Differential Effects of Bacterial Lipopolysaccharide (LPS) and Tumor Necrosis Factor-Alpha on the Functions of Human Osteoblast Cells.

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Introduction

Inflammation can be developed in either sterile microenvironment (e.g. rheumatoid arthritis) or after bacterial recruitment (e.g. bacterial arthritis). In both cases, bone erosion is observed as the disease progresses suggesting similar pathophysiological events that is the disruption of the balance between osteoclast and osteoblast functions. TNF-α is believed to be one of the key regulators in the development of arthritis and arthritis-related bone resorption. The role of TNF-α in Gram negative bacteria-induced inflammation has also been studied. The initiating signaling mechanism in the presence of bacteria or especially, the cell response to bacterial products, such as endotoxins/lipopolysaccharides (LPS), still unclear, whereas these products may provoke cells to produce “bone-resorbing” agents (cytokines, prostaglandins, etc.). A number of studies have shown TNF-α and/or LPS-induced vigorous effects on macrophages and osteoclasts, but only limited information is available on the effects of these mediators on osteoblast functions, especially on human osteoblasts.

Methods

Osteoblast-like MG-63 cells were purchased from American Type Culture Collection. Primary human osteoblasts were isolated from bone marrow of patients undergoing surgical procedures. Osteoblastic phenotype in bone marrow-derived cultures was characterized by alkaline phosphatase (ALP) positivity and only cultures showing more than 80% positivity for ALP were used for experiments. Cells were grown to confluency and then used for experiments. Cells were left untreated or treated with various compounds for different periods. TNF-α (20 ng/ml), LPS (types O127:B5, O55:B5, from 10 µg/ml to 2 mg/ml) were used alone or in different combinations. All of the above listed concentrations were selected after serial dilutions of each compound tested in cell culture and only the viable range and the most effective concentration of each compound was used for experiments. Cell layers were utilized for proliferation assay, viability tests, RNA isolation, protein extraction and flow cytometry. Cell viability was analyzed by trypan-blue exclusion. Cell proliferation was examined by [3H]-thymidine incorporation into the DNA. The levels of IL-1β, IL-6, soluble IL-6 receptor (sIL-6R) and monocyte chemoattractant protein-1 (MCP-1) in conditioned media were analyzed by enzyme-linked immunosorbent assay. RNase protection assay (RPA) was used to assess the expression of IL-6, IL-6 receptor (IL-6R) and glycoprotein 130 (gp130, the signal transducing component of the IL-6R complex) mRNAs. The expression of procollagen α[1] mRNA was analyzed by Northern blot hybridization. The cell surface expression of IL-6R and gp130 was determined by flow cytometry. Immunoprecipitation-Western blot analysis was used to measure the total amounts of IL-6R and gp130. Descriptive statistics were used to determine group means and standard deviations. Paired Student’s t tests were performed between groups of interest. The level of significance was set at p<0.05.

Results

Osteoblasts exhibited significantly suppressed proliferation in TNF-α-treated cultures, while LPS (up to 2 µg/ml concentration) had only marginal inhibitory effect on cellular proliferation. Both TNF-α and LPS were potent inducers of interleukin-6 (IL-6), IL-8 and MCP-1 production. In addition to IL-6, IL-8 and MCP-1, LPS also induced the release of IL-1β. As IL-6 has a central role in bone metabolism, we also tested the effect of LPS and TNF-α on the expression of the components of IL-6 receptor complex (gp80 [IL-6R] and gp130) in osteoblasts. Human osteoblasts expressed constitutively mRNAs of both IL-6R and gp130 and this was not modified by either LPS or TNF-α. In contrasts, TNF-α significantly inhibited the cell surface expression of IL-6R (gp80), which process was due to the inhibition of receptor synthesis analyzed by Western blots. The expression of gp130 was not changed. LPS practically had no effect on the expression of either IL-6R or gp130. These results indicate that TNF-α-induced inhibition of IL-6R synthesis was due to inhibition of translational and posttranslational events, but no similar response was achieved by LPS. Similarly, the expression of procollagen α[1] mRNA and type I collagen synthesis were significantly inhibited by TNF-α, but remained at normal level in LPS-treated cultures (Fig. 1).

Discussion

Bone erosion occurs, when either bone formation diminished or bone resorption is increased i.e. the normal bone turnover and remodeling is disrupted. Inflammatory joint diseases are frequently associated with osteolysis due to increased osteoclastic bone resorption. In this case the osteoblastic bone formation is unable to compensate the osteoclast-mediated bone resorption. Here we showed that TNF-α, a key proinflammatory cytokine, had a substantial negative impact on bone forming capacity of osteoblast (i.e. suppression of both cell proliferation and synthesis of type I collagen). Moreover, TNF-α induced the release of osteostat-activating IL-6 contributing to the negative turnover of bone metabolism. Interestingly, osteoblasts reduced their sensitivity via the TNF-α-induced suppression of IL-6R. LPS affected mainly the osteostat activating side of osteoblast functions by inducing the release of IL-6 and IL-1β, but neither the IL-6R expression (i.e. IL-6 mediated signaling) nor the type I collagen production was affected. These results suggest that both TNF-α and LPS alter osteoblast functions by which osteoblasts may recruit leukocytes due to the effect of IL-8 and MCP-1, and either activate osteoclasts in a paracrine manner, or produce significantly reduced amount of bone matrix leading to bone loss.

Figure 1: The expression of procollagen α[1] mRNA analyzed by Northern blot in TNF-α- or LPS-treated osteoblast cultures after 24 hours in various concentrations as indicated. Level of significance is shown: ** p<0.01

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