A major predisposition of atopic dermatitis biology essay

Science, Biology

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\n[/toc]\n \nProperties of the key protein involved in atopic dermatitis - filaggrin - in organotypic cultures to look at how this is modulated by an inflammatory stimuli.

BSc (Hons) Medical Sciences

Project Supervisors:

Professor Sarah Howie

Dr. Richard Weller

ABSTRACT – Filaggrin mutation is a major predisposition of Atopic dermatitis (AD). Filaggrin is formed in the terminally differentiated keratinocytes (KCs) in epidermis of skin. Filaggrin has a role in skin barrier function. This includes n hydration of the stratum corneum layer and filaggrin breakdown product, urocanic acid (UCA) has been associated with local immunotolerance in skin models. Filaggrin expression is modulated by cytokines such as IL-4 & IL-13 and therefore individuals can also acquire filaggrin-deficiency. It is also accepted that environment plays a big role in outcome of AD phenotype in individuals. In this investigation, we look at the effects of inflammatory stimuli LPS and DERp1 on differentiated and non-diffentiated KC cell lines

(HaCats). Effects we are looking at are change expression of filaggrin and cytokine profile in HaCats. Atopic dermatitis (AD) is a chronic inflammatory skin disorder associated with genetic and environmental predisposition. Symptoms of AD involve dry, red, scaly and itchy rashes, and in severe cases, lesions may weep, and bleed. (1) AD usually affects children as early as 2 to 6 months and most of them grow out of the condition by early adulthood. However, small percentage may experience persistent AD throughout life. Pathogenesis of AD is due to a dysfunctional interaction between skin barrier and immune function. (1) Clinical symptoms arise due to an impaired protective skin barrier which has a role of retaining moisture in stratum corneum (SC) layer of skin and preventing ingress of pathogens. (2) AD is thought to be a complex polygenic disorder and a long standing hypothesis of pathogenesis of AD proposes that, AD is primarily driven by chronic type 1 hypersensitivity, mediated by allergen-specific immune response to produce a type 2 CD4+ T-lymphocyte (Th2 response) driven atopic inflammation. (1)(3)(4) And, defect in skin barrier was secondary phenomenon due to the above immune abnormalities. In recent years, genetic linkage studies underscore the importance of chromosome 1q21, which contains a group of genes, collectively known as epidermal differentiation complex (EDC). (2) So far, 27 genes have been identified in EDC that encodes proteins that are important in maturations of the human epidermis to form a structural barrier. (5) (17) One gene of particular interest located in EDC, encodes for a key protein, filaggrin. Loss-of-function (null) filaggrin gene (FLG) mutations have been identified to be a strong predisposing factor for AD. (7) Up to 42% of patients with atopic dermatitis in

European population have one or more null filaggrin allele. (8) FLG is not only associated in pathogenesis of AD, but also thought to be linked to itchyosis vulgaris, an inherited skin disease characterised by non-inflamed chronically dry and scaly skin. (9) The identification of FLG in relation to eczematous skin disorders has enlivened the research in this area of study. A new hypothesis in pathogenesis of AD has been proposed, that the ingress of allergens and irritants together with moisture loss through a primary defect in epidermal skin barrier function due to filaggrin precipitates an immune response which develops into a chronic Th2 response. Filaggrin and keratinocytes – what do we know about them? Filaggrin is formed in keratinocytes (KCs) in the epidermis. The prominent keratohyalin granules in KCs of the stratum granulosum of epidermis are predominantly composed of a 400-kDa profilaggrin polypeptide made up of 10 – 12 filaggrin monomers. (6) Upon terminal differentiation of KCs, profilaggrin is dephosphorylated and proteolytically cleaved by serine proteases into monomers of 37-kDa filaggrin protein. (6) Filaggrin protein then aggregates the intermediate keratin filament cytoskeleton system, which then collapses to form a dense lipid matrix that eventually forms the cornified envelope (CE) of the SC, a process known as cornification. (6) CE provides a physical barrier which is essential in preventing ingress of pathogens. Filaggrin protein is progressively degraded by enzymes into free hydrophilic amino acids, urocanic acid (UCA), pyrrolidone carboxylic acid (PCA), and alanine, collectively known as natural moisturising factor (NMF). NMF contributes to the retention of water in SC and may additionally regulate pH in SC. (6) (13) A reduction in NMF is a likely contributor to xerosis (dry and flaky skin), a

common phenomenon in null FLG mutation individuals. The concentration of NMF is dependent on two factors, filaggrin genotype and AD severity. (16) Homozygotes for null FLG mutations who have AD, have extremely low concentrations of NMF compared to heterozygote for null FLG mutations that have AD and highest concentrations of NMF was found in wild-type with AD. (16) This results show that, (i) FLG genotype and filaggrin expression correlates to the concentrations of NMF and (ii) filaggrin is the main source for NMF, but is not the only source as homozygotes for null FLG mutations did show low but traceable concentrations of NMF. (16) Despite conferring an overall drier skin, it was showed that a reduction of NMF concentration did not confer AD phenotype. To look at structural importance of filaggrin, investigation by Howie and team measured epidermal barrier integrity, by measuring trans-epidermal water loss (TEWL), capacitance and effects of challenges to epidermal barrier, which include tape stripping of SC and chemical irritation with sodium lauryl sulphate in (i) null FLG mutation skin with and without AD and (ii) wild-type with AD and normal skin. They found no significant difference between measured parameters in group with FLG mutations without AD and wild-type without AD. (unpublished data) This shows that even though, the central role of filaggrin in the formation of tough CE is generally accepted, it should be looked with caution as an initiation factor of pathogenesis of AD as up to 40% of patients with null FLG mutation do not have clinical evidence of AD and has a normal phenotype of skin epidermal barrier function. (7) There also seem to be a redundancy in proteins (e. g. involucrin and loricrin) that can cause aggregation of intermediate keratin filament in KC and subsequent CE formation. (10) These

are contradictory reports to the newer hypothesis of pathogenesis of AD, and suggest that null FLG mutations alone do not confer resultant AD phenotype. Xerosis may exacerbate the permeability of the SC. Recently Howie and team the hypothesis that people with null FLG mutations individuals have increased exposure of pathogens, allergens and irritants to antigen presenting cells (APCs) (e. g. resident DCs and langerhans cell) in skin. (unpublished data) And their response would determine whether individual developed immunological changes of AD or normal phenotype. They found that DCs in skin with null FLG mutations had an increased proportion of CD11c+ DCs; an integrin, expression of which is increased during inflammation - indicating an increased exposure through a more permeable epidermal skin barrier. (unpublished data) This suggests that genotypic or acquired filaggrin deficiency does increase the permeability of SC but it was shown that even this did not confer AD phenotype. In filaggrin-deficient organotypic skin-equivalent models, a decrease in immunotolerance was seen. (11) This was measured the percentage of UVB-induced apoptosis – suggesting that filaggrin or its breakdown product may have an active role in immune response. Particularly interesting is the filaggrin breakdown product, UCA. It was found that a reduction in UCA concentrations increased UVB-induced apoptosis proportionally. (11) Trans-UCA is isomerised to cis-UCA by ultraviolet radiation particularly by UVB wavelengths. Cis-UCA has a role as an immunosuppressor and photoprotection in human epidermis, seen by conferred protection of skin from DNA damage and apoptosis of KCS in organotypic skin models upon cutaneous sensitisation by dinitrochlorobenzene (DNCB) and UVB. (12) Cis-UCA modulates immune

response in a complex manner and the mechanism is not yet clear. (11) This lack of immunosuppression is believed to contribute to the decreased tolerance and increased atopic inflammation associated in AD. (13)Previously KCs was thought to be only important in maturation and formation of a tough epidermal skin barrier in human. There is an Increasing compilation of evidence suggesting that KCs participate actively in skin pathology. (15) KCs are able to act as primary sensors of invading pathogen by recognition of various pathogen-associated molecular patterns (PAMPs) via Toll-like receptors (TLRs) to produce an array of pro-inflammatory cytokines and chemokines such as tumor necrosis factor alpha (TNF-alpha), IL-1, IL-15, CXCL10, CCL2, and CCL22. (15) There have been studies conducted by various groups which show that, epithelial cells (in lungs and gut) have a role in maintaining a local tolerogenic environment by suppression of CD4+ T-lymphocytes activations. (19)(20) KCs are believed to be playing this role in skin. CD4+ T-lymphocyte-KC interaction is poorly understood. In study conducted by Lanning et al 1997, this CD4+ T-lymphocyte-KC interaction are seen to behave differently between differentiated and non-differentiated KCs. (18) Non-differentiated KCs are able to inhibit T-lymphocyte proliferations by production of prostaglandin E2 (PGE2), as this suppression is inhibited by PGE2 inhibitor.(18) Differentiated KCs on the other hand, are able partially suppress T-lymphocyte in presence of PGE2 inhibitor and also did not require cell-cell contact. (18) This suggests that KCs are able to secrete other soluble factor to suppress T-lymphocyte activation. This other soluble factor is hypothesised filaggrin breakdown product, UCA by Howie and team. If filaggrin protein is thought to be essential in pathogenesis of

AD, there must be an explanation for why over 50% of AD patients are homozygous for functional FLG and yet a skin epidermal barrier defect still persists. To investigate this phenomenon, Howell et al in 2007 conducted a study by subjecting various cytokines to KCs in skin cultures and skin biopsy of samples with or without null FLG mutations. (14) They found a significant reduction in expression of filaggrin when treating skin cultures with IL-4 and IL-13. (14) These cytokines (IL-4 and IL-13) are key mediators in Th2 responses and are over-expressed in AD, particularly in acute lesions. A very interesting results was found from this investigation, which shows that filaggrin deficiency is not restricted to genotype (null FLG mutations) of an individual but can be acquired as a result of Th2 cytokine modulation and suggests that aberrant immune response may be an initiation factor in patients wild-type patients. Summary of key findings that lead up to the question being asked in my dissertation project: 1. Most patients outgrow AD – suggesting pathogenesis may be reversed or modulated. 2. Null FLG mutation and environmental factors (e. g. allergens, pathogens and etc) contribute to the resultant AD phenotype. 3. Keratinocytes are immunologically active – has a sensory receptors and effectors pathways to respond to pathogens and allergens. As such, during inflammation KCs senses presence of IL-4 and IL-13 and responds by a reduction in filaggrin expression. (14) (15)4. UCA functions to confer local immunotolerance to decrease inflammation but a reduction in UCA did not confer a definite AD phenotype. (11) (18)5. Decrease of filaggrin expression did increase permeability of epidermal barrier – subsequent increase in inflammatory response but did not confer AD phenotype. 6. AD phenotype is independent

of concentrations of filaggrin protein and its breakdown product. 7. Any of the known roles/functions of filaggrin or its breakdown products in association with immune responses do not seem to confer a definite AD phenotype – suggesting an external factor might be in play? As to date, most investigations look at interaction of filaggrin and immune responses, and missed out a key predisposition of AD, environmental factors. In my dissertation project we shall look at interaction of environment (inflammatory stimuli) to expression of filaggrin and immune response. My dissertation projectFilaggrin is thought to have roles in immunological processes and formation of skin epidermal barrier. As such it may be possible to link the both hypotheses of pathogenesis of AD through filaggrin. That is why it is necessary to widen the current understanding normal immunological functions of filaggrin. Reports of filaggrin-deficiency may be acquired due to cytokine modulation led up to a key question being asked in my project, that if external/environmental factors which include inflammatory stimuli, may be able to dictate the expression of filaggrin and the cytokines produced to dictate the immune response. Coming to my dissertations project, a hypothesis is made, that an inflammatory stimulus may modulate the expression of filaggrin which may have an effect in overall immune responses and this may be altered between differentiated and non-differentiated KCs. Inflammatory stimuli may change the properties (if present) of filaggrin, and may not be able to maintain a normal skin and confer immunological changes that may result in AD-associated skin phenotype. In this investigation, lipopolysaccharides (LPS) and house dust mite protein, DERp1 and a combination of LPS + DERp1 will be used as

inflammatory stimuli at varying duration and concentration and are subjected to differentiated and non-differentiated, immortal KC cell lines (HaCats). LPS is an endotoxin and a strong activator of immune response, which had contradictory reports as to be beneficial to AD patients. And, DERp1 is a common allergen and confers Th2 immune response in majority of AD patients. We hypothesised that both LPS and DERp1 may be able to modulate expression of filaggrin in KCs. The effects of LPS and DERp1 on keratinocytes will be assessed by a change in phenotype and function of KCs which corresponds to the expression of filaggrin and cytokine released (i. e. IL-1 and IL-8) respectively. Expression of filaggrin and cytokine released are measured using western blotting and ELISA techniques. These results will hopefully allow us to extend our understanding of roles of filaggrin. Importance this investigationResults from this experiment will provide us a compilation data of significance of (i) DERp1 and LPS individually, (ii) combination of DERp1 + LPS and (iii) IL-1 and IL-8 in association with expression of filaggrin and inflammatory stimuli. This result may be used as reference in future experiments to investigate if filaggrin may dictate the immune response outcome upon simulation by DERp1 and LPS. If LPS is proved to induce a Th1 response in HaCats as some previous reports, there may be a therapeutic use of LPS for AD, as cytokines released in Th1 response (i. e IFN-gamma) inhibit Th2 response and subsequently may inhibit acquired-filaggrin deficiencies in individuals. Findings from this investigation may be used as a reference for future investigations which may lead to a discovery of new functions of filaggrin in association with response to varying inflammatory stimuli on KCs. This may contribute to the overall

understanding of roles of filaggrin and answer why null FLG mutations are common in patients with AD. Properties of filaggrin specific to an inflammatory stimulus may lead to provide an understanding of immunological pathways which are necessary in initiation of pathogenesis of AD. These immunological pathways (for example activation of key secondary signaling molecules) may provide a convergence between null FLG mutation group with AD patients and wild-type group with AD patients. AD patients have a reduced quality of life. The current treatment plan for AD is only to reduce the arising symptom with moisturisers and immunosuppressors. The discovery of key property of filaggrin in relations to immunological profile in KCs which confers resultant AD phenotype may provide pharmacological target that may lead to synthesis of drugs that inhibit/potentiate function of target molecule to act as a cure for AD. In conclusion, this project is a necessary investigation to understand the pathway or mechanism by which interaction of environment, filaggrin and immune response in KCs occurs. The results from this investigation may be a stepping stone and be used as reference to a variety of other investigations on possible roles of filaggrin and initiating factor of AD.