

These articles have been accepted for publication in the *British Journal of Dermatology* and are currently being edited and typeset. Readers should note that articles published below have been fully refereed, but have not been through the copy-editing and proof correction process. Wiley-Blackwell and the British Association of Dermatologists cannot be held responsible for errors or consequences arising from the use of information contained in these articles; nor do the views and opinions expressed necessarily reflect those of Wiley-Blackwell or the British Association of Dermatologists

Accepted Date : 12-Nov-2012

Article type : Review Article

Why does allergic contact dermatitis exist?

J.P. McFadden¹, P.Puangpet¹, D.A. Basketter², R.J. Dearman³ and I. Kimber³

¹Department of Cutaneous Allergy, St John's Institute of Dermatology, St Thomas' Hospital, London SE1 7GN, UK (for correspondence john.mcfadden@kcl.ac.uk)

²DABMEB Consultancy Ltd., Sharnbrook MK44 1PR, UK

³Faculty of Life Sciences, University of Manchester, Michael Smith Building, Oxford Road, M13 9PT, UK

Key words: allergic contact dermatitis, hapten, microbial commensals, microbial pathogens, acne vulgaris, hidradenitis suppurativa, acne rosacea

Abstract

The skin immune system's propensity to produce allergic contact dermatitis (ACD) to harmless chemicals, whilst otherwise being an efficient defence system, represents a

dermatological paradox. We postulate that a major role in signaling in ACD is played by Toll-like receptor (TLR) 2 and TLR4 that arises from their activation by extracellular DAMPs (danger associated molecular patterns). Ligand activation of TLR4/2 results in the expression of interleukins (IL) IL-1 β , IL-6, IL-12, IL-18 and IL-23, tumour necrosis factor- α (TNF- α) and interferon- α (IFN- α). These cytokines promote acquisition of sensitisation, and facilitate elicitation of contact allergy, via multiple mechanisms, including the recruitment of CD4+ T helper (Th) cells, Th1 and Th17 cells. As Th1 cells secrete large amounts of Danger Associated Molecular Pattern molecules (DAMPs), a DAMP immune circuit (positive feedback loop) is created. This is an important driver of skin sensitisation and skin inflammation. Pathogenic extracellular bacteria, but not commensal bacteria, produce PAMPs (Pathogen-Associated Molecular Pattern molecules), that stimulate the expression of Th1 and Th17-promoting cytokines via TLR 2 and 4. This also induces an immune circuit. The ability of the skin immune system to activate host defence mechanisms and to distinguish between pathogenic bacteria and commensals provides an explanation why skin sensitisation and ACD develops; processes that rely on the same biological pathways. These pathways may also shed light on pathogenesis of chronic pustular inflammatory dermatoses (e.g. acne vulgaris). The existence of safety signals from commensal bacteria that prevent initiation of these pathways may represent opportunities for novel therapeutic approaches to the treatment of inflammatory skin diseases.

Introduction

The skin immune system has evolved into an extremely effective mechanism for providing host resistance to infectious and malignant disease. One consequence of possession of a finely tuned adaptive immune system is that the ability to distinguish between harmless or self antigens, and those that signal invasion by pathogenic microorganisms or malignant cells is imperfect. The result is allergy or autoimmunity. The skin is no exception, and an important and prevalent form of skin allergy is allergic

contact dermatitis (ACD) that results from the cutaneous immune system ‘mistaking’ encounter at skin surfaces with what are commonly innocuous chemicals for incursions that threaten health. In simplistic terms, therefore, skin sensitisation, in common with most other forms of allergy, can be described as an immune response of little or no value to the host, to an antigen from which protection is not required, and that results in adverse health effects. Allergic contact dermatitis to agents such as nickel, hair dyes and plant products is morbidity without benefit, and apparently a price that must be paid for possession of an alert and responsive cutaneous immune system. To explore the balance between the critical role the skin immune system plays in host defence, and how those functions become subverted in ACD, requires an appreciation of the relevant biological mechanisms. Such an exploration is now timely because our understanding of the events and processes that are associated with, and necessary for, the induction of skin sensitisation, and elicitation of ACD is becoming more sophisticated.

A key consideration in addressing this subject is the role that ‘danger signals’ play in modulating adaptive immune responses, and in attempting to focus the attentions of the immune system where it is most needed. In general terms, danger signals, that may derive from either endogenous or exogenous sources are required as a supplementary signal, together for antigen, for the initiation of an immune response. The argument is that antigen, in the absence of ‘danger’, does not provide a health threat, and is therefore not worthy of the attention of the immune system.¹ Moreover, the inability to respond to antigen in the absence of other signals provides one mechanism through which autoimmune responses are avoided.

Against that background we and others have postulated that the recognition of haptens (contact allergens) in the absence of danger signal leads to immunological tolerance, whereas the in the presence of appropriate danger signals allergic sensitisation will be acquired.^{2,3} Because chemical allergens are commonly able – at least at concentrations

that will result in sensitisation – to deliver both an antigenic stimulus and danger signal(s), skin sensitisation and subsequently ACD are able to develop without the need for any additional intervention. We and others have proposed that the most important route for the expression and delivery of danger signals resulting from topical exposure to chemical allergens is secondary to cell and tissue trauma and the availability of “injury” extracellular matrix proteins such as fibronectin, hyaluronan and β -defensins that serve as endogenous ligands for the extracellular Toll like receptors (TLR), and in particular TLR4.^{4, 5, 6} These injury proteins are part of the ‘Danger Associated Molecular Pattern proteins’ (DAMPs) family. Stimulation of the TLR receptors triggers the production of proinflammatory cytokines that in turn promote the acquisition of skin sensitisation, and subsequently the elicitation of ACD.

There has been recent interest in common innate signalling pathways of the skin immune system shared between the response to microbial infections and allergic contact dermatitis.^{7, 8, 9} An important aspect of the skin immune system, and indeed of the immune system *per se*, is that the ability to identify and respond to pathogenic microorganisms must exist in tandem with tolerance toward commensal microbes. Allergens from extracellular microbes have not been well studied, but examples of these, such as neomycin and fucidin, differ from chemical allergens in that, despite being antigenic, they commonly do not elicit appropriate danger signals. It may be, therefore, that potentially pathogenic bacteria differ from their commensal counterparts in terms of their ability to induce and deliver the necessary danger signals that will support immune activation and the development of protective immune responses. Such PAMPs (Pathogen Associated Molecular Pattern molecules) are ligands for plasma membrane TLR4 and TLR2 (such as lipopolysaccharide, peptidoglycan, leipotechoic acid), and commensal bacteria do not normally express these. Thus, the argument is advanced that the basis for the ability of the skin immune system to distinguish between potentially pathogenic microorganisms and commensal bacteria resides in the differential

availability of appropriate danger signals. This model would also predict that common inflammatory pustular conditions such as, for instance, acne vulgaris, acne rosacea and hidradenitis suppurativa, may in reality be the consequence of the failure of normal immunological tolerance to commensal skin bacteria. Thus *P.acnes* is a skin commensal present in sebaceous glands but the presence in acne sufferers of high sebum levels of immunostimulatory saturated fatty acids, which stimulate TLR4, could deliver a danger signal that leads to aberrant immune response to this commensal bacteria.

An efficient skin immune system

The skin immune system has evolved to act as an efficient (defensive) asset. Its main purpose is to combat pathogenic microorganisms (including bacteria), as well as malignant disease.¹⁰ Thus, for instance, a declining effectiveness of the cutaneous immune system associated with ageing, as part of a generalised immunosenescence, is associated with an increased prevalence of skin infections and cutaneous malignant disease.¹¹ The skin immune system is also involved in other activities that contribute either directly, or indirectly, to host defence, including wound healing, angiogenesis and regulation of cell growth and differentiation. However, as well as reacting to hostile microbes it also has to tolerate benign commensal microbes, and appears to do this with remarkable precision.

An efficient skin immune system and allergic contact dermatitis: an immunological paradox?

A conspicuous exception to the efficiency of the skin immune system is allergic contact dermatitis. Many hundreds of chemicals are known to have the potential to cause acquisition of skin sensitisation, secondary to the induction of a cutaneous immune response. If a sensitised subject is exposed subsequently, at the same or a different skin site, to the inducing chemical allergen then an accelerated and more aggressive

secondary immune response will be provoked that causes the local inflammatory reaction in the skin that is recognised clinically as ACD. The chemicals which are able to cause the acquisition of skin sensitisation are known as contact allergens or haptens. By definition, these are low molecular weight molecules which can gain access to the viable epidermis/dermis and trigger skin immune responses. However, haptens are too small to trigger an immune response, so to achieve that they must associate in stable fashion (usually covalently) with proteins. Thus, skin sensitising chemicals are naturally electrophilic, or can be converted to an electrophilic species in the skin, such that binding to nucleophilic amino acids (usually, but not exclusively, cysteine and lysine).¹² Binding to the skin confers immunogenicity. Examples of skin sensitising chemicals include fragrance materials, certain metals, preservatives used in toiletries and personal care products, resins, medicated materials and plant chemicals. Allergic contact dermatitis to these chemicals is common, and it has been estimated to affect 20% of the European adult population^{13, 14} and that 34% of adult Thai women are allergic to nickel.¹⁵ In addition, the reported annual incidence of work related allergic contact dermatitis is reported to be 0.5-1.9%.¹³ Consistent with these high rates of ACD is the evidence that 100% of adults can be sensitised experimentally to the more powerful contact allergens.¹⁶ The ability to acquire skin sensitisation and to support the elicitation of ACD appears therefore to be integral properties of the skin immune system. However, by inducing inflammatory reactions to these commonly harmless chemicals the skin immune system appears to cause unnecessary tissue damage and significant morbidity for the human host without providing any obvious benefit.

Unravelling this paradox requires an appreciation that the recognition of, and response to, contact allergens, are fundamental characteristics of the skin immune system. Required also is an awareness of recent advances in our understanding of the immunological mechanisms that underlie ACD.

Immune mechanisms of allergic contact dermatitis incorporating danger theory

We have previously outlined models of allergic contact dermatitis aligned with the danger theory.^{2,3} In such models immune recognition of antigen in the absence of appropriate danger signals leads to immunological tolerance (or at least a failure to elicit an immune response) rather than immunity and sensitisation. However, in the presence of danger signals immune recognition leads to a cutaneous immune response and the acquisition of skin sensitization (Figure 1). Most contact allergens have the ability to deliver both antigenic and danger signals, and these can be considered to represent 'complete allergens'. In such instances the danger signals are related to irritant-type properties of the inducing chemical allergens² results in cell and tissue trauma and the elaboration of DAMPs. Indeed, there is some relationship between irritancy and skin sensitization potential of contact allergens¹⁷ and others have also reported a relationship between sensitizing activity and irritancy.¹⁸

The innate immune system is equipped with receptors that permit recognition of PAMPs that derive from bacteria, fungi and viruses. Of this family of receptors TLR are the most prominent. The extracellular TLR2 and TLR4 recognise bacterial proteins such as lipopolysaccharide and peptidoglycans, and thereby support the adaptive immune system in elaborating responses against invading pathogenic microorganisms.^{14, 15} However these TLRs are equally well equipped to recognise and respond to DAMPs that derive from the traumatic changes that may be associated with topical exposure to skin sensitizing chemicals.¹⁹ Among the endogenous TLR ligands (danger signals) that may be elaborated in response to cell or tissue damage are: (extradomain A+) fibronectin, hyaluronan, cathelicidin and heparin sulphate.^{21, 22, 23, 24} Ligand-activation of TLR4 and TLR2 leads to increased expression of a variety of proinflammatory cytokines including: IL-1, TNF α , IL-6, IL-12, IL-18, IL-23 interferon- α (IFN- α).^{4, 5, 6}

Cutaneous insult in the form of irritant chemicals results in expression of these DAMP ligands of extracellular TLRs, particularly TLR4 such as hyaluronan, fibronectin and heparan sulphate.^{4, 21, 22, 23, 24} Similarly expression of several endogenous ligands of TLR4 are recorded following cutaneous exposure to contact allergens, including fibronectin, HSP70, hyaluronan and β -defensin.^{4,5}

As Martin and colleagues²⁵ have shown experimentally that TLR4 and TLR2 activation is essential for skin sensitization it would appear that stimulation of membrane-expressed TLR via the elaboration of endogenous ligands is the key event for successful danger signalling by contact allergens.

Other innate receptors appear to contribute to the signaling pathways in ACD and have recently been reviewed.^{7, 26} Several papers have shown an important role for NLR(Nucleotide-binding domain leucine-rich repeat containing receptors) signaling in ACD through activation of caspase-1 and subsequent expression of IL-1 β .^{27, 28, 29, 30} For example NLRP3 deficient mice display an impaired response to skin sensitizers.²⁹ Signalling of other parts of the innate immune system, for example C-type lectin receptors may also play a role.²⁶

Martin's group demonstrated that contact sensitizers induce reactive oxygen species which in turn generated DAMP molecules such as low molecular weight fragments of hyaluronic acid which can activate TLR4.⁴ Activation of TLR4 in this way will result in the induction or increased expression of IL-1 β and TNF- α , both of these cytokines are known to be required for contact allergen induced mobilization and migration of epidermal Langerhans cells (and possibly of dermal dendritic cells) as an important first step in the delivery of antigen to skin-draining regional lymph nodes.³¹ In addition, the cytokines up-regulated by the activation of TLR4 will, collectively, also stimulate

vascular endothelial adhesion molecule expression and the production of various chemokines that will in turn favour Th1/Th17 cell responses.^{5, 6}

Three other factors are worthy of note:

- 1) Recruited Th1 cells can secrete substantive amounts of the DAMP protein and endogenous ligand of TLR4, extracellular matrix A+ fibronectin, thereby triggering a positive feedback mechanism that will further reinforce the process of innate and adaptive immune activation.³² Furthermore Th1-associated cytokines stimulate production of another TLR4 ligand, hyaluronan, thereby reinforcing this feedback loop.³³
- 2) Th2 cytokines reduce TLR4 function, thus antagonising this pathway in atopic inflammation.⁶
- 3) Repeated topical application of chemical allergens in experimental systems can result in a switch from a Th1-selective response to a Th2-type response.³⁴

Thus, chemical allergens normally deliver 2 independent signals. One is an antigenic signal that is based upon the chemical itself. The second is a danger signal that results usually from traumatic changes to cells and tissues local to the site of encounter with chemical allergen. The presence or absence of the danger signal determines whether a cutaneous immune response and skin sensitization develops, or whether instead there is either a failure to initiate an immune response, or the development of immunological tolerance occurs.

In allergic contact elicitation reactions (Figure 2) the haptens will release DAMP molecules which cause TLR4/2 stimulation. Release of proinflammatory cytokines such as IL-1 β and TNF α will lead to vascular and chemokine expression with recruitment of Th1/Th17 cell recruitment.^{4, 5, 6} As at least Th1 release significant quantities of DAMP³² a DAMP/TLR4/Th1Th17 or DAMP immune circuit is created to drive the inflammation. (Figure 3).

Microbial haptens differ from chemical haptens

Microbial haptens differ from chemical haptens in delivering little or no danger signals.

Although not well studied there are two examples which demonstrate this phenomenon;

a) *contact allergens*: Aminoglycosides are used as topical antibiotics to treat skin infection. Most of them are naturally occurring, produced by the actinomycetes group of antibiotics.³⁵ By experimental evaluation of skin sensitizing properties (using the occluded patch test of Buehler) these chemicals were found to have very weak sensitizing potential.³⁶ Fusidic acid, derived from the fungus *Fusidium coccineum*, is another microbial hapten used principally as a topical antibiotic that has been classified as a contact allergen. However, when tested experimentally fusidic acid failed to display skin sensitizing potential.³⁶ These agents would only appear to act as contact allergens because they are applied to already inflamed skin, where danger signalling is already in place.

b) *vaccines*: Microbial haptens/antigens used for vaccines are generally at best weak sensitisers.^{37, 38} Adjuvants have long been used to not only achieve and/or increase success of vaccination, but also to target more efficiently the selected pathogens.³⁹ Although alum has traditionally been favoured as an adjuvant, microbially derived preparations have been developed that engage with the innate immune system, particularly through TLR activation.^{39, 40}

A pattern emerges whereby microbial haptens differ from chemical haptens in not provoking significant danger signals.

Microbial ligands for Toll-like receptors; a potential for the skin immune system to discriminate between microbial pathogens and commensals.

As haptens derived from microbes are apparently delivering no, or only very weak,

danger signals, this leaves the skin immune system with the need to differentiate between commensals and pathogenic bacteria. This is achieved due to the possession by pathogens of PAMPS that are recognised readily by the innate immune system. There are 10 TLRs in humans and the microbial proteins they recognise are determined to some extent by their cellular localisation (intracellular receptor or those displayed at the plasma membrane).⁴¹ The two extracellular/plasma membrane TLRs, TLR2 and TLR4, represent the main innate receptors that are triggered by bacterial cell wall components to combat extracellular bacteria.⁴¹

Lipopolysaccharide, a component of the outer wall of gram-negative bacteria, is recognised by TLR4.⁴² This receptor also recognizes respiratory syncytial virus fusion proteins and *Strep. pneumoniae* pneumolysin.⁴² In contrast, TLR2 recognizes lipopeptides, peptidoglycans and lipoteichoic acid from gram positive bacterial pathogens, lipoarabinomannan from mycobacteria, zymosan from fungi, and tGP-1-mucin from *Trypanosoma cruzii*.⁴²

Again a pattern emerges. The skin immune system recognises danger signals using innate immune receptors, and in particular the TLRs. The microbial ligands for TLRs appear to derive from microorganisms that are pathogens, or that have the ability to become pathogens. In common with skin sensitization, it is hypothesized that recognition of antigens in the absence of appropriate danger signals will fail to result in the initiation of a protective immune response, and may cause immunological tolerance. The principal difference between the two is that 'complete' chemical allergens deliver both the antigen and (indirectly) the danger signal, whereas in the case pathogenic microorganisms the antigenic signal and the danger signal are delivered by different molecules microbial proteins.

Further proof that shared mechanisms exist between the skin immune response to extracellular pathogens and contact allergens exist can be found in the observation that

that the level of experimental allergy-inducing nickel concentrations is markedly lowered by the TLR4-recognising bacterial PAMP lipopolysaccharide at both the sensitization and elicitation steps.⁴³ Another study showed that patients bearing metal orthopaedic implants were more than twice as likely to have contact sensitivity to metal contained within the implant when there had been infection.⁴⁴

Applying ‘skin sensitization danger theory’ to chronic pustular skin conditions. (Figures 4 and 5)

The danger signal models also provides a compelling explanation for why certain inflammatory pustular skin conditions occur. Acne vulgaris, pustular rosacea and hidradenitis are characterized by inflammatory pustular reactions that one would expect to be associated with an anti-bacterial immune response. However unlike suppurative responses to bacterial pathogens, the inflammation in these conditions is chronic and can be destructive. Importantly, cultures from acneiform chronic inflammatory pustular conditions tend to show the presence only of commensals, and the presence of the commensal *P. acnes* is a consistent finding in microbiological studies of acne vulgaris.⁴⁵ There is a very significant increase in isolation of the skin commensal *Staph.epidermis* in pustular rosacea.⁴⁶ *Demodex*, another skin commensal, has also been associated with pustular rosacea.⁴⁷ Recently characterization of deep swabs from resistant hidradenitis suppuritiva has been reported.⁴⁸ All swabs grew the commensal *Staph.epidermis*, but none grew the pathogen *Staph.aureus*.

A clue to the pathogenesis may come from reviewing the sebum environment in acne vulgaris. In comparison with healthy controls, acne sufferers produce higher ratios of certain fatty acids such as palmitic acid and palmitoleic acid in the comedonal sebaceous glands.⁴⁹ Saturated fatty acids such as these acids are known to stimulate TLR4 and therefore elicit danger signaling either directly or through stimulating the production of endogenous TLR ligands.⁵⁰ The argument is, therefore, that in acne, danger signalling

from the sebum, or from other local agents/conditions, could cause the skin immune system to mistake commensals for pathogenic bacteria causing an immune response to be mounted resulting in a severe inflammatory reaction. As the commensals are programmed to inhabit the skin removing them is unsuccessful and a chronic pustular inflammatory rash results. Consistent with this hypothesis is the fact that certain TLR4 polymorphisms are known to protect against acne conglobata.⁵¹ Moreover, some effective treatments for acne have been shown to down-regulate local TLR2 and TLR4 expression.⁵² Likewise the local environment in hidradenitis and rosacea may provide danger signalling that results in pustular reaction to commensals. TLR2 has been reported to be up-regulated in both hidradenitis and rosacea.^{41, 42}

Danger signals and safety signals

The danger hypothesis argues that it is the presence or absence of danger signals that determines whether a hostile immune response will be initiated in response to encounter with an antigen. However, as a protective measure, commensal bacteria may actively induce signals to indicate the opposite of danger, that is 'anti-danger' or 'safety' signals. The argument is that rather than relying solely on the absence of appropriate danger signals to maintain tolerance, and avoid initiation of an immune response, positive action is taken. There are intriguing recent reports indicating that some cell wall proteins derived from symbiotic bacteria actively antagonize TLR4 signalling.⁵⁵ These have the ability to antagonise TLR4 stimulation at micromolar concentrations.⁵⁶ The discovery of these 'safety signals' could have important therapeutic potential. For example, it is tempting to speculate that conventional encounter with contact allergens in the absence of danger signals, but in the presence of safety signals, would not only prevent the acquisition of skin sensitization, but also result in the induction of immunological tolerance.

Concluding remarks; Why does allergic contact dermatitis exist?

A note of caution should be given regarding this review. Our current knowledge of the role of TLRs in allergic contact dermatitis rest with metal ions and experimental contact allergens such as oxazolone.^{25,57} Some contact allergens may bypass the need for expression of DAMPs by binding directly to TLR4.⁵⁷ However there is a consensus developing for a pivotal role for the extracellular TLRs in allergic contact dermatitis. The question posed initially is why does allergic contact dermatitis exist? Lifestyle in Western Society is characterized by a personalized environment rich in chemical allergens/haptens. We postulate here that the ability to acquire skin sensitization and to subsequently elicit ACD is a fundamental feature of the mammalian immune system because in most instances inducing chemical allergens are able to combine delivery of antigen with the elicitation of appropriate danger signals. That is contact allergy 'makes use' of the conserved mechanism that permits the mammalian immune system to distinguish between harmless and beneficial commensal bacteria and potentially dangerous pathogenic microorganisms. An understanding of the danger model of skin sensitization, may provide the basis for development of novel therapeutic strategies for combating allergic contact dermatitis.

References

- 1) Matzinger P. Tolerance, danger, and the extended family. *Ann Rev Immunol* 1994; **12**: 991-1045.
- 2) McFadden JP, Basketter DA. Contact allergy, irritancy and 'danger'. *Contact Dermatitis* 2000; **42**: 123-127.
- 3) Kimber I, Cumberbatch M, Dearman RJ, Griffiths CE. Danger signals and skin sensitization. *Br J Dermatol* 2001; **147** (3): 613-614.

- 4) Esser PR, Wolfle U, Durr C *et al.* Contact sensitizers induce skin inflammation via ROS production and hyaluronic acid degradation. *PLoS One* 2011; **7** (7): e41340. Epub
- 5) McFadden JP, Dearman RJ, White JML *et al.* The hapten-atopy hypothesis II: the 'cutaneous hapten paradox'. *Clin Exp All* 2011a; **41**: 327-337.
- 6) McFadden JP, Basketter DA, Dearman RJ, Kimber IR. Extra domain A-positive fibronectin-positive loops and their association with cutaneous inflammatory disease. *Clin Dermatol* 2011b; **29**: 257-265.
- 7) Martin SF. Contact Dermatitis: from pathomechanisms to immunotoxicology. *Experimental Dermatology* 2012; **21**: 382-389.
- 8) Martin SF. Allergic contact dermatitis: xenoinflammation of the skin. *Curr Opin Immunol* 2012; **24**: 1-10.
- 9) Freudenberg MA, Esser PR, Jakob T *et al.* Innate and adaptive immune responses in contact dermatitis : analogy with infections. *G Ital Dermatol Venereol* 2009; **144** (2): 173-85.
- 10) Di Meglio P, Perera GK, Nestle FO. The multitasking organ: recent insights into skin immune function. *Immunity* 2011; **35** (6): 857-869.
- 11) Rymkiwicz PD, Heng YX, Vasudev A, Larbi A. The immune system in the aging human. *Immunol Res* 2012; **53** (1-3): 235-250.
- 12) Karlberg A-T, Bergstrom MA, Borje A *et al.* Allergic contact dermatitis - formation,

structural requirements, and reactivity of skin sensitisers. *Chem Res Toxicol* 2008; **21**: 53-69.

13) Peiser M, Tralau T, Heidler J *et al.* Allergic contact dermatitis: epidemiology, molecular mechanisms in vitro methods and regulatory aspects. *Cell Mol Life Sci* 2012; **69**: 763-781.

14) Coenraads P-J, Uter W, Diepgen T. (2011) Epidemiology. In “Contact Dermatitis”, 5th edition, Eds Johansen J D, Frosch PJ, Lepoittevin J-P, Springer, Berlin., 193-214.

15) White JML, Gilmour NJ, Jeffries D *et al.* A general population from Thailand: incidence of common allergens with emphasis on para-phenylenediamine. *Clin Exp Allergy* 2007; **37**: 1848-1853.

16) Kligman AM. The identification of contact allergens by human assay. The maximisation test; a procedure for screening and rating contact sensitisers. *J Invest Dermatol* 1966; **47**: 393-409.

17) Basketter DA, Kan-King-Yu D, Dierkes P, Jowsey IR. Does irritation potency contribute to the skin sensitization potential of contact allergens? *Cutan Ocul Toxicol* 2007; **26** (4): 279-286.

18) Jacobs JJ, Lehe CL, Cammans KD *et al.* Assessment of contact allergens by dissociation of irritant and sensitizing properties. *Toxicol in Vitro* 2004; **18** (5): 681-690.

19) Erridge C. Endogenous ligands of TLR2 and TLR4: agonists or assistants? *J Leuk Biol* 2010; **87**: 989-999.

20) Cristofaro P, Opal SM. Role of toll-like receptors in infection and immunity. *Drugs* 2006; **66** (1): 15-29.

21) Kim JE, Kim B, Kim H *et al.* Retinyl retinoate induces hyaluronan production and less irritation than other retinoids. *J Dermatol* 2010; **37** (5): 448-454.

22) Dorner RW. Effect of nitrogen mustard on hyaluronate. *Biochem Pharmacol* 1969; **18** (10): 2339-2246.

23) Poulsen JH, Cramers MK. Dermal glycosaminoglycans characterise the primary irritant dermatitis in psoriatics and healthy individuals. *Clin Chim Acta* 198; **130** (3): 305-315.

24) Martin AP, Ortiz S, Cabalier ME *et al.* Vascular endothelium express CS-1 fibronectin in allergic contact dermatitis. *J Cutan Pathol* 2002; **29** (6): 347-353.

25) Martin SF, Dudda JC, Bachtanian E *et al.* Toll-like receptor and IL-12 signaling control susceptibility to contact hypersensitivity. *J Exp Med* 2008; **205** (9): 2151-2162.

26) Ainscough JS, Gerberick GF, Dearman RJ, Kimber I. Danger, intracellular signalling and the orchestration of dendritic cell function in skin sensitization. *J Immunotoxicol*, 2012; Early online: 1-12. Epub.

27) Kaplan DH, Igyarto BZ, Gaspari AA. Early immune events in the induction of allergic contact dermatitis. *Nat Rev Immunol* 2012; **12** (2): 114-124.

- 28) Sutterwate FS, Ogura Y, Szczepanik M *et al.* Critical role for NALP3/CIAS1/Cryopyrin in innate and adaptive immunity through its regulation of Caspase-1. *Immunity* 2006; **24**: 317-327.
- 29) Watanabe H, Gaide O, Petrilli V *et al.* Activation of the IL-1 β -processing inflammasome is involved in contact hypersensitivity. *J Invest Dermatol* 2007; **127** : 1956-1963.
- 30) Weber FC, Esser PR, Muller T *et al.* Lack of the prurinergeric receptor P2X7 results in resistance to contact hypersensitivity. *J Exp Med* 2010; **207** (12): 2609-2619.
- 31) Cumberbatch M, Dearman RJ, Kimber I. Langerhans cells require signals from both tumour necrosis factor-alpha and interleukin-1 beta for migration. *Immunology* 1997; **92** (3): 388-395.
- 32) Sandig H, McDonald J, Gilmour J *et al.* Fibronectin is a Th1-specific molecule in human subjects. *J Allergy Clin Immunol* 2009; **124**: 528-535.
- 33) Bollyky PL, Evanko SP, Wu RP *et al.* Th1 cytokines promote T-cell binding to antigen-presenting cells via enhanced hyaluronan production and accumulation at the immune synapse. *Cell Mol Immunol* 2010; **7** (3): 211-220.
- 34) Man MQ, Hatano Y, Lee SH *et al.* Characterisation of a hapten-induced, murine model with multiple features of atopic dermatitis: structural, immunologic and biochemical changes following single versus multiple oxazolone challenges. *J Invest Dermatol* 2008; **128**: 79-86.
- 35) Padilla IM, Burgos L. Aminoglycoside antibiotics: structure, functions and effects

on in vitro plant culture and genetic transformation protocols. *Plant cell Rep* 2010; **29** (11): 1203-1213.

36) Goh CL. Contact sensitivity to topical antibiotics. (II) Sensitizing potential of some topical antibiotics. *Contact Dermatitis* 1989; **21** (3): 166-171.

37) Smith RH, Messner P, Lamontagne L *et al*. Induction of T-cell immunity to oligosaccharide antigens immobilized on crystalline bacterial surface layers (S-layers). *Vaccine* 1993; **11** (9): 919-924.

38) Bessler WG, Heinevetter L, Wiesmuller KH *et al*. Bacterial cell wall components as immuno modulators- 1. Lipopeptides as adjuvants for parenteral and oral immunization. *Int J Immunopharmacol* 1997; **19** (9-10): 547-550.

39) Coffman RL, Sher A, Seder RA. Vaccine adjuvants: putting innate immunity to work. *Cell* 2010; **33**: 492-503.

40) de Hora VP, Conceicao FR, Dellagostin OA, Doolan DL. Non-toxic derivatives of LT as potent adjuvants. *Vaccine* 2011; **29** (8): 1538-1544.

41) Knapp S. Update on the role of Toll-like receptors during bacterial infections and sepsis. *Wienn Med Wochenschr* 2010; **160** (5-6): 107-111.

42) Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. *Nature Immunol Rev* 2010; **11**: 373-384.

- 43) Kinbara M, Sato N, Kuroishi T *et al.* Allergy-inducing nickel concentration is lowered by lipopolysaccharide at both the sensitization and elicitation steps in a murine model. *Br J Dermatol* 2011; **164** (2): 356-362.
- 44) Hierholzer S, Hierholzer G. Allergy to metals following osteosynthesis. *Unfallchirurgie* 1982; **8** (6): 347-352.
- 45) Williams HC, Dellavalle RP, Garner S. Acne vulgaris. *Lancet* 2012 Jan 28; **379** (9813): 361-372.
- 46) Whitfeld M, Gunasingam N, Leow LJ *et al.* Staphylococcal epidermis: a possible role in the pustules of rosacea. *J Am Acad Derm* 2011; **64** (1): 49-52.
- 47) Lazaridou E, Giannopoulou C, Fotiadou C *et al.* The potential role of microorganisms in the development of rosacea. *J Dtsch Dermatol Ges* 2011; **9** (1): 21-25.
- 48) Satorius K, Killasli H, Oprica C *et al.* Bacteriology of hidradenitis suppuritiva exacerbations and deep tissue culture during carbon dioxide laser treatment. *Br J Dermatol* 2012; **166** (4): 879-883.
- 49) Akamatsu H, Niwa Y, Matsunaga K. Effect of palmitic acid on neutrophil function. *Int J Dermatol* 2001; **40** (10): 640-3.
- 50) Nakatsuji T, Kao MC, Zhang L *et al.* Sebum free acids enhance the innate immune defence of human sebocytes by upregulating beta-defensin 2 expression. *J Invest Dermatol* 2010; **130** (4): 985-994.

51) Grech I, Giatrakou S, Damoraki G *et al.* Single nucleotide polymorphisms of toll-like receptor-4 protect against acne conglobata. *J Eur Acad Dermatol Venereol* 2011 Nov 16; Epub

52) Jeong E, Hong JW, Min JA *et al.* Topical ALA-photodynamic therapy for acne can induce apoptosis of sebocytes and down-regulate their TLR2 and TLR-4 expression. *Ann Dermatol* 2011; **23**: 23-32.

53) Yamasaki K, Kanad A, Macleod DT *et al.* TLR2 expression is increased in rosacea and stimulates enhanced serine protease production by keratinocytes. *J Invest Dermatol* 2011; **131**: 688-697.

54) Hunger RE, Surow AM, Hassan AS *et al.* Toll-like receptor 2 is highly expressed in lesions of acne inverse and colocalises with C-type lectin receptor. *Br J Dermatol* 2008; **158** (4): 691-697.

55) Sansonetti PJ. To be or not to be a pathogen: that is the mucosally relevant question. *Mucosal Immunol* 2011 ; **4**: 8-14.

56) Dunn-Siegrist I, Tissieres P, Drifte G *et al.* Toll-like receptor activation of human cells by synthetic triacylated lipid A-like molecules. *J Biol Chem* 2012; **287** (20): 16121-16123.

57) Schmidt M, Raghaven B, Muller V *et al.* Crucial role for human Toll-like receptor 4 in the development of contact allergy to nickel. *Nature Immunol* 2010; **11** (9): 814-819.

Figure 1: Allergic contact dermatitis; sensitisation. Hapten chemical provides two immune signals. Firstly an antigenic signal is established through haptenic binding to host peptide in the skin. Secondly, it causes release of damage associated molecular pattern (DAMP) molecules which act as ligands for TLR2 and 4. Ligand stimulation of TLR4 (and 2) leads to expression of pro-inflammatory cytokines IL-1 β , TNF α , IL-6, IL-12, IL-18, IL-23 and α IFN, thus providing a 'danger' signal. TNF α and IL-1 β are essential for antigen presenting cell maturation and migration to the local lymph node to produce hapten sensitisation.

Figure 2: Allergic contact dermatitis; elicitation. As with figure 1 hapten produces antigenic signal and also danger signal through release of DAMP molecules, ligand stimulation of TLR4 (and 2) and pro-inflammatory cytokine release. The cytokines produce expression of vascular adhesion molecules and chemokines which lead to recruitment of Th1 and Th17 cells. Th1 cells produce significant quantities of DAMP molecules (EDA+ fibronectin) which will further stimulate TLR4. A DAMP/TLR4/Th1Th17 or 'DAMP' immune circuit is therefore established (highlighted in red)

Figure 3: A simplified illustration of 'DAMP' immune circuit.

Figure 4: Immune response to commensals versus pathogens. Extracellular microbial pathogens deliver antigenic signal(s). The 'danger' signal is, however, delivered through separate cell wall protein(s) known as pathogen associated molecular patterns or PAMP molecules. These act as ligands for TLR2 and 4 with subsequent expression of pro-inflammatory cytokines and 'danger' signalling. This will lead to a hostile host immune response. With commensals antigen signal is delivered in the absence of 'PAMP' danger signalling. A tolerance and non-hostile response occurs.

Figure 5: Acne vulgaris-proposed immunopathogenic pathway. Commensal

microorganisms in the pilosebaceous unit deliver antigenic signal(s). However the high concentrations of saturated fatty acids observed in the comedonal sebaceous glands of acne sufferers can lead to stimulation of TLR4 (and 2). Proinflammatory cytokine expression and 'danger' signalling leads to a hostile host immune response to commensal micro-organisms resembling that to a pathogenic extracellular microbe.









