Expression of Heat Shock Proteins in Periapical Granulomas

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Abstract

Introduction: Cells from virtually all organisms respond to a variety of stresses by the rapid synthesis of a highly conserved set of polypeptides termed heat shock proteins (HSPs). HSPs protect cells under adverse conditions such as infection, inflammation, and disease. We hypothesize that endodontic infection might result in an imbalance in the expression of heat shock genes, accounting for different clinical outcomes in periapical lesions. **Methods:** We analyzed the expression of 44 HSPs genes using a pathway-specific real-time polymerase chain reaction array in 93 human periapical granulomas and 24 healthy periodontal ligament tissues collected postoperatively. Observed variations in the expression of HSP genes were also analyzed based on the classification of periapical granulomas as active or inactive. In addition, U937 cells were differentiated into macrophages, infected with different concentrations of purified *Escherichia coli* lipopolysaccharide (LPS), and used as templates for the HSP gene array. Protein expression was assessed by immunohistochemistry. **Results:** The expression of HSP genes was significantly increased in granulomas compared with healthy periodontal ligament ($P < .00001$). Among the 44 HSP genes, *DNAJC3*, *HSPA4*, *HSPA6*, and *HSPB1* showed the highest expression levels in both granulomas and LPS-treated macrophages. *DNAJC3*, *HSPA6*, and *HSPB1* were highly expressed in active lesions, whereas *HSPA4* expression was higher in inactive lesions ($P < .005$). Higher concentrations of LPS led to increased HSP expression in macrophages ($P < .0001$). Immunocytochemistry confirmed the expression and colocalization of *HSPB1* and *HSPA6* proteins in the cytoplasm of LPS-infected macrophages. **Conclusions:** The observed differential expression patterns of HSPs in periapical granulomas and LPS-infected macrophages suggest that HSP genes and proteins are involved in periapical lesion development and may account for different clinical outcomes. Understanding the role of the heat shock response might provide additional insights into the process of periapical lesion development. *(J Endod 2014;40:830–836)*

Key Words

Apical periodontitis, gene expression, heat shock protein, macrophages, protein expression

The most primitive mechanism of cellular protection involves the expression of a polypeptide family called heat shock proteins (HSPs). Although the description of HSPs as "stress proteins" was generated based on initial descriptions, individual HSPs fulfill different biological functions (1). Some HSPs are present in unstressed cells and play important roles in the folding and translocation of polypeptides across the cell membrane (1). However, HSPs are characteristically induced by stress signals such as elevated temperature, reduced oxygen supply, infectious agents, and inflammatory mediators (2). Therefore, HSPs also exert a protective role against harmful environmental conditions and pathogens (1). A brief overview of the role of HSPs is presented in Figure 1.

According to their molecular weight, HSPs are subdivided into the following groups: HSPH (HSP110), HSPC (HSP90), HSPA (HSP70), DNAJ (HSP40), HSPD (HSP60), and HSPB (HSP27) (3). HSPs are especially effective in triggering the innate immune response by activating macrophages and macrophage-like cells (4). HSPs can also increase the cellular response to lipopolysaccharide (LPS) to stimulate the production of prototypic proinflammatory cytokines such as tumor necrosis factor alpha (TNF-α) (4).

Macrophages are key players in innate immunity, respond rapidly to danger signals generated from inflamed sites, and have 3 major functions once activated: antigen presentation, phagocytosis, and immunomodulation through the production of various cytokines and growth factors (5).

In the context of periapical lesions, macrophages apparently have a key role in lesion expansion and subsequent bone resorption (6, 7). Macrophage activation will elicit a prominent proinflammatory action mediated by lymphocyte-recruiting chemokines, the production of tissue-degrading enzymes, and osteoclastogenic effects (8). However, the differential activation of macrophages that may stimulate the production of diverse intrinsic HSPs can induce the development of tolerizing phenotypes, thus suppressing cellular immune reactions (4).

Therefore, considering that HSPs can exert active roles on the modulation of the immune response (9), especially on determining the functional heterogeneity of macrophages (10), we hypothesized that the differential expression of HSPs can be associ-

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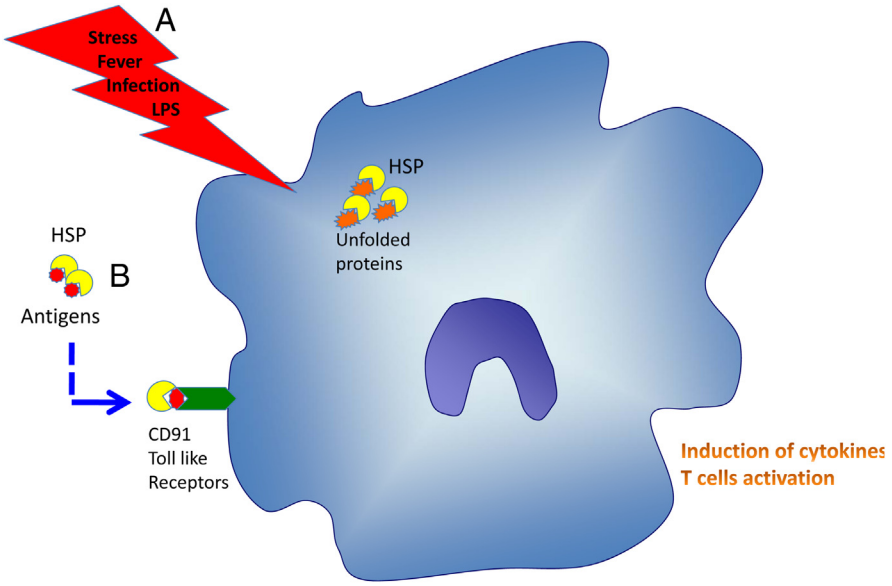

Figure 1. A schematic representation of the various roles of HSPs. A, HSPs function to repair protein damage caused by oxidative stress (ie, fever and infection). B, HSPs bind to antigens and present them to immunoregulatory cells via CD91 and toll-like receptors. Both mechanisms will contribute to cytokine release and T-cell activation.

ated with the outcome of periapical lesions and that macrophages may be considered a source of HSPs in the periapical lesion microenvironment.

Materials and Methods

Subjects and Samples

This study was approved by the Committee for Protection of Human Subjects at the University of Texas Health Science Center at Houston, Houston, TX. Subjects were patients (age, 15–57 years; average = 38.2 years) presenting with periapical lesions characterized radiographically as rarefaction lesions with the disappearance of the periodontal ligament space and discontinuity of the lamina dura; patients were referred for endodontic surgery when teeth failed to heal after conventional root canal treatment. Patients with medical conditions requiring the use of systemic modifiers of bone metabolism or other assisted drug therapy (ie, systemic antibiotics, anti-inflammatory, or hormonal therapy) during the last 6 months before the initiation of the study, patients with pre-existing conditions such as periodontal disease, and pregnant or lactating women were excluded from the study.

Periapical lesion samples were collected and divided into 2 roughly similar fragments and stored in formalin and RNALater (Ambion, Austin, TX) solutions. Samples stored in formalin were submitted to routine histologic processing (formalin-fixed paraffin-embedded tissues) and sectioned for histopathologic analyses. Only cases of periapical granulomas, represented by the presence of capillaries, inflammatory cells, fibroblasts, collagen, and macrophages and without the presence of an epithelial lining, were selected for the study. Periapical cysts in which cavities were further developed and lined by stratified squamous epithelium were excluded. A total of 93 periapical granulomas were selected for the study. Healthy periodontal ligament tissue samples (n = 24) obtained from premolars extracted for orthodontic purposes (patients aged 17–23 years) were stored in RNALater solution (Invitrogen, Carlsbad, CA) and used as control specimens. Only 1 sample per individual was used in the study, either as a case or control sample.

Cell Culture

U937 cells (ATCC, Manassas, VA), a human monocyte cell line, were cultured in RPMI-1640 Medium (ATCC) supplemented with 10% fetal bovine serum (Atlanta Biologicals, Lawrenceville, GA) and Penicillin Streptomycin (VWR, Radnor, PA). The cells were incubated at 37°C in a humidified atmosphere of 95% air and 5% CO_2. For experiments, 2.5×10^5 cells/well were seeded and subjected to phorbol-12-myristate-13-acetate (VWR) treatment at a final concentration of 100 nmol/L for 72 hours for differentiation into macrophages. The cells were then washed with phosphate-buffered saline (PBS) and incubated in normal growth medium without phorbol-12-myristate-13-acetate for 24 hours before treatment with purified *Escherichia coli* LPS (Sigma, St Louis, MO). LPS was used at a final concentration of 1 μg/mL and 10 μg/mL for 24 hours. By the end of the process, cell density increased up to 80% of confluence.

Gene Expression Analyses

Total RNA was extracted from the tissue samples using TRIZOL reagent (Life Technologies, Grand Island, NY) and from the cells using TaqMan Cells-to-CT Control Kit (Life Technologies) following manufacturer's instructions. Next, the RNA pellet was dried under a vacuum and resuspended in 50 mL diethyl pyrocarbonate–treated water. The integrity of RNA samples was checked by analyzing 1 μg total RNA on a 1.2% (w/v) denaturing formaldehyde-agarose gel. After RNA extraction, complementary DNA was synthesized using 3 μg RNA through a reverse-transcription reaction using SuperScript III Reverse Trancriptase (Invitrogen). Sample pools of the 93 human periapical granulomas (cases) and the 24 human healthy periodontal ligament tissue samples (controls) were obtained and used for posterior analyses.

We investigated the messenger RNA (mRNA) expression of 44 HSP genes in a pool of the periapical granuloma samples and controls and in the macrophages with and without LPS treatment (1 μg/mL and 10 μg/mL) using a TaqMan Human Heat Shock Proteins Array (Life Technologies, Foster City, CA) in a quantitative real-time reverse-transcription polymerase chain reaction. *18S*, *GAPDH*, *HPRT1*, and *GUSB* were used as internal control genes for normalization as provided in the