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MEETING REPORT

4th Georg Rajka International Symposium on Atopic Dermatitis

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In 1979 Georg Rajka initiated in Oslo a series of international meetings on atopic dermatitis (AD) to take place approximately every third year. The 10th meeting was held in Arcachon, France on 15–17 September 2005, and was organized by Professor Alain Taieb and colleagues from Bordeaux. The following is the authors' summary from the meeting, giving a highlight of events and "what's new" in AD.

FROM HISTORY TO GENETICS

Dr D. Wallach summarized the history of the disease we today call "atopic dermatitis". Since Hippocrates' day skin has been regarded as an "excretory organ", in which oozing was seen as a symptom of discharge of dangerous material. It is highly likely that many oozing patients had skin infections. The belief was that stopping oozing would be dangerous for the patient, since the "toxic" material would then stay inside the body. Around 1800 Willan & Bateman introduced a new dermatological "disease classification" according to recognizable symptoms and described skin disorders, comparable with our current classification of AD, characterized by oozing, vesicular/papular/pruritic rash appearing on the head and scalp in suckling children. As Dr Wallach stated: "Doctors name the disorder as they see it and it leads to the way they treat the patient". In 1892, Besnier considered the symptoms to be "diatethique", meaning "coming from the inside". However, following the recognition of "anaphylaxis" in 1902 and Blackfan's 1916 documentation of food allergy in some patients, in 1933 Wise & Sulzberger termed the condition "AD".

More detailed definitions of "AD" came in 1980, with Hanifin & Rajka's well-known lists of major (3 of 4) and minor (3 of 24) criteria, followed in 1994 by the British Working Group's simple definition of "AD", excluding IgE among the parameters for its diagnosis. This is in contrast to the "extrinsic", i.e. documented presence of IgE allergy, versus the "intrinsic", i.e. no type I allergy, "classification" of AD. Further comments on dermatitis and IgE are given below.

According to Dr Wallach, the main historical doctrines of AD were therefore: (*i*) a reflection of an internal disorder with "secretion"; (*ii*) a skin disease with specific and recognizable symptoms; (*iii*) a "diathetique, i.e. an internal derangement in the body and, finally, (*iv*) "an immune disease".

Dr W. Cookson gave an overview of the genetics of AD. He stated that genes interacting with the environment are highly polymorphic and that AD is a disease that results from interactions between an unknown number of genes and environmental factors. The genetic imprinting in AD, i.e. the parental phenotypes influencing the immune status of the child, is now known to come primarily from the mother and not the father. He stressed the necessity for more stringency in future studies, e.g. not just recording a patient with AD, but details about their immune status. Interestingly, it is now clear that many gene loci are associated with AD, asthma and psoriasis, see Cookson's review (1) and, surprisingly, there are no genetic overlaps between AD and asthma. In contrast, several associations are similar between AD and psoriasis. A further example: the "psoriasis gene" on chromosome 20 is associated with psoriasis, AD and leprosy, indicating the existence of "gene-sets" related to "inflammation". He questioned whether the "atopic march" exists, which is the observed change over time of disease expression, as AD is seen at an earlier age than asthma and ensuing allergic rhinitis. This observation is true, but the patients developing the different disorders may not develop the one disease as consequence of the other.

Using an *in vitro* culture system of normal human keratinocytes and Affymetrix gene chips Cookson et al. have found that during a 10-day observation period of developing "epidermis" *in vitro*, 1283 different genes are either up- or down-regulated. Putting them into "clusters", 11 clusters could be defined, ranging from cell motility/adhesion and coagulation to cell motility and DNA replication, RNA processing and protein folding.

Dr H. Sugiura could demonstrate by similar studies on AD epidermis that only 10 genes were 5-fold changed, among them calcium-binding proteins being upregulated, loricrin and filaggrin being downregulated and a significant variation in keratins being either up- or downregulated.

Dr M. Moffett looked at genes lying within the 1q2.11 region, which spans approximately 2 Mb and observed that "gene B" seems to be involved in the aetiology of AD.

Dr M. Bradley observed an upregulation of the SOCS3 gene (17q25) using a 42,000 cDNA gene chip in AD apart from another 3931 cDNA, which were differently expressed. The SOCS3 protein is observed in epidermal dendritic cells and seems to be involved in cytokine signalling

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with inhibitory effects on the STAT-JAK pathway. Recent studies point to STAT3 as being involved in AD.

In summary, no specific "AD gene(s)" have been found, but many genes are certainly involved. There is no overlapping gene association between AD and asthma. Instead there are associations or overlaps with psoriasis ("inflammatory genes"). The genetic field is currently very complex and future studies must include a meticulous investigation of patients studied, not just recording them as having AD. Looking at just one gene association is not permissible. This is a difficult and resource-demanding area. Finally, the dynamic aspect seems important, as it is very likely that different genes are upregulated at different time points. Following the meeting a publication in the March issue of Nature Genetics have shown common "loss-of-function" variants in the epidermal barrier protein filaggrin to be a major predisposing factor for atopic dermatitis (2).

EPIDEMIOLOGY

Dr T. Diepgen reviewed predisposing factors for AD in 2184 German children, being on average 18 months old. Living with cats predispose to AD, but dogs do not ("the dog effect" is actually protective against AD even though living with dogs causes a high exposure to house dust mites). Food sensitizations diminish with age in contrast to external allergens. The frequency of sensitized children is associated with age, not severity of AD, which is rather surprising. Finally, vaccinations, antibiotics and infections were not observed to be predisposing to AD. In the discussion Dr Yamamoto remarked that Mongolian children living in Ulan Bator may have AD, but those living in tents with high exposure to animals do not. Dr *C. Flohr* had studied whether flexural dermatitis is associated with type I allergy; in affluent countries it is, but in non-affluent countries rarely. His conclusion was that the IgE association with AD is probably over-emphasized. Still, the presence of type I allergy in AD is high.

Finally, *Dr H.C. Williams* presented data on 298,080 (13–14-year-old) and 185,891 (6–7-year-old) children from UK, studied approximately 7 years apart for "dermatitis" and came to the conclusion that the dermatitis epidemic seems to level off or is even falling in countries with previous high incidences. Changes seen with 7 years difference are probably caused by environmental factors.

MATURATION OF THE IMMUNE SYSTEM

Dr P. Holt stressed that the immune system changes with time, and he documented how children who develop atopy (presence of IgE to allergens), change from a Th2-predominant immune profile to a Th1-predominant immune one, which he termed a "developmental overshoot". He could also document hypomethylation of CPG sites (3) in the CD8 T cell population influencing the interferon (IFN)-γ production of these cells. Neonatal CD8+ T cells, which do not have hypermethylation of their IFN-γ promoter, produce low amounts of IFN-γ, but within a few years this changes to increased production. Thus, he stressed that the "time shift" of the immune system seems extremely important in "atopy", indicating that maturation of the immune system is a highly dynamic process. Clearly, in atopics something goes wrong. Why some develop dermatitis and others atopy is unknown, although the predominant Th2 profile seems to be responsible for the atopy part.

Dr H. Hashizume showed that atopics have a higher amount of plasmacytoid dendritic cells defined by the CD11c-CD123+ phenotype being correlated to IgE, SCORAD and the IFN-γ:IL4 ratio. However, besides IL5 these cells can produce large quantities of IFN-γ. They observed the existence and function in AD of T_{reg} cells, which are T cells having a regulatory function on inflammation. In foxp3 –/– mice lacking T_{reg} cell function, dermatitis and increased IgE levels are seen. Still, a clear definition of T_{reg} cells is lacking as some use the CD+CD25+ phenotype, others say IL10 production is a significant marker.

Dr H. Just showed that recent thymic emigrant cells, as measured by the TREC technique, have a larger variation among AD T lymphocytes than among healthy controls, indicating that the thymus sends out cells in bursts, especially within the CD8 population. Surprisingly few studies were presented on T lymphocytes and their biology in AD.

PROBIOTICS

Probiotics stem from *Lactobacilli* found in milk products and carries a large number of strains. They were discussed in relation to the maturation of the immune system. *Dr E. Isolauri* mentioned that probiotics work best in infancy, but probably not later in life, corresponding to the "shift" happening in the immune system during development as mentioned by *Dr P. Holt*. They work on the gut immune system and can delay the onset of AD in infants. *Dr S. Weston* confirmed this from an Australian study, although the statistics of her study were questioned. *Dr J.H. Kim* had a similar experience among Korean patients. The therapeutic window for obtaining a positive effect of probiotic treatment seems to be narrow and the effect is also dependent on the strain of bifidobacteria chosen. Still, the improvements were small and probiotics are not yet a standard treatment in AD.

INFECTIONS AND ATOPIC DERMATITIS

Drs D. Leung and *T. Werfel* reviewed the topic of *S. aureus* and AD. The innate immunity of patients with

AD is lacking, although a Japanese investigation indicated increased amount of beta-defensin 2 (reported by S. Asano et al.). According to *in vitro* studies, staphylococcal enterotoxins, named "superantigens" because they stimulate a wide range of different T lymphocytes, can change many aspects of the dermatitis inflammation, from breaking the effect of steroids on lymphocyte proliferation, to the subversion of CD4+CD25+ T_{reg} cell activity. However, the possibility of superantigen being suppressive on T_{reg} function is attractive. However, if IL-10 production is a marker for T_{reg} cells, then plenty of T_{reg} cells must be present, in accordance with results from Hanifin's group, who years ago described the significant upregulation of IL-10 in AD. Many of the experiments supporting the statements of these authors are based on *in vitro* models and should be evaluated with care. However, the clinical experience that antibiotic therapy, be it systemic or topical, improves AD is evident, but anti-microbial therapy does not bring dermatitis into full remission. The bottom-line is that superantigens may very well be important in supporting and/or augmenting the immune inflammation of AD.

Besides *S. aureus*, *Dr M. Howell* showed how AD keratinocytes have different expression of cathelicidin (a peptide involved in the innate immunity of skin) compared to keratinocytes from psoriasis and normal skin, leading to their much higher susceptibility to vaccinia virus replication, and probably explaining the clinical fact that AD is susceptible to herpes virus infection. Practical implications are that smallpox vaccination in the USA is not recommended for those with previous AD.

CHEMOKINES

Dr B. Homey gave a review on chemokines in AD. He showed how keratinocytes express and secrete cytokines, which attract and ignite T lymphocytes. Many chemokines seem to be involved and the most "specific" one is probably not existent, although CCL1 (previously known as I-309) is associated with AD. These cytokines can be induced by a range of stimulators, such as allergens, superantigens and microbial products, at least *in vitro*. Therefore, the keratinocytes seem to be important for profiling the immunological inflammation of AD. The significance of I-309 (CCL1) was confirmed by *Dr N. Higashi*, who noted a significant increase in patients with AD similar to what has been observed for thymus and activation-regulated chemokine (TARC), macrophage migration inhibitory factor (MIF) (*Dr. J.-S. Kim*) and IL16 (*Dr B. Pigozzi*). In his time-course studies, Dr Higashi made the interesting observation that, although SCORAD diminished, the levels of I-309 (CCL1) stayed high, leading to the question: Is AD only a skin disease, or is it a systemic disease, as the immune system is still activated even though skin inflammation is reduced? Finally, *Dr J.K. Park* demonstrated that thymic stromal lymphopoietin (TSLP) is highly upregulated in keratinocytes in AD. TSLP augments dendritic cells to produce TARC and MDC, but not IL12, leading towards a Th2 response.

NEURO-IMMUNOLOGY

Dr U. Gieler summarized the knowledge of how mast cells and Langerhans' cells have close anatomical correlations with nerve fibres containing mediators such as substance P, neutrophine growth factor and vasoactive peptide. Recent studies have shown an interplay between stress and the skin barrier function (4) and *Dr U. Raap* could show increased neurotrophine receptors on eosinophils. *Dr M. Takigawa* could demonstrate that a serotonin 1A receptor agonist with anti-anxiety effects could improve the clinical status of the patients. This area is still complex and very difficult to study given the present biological techniques.

ANIMAL MODELS AND ATOPIC DERMATITIS

Dr T. Olivry described a dog strain that develops dermatitis resembling the evolution of AD with increased levels of IgE. The disease seems to be provoked by environmental factors. The symptoms can be suppressed by the remedies known from human medicine (steroids, calcineurin inhibitors). He also noted that dogs are very hard to sensitize with dinitrochlorobenzene or poison ivy, which means they cannot develop allergic contact dermatitis. Thus, may AD not be comparable to allergic contact dermatitis? *Dr L. Nagelkerken* demonstrated a mouse model with an overexpression of apolipoprotein C1 having increased levels of cholesterol, triglycerides and free fatty acids in serum, lipids that are partially deficient in keratinocytes of AD. The mice developed an AD-like skin with pruritus. Their TEWL was 5-fold increased and in addition their skin had increased neutrophils, CD4+, CD8+, mast cells, but no eosinophils in the skin. IgE was increased 6–9-fold, IL13 expressed in basal keratinocytes and with epidermal hyperplasia. Topical corticosteroids diminished itch, but emollients did not. *Dr A. Henino* demonstrated an allergic contact dermatitis mouse model with house dust mite antigen and could show that the "dermatitis" was transferable to healthy mice using CD8+ T cells, indicating that the inflammation involves cytotoxic responses. Finally, *Dr H. Mizutani* showed a method of measuring itch based on sounds (in Japanese "pori-pori") – the sound of a mouse scratching, which it can do up to 27×per second, which is not registrable by the human eye.

THE SKIN BARRIER AND ATOPIC DERMATITIS

Dr A. Hovnanian reviewed his very significant observations in Netherton's syndrome, where the SPINK5 gene

was documented to be the pathogenic factor for this condition, which highly resembles (or includes) AD. The SPINK5 encodes the LEKTI protein, which is a serine protease inhibitor (kazal type 5). The gene is located on chromosome 5.32. The LEKTI protein is also expressed in the Hassell's corpuscles in thymus. LEKTI is a key inhibitor of many epidermal proteolytic enzymes, including stratum corneum tryptic enzyme and similar enzymes. If mutations in SPINK5 occur the lack of LEKTI activity will lead to a range of changes in keratin and loricrin expression together with a deficiency of desmoglein 1 packing. Thus, if you lose an inhibitor of proteolytic enzymes in the epidermis, you can develop an AD-like condition. This affects the epidermal function in many ways and *Dr J.-P. Hachem* showed how it changed the permeability of the epidermis.

Dr M. Brattsand talked about another group of proteolytic enzymes in epidermis, the kallikreins, which are located on chromosome 9q13.3–13.4. The kallikreins are probably involved in the normal desquamation process and any change in them could lead to keratinocytes becoming "immunologically" active.

Dr M. Cork gave an interesting lecture showing that topical corticosteroids remove almost all cell layers of the stratum corneum, opening the way for irritants and allergens in AD. Confirming the work of other investigators, he had shown that stratum corneum chymotryptique enzyme (SCCE) is very important for the normal scaling of skin. There is increased SCCE activity in AD (5). The problem is how we can deliver anti-protease, which should then be tried in emollients. *Dr J.-P. Hachem* described the acidic mantle of the skin, which is important as a regulator of barrier permeability. The acidic mantle has antimicrobial properties and encompasses free fatty acids and a sodium proton antiporter (NHE1) enzyme localized in the outermost nucleated layers of epidermis. The enzyme activity is regulated by skin pH. Other pH dependent enzymes, such as β-glucocerebrosidase and acidic sphingomyelinase, are involved in ceramide in the value of atopy patch testing (APT) synthesis. The enzymes can be inactivated by serine proteases present in bacteria, and activity can be normalized by serine protease inhibitors, possibly leading to a new therapeutic approach.

ATOPY PATCH TESTING IN ATOPIC DERMATITIS

This section of the meeting had the most heated discussions, as there seem to be "believers" and "non-believers". *Dr U. Darsow* presented an overview and could demonstrate that APT reactivity is always lower than skin prick tests and RadioAllergoSorbent Test (RAST) to environmental allergens. Of those patients having a positive APT very few are multi-allergic. Patients with atopy with respiratory symptoms only, never react in the APT. However, among skin prick test negative and RAST negative AD patients only 7% will have a positive APT. He recommended the use of standardized patch test materials in petrolatum and large Finn chambers for application to the skin. When it comes to determination of the clinical relevance of a positive test, it is a problem that there is no general consensus on how to define a positive delayed challenge response. Dr Darsow has recently published a review (6) for readers wanting more information.

The issue of food allergen patch testing was presented by *Dr K. Turjanmaa* and heavily discussed. Also, other contact allergens were investigated, such as benzalkonium chloride, which is an irritant even in the recommended patch test concentration of 0.1%. The "excited skin syndrome" or "irritancy" was not discussed, although it cannot be excluded. Therefore, the current conclusion is that the APT is not sufficiently standardized for routine use and tests with food allergens will lead to many "reactions", but the therapeutic consequences are hard to prove.

AUTOIMMUNITY AND ATOPIC DERMATITIS

Dr P. Schmid-Grendelmaier presented evidence that patients with AD have IgE antibodies and T cells reacting against manganese superoxide dismutase, which is an enzyme found in many cell types ranging from moulds (*Aspergillus*), and yeasts, (*Malassezia furfur*) to human cells. The question is whether patients with AD react to these antigens, and approximately half of patients seem to do. In another lecture Dr A. Scheynius gave an overview of the *M. furfur* (*Pityrosporum orbiculare*) story, and demonstrated the many antigens of *M. sympodialis*, which colonizes normal and atopic skin. Still, the question is: Are these antigens responsible for an allergic contact dermatitis (which we call AD) – or not. The problem with this "antigen" has been standardization.

TREATMENT

Dr H.E. Williams reviewed progress in evidence-based treatments of AD. The information is available on the website: http://www.ncchta.org/execsumm/summ437.htm.

Based on the available evidence he has stopped routine use of topical antimicrobial-corticosteroid combinations for treatment and there is good evidence for the positive effect of focussing on patient information through the establishment of so-called "dermatitis schools".

SUMMARY

The ISAD2005 meeting presented the following highlights:

- It brought the keratinocyte back into focus, both by showing the various proteolytic enzymes and their inhibitors involved in epidermal shedding.

- The importance of skin barrier, as indicated above, and the ability of keratinocytes to be immunologically active.
- That there is an innate immune deficiency of AD epidermis.
- That superantigens, skin irritation and environmental allergens can induce "inflammation".
- That animal models of AD are emerging.
- That although IgE and specific atopy are present, they seem not to be causatively associated with AD, which leads us to question the use of the word "atopic" for this disease.
- That topical corticosteroids may dramatically alter stratum corneum.
- The continued focus on establishing evidence-based treatments for AD.

ISADs are small meetings, but all participants are directly involved in basic or clinical research on AD. One of the major charms of the meetings is that they allow participants to meet and hold discussions in an informal way. The next ISAD is planned for 2008 in Japan. Specific dates will appear on the ISAD2005.org website. In his concluding remark Georg Rajka said: "The techniques have improved, but the results are the same". We have more pieces to the puzzle called AD, but we still have no real understanding as to why this disease develops. Therefore, we need more research and to meet again.

It is recommended to use the names of the conference speakers via the PubMed system in order to access the latest publications. Further details about the next meeting will be announced within the next 6 months.

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