Bactericidal/Permeability-Increasing Protein Is Expressed by Human Dermal Fibroblasts and Upregulated by Interleukin 4

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The bactericidal/permeability-increasing protein (BPI) is an antibiotic- and endotoxin-neutralizing protein of granulocytes and epithelial cells. Constitutive expression of BPI, which increases upon interleukin 4 stimulation, by human dermal fibroblast was demonstrated, suggesting an important role of BPI in gram-negative bacterial clearance and a dampened response to endotoxin in the skin.

The skin as a major organ of host defense not only acts as a mechanical barrier but also controls bacterial colonization and infection by producing an antibiotic layer (6, 15). Yet, this shield is often broken by microscopic lesions, e.g., in atopic dermatitis, that allow microorganisms to reach the underlying tissue (7). Gram-negative bacteria and their lipopolysaccharides are especially potent stimuli for an inflammatory response (1). The absence of chronic skin infections indicates that there may be effective local antibiotic- and endotoxin-neutralizing mechanisms that minimize the host's response. The bactericidal/permeability-increasing protein (BPI) produced by granulocytes and epithelial cells (3) kills gram-negative bacteria and neutralizes their endotoxin (9, 13). Thus, the question was raised whether BPI can also contribute to host defense against gram-negative bacteria and lipopolysaccharides in the skin. We have investigated the constitutive expression of BPI on mRNA and protein levels as well as the modulating effects on its expression by cytokines (tumor necrosis factor alpha [TNF-α], interleukin 1β [IL-1β], and IL-4), immunosuppression (4-hydroxycyclophosphamide [4-HC]), and infectious agents (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Candida albicans*).

Human dermal fibroblasts (Cambrex, East Rutherford, N.J.) were grown in Dulbecco's minimal essential medium–F-12 medium (10% fetal calf serum, 1 mM L-glutamine, 0.05 g of Na-ascorbate/liter, 1,000 U of penicillin/liter, 1 g of streptomycin/liter; Invitrogen, Carlsbad, Calif.), stimulated for 72 h with TNF-α, IL-1β, IL-4 ([C] = 0.1 to 10 ng/ml; Tebu, France), 4-HC ([C] = 10^{-9} to 10^{-7} M; Asta Medica, Frankfurt au Main, Germany), or heat-inactivated *S. aureus*, *P. aeruginosa*, and *C. albicans* (ATCC 25923, 27853, and 10231, [C] = 10^5 to 10^7 microorganisms/ml), trypsinized, and used for total RNA extraction. Supernatants were stored at −20°C. Total RNA was isolated after DNase digestion by using the RNeasy kit (Qiagen, Valencia, Calif.). Reverse transcription (RT)-PCR was carried out with forward primer 5′-TTC CAG CTT CCC AGT

TCC CAG ATA-3′ and reverse primer 5′-CAT CCA CGG CAG GGT AGA AGG TAA-3′ (Invitrogen). Thirty-five amplification cycles were carried out. PCR products were loaded on 1.5% agarose gels, and bands were visualized by ethidium bromide staining. DNA sequencing was performed with an automated sequencer (ABI 377 PRISM; Perkin Elmer, Norwalk, Conn.) and revealed >98% sequence similarity to the BPI-encoding DNA sequence (2). Culture supernatants were concentrated (YM30 filter membrane; Amicon, Danvers, Mass.), diluted in 0.01 M Tris-citrate buffer (pH 8), and applied to a heparin column (HiTrap 1 ml, 7 by 25 mm, 34-μm particle size; Pharmacia, North Peapeck, N.J.), attached to a SpectraSystem P 4000 high-performance liquid chromatography (HPLC) apparatus (Spectra-Physics, Mountainview, Calif.), and eluted with 2 M NaCl in dilution buffer. Cationic fractions were concentrated, rediluted in 20 mM ammonium formate (pH 4) containing 25% (vol/vol) acetonitrile, and applied to a micro-Mono S HPLC column (Mono S PC 1.6/5, 2.1 by 100 mm, 5-μm particle size), attached to a Smart-Micro-HPLC apparatus (Pharmacia), and equilibrated with buffer. Proteins were eluted with an NaCl gradient (maximum, 1 M NaCl) in equilibration buffer. Fractions were tested for BPI content by Western blotting with P1G8 monoclonal mouse immunoglobulin G1 (IgG1) anti-BPI antibody (a kind gift from Anne Pereira, Department of Pathology, University of Oklahoma) and a horseradish peroxidase staining kit (Opti-4CN substrate kit; Bio-Rad, Hercules, Calif.) according to the manufacturer's instructions. BPI concentrations were determined by capture enzyme-linked immunosorbent assay (ELISA) performed as previously described (14) with 6C2 monoclonal mouse IgG1 anti-BPI antibody and polyclonal rabbit anti-BPI antibody visualized by alkaline phosphatase-conjugated secondary antibodies. Recombinant BPI (rBPI; Xoma) served as the standard ([C] = 0.2 to 500 ng/ml). Cell lysates of neutrophil granulocytes served as positive controls. For both Western blotting and ELISA, isotypic control staining was done with irrelevant mouse IgG1 antibodies from Pharmingen (San Diego, Calif.).

BPI is constitutively expressed by human dermal fibroblasts on a transcriptional level. This could be demonstrated by BPI-specific RT-PCR as well as sequencing of PCR products (Fig.

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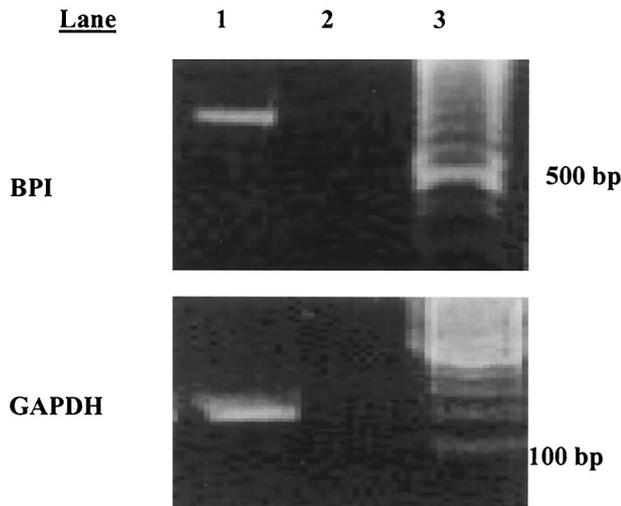
FIG. 1. Constitutive BPI mRNA expression in human dermal fibroblasts. Shown is the RT-PCR product from unstimulated human dermal fibroblasts as described in the text. In the upper image, the PCR product of BPI-specific RT-PCR (size, 841 bp) is shown. The lower image shows the product of GAPDH RT-PCR (housekeeping gene; size, 233 bp). Lane 1, human dermal fibroblasts; lane 2, negative-control sample (containing no RNA); lane 3, 100-bp marker.

1). In fibroblast cell cultures, BPI is released spontaneously into the medium at a concentration of approximately 1 ng/10^6 cells. Stimulation with TNF-α and IL-1β had no effect on the expression of BPI, in contrast to IL-4, which increased the release of BPI 20-fold to 20 ng/10^6 cells (Fig. 2). After direct exposure to *S. aureus*, *P. aeruginosa*, or *C. albicans*, an increase of BPI secretion could not be detected. Moreover, incubation with the immunosuppressive drug 4-HC also changed neither transcription nor release of BPI.

The epidermal and dermal components of the skin are a dynamic network of cells capable of detecting and reacting to a variety of traumata and infectious agents (6, 15). The demonstrated constitutive expression of BPI by ubiquitously present dermal fibroblasts and upregulation upon IL-4 stimulation indicates an active role of these cells in defense against gram-negative infections on a subepithelial level. Recurrent skin lesions due to fissures or scratching are typical clinical findings in atopic dermatitis (7). Although gram-positive bacteria, such as *S. aureus*, and gram-negative bacteria inhabit the skin, the former are involved in infection and promoting inflammation in atopic dermatitis (8, 10). As a Th2-type immune reaction with a predominance of IL-4 is a characteristic feature of atopic dermatitis and a peptidoglycan-induced, TLR2-depen-
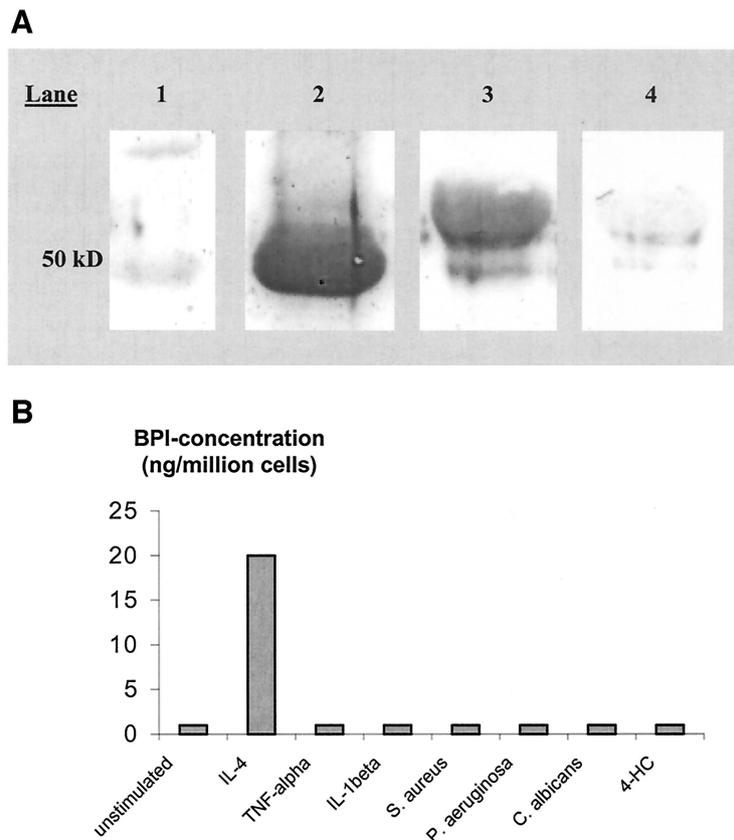

FIG. 2. BPI total protein production by human dermal fibroblasts. (A) Shown is the Western blot band from culture supernatants from unstimulated human dermal fibroblasts as described in the text. Lane 1, molecular weight marker; lane 2, rBPI$_{50}$, where the subscript number indicates molecular size); lane 3, culture supernatant from unstimulated human dermal fibroblasts; lane 4, isotypic control staining of cell culture supernatant from unstimulated human dermal fibroblasts with an irrelevant mouse IgG1 antibody. (B) Shown are the results of quantitative BPI detection in culture supernatants from human dermal fibroblasts by capture ELISA. In culture supernatants from resting fibroblasts, BPI was found in concentrations of 1 ng/1 million cells. These levels were increased by 20-fold after IL-4 stimulation but were not changed by stimulation with IL-1β, TNF-α, *S. aureus*, *P. aeruginosa*, *C. albicans*, and 4-HC.

dent IL-4 release of dermal mast cells has recently been reported (12), it can be hypothesized that in this setting an increased secretion of BPI is induced in dermal fibroblasts and contributes to gram-negative bacterial killing and endotoxin neutralization (9, 13). Moreover, in burn injuries, patients are threatened by gram-negative infections of the destroyed skin, endotoxin release, and a systemic inflammatory response syndrome (5). One surgical counterstrategy is the debridement and coverage of disrupted skin by autografts composed of the patients' keratinocytes and dermal fibroblasts (4). Knowledge about the detailed regulation of BPI expression could facilitate genetic modification of these fibroblasts. For example, by increasing IL-4 receptor density and thus making them more responsive to IL-4 secreted by activated T cells within the characteristic Th2 response (11) or by stimulation with drugs, such as lipoxins (3), graft infections and systemic stimulation by lipopolysaccharides could be diminished (9, 13).

In summary, the pluripotent BPI is constitutively produced by dermal fibroblasts and may contribute to gram-negative bacterial clearance and a dampened response to endotoxin in the skin.

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