cell proliferative responses in these experiments (Figure 6).

## DISCUSSION

The present studies demonstrate that cultured type B fibroblastic synoviocytes from patients with RA can function as accessory cells for T lymphocyte responses to superantigen and lectin. The conclusion that synoviocytes, and not a contaminating antigen-

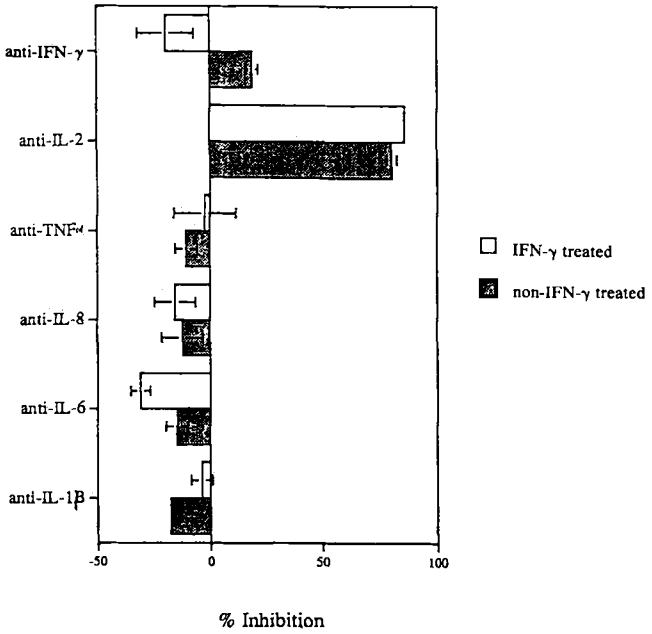


**Figure 6.** Effects of anticytokine antibodies. Antibodies to IFN-γ, interleukin-1β (IL-1β), IL-2, IL-6, IL-8, and tumor necrosis factor α (TNFα) (10 μg/ml) were incubated with purified T lymphocytes ($5.0 \times 10^4$) and synoviocytes ($1.0 \times 10^4$) in 96-well round-bottomed plates at a final volume of 200 μl in the presence or absence of SEA (100 ng/ml). The anti-TNFα antibody is a murine monoclonal reagent. Identical results were obtained with a chimeric murine/human anti-TNF antibody known to neutralize TNF function in vivo (data not shown). Additional control reagents which had no functional effects in this system included mouse IgG and a monoclonal mouse anti-human von Willebrand factor antibody (results not shown). Values are the mean ± SD. See Figure 2 for other definitions.

presenting cell population such as synovial monocytes, provided the observed accessory function is supported by several lines of evidence. First, excellent accessory cell function was seen with late-passage cultures (passages 10–12), at which time no monocytes or negligible numbers of monocytes are detectable using flow cytometric analysis of surface markers. Excellent responses were observed with 5,000 cells per well, such that the absolute numbers of contaminating cells would be very low. Furthermore, accessory cell function was actually more potent with later, rather than earlier, passages of the same synoviocyte lines, with an inverse correlation between the percentage of cells with monocyte surface markers present and the strength of the responses to superantigen or lectin upon coculture with T lymphocytes (Diaz L, Fox D: unpublished data). Finally, in most of the experiments, the T cell response to allogeneic synoviocytes was no greater than a few hundred counts per minute above background (occasionally slightly higher, as in Figure 3), inconsistent with the presence of substantial numbers of professional antigen-presenting cells, which are typically excellent stimulators of allogeneic responses. Poor allostimulatory capacity of mixed synoviocyte populations has been observed previously (30). The T cell populations used were sufficiently depleted of accessory cell populations such that they could not respond to superantigen or anti-CD3, although partial responses to PHA were sometimes seen (Figure 3).

Consistent with this evidence for low levels of contamination by professional antigen-presenting cells was the absence of significant levels of MHC class II expression on synoviocytes unless these cells were cultured with IFNγ. Other published studies have shown that a variety of important cell interaction molecules, including MHC class II, ICAM-1 (CD54), and VCAM-1 (CD106), are inducible on synoviocytes with IFNγ, and such molecules have been detected in vivo and implicated in the pathogenesis of RA (31). An interesting finding in the present studies was that IFNγ was not necessary for accessory cell function of synoviocytes, although it boosted such function in most experiments. A spectrum of results was obtained, ranging from minimal but clearly detectable accessory cell function in the absence of IFNγ (Figure 2a) to modest augmentation of accessory cell function following treatment with IFNγ (Figure 5) to equivalent accessory cell function in the presence and absence of IFNγ (Figure 3).

The observation that synoviocytes can present superantigen in the absence of baseline MHC class II expression detectable by flow cytometric analysis suggests several potential explanations. One possibility is that synoviocyte molecules other than MHC class II are responsible for presenting superantigen, as has been suggested in other experimental systems (32,33). Alternatively, MHC class II molecules may be expressed at functionally significant levels by synoviocytes, but at a surface density too low to be detected by flow cytometry. Finally, it is plausible that MHC class II is significantly up-regulated on synoviocytes early during coculture with T cells, facilitating presentation of superantigen. In support of either or both of these latter 2 possibilities, antibody against MHC class II effectively blocked superantigen presentation (Figure 5). In other experiments (results not shown), lower concentrations of anti–MHC class II more effectively blocked antigen presentation by non–IFNγ-pretreated

cells compared with IFN$\gamma$-pretreated synoviocytes. These results are consistent with functionally relevant low-level MHC class II expression on non–IFN$\gamma$-pretreated synoviocytes. It appears likely that functional assays using blocking effects of antibodies are more sensitive for demonstrating a role of MHC class II in these experiments than is baseline flow cytometric staining.

The foregoing considerations suggest that in experiments in which IFN$\gamma$ boosted accessory cell function of synoviocytes, superinduction of expression of MHC class II antigens might not necessarily be the principal reason for the observed functional changes. A variety of other accessory cell surface molecules may serve as costimulatory ligands for T cell surface structures in the response to superantigen, as in other T cell responses. The role of specific costimulatory structures in SAg responses may be complex, with distinct effects on transcription and secretion of various T cell cytokines (34). RA type B synoviocytes express ICAM-1 in vivo, but ICAM-2 and ICAM-3 are expressed only on endothelial cells and leukocytes in synovium (31,35). This distinction appears to be maintained in vitro on type B synoviocytes. CD28 ligands are absent on or barely expressed by type B synoviocytes (Table 1). The role of these molecules in T cell proliferative responses to SAg is controversial (36–38), but interaction of CD28 with its ligands may be involved in SAg-triggered T cell–B cell cooperation (39).

In order to determine the necessary costimulatory receptors and ligands for T cell responses to SAg presented by synoviocytes, antibodies were used in blocking experiments. When used at high concentrations (10 $\mu$g/ml), purified antibodies directed at LFA-1 (CD11a/CD18) and CD2 strongly inhibited superantigen responses of T lymphocytes in the presence of either IFN$\gamma$-pretreated or untreated synoviocytes. In other experiments (results not shown), lower concentrations of antibody showed more variable inhibitory effects, and inhibition was less effective with IFN$\gamma$-pretreated synoviocytes. Such results are compatible with higher expression of costimulatory ligands for CD2 and LFA-1 on IFN$\gamma$-treated synoviocytes, such that blocking effects of monoclonal antibodies are difficult to obtain at lower antibody concentrations. Consistent with this interpretation was the finding that expression of CD54 and CD58, ligands for LFA-1 and CD2, respectively, were up-regulated with IFN$\gamma$ treatment. Even when a majority of cells expressed these molecules at baseline, surface density was increased following exposure to IFN$\gamma$.

It was noted that anti-CD58 did not block T cell responses despite blocking effects of anti-CD2. This may indicate insufficient affinity of the anti-CD58 reagent, or alternatively, may suggest that anti-CD2 has effects on T cell function other than inhibition of CD58 binding. These could include inhibition of binding to other CD2 ligands or direct regulatory signals generated through CD2. Anti-CD98 antibody did not block T cell proliferation in response to SEA presented by IFN$\gamma$-treated or non–IFN$\gamma$-treated synoviocytes. However, it has been demonstrated to block the T cell response to SEA with PBMC acting as antigen-presenting cells (40). These discrepancies are probably due to differences in the antigen-presenting cell populations used in these 2 systems.

Effects of antibodies directed at cell–cell interaction structures suggest that the observed T cell responses required cell–cell contact, not merely factors secreted by accessory cells. Considerable binding of T cells to synoviocytes was observed, particularly in the presence of superantigen (Figure 1). Since superantigen-stimulated synoviocytes have been found to secrete cytokines such as IL-6 and IL-8 (22), it was important to establish that supernatants of superantigen-treated synoviocytes could not by themselves support T cell proliferation, and this was demonstrated by the data shown in Figure 4. These data do not, however, exclude an important role for cytokines in costimulating the observed T cell responses, and effects of anticytokine monoclonal antibodies were therefore investigated. Individual antibodies to IFN$\gamma$, IL-1$\beta$, IL-6, IL-8, and TNF$\alpha$ did not down-regulate synoviocyte/SEA-induced T cell proliferation. These results must be interpreted with caution, however. Although the antibodies were selected because of documented neutralizing properties (refs. 41 and 42, and manufacturers' data), massive antibody concentrations might be needed to block the responses examined in this study. If membrane-bound cytokines play a role in regions of close cell contact, antibody blocking might be inefficient. Further studies using combinations of antibodies seem warranted. A blocking antibody directed at human IL-2 did inhibit T cell proliferation significantly, consistent with an IL-2/IL-2 receptor–dependent T cell activation pathway triggered by superantigen presented by synoviocytes.

Although type B fibroblastic synoviocytes were potent accessory cells for T cell responses to superantigen and lectin, such cells may not exhibit a full

spectrum of accessory cell functions. Thus, responses to anti-CD3 antibody were not well supported by synoviocytes, possibly relating to poor expression of Fc receptors on these cells or a requirement for CD28 ligands on accessory cells for this response. A recent study has suggested that synoviocytes can play a role in autologous presentation to T cells of a nominal antigen, tetanus toxoid, which is MHC class II restricted (43). Furthermore, synoviocytes were able to function as antigen-presenting cells for T cell clones specific for mycobacterial antigens, also in an MHC class II–restricted system (43). However, the proliferative responses observed were relatively modest. In contrast, strong proliferative responses were observed in the present studies with low SAg concentrations, comparable with SAg responses supported by professional antigen-presenting cells, and in contrast to responses supported by the murine fibroblast L cell line transfected with MHC class II molecules, which require much higher SAg concentrations (37).

Synoviocytes are not necessarily the only type of fibroblastic cell capable of functioning in antigen presentation. A recent report documents the capacity of fibroblasts transfected with viral proteins to induce antiviral cytotoxic T lymphocyte responses independent of other professional antigen-presenting cells, but only in the environment of lymphoid organs (44). In this regard, our findings with RA type B synoviocytes could be relevant to cell–cell interactions in vivo, given the histologic resemblance of RA synovial inflammation to lymphoid tissue in patients with active disease.

A role for superantigens in the pathogenesis of RA would depend on the functional properties of accessory cells found in the synovium (and possibly elsewhere) and on the responses of T lymphocytes to such antigens. Some recent reports have suggested that the V$\beta$ repertoire in RA reflects SAg responses (23,24), but other studies have yielded somewhat contradictory results (45,46). No reports, to our knowledge, have documented the ability of synovial tissue cells to present SAg to T lymphocytes.

Superantigens have been implicated in various infectious diseases due to staphylococci and mycobacteria (47,48). Endogenous superantigens have been identified in mice (for review, see ref. 49) but not yet in humans. In animal models, SAg responses can initiate joint inflammation (50) or exacerbate autoimmune responses in the synovium (51) or in the central nervous system (52,53). If SAg have a role in autoimmune diseases with organ-specific manifestations, SAg presentation by cells localized to such organs could be important. Pertinent findings in this regard include the recent demonstration that keratinocytes can present SAg in an LFA-1/ICAM-1–dependent manner (54,55) and a report of SAg presentation by intestinal epithelial cells (56). Dendritic cells localized to the skin (57), the joint (58), or other tissues could also be important in localized SAg presentation. Recently, evidence for a SAg effect on lymphocytes infiltrating the pancreas in early type I diabetes has been described (59).

The data in this report demonstrate that cultured rheumatoid type B fibroblast-like synoviocytes are capable of supporting T lymphocyte proliferation in response to SAg and lectin. This system, while not establishing a role for SAg in RA, provides a new and useful model for examining synoviocyte–T cell interactions in induction of T cell responses that could contribute to the pathogenesis of this disease. These findings, together with evidence that RA type B synoviocytes can support differentiation of B cells into plasma cells (60), begin to define a role for synoviocytes in the induction of immune responses.

**Addendum.** A recent report by Kraft and colleagues (61) also documents accessory cell function of synovial fibroblasts in T cell responses to superantigen.

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