Direct Contact between T Lymphocytes and Human Dermal Fibroblasts or Synoviocytes Down-regulates Types I and III Collagen Production via Cell-associated Cytokines*

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In many inflammatory diseases where tissue remodeling occurs, T cells are in close contact with mesenchymal cells. We investigated the effect of direct cell-cell contact between peripheral blood T lymphocytes or HUT-78 lymphoma cells and dermal fibroblasts or synoviocytes on the deposition of the major extracellular matrix components: types I and III collagen. Incubation of dermal fibroblasts and synoviocytes with plasma membrane preparations from resting T cells slightly increased the production of collagen I but did not significantly affect that of collagen III. Conversely, direct contact with either plasma membranes or fixed phorbol myristate acetate-activated T cells markedly inhibited the synthesis of types I and III collagen by 60–70% in untreated dermal fibroblasts and synoviocytes and by 85% in transforming growth factor β-stimulated fibroblasts. This decrease of collagen synthesis was abrogated when fixed T cells were separated physically from fibroblasts, demonstrating that direct contact between the two cell types was necessary. This inhibition was associated with a marked decrease in steady-state levels of pro-α1(I) and pro-α1(III) collagen mRNAs. T cell contact decreased the transcription rate but did not significantly alter the stability of the α1(I) and α1(III) transcripts. Finally, using neutralizing antibodies or cytokine inhibitors we provide evidence that this inhibition of extracellular matrix production mediated by T cell contact was partially due to additive effects of T cell membrane-associated interferon γ, tumor necrosis factor α, and interleukin-1α.

Regulation of connective tissue metabolism is an important event in a number of biological and pathophysiological processes such as embryonic organogenesis, wound healing, inflammation, tissue destruction, fibrosis, tumor invasion, and metastasis. Fibroblasts are mesenchymal cells which play a crucial role in the remodeling of extracellular matrix (ECM) by synthesizing and organizing connective tissue components, predominantly constituted of types I and III fibrillar collagens. Type I collagen is abundant in the skin, tendons, and bones and it is also the main collagen type produced by dermal fibroblasts in culture (1, 2).

Fibroblasts respond to various microenvironmental signals including soluble cytokines and growth factors as well as cell-matrix or cell-cell interactions which intervene notably in the control of the balance between synthesis and degradation of ECM (3). Alterations in this balance can lead to pathological events such as the invasion of tissue by malignant cells (4), abnormal ECM deposition in fibrotic diseases (5) or, conversely, to tissular destruction in chronic inflammation (6). Furthermore, in some pathological conditions there is evidence for a lack of ECM neosynthesis (i.e. proteoglycans) and consequently a lack of repair (7, 8).

It is highly suggested that T cells may play an important role in the pathogenesis of some chronic inflammatory diseases (i.e. rheumatoid arthritis, scleroderma) not only through the release of soluble factors but also through direct contact with fibroblasts or fibroblast-like cells (i.e. synoviocytes) (9–13). This contact can induce the production of cytokines, matrix metalloproteinases (MMP), prostaglandins (PGE2) and control the expression of adhesion molecules, substantiating the assumption that the interaction between fibroblasts and inflammatory cells might influence both fibroblast activation and inflammatory response (14–18).

A wide range of cytokines exert profound effects on fibroblast migration, proliferation, and ECM production. Transforming growth factor-β (TGFβ), IL-4, IL-6, IL-13, platelet-derived growth factor, epidermal growth factor, and basic fibroblast growth factor are fibrogenic cytokines and growth factors while IFN-α, IFN-β, IFN-γ, IL-10, relaxin, and leukoregulin suppress collagen synthesis (5, 19–26). The effects of TNFα and IL-1 on the production of collagen by fibroblasts appear much more controversial and depend on the cell type (19, 27–30).

Collagen is known to control its own synthesis by fibroblasts through a negative feedback loop via interaction with the α1β1 integrin (31, 32). In contrast to these well documented fibroblast-ECM interactions, little information is available concerning the role of cell-cell contact in the regulation of ECM deposition. Previous in vitro studies suggest that the interaction between inflammatory cells and fibroblasts can modulate several fibroblast functions including collagen production (33–35). However, these studies do not distinguish the effects of monocytes/macrophages from the action of T lymphocytes. Nor do they take into account the respective role of soluble factors released by mononuclear cells and those requiring direct cell-cell contact in the mediation of this phenomenon.
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The aim of the present study was to shed light on the regulation of ECM deposition, mainly types I and III collagen, operated by direct cell-cell interaction between inflammatory cells, namely T lymphocytes or for convenience and standardization the HUT-78 T lymphoma cell line, and mesenchymal cells such as dermal fibroblasts and synoviocytes.

We provide evidence that the physical interaction between PHA/PMA-activated T cells and dermal fibroblasts or synoviocytes reduces markedly the production of both types I and III collagen by these cells. We also demonstrate that this inhibition is regulated at the transcriptional level and that it is mainly due to an additive effect of T cell-associated IFN-γ, TNFα, and IL-1α.

EXPERIMENTAL PROCEDURES

Reagents—Dubelco’s modified Eagle’s medium, RPMI 1640 medium, phosphate-buffered saline, penicillin, streptomycin, t-glutamine were supplied by Life Technologies (Paisley, United Kingdom) and fetal calf serum from Seromed (Biochrom KG, Berlin, Germany). Cycloheximide (CHX), 5,6-dichlorobenzimidazole riboside (DRB), β-aminopropionitrile, α-ketoglutaric acid, t-aspartic acid, iodoacetamide, indomethacin, and PMA (phorbol myristate acetate) were purchased from Sigma. [35S]fucose was from Du Pont NEN and [3H]thymidine was from Hartmann Analytic GmbH (Braunschweig, Germany). Parafomaldehyde was from Merck (Darmstadt, Germany). Phascolus vulgaris leucytothemaglutinin (PHA) was from E-Y Laboratories Inc. (San Mateo, CA). Human recombinant TGFβ (hTGFβ1) was from R & D Systems (Minneapolis, MN). IFN-γ, TNFα, and IL-1β were from BioGenex (Geneva, Switzerland). Human recombinant soluble TNF receptor p55 (rsTNF-p55-human) was from Genzyme (Minneapolis, MN). IFN-α, IFN-β, IL-1Ra was obtained from Hoffmann-La Roche (Basel, Switzerland). Human recombinant soluble TNF receptor p55 (rsTNF-p55-human) was from Hoffmann-La Roche (Basel, Switzerland). Human recombinant soluble TNF receptor p55 (rsTNF-p55-human) was from Hoffmann-La Roche (Basel, Switzerland).

Dermal Fibroblasts and Synoviocytes—Human dermal fibroblasts and synoviocytes were isolated by protease treatment of foreskin and synovial surgical synovectomy specimens from rheumatoid arthritis patients, and synoviocytes were isolated by protease treatment of foreskin and provided by Dr. G. Garotta (Hoffmann-La Roche, Basel, Switzerland).

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Production during Direct Cell-Cell Contact with Human Dermal Fibroblasts and Synoviocytes—The role of direct cell-cell interactions between resting or activated T lymphocytes and dermal fibroblasts or synoviocytes on collagen deposition was assessed by determining the concentration of NH₂-terminal propeptides of procollagens I and III (PINP and PIINP) in culture supernatants.

RESULTS

Freshly Isolated Peripheral Blood T Lymphocytes and HUT-78 T Lymphoma Cells Modulate Types I and III Collagen Production during Direct Cell-Cell Contact with Human Dermal Fibroblasts and Synoviocytes—The role of direct cell-cell interactions between resting or activated T lymphocytes and dermal fibroblasts or synoviocytes on collagen deposition was assessed by determining the concentration of NH₂-terminal propeptides of procollagens I and III (PINP and PIINP) in culture supernatants.

Confluent dermal fibroblasts and synoviocytes constitutively produced type I collagen at 63 ± 25 (n = 24) and 90 ± 29 ng/ml (n = 8), respectively, and to a lower extent type III collagen at 13.6 ± 2.8 and 18.7 ± 5.5 ng/ml, respectively. Co-cultures of dermal fibroblasts or synoviocytes and paraformaldehyde-fixed unstimulated PBTL or HUT-78 cells slightly increased the production of type I collagen but did not significantly affect type III collagen production (Fig. 1). Conversely, incubation with fixed PHA/PMA-activated PBTL or HUT-78 cells markedly decreased types I and III collagen production both in dermal fibroblasts and synoviocytes. Interestingly, in all experiments the inhibition of PINP and PIINP production mediated by cell-cell contact was more efficient than that induced by a potent soluble anti-fibrogenic effector such as interferon-γ (1000 units/ml). This inhibitory effect was also observed on three other dermal fibroblast cell lines (data not shown).

The inhibition of collagen I production was also observed when dermal fibroblasts were incubated with plasma membrane preparations from PHA/PMA-activated PBTL or HUT-78 of Dubelco’s modified Eagle’s medium supplemented with 1% FCS, 50 µg/ml β-aminopropionitrile, 3.5 µg/ml α-ketoglutaric acid, and 25 µg/ml t-aspartic acid. The culture supernatants were frozen at −20 °C for further determination of types I and III collagen contents.

In some experiments, 24-well cluster plates with 6.5-mm Transwell inserts (Costar) were used. The wells were separated into upper and lower chambers by a layer of nuleopore membrane with 3-µm pores. T cells and dermal fibroblasts were seeded into the upper and lower chambers, respectively. In blocking experiments, activated T-cell membranes were preincubated for 30 min at 4 °C with the designated blocking antibodies or cytokine inhibitors, and then co-cultured for a further 24 h with dermal fibroblasts or synoviocytes. Statistical comparison of means was performed using the Student’s t-test.

Determination of Types I and III Collagen Production—The production of types I and III collagen was estimated in 48 h culture media by measuring the concentration of the amino-terminal propeptides of procollagen I and III (PINP and PIINP) by competition-based radioimmunoasays (Orion Diagnostica, Espoo, Finland). The threshold of the radioimmunoassays for PINP and PIINP were 2 and 0.2 ng/ml, respectively.

RNA Extraction and Northern Blot Analysis—Total RNA was isolated from confluent fibroblast monolayer cultures (in 60-mm Petri dishes) by lysing the cells with TRIzol® reagent (Life Technologies) according to the manufacturer’s procedures. RNAs (5–10 µg) were separated on 1% agarose gels, transferred onto nylon Hybond N membrane (Amersham), and hybridized to [32P]-labeled cDNA probes specific for pro-α(I) collagen (HR677) (41), pro-α(III) collagen (HR934) (42), pro-α(II) collagen (HR1131) (43), MMP-1 (44), and GAPDH (45). Autoradiographs were quantified by densitometric scanning using a laser densitometer equipped with ImageQuant software (Molecular Dynamics) and values were normalized to GAPDH signals.

Nuclei Isolation and Run-on Transcription Assay—Preparation of nuclei from dermal fibroblast (2 × 10⁶ cells), transcription assay, and hybridization were performed as described previously (46). To ensure that comparable amounts of nuclei would be present in each condition, the DNA content in lysed aliquots was determined. Biosynthetically radioactively labeled [3H]uridine (5 × 10⁶ cpm) was hybridized onto slot-blotted cDNA (4 µg/slot of linearized HR677, pBSGAPDH, or pBR322, as a control) for 48 h at 65 °C in 6 × SSC, 0.5% SDS, 0.1% polyvinylpyrrolidone, 0.1% Ficoll, 50 mM sodium phosphate, pH 6.5, and 100 µg/ml denaturated salmon sperm DNA, washed, and treated with RNase A. Filters were dried and exposed to Amershan Hyperfilms MP at −80 °C.

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cells. Indeed, co-incubation of dermal fibroblasts and membranes of activated T cells led to a dose-dependent down-regulation of the basal level of collagen I synthesis (Fig. 2). Furthermore, membranes from activated HUT-78 cells seemed to be more efficient in inhibiting PINP production than membranes from activated PBTL. Maximal inhibition (70–75%) was obtained with a 2–4-fold lower amount of HUT-78 membranes (T cell/fibroblast ratio, 10–20) than PBTL membranes (T cell/fibroblast ratio, 40). This dose-dependent inhibitory effect was also observed on PIINP production (data not shown). In contrast, as observed with resting fixed T cells, direct contact with membrane preparations of unstimulated freshly isolated PBTL or unstimulated HUT-78 cells slightly increased in a dose-dependent manner the basal level of type I collagen production on dermal fibroblasts (Fig. 2).

As already described (47), incubation of dermal fibroblasts with TGFβ (5 ng/ml) led to a 2–4-fold increase in the basal level of type I collagen production (Fig. 1, panel A), this up-regulation being less pronounced in synovial cells. Interestingly, this effect of TGFβ was strongly inhibited by the addition of membranes of PHA/PMA-activated PBTL or HUT-78 cells (Fig. 3). Indeed, the TGFβ-induced production of type I collagen in dermal fibroblasts was decreased in a dose-dependent manner and raised a maximal inhibition with a T cell/fibroblast ratio of 10 for HUT-78 cells and 40 for PBTL. Like in the case of the constitutive production of collagen I, activated HUT-78 cells were again more efficient than PBTL in inhibiting TGFβ-stimulated PINP production. Together these data indicate that the contact between activated T cells and fibroblasts down-regulates both constitutive and TGFβ-induced types I and III collagen production in dermal fibroblasts and synoviocytes.

In order to demonstrate that the down-regulation of collagen production was due to a contact-mediated signal and to exclude the implication of soluble factors released by either membrane preparations or fixed T cells, co-cultures of T cells and fibroblasts were carried out in a double-chamber system. In this system the culture well was divided into an upper and lower chamber by a porous nucleopore membrane which physically separated the two cell types but allowed free diffusion of soluble factors. As shown in Table I, down-regulation of PINP production mediated by direct contact with fixed PHA/PMA-stimulated PBTL or HUT-78 cells was drastically reduced when the two cell populations were physically separated. Indeed, when dermal fibroblasts and fixed activated PBTL or HUT-78 cells were seeded into the same chamber, PINP production was, respectively, 26 ± 11 and 29 ± 5% of the basal value, a result comparable to that depicted in Fig. 1. When fixed stimulated PBTL and HUT-78 cells were added to the upper chamber, PINP production reverted, respectively, to 82 ± 7 and 94 ± 10%. Conversely the slight increase in PINP production induced by fixed unstimulated PBTL or HUT-78 cells was not abrogated by separating the two cell types, indicating that this effect probably involved soluble factors. Similar results were obtained with membrane preparations from unstimulated or activated HUT-78 cells (Table I) and using synoviocytes as target cells (not shown).

Taken together these results demonstrate that signals mediated by direct contact with activated T cells were responsible
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for the down-regulation of types I and III collagen production, whereas soluble factors released by unstimulated fixed T cells or membrane preparations induced a slight up-regulation of the basal production of collagen I by dermal fibroblasts and synoviocytes.

To ascertain whether the expression of surface molecules responsible for the down-regulation of collagen production would be dependent on the time of stimulation of T lymphocytes, PBTL were incubated with PHA/PMA for different periods of time, then plasma membranes were prepared and incubated with either dermal fibroblasts or synovial cells for a further 48 h. PBTL mediated inhibition of PINP production as early as 1 h after PMA/PHA addition (Fig. 4). Inhibitory capacity was maximal after 3–6 h of PMA/PHA treatment and persisted for up to 48 h of activation. The data suggest that cell-surface molecules responsible for collagen down-regulation were expressed at an early stage of T-cell activation.

Direct Contact with Activated T Cells Decreases Steady-state Levels of Procollagen I and III mRNAs in Both Human Dermal Fibroblasts and Synoviocytes—To further investigate the molecular mechanisms underlying the inhibition of types I and III collagen, we focused on the regulation of procollagen mRNAs by cell-cell contact. For this purpose dermal or synovial fibroblasts were incubated for 14 h with TGFβ or membranes of unstimulated or activated PBTL and HUT-78 cells, and expression of α1(I), α2(I), and α1(III) collagen chain genes was estimated by Northern blot hybridizations with specific probes.

As shown in Fig. 5, pro-α1(I), pro-α2(I), and pro-α1(III) collagen mRNAs were constituatively expressed in dermal fibroblasts (panel A) and synoviocytes (panel C). Incubation of dermal fibroblasts and synoviocytes with membrane preparations from PHA/PMA-activated PBTL or HUT-78 cells resulted in a marked decrease (2–4-fold) in the levels of pro-α1(I), pro-α1(III), and to a lesser extent of pro-α2(I) transcripts. In contrast, when membranes from unstimulated PBTL or HUT-78 cells were added to dermal fibroblasts or synoviocytes, the steady-state levels of procollagen mRNAs increased slightly. However, this effect was less marked in synovial cells, probably due to the fact that the constitutive pro-α1(I), pro-α2(I), and pro-α1(III) mRNAs levels were higher than in dermal fibroblasts.

These data demonstrate that the down-regulation of collagens I and III production mediated by direct contact took place at the pretranslational level. They also indicate that this inhibition was specific for collagen genes. Indeed, at variance with collagen transcripts, MMP-1 mRNA level was strongly up-regulated in dermal and synovial fibroblasts treated with membranes of activated T cells (Fig. 5).

Cell Contact Inhibited the Transcription of Pro-α1(I) Collagen—In order to determine the mechanism underlying the inhibition of procollagen I transcripts level we measured the effect of cell contact on the transcription of pro-α1(I) collagen gene. Nuclei were isolated from dermal fibroblasts cultured for 4 h in the absence or presence of membranes from unstimulated or PHA/PMA-activated HUT-78 cells, and run-on experiments were performed. According to the results presented in Fig. 6, the relative de novo mRNA synthesis of pro-α1(I) collagen was increased 1.5-fold in dermal fibroblasts treated with unstimulated HUT-78 cells. Conversely, this transcription rate was decreased 4-fold in dermal fibroblasts incubated with PHA/PMA-activated HUT-78 cells. These data are in total agreement with results obtained from Northern blots and thus imply that contact-induced down-regulation of collagen I production was controlled by a transcriptional mechanism sufficient to result in a 4-fold reduction in pro-α1(I) collagen mRNA level in dermal fibroblasts.

Although these results argue for a purely transcriptional inhibitory effect of cell-cell contact on collagen production, we could not rule out a parallel post-transcriptional regulation of procollagen mRNAs. To make sure, we measured the stability of procollagens mRNAs in dermal fibroblasts incubated or not

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**Table 1**

Contact between activated T cells and dermal fibroblasts is required to down-regulate collagen I production

Cultures were set in double-chamber plates separated by a 3-μm pored membrane. Dermal fibroblasts (DF) (10^5 cells/well) were plated into the lower chamber 48 h before the contact experiment was performed. PFA-fixed cells or membrane preparations from unstimulated (ns) or PHA/PMA-activated (s) PBTL or HUT-78 cells were added either to the upper or lower chamber. PINP production was measured after 48 h of culture. T cell/fibroblast ratio was 16:1. Data are mean ± S.D. of three distinct experiments.

| Lower Chamber | Upper Chamber | PINP production (%) |
|---------------|---------------|---------------------|
| DF            | fixed sHUT-78 cells | 100                 |
| DF + fixed sHUT-78 cells | sHUT-78 membranes | 60                  |
| DF + fixed sHUT-78 cells | sHUT-78 membranes | 40                  |
| DF + fixed sPBTL | sHUT-78 membranes | 20                  |
| DF + fixed sPBTL | sHUT-78 membranes | 0                   |
| DF + mHUT-78 membranes | sHUT-78 membranes | 15                  |
| DF + mHUT-78 membranes | sHUT-78 membranes | 5                   |
| DF + fixed mHUT-78 cells | sHUT-78 membranes | 5                   |

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**Fig. 3.** Dose-dependent inhibitory effect of activated T cells on TGFβ-induced collagen I production in dermal fibroblasts. Dermal fibroblasts were co-cultured for 48 h with increasing amounts of membrane preparations from either PHA/PMA-activated PBTL (●) or PHA/PMA-activated HUT-78 cells (● in the presence of TGFβ (5 ng/ml). The T cell/fibroblast ratio was 16:1. PINP production was determined as previously mentioned. Dotted line represents PINP production in medium containing 1% FCS in the absence of TGFβ. Data are mean ± S.D. from triplicates of one experiment representative of two different experiments.
with activated T lymphocytes. For this experiment, dermal fibroblasts were treated with the transcription inhibitor DRB in the presence or absence of membrane from PHA/PMA-activated PBTL, and the decay of pro-α1(I) and pro-α1(III) mRNAs was followed as a function of time by quantitative blot hybridization analyses (Fig. 7). The half-life of pro-α1(I) collagen mRNA estimated by densitometric scanning (Fig. 7A, right panel) was 3.5–4 h when transcription was blocked by DRB alone, and 4.5–5 h in the presence of DRB and activated PBTL membranes. Similarly, the half-life of α1(III) mRNA was estimated at 4.5–5 h in the absence of activated-PBTL membranes, and 5.5–6 h in the presence of membranes (Fig. 7B). Thus the half-lives of procollagen mRNAs were slightly increased when membrane preparations were added to DRB-treated fibroblasts. Similar results were obtained using actinomycin D (5 μg/ml) (not shown). Therefore, these data demonstrate that the down-regulation of fibroblast collagen production induced by direct contact with activated T cells was exclusively controlled at the transcriptional level and did not result from a post-transcriptional destabilizing effect.

CHX was used to determine whether inhibition of protein synthesis might influence the inhibitory effect of membranes of activated T cells on the expression of procollagen mRNAs. Dermal fibroblasts preincubated for 1 h with or without CHX (10 μg/ml) were cultured for 14 h in the presence of membranes of PHA/PMA-activated PBTL or HUT-78 cells or without either, and types I and III procollagen mRNAs were measured by Northern blot analyses. As shown in Fig. 8, the addition of CHX alone inhibited the expression of the low migrating species of pro-α1(I) and pro-α1(III) transcripts, probably by interfering with the polyadenylation process, but did not affect the steady-state levels of the fast migrating forms. Interestingly, CHX abolished the down-regulation of pro-α1(I) mRNA level elicited by the direct contact with activated T cells, whereas it did not have a significant effect on the inhibition of pro-α1(III) mRNA.
level, suggesting that the inhibition of pro-α1(I) and pro-α1(III) mRNAs are regulated by distinct mechanisms. In these conditions, CHX treatment was efficient since it abrogated the stimulation of MMP-1 mRNA level induced by membranes of activated T lymphocytes. Thus, the inhibition of pro-α1(I) collagen mRNA level mediated by the interaction with activated T cells appears to require de novo protein synthesis.

Role of Membrane-associated Cytokines in the Down-regulation of Type I Collagen Production—In order to identify the molecules present on activated T cells and likely to control the down-regulation of collagen I production, we first used blocking mAbs raised against CD2, CD11a, CD11b, CD11c, CD18, CD40, CD54, CD58, and CD106. Since these antibodies failed to reverse the inhibition of collagen I synthesis (not shown), we next tested the effect of specific inhibitors or neutralizing mAbs raised against cytokines (IFN-γ, TNFα, and IL-1), known to decrease in vitro the production of collagen I by dermal fibroblasts. The blocking agents used in these experiments were IL-1 receptor antagonist (IL-1Ra), human recombinant soluble TNF receptor p55 (rsTNF-p55-h), and a neutralizing anti-IFNγ monoclonal antibody.

As shown in Table II, these molecules abrogated the inhibitory effect of soluble IFN-γ, TNFα, and IL-1β on collagen I production. Furthermore, the decrease of PINP production induced by membranes from PHA/PMA-activated PBTL on dermal fibroblasts (37.2 ± 7.1% of basal value) was significantly reversed by anti-IFN-γ mAb, rsTNF-p55-h, and IL-1Ra, amounting to 58.4 ± 8.4, 46.3 ± 9.7, and 44.9 ± 6.1%, respectively. Inhibition of PINP production was also partially reversed when fibroblasts were incubated with membranes from activated HUT-78 cells: 35.1 ± 6.2 versus 45 ± 10, 42.9 ± 7.5, and 40.4 ± 8.4%, in the presence of anti-IFN-γ mAb, rsTNF-p55-h, and IL-1Ra, respectively. Similar results were obtained on synoviocytes except that the anti-INF-γ mAb was less efficient in reversing the inhibitory effect of activated PBTL or HUT-78 cells membrane preparations (Table II). Interestingly, when combined these blocking agents induced a stronger blocking effect amounting to 81 ± 6.3 and 66.7 ± 7.9% in dermal fibroblasts cultured with PBTL and HUT-78 cells, respectively. Similarly, the inhibition of collagen I production was reversed to 86.2 ± 12 and 63.2 ± 12.7% in synovial fibroblasts. Moreover, the decrease of collagen production is in accordance with the amount of cytokines measured by enzyme-linked immunosorbent assay in plasma membrane preparations of PBTL and HUT-78 cells activated with PHA/PMA for 48 and 16 h, respectively. Indeed, membranes of activated PBTL and HUT-78 cells contained IFN-γ amounting to 3.6 ± 0.85 and 0.453 ± 0.1 ng/mg of proteins, respectively (i.e. 288 ± 70 and 145 ± 26 pg/ml of membranes; 50 × 10⁶ cell equivalent/ml), but no IFN-γ was detected in membrane preparations of resting T cells. In addition, TNFα was detected in membrane preparations of both activated PBTL and HUT-78 cells (9.23 ± 0.64 and 13.32 ± 0.76 ng/mg of proteins, respectively, i.e. 1043 ± 72 and 5503 ± 314 pg/ml of membranes), whereas IL-1α was only detected in membranes of activated PBTL (380 ± 26 pg/mg of proteins, i.e. 43 ± 3 pg/ml of membranes). Taken together, these data indicate that the decrease of fibroblast collagen synthesis mediated by contact with activated T cells can mainly be accounted for an additive inhibitory action of plasma membrane-associated IFN-γ, TNFα, and IL-1.

DISCUSSION

In the present study we investigated the effect of direct cell-cell interactions on the production of types I and III collagen by human dermal fibroblasts and synoviocytes. We demonstrated that co-culturing fixed PHA/PMA-activated PBTL or HUT-78 lymphoma cells with dermal fibroblasts or synoviocytes results in a marked decrease in the basal production of types I and III collagen. In contrast, contact with unstimulated fixed T cells slightly increased the production of collagen I while that of collagen III was not significantly affected. These results suggest that cell-cell contact modulates connective tissue metabolism. However, it is well established that soluble factors may be released by fixed T cells. Thus, authors have shown that significant levels of TNFα (4–234 pg/ml) can be detected in culture supernatants from fixed T cells (48). To rule out the possible involvement of signals generated by both cell-cell contact and soluble factors in the regulation of collagen synthesis, experiments were set up first using plasma membrane preparations from unstimulated or activated T cells, and then in double-chamber culture systems. Results showed that contact-mediated signals are responsible for the inhibitory action of activated T cells on the production of collagen I and III, while diffusing mediators released from unstimulated T cells slightly up-regulate collagen I synthesis.

Using specific neutralizing antibody we identified TGFβ as the diffusing factor responsible for the increase in collagen I production mediated by unstimulated T cell membranes or fixed cells (not shown). This could explain why collagen III production is not up-regulated by incubation with resting T cells since in this system production of type III collagen appeared less sensitive to TGFβ than did that of collagen I.

In vitro studies revealed that human T cells have the ability to adhere to synoviocytes as well as to dermal fibroblasts via CD2/CD58 (LFA-3) and LFA-1 (CD11a/CD18)/CD54 (ICAM-1) interactions (12, 13, 49). Furthermore, the cellular adhesion between T cells and synoviocytes induces the production of IL-1β by synovial cells in part through LFA-1-ICAM-1 interaction (17). Consequently, the putative implication of these adhesion molecules in the inhibition of collagen I production mediated by contact with activated T cells was investigated. In the presence of blocking mAbs raised against CD2, CD11a, CD11b, CD11c, CD18, CD40, CD54, CD58, and CD106, the decrease of collagen I production was not reversed (data not
shown), thus ruling out the involvement of these surface molecules in the mediation of cell-contact mediated inhibition of collagen synthesis.

Next, we investigated the implication of cell-associated cytokines on the inhibition of collagen deposition. Several cytokines are known to decrease in vitro the production of collagen I by fibroblasts i.e. IFN-γ, TNFα, and in some cases IL-1α and IL-1β (29, 30, 50). Moreover, membrane-associated TNFα expressed by activated T cells has been shown to provide stimulatory signals for the activation of human B cells, monocytes, and endothelial cells (51–53). Membrane-associated IL-1 has also been described (54), and detectable levels of IL-1α and TNFα measured in plasma membrane preparations of PHA/PMA-activated PBTL have been shown to stimulate MMP-1 and PGE2 production by dermal fibroblasts and synoviocytes (18). Furthermore, it has recently been reported that IFNγ is expressed on the surface of Th1 cells (55). Therefore, we used antibodies or cytokine inhibitors for these cytokines and demonstrate that the concomitant neutralization of IFN-γ, TNFα, and IL-1 results in a marked reversion of the inhibition of collagen I production amounting to 81 and 66.7% in dermal fibroblasts cultured with activated PBTL and HUT-78 cells, respectively. However, since cytokine blocking agents did not prompt the complete reversion of collagen down-regulation, particularly in the case of activated HUT-78 cells, we propose that other still unidentified cell-surface molecules might be implicated in this

![Diagram](image1)

**FIG. 7.** Effect of T cell contact on the stability of α1(I) and α1(III) procollagens mRNAs levels in dermal fibroblasts. Northern blot analysis of the decay of pro-α1(I) (panel A) and pro-α1(III) (panel B) collagen mRNAs in dermal fibroblasts during contact with activated PBTL. Dermal fibroblasts were cultured for the indicated times with (Δ, ●) or without (△, □) membrane preparations from PHA/PMA-activated PBTL (sPBTL) in the presence (■, ○) or absence of DRB (60 μM) (Δ, Δ). DRB was added 30 min prior to membrane preparations. Diagrams show the densitometric scanning quantification of α1(I) and α1(III) procollagen Northern analysis normalized to GAPDH. The figure represents autoradiograms exposed for 15 (α1(I)) and 24 h (α1(III)), respectively. These data represent one of three separate determinations which yielded similar results.

![Diagram](image2)

**FIG. 8.** Effect of cycloheximide treatment on contact-induced down-regulation of procollagens I and III gene expression in dermal fibroblasts. Dermal fibroblasts were incubated in medium containing 1% FCS with membranes of either PHA/PMA-activated PBTL or HUT-78 cells in the presence (+) or absence (−) of CHX (10 μg/ml). Membranes were added 1 h after the addition of CHX. Total RNA (8 μg/lane) was extracted after 14 h of incubation and analyzed by Northern hybridizations with cDNA probes as described in the legend to Fig. 5.
TABLE II

Effect of neutralizing antibodies or cytokine inhibitors on contact-induced down-regulation of fibroblast collagen I production

Dermal fibroblasts were cocultured for 48 h with 12 μl of membranes (6 × 10⁵ cells) of either stimulated PBTL or HUT-78 cells in the presence or absence of anti-IFN-γ mAb (10 μg/ml), rsTNF-p55-hy (1 μg/ml), or IL-1Ra (2 μg/ml). PBTL and HUT-78 cells were activated with PHA/FMA for 48 and 16 h, respectively. IFN-γ, TNFα, and IL-1β were used at 2000 units/ml, 5 ng/ml, and 250 pg/ml, respectively. Data are expressed as percentage of basal PINP production and represent the mean ± S.D. of several experiments (number of experiments is presented in parentheses).

| Stimuli       | Anti-IFN-γ | rsTNF-p55 | IL-1Ra |
|---------------|------------|-----------|--------|
| Medium        | −          | −         | −      |
| Medium        | +          | +         | +      |
| Medium        | −          | +         | +      |
| Medium        | +          | −         | +      |
| sPBTL         | −          | −         | +      |
| sPBTL         | +          | +         | +      |
| sHUT-78       | −          | −         | +      |
| IFN-γ         | −          | +         | +      |
| IFN-γ         | +          | −         | +      |
| TNFα          | −          | +         | +      |
| TNFα          | +          | −         | +      |
| IL-1β         | −          | +         | +      |
| IL-1β         | +          | −         | +      |
| IFN-γ/TNFα/IL-1β | −     | −     | +      |
| IFN-γ/TNFα/IL-1β | +     | +    | +      |

| PINP production (%) in Dermal fibroblasts | SYN | Synoviocytes |
|-------------------------------------------|-----|-------------|
| Medium                                    | 100 | 100         |
| sPBTL                                    | 97.4 ± 4.9 (6) | 101 ± 2.1 (2) |
| sPBTL                                    | 94.8 ± 8.7 (6) | 104 ± 1.8 (2) |
| sPBTL                                    | 89.7 ± 13.6 (6) | 103 ± 12.2 (2) |
| sPBTL                                    | 86.0 ± 14.1 (4) | 105 ± 5.6 (2) |
| sPBTL                                    | 37.2 ± 7.1 (12) | 39.9 ± 7.2 (4) |
| sPBTL                                    | 58.4 ± 8.4 (12) | 49.0 ± 2.6 (4) |
| sPBTL                                    | 46.3 ± 9.7 (9) | 49.5 ± 3.5 (4) |
| sPBTL                                    | 44.9 ± 6.1 (8) | 50.0 ± 4.0 (4) |
| sPBTL                                    | 81.0 ± 6.3 (6) | 86.2 ± 12.4 (4) |
| sHUT-78                                   | 35.1 ± 6.2 (11) | 42.1 ± 4.2 (4) |
| sHUT-78                                   | 45.0 ± 10 (11) | 48.7 ± 1.5 (4) |
| sHUT-78                                   | 42.9 ± 7.5 (9) | 51.7 ± 13.7 (4) |
| sHUT-78                                   | 40.4 ± 8.4 (8) | 46.7 ± 3.7 (4) |
| IFN-γ                                     | 66.7 ± 7.9 (6) | 63.2 ± 12.7 (4) |
| IFN-γ                                     | 34.6 ± 9.1 (8) | 54.0 ± 7.4 (3) |
| IFN-γ                                     | 82.0 ± 17 (8)  | 106 ± 2.1 (2)  |
| IFN-γ                                     | 65.3 ± 7.9 (6) | 59.0 ± 8.4 (3) |
| IFN-γ                                     | 96.0 ± 7.3 (6) | 101 ± 2.1 (2)  |
| IFN-γ                                     | 57.3 ± 4.3 (6) | 84.0 ± 5.4 (3) |
| IFN-γ                                     | 91.2 ± 4.3 (4) | 107 ± 1.2 (2)  |
| IFN-γ                                     | 16.5 ± 4.5 (4) | 28.0 ± 3.2 (2) |
| IFN-γ                                     | 92.5 ± 15 (4)  | 95.0 ± 6.7 (2)  |

a p < 0.001 with respect to sPBTL stimulation.
b p < 0.02 with respect to sPBTL stimulation.
c p < 0.005 with respect to sHUT-78 stimulation.
d p < 0.02 with respect to sHUT-78 stimulation.

Inhibitory effect. Besides, since IL-1α was only detected in the membrane preparations of activated PBTL and not in those of HUT-78 cells, we cannot rule out the possible implication of a cytokine autocrine loop produced by fibroblasts following contact with activated PBTL or HUT-78 cell membranes. Indeed, co-cultures of T cells and dermal fibroblasts or synoviocytes have been reported to stimulate the production of IL-1β by fibroblasts (16, 17), and Bombara et al. (14) have reported that co-culturing T cells and synovial fibroblasts results in the accumulation of cytokines in the culture supernatant, notably of IFN-γ and TNFα (14). However, according to RNase protection assay analysis, in our system mRNAs encoding for IFN-γ and TNFα were not expressed in dermal fibroblasts or synoviocytes incubated with membranes of activated T cells (not shown). These data confirm that inhibition of collagen production mediated by IFN-γ and TNFα is due to membrane associated forms of these cytokines.

The involvement of membrane-associated cytokines in the down-regulation of collagen deposition is consistent with the time course of induction by PHA/FMA of the ability of PBTL to inhibit the production of collagen. Indeed, TNFα which is the best characterized membrane-associated cytokine, was shown to be expressed in the surface of CD4⁺ T cell clones within 2 h after activation (51).

Previous reports have shown that soluble IFN-γ and TNFα inhibit collagen synthesis both by transcriptional and post-transcriptional mechanisms (28, 56). We demonstrate here that contact-mediated decrease of types I and III collagen takes place exclusively at the transcriptional level. Indeed, we provide evidence that no post-transcriptional modifications likely to destabilize pro-α1(I) and pro-α1(III) mRNAs take place. Furthermore, this inhibition was specific for collagen genes since the expression of mRNA coding for MMP-1 was up-regulated during T cell-fibroblast interaction. However, the inhibition of types I and III collagen probably involves distinct molecular mechanisms. Indeed, the decrease of pro-α1(I) mRNA required de novo protein synthesis whereas that of pro-α1(III) did not.

Finally, having observed that direct contact with activated T cells induced the production of PGE₂ in dermal fibroblasts (18), and PGE₂ having proved to inhibit fibroblast collagen synthesis (57), we studied the effect of activated T cell membranes on collagen I production in the presence of indomethacin. The latter, however, did not reduce the decrease of collagen I production mediated by contact with activated T cells (not shown). These data match previous reports on the inhibitory action of soluble IL-1, TNFα, and IFN-γ on collagen I and III accumulation in lung fibroblasts (58). Consequently, the additive inhibitory effect of cell-associated TNFα, IFN-γ, and IL-1 on collagen I and III production by dermal fibroblasts and synoviocytes takes place at the transcriptional level by a PGE₂-independent mechanism.

Our observations demonstrate for the first time to our knowledge that collagen production might be regulated in vivo by close contact between mesenchymal cells and T lymphocytes. This work also indicates that membrane-associated IFN-γ might be biologically active as already described for surface TNFα. The nature of the binding of IFN-γ to activated T cell membranes is still unclear. However, as proposed by Assenmacher et al. (55) it would not be IFN-γ bound to IFN-γR since surface-bound IFN-γ was detected with several mAbs which block the binding of mouse IFN-γ to its receptor. Further experiments are at present being conducted in our laboratory using CD4⁺/CD8⁺ as well as Th1 and Th2 T cell clones activated by an immobilized anti-CD3 antibody to specify the role of membrane-associated IFN-γ in the contact-mediated inhibition of collagen production. Current works are also under progress to study this contact-mediated inhibition of collagen deposition in diffuse systemic scleroderma, a fibrotic disease characterized by abnormal deposition of collagen. In these conditions, the decrease of type I collagen seem to be significantly...
less pronounced in fibroblasts from systemic scleroderma patients than normal individuals, suggesting that resistance of systemic scleroderma fibroblasts to inhibition might play a pathogenic role in systemic scleroderma (59).

In conclusion, remodeling of ECM occurs in many biological processes and involves the controlled degradation and neosynthesis of collagenous and non-collagenous components. Types I and III collagen are the main constituents of connective tissue and their homeostasis is finely regulated by various signals including soluble factors, cell-matrix, and cell-cell interactions (5).

We have reported that direct contact between activated T cells and dermal fibroblasts or synoviocytes induced an imbalance between the production of interstitial collagenase and dermal fibroblasts or synoviocytes induced an imbalance between the production of interstitial collagenase (TIMP-1), favoring matrix catabolism (18). We demonstrate that the production of interstitial collagenase between the production of interstitial collagenase (TIMP-1), favoring matrix catabolism (18).

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