Regulation of Collagen Deposition and Lysyl Oxidase by Tumor Necrosis Factor-α in Osteoblasts*

Nicole Pischon‡§, Laurent M. Darbois‡, Amita H. Palamakumbura‡, Efrat Kessler¶, and Philip C. Trackman¶¶

From the Department of Oral Biology, Goldman School of Dental Medicine, Boston University, Boston, Massachusetts 02118 and Maurice and Gabriela Goldschleger Eye Research Institute, Tel Aviv University Sackler Faculty of Medicine, Sheba Medical Center, Tel-Hashomer 52621, Israel

Received for publication, April 15, 2004, and in revised form, May 6, 2004 Published, JBC Papers in Press, May 10, 2004, DOI 10.1074/jbc.M404208200

Tumor necrosis factor-α (TNF-α) inhibits osteoblast function in vitro by inhibiting collagen deposition. Studies generally support that TNF-α does not inhibit collagen biosynthesis by osteoblasts but that collagen deposition is in some way diminished. The study investigated TNF-α regulation of biosynthetic enzymes and proteins crucial for posttranslational extracellular collagen maturation in osteoblasts including procollagen C-proteinases, procollagen C-proteinase enhancer, and lysyl oxidase. The working hypothesis is that such regulation could inhibit collagen deposition by osteoblasts. We report that in phenotypically normal MC3T3-E1 osteoblasts, TNF-α decreases collagen deposition without decreasing collagen mRNA levels or procollagen protein synthesis. Analyses of the cell layers revealed that TNF-α diminished the levels of mature collagen cross-links, pyridinoline and deoxypyridinoline. Further analyses revealed that the mRNA expression for lysyl oxidase, the determining enzyme required for collagen cross-linking, is down-regulated by TNF-α in a concentration- and time-dependent manner by up to 50%. The decrease was accompanied by a significant reduction of lysyl oxidase protein levels and enzyme activity. By contrast, Northern and Western blotting studies revealed that procollagen C-proteinases bone morphogenetic protein-1 and mammalians Tolloid and procollagen C-proteinase enhancer were expressed in MC3T3-E1 cells and not down-regulated. The data together demonstrate that TNF-α does not inhibit collagen synthesis but does inhibit the expression and activity of lysyl oxidase in osteoblasts, thereby contributing to perturbed collagen cross-linking and accumulation. These studies identify a novel mechanism in which proinflammatory cytokine modulation of an extracellular biosynthetic enzyme plays a determining role in the control of collagen accumulation by osteoblasts.

TNF-α is an inflammatory cytokine produced primarily by monocytes and macrophages and also by a variety of mesenchymal cells. TNF-α levels are elevated in various bone disorders such as rheumatoid arthritis, osteoporosis, and periodontitis (1–4). In bone tissue, TNF-α inhibits osteoblast function and increases osteoclastogenesis, thus favoring net matrix destruction (5, 6) and the collagenous matrix structure is disrupted by TNF-α (7–9). Reports indicate that TNF-α somehow inhibits collagen deposition while having little effect on collagen synthesis, although the mechanisms that contribute to this phenomenon have not been elucidated (6, 7).

Type I collagen is the major structural protein in the extracellular matrix of bone tissue. Normal collagen structure and the balance between production, deposition, and degradation of collagen are important in the development and maintenance of skeletal tissue (1). Collagen biosynthesis is a multistep process that involves intracellular posttranslational modifications, assembly of procollagen chains, secretion, extracellular processing, and cross-linking to form a mature functional matrix (10). The mechanisms that control collagen deposition in osteoblasts are not well understood but seem likely to include the regulation of extracellular collagen-biosynthetic enzymes and proteins (11). Moreover, TNF-α regulation of enzymes and proteins crucial for posttranslational extracellular collagen maturation such as procollagen C-proteinases and the cross-linking enzyme lysyl oxidase have not been studied directly to our knowledge.

Tropocollagen molecules assemble into collagen fibrils and are subsequently stabilized as a result of oxidative deamination of ε-amino groups of peptidyllysines and hydroxylysines residues by lysyl oxidase, forming the aldehyde moieties. The resulting peptidyl-ε-aminoacyclic-δ-semialdehyde and peptidyl-δ-hydroxy-ε-aminoacyclic-δ-semialdehyde residues are highly reactive and form covalent cross-links that are critically required for the formation of functional mature and insoluble collagen (12, 13). Deficient levels of lysyl oxidase-derived cross-links are associated with osteoporosis and weak bones (14–16). Lysyl oxidase biosynthesis requires extracellular proteolytic processing of the 50-kDa pro-lysyl oxidase to form the mature 32-kDa enzyme and an 18-kDa propeptide (17–19). Proenzyme processing is catalyzed by bone morphogenetic protein-1 (BMP-1) and related procollagen C-proteinase mammalians Tolloid (mTLD), TLL-1 and TLL-2 (18, 19). The cleavage of gloabal carboxyl-terminal procollagen I-III propeptides by procollagen C-proteinases results in a 1,000-fold decreased collagen solubility (10). In the collagenous matrix procollagen, C-proteinases are only present in limited quantities. However, procolla...
Regulation of Collagen Deposition and Lysyl Oxidase by TNF-α

Regulation of Collagen Deposition and Lysyl Oxidase by TNF-α

The decrease in lysyl oxidase mRNA levels was accompanied by up to 50%, and this was time- and concentration-dependent. TNF-α inhibited lysyl oxidase steady-state mRNA expression by up to 50%, and this was time- and concentration-dependent. Although collagen synthesis was not inhibited, collagen accumulated in the extracellular matrix in the presence of TNF-α and the deposited collagen contained diminished levels of mature cross-links, pyridinolines (Pyd) and deoxypyridinolines (Dpd). By contrast, BMP-1, mTLD, and their biological enhancer, PCPE, were not regulated by this cytokine.

These findings identify a novel mechanism in which down-regulation of an extracellular collagen biosynthetic enzyme by TNF-α contributes to the deposition of abnormal collagen more likely to be degraded. This mechanism could contribute to net bone resorption that occurs in vivo as a consequence of inflammation.

EXPERIMENTAL PROCEDURES

Cell Line Hydroxyproline and Collagen Cross-links—200,000 MC3T3-E1 cells were plated on 100-mm tissue cultures plates and grown until confluency. Cells then were grown in differentiation medium (α-MEM) supplemented with 10% fetal bovine serum, 50 μg/ml ascorbate, and 10 mM β-glycerophosphate with or without 10 ng/ml or 100 ng/ml TNF-α for 12 days. Cell layers were harvested after 24 h, washed in phosphate-buffered saline, and fragmented to 100 μl of vacuum-dried and then used to determine hydroxyproline levels by colorimetric assays (25) and for mature type I collagen cross-links Pyd and Dpd using the Metra PYD enzyme immunoassay kit (Quidel, San Diego, CA). The cross-link assay is a competitive enzyme-linked immunoassay in which Pyd and Dpd in hydrolyzed cell extracts compete with Pyd coated on a microtiter plate for soluble monoclonal anti-pyridinyl cross-link antibody conjugated to alkaline phosphatase. Following a washing step, the substrate p-nitrophenyl phosphate was added for 60 min. The reaction then was stopped by adding 1 N NaOH to each well, and the optical density was measured at 405 nm. The Pyd plus Dpd concentration in each sample was determined from a standard curve run on the same microtiter plate.

Collagen Synthesis Assay—Subconfluent cells were treated with or without TNF-α in serum-free α-MEM cell culture medium for 24 h. After removing the medium plus or minus TNF-α, cell layers were harvested after 24 h, washed in phosphate-buffered saline, and scraped in 500 μl of sample buffer (0.1 mM Tris-HCl, 4% SDS, 10% glycerol, 5% β-mercaptoethanol). Media samples were collected (20 μl) and concentrated to 1 ml (10 kDa, Centricon, Amicon, Bedford, MA) and suspended in 500 μl of sample buffer. Protein concentration in the samples was measured using a fluorometric protein assay (NanoOrange, Molecular Probes, Eugene, OR). 25 μg of protein of the cells and 25 μg of protein of the cell lysates were subjected to 10% SDS-PAGE and transferred to polyvinylidene difluoride transfer membranes (PerkinElmer Life Sciences) by electroblotting in 25 mM Tris, 192 mM glycine, and 10% methanol. Blots were blocked in BlockHalt II (Aves Labs, Portland, OR) for 1 h and incubated with antibodies to alkaline phosphatase-conjugated secondary antibody. Western Blue substrate (Promega, Madison, WI) was applied for detection. A BMP-1-specific antibody was raised in rabbit against a synthetic peptide (GRPRLQKLKFRVQKRNRTPQC) corresponding to the carboxy-terminal sequence of human BMP-1. The cytochrome residue was added to allow coupling to ovalbumin using the Immject maleimide-activated immunogen kit ( Pierce). An IgG fraction was prepared from the immune serum by ammonium sulfate precipitation and used for detection of BMP-1. PCPE antibody was described previously (34). Lysyl oxidase antibody was raised in rabbit against a peptide corresponding to the unique carboxy-amino terminal 20 amino acids of lysyl oxidase (32). For normalization, blots were stripped and rehybridized with a radiolabeled 18 S rRNA probe (33). Blots were washed in 2× SSC, 0.05% SDS for 30–40 min at room temperature and then twice in 0.1× SSC, 0.1% SDS for 40 min at 50 °C. Washed membranes then were exposed to Kodak X-Omat AR film with intensifying screens at −80 °C. Autoradiograms were quantitated by the scanning densitometry of autoradiograms exposed for varying lengths of time.

Western Blot Analysis—Subconfluent cells were treated with or without TNF-α in serum-free α-MEM cell culture medium for 24 h. After removing the medium plus or minus TNF-α, cell layers were harvested after 24 h, washed in phosphate-buffered saline, and scraped in 500 μl of sample buffer (0.1 mM Tris-HCl, 4% SDS, 10% glycerol, 5% β-mercaptoethanol). Media samples were collected (20 μl) and concentrated to 1 ml (10 kDa, Centricon, Amicon, Bedford, MA) and suspended in 500 μl of sample buffer. Protein concentration in the samples was measured using a fluorometric protein assay (NanoOrange, Molecular Probes, Eugene, OR). 25 μg of protein of the cells and 25 μg of protein of the cell lysates were subjected to 10% SDS-PAGE and transferred to polyvinylidene difluoride transfer membranes (PerkinElmer Life Sciences) by electroblotting in 25 mM Tris, 192 mM glycine, and 10% methanol. Blots were blocked in BlockHalt II (Aves Labs, Portland, OR) for 1 h and incubated with antibodies to alkaline phosphatase-conjugated secondary antibody. Western Blue substrate (Promega, Madison, WI) was applied for detection. A BMP-1-specific antibody was raised in rabbit against a synthetic peptide (GRPRLQKLKFRVQKRNRTPQC) corresponding to the carboxy-terminal sequence of human BMP-1. The cytochrome residue was added to allow coupling to ovalbumin using the Immject maleimide-activated immunogen kit ( Pierce). An IgG fraction was prepared from the immune serum by ammonium sulfate precipitation and used for detection of BMP-1. PCPE antibody was described previously (34). Lysyl oxidase antibody was raised in rabbit against a peptide corresponding to the unique carboxy-amino terminal 20 amino acids of lysyl oxidase (32). For normalization, blots were stripped and rehybridized with a radiolabeled 18 S rRNA probe (33). Blots were washed in 2× SSC, 0.05% SDS for 30–40 min at room temperature and then twice in 0.1× SSC, 0.1% SDS for 40 min at 50 °C. Washed membranes then were exposed to Kodak X-Omat AR film with intensifying screens at −80 °C. Autoradiograms were quantitated by the scanning densitometry of autoradiograms exposed for varying lengths of time.

Western Blot Analysis—Subconfluent cells were treated with or without TNF-α in serum-free α-MEM cell culture medium for 24 h. After removing the medium plus or minus TNF-α, cell layers were harvested after 24 h, washed in phosphate-buffered saline, and scraped in 500 μl of sample buffer (0.1 mM Tris-HCl, 4% SDS, 10% glycerol, 5% β-mercaptoethanol). Media samples were collected (20 μl) and concentrated to 1 ml (10 kDa, Centricon, Amicon, Bedford, MA) and suspended in 500 μl of sample buffer. Protein concentration in the samples was measured using a fluorometric protein assay (NanoOrange, Molecular Probes, Eugene, OR). 25 μg of protein of the cells and 25 μg of protein of the cell lysates were subjected to 10% SDS-PAGE and transferred to polyvinylidene difluoride transfer membranes (PerkinElmer Life Sciences) by electroblotting in 25 mM Tris, 192 mM glycine, and 10% methanol. Blots were blocked in BlockHalt II (Aves Labs, Portland, OR) for 1 h and incubated with antibodies to alkaline phosphatase-conjugated secondary antibody. Western Blue substrate (Promega, Madison, WI) was applied for detection. A BMP-1-specific antibody was raised in rabbit against a synthetic peptide (GRPRLQKLKFRVQKRNRTPQC) corresponding to the carboxy-terminal sequence of human BMP-1. The cytochrome residue was added to allow coupling to ovalbumin using the Immject maleimide-activated immunogen kit ( Pierce). An IgG fraction was prepared from the immune serum by ammonium sulfate precipitation and used for detection of BMP-1. PCPE antibody was described previously (34). Lysyl oxidase antibody was raised in rabbit against a peptide corresponding to the unique carboxy-amino terminal 20 amino acids of lysyl oxidase (32). For normalization, blots were stripped and rehybridized with a radiolabeled 18 S rRNA probe (33). Blots were washed in 2× SSC, 0.05% SDS for 30–40 min at room temperature and then twice in 0.1× SSC, 0.1% SDS for 40 min at 50 °C. Washed membranes then were exposed to Kodak X-Omat AR film with intensifying screens at −80 °C. Autoradiograms were quantitated by the scanning densitometry of autoradiograms exposed for varying lengths of time.
Regulation of Collagen Deposition and Lysyl Oxidase by TNF-α

RESULTS

TNF-α Inhibits Collagen Accumulation—Osteoblasts deposit abundant amounts of collagen as they differentiate in culture to ultimately form a bone-like extracellular matrix. Previous data reveal that maximum collagen accumulation in the cell layer of MC3T3-E1 cells occurs between days 12 and 15 (11). To understand the regulation of extracellular collagen biosynthetic enzymes by TNF-α, we first investigated how TNF-α controls collagen deposition in MC3T3-E1 cells under these culture conditions. Therefore, MC3T3-E1 cells were cultured in ascorbate- and β-glycerophosphate-supplemented medium for 12 days in the presence and absence of 10 and 100 ng/ml TNF-α. Hydroxyproline levels as an index of collagen deposition then were measured in hydrolyzed total cell layers. The results in Fig. 1A show that in the presence of TNF-α cell layer collagen accumulation was inhibited and was dose-dependent and significant (p < 0.01). Cell layer hydroxyproline levels in cultures treated with 100 ng/ml TNF-α were reduced to 34% of untreated control cultures (Fig. 1A).

TNF-α Does Not Alter Collagen Synthesis—Since hydroxyproline analysis indicated that TNF-α inhibits collagen accumulation, we further studied the effects of TNF-α on new collagen synthesis in MC3T3-E1 cells. MC3T3-E1 cells were treated for 24 h with or without TNF-α and then pulsed with [2,3-3H]proline for 2 h. A previous study (38) indicates that labeling periods of 1–2 h are optimal for measuring the synthesis of newly formed procollagen. Thus, collagen synthesis was determined in the cell layer by measuring the amount of tritiated proline incorporated into collagenase-sensitive protein. As shown in Fig. 1B, the exposure of MC3T3-E1 cells to TNF-α did not affect collagen synthesis significantly, although a trend toward decreased collagen synthesis at 100 ng/ml TNF-α was observed that was not significant statistically (p = 0.515). Normalization to DNA levels/plate did not change these findings, because DNA levels were the same in all of the treatment groups: 564.6 ± 71.22 μg/plate (mean ± S.D.).

TNF-α Decreases Total Mature Cross-link Formation—The data indicate that TNF-α down-regulates collagen accumulation but hardly affects collagen synthesis and steady-state collagen type I mRNA levels. This finding strongly suggests that TNF-α primarily controls extracellular collagen modifications rather than procollagen biosynthesis. Therefore, we wished to further examine the collagenous matrix deposited under the influence of TNF-α to investigate whether cross-linking might be inhibited. MC3T3-E1 cells were cultured in ascorbate- and β-glycerophosphate-supplemented cell culture medium for 12 days in the presence and absence of 10 and 100 ng/ml TNF-α. The amount of mature cross-links Pyd and Dpd then was assessed in the total cell layer by enzyme immunoassay as described under “Experimental Procedures.” TNF-α inhibited collagen cross-links in doses of 25% found in control cultures. This is a greater reduction compared with that found for TNF-α-dependent reduction of total collagen deposition as determined above in Fig. 1A. This finding points to the possibility that lysyl oxidase-dependent cross-linking could be a primary target of TNF-α.

TNF-α Reduces Lysyl Oxidase Steady-state mRNA Levels, Protein Expression, and Enzyme Activity in MC3T3-E1 Cells—As TNF-α decreases mature collagen cross-links, we wished to assess whether TNF-α regulates lysyl oxidase. MC3T3-E1 cells were treated in serum-free α-MEM supplemented with 0.1% BSA in the absence or presence of TNF-α for varying periods of time. Total RNA was isolated and subjected to Northern blot analyses. Results presented in Fig. 2, A and B, revealed that treatment of osteoblasts with TNF-α for 24 h inhibits steady-state lysyl oxidase mRNA levels and is dose-dependent. Diminished lysyl oxidase mRNA levels were observed at 10 and 100 ng/ml TNF-α compared with the untreated control (Fig. 2B). In multiple experiments, down-regulation of lysyl oxidase mRNA levels by TNF-α ranged between 20 and 50% compared with untreated control cultures. Time-dependent regulation of steady-state lysyl oxidase mRNA levels was tested as well. The data indicate that 10 ng/ml TNF-α down-regulated lysyl oxidase mRNA levels slowly and was first ob-
Regulation of Collagen Deposition and Lysyl Oxidase by TNF-α

The present studies demonstrate that, in the presence of TNF-α, less collagen accumulates in the extracellular matrix of osteoblast cultures and that the deposited collagen contains diminished levels of mature collagen cross-links, Pyd and Pdp. These data support the proposition that these effects are caused in part by a significant down-regulation of lysyl oxidase expression and activity by TNF-α.

MC3T3-E1 cells derived from newborn mouse calvaria are capable of fully differentiating into osteoblast-like cells during culture, forming an extensive collagenous extracellular matrix that mineralizes in later stages through a process similar to that in bone formation in vivo (39). This study shows that TNF-α decreases collagenous protein accumulation in the extracellular matrix and that type I collagen mRNA expression and collagen synthesis are not affected significantly in agreement with other studies (6, 40). Most important, these findings...
suggest that TNF-α regulates extracellular rather than intracellular events of collagen biosynthesis. In fact, we show that TNF-α affects the posttranslational extracellular cross-linking of collagen by down-regulating lysyl oxidase. Insufficiencies in the post-translational modification of type I collagen in bone in vivo affect the mineralization density and crystal structure (41), and TNF-α negatively affects bone formation (1, 4).

The data now presented show that TNF-α reduced lysyl oxidase steady-state mRNA expression, and this regulation is concentration-dependent as 10–100 ng/ml TNF-α down-regulated lysyl oxidase mRNA levels by up to 50%. The TNF-α concentrations used are in a physiologically important range. For example, in vivo, the TNF-α concentrations of 10 ng/ml TNF-α were found in chronic inflammatory lesions (9). Decreased lysyl oxidase gene expression was detected after 16 h of treatment, and decreased lysyl oxidase steady-state mRNA levels were accompanied by a reduction in the amount of a 32-kDa lysyl oxidase protein. Regulation of lysyl oxidase enzyme activity can occur in parallel with mRNA changes (42, 43), and a decrease in lysyl oxidase enzyme activity was found following TNF-α treatment.

Lysyl oxidase catalyzes the oxidative deamination of lysine and hydroxylysine residues in tropocollagen to generate reactive peptidyl aldehydes that undergo condensation reactions to ultimately form lysine-derived cross-links (12, 13). Proper functioning of lysyl oxidase is crucial for collagen cross-linking and subsequent accumulation of insoluble collagen. Disruption of the cross-linking process can result in severe structural collagen changes and dysfunction of the tissue. For example, inhibition of lysyl oxidase activity leads to osteolathrysm when bones are thickened, extremely fragile, and soft leaving them with an increased risk for deformities and fractures (44, 45). Inhibition of lysyl oxidase enzyme activity negatively affects bone and cartilage formation and function in vivo and in vitro (44, 46, 47). As we have published earlier (11), experimental inhibition of lysyl oxidase enzyme activity with the specific inhibitor β-aminoproprionitrile fumarate increased the accumulation of abnormal collagen fibrils in MC3T3-E1 cells. β-Aminoproprionitrile fumarate treatment abnormally increased fiber diameters, and the deposited collagen was characterized by enhanced solubility.

Pyridinium cross-links, Pyd and Dpd, are the primary cross-links of mature type I collagen in bone (48, 49). Their formation depends directly on lysyl oxidase activity. As noted, we found that significantly less Pyd and Dpd collagen cross-links were formed in the matrix of TNF-α-treated MC3T3-E1 cell cultures. A decrease of mature collagen cross-links affects the biomechanical integrity, rigidity, and strength of bone (41), and TNF-α disrupts the extracellular matrix structure of osteoblasts (7). Electron microscopic studies of limb bud cultures reveal that sustained TNF-α treatment for 12 days leads to loosely packed collagen fibrils (8), and less cross-striated collagen fibrils were observed. In chronic inflammatory lesions where significant levels of TNF-α are found, accumulated collagen is disorganized (9). This study demonstrates for the first time that diminished lysyl oxidase and diminished collagen cross-linking may be important contributors to the effects of TNF-α on osteoblast extracellular matrix alterations.

Our data suggest that down-regulation of lysyl oxidase by TNF-α contributes to diminished deposition of mature collagen. It is known that TNF-α induces the synthesis of gelatinases in MC3T3-E1 cells, which preferentially cleave denatured collagen chains (50–54). In addition, it has been shown that MMP-13 is expressed by MC3T3-E1 cells and is stimulated by TNF-α (53, 55). MMP-13 is the major interstitial collagenase produced by osteoblasts (56). The lack of proper collagen maturation because of inhibited lysyl oxidase expression most probably results in a higher capability of collagensases and gelatinases to degrade collagen molecules and promote matrix degradation in the course of inflammation (12). Indeed, we suspect that diminished collagen accumulation in response to TNF-α reported here depends partly on stimulation of proteolytic activity. This notion is based on our findings that inhibition of lysyl oxidase in MC3T3-E1 cultures with β-aminoproprionitrile fumarate that does not affect collagenase or gelatinase activity resulted in increased total collagen deposition, but the collagen was abnormal in structure (11). The ability of TNF-α to simultaneously inhibit lysyl oxidase biosynthesis and stimulate proteolytic activity seems likely to account for the decreased total amount of collagen deposited reported here.

Currently, little is known regarding procollagen C-proteinases and PCPE regulation, in general, and in osteoblast-like cells, in particular. As we have recently shown, BMP-1 and mTLD are expressed constitutively during the differentiation of phenotypically normal murine osteoblasts (11). It is unknown whether the same factors that regulate collagen biosynthesis also control the expression of procollagen C-proteinases and PCPE. As noted, we wished to investigate whether TNF-α, which regulates collagen accumulation, also controls the expression of procollagen C-proteinases and PCPE. It has been shown previously that transforming growth factor-β increases the levels of BMP-1 and mTLD in fibrogenic cells and keratinocytes and that PCPE remained unchanged (57, 58). In a study by Ogata et al. (34) conducted in lipocyte-like liver stellate cells, TNF-α down-regulated PCPE mRNA after 24 h. The data now presented show that in phenotypically normal MC3T3-E1 cells, TNF-α did not alter steady-state mRNA levels of BMP-1, mTLD, or PCPE. The cognate protein expression was not affected by TNF-α in these cells. Correspondingly, the processing of procollagen type I C-propeptides remained unaltered in our cell cultures (data not shown). As procollagen C-proteinases, especially BMP-1, are involved in the processing of numerous extracellular matrix components, stable BMP-1 and mTLD expression seems to be essential and is not affected by TNF-α. This stable expression seems to assure the precise regulation of the deposited collagenous matrix and maintain bone tissue homeostasis.

In conclusion, we demonstrated that TNF-α regulates lysyl oxidase expression and activity in MC3T3-E1 cells. Lysyl oxidase is the key enzyme required for collagen cross-linking in the extracellular matrix and is essential for the accumulation of a functional collagen matrix. Less lysyl oxidase activity leads to perturbed collagen deposition that is prone to degradation by proteinases. As it is probable that these effects of TNF-α occur in vivo, this inflammatory cytokine secreted in excess by activated monocytes and macrophages could contribute to net bone resorption by these mechanisms in inflamed mineralized tissues. In fracture healing where TNF-α is secreted during the initiation of the repair process as well as in later stages of bone formation (59), structural alterations of the collagen molecule might promote tissue remodeling during different phases of the healing process. Therefore, elucidation of how TNF-α inhibits the posttranslational extracellular processing of the fibrillar collagens provides new insights into the mechanisms that could contribute to diminished osteoblast function, bone remodeling, and bone resorption.

REFERENCES
1. Pacifi, R. (1996) J. Bone Miner. Res. 11, 1043–1051
2. Beutler, B., and Cerami, A. (1988) Endocr. Rev. 9, 57–66
3. Bertolami, D. R., Nedwan, G. E., Bringman, T. S., Smith, D. D., and Mundy, G. R. (1986) Nature 319, 514–516
4. Graves, D. T. (1999) Clin. Infect. Dis. 28, 482–490
5. Canalis, E. (1987) Endocrinology 121, 1596–1604
Regulation of Collagen Deposition and Lysyl Oxidase by TNF-α

6. Centrella, M., McCarthy, T. L., and Canalis, E. (1988) Endocrinology 123, 1442–1448
7. Panagakos, F. S., Fernandez, C., and Kumar, S. (1996) Mol. Cell. Biochem. 158, 81–89
8. Mohamed-Ali, H., Scholz, P., and Merker, H. (1996) Cell Tiss. Res. 284, 509–515
9. Chou, D., Lee, W., and McCulloch, C. (1996) J. Immunol. 156, 4354–4362
10. Prockop, D. J., and Kivirikko, K. I. (1995) Ann. Rev. Biochem. 64, 403–434
11. Hong, H., Pischeda, N., Santana, R., Palamakumbura, A., Babahkandelou Chase, H., Gantz, D., Guo, Y., Uzel, M., Ma, D., and Trackman, P. (2004) J. Cell. Physiol. 209, 59–62
12. Kagan, H. (1986) in Biology and Regulation of Extracellular Matrix: A Series. Regulation of Matrix Accumulation (Mecham, R. P., ed) Vol. 1, pp. 321–398, Academic Press, Orlando, FL
13. Kagan, H. M., and Trackman, P. C. (1991) Am. J. Resp. Cell Mol. Biol. 5, 206–210
14. Bailey, A. J., Wotton, S. F., Sims, T. J., and Thompson, P. W. (1993) Connect. Tissue Res. 29, 119–132
15. Oxlund, H., Mosekilde, L., and Ortoft, G. (1996) Bone 21, 479–484
16. Oxlund, H., Barckman, M., Ortoft, G., and Andreassen, T. T. (1995) Bone 17, S365–S571
17. Trackman, P. C., Tang, J., Bedell-Hogan, D., and Kagan, H. M. (1992) J. Biol. Chem. 267, 8666–8671
18. Panchenko, M. V., Stetler-Stevenson, W. G., Trubetskoy, O. V., Gacheru, S. N., and Kagan, H. M. (1996) J. Biol. Chem. 271, 7113–7119
19. Uzel, M. I., Scott, I. C., Babahkandelou-Chase, H., Palamakumbura, A. H., Pappano, W. N., Hong, H. H., Greenspan, D. S., and Trackman, P. C. (2001) J. Biol. Chem. 276, 25237–25243
20. Hojima, Y., van der Rest, M., and Prockop, D. J. (1985) J. Biol. Chem. 260, 15996–16003
21. Kessler, E., Mould, A. P., and Hulmes, D. J. S. (1990) Biochem. Biophys. Res. Commun. 173, 81–86
22. Takahara, K., Kessler, E., Biniaminov, L., Brusel, M., Eddy, R. L., Jani-Sait, S., Shows, T. B., and Greenspan, D. S. (1984) J. Biol. Chem. 269, 26280–26285
23. Hulmes, D. J., Mould, A. P., and Kessler, E. (1997) Matrix Biol. 16, 41–45
24. Bernocco, S., Steiglitz, B., Svergun, D., Petoukhov, M., Ruggiero, F., Ricard-Blum, S., Ebel, C., Gourjon, C., Delage, G., Font, B., Kiechberger, D., Greenspan, D., and Hulmes, D. (2003) J. Biol. Chem. 278, 7199–7205
25. Edwards, C. A., and O’Brien, W. D., Jr. (1980) Clin. Chim. Acta 104, 161–167
26. Peterkowsky, B., and Diegelmann, R. (1971) Biochemistry 10, 988–994
27. DeRenzi, F. A., and Schechter, A. (1973) Strain Technol. 48, 135–136
28. Kosteniuk, P. F., Halloran, B. P., Moriy-Holton, E. R., and Bille, D. D. (1997) Am. J. Physiol. 273, E1133–E1139
29. Feinberg, A. P., and Vogelstein, B. (1983) Anal. Biochem. 132, 6–13
30. Kenyon, K., Contente, S., Trackman, P. C., Tang, J., Kagan, H. M., and Friedman, R. P. (1991) Science 253, 802
31. Kessler, E., Takahara, K., Biniaminov, L., Brusel, M., and Greenspan, D. S. (1996) Science 271, 360–362
32. Scott, I. C., Clark, T. G., Takahara, K., Hoffman, G. G., and Greenspan, D. S. (1999) Genomics 55, 229–234
33. Hillis, D., and Dixon, T. (1991) Q. Rev. Biol. 66, 411–453
34. Ogata, I., Auster, A. S., Matsui, A., Greenwell, P., Geerts, A., D’Amico, T., Fujikawa, K., Kessler, E., and Rokujin, M. (1997) Hepatology 26, 611–617
35. Trackman, P. C., Pratt, A. M., Wolanski, A., Tang, S.-S., Offner, G. D., Treuder, R. F., and Kagan, H. M. (1990) Biochemistry 29, 4863–4870
36. Palamakumbura, A., and Trackman, P. (2002) Anal. Biochem. 306, 245–251
37. Vyas, R. (1982) Anal. Biochem. 120, 243–248
38. Beldekas, A., and Kagan, H. M. (1981) Biochemistry 20, 2162–2167
39. Oxlund, H., Mosekilde, L., Ortoft, G., and Andreassen, T. T. (1995) Bone 16, 3–82
40. Siegel, R. C. (1979) Int. Rev. Connect. Tiss. Res. 8, 73–118
41. Gerstenfeld, L. C., Riva, A., Ogata, I., Geller, A., Ly, D. B., and Landis, W. J. (1993) J. Bone Miner. Res. 8, 1031–1045
42. Seyein, S., and Rosen, D. (1990) Curr. Opin. Cell Biol. 2, 914–919
43. Delmas, P. (1995) in Osteoporosis: Etiology, Diagnosis, and Management. (Riggs, B., and Melton, L., eds) 3rd Ed., pp. 319–333, Lippincott Williams & Wilkins, Philadelphia
44. Panagakos, F. S., and Kumar, S. (1995) Inflammation 19, 423–443
45. Panagakos, F. S., and Kumar, S. (1994) Inflammation 18, 263–265
46. Lorenzo, J. A., Pilibean, C. C., Kalinowski, J. F., and Hibbs, M. S. (1992) Matrix 12, 282–290
47. Minnati, A., Sugiyama, Y., Uedo, S., Matsunaga, S., and Tsukagoshi, N. (2001) J. Bone Miner. Res. 16, 2043–2049
48. Goldberg, G. I., Strongin, A., Collier, I. E., Genrich, L. T., and Marmer, B. L. (1992) J. Biol. Chem. 267, 4585–4591
49. Uchida, M., Shima, M., Shimoaka, T., Fujieda, A., Obara, K., Suzuki, H., Nagai, Y., Ikeda, T., Yamato, H., and Kawaguchi, H. (2000) J. Cell. Physiol. 185, 207–214
50. Kanzaki, N., Rydziel, S., Delany, A. M., and Canalis, E. (1997) J. Biol. Chem. 272, 12144–12150
51. Lee, S., Solow-Cordere, D. E., Kessler, E., Takahara, K., and Greenspan, D. S. (1997) J. Biol. Chem. 272, 19059–19066
52. Takahara, K., Breward, R., Hoffman, G. G., Suzuki, N., and Greenspan, D. S. (1996) Genomics 34, 157–165
53. Kon, T., Cho, T. J., Aizawa, T., Yamazaki, M., Nooh, N., Graves, D. T., Gerstenfeld, L. C., and Einhorn, T. A. (2001) J. Bone Miner. Res. 16, 1004–1014
54. Ahsan, T., Lottman, L. M., Harwood, F., Amiel, D., and Sah, R. L. (1999) J. Orthop. Res. 17, 850–857
Regulation of Collagen Deposition and Lysyl Oxidase by Tumor Necrosis Factor-α in Osteoblasts
Nicole Pischon, Laurent M. Darbois, Amitha H. Palamakumbura, Efrat Kessler and Philip C. Trackman

J. Biol. Chem. 2004, 279:30060-30065.
doi: 10.1074/jbc.M404208200 originally published online May 10, 2004

Access the most updated version of this article at doi: 10.1074/jbc.M404208200

Alerts:
• When this article is cited
• When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 59 references, 14 of which can be accessed free at http://www.jbc.org/content/279/29/30060.full.html#ref-list-1