SCOTTISH SKIN BIOLOGY CLUB

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Agenda of the 85th SSBC Business Meeting, Thursday 23rd June, Glasgow Caledonian University

1. *Apologies:*
2. *Approval of Minutes of 84th meeting*:
3. *Finances:*
4. *New members*:
5. *Matters arising:*
6. *AOCB*:
7. *Next meeting*:

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Minutes of the 84th SSBC Business Meeting, Thursday 4th November, University of Dundee.

1. *Apologies from the 84th Meeting:*
   Received from Mike Edward, Mary Norval, James Ferguson, Malcolm Hodgins, Patricia Martin, David McEwan Jenkinson, Sue Lewis Jones.

2. *Approval of Minutes of 83rd meeting*:
   These were adopted. Approved by David Greenhalgh, seconded by Richard Weller.

3. *Finances from the 83rd Meeting:*
   Income from the Edinburgh meeting was £375 (25 x £15) and one person paid membership fees (£10). Total income from the meeting was £385.
   Expenditure was: £221.49 for catering.
   As of 19th May 2010 the treasurer account contained £1099.66

4. *New members*:
   At the Dundee meeting, new members proposed were Christabelle Goh, Zoe Venables, Yu Chunshi, Gemma Barron, Silvia Auxilia and Sara Brown. New members were proposed by Julie Woods/Adrian Mason/Hilary Jackson and seconded by David Greenhalgh.

5. *Matters arising from the 84th Meeting*
   Dr Ryan O'Shaughnessy from UCL Institute of Child Health gave a fascinating talk on epidermal barrier acquisition and dysfunction.

6. *AOCB*:

7. *Next meeting*:
   GCU have offered to host the spring meeting in 2011.

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Programme for the 85th SSBC, Thursday 23rd June, Glasgow Caledonian University

10.00-10.25: Registration & Coffee
10.25-10:40 Business Meeting. Chair: Richard Weller

10.40-12.20: Morning Session: David Greenhalgh

10.40-11.00:

Repeat number variation within the profilaggrin gene positively correlates with the risk of developing atopic dermatitis

Karin E. Kroboth,[1] Sara J. Brown,[1,2,3] Aileen Sandilands,[1] Linda E. Campbell,[1] Elizabeth Pohler,[1] Sanja Kezic,[4] Heather J. Cordell,[5] Alan D. Irvine[3] and W.H. Irwin McLean[1]
[1]Division of Molecular Medicine, University of Dundee; [2] Department of Dermatology, Ninewells Hospital & Medical School; [3] Department of Clinical Medicine, Trinity College Dublin, Ireland; [4] Academic Medical Center, Coronel Institute, Amsterdam, The Netherlands; [5] Institute of Genetic Medicines, Newcastle University.

11.00-11.20:

Filaggrin mutations are associated with altered epidermal barrier and antigen presenting cell immunophenotypes in atopic eczema patients.

Sharizan Abdul-Ghaffar(2), Nayani Madarasinga* (1) (2), Zoe Venables (1) Roland Chu (1) Siao Pei Tan (1), Andrew Muinonen-Martin (3), Colin Munro, Jurgen Schwarze* (5) (1), Sarah Howie (5) (1) RichardWeller (2) (5)
(1) University of Edinburgh, Edinburgh, UK; (2) Dermatology Dept, Lauriston Building, Edinburgh, UK; (3) Dermatology Dept, Southern General Hospital, Glasgow, UK; (4) College of Life Sciences, University of Dundee, Dundee, UK; (5) Centre for Inflammation Research, Queen's Medical Research Institute, Edinburgh, UK

11.20-11.40:

Intracellular cholesterol trafficking proteins, STARD4 and D5, regulate cholesterol homeostasis and differentiation status in HaCaT keratinocytes.

H. Elbadawy, P.E. Martin, A. Graham
Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow G4 0BA

11.40-12.00:

Skin-targeted inhibition of PPARβ/δ by selective antagonists to treat PPARβ/δ - mediated psoriasis like skin disease *in vivo.*

John Foerster et al.
University of Dundee

12.00-12.20:

P2Y receptor subtypes and contribution to calcium signalling in equine sweat gland epithelial cells from normal and anhidrotic animals.

S Moran, WH Ko and D Bovell
Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, United Kingdom

12.20-13.10: Lunch

Cont.....

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Afternoon Session: 13.10-15.40: Richard Weller

13.10-14.00: Invited Speaker

Cutaneous xenobiotic metabolism – progress in the 21st Century.
Dr Simon Wilkinson

Medical Toxicology Centre, Wolfson Unit, School of Clinical & Laboratory Sciences, Medical School University of Newcastle upon Tyne.

14.00-14.20:
RNAi depletion of PRSS21 induces apoptosis in Cutaneous Squamous Cell Carcinoma keratinocytes.
K.S. Robinson, S.A. Watt, I.M. Leigh and A.P. South
Centre for Oncology and Molecular Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee

14.20-14.40: Short refreshment break

14.40-15.00:
A discrete point mathematical model for the role of connexins in cell migration events.
Patricia Martin[1], Catherine Wright[1], Steven Webb [2], Michael Watson[3] and Steven MacDougall[3]
[1]Department of Biological and Biomedical Sciences, Institute of Health and Life Sciences, Glasgow Caledonian University, Glasgow; [2] Department of Mathematics and Statistics, University of Strathclyde, Glasgow; and [3]Institute of Petroleum Engineering and Department of Mathematics, Heriot-Watt University, Edinburgh.

15.00-15.20:
KID Syndrome Cx26 Mutations Show and Altered Responses to Bacterial Cell Wall Components.
S. DONNELLY, M.A. VAN STEENSEL, M.B HODGINS AND P.E. MARTIN
Department of Biological and Biomedical Sciences, Institute of Health and Life Sciences, Glasgow Caledonian University, Glasgow.

15.20-15.40
Mechanistic insight how human beta defensin-2 protected skin barrier by neutralizing *Staph. aureus* secreted proteases.
A- Ayub Qureshi. B-Richard Weller **.C** Simon Brown
[1]MRC Center for Inflammation Research, Queens Medical Research Institute
and [2]Department of Dermatology University of Edinburgh.

Close.

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Repeat number variation within the profilaggrin gene positively correlates with the risk of developing atopic dermatitis.

Karin E. Kroboth,[1] Sara J. Brown,[1,2,3] Aileen Sandilands,[1] Linda E. Campbell,[1] Elizabeth Pohler,[1] Sanja Kezic,[4] Heather J. Cordell,[5] Alan D. Irvine[3] and W.H. Irwin McLean[1]

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Atopic dermatitis (AD) commonly known as eczema is a highly irritating and distressing inflammatory skin disorder affecting 10-20% of children and 1-3% of adults worldwide. With the identification of loss of function mutations in the gene encoding for profilaggrin, *FLG*, a highly significant risk factor for AD was found.
Here we looked at three different *FLG* size variants (10, 11, and 12 repeats), their allelic frequency and their contribution to the production of the filaggrin breakdown-derived natural moisturising factor (NMF). We hypothesized that individuals carrying the longest variant of *FLG* express more filaggrin monomers, producing higher amounts of NMF, leading to a protective effect against the development of AD.
The three different *FLG* size variants were screened with long range PCR and SNP genotyping assays, in a case-control study of 876 Irish paediatric AD cases and 928 controls, genotyped previously for the four common *FLG* mutations. The most prevalent *FLG* size variant was 11 repeats (allele frequency 51.5%) then 10 (33.9%) and 12 (14.6%). Statistical analysis revealed a significantly higher number of repeats (Chi-square p=0.043) in the control population. Odds ratio of disease, linking the repeat number with the risk of developing AD, was reduced by a factor of 0.88 for every further expressed *FLG* repeat. Urocanic acid levels, analyzed in 31 patients, showed a small but statistical significant positive correlation between repeat number and amount of filaggrin breakdown product. These findings illustrate the correlation between *FLG* repeat number, the amount of filaggrin protein expressed and the risk of developing AD, highlighting the protective effect of filaggrin.

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Filaggrin mutations are associated with altered epidermal barrier and antigen presenting cell immunophenotypes in atopic eczema patients.

Sharizan Abdul-Ghaffar(2), Nayani Madarasinga* (1) (2), **Zoe Venables** (1) Roland Chu (1) Siao Pei Tan (1), Andrew Muinonen-Martin (3), Colin Munro, Jurgen Schwarze* (5) (1), Sarah Howie (5) (1) RichardWeller (2) (5)

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Filaggrin null mutations (FLG) are associated with atopic dermatitis (AD) and skin barrier defects. We assessed the role of FLG on the skin barrier phenotype and dendritic cell (DC) subtypes. 94 subjects were genotyped for common European FLG variations (R501X and 2282del4) and phenotyped; 62 subjects with AD (13 FLG heterozygotes, 2 homozygote), 32 without AD (8 FLG heterozygotes). We measured trans-epidermal-water-loss (TEWL), hydration, number of tape-strips to raise TEWL>20g/h/m2, SLS irritation and epidermal DC immunophenotypes by flow-cytometry. TEWL was highest in FLG AD 8.4±0.6; WT AD 8.1±0.8; FLG non-AD 7.9±0.7; WT non-AD 7.2±0.4 ($p<0.05$ for FLG vs. WT and FLG AD vs. WT AD). No. tape-strips to reach TEWL>20g/h/m2 was lowest in FLG AD 11.3±1.9; WT AD 14.2±5.7; FLG non-AD 15.3±6.2; WT non-AD 18.9±5.9 ($p<0.05$ for FLG vs. WT, AD vs. non-AD and FLG AD vs. WT AD). The amount of protein removed per strip was greatest in FLG AD 31.2±8.27; WT AD 18.58±6.38; FLG non-AD 3.78±1.01 and WT non-AD 4.04±1.9mg.cm-2. TEWL rose dose-dependently following 24hr application of SLS (0.06-4%); and significantly higher in FLG AD vs. WT AD patients at 1% and 2%. FLG AD subjects had a significantly higher proportion of IDEC and mature DC in their epidermal samples than WT AD subjects. This data infers a complex aetiology of AD where non-AD FLG carriers appear not to develop significant defects. In AD, our study supports the role of filaggrin mutations in defective corneocyte adhesion and epidermal barrier integrity, and a resultant alteration in antigen presenting cells.

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Intracellular cholesterol trafficking proteins, STARD4 and D5, regulate cholesterol homeostasis and differentiation status in HaCaT keratinocytes.

H. Elbadawy, P.E. Martin, A. Graham

Department of Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow G4 0BA

This study explored the hypothesis that cytosolic cholesterol trafficking/sensing proteins, STARD4 and STARD5, members of the START family of lipid trafficking proteins, impact on keratinocyte cholesterol homeostasis and differentiation status, providing a novel strategy to target defects in lipid metabolism causative of a range of skin disorders.

Human immortalised keratinocytes (HaCaT) were transiently transfected (48h) with pCMV.6, pCMV.6_*STARD4* or pCMV.6_*STARD5*. Incorporation of [^{14}C]acetate was used to measure *de novo* lipid biosynthesis, and quantitative polymerase chain reaction and immunoblotting to determine steady state mRNA and protein levels of transcription factors, enzymes, receptors and transporters implicated in cholesterol homeostasis, and markers of keratinocyte differentiation.

Overexpression of each of these cytosolic cholesterol transporters repressed cholesterol and cholesteryl ester biosynthesis from [^{14}C]acetate, maximally by ≥32% ($p<0.05$). However, these changes in endogenous cholesterol biosynthesis were associated with distinct changes in gene and protein expression. For example, *STARD4* overexpression induced a specific increase in *SREBF2* mRNA levels (4.5-fold; $p<0.01$) without significant alteration in gene expression of other lipid-responsive transcription factors, such as *NR1H3* or *PPARA/D/G* and reciprocal regulation of expression of ABC transporters, *ABCA1* (repression) and *ABCG4* (induction). Notably a significant increase (2.6-fold; $p<0.05$) in gene expression of late differentiation marker loricrin in *STARD4* overexpressing keratinocytes was reflected in a small but detectable increase in protein expression.

By contrast, overexpression of *STARD5* increased the expression of *PPARD* (28 fold; $p<0.001$), *PPARG* (1.3 fold; $p<0.01$) and repressed gene expression of *SREBF2* (1.8 fold; $p<0.05$). Downstream, these changes were reflected in substantive increases in gene expression of ABCA (9-fold; $p<0.001$) and repression of LDL receptor (5.6-fold; $p<0.01$); levels of mRNA encoding early differentiation marker keratin 1 (KRT1) also fell by 2.7-fold ($p<0.001$).

Thus, cytosolic cholesterol transporters, STARD4 and STARD5, regulate keratinocyte cholesterol homeostasis and differentiation status, and may provide novel therapeutics for treatment of lipid-related clinical skin disorders.

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Skin-targeted inhibition of PPARβ/δ by selective antagonists to treat PPARβ/δ - mediated psoriasis like skin disease *in vivo.*

John Foerster et al.

University of Dundee

Abstract: Peroxisome proliferator activating receptor β/δ (PPARβ/δ) is overexpressed in psoriasis. PPARβ/δ is not present in adult epidermis of mice. Targeted expression of PPARβ/δ and activation by a synthetic ligand is sufficient to induce an inflammatory skin disease resembling psoriasis. Several signalling pathways dysregulated in psoriasis are replicated in this phenotype, suggesting that PPARβ/δ activation contributes to psoriasis pathogenesis. Thus, inhibition of PPARβ/δ might harbour therapeutical potential. Since PPARβ/δ has pleiotropic functions in metabolism, skin-targeted inhibition harbours the potential of reducing systemic adverse effects. Here, we report that two selective PPARβ/δ antagonists, GSK0660 and compound 3h, can be formulated for topical application to the skin and that their skin concentration can be accurately quantified using ultra-high performance liquid chromatography (UPLC)/ mass spectrometry. These antagonists show efficacy in our transgenic mouse model. Importantly, PPARβ/δ antagonists do not exhibit systemic drug accumulation after prolonged application to the skin, nor do they induce any inflammatory or irritant changes upon application to shaved mouse skin. Our data suggest that topical inhibition of PPARβ/δ to treat psoriasis may warrant further exploration.

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P2Y receptor subtypes and contribution to calcium signalling in equine sweat gland epithelial cells from normal and anhidrotic animals.

S Moran, WH Ko and D Bovell

Biological and Biomedical Sciences, Glasgow Caledonian University, Glasgow, United Kingdom

Equine sweat secretion is mainly under the control of β_2-adrenoceptors, however P2Y receptors are also involved in epithelial transport in varying tissue types, including sweat gland secretion via an increase in chloride permeability and changes in intracellular calcium ($[Ca^{2+}]_i$). Epithelial cells from isolated sweat glands of normal horses release $[Ca^{2+}]_i$ in response to purinergic agonists such as ATP and UTP, suggesting the presence of $P2Y_2$ and $P2Y_4$ receptors. Anhidrosis, or lack of ability to sweat, is a debilitating condition affecting humans and horses thought to be caused by a failure in the secretory process. Little is known about the effect of this condition on equine sweat gland cells as the physiology of sweating in horses is not fully understood. Therefore this study examined the effects of ATP and UTP on $[Ca^{2+}]_i$ and investigated the presence of P2Y receptors in cells from normal and anhidrotic animals.
Epithelial cell lines were obtained from horse skin samples of normal and anhidrotic racing thoroughbreds in Hong Kong. Cells were cultured using standard tissue culture techniques. Epithelial cell lysates (3 normal, 6 anhidrotic) were used for Western blotting using antibodies against $P2Y_2$ and $P2Y_4$ receptors. Cells were examined for changes in $[Ca^{2+}]_i$ using the calcium-sensitive dye Fura-2 AM and calcium imaging techniques. Statistical analyses were carried out using one-way ANOVA and Bonferroni multiple comparison test, where $P<0.01$ was considered significant.
Western blot analysis showed bands for $P2Y_2$ and $P2Y_4$ in both normal and anhidrotic cell lines, with no difference in band density observed. ATP and UTP evoked significantly higher $[Ca^{2+}]_i$ responses in anhidrotic cells (ATP 0.417±0.140; UTP 0.549±0.201 ratio units) in comparison to normal cells (ATP 0.185±0.066; UTP 0.173±0.064 ratio units). Removal of extracellular calcium in the normal cells did not evoke a significant change in response to either ATP or UTP. However, in the anhidrotic cells, the absence of external calcium caused a significant change in both the ATP (60% reduction; $P<0.0001$) and UTP (74% reduction; $P<0.0001$) responses.
Western blot results have confirmed the presence of $P2Y_2$ (ATP & UTP-sensitive) and $P2Y_4$ (UTP-sensitive) receptors in both normal and anhidrotic cells from equine sweat glands, which is supported by the increase in $[Ca^{2+}]_i$ in response to ATP and UTP in both cell types. The significant reduction in $[Ca^{2+}]_i$ upon removal of extracellular calcium in response to both ATP and UTP in anhidrotic cells indicates that influx of calcium has a role to play in the anhidrotic condition, although whether it is a cause or symptom of the condition remains to be discovered. Increased $[Ca^{2+}]_i$ has been shown to increase apoptosis in pancreatic acinar cells, thus it is possible that apoptosis could be involved in anhidrosis.

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RNAi depletion of PRSS21 induces apoptosis in Cutaneous Squamous Cell Carcinoma keratinocytes.

K.S. Robinson, S.A. Watt, I.M. Leigh and A.P. South

Centre for Oncology and Molecular Medicine, Ninewells Hospital and Medical School, University of Dundee, Dundee

In the UK, over 1 in 4 skin cancer deaths can be attributed to non-melanoma skin cancer, principally cutaneous squamous cell carcinoma (cSCC) – the most common skin neoplasm with malignant potential. High risk groups exist, including immunosuppressed and recessive dystrophic epidermolysis bullosa patients, where cSCC is a major complication resulting in increased morbidity and mortality. Targeted therapies capable of halting growth and metastatic potential of cSCC remain an unmet clinical need.

Protease, Serine, 21 (Testisin encoded by the gene *PRSS21*) is a GPI-linked membrane anchored protein frequently overexpressed in malignant tissues and shown to promote tumour progression and metastasis. We show that PRSS21 is over expressed in cSCC in vitro (7.4 fold, $p<0.0005$) and in vivo (averaged fold change of 1.5 over multiple data sets) compared with normal skin. RNAi knockdown of *PRSS21* significantly ($P<0.005$) increases cytotoxicity (20%±5% *PRSS21* vs non-targeting control siRNA n=4) as measured by LDH release and decreases cell viability (40%±5% of non-targeting control siRNA, n=3) in cSCC cells without affecting normal primary keratinocytes as determined by MTS assay. Depletion of *PRSS21* resulted in induction of apoptosis in cSCC as detected by a 2.3 (SD±0.4) fold increase in cytoplasmic nucleosomes and a 25% (SD ± 5) increase in annexin V/7AAD positive cells. Additionally, *PRSS21* depletion resulted in a 60% increase in expression of *maspin*, a novel serine protease inhibitor and known tumour suppressor. In conclusion, we have identified a potential therapeutic cancer target - *PRSS21* which is overexpressed in cSCC and whose siRNA-mediated knockdown results in induction of cell death and reduction of cell viability *in vitro*. Further studies will continue to evaluate *PRSS21* as a viable therapeutic target.

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A discrete point mathematical model for the role of connexins in cell migration events

Patricia Martin[1], Catherine Wright[1], **Steven Webb** [2], Michael Watson[3] and Steven MacDougall[3]

[1]Department of Biological and Biomedical Sciences, Institute of Health and Life Sciences, Glasgow Caledonian University, Glasgow; [2] Department of Mathematics and Statistics, University of Strathclyde, Glasgow; and [3]Institute of Petroleum Engineering and Department of Mathematics, Heriot-Watt University, Edinburgh.

Central to skin wound repair is deposition of extracellular matrix (ECM) and the rate at which dermal fibroblasts and keratinocytes move to fill in the wound 'gap'. These events are altered during diabetes, resulting in slowly healing wounds susceptible to sustained inflammatory responses and infection. The ability of cells to communicate directly with each other is critical in organising such cellular activities. One way cells do this is via a gap junction network of intercellular communication channels composed of a family of proteins the connexins, of which connexin43 (Cx43) is the most widely expressed. Dynamic and localised modulation of Cx43 during wound repair in both dermal fibroblasts and epidermal keratinocytes occurs. In normal wounds, Cx43 is down-regulated at the regenerating wound edge, while in chronic wounds, Cx43 expression is increased at the wound edge; and in diabetic rodent models the protein is abnormally expressed at wound edges and proposed to be associated with delayed wound closure (Brandner *et al*., 2004). Using a range of *in vitro* organotypic models we have determined highly specific peptides targeted to Cx43 improve *in vitro* wound closure events and have potential as therapeutic tools to improve wound healing events (Wright *et al*., 2009, Pollock *et al*., 2010). Time-lapse microscopy analysis reveals that application of the peptides modifies rates and directionality of cell movement.

To enable us to better predict and optimise experimental parameters we have begun to develop a mathematical model to produce an accurate and validated description of the role of connexins and the impact of the peptide on the wound healing process.

Using a point-based discrete cell modelling approach similar to that developed by Dallon *et al.* (2000) we will discuss the merits of how such mathematical models can be used to examine the differential role of connexins in normal and chronic wounds with a view to ultimately suggesting a novel therapeutic protocol.

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KID Syndrome Cx26 Mutations Show and Altered Responses to Bacterial Cell Wall Components.

S. DONNELLY, M.A. VAN STEENSEL, M.B HODGINS AND P.E. MARTIN
Corresponding author: Steven.Donnelly@gcal.ac.uk

Connexin (Cx) mediated intercellular communication is essential for the development and maintenance of the epidermis but the functional role is not yet fully understood. There are a wide range of genetically inherited skin disorders associated with mutations in Cx26, illustrating the important role of Cxs in the epidermis. Mutations such as G12R, N14K and D50N give rise to Keratitis-ichthyosis-deafness (KID) syndrome, a hyperkeratotic skin disease associated with chronic inflammation. Recurrent bacterial and fungal infection in these patients suggests that Cx26 has a role in maintaining the epidermal barrier and may have some function in innate immunological responses. Protein trafficking studies, using GFP linked connexins, show that KID syndrome mutants have the ability to traffic to the plasma membrane, unlike other Cx26 syndromic mutations (e.g. D66H, associated with Vohwinklel syndrome), where they may form "leaky" connexin hemichannels that maybe partially be responsible for the pathogenesis of KID syndrome. Hemichannel activity in cells expressing KID mutants was confirmed, by measuring ATP concentration in the media following a switch from high calcium to low calcium media, a response that was blocked using the gap junction blocker carbenoxelone (CBNX). Non KID syndrome Cx26 mutants showed no hemichannel activity. Further analysis showed that KID mutant hemichannel activity was more sensitive to the bacterial cell wall component peptidoglycan (PGN), 10ug/ml, from *S. aureus* and *S.epidermidis* in HeLa Ohio cells than in cells transfected to express Cx26 wild-type (Wt) or non-KID Cx26 mutants (n=3, P=<0.001) . This effect was blocked with CBNX. Interestingly PGN from *S. aureus* and *S.epidermidis* stimulated hemichannel activity in in HeLa Ohio cells but only *S. aureus* PGN showed a response in HaCat cells, a keratinocyte cell line.. Quantitative PCR analysis revealed increased expression of the pro-inflammatory cytokine IL-6 following 6 hr PGN challenge of KID syndrome mutants (n= 3, P=<0.01). Increased ATP release through "leaky" hemi channels may affect downstream signalling cascades, such as IL-6, and KID syndrome pathology may be as a result of altered signal transduction cascades initiated by these faulty hemichannels.

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Mechanistic insight how human beta defensin-2 protected skin barrier by neutralizing *Staph. aureus* secreted proteases.

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We are interested in better understanding why atopic dermatitis (AD) patient's exhibit increased susceptibility to viral and microbial infections, especially *Stahlococcus aureus (S.aureus)*. Knowing that *S.aureus* secrete a variety of proteases, we reasoned that increased susceptibility and colonization in AD patients may reflect on antiprotease imbalance. where increased breakdown of junctional and desmosomal adhesions may contribute to a breakdown in skin barrier function. We therefore examined the effect of *S. aureus* secreted proteases on an intact monolayer of HaCat cells, a keratinocyte cell line. Damage to the HaCat monolayer was observed as a loss in junctional and desmosomal contacts, cell membrane integrity and the widespread appearance of apoptosis throughout the monolayer. The key agents secreted by *S. aureus* responsible for the gross effects on HaCaT monolayer's were a pore forming agent, presumably PVL, and V8 protease. We also discovered that pre-treatment of HaCaTs with both IL-1β and LTA protected the monolayer and that this could be attributed to the induced expression of human β-defensin, we further demonstrated that hBD2 is a novel antiprotease that inhibit both V8 & staphopain. Importantly, we could replicate the deleterious effect of *S. aureus* conditioned media with purified V8 protease in the presence of either perforin or saponin as substitutes for PVL which is not commercially available. While V8 protease, apoptosis and corneodesmosomal breakdown was blocked with human β-defensin, cell permeability was unaffected as measured by either Trypan blue staining or cytoplasmic release of lactate dehydrogenase . Thus, we conclude that V8 protease and PVL, virulence factors for *S.aureus*, may function analogously to Granzyme B and perforin at inducing apoptosis in HaCaT cells, where Granzyme B and V8 are both aspartase proteases while both perforin and PVL are cholesterol dependent cytolysins (CDC).