The integrin-mediated stress relaxation as it occurs in a retracting three-dimensional collagen gel (RCG) is accompanied by a large up-regulation of the interstitial collagenase, matrix metalloproteinase 1 (MMP-1, EC 3.4.24.7), regulated notably by interleukin-1 (IL-1), phorbol esters, and cytoskeleton-disrupting drugs as cytochalasin D (CD). The repression of MMP-1 up-regulation in RCG by cycloheximide suggested the participation in the regulation process of a de novo synthesized intermediary component. We demonstrate here that culture of human skin fibroblasts in RCG or in CD- and 12-O-tetradecanoylphorbol-13-acetate (TPA)-treated monolayers resulted in the activation of an IL-1 autocrine feedback loop that was switched off by the naturally occurring IL-1 receptor antagonist (IL-1RA), a blocker of the common IL-1 receptor. The IL-1RA did not suppress the MMP-1 up-regulation induced in RCG nor in CD-treated cells, indicating that the up-regulation of MMP-1 and the IL-1 autocrine loop occurred in an independent way, while the TPA-induced MMP-1 expression was suppressed by the receptor antagonist. The RCG- as well as the TPA-, IL-1-, and CD-induced up-regulation of both MMP-1 and IL-1 was totally suppressed by protein tyrosine kinases inhibitors. In contrast bisindoylmaleimide, at a concentration (5 μM) that inhibits the TPA-induced protein kinase C activity, suppressed the CD-induced MMP-1 expression but did not or barely altered that induced in RCG or by IL-1. None of the other tested inhibitors of a variety of signaling pathways including those used by integrins was able to suppress the RCG or CD-induced MMP-1. These results point to a potent regulation of MMP-1 by mechanical stress relaxation, a process depending on de novo protein synthesis and occurring independently of the activation of an IL-1 autocrine feedback loop.

Integrins are transmembrane heterodimeric proteins providing a structural link between the extracellular matrix and the cytoskeleton. They are responsible for the transduction to the cell of information arising from specific sequences within the macromolecules and/or triggered by the mechanical properties of their polymers. Both messages, chemical and/or mechanical, are responsible for controlling signaling processes that involve assembly of multiple proteins in the focal adhesion plaque, organization of cytoskeletal actin polymers, and their anchorage to the cell membrane, protein phosphorylation and activation of signaling cascades leading to regulation of genes expression through several potential pathways (1). The interactions of cells with the extracellular matrix are essential in many biological and pathophysiological processes as embryonic development, wound healing, fibrosis, tumor invasion, and metastasis.

Most of the integrin-mediated regulations and signaling by mechanical forces have been investigated in cells under stress (1, 2). Much less is known about the messages that the cells receive and the signaling that is triggered when stress is released. This event is relevant to investigate since it participates in physiological and pathological processes observed during extracellular matrix degradation in remodeling, cancer and inflammatory diseases, mammary epithelium differentiation (3), and osteoporosis induced by suppression of muscular activity or in weightlessness conditions (4). Furthermore, reduced mechanical tension is actually regarded as a signal inducing programmed cell death (5, 6).

The culture of cells within a free floating gel of collagen polymers offers a model of integrin-mediated stress relaxation. When fibroblasts are cultured in such a gel, they attach to the fibers and retract the gel. According to the “tenesgity” concept proposed by Ingber (7), the dissipation of mechanical tension upon retraction of the malleable collagen gel results in alteration of the dynamic balance of the cytoskeleton. It is visualized by the reorganization of the actin network with the disappearance of the stress fibers (8) and the assembly of actin clusters along the cell periphery (9). This process is accompanied by a profound reprogramming of the cell phenotype. We, and others, have demonstrated an arrest of cell division, an extensive reduction of collagen and other structural macromolecules expression (10, 11), whereas a large up-regulation of interstitial collagenase (MMP-1,1 EC 3.4.24.7) was observed (12, 13). It was recently demonstrated that α2β1 is the major determinant of the contraction of the collagen gel (14, 15) and the mediator of the regulation of the MMP-1 gene in fibroblasts cultured in a retracting collagen gel while α1β1 mediates the COL1A1 gene down-regulation (16, 17).

MMP-1 expression is modulated by many growth factors and potent cytokines such as IL-1, by pharmacological agents such as phorbol esters, and by cytoskeleton-disrupting drugs such as

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cytochalasin D (8, 18). CD and TPA have been shown to induce the MMP-1 expression in rabbit corneal fibroblasts through the activation of an autocrine loop of the inflammatory cytokine IL-1α (19–21). We describe here a similar IL-1α and IL-1β loop in human fibroblasts embedded in RCG and upon treatment by CD and TPA. The stress relaxation-induced MMP-1 up-regulation in RCG- and CD-treated human fibroblasts occurred, however, independently of the IL-1 loop. The signaling in RCG- and CD-induced regulations used, at least partly, a different pathway.

**EXPERIMENTAL PROCEDURES**

**Cell Culture—**Normal human dermal fibroblasts were obtained from a healthy 18-year-old donor and used at passage 7–13. Cells were cultured at 37 °C under 5% CO2 in DMEM supplemented with 10% fetal calf serum (Life Technologies, Inc.) and were mycoplasma-free. The three-dimensional cultures in the collagen gel were prepared mainly as described earlier (22). Coated dishes with fibrillar collagen (20 μg/cm²) were prepared by polymerizing bovine type I collagen in phosphate-buffered saline at 37 °C prior to air-drying and washing with distilled water. Fibroblasts treated by CD after trypsination remained rounded, but attached to the dish. Repleting culture medium without CD resulted in the spreading of the cells within a few hours.

**Chemicals and Probes—**Genistein and HbA were purchased from Life Technologies, Inc.; CHX, CD, tyrophostin 23, neomycin sulfate, wortmannin, HA1004, H8, W-7, pertussis toxin, induomethacin, TPA, and MTT from Sigma; BIM I (GF 109263X) from Boehringer Mannheim (Mannheim, Germany); and mevastatin from Biomol Research Laboratories (Plymouth Meeting, PA). D609 was a generous gift from Prof. D. G. Sauer (Deutsches Krebsforschungszentrum, Heidelberg, Germany) or purchased from Biomol Research Laboratories. The recombinant human IL-1α was from Peprotech (Canton, PA) and Genzyme (Cambridge, MA), recombinant human IL-1β from Genzyme, and IL-1RA from Genzyme. The MMP-1 cDNA probe was kindly provided by Dr. G. I. Goldberg (Washington University School of Medicine, St. Louis, MO).

**Cytotoxicity Assays and Measurements of Protein Synthesis**—The cytotoxicity of the various agents used in this study was tested by measuring the activity of the mitochondrial succinate dehydrogenase as described previously (23) and expressed as the loss in percent of the activity as compared with untreated cells. Protein synthesis measurements were performed in triplicate by incubating cells with [3H]proline (10 μCi/ml, 38 Ci/mmol, NEN Life Science Products) for 24 h and measuring the trichloroacetic acid-insoluble radioactivity by liquid scintillation spectrometry.

**PKC Assay in Crude Membrane Fraction**—Crude membrane fractions were prepared and the associated PKC activity measured in triplicate using the PKC assay kit (Life Technologies, Inc.) as described by the manufacturer and corrected for the concentration of proteins (MicroBCA assay, Pierce).

**IL-1 Measurement**—IL-1α and IL-1β were measured in duplicate by ELISA using a kit from Amersham Pharmacia Biotech (Rainham, UK) for IL-1α and from Genzyme for IL-1β. Monolayers were washed with PBS and harvested in 1 ml of DMEM containing 10% fetal calf serum. The cell suspension was submitted to five cycles of freezing and thawing and centrifuged at 6000 × g for 1 min, and the supernatant was used for ELISA. Collagen gels were dissolved by bacterial collagenase (clodrindopeptidase, Sigma type IA, 0.5% in PBS) for 15 min at 37 °C. Cells were pelleted by centrifugation at 6000 × g for 1 min and treated as the monolayers (fetal calf serum 10% final concentration) was added to the cell lysate prior to assay. A standard curve was obtained by diluting the IL-1 standards in DMEM containing 10% fetal calf serum.

**Specific mRNA Measurement**—Total RNA was prepared as described previously (24) or using the High Pure RNA isolation kit (Boehringer Mannheim). IL-1α, IL-1β, MMP-1 mRNA, and 28 S rRNA were measured in 19-ng aliquots of total RNA by RT-PCR using the GeneAmp Thermocycler RNA PCR kit (Perkin-Elmer, Foster City, CA). An external control RNA template, pAW109 (Perkin-Elmer), was introduced into each sample to monitor the assays of IL-1α and IL-1β. The mRNA of MMP-1 was also measured by hybridization of Northern blots as detailed elsewhere (13).

**RESULTS**

The Expression of MMP-1 Induced in the RCG and on Treatment of Monolayers with CD and IL-1, but Not by TPA Requires de Novo Protein Synthesis—The expression of MMP-1 by normal human skin fibroblasts was low in monolayer on plastic (Fig. 1a) and was barely increased on a fibrous collagen-coated versus uncoated plastic (2.0 ± 0.2-fold). It was largely increased in CD-, IL-1-, or TPA-treated monolayer cultures and in RCG. The maximum level of MMP-1 induction was observed after 24 h of treatment with IL-1 or TPA, but only after 48 h with CD and in RCG. The requirement for de novo protein synthesis was investigated by using CHX at a concentration (70 μg/ml) that was largely increased on a fibrous collagen-coated RCG (for 48 h). When indicated by (+), cells were pretreated with CHX (70 μg/ml) for 3 h before trypsination and/or treated for 24 h with IL-1α (10 ng/ml, i.e. 100 units/ml) or β (0.5 ng/ml, i.e. 100 units/ml), TPA (20 nm, 24 h), or CD (10 μg/ml, 48 h). EtBr, ethidium bromide staining of the gel before transfer. b, RT-PCR measurements of IL-1α and IL-1β mRNA. Conditions as in a. pAW109, co-amplified internal standard; 28S, 28 S rRNA amplification product.
24 h of culture while IL-1α mRNA remained undetectable. Exogenously added IL-1α or IL-1β induced the expression of their own mRNA. In all instances, CHX resulted in a highly increased level of both IL-1 mRNAs (Fig. 1b). The only exception was observed in the CD-treated monolayers and mainly concerned IL-1β. Addition of CHX resulted first in an increased level of IL-1 mRNA after 24 h of treatment (not shown) followed by a decreased steady-state level after 48 h (Fig. 1b).

As shown in Table I for one representative experiment, the IL-1s were measured in the untreated and TPA- and CD-treated monolayers or in RCG by ELISA in the cell lysates, as secreted proteins in the culture medium, or associated with the collagen gel in RCG, at 24 and 48 h of culture. Low to undetectable levels of IL-1α and IL-1β (<100 pg/ml) cells were found in untreated monolayer cultures on plastic or on a fibrillar collagen coat. IL-1α and mainly β were slightly increased by TPA at 24 h while only IL-1β remained increased at 48 h. CD and, to a lesser extent, the culture in RCG induced the production of large amounts of IL-1α and IL-1β, the latter being by far the most abundant. Although most of the IL-1α and β was associated with the cells, significant amounts were found in the culture medium in the CD-treated cultures or associated with the collagen gel and released by collagenase digestion of the RCG.

The Induction of MMP-1 Expression by TPA, but Not by CD or in RCG, Is Mediated by IL-1—The potential involvement of both endogenous IL-1α and IL-1β in the TPA-, CD-, and RCG-induced overexpression of MMP-1 was assessed by adding the naturally occurring antagonist IL-1RA to the culture (Fig. 2). The overexpression of the mRNA of IL-1α, IL-1β, and MMP-1 induced by exogenous IL-1 (10 ng/ml of IL-1α, 0.5 ng/ml of IL-1β, i.e. 100 units/ml each) was suppressed by IL-1RA, demonstrating its efficiency in blocking the effect of both cytokines.

A similar inhibition of the expression of the three genes was observed in TPA-treated cells. The expression of the mRNA of both IL-1s in CD-treated cells or in RCG was also inhibited or largely reduced by IL-1RA. In contrast, IL-1RA did not influence the expression of MMP-1 in these conditions. These data strongly suggest that the autocrine IL-1 loop activated by CD and in RCG is not the unique regulatory event inducing the MMP-1 overexpression in human dermal fibroblasts.

Protein Tyrosine Kinases Participate in the TPA-, IL-1α, CD-, and RCG-induced Overexpression of MMP-1 and IL-1—The involvement of protein tyrosine kinase in the regulation of MMP-1 and IL-1 expression was tested by using the inhibitors HbA and genistein. Fibroblasts in monolayer were treated overnight with increasing concentrations of HbA or genistein, trypsinized, and subcultured in monolayers supplemented with TPA, IL-1, or CD or in RCG in the presence of the same concentration of the inhibitors. The steady-state level of MMP-1 mRNA in cells cultured in monolayers supplemented with TPA, IL-1, or CD or cultured in RCG was extensively reduced by HbA at 260 nm (Fig. 3a) to levels close to those found in control monolayers. These data were confirmed in CD-treated monolayer or RCG by using genistein (Fig. 3b). HbA also suppressed the autoinduction of IL-1α and IL-1β expression (not illustrated) as well as their up-regulation upon treatment by CD and TPA and in RCG (Fig. 3c).

PKC Is Involved in the TPA- and CD- but Not in the IL-1- or RCG-induced Regulation of the MMP-1 and IL-1 Gene Expression—The potential involvement of PKC in the increased expression of MMP-1 and IL-1 induced by CD and IL-1 and in RCG was investigated by using the PKC inhibitor BIM. Treatment with BIM at 5 μM decreased the TPA-induced PKC activity by a factor of 6.0 ± 1.5, i.e. below the basal PKC activity of untreated cells, a result supporting the efficiency of the inhibitor. The TPA-induced overexpression of the mRNA of MMP-1 and IL-1β (Fig. 4, a and b) was suppressed by BIM as expected. Similar experiments were performed on cells cultured in monolayers treated with IL-1α or β for 24 h and with CD or in RCG for 48 h. In the two latter conditions the medium and BIM were renewed at day 1. The rate of contraction of the collagen gel was slightly reduced by BIM (Table II). Treatment by BIM resulted in an increased level of the mRNA of MMP-1 (Fig. 4a) in IL-1α- or β-treated cells. The CD-induced up-regulation of MMP-1 (Fig. 4a) and IL-1β (Fig. 4b) was largely reduced by BIM. By contrast, the RCG-induced overexpression of MMP-1 was not modified while that of IL-1β was partially (Fig. 4b) or

| IL | Time | Monolayer on plastic | TPA treated monolayer | CD treated monolayer | RCG |
|---|---|---|---|---|---|
|   | h | Cell | Medium | Cell | Medium | Cell | Medium | Cell | Medium | Cell | Medium |
| IL1-α | 24 | 58 | 0 | 338 | 0 | 3,530 | 28 | 312 | 17 | 9 |
|   | 48 | 6 | 50 | 74 | 0 | 6,800 | 74 | 1,730 | 112 | 1 |
| IL1-β | 24 | 60 | 170 | 600 | 1,430 | 40,000 | 15,930 | 4,000 | 2,117 | 30 |

*Values are expressed as picograms of the cytokine per 10^6 cells.

**Fig. 2.** Modulation of the MMP-1, IL-1α, and IL-1β mRNA level in TPA-, IL-1α, and CD-treated monolayers and in RCG by IL-1RA. RT-PCR measurements of MMP-1, IL-1α, and IL-1β mRNA. Cells were trypsinized and seeded on a fibrous collagen coat (MC, 24 or 48 h) or in RCG. When indicated, the cells were treated with TPA (20 nM, 24 h), IL-1 (IL-1α 10 ng/ml and IL-1β 0.5 ng/ml, 24 h), or CD (10 μg/ml, 48 h), in the absence or presence of IL-1RA (1 μg/ml). pAW109, co-amplified internal standard; 28S, 28 S rRNA amplification product.
not reduced by BIM in five separate measurements. Similarly, BIM suppressed the TPA- and CD-induced expression of IL-1β as measured by ELISA (not shown).

**RCG- and CD-induced MMP-1 Regulation Does Not Depend on Phospholipase C, Protein Kinase A, Protein Kinase G, Calmodulin, Prostaglandin E2, Receptor Tyrosine Kinases, Ras, or Protein Gi**—To further characterize the cascade of events involved in the RCG and CD-induced regulation of MMP-1, a panel of agents known to interfere with a variety of signaling pathways were tested for their ability to block this effect. D609, neomycin, indomethacin, pertussis toxin, wortmannin, HA1004, H8, W-7, tyrphostin 23, and mevastatin were used at nontoxic concentrations close to or exceeding the described IC50 (see Table II for the working concentrations and molecular targets). Tyrphostin 23, D609, wortmannin, and W7 slowed down the initial rate of contraction of the gels, although the final contraction measured after 2 days was not modified. The disruption of the cytoskeleton by CD was not impaired by any of these agents. None of them was able to modify the up-regulation of the MMP-1 gene induced by CD or in RCG.

**DISCUSSION**

The retracting collagen gel is a useful model for investigating the mechanism(s) by which fibroblasts sense the release of mechanical stress in their physiological support of collagen fibers and transduce this signal into a biochemical response. In the free-floating collagen gel the actin stress fibers are progressively disrupted, and filamentous actin is relocated to the cell periphery (8, 9). This is accompanied by a profound reprogramming of the cell phenotype and, notably, by an extensive expression of MMP-1 (8, 12, 13), a matrix metalloproteinase barely expressed in monolayer on a rigid support or under physiological conditions in vivo. In vitro, MMP-1 can be stimulated by detachment of cells from their support using trypsin or EGTA (40) and by a wide range of physiologically relevant agents, such as inflammatory cytokines, notably IL-1 (41), growth factors, and pharmacological agents, such as PKC activators, and actin stress fiber-disrupting drugs, such as cytochalasin B or D (8, 18, 20, 21).
It has been proposed that the up-regulation of MMP-1 in TPA- and CD-treated corneal, synovial, and tendon fibroblasts of the rabbit is mediated by IL-1α through the activation of an autocrine feedback loop (19–21). The existence of a similar regulatory pathway in human fibroblasts within the RCG could indicate that the up-regulation triggered by disruption of the cytoskeleton is induced through the dominant activity of newly synthesized intermediary protein(s). The IL-1α and β mRNA steady-state level is increased by CHX. These transcripts are characterized by an AUUUA-rich sequence in the 3′-noncoding region that is thought to target the mRNAs for degradation (44), in a process coupled to active translation (45) and blocked by CHX.

The effective suppression of the IL-1 autocrine loop by IL-1RA that blocks their common receptor and the lack of effect by immunologically blocking IL-1α alone (not illustrated) suggested the participation of active IL-1β in the establishment of the autocrine loop. This is another point of divergence with the animal fibroblasts model. The IL-1β converting enzyme, responsible for processing the inactive pro-IL-1β into its active and secreted form in monocytic cells, is lacking in all the fibroblastic lines tested (46, 47). The finding that a significant amount of IL-1β was found in the culture medium of CD-treated fibroblasts or associated with the collagen gel in RCG suggests that an alternative processing pathway might exist in fibroblasts. It is noteworthy that MMP-1 has been reported to be able to process proIL-1β in vitro (48). Experiments are currently underway to clarify this issue.

The engagement of integrins with their cognate ligands generates a number of signaling events including phosphorylation of pp125FAK and pp60src and activation of phospholipase C, protein kinase C, and phosphatidylinositol 3-kinase (1). Abrupt stress relaxation of tethered collagen gels has been shown to activate phospholipase D, prostaglandins, and the cAMP path-

| Agents | Targets | IC_{50} | References |
|--------|---------|---------|------------|
| None   |         |         |            |
| CD     |         |         |            |
| Cycloheximide | Protein synthesis | 10 µg/ml | 38 |
| Anti-IL-1α | IL-1α | 70 µM | 99 |
| IL-1RA | IL-1 receptor | 50 µg/ml | nd |
| Genistein | Tyrosine kinases | 2.6–20 µM | 25 |
| Tyrohydrocinnam A | Tyrosine kinases | 26 |
| Tyrophanol 23 | EGFR and PDGF-R | 35 and 25 µM | 27 |
| BIM | Protein Kinase C | 0.2 µM | 28 |
| D609 | PLC and PLD | 10 µM | 34, 35 |
| Neomycin | PLC and PLD | <600 and 65 µM | 30, 31 |
| Wortmannin | PI3K, PI4K, and MLCK | 3500 µM | 41 |
| HA1004 | PKA, PKG and CaMK | 2, 3, 13 and 13 µM | 34, 35 |
| H8 | PKA and PKG | 1.2 and 0.48 µM | 34 |
| W7 | Calmodulin | 31 µM | 36 |
| Pertussis toxin | Gt | 0.2 ng/ml | 37 |
| Indomethacin | COX and 1, 2, 700 and 970 nm | 38 |
| Mevastatin | HMG-CoA reductase | <1 µM | 39 |

a RGF-R, epidermal growth factor receptor; PDGF-R, platelet-derived growth factor receptor; PLC, phospholipase C; PLD, phospholipase D; PI3K, phosphatidylinositol 3-kinase; PI4K, phosphatidylinositol 4-kinase; MLCK, myosin light chain kinase; PKA, protein kinase A; PKG, protein kinase G; CaMK, calmodulin-dependent kinase; COX, cyclooxygenase; HMG, 3-hydroxy-3-methylglutaryl.  
b HbA at 87–870 nM reverses the v-Src-induced morphological transformation in NRK cells.  
c This concentration of neomycin inhibits the thrombin-induced PLC activation in Syrian hamster fibroblasts.  
d Cells pretreated with W7 and treated with both W7 and CD after trypsinization failed to reattach.

The mitochondrial activity was measured as the activity of the succinate dehydrogenase using the MTT assay described under "Experimental Procedures." The results are expressed as loss of activity in percents of control cultures. The contraction rate of the collagen gel was determined by measuring its diameter as a function of time. Measurements were performed in triplicate, and the standard deviation was less than 5% of the mean values. IC_{50} refers to half-inhibitory concentrations reported in the literature.
ways (49). PKC activation is involved in TPA-induced MMP-1 and IL-1 regulation (50, 51). The participation of these signaling enzymes in the RCG- and CD-induced regulation of MMP-1 and IL-1 was investigated by using selected inhibitors. Their concentration was chosen by taking into account their cytotoxicity determined by the MTT assay that displays a threshold response lower than the lactate dehydrogenase release used by others (16, 52). The effective inhibiting concentration was based either on data from the literature or tested on known mediators (see Table II). None of the inhibitors at the indicated concentration significantly reduced the contractile activity of the fibroblasts. The involvement of protein tyrosine kinase in the induction of MMP-1 by CD and in RCG is demonstrated by the down-regulation of its mRNA by HbA or genistein. A similar reduction of IL-1 overexpression in both models is also observed using HbA. The suppression by HbA of the regulation introduced by disruption of the actin stress fibers, either in RCG- or in CD-treated cells, supports a role for c-Src or related members of the family although the selectivity of HbA toward Src or Src-like kinases is not absolute. The results with HbA are in agreement with data from Broberg and Heino (53), but contrast with those of Langholz et al. (16) who found that HbA was inactive even at a dose that largely exceeds that used in this study. This divergence might come from differences in the experimental procedure since we preincubated cells with the inhibitor overnight before seeding them in the RCG.

The overexpression of the MMP-1 and IL-1 induced by CD and in RCG obviously uses, at least partly, divergent signaling pathways since BIM (5 μM) suppresses the CD-induced MMP-1 up-regulation but does not alter that induced in RCG or by IL-1. Xu and Clark (52) suggested that the induction of MMP-1 in RCG was dependent on the atypical TPA-independent PKC ζ activity that could be suppressed by higher concentrations of BIM (20 μM). At higher concentrations, we did not suppress the MMP-1 overexpression in RCG but found that 10 and 20 μM BIM resulted in a loss of 35 and 48%, respectively, of cell viability in our normal human fibroblasts (not shown). None of the other inhibitors listed in Table II was able to suppress the MMP-1 up-regulation induced either by RCG or by CD.

Type I collagen is recognized by the integrins α1β1 and α2β1. The latter is the major integrin involved in gel contraction as up-regulation does not occur when the former integrin is inhibited (58) and up-regulates MMP-1 and IL-1. The overexpression of the MMP-1 and IL-1 induced by CD and in RCG obviously uses, at least partly, divergent signaling pathways since BIM (5 μM) suppresses the CD-induced MMP-1 up-regulation but does not alter that induced in RCG or by IL-1. Xu and Clark (52) suggested that the induction of MMP-1 in RCG was dependent on the atypical TPA-independent PKC ζ activity that could be suppressed by higher concentrations of BIM (20 μM). At higher concentrations, we did not suppress the MMP-1 overexpression in RCG but found that 10 and 20 μM BIM resulted in a loss of 35 and 48%, respectively, of cell viability in our normal human fibroblasts (not shown). None of the other inhibitors listed in Table II was able to suppress the MMP-1 up-regulation induced either by RCG or by CD.

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