Hyaluronic acid-specific regulation of cytokines by human uterine fibroblasts

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Kobayashi, Hiroshi, and Toshihiko Terao. Hyaluronic acid-specific regulation of cytokines by human uterine fibroblasts. Am. J. Physiol. 273 (Cell Physiol. 42): C1151–C1159, 1997.—The physiological inflammatory response can provide an effective mechanism for delivering the baby at the time of parturition. We characterized the mechanisms by which hyaluronic acid (HA) regulates interleukin-1 (IL-1β), tumor necrosis factor-α (TNF-α), and interleukin-8 (IL-8) production in human uterine fibroblasts. A dose-dependent increase in cytokine release was observed over an HA concentration range of 10 μg/ml to 1 mg/ml. The action of HA on the cytokine production is mediated by CD44. Under serum-free conditions, HA-induced cytokine generation was significantly less compared with production in the presence of serum, suggesting involvement of serum proteins. Addition of inter-α-trypsin inhibitor (ITI) under serum-free conditions enhanced the HA-induced synthesis of TNF-α, which stimulated the temporary release of IL-8. In addition, HA and IL-1β stimulated the release of hyaluronidase by the fibroblasts. These results indicate that cytokine production in human uterine fibroblasts is regulated in a CD44-HA-ITI-specific fashion. HA may be involved in the regulation of delivery in part through the selective release of cytokines that contribute to uterine cervical ripening.

Cytokines such as interleukin-1β (IL-1β) and tumor necrosis factor-α (TNF-α) are known to exert numerous effects on various target tissues. It is well recognized that various substrates, including lipopolysaccharides, can stimulate macrophages and/or fibroblasts to produce IL-1β (11, 12). IL-1β is considered to be a potent stimulator of hyaluronic acid (HA) production by fibroblasts in vitro (17). The local accumulation of HA concentrations could function to facilitate the migration of inflammatory cells into the reaction site (4, 21). HA is able to retain large amounts of water within its molecular domain on surrounding cells and matrix constituents. HA is synthesized early during the course of an inflammatory reaction and is later degraded by fibroblasts during the healing phase of inflammation (15, 27).

The cell surface adhesion molecule CD44 is the principal receptor for HA (3, 23). The CD44 family has several different isoforms that are derived from a single gene by alternative splicing of the mRNA. The NH2-terminal domain of CD44, which shows homology to the link protein of cartilage, binds to a six-sugar sequence of HA. Antibodies that block the interaction between HA and CD44 prevent the binding of HA to the cell surface, its subsequent uptake into the cytoplasm, and its eventual degradation in lysosomes. CD44 is an extremely versatile protein that may carry out several functions through HA binding (14).

It has been reported that a serum factor is required to organize HA within the extracellular matrix (5, 7). The intercellular space between fibroblasts increases due to the production of a mucopolysaccharide extracellular matrix in HA. This factor, which is important for organizing HA within the extracellular matrix, has been recently identified as a protein belonging to the inter-α-trypsin inhibitor (ITI) family (6, 8, 16, 25, 31). Purified ITI was able to replace serum in supporting pericellular matrix coats, and it was proposed that ITI provides an integral structural component of the matrix by interacting directly with HA. Previous studies showed that hyaluronidase (HAase) and protease disorganized and partially degraded the microstructure of the matrix, indicating that interactions between proteins and HA are important for stability of the extracellular network between the cells (5–8, 16, 25, 31).

At the time of parturition, the physiological inflammatory response can provide an effective mechanism for delivering the baby. With uterine cervical dilatation and softening (ripening), increases of HA concentrations and collagenase activity and a conspicuous decrease of collagen in the uterine cervix have been reported (9). The ripening of the cervix is a necessary prerequisite for a normal labor. It is an interesting fact that IL-1β promotes collagenase production and HA synthesis as well as a depression of collagen synthesis, since these actions could be associated with the uterine cervical ripening process at parturition. HA synthesis is accelerated soon after onset of labor. Cellular migration has been correlated with elevations of HA concentrations in uterine cervix. However, endogenous cytokine inducers during the ripening of the uterine cervix are not fully clarified.

Here, we report that HA induces cytokine production in human uterine fibroblasts and may contribute to pathological and physiological changes in connective tissues. HA stimulates the release of IL-1β and TNF-α from uterine fibroblasts. IL-1β could stimulate neighboring connective tissue fibroblasts to produce increased quantities of HA. IL-1β and TNF-α synthesized by fibroblasts after stimulation by endogenously produced or exogenously applied HA could act directly as a positive feedback to release additional HA from fibroblasts.

MATERIALS AND METHODS

Materials. Dulbecco’s modified Eagle’s medium (DMEM), RPMI 1640 medium, Hanks’ balanced salt solution, fetal calf serum (FCS), trypsin-EDTA, and gentamicin were obtained from Gibco (Grand Island, NY). HA, with molecular masses...
of 800 and 2,000 kDa, were kindly supplied by Chugai Pharmaceutical and Kaken Chemical, respectively; both had a purity of at least 99%. Intra-articular injection therapy with HA is widely used in Japan for degenerative gonarthrosis, and it has shown favorable clinical results in terms of improving articular symptoms. The HA used in the present experiments was derived from chick’s comb. HA is a pure polysaccharide. Combining 12,500 disaccharide units produces an HA molecule of ~5 x 10^9 molecular weight, similar to the HA present in cartilaginous tissues. The normal size range of HA secreted by uterine fibroblasts is also ~3–5 x 10^9 molecular weight. When HA is dissolved in physiological saline, every molecule touches another one and a continuous molecular network is formed. HA is not subjected to conformational changes by physiological saline. HA binding protein (HABP, 40 kDa) was also kindly supplied by Chugai Pharmaceutical. A monoclonal antibody (MAb) against IL-1β and an MAb against TNF-α were obtained from Genentech. Heparan sulfate, dermatan sulfate, chondroitin sulfate, and HAase (which was from Streptomyces hyaluronicus) were purchased from Sekagaku Kogyo (Tokyo, Japan). Anti-CD44 antibody was obtained from Cosmo Bio (Tokyo, Japan). N-acetyl-D-glucosamine, glucuronic acid, N-acetylgalactosamine, N-acetyl-D-galactosamine, and D-glucosamine were obtained from Sigma Chemical (St. Louis, MO).

Cell culture. Human uterine fibroblasts were obtained from surgery specimens using an explant culture. Cells were maintained in vitro in 75-cm² flasks (Nunc, Tokyo, Japan) in DMEM supplemented with 10% FCS and antibiotics (50 µg/ml gentamicin and 2.5 µg/ml amphotericin). The cells were maintained at saturated humidity in an atmosphere of 95% air-5% CO₂. Fibroblasts were studied during passages 2–5.

Confluent fibroblasts were washed in phosphate-buffered saline (PBS), trypsinized (0.05% trypsin, 0.1% EDTA) for 2 min at 37°C, diluted in medium containing 10% FCS to inactivate the trypsin, and centrifuged for 10 min at 250 g. Cell pellets were resuspended in culture medium (DMEM containing 10% FCS), and quadruplicate samples of 1 ml aliquots of cell suspension were added to 24-well plates (Costar). The cell number added to each well was ~2 x 10⁵ cells.

After 24 h, the cells were washed three times with Hank’s balanced salt solution and serum-free DMEM supplemented with 0.1 mg/ml bovine serum albumin (BSA). The cells were maintained in fresh complete medium containing either no additions (control) or HA in the presence or absence of anti-CD44 antibody. All conditions were examined in triplicate. After the indicated periods of time, the culture supernatants were harvested and stored at ~20°C for measurement of IL-1β and TNF-α.

Fibroblasts in the corresponding fourth well of each sample were trypsinized and counted in a hemocytometer. The obtained cell counts were then used to correct the measured amounts of IL-1β and TNF-α for cell numbers.

In a parallel experiment, fibroblasts were incubated in quadruplicate samples in 96-well flat-bottom microtiter plates to determine spontaneous as well as HA-induced interleukin-8 (IL-8) synthesis. The cell number added to each well was ~2 x 10⁵ cells/200 µl.

Protein levels were measured in cell wells at the end of the assay, and these were remarkably constant across all treatments, indicating that the cell numbers from well to well were not significantly different at the end of the assay period.

Enzyme-linked immunosorbent assays for IL-1β, TNF-α, and IL-8. Cytokines were measured in fibroblast culture supernatants by specific enzyme-linked immunosorbent assays (ELISA; TFB, Tokyo, Japan). The ELISA had a lower detection limit of 6.3 pg/ml (IL-1β), 1.9 pg/ml (TNF-α), or 3.1 pg/ml (IL-8). The intra-assay variations of these assays were <10%.

Flow cytometric analysis. Fibroblast cells growing as monolayers were detached with 0.02% EDTA in PBS. Complete detachment was confirmed by direct visualization. Single cell suspensions were made by repeated pipetting through a 0.4-mm-diameter cannula. Cells were washed with ice-cold PBS containing 0.1% BSA and resuspended. Aliquots of cells were incubated with anti-CD44 antibody for 30 min at 4°C. After two washes, cells were incubated with 100 µg/ml of HA (30 min, 23°C) and then stained with fluorescein isothiocyanate (FITC)-labeled HABP (5 µg/ml; 30 min, 23°C). HABP was used as a specific probe for HA. The cells were analyzed by flow cytometry (Coulter, Miami, FL). Cells were gated using forward vs. side scatter to select for HA expression on fibroblasts, excluding dead cells and debris. Net mean fluorescence intensity was expressed as channel number of HA expression after subtraction of the background fluorescence.

Preparation of ITI and its derivatives. Human ITI was isolated from serum according to the method of Salier et al. (28). ITI is comprised of three genetically different peptides: two heavy chains (HC1 and HC2) and a light chain (urinary trypsin inhibitor; UTI). The trypsin inhibitor activity of ITI is localized with UTI (10, 13). A highly purified preparation of human UTI with an activity of 2,330 U/mg protein and a molecular mass of 40 kDa (by sodium dodecyl sulfate (SDS)-polyacrylamide gel electrophoresis) was kindly supplied by Mochida Pharmaceutical (Tokyo, Japan). The covalent structure of the polypeptide chain of the physiological inhibitor UTI has already been determined by Wachter and Hochstrasser (29).

HAase activity. HA-unstimulated and HA-stimulated fibroblast culture supernatants (100 µl of each) were dialyzed and then lyophilized. Samples to be examined for the presence of HAase were electrophoresed on 8.0% SDS acrylamide gel. HAase activity was determined by zymography using HA-impregnated gels as described previously (22). Fragments of HA. Oligosaccharides of HA were prepared by degradation of HA with HAase and characterized by gel chromatography according to the manufacturer’s instructions.

Statistical analysis. All data were analyzed by comparing the samples with a control group by the Student’s t-test.

RESULTS

HA-induced release of TNF-α and IL-1β by fibroblasts. Many cytokines are released either spontaneously or in response to cellular activation. To determine whether HA (molecular mass of 800 kDa) affects cytokine production, IL-1β and TNF-α proteins released into the conditioned medium of fibroblasts were quantitated by ELISA. These data enabled an estimate to be made of the relative quantities of newly synthesized cytokines released into the incubation medium.

Fibroblast monolayers were cultured by 48 h. ELISA analyses of culture supernatants shown in Figs. 1 and 2 confirmed the stimulation of cytokine synthesis resulting from the addition of HA.

Fibroblast monolayers were exposed to soluble HA in the presence of complete medium containing 10% FCS. Unstimulated cells released detectable levels of IL-1β and TNF-α proteins. Stimulation with HA (1 mg/ml) resulted in significantly higher levels of released cyto-
kines compared with unstimulated cells. Figure 1 demonstrates the time course for cytokine release after incubation with 1 mg/ml HA. During the culture period, the concentrations of IL-1β and TNF-α in supernatant gradually increased spontaneously as well as when stimulated with HA, reaching plateau values after 12 h. Cytokine release remained elevated at 48 h. In addition, IL-1β and TNF-α were measured in supernatant derived from fibroblasts cultured in the presence of different concentrations of HA. A dose-dependent increase in cytokine release was observed over an HA concentration range of 10 µg/ml to 1 mg/ml (Fig. 2). TNF-α release reached plateau values at a concentration of 0.1–1.0 mg/ml HA. Release of TNF-α was enhanced when activated with HA. However, HA-induced IL-1β release was only minimally increased compared with the release of TNF-α induced by HA.

In a parallel experiment, we investigated the molecular basis for the effect of HA (800 and 2,000 kDa). The 2,000-kDa HA also demonstrated the stimulatory effect on cytokine generation with almost similar results (data not shown).

Involvement of CD44 in HA-induced cytokine release. We tested whether the anti-CD44 antibodies block the binding of HA to the cell surface CD44 (Fig. 3). A direct binding assay of HA and a competitive binding study between HA and the anti-CD44 antibodies to uterine

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**Fig. 1.** Enhanced release of interleukin-1β (IL-1β; A) and tumor necrosis factor-α (TNF-α; B) by fibroblasts after stimulation with hyaluronic acid (HA). Cells were cultured in DMEM supplemented with 10% fetal calf serum (FCS) in the absence (○) or presence (●) of HA (1 mg/ml). After the indicated periods of time, IL-1β and TNF-α concentrations were measured from culture supernatants by enzyme-linked immunosorbent assays (ELISA). Values are means ± SD from 3 experiments.

**Fig. 2.** Effect of HA concentrations on cytokine release by fibroblasts. After a 24-h culture period of fibroblasts in DMEM containing 10% FCS with different concentrations of HA (0–1,000 µg/ml), IL-1β (A) and TNF-α (B) concentrations in supernatant were determined by ELISA. *Statistically significant (P < 0.05) levels of stimulation. Values are means ± SD from 3 experiments.
Fibroblasts were demonstrated. In the first experiment, direct binding assays using flow cytometry were performed. Binding of HA to the fibroblasts was determined by FITC-conjugated HABP as a specific probe. Binding of HA to the fibroblasts was dose dependent and saturable. In the next experiment, competitive binding assays were performed in which increasing amounts of anti-CD44 antibodies were preincubated with the cells. The binding of HA (10 µg/ml) was competed by the anti-CD44 antibodies but not by nonimmune immunoglobulin G (IgG; 50 µg/ml). 50% inhibition of HA binding to the cells was obtained with 4 µg/ml anti-CD44 antibodies. This firmly confirms the data from Fig. 4.

To study the role of CD44 in the HA-induced cytokine release, fibroblasts were stimulated for 12 h with HA alone or in combination with anti-CD44 MAb in the presence of complete medium containing 10% FCS (Fig. 4). To determine whether HA induces cytokine production by activating the cell surface receptor CD44, fibroblasts were exposed to HA in the presence of 10 µg/ml anti-CD44 MAb. The HA-induced TNF-α release was abrogated by anti-CD44 MAb at concentrations ranging from 10 to 50 µg/ml. The inhibiting effect of...
anti-CD44 MAb was specifically directed against stimulation by HA. HA-induced TNF-α release was not affected by nonimmune IgG. Thus HA-stimulated release of TNF-α appears to be mediated by CD44. The HA-induced IL-1β release was also inhibited by anti-CD44 MAb (data not shown).

Effect of serum or ITI and its derivatives on HA-induced cytokine release. We investigated whether FCS affected HA-induced cytokine release (Fig. 5). In the absence of serum, the release of cytokine during a 24-h culture period was strongly diminished compared with the release in the presence of 10% FCS.

We studied the effect of ITI and of exogenous HA on the cytokine generation from cultured fibroblasts (Fig. 5). To investigate the nature of the action by HA and ITI, the TNF-α generation from fibroblasts preincubated with HA and ITI for 24 h in the absence of serum was tested. Addition of ITI, but not UTI, under serum-free conditions enhanced the release of cytokines using optimal concentrations of HA. However, enhancement by ITI was not observed in the presence of serum.

Furthermore, fibroblasts were 1) preincubated with HA (1 mg/ml) and ITI (100 µg/ml) for 12 h and thereafter washed and incubated for 12 h, 2) preincubated with HA (1 mg/ml) for 12 h, washed, and thereafter substituted with ITI (100 µg/ml) for 12 h, and 3) preincubated with ITI (100 µg/ml), washed, and thereafter substituted with HA (1 mg/ml); the TNF-α concentrations were then measured. The stimulatory effect of HA and ITI disappeared after these agents had been washed away from the cultured fibroblasts (data not shown). The stimulatory action of HA and ITI probably worked only when the agents were added simultaneously. This suggests that cooperative binding of ITI to HA is, at least in part, required to stabilize the extracellular matrix, which is followed by cytokine generation.

Enhanced release of IL-8 by fibroblasts after stimulation with HA. Fibroblast monolayers also showed a significant stimulation of IL-8 release when the cells were exposed to HA (1 mg/ml) (Fig. 6). IL-8 release peaked at 24 h and remained elevated at 48 h. A dose-dependent increase in IL-8 release was observed over an HA concentration range of 100 µg/ml to 1 mg/ml (data not shown).

Release of IL-1β and TNF-α preceded IL-8 protein production, suggesting the possibility that these cytokines may be involved in regulating the pathway leading to IL-8 production. To determine if IL-1β and TNF-α play a role in regulating IL-8 production, fibroblasts were stimulated with HA (1 mg/ml) in the presence of anti-IL-1β or anti-TNF-α antibody. HA stimulation of IL-8 production, at least in part, is inhibited in the presence of anti-IL-1β or anti-TNF-α antibody. Thus endogenous IL-1β or TNF-α production may be critical for IL-8 production.

Cytokine generation from fibroblasts after incubation with glycosaminoglycans. The effects of glycosaminoglycans and their components on cytokine generation were tested by incubation in cultured fibroblasts (Fig. 7). Fibroblast monolayers were incubated with soluble, structurally related glycosaminoglycans to provide some assessment of specificity for HA. The stimulatory effect was specific for HA and chondroitin sulfate to some extent. Other glycosaminoglycans, such as heparan sulfate and dermatan sulfate, had no significant effects.

Also, there was no significant increase in cytokine release in response to N-acetyl-D-mannosamine, N-acetyl-D-galactosamine, and D-glucosamine, and N-acetyl-D-glucosamine at the high concentration of 1 mg/ml (data not shown).

HA regulation of fibroblast HAase activity. We measured the HAase activity of unstimulated and HA-stimulated fibroblast culture supernatants (Fig. 8). Unstimulated fibroblasts manifest modest levels of HAase activity. HA caused a significant increase in HAase activity of unstimulated and HA-stimulated fibroblast culture supernatants (Fig. 8). Unstimulated fibroblasts manifest modest levels of HAase activity. HA caused a significant increase in HAase activity. The levels of HAase activity in cells stimulated with HA were significantly greater than the

![Fig. 5. Effect of serum or inter-alpha-trypsin inhibitor (ITI) and its derivative on HA-induced TNF-α release. Serum enhanced the HA (1 mg/ml)-induced TNF-α release. Fibroblasts were incubated in DMEM for 24 h under serum-free conditions. Cells were then cultured in DMEM with different concentrations of FCS (0–10%) at an HA concentration of 1 mg/ml. In addition, cells were incubated in DMEM at an HA concentration of 1 mg/ml with different concentrations of ITI (10 or 100 µg/ml) or urinary trypsin inhibitor (UTI; 10 or 100 µg/ml) under serum-free conditions. Supernatant was collected and TNF-α release was measured by ELISA. At the concentrations employed (10% FCS, 100 µg/ml ITI, and 100 µg/ml UTI), these compounds did not interfere during the assay. * Statistically significant (P < 0.05) levels of stimulation. Values are means ± SD from 3 experiments.](http://ajpcell.physiology.org/issue)
levels seen in cells incubated with HA in the presence of anti-IL-1β antibody. Treatment of fibroblast monolayers with IL-1β caused a significant increase in HAase activity. These studies suggested the possibility that HA-stimulated IL-1β release may be involved in regulating the pathway leading to HAase production.

Cytokine release from fibroblasts incubated with HA fragments of different molecular masses. We investigated the molecular basis for the effect of HA (Fig. 9). HA fragments ranging from 800 to 2,000 kDa all stimulated TNF-α generation. HA fragments at lower molecular masses (10–100 kDa) stimulated the cytokine generation significantly less. HA fragments of <5 kDa were without effect, as were the glycosaminoglycans, N-acetyl-d-glucosamine, glucuronic acid (these two are the components from which HA is built up), N-acetyl-d-mannosamine, N-acetyl-d-galactosamine, and d-glucosamine. These results demonstrated that the stimulatory effect of HA on cytokine generation was restricted to HA of high molecular mass (≥800 kDa).

DISCUSSION

HA is a potent, cell-derived bioactive macromolecule thought to be involved in many cellular functions, such as cell migration, invasion, proliferation, transformation, and mitosis (19, 20, 30, 32). Dilatation of the uterine cervix at parturition is associated with an increase in cervical HA content. It has been reported that circulating HA levels increase significantly at parturition in the guinea pig (26). An accumulation of HA in the extracellular matrix may result in the observed softening and swelling of the uterine cervix.
because of its unique viscoelastic properties and its high avidity for water (26). More recently, it has been reported that HA acts on intracellular signal transduction via protein kinase C by the binding of HA to CD44 or its entry into the cytoplasm (internalization), suggesting that HA also exerts chemical effects, activating fibroblast metabolism at the cell level (2).

In the present study, we showed that the action of HA on the production of cytokines, including IL-1β, TNF-α, and IL-8, by uterine fibroblasts is mediated by CD44 and that HA and IL-1β stimulate the release of HAase by the fibroblasts. In addition, ITI enhanced the HA-induced TNF-α synthesis under serum-free conditions. These findings seem to be useful for understanding the roles of CD44, HA, and ITI in uterine cervical ripening.

Cytokine production is regulated in an HA-specific fashion. However, HA fragments of molecular weight of <5,000 were without effect. Furthermore, the HA-enhanced generation of cytokines leads to postulated temporary release of HAase. These data are in accord with prior observations that HA is synthesized early during the course of an inflammatory reaction and is later degraded by fibroblasts during the healing phase of inflammation (15).

HA accumulation is regulated by the balance of biosynthesis and degradation by HAase. This balance is altered in physiological (normal delivery) and pathological (premature delivery) states. Increased amounts of HA have been noted in uterine cervical tissues at parturition (9, 26). Studies from several laboratories have shown that a variety of inflammatory cytokines, including interferon-γ, TNF-α, IL-1, and transforming growth factor-β, regulate fibroblast HA production. These cytokines interact to alter the size as well as the amounts of HA produced by fibroblasts. These alterations may be mediated by alterations in fibroblast HA degradation. An interesting aspect of this study is the demonstration that HA itself stimulates fibroblast-derived HAase production. Cytokines may regulate fibroblast HA degradation by altering fibroblast HA binding, and CD44 may also play a role in this process. It is likely that exogenously applied HA is depolymerized into lower-molecular-weight forms by fibroblast-derived HAase. The effects of the molecular weight of HA on binding to CD44 in fibroblasts have not been investigated, but depolymerization of HA may affect binding and signal transduction.

Under serum-free conditions, HA-induced cytokine generation was significantly less compared with production in the presence of serum, suggesting involvement of serum proteins. The serum factor has been recently identified as a protein belonging to the ITI family (5–8, 16, 25, 31). It has been reported that the presence of ITI in the culture medium is necessary for organizing HA in the extracellular matrix. The ITI family responsible for this effect has been identified by Chen et al. (8). We studied the effect of ITI and its derivative, UTI, on the HA-induced cytokine generation from cultured fibroblasts. Addition of ITI enhanced the HA-induced cytokine synthesis under serum-free conditions but had no effect in the presence of serum. On the other hand, UTI, known to possess protease-inhibiting activity within the ITI molecule, did not enhance HA-induced cytokine synthesis. Some structural components, such as ITI or heavy chains of ITI, that bind to HA with specificity for the HA oligomers are required to organize and stabilize HA within the matrix. The stabilization of the pericellular matrix with HA-ITI complexes may be required for cytokine generation from the cultured fibroblasts.

The cell-associated HA matrix dissociates in the absence of serum (7). Initially, the process is rapid. After the disaggregation process (after most of the HA has already been released into the culture medium), cytokine generation slowly declined and was completely abolished by 24 h of total culture (7). Once the fibroblast matrix is formed, then cooperation of several structural elements may be responsible for the stability of the pericellular matrix and the generation of cytokines. When ITI and HABP (the link protein family) cooperatively bind to HA on the surface of fibroblasts, the ternary aggregates (ITI-HA-link protein) may not be dissociated in the presence of exogenously applied HA. Cell-matrix interactions may be important to prime the cells so that they can respond to HA.

Uterine cervical ripening at parturition is a particularly interesting state with regard to HA metabolism (9). The uterine cervix is infiltrated with inflammatory cells that produce exaggerated amounts of IL-1β, TNF-α, and IL-8. These cytokines are, at least partly, respon-
sible for the abnormal HA accumulation noted during this condition. Our finding that HA can stimulate the secretion of cytokines has important implications for our understanding of the regulation of cervical ripening. When pregnancy is close to term, the HA component increases, leading to greater water retention and the soft, swollen appearance of the term cervix. The dilatation and effacement (ripening) at the cervix are necessary prerequisites for a normal labor (9). The HA-enhanced generation of TNF-α that leads to postulated temporary activation of IL-8 may share in a physiological mechanism for ripening of the uterine cervix. If cervical ripening is induced by a neutrophil infiltrate, one of the mediators is IL-8. An action of HA with a relatively small constitutive production of IL-8 within the cervix is a sufficient explanation of the ripening effect of endogenously produced HA. A corollary of such a mechanism is that HA or IL-8 as vaginal suppositories could also be used to ripen the cervix, with the advantage that such an approach would be less likely to result in hyperstimulation of the myometrium. IL-1β and TNF-α synthesized by fibroblasts after stimulation by endogenously produced HA could act directly as a positive feedback of further generation of HA from neighboring connective tissue fibroblasts. The production of cytokines in HA-stimulated fibroblasts implies that the autocrine stimulatory loops may play a role in ripening of the uterine cervix. Because HA is a physiological potent stimulator of cytokines from uterine fibroblasts and may contribute to cervical ripening, followed by dilatation of the uterine cervix, HA could be used to control delivery.

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