Comparative proteomic analysis of peripheral blood mononuclear cells from atopic dermatitis patients and healthy donors

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Atopic dermatitis (AD) is a chronic inflammatory skin disease that induces changes in various inflammatory skin cells. The prevalence of AD is as high as 18% in some regions of the world, and is steadily rising. However, the pathophysiology of AD is poorly understood. To identify the proteins involved in AD pathogenesis, a comparative proteomic analysis of protein expression in peripheral blood mononuclear cells isolated from AD patients and healthy donors was conducted. Significant changes were observed in the expressions of fourteen proteins, including the vinculin, PITPNB, and Filamin A proteins. Among the proteins, α-SNAP and FLNA decreased significantly, and PITPNB increased significantly in AD patients compared with control subjects; these findings were further confirmed by real-time PCR and Western blot analysis. The comparative proteome data may provide a valuable clue to further understand AD pathogenesis, and several differentially regulated proteins may be used as biomarkers for diagnosis and as target proteins for the development of novel drugs. [BMB reports 2008; 41(8): 597-603]

INTRODUCTION

Atopic dermatitis (AD) is a highly pruritic, chronic inflammatory skin disease that affects children and adults worldwide. Most manifestations of AD result from a complex interplay of susceptible genes, environmental factors, pharmacological abnormalities, skin barrier defects, and immunological responses (1, 2).

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The skin lesions of AD patients are characterized by increased numbers of IgE-bearing Langerhans cells, inflammatory dendritic cells, macrophages, eosinophils, activated T lymphocytes, and mast cells (3-5). In particular, peripheral blood mononuclear cells (PBMCs) act as the main effectors via the functional regulation of cytokines such as interleukin and interferon-γ. This important role of PBMCs is well established, and has been demonstrated in experimental models. Specifically, the PBMCs in AD patients have a decreased capacity to produce interferon-γ, which is inversely correlated with serum IgE concentrations (1, 2, 6).

Proteomics can provide a global, systemic, and comprehensive approach to the identification and description of the biochemical processes, pathways, and networks involved in both normal and abnormal physiological states at the protein level (7-10). As a typical proteomic analysis, two-dimensional electrophoresis (2-DE) coupled with mass spectrometry (MS) has been used widely in research (11-13). 2-DE can be used to detect differences in protein expression levels in cell states between healthy and diseased cells, and is also a promising tool for use in identifying disease markers and candidates for therapeutic intervention (8, 14).

The number of AD patients has increased with modernization and industrialization. Therefore, further insight into the complex pathogenesis of AD, determining its detailed mechanisms and its regulatory proteins, is needed (10, 15). Although proteomic studies can be employed to identify new target proteins of interest in AD, proteomic approaches have been used less often in the study of AD than have genomic approaches (11, 15-17). Moreover, a comparative analysis of human PBMCs between AD patients and healthy donors has not yet been reported. In this study, 2-DE combined with MS has been used to investigate the protein expression profiles in PBMCs isolated from both AD patients and healthy donors.

RESULTS AND DISCUSSION

PBMCs are a heterologous cell population composed of approx-
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Fig. 1. Representative 2-DE image of PBMCs from an AD patient. The protein samples (500 μg) were applied to a first dimension of pH 4-7 nonlinear IEF strips and a second dimension of 12.5% SDS-PAGE visualized by CBB-G250 staining. The rectangles and circles mark spots that show significant changes in expression levels. The indicated spot numbers in Table 1 correspond to the spots shown in Fig. 1.

Fig. 2. Comparative analysis of differentially expressed protein spots in normal healthy donors and AD patients. (A) Zoom of the 2-DE gel on up- and down-regulated spots. (B) Quantification of differentially expressed protein spots in AD patients compared to healthy donors (Fig. 1). Among these, a total of fourteen proteins were identified via mass spectrometry (Fig. 2 and Table 1). These proteins were classified into distinct functional groups of cell motility, regulation of signal transduction, transport, organismal physiological processes, and proteins of unknown function.

In order to verify the results from the 2-DE analysis, Western...