Influences of Gamma Interferon on Synovial Fibroblastlike Cells

Ia Induction and Inhibition of Collagen Synthesis

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Abstract

The shape and function of adherent cells cultured from rheumatoid synovial membranes are influenced by immune cells, and their products. The synovial cells produce collagenase and prostaglandin E_2 (PGE_2), the levels of which are increased when the cells are incubated with the monokine, mononuclear cell factor/interleukin 1. The majority of adherent synovial cells are fibroblastlike in appearance and synthesize collagens and fibronectin; the synthesis of collagens and fibronectins are also increased by a monocyte factor. In the present study we found that the fibroblastlike cells expressed major histocompatibility complex class II (Ia-like) antigens after initial dispersion from the synovial membrane. Monocyte lineage antigens were detected on some round cells in early passage, but no T lymphocytes were identified in established cultures. There was loss of Ia expression on the fibroblastlike cells with age and passage in culture. The addition of the lymphokine, gamma interferon (recombinant), induced class II antigen (DR and DS/DQ) expression in early or late passage cells in a time- and dose-dependent manner and required protein synthesis. Furthermore, the adherent synovial fibroblastlike cells continued to be Ia-positive when examined as long as 10 d after the removal of gamma interferon. Ia expression was also induced by gamma interferon in normal skin fibroblasts. Synovial cells that could be induced to express Ia also bound a monoclonal antibody to type III collagen (a fibroblast marker). Gamma interferon, while inducing Ia expression, decreased the binding of type III collagen antibody on unstimulated as well as monokine-stimulated cells. Analysis of [^{3}H]proline-labeled medium by SDS polyacrylamide gel electrophoresis showed that gamma interferon decreased the synthesis of type I and III collagens and fibronectin by adherent synovial cells in a dose-dependent manner.

These findings suggest that Ia expression by synovial tissue cells is not cell-specific, but reflects one or several related events, such as the degree of T lymphocyte infiltration, the presence of factors that stimulate gamma interferon release, or an increased sensitivity of the cells to gamma interferon. Whereas the synthesis of class II antigens is enhanced by the lymphokine gamma interferon, and a monocyte factor(s) stimulates collagen, collagenase and PGE_2 synthesis by the same cells, gamma interferon inhibits basal and monokine-induced collagen synthesis. Thus, lymphokines and monokines may influence the extent of fibrosis as contrasted to matrix destruction at various stages of the rheumatoid lesion by affecting the function of fibroblastlike synovial cells.

Introduction

The inflammatory synovial membrane of rheumatoid arthritis includes among other cells, T and B lymphocytes, monocyte/macrophages, and fibroblastlike cells (1). One of the earliest lesions described in rheumatoid arthritis is a proliferation of the synovial lining cells (2, 3). Although there is marked variability in the distribution of cells even within a single synovial specimen from patients with active disease, a frequent finding is that of activated T lymphocytes clustered around monocytes which suggests a cell-mediated immune response to an antigen as yet unidentified (4, 5). The association of the proliferating inflammatory synovium contiguous with bone erosions and disrupted capsules, ligaments, and tendons, which are the hallmark of the disease, suggests that proteolytic enzymes are involved in the extracellular degradation of the connective tissue matrix of the joint. We have previously shown that when cultures of the synovial lining membrane from patients with rheumatoid arthritis are established, the adherent cells produce latent collagenase among other proteases as well as prostaglandin E_2 (PGE_2)[1] (6).

With age in culture and cell passage the spontaneous production of PGE_2 and collagenase decreases, but can be augmented by the addition of a soluble factor from monocytes, which we have termed mononuclear cell factor. Mononuclear cell factor copurifies with interleukin 1 and has similar chemical properties (7). In addition, partially purified conditioned medium from monocyte-enriched cells that contains mononuclear cell factor/interleukin 1 activity stimulates the synthesis of fibronectin as well as type I and III collagens by the synovial fibroblastlike cells (8). T lymphocytes produce a soluble factor that stimulates the synthesis and release of mononuclear cell factor/interleukin 1 by monocytes, which in turn influences the function of the presumably nonimmune synovial cells (9–11). Fibroblasts in the area of a cell-mediated immune response could therefore be influenced by a variety of soluble signals elaborated by adjacent monocyte/macrophages and T lymphocytes.

The development of monoclonal antibodies to cell surface antigens has permitted a more precise evaluation of the cell

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1. *Abbreviations used in this paper:* PAGE, polyacrylamide gel electrophoresis; PGE_2, prostaglandin E_2.

types present in normal and abnormal tissues. By use of these antibodies, cells have been analyzed in blood, synovial fluid, and synovial tissue from patients with rheumatoid arthritis. Alterations in the ratio of T lymphocyte subsets, patterns of cell clustering in situ and expression of major histocompatibility complex class II (Ia-like) antigens have been observed (4, 12–17). The results of these studies have indicated several potential cellular interactions that might be involved in the synovial inflammation (18, 19), but have not defined functional relationships among the cells.

We undertook the present study by using our cell culture system to address the question of possible functional interrelationships, by attempting to define the phenotype of the cultured synovial cells and to evaluate the influence of immune cell products on the adherent synovial cells. Our results show that adherent synovial fibroblastlike cells continue to synthesize type I and III collagens while the expression of Ia antigens decreases in culture. Recombinant DNA-derived gamma interferon inhibits basal collagen synthesis as well as monocyte-stimulated collagen synthesis by these cells while at the same time inducing the de novo expression of class II (DR and DQ) antigens. Therefore, gamma interferon is not only a specific T lymphocyte factor for Ia expression by synovial fibroblasts but may also be responsible for lymphokine-mediated inhibition of collagen synthesis.

Methods

Cell culture. Adherent rheumatoid synovial cells were maintained in culture after enzymatic dispersion of the lining cells from synovial tissue obtained during joint surgery as previously described (6). Cells were grown in 100-mm diam tissue culture plates (Falcon Labware, Div. of Becton, Dickinson & Co., Oxnard, CA) in Dulbecco's modified Eagle's (DME) medium (Gibco Laboratories, Grand Island, NY), supplemented with 10% fetal calf serum (Bioproducts, Inc. Warrenton, OR), 100 U/ml penicillin, and 100 μg/ml streptomycin (Gibco Laboratories) at 37°C in a humidified atmosphere of 5% CO_2 and 95% air. Human foreskin fibroblasts (kindly provided by Dr. Richard Erbe, Genetics Unit, Massachusetts General Hospital) were cultured under similar conditions. Cells were passaged with trypsin (trypsin-EDTA, Gibco Laboratories).

Analysis of cell-associated antigens. A portion of rheumatoid synovial membrane was snap-frozen in liquid nitrogen and placed in OCT compound (Ames Co., Division of Miles Laboratories, Elkhart, IN) for tissue sections. Similar fragments of the synovial membrane were placed in formalin and processed for staining with hematoxylin and eosin. Dispersed cells were either cultured initially in four chamber tissue culture slides (Lab-Tek, Division of Miles Laboratories, Westmont, IL) or transferred to these chambers after trypsinization from 100-mm diam dishes. Cultures were allowed to recover from trypsinization for at least 24 h before analysis. The adherent synovial cells were analyzed using the following monoclonal antibodies: OKT3, OKT8, OKM1, OKIa (Ortho Pharmaceutical, Raritan, NJ) (20), anti-Leu-1, anti-Leu-4, anti-HLA-DR (21), anti-Leu-M3 (22), anti-Leu-10 (DC/DS, now designated DQ[23]) (24) (Becton-Dickinson Monoclonal Center, Mountain View, CA), anti-T4, anti-B1, anti-B2 (25) (Coulter clone, Coulter Diagnostics, Inc., Hialeah, FL), antivimentin (Labsystems, Inc., Chicago, IL) and anti-HLA (Bethesda Research Laboratories, Inc., Gaithersburg, MD) (26). In addition, the presence of collagen was assessed using a monoclonal antibody to human type III collagen. The antibody (IgG_{2b}) recognizes a helical, conformation-independent domain within the carboxyl-cyanogen bromide peptide α1(III)CB5 or α1(III)CB9 of human type III collagen. It does not cross-react with human types I, IV, and V collagens or bovine type II collagen (Moorhouse, C. M., A. L. Cook, K. J. Thompson, K. M. Dawson, G. J. O'Neill, C. H. J. Sear, and K. G. McCullagh, manuscript submitted for publication). Monoclonal antibody binding was assessed by avidin–biotin immunóperoxidase staining and quantitated using ^{125}I-labeled second antibody. Immunoperoxidase staining was performed by use of the avidin–biotin complex method as previously described on air-dried and acetone-fixed cells (27, 28). Acetone-fixed frozen tissue sections or cells on tissue culture slides were incubated with normal horse serum for 10 min, followed by optimal dilutions of the monoclonal antibodies. After 30 min of incubation and washes, biotinylated horse anti-mouse IgG (Vector Laboratories, Burlingame, CA) diluted at 1:100 (previously absorbed with 10 mg each of human liver and kidney powders) was added and incubation continued for an additional 3 min followed by further washes. Endogenous peroxidase activity was blocked by incubating the slides in 0.3% hydrogen peroxide in phosphate-buffered saline for 30 min following which the avidin–biotinylated peroxidase complex diluted 1:120 was added for 45 min. The sections were stained by incubation in a solution of 3-amino-9-ethyl-carbazole (Aldrich Chemicals Co., Inc. Milwaukee, WI) and postfixed in 4% formaldehyde for 5 min, counterstained with hematoxylin, and mounted in Gelvatol 20/30 (Monsanto, St. Louis, MO).

HLA-DR expression was quantitated on intact cultured cells grown in 24-well trays (Costar) using ^{125}I-labeled intact or $F(ab')_2$ goat anti-mouse IgG (Amersham Searle, Arlington Heights, IL). Cells incubated under the various conditions were washed three times with Tris-balanced salt buffer supplemented with 0.2% bovine serum albumin (washing buffer) and incubated with the monoclonal antibody HLA-DR (Becton-Dickinson Monoclonal Center) diluted 1:100 with washing buffer for 1 h on a rotating platform. Wells were then washed three times and incubated with ^{125}I-labeled goat anti-mouse antibody for an additional h. After three washes, cells were removed in 1 ml of 1 M NaOH and bound antibody measured with a gamma counter.

To determine effects of inhibition of protein synthesis on Ia expression, cycloheximide (Sigma Chemical Co.) was added at varying concentrations to adherent synovial cells in four-chamber tissue culture slides with or without gamma interferon. Ia induction was assayed after 2 d of culture by immunoperoxidase staining.

Collagen synthesis. Cultures were incubated with or without gamma interferon (recombinant DNA-derived) in 3.5-cm diam six-well trays with DME medium, 10% fetal calf serum, for 2 d at 5×10^5 cells/ml (confluent cultures). The medium was removed, cells were washed three times and 1.0 ml of fresh medium without fetal calf serum was added which contained 50 μg/ml β-aminopropionitrile, 50 μg/ml ascorbic acid, 2 mM glutamine, and 20 μCi L-[5-^3H]proline (30 Ci/mmol, Amersham Searle, IL). The cultures were then incubated for an additional 24 h. The medium was then removed and aliquot portions analyzed by SDS-polyacrylamide gel electrophoresis (PAGE) (5% acrylamide) (29) with and without reduction with 0.5% β-mercaptoethanol. ^{14}C-labeled rat tail tendon collagen (30) was used for molecular weight markers. The labeled collagen in the cell culture medium was further characterized after pepsinization at 4°C, and the collagens were analyzed by SDS-PAGE with delayed reduction to distinguish α1(I) from α1(III) chains (31). Fluorograms of the gels were prepared as described (32).

Gamma interferon. Biologically active, recombinant gamma interferon (sp act $\sim 2 \times 10^6$ U/mg of protein), kindly provided by Genentech, Inc. (South San Francisco, CA) was stored at 4°C in concentrated form and diluted immediately before use.

Monocyte-conditioned medium. Mononuclear cells were obtained from buffy coat preparations prepared by centrifugation on Ficoll/metrizoate (Lymphoprep; Nyegaard, Oslo, Norway) gradients at 400 *g* for 40 min at 20°C. Cultures were enriched for adherent cells by incubation for 1 h at 37°C in plastic culture dishes (Falcon) followed by multiple washes to remove the nonadherent cells. The adherent cells were then incubated for 3 d with Iscove's modified Dulbecco's medium containing 10% fetal calf serum. The cell-free conditioned medium was dialyzed against H_2O (Spectropor membrane tubing, nominal molecular weight cutoff ~ 3,500; Spectrum Medical Industries, Inc., Los Angeles, CA) and lyophilized. This crude conditioned medium

from monocyte-enriched cells was used as the source of the collagen-inducing factor for fibroblasts in these experiments. Previous studies have shown that fibroblast collagen-inducing activity is present in AcA54 column fractions which also contain mononuclear cell factor/interleukin 1 activity (8).

Mononuclear cell factor assays. Mononuclear cell factor assays were performed as previously described (6). After enzymatic dispersion of the synovial lining cells, the adherent cell population was prepared and maintained in culture as described above. Before bioassay adherent synovial cells were harvested with trypsin (trypsin-EDTA, Gibco Laboratories) and placed in 24-well trays at 5×10^4 cells per well. Samples to be tested were then diluted in DME medium (Gibco Laboratories) which contained 10% fetal calf serum and penicillin/streptomycin and incubated with the adherent synovial cells for 3 d. PGE_2 production by the synovial cells was determined by radioimmunoassay (33) that used an antibody provided by Dr. L. Levine, Brandeis University, Waltham, MA.

Results

Analysis of synovial cells. The histologic appearance of the nine synovial samples used in these studies was consistent with the clinical diagnosis of rheumatoid arthritis with a prominent mononuclear cell infiltrate and synovial lining cell hyperplasia. Analysis with a panel of monoclonal antibodies and a two-step avidin–biotin immunoperoxidase stain confirmed previous reports describing clusters of T lymphocyte and monocytes (4, 14). The adherent synovial cells cultured from these specimens and used in the mononuclear cell factor assay were then analyzed from the time of their initial culture through serial passages.

Fig. 1 is a phase-contrast photomicrograph of adherent synovial cells in their second passage incubated with preparations containing mononuclear cell factor/interleukin 1. Several large stellate cells are present in this field. We have previously demonstrated that the morphology of this cell is dependent upon the concentration of PGE_2 in the medium (34). When the adherent synovial cells were examined by using the monoclonal antibodies, we found, as expected, that in primary cultures the small round cells that attached to the adherent fibroblastlike cells and to the plastic surface of the culture dish bound antibodies to monocyte-related antigens (OKM1, Leu-M3). By the second cell passage no cells were present that expressed monocyte, B (B1, B2) or T lymphocyte (Leu-1, T3) antigens whereas all were stained with antibodies directed against class I antigens (HLA) and the intermediate filament vimentin. Of considerable interest is that many of the adherent fibroblastlike synovial cells in primary culture expressed Ia antigens (Fig. 2 *A*). Most of the small round cells apparently attached to the fibroblastlike cells were also Ia positive. With age in culture and cell passage, the round cells could no longer be detected (Fig. 2, *B* and *C*). Coincidental with this loss of the round cells was a marked decrease in the number of fibroblastlike cells that expressed Ia antigens. Occasionally an Ia-positive cell was found in the third cell passage cultures, which had the appearance of an elongated bipolar cell (Fig. 3 *A*). On the other hand, late passage stellate cells were all Ia antigen-negative (Fig. 3 *B*).

Effects of gamma interferon on Ia expression. This loss of Ia antigen expression with cell passage suggested among other possibilities that: (*a*) the large cells that had a fibroblast appearance and lacked other immune cell markers were not

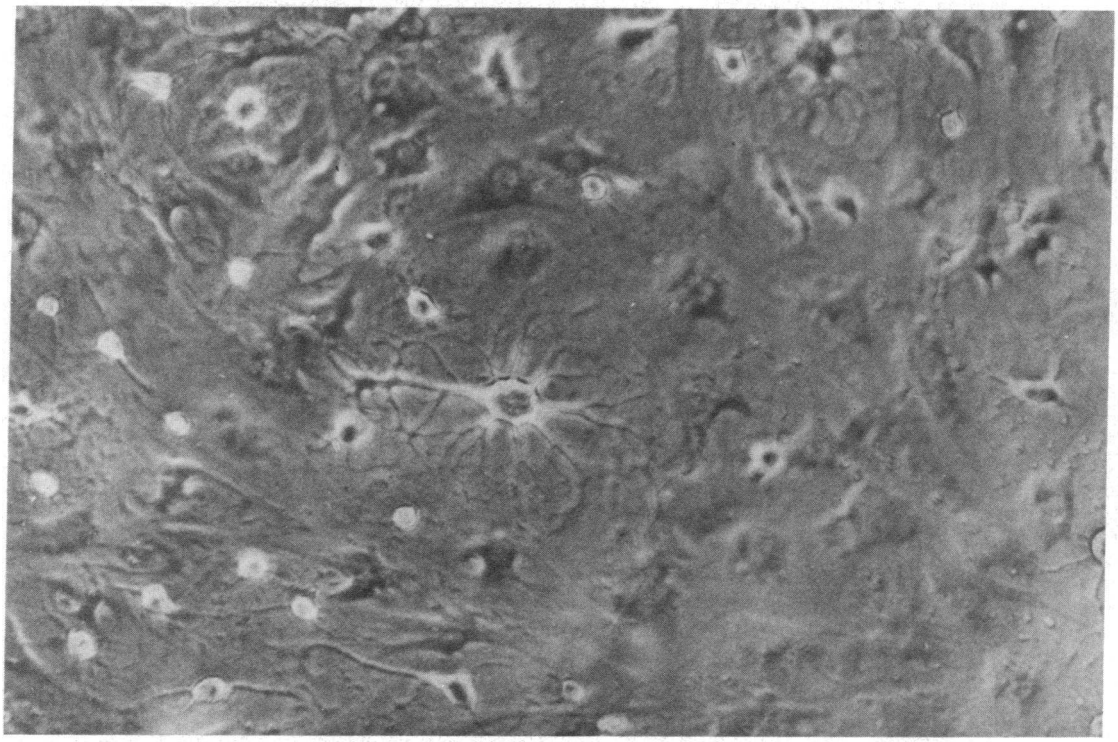

Figure 1. Phase-contrast photomicrograph of mononuclear cell factor/interleukin 1-stimulated adherent synovial cells. Adherent synovial cells in their second passage were plated at 5×10^4/ml for 3 d. They were then incubated with mononuclear cell factor/interleukin 1-containing conditioned medium for an additional 3 d. Wet preparations, were photographed through an inverted-stage microscope (× 688).

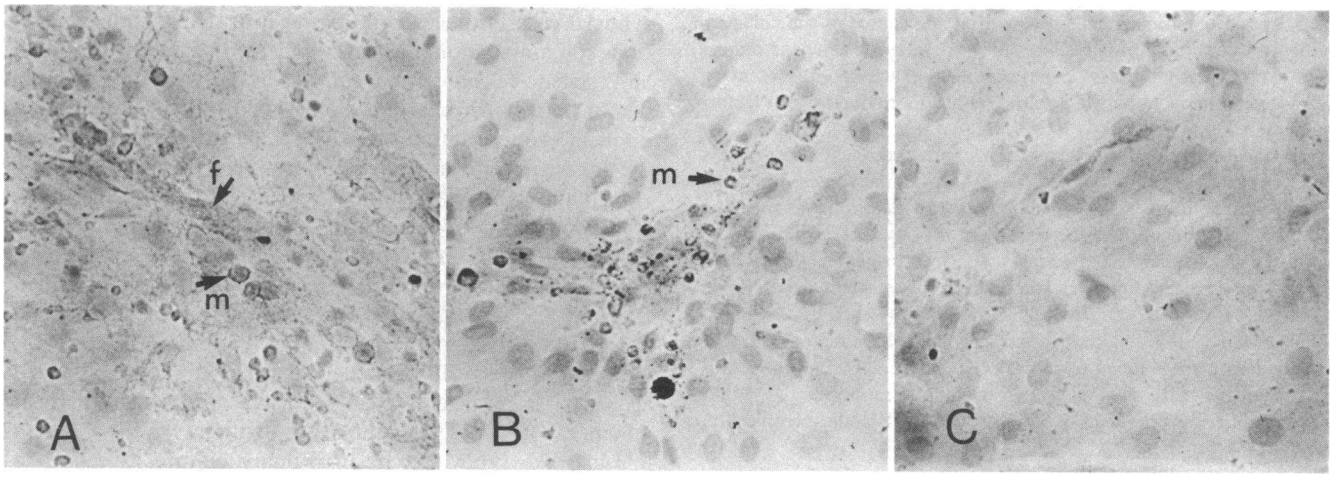

Figure 2. Serially passaged adherent synovial cells stained with anti-HLA-DR antibody. Synovial cells after enzymatic dispersion were initially cultured in four-chamber tissue culture slides or transferred to them after serial passages. 24 h after plating, nonadherent cells were removed and the adherent cells air dried and frozen or maintained in culture for analysis following the cell passages. (*A*) Adherent round cells (*m*) and fibroblastlike cells (*f*) stained with the anti-HLA-DR antibody 48 h after initial cell dispersion. (*B*) After the first cell passage, 7 d later, the number of HLA-DR-positive round cells (*m*) were reduced in number and the fibroblastlike cells stained with less intensity. (*C*) By the second passage 35 d later, no round cells were detected and the fibroblastlike synovial cells did not stain with the anti-HLA-DR antibody (× 228).

fibroblasts; (*b*) a subpopulation of Ia-bearing fibroblasts was lost with cell passage; (*c*) the Ia antigens associated with the fibroblastlike cells were shed by the adherent immune cells; or (*d*) the in vivo environment from which the fibroblastlike cells were removed influenced their expression of Ia antigens, possibly mediated through locally secreted soluble factors.

We favored the latter possibility because previous work indicated that the lymphokine, gamma interferon induced Ia expression by many cell types including among others, monocytes, endothelial cells, a variety of neoplastic cells, and skin fibroblasts (35–38). Although we had shown that a monocyte product augmented collagen and fibronectin synthesis by adherent rheumatoid synovial cells, the cells that were synthesizing these macromolecules were not further characterized (8). Therefore, using the monoclonal antibody to type III collagen, we attempted to correlate collagen content of the intact, adherent synovial cells with expression of Ia antigens in the presence and absence of the monocyte-derived factor and

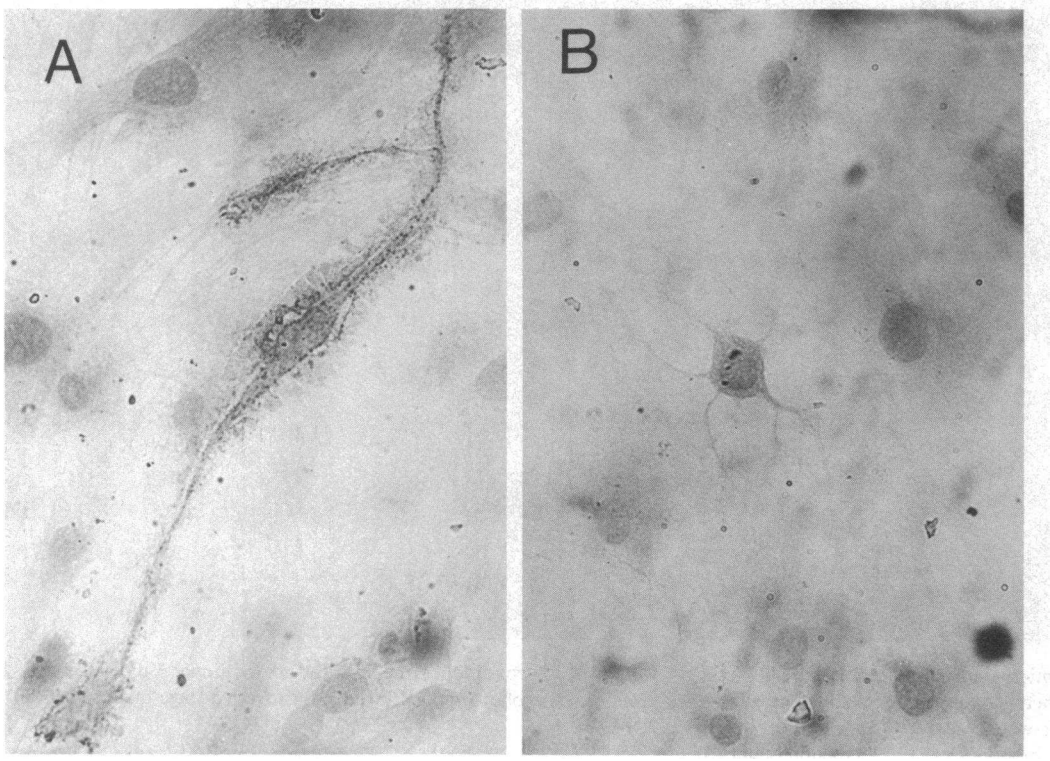

Figure 3. Adherent synovial cells stained with anti-HLA-DR antibody. (*A*) A bipolar HLA-DR-positive adherent synovial cell, rarely seen at the third cell passage. (*B*) In another synovial sample a stellate cell is HLA-DR-negative (× 313).

gamma interferon. As shown in Fig. 4, *A* and *B*, recombinant DNA-derived gamma interferon induced DR antigen expression on all adherent fibroblastlike synovial cells at a time when control cells had no detectable DR antigens.

To investigate the possibility that only a subgroup of cells were responsive to gamma interferon, similar preparations of adherent synovial cells were maintained in culture for periods up to 4 mo and passaged six times before repeating the study. Incubation with gamma interferon again induced DR and DQ antigen expression on all cells. Similar results were obtained using normal skin fibroblasts (Fig. 4, *C* and *D*). In addition, although the induction of DQ antigen (Leu-10) was detected in early- and late-passage adherent synovial cells and dermal fibroblasts, the staining intensity was less than for the DR antigen. The induction of Ia antigen expression by gamma interferon could be quantitated using an ^{125}I-labeled second antibody. The effect of gamma interferon on DR expression was dose-dependent and was readily discernible at concentrations as low as 0.1 U/ml when assayed after 48 h of incubation (Table I). DR expression was detected after 16 h of incubation, but was not evident at 8 h. The induction of DR antigens by gamma interferon was blocked by cycloheximide suggesting that new protein synthesis was necessary for this event as has been previously reported with other cell types (39).

Ia expression by most cells is a transient event after induction. For example, murine monocyte/macrophages (40) and gut epithelial cells (39) do not retain detectable Ia antigens within 3 d of removal of gamma interferon from incubation medium. After only 24 h of exposure to gamma interferon, however, adherent synovial cells continued to express Ia antigens at maximum intensity as long as 10 d after the removal of gamma interferon as assessed by immunoperoxidase staining. In similar experiments, cultured skin fibroblasts expressed Ia antigens for 8 d after 24 h of incubation with gamma interferon. The prolonged expression of Ia antigens could be quantitated as shown in Table II which represents a time point 6 d after the removal of gamma interferon.

Presence of type III collagen on synovial cells. Cells passaged at the same time as those described above in adjacent wells also stained with the monoclonal antibody to type III collagen. This staining increased when the cells were incubated with conditioned medium from monocyte-enriched cells (Fig. 5, *A* and *B*) while Ia staining remained negative. The inability of monocyte conditioned medium to induce Ia expression by synovial fibroblasts could be demonstrated using a ^{125}I-labeled second antibody as well. (Cells alone 1,600±1,146, monokine 1,878±330, and gamma interferon 100 U/ml 15,182±1,619). Therefore, cells that had a fibroblast phenotype (cell-associated collagen) and were DR-negative could also be induced to express DR antigen with the addition of recombinant DNA-derived gamma interferon.

Several groups have described an influence of mononuclear

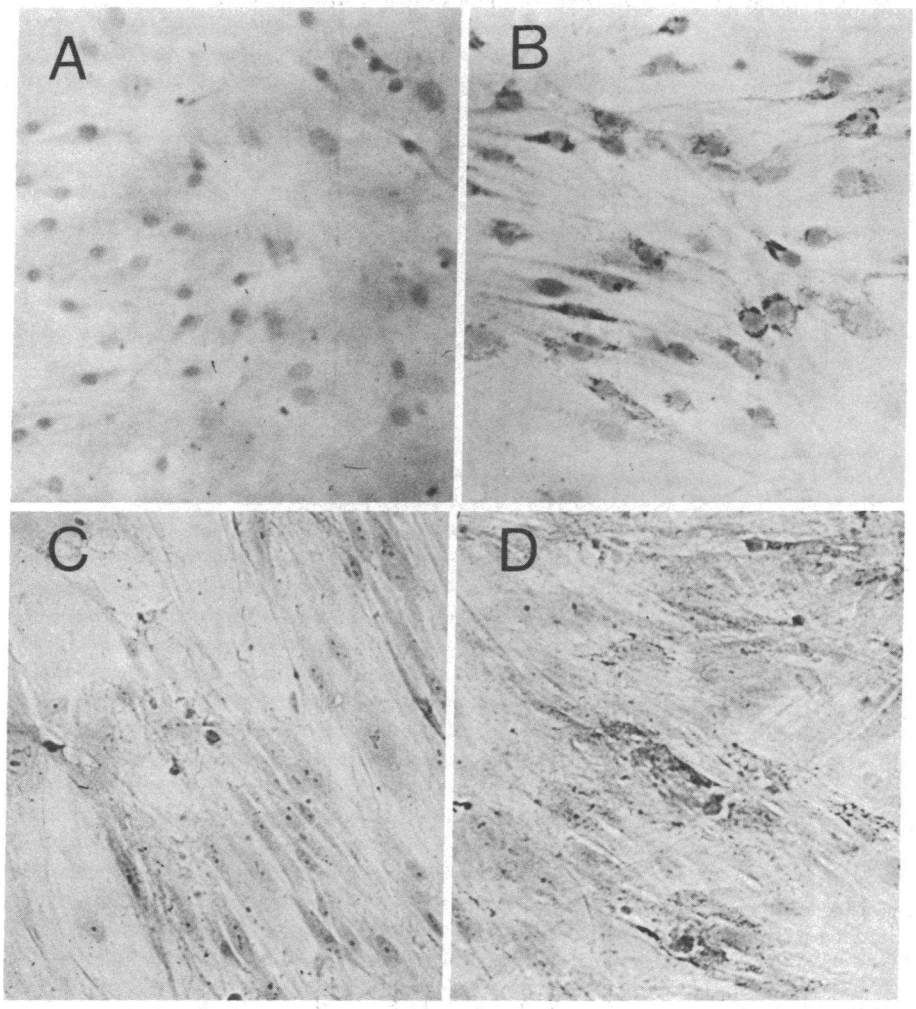

Figure 4. Induction of DR antigen expression on adherent synovial cells and dermal fibroblasts by gamma interferon. Adherent synovial cells (*A* and *B*) and dermal fibroblasts (*C* and *D*) at 1×10^4 cells/well were incubated alone (*A* and *C*) or with gamma interferon (100 U/ml) (*B* and *D*) for 3 d. All of the synovial cells (*B*) and most of the dermal fibroblasts (*D*) are HLA-DR positive (× 160).

Table I. Induction of HLA-DR Antigen on Adherent Synovial Cells by Gamma Interferon

Addition	^{125}I-anti-mouse IgG bound	
	Anti-HLA-DR	Anti-Leu-2a
	cpm	*cpm*
None	545±24*	251±92
Gamma interferon (*U/ml*)		
0.01	615±106	218±31
0.1	1,708±181	265±22
1.0	3,589±1,101	387±40
10	4,899±177	385±17
100	5,865±465	468±22
1,000	5,297±360	495±22

Adherent synovial cells were plated at 3×10^4 cells/well 5 d before assay. They were then incubated alone or with gamma interferon for 2 d and assayed for the appearance of DR antigen. The binding of the monoclonal antibody anti-HLA-DR (Becton, Dickinson & Co.) to synovial cells was assessed using ^{125}I-labeled F(ab')$_2$ goat anti-mouse IgG. Nonspecific binding was determined using the monoclonal antibody anti-Leu-2a.

* Values are means±SD for triplicate samples.

cell factors or lymphokines on collagen synthesis (41–44). Inasmuch as gamma interferon had such profound effects on the synovial cells, we asked whether it was the specific lymphokine responsible for the previously described inhibition of collagen synthesis. Indeed, as shown in Fig. 5, *C* and *D*, gamma interferon inhibited not only the type III collagen content of synovial cells in control cultures but prevented the monokine-induced increase in collagen content. Similar results were obtained using skin fibroblasts (Fig. 6 *A–D*).

Effects of gamma interferon on collagen synthesis. To examine this effect in greater detail, skin fibroblasts were incubated with [^{3}H]proline for the last 24 h of a 72-h culture and the medium was analyzed by SDS-PAGE and fluorography. As shown in Fig. 7, preparations of monocyte-conditioned medium that contained mononuclear cell factor activity increased amino acid incorporation into proteins found in bands corresponding to fibronectin as well as partially processed and unprocessed procollagens as previously described (8), whereas gamma interferon inhibited basal as well as monokine-induced collagen and fibronectin synthesis. Further analysis of the collagens produced was performed by pepsin digestion of the [^{3}H]proline-labeled conditioned medium followed by SDS-PAGE and delayed reduction which permitted separation of α1(I) from α1(III) chains (31). As shown in Fig. 8, gamma interferon inhibited basal as well as monokine-induced synthesis of α1(I), α2(I), and α1(III) chains. The effect of gamma interferon on the inhibition of collagen synthesis by adherent synovial fibroblastlike cells was dose-dependent as demonstrated in Fig. 9.

Table II. Retention of DR Antigen Expression by Adherent Synovial Cells after the Removal of Gamma Interferon

Addition	^{125}I-anti-mouse IgG bound	
	Anti-HLA-DR	Anti-Leu-1
	cpm	*cpm*
None	544±38*	373±28
Gamma interferon (*100 U/ml*)	18,738±1,537	1,034±161
6 d after removal of gamma interferon	15,742±412	1,235±389

Adherent synovial cells were plated at 5×10^4 cells/well 3 d before assay. They were then incubated alone, or with gamma interferon for 8 d, or with gamma interferon for 2 d followed by removal of the gamma interferon by thorough washing (five times) and culture continued in fresh medium for an additional 6 d. DR antigen expression was assayed as described in Table I.

* Values are means±SD for triplicate samples.

Because gamma interferon had profound but opposite effects on the synthesis of Ia antigens and collagens, we also evaluated the effect of this lymphokine on PGE_2 production by the adherent synovial cells. Gamma interferon neither stimulated production of PGE_2 by adherent synovial cells nor influenced mononuclear cell/interleukin 1-stimulated production (Table III).

Discussion

The adherent synovial cells derived from the rheumatoid synovial membrane that persist in culture are fibroblastlike but are under the influence of multiple factors released by cells of the immune system. In the course of studies designed to characterize these adherent synovial cells, we have identified at least two discrete influences of the lymphokine gamma interferon on synovial fibroblasts, i.e., the induction of major histocompatibility complex class II antigens and the inhibition of collagen synthesis. The ability of gamma interferon to induce Ia antigens by skin fibroblasts has recently been described (37). Our observations that synovial fibroblastlike cells upon initial isolation exhibited Ia antigens, the expression of which decreased with cell passage coincident with the loss of monocytes and lymphocytes, suggested an in situ immune-related event. The incubation of DR-negative cells with gamma interferon induced all of the adherent synovial cells to become HLA-DR-positive. These results suggest that the initial expression of Ia antigen by recently isolated adherent synovial cells is not an artifact of cell dispersion or culture in vitro.

The previous demonstration of Ia antigens on all synovial lining cells of the tissue of patients with rheumatoid arthritis is consistent with our findings (15–17). In contrast to the synovial lining cells from patients with rheumatoid arthritis, approximately one-half of the lining cells in synovium from normals, osteoarthritis, or traumatic arthritis were Ia-positive. The ability to induce Ia antigen on late-passage synovial cells and skin fibroblasts suggests, in addition, that shed Ia antigen does not account for the observations made with intact tissues or early passage cells. Furthermore, observations that HLA-DR antigens were induced in a time- and dose-dependent-manner and required new protein synthesis, are consistent with reports indicating that gamma interferon induces mRNA for Ia antigens (45).

It is becoming evident that specific immune signals can influence specific functions of the nonimmune synovial fibroblastlike cells. The monokine, mononuclear cell factor/interleukin 1, augments collagenase and PGE_2 production by adherent synovial cells (7, 9, 46). A lymphokine, distinct from

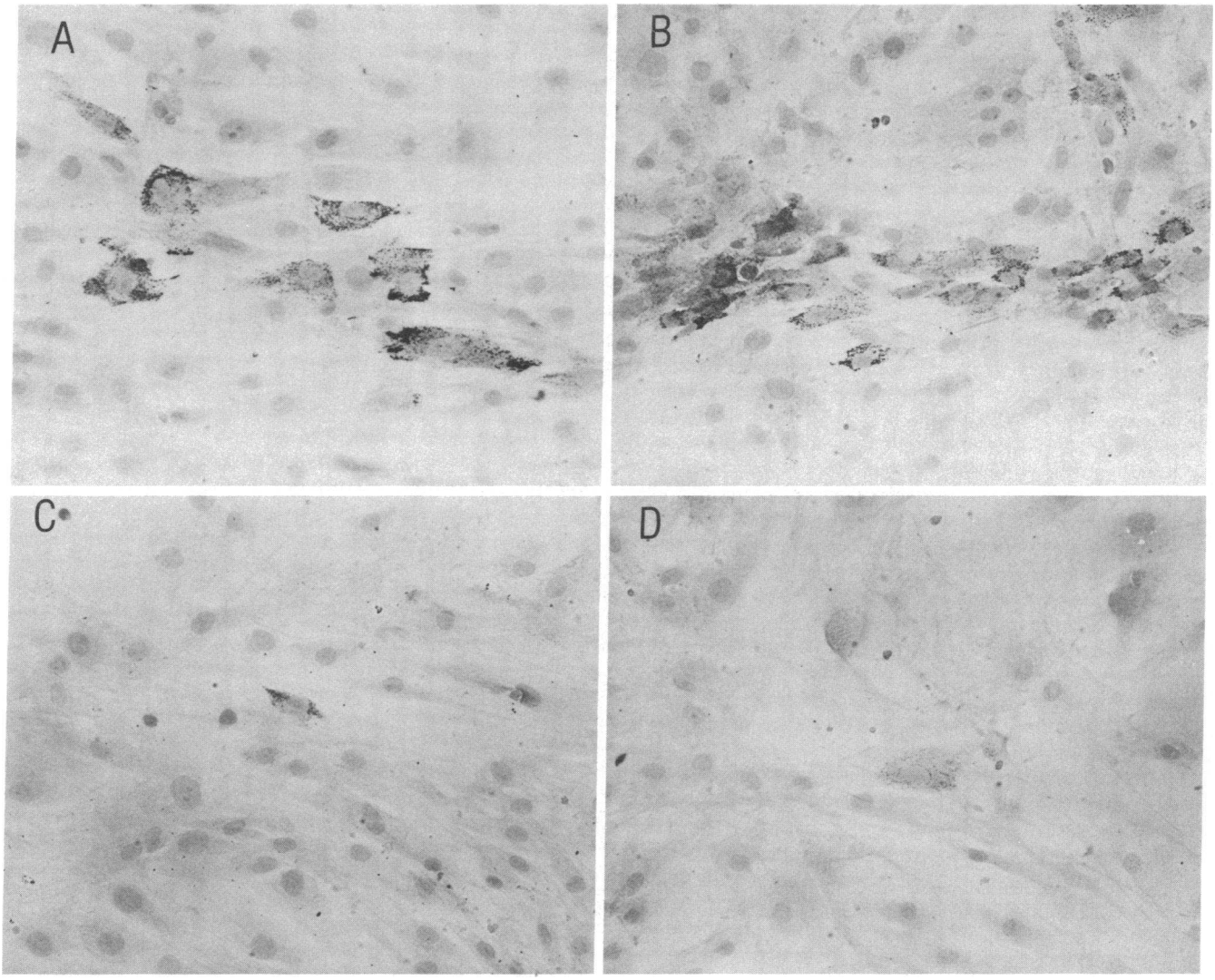

Figure 5. Effects of gamma interferon on adherent synovial cell-associated type III collagen. Adherent synovial cells at 2.5×10^4 cells/well were incubated alone (*A*), with conditioned medium from monocyte-enriched cultures (*B*), with gamma interferon (100 U/ml) (*C*), or with a combination of monokine and gamma interferon (*D*) for 3 d, and stained with a monoclonal antibody to type III collagen (× 211).

gamma interferon indirectly affects the synovial cells by stimulating secretion of mononuclear cell factor/interleukin 1 by the monocytes (11, 47). The factor present in monocyte-conditioned medium which contains mononuclear cell factor/interleukin 1 activity also stimulates the synthesis of types I and III collagens and fibronectin (8) but does not induce Ia expression. The T lymphocyte product, gamma interferon, activates tumoricidal capacity (macrophage-activating factor activity) (48, 49) and in addition increases Fc and fibronectin receptors and increases phagocytic capacity of monocyte/macrophages (50–52). Gamma interferon does not have mononuclear cell factor activity, however, nor does it alter mononuclear cell factor-stimulated PGE_2 production by the synovial cells. Yet, gamma interferon, which stimulates cells to inhibit viral replication, has other diverse affects on the adherent fibroblastlike synovial cells. These studies clearly show that gamma interferon is a regulatory lymphokine that induces the de novo synthesis of Ia antigens in the same cells in which it inhibits the synthesis of collagen.

In their characterization of dispersed cells from rheumatoid synovial membranes, Burmester et al. (53) identified three major cell populations by using several monoclonal antibodies: (*a*) monocyte antigen-, Fc receptor-, and Ia-positive, (*b*) monocyte antigen- and Fc receptor-negative, but Ia-positive and (*c*) monocyte antigen- and Ia-negative. On the basis of our observations, we suggest that some of the synovial fibroblastlike cells are included among the Ia positive-, Fc receptor-, and monocyte antigen-negative cells. These cells can assume diverse morphologic appearances varying from those of typical fibroblasts to those with a branched stellate character (34), depending upon environmental conditions, such as ambient levels of PGE_2. Cells with a similar branching morphology have been identified in tissue sections of rheumatoid synovium (54).

Cells similar to those described by Winchester and Burmester (55) as elongate or dendritic cells of synovial origin that lose Ia expression with time in culture are seen in our cultures of adherent cells dispersed from synovial lining as well. In that the stellate appearance can be reinduced in the fibroblasts by

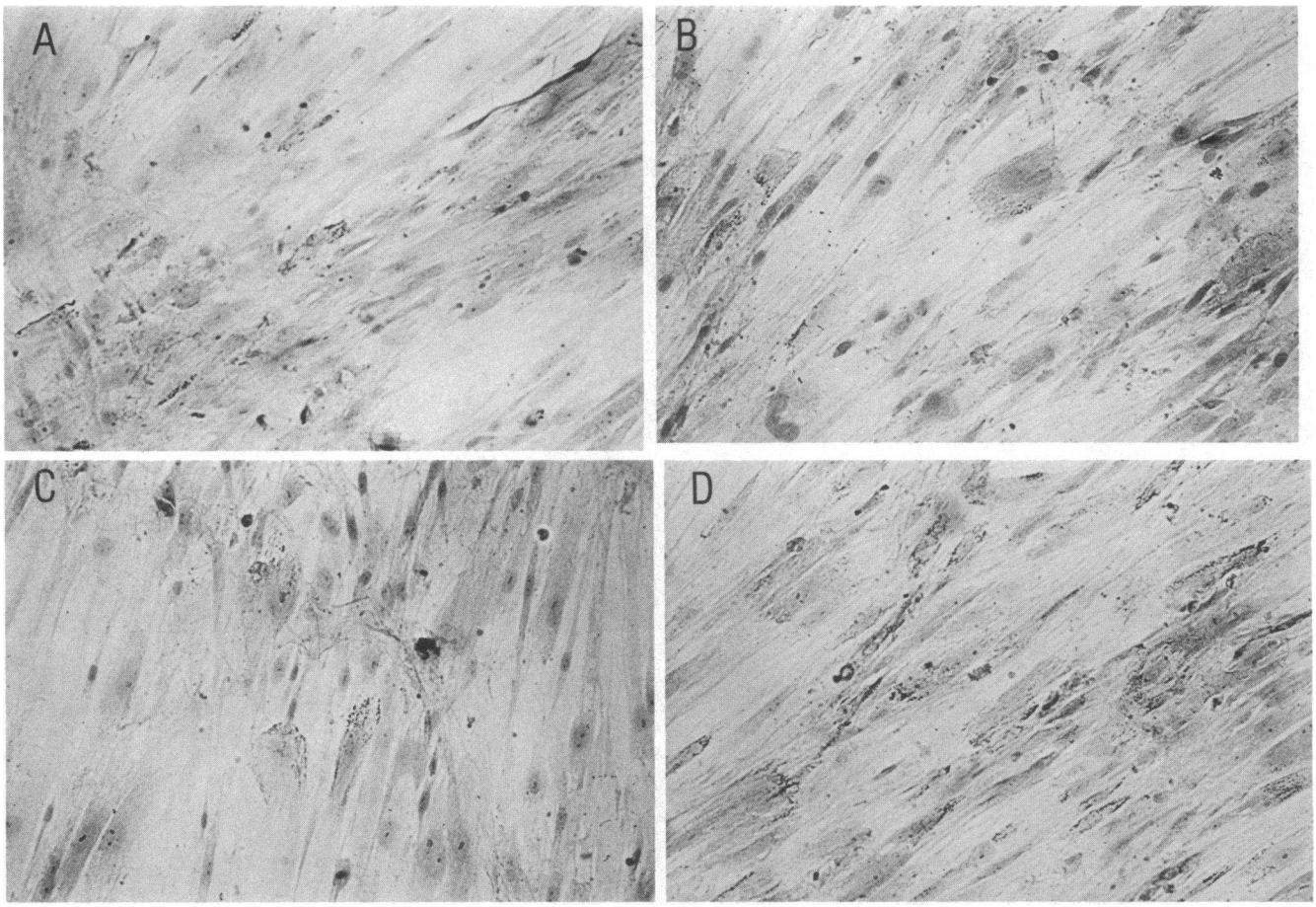

Figure 6. Effects of gamma interferon on dermal fibroblast-associated type III collagen. Dermal fibroblasts were cultured as described in Fig. 5 either alone (*A*), with monocyte-conditioned medium (*B*), with gamma interferon (100 U/ml) (*C*), or with a combination of monokine and gamma interferon for 3 d and (*D*) stained with a monoclonal antibody to type III collagen (× 186).

incubation with mononuclear cell factor/interleukin 1 or exogenous PGE_2 (34), it is not likely that these cells are the equivalent of the circulating dendritic cells described by Van Voorhis et al. (56). The latter are antigen-presenting cells that are not phagocytic and lack monocyte-related surface antigens and Fc receptors but are Ia-positive. Such cells could be present in the inflammatory synovial membrane. The separation technique that we have utilized which is dependent upon adherence for selection would not be expected to enrich for the poorly adherent dendritic cells.

The presence of DR antigens on many of the cells found in rheumatoid synovial tissue is consistent with the hypothesis that Ia expression in vivo is an indication of heightened local gamma interferon production. This enhanced Ia expression could function in immunological processes such as antigen presentation (57, 58), autologous lymphocyte proliferation (5, 18, 59), or as presumed viral receptors (60). Alternatively, the heightened expression of Ia antigens by synovial fibroblastlike cells could be a consequence of one or several factors in addition to the local production of gamma interferon such as an increased sensitivity of synovial fibroblasts to effects of gamma interferon, or alterations in the ability of the synovial fibroblasts or adjacent cells to dampen Ia expression.

Recent work suggests that interleukin 2 may influence the production of gamma interferon by T lymphocytes (61, 62). If increased expression of Ia antigens on cells in the inflammatory synovium is due to the local production of gamma interferon, then we must also account for observations of others of decreased production of gamma interferon by peripheral blood T lymphocytes from patients with rheumatoid arthritis (63). Cells in peripheral blood cells may not, however, be representative of the population in the synovial lesion as shown, for example, by the analysis of distribution of surface antigens on T lymphocytes (64). In addition, cells from peripheral blood may function normally after growth in vitro as has been reported for the mRL-*lpr*/*lpr* mouse (65). Indeed, when T lymphocytes from patients with rheumatoid arthritis are stimulated with allogeneic rather than autologous cells gamma interferon production is normal (63). Thus these studies suggest that other factors in vivo may alter the apparent function of circulating cells.

The degree of Ia expression by cells, especially the DQ antigen, has been associated with the ability of cells to present antigen (66). Of interest, therefore are our findings that skin fibroblasts and synovial fibroblastlike cells continue to express Ia (DR and DQ) antigens for >10 d after incubation with gamma interferon. Although human B lymphocytes and monocytes express Ia antigens constitutively, most other cells after induction of Ia antigens lose these antigens rapidly. The prolonged expression of Ia antigens by synovial fibroblastlike

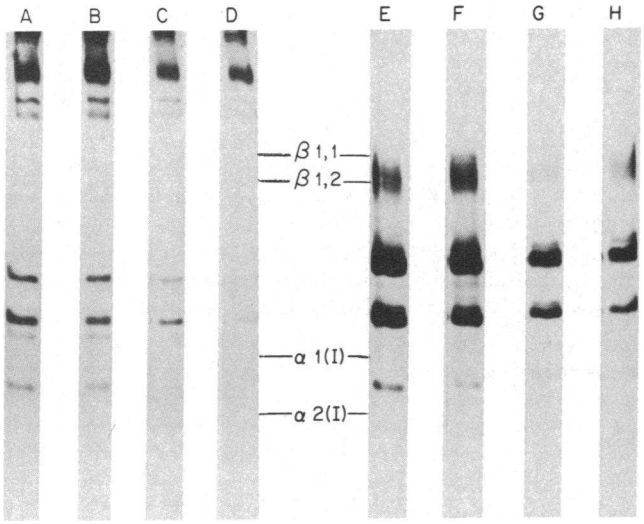

Figure 7. Effects of gamma interferon on collagen synthesis by dermal fibroblasts. Medium conditioned by dermal fibroblasts incubated for 24 h with L-[5-^{3}H]proline was examined by SDS-PAGE and fluorograms prepared. Samples applied to lanes *A* and *E* were from dermal fibroblasts incubated alone; lanes *B* and *F* from fibroblasts incubated with monocyte conditioned medium; lanes *C* and *G* from fibroblasts incubated with gamma interferon (100 U/ml); and lanes *D* and *H* from fibroblasts incubated with monocyte conditioned medium plus gamma interferon. Lanes *A–D* were run under nonreducing conditions and lanes *E–H* were run under reducing conditions in the presence of 0.5% β-mercaptoethanol. Position of labeled protein was determined by fluorography with ^{14}C-rat tail tendon collagen as molecular weight marker. Fibronectin migrates in the region of the β-components under reducing conditions. The major bands migrating between the β-components and α-chains under reducing conditions are processed type I and III procollagens.

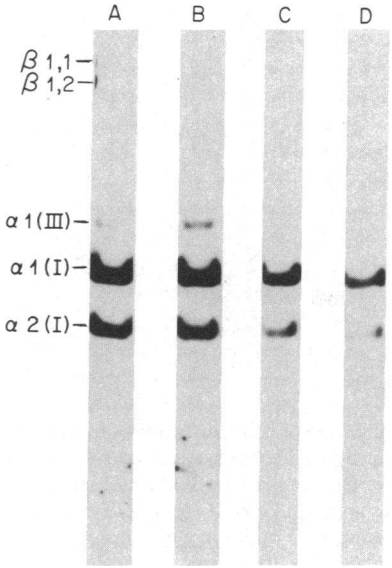

Figure 8. Effects of gamma interferon on collagen synthesis by dermal fibroblasts. Medium conditioned by dermal fibroblasts incubated for 24 h with L-[5-^{3}H]proline was examined by SDS-PAGE after pepsinization at 4°C. Samples applied to lane *A* were from dermal fibroblasts incubated alone; lane *B* from fibroblasts incubated with monocyte-conditioned medium; lane *C* from fibroblasts incubated with gamma interferon (100 U/ml); and lane *D* from fibroblasts incubated with monocyte-conditioned medium plus gamma interferon. The collagens were analyzed with delayed reduction with β-mercaptoethanol to distinguished α1(I) from α1(III) chains.

cells may therefore imply a potential for continuous involvement of these cells in an immune response. Control mechanisms for Ia expression may be important for the immune response in local areas, not only in the regulation of gamma interferon production but also in dampening the expression of Ia antigens once they are induced. For example, it has been found that Ia expression by macrophages is decreased by the addition of PGE_2 and stimuli such as endotoxin and C_{3b} that augment endogenous PGE_2 production (67–69). On the other hand, α-fetoprotein decreases Ia expression through a PGE_2-independent mechanism (70). In contrast to our findings in fibroblastlike cells, intestinal epithelial cells and murine monocyte/macrophages once induced rapidly lose Ia expression after removal of the gamma interferon (39, 40). Therefore, local synthesis of gamma interferon and/or enhanced sensitivity of the synovial fibroblastlike cells to gamma interferon with prolonged expression of Ia antigens may be critical in perpetuation of the chronic inflammatory state and in preventing local healing.

Our results also indicate that the same cells that can be induced to express Ia antigens stain positively with an antibody to type III collagen. On the basis of our previous work this type of cell can be induced with prostaglandins to undergo morphologic changes and become stellate in appearance (34). Using the monoclonal antibody to type III collagen and immunoperoxidase staining, we confirmed our previous observations that a monocyte-derived factor augments collagen synthesis by direct staining of the target cells (8). This technique also permitted us to directly examine the influence of gamma interferon on cell-associated collagen. We found that the intensity of staining with the monoclonal antibody to type III collagen of control cells, as well as cells incubated with the monokine, was reduced after incubation with gamma interferon. Furthermore, gamma interferon inhibited basal and monokine-induced incorporation of [^{3}H]proline into type I and III collagens and fibronectin. Lymphokines generated by mixed lymphocyte reactions or lectin stimulation have been previously reported to inhibit collagen synthesis (41–44). Jiminez et al. (71), recently found that affinity-purified gamma interferon inhibited collagen synthesis by skin fibroblasts. Our studies using recombinant DNA-derived gamma interferon and direct analysis of cell-associated collagen enabled us to demonstrate that gamma interferon inhibited collagen synthesis by the same synovial cell population that was induced to express Ia antigens. Furthermore, gamma interferon also inhibited monokine-stimulated collagen synthesis. Further investigation is necessary to understand the mechanisms by which gamma interferon inhibits collagen synthesis.

An initial approach which we and others have used has been to measure cellular levels of type I and III procollagen mRNAs (72, 73). We have found that the decrease in collagen synthesis by skin and synovial fibroblasts after incubation with gamma interferon is associated with decreased levels of type I and III procollagen mRNAs as measured by dot-blot hybridization (73). In contrast, the induction of Ia antigens by gamma interferon has been accompanied by the synthesis of new mRNAs for Ia antigens (45). The inhibitory effects of the

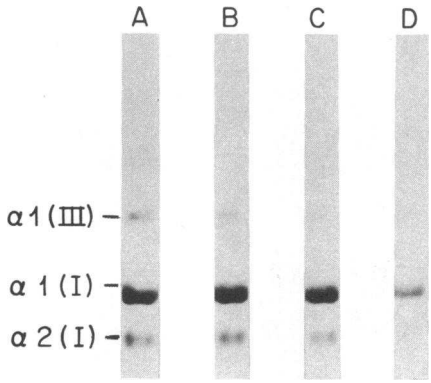

Figure 9. Dose-dependent inhibition of collagen synthesis by gamma interferon. Medium conditioned by adherent synovial cells incubated for 24 h with L-[5-^{3}H]proline was examined by SDS-PAGE after pepsinization at 4°C. Sample applied to lane *A* was from synovial fibroblasts incubated alone; lanes *B–D* from synovial fibroblasts incubated with gamma interferon at 1 (*B*), 10 (*C*), or 100 (*D*) U/ml. The collagens were analyzed with delayed reduction with β-mercaptoethanol as in Fig. 8.

lymphokine, gamma interferon, in the presence of the collagen synthesis-stimulating activity of a monokine suggests a hierachy of molecular control of collagen synthesis. Matrix destruction may therefore be mediated not only by monokine-influenced collagenase secretion by the fibroblastlike synovial cells but also by inhibition of collagen synthesis mediated by a T lymphocyte product gamma interferon. Observations that gamma interferon induces Ia expression by synovial fibroblastlike cells suggests that these modified cells could also participate in immune-related events such as antigen presentation to T lymphocytes, triggering of T lymphocytes to self Ia antigens, or in yet unknown T lymphocyte–fibroblast interactions. At the cellular and tissue level therefore the degree of T lymphocyte infiltration and signals for gamma interferon release may influence not only the degree of Ia expression but also the synthesis of collagen. Thus lymphokines and monokines may balance the extent of fibrosis on one hand and matrix destruction on the other at various stages of the rheumatoid lesion by affecting the function of fibroblastlike synovial cells.

Table III. Effects of Mononuclear Cell Factor/Interleukin 1 and Gamma Interferon on PGE_2 Production by Adherent Synovial Cells

Additions	PGE_2*
	ng/ml
None	3.3±1.1
Gamma interferon	
0.01 U/ml	1.5±0.4
0.1	2.2±0.5
1.0	1.5±0.4
10	1.6±0.6
100	1.5±0.4
Mononuclear cell factor/interleukin 1	176±45
Mononuclear cell factor/interleukin 1 plus gamma interferon	
0.01 U/ml	188±12
0.1	247±45
1.0	173±17
10	190±29
100	197±33

Synovial cells in their second passage were plated at 5×10^4 cells/well 3 d before bioassay. They were then incubated for 3 d alone, or with gamma interferon, or with mononuclear cell factor/interleukin 1-containing medium or a combination of mononuclear cell factor/interleukin 1 and gamma interferon in a final volume of 0.4 ml/well.
* Values are means±SD for triplicate samples.

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