Innate Immune Proteins In Atopic Skin Disease Individuals

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Abstract

Barrier dysfunction is increasingly established in its role in many diseases. Atopic Dermatitis (AD) is a chronic inflammatory disease that is caused by interactions between gene, environment and functionality of the skin. The stratum corneum layer of the skin is most crucial in the epidermal barrier. Therefore, innate immune proteins found on the human skin play modulatory roles on the skin barrier. In this study, the main objective is to identify whether innate immune proteins on human skins are differentially expressed in atopic dermatitis individuals as compared to the control individuals. Skin sample was collected via adhesive tape from individuals. This is then followed by the processing and extraction of the protein. Accordingly, the proteins are assayed against primary antibodies of interest. Results obtained show that some proteins do have significant differential expression in the AD as compared to the non-AD group. These proteins show rather promising ability in establishing concrete link between barrier dysfunction and AD. More research should be performed in the need of further validation for a more through understand of the candidates’ innate immune proteins.

Introduction

Atopic Dermatitis (AD) is a chronic inflammatory skin disorder that is multi-factorial. The complex interaction between the effects of gene, environment and functionality of the skin barrier on AD is remarkable. Genetic predisposition causes amplified production of stratum corneum chymotryptic enzyme resulting in the increased breakdown of comeodesosomes (Cork et al. 2006). The coexistence of certain environmental factors such as soap and detergent would further aggregate the condition by up-regulation of the protease enzyme (Cork et al. 2006). The stratum corneum layer of the skin is most essential in the functionality of the epidermal barrier. It constitutes mainly protein-enriched cells, the corneocytes as well as lipid-enriched domains (Proksch et al. 2006). The lipid matrix specifically prevent entry of irritants to the living epidermal layers and thus damage keratinocytes. Thus, barrier dysfunction leads to increase infiltration of external allergens into the skin and succeeding inflammatory reaction, thus importantly involved in the pathogenesis of AD.
Materials and Methods

Adhesive tapes were utilized for the extraction of stratum corneum cell layers of the skin. This was done by 50 times of successive tape sampling at the flexor area of the right and left arm of participants respectively. Consequently, protein extraction is carried out to disrupt cell membranes for the release of cellular contents. Sonication can loosen the cells and protein adhering to the tape. Subsequently, they were lyophilized overnight to dryness and solubilized using 50mM of Tris HCl (pH 8.5) for later protein quantification and dot blotting assay. Addition of chemiluminescent substrate (Invitrogen) was for the detection of signals by the enhanced chemiluminescene system. Micro Image is utilized for the analyzing of the membranes.

490 random volunteers from National University of Singapore have been gathered for the sample collection. Out of this population, 63 controls (non-AD) and 39 cases (AD) have been identified by the questionnaire. The group consists of 40 female and 62 male. In addition, all the cases chosen have positive responses to the skin-prick test as opposed to the controls which have no reaction to the skin prick test. The average age for both the case and control group selected is 23 years old.

Results

Zinc alpha 2-glycoprotein (p=0.027), Immunoglobulin M (p=0.009) and Immunoglobulin E (p=0.017) are statistically differentially expressed in the case and control groups by the Mann Whitney U test. The other proteins (Cathelicidin, Dermcidin, Psoriasin, Superoxide Dismutase 1, Lactotransferrin, Calgranulin B) screened shown no statistical difference between the case and control groups at 5% significance level by the Mann Whitney U test. Therefore, ROC curves were constructed for ZAG, IgM and IgE (Figure 3). In a ROC curve, the true positive rate is plotted in function of the false positive rate for different cut-off points. Therefore, every single point present in the curve shows a sensitivity/specificity pair which is parallel to a decision threshold. Hence, the closer the ROC plot is to 100% sensitivity and 100% specificity, the higher the overall accuracy of the diagnostic test (Zweig and Campbell 1993). Thus, the area under the curve serves as the basis of the overall effectiveness of the selected single proteins’ diagnostic validity. From Figure 3 and Table 3, the three identified proteins show reasonable diagnostic validity of approximately AUC of 0.60. However, a normal good marker would be of estimated AUC of 0.75. Therefore, the computer program mROC could be utilized for a combinational of uncorrelated proteins to increase the strength of the test. Hence, Spearman’ correlation coefficient test was carried out. The scatter plots constructed (Figure 4) showed no observable linear trend for the paired proteins. Spearman’ correlation coefficient further confirmed that the proteins are not highly linearly correlated with each other.
Discussion

ZAG (p=0.027), IgM (p=0.009) and IgE (p=0.017) are statistically differentially expressed in the case and control groups by the Mann Whitney U test. ZAG is present in elevated levels in the control group as compared to the case group. It is a soluble protein established to known as a fat-depleting factor of adipocytes in cancer patients (Sanchez et al. 1999). The organization and fold of ZAG is similar to major histocompatibility complex (MHC) class I antigen-presenting molecule (Sanchez et al. 1999; Hassan et al. 2008). Ketelaar et al. (1988) reported that fibroblastic cell line will differentiate along mesodermal cell lineage to form adipocytes. Fibroblasts play a critical role in wound healing (Grillo et al. 1961). AD patients have dry, scaly skin often companied with broken skin. This can be due to the deregulation of the wound healing process. Hence, the decreased in ZAG could lead to decreased rate in wound healing procedures that lead to the manifestation of common symptoms seen in AD patients.

Immature B cells, which have never been exposed to an antigen, are known as naïve B cells and express only the IgM isotype in a cell surface bound form (Goding 1978). Therefore, IgM is the basic antibody of the body. A possible explanation for the significance increased in the level of IgM in the skin of AD individuals could be due to the broken skin of the individuals at the site of sampling.

IgE is expressed to a higher level in the case group as opposed to the control group. The production of IgE is often involved in defense against parasites infection and allergic reactions. Mast cells are present abundantly in connective tissue around the body can drive IgE production. This is linked to increased local or systematic hypersensitivity reactions due to mast cell activation and degranulation. Therefore, it is a norm that IgE is elevated in atopic individuals compared to non-atopic individuals. Studies have provided substantial evidence that allergens can trigger acute IgE-mediated mast-cell dependent exacerbations of eczema in AD patients (D Y Leung 1993). Hence, this is a possible explanation why IgE is present at higher level in the innate immune proteins present in skin in case group as opposed to control group.

Moreover, Adachi and Aoki (1989) reported that out of 45 AD patients that are tested for skin reactions to their own sweat, 43 showed positive reactions. The IgE antibody that responds to sweat did not exhibit cross-reactivity with the mite extract, Dermatophagoides farina, or Staphylococcus aureus. These results indicate that there might be specific IgE antibody that reacts to sweat.

Auto-allergy is another potential basis for the up regulation of total IgE levels in AD individuals. This is brought up due to the observation that AD patients frequently exhibit worsening of the disease condition despite obvious contact with exogenous allergens. The discovery of several environmental allergens that are structurally and immunologically very similar with human proteins is one supporting evidence (Valenta et al. 2000). The proposed mechanism of auto-allergy in AD could be that the contact of exogenous allergens leads to damage of tissues which could cause release auto antigens. These antigens that activate mast cell-bound IgE auto-antibodies and cause mast cell degranulation (Valenta et al. 2000).

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Generally, IgM and IgE play centrally important roles in immune responses. At the same time, this is in conjunction with the large immune responses and inflammation in AD. Therefore, this could be a probable cause of the elevated level of both IgM and IgE.

References


