The immunology of allergy. And how immunotherapy can change it

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"The more I learn, the more I realize how much I don't know." Albert Einstein

Caveats:

1. Please focus on the learning objectives as you study for the exam. I always add extra detail for these reviews because they fascinate me!
2. As you deepen your understanding of atopic dermatitis, the chronic inflammatory disease we see the most, think about how what you learn about the human disease can inform your thoughts about the canine disease.
3. You can't really talk about the immunology of atopic dermatitis without talking about the skin barrier and the microbiome.

Learning objectives:

1. Be familiar with the concept of phenotypes and endotypes as one explanation for the heterogeneity of this disease.
2. Be able to differentiate between genetic and epigenetic changes.
3. Understand how epidermal barrier defects contribute to the pathogenesis of atopic dermatitis.
    a. How does a damaged barrier promote inflammation?
    b. What are epidermal alarmins?
    c. How do epidermal alarmins promote the Type 2 immune response?
    d. What physical changes to the barrier promote antigen penetration?
4. What happens to the immune system in atopic patients?
    a. Be able to characterize the Type 2 response. What cells are involved? What cytokines?
    b. Trace the path of an allergen from the skin to the lymph node.
    c. How does the Type 2 response affect the skin barrier?
5. Be familiar with the hygiene hypothesis and how its complexity has increased now that we know about dysbiosis. How does skin dysbiosis contribute to allergy? How does gut dysbiosis contribute to allergy? Is there anything we can do about it?
6. How does the environment contribute to atopic dermatitis? What do we know about the canine disease?
7. How are target therapeutics helping to clarify what molecules are important in the pathogenesis of atopic dermatitis? If you could snap your fingers and have a new monoclonal antibody therapy for dogs, what would you target?
8. Mechanisms for how allergen immunotherapy works.
    a. Be familiar with the 4 major mechanisms

b. With regard to regulatory cells, be aware that there are more than just Treg.
c. I would not recommend memorizing all the different types of Treg. Rather, be aware of how they repress the allergic reaction, what cytokines are inhibitory (IL-10, TGFβ, IL-35),P3 and what FoxP3 is.

Atopic dermatitis is a disease of great complexity. It has been suggested that the canine disease has many similarities to the human disease; therefore, following the human literature is immensely helpful to us as veterinary dermatologists. We can determine what needs to be verified, then move on to apply what we learn to our own patients. Regrettably, we don't know enough about the disease in cats, horses, or the other species with which we interact, so application of this knowledge to those species can sometimes be a little tricky. In general, the mammalian immune system is quite similar among species, so thinking by analogy can often be helpful.

It is useful to consider the history of veterinary allergic disease, because its history has dictated some of the treatments we have used in the past. While allergic skin disease has been recognized in dogs since at least the early 20th century, it is only recently that we have appreciated just how complex atopic dermatitis can be. The complexity derives not only from the immune response, but also from the influence of the skin barrier, environmental triggers, and the skin and gut microbiome. It is also important to think about the concept of atopic-like dermatitis. The diagnosis of atopic-like syndrome relies on a failure to identify allergen-specific IgE by intradermal or serum testing; it has been compared to intrinsic atopic dermatitis in humans. (Czarnowicki et al). Some of the questions I have about canine atopic-like dermatitis:

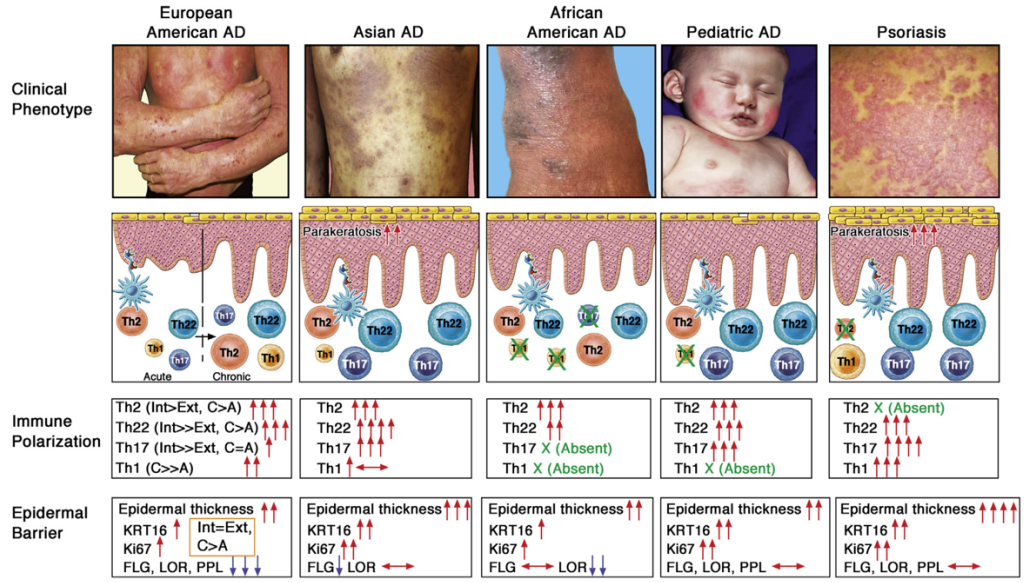
1. What if these dogs are allergic to something for which we don't test?
2. What if the sensitivity of our tests is not high enough to pick up all true positives?

Hopefully continued research will help us understand these diseases better.

In the mid-20th century, we believed that the canine skin disease we now call atopic dermatitis resulted from inhalation of allergens. We called it allergic inhalant dermatitis, and it was believed that allergenic proteins were transmitted by the bloodstream to the skin. They were hypothesized to percolate out into the dermis, where they bound to mast cell-bound allergen-specific IgE triggering degranulation. There is no doubt that mast cells continue to play an important role in atopic dermatitis, but we have learned that histamine is only one of many mediators involved in itch and inflammation, explaining why atopic patients, whether human or canine, respond poorly to H1 antihistamines.

The prevalent model for disease pathogenesis in the late 20th century was an imbalance in the ratio of Th2 to Th1. It was a useful model at the time, but we now appreciate that the immunological situation is much more complex and dynamic. It is now acknowledged that the immunologic input to this disease can change over time; however, it is also well acknowledged that Type 2 cytokines remain important throughout. The input of Type 1, Type 17, and other cytokines may be determined by

the genetic background of the individual. It is very clear in humans that specific phenotypes and endotypes are associated with these specific cytokine profiles.



What do we know?

- **AD is a heterogenous inflammatory skin disease characterized by different subtypes/phenotypes based on age, disease chronicity, ethnicity, FLG and IgE status, and underlying molecular mechanisms/endotypes.**
- **Endotype variances make the "one-size-fits-all" therapeutic approach irrelevant for patients with AD.**

What is still unknown?

- **Are there endotypes related to microbiome diversity or specific bacterial strains?**
- **Is there an allergic endotype related to specific allergens, such as food versus environmental antigens?**
- **Is there an endotype related to distinct autoantigens/T-cell autoreactivity?**
- **What is the relative role of different cytokine axis activation in driving disease pathogenesis across AD endotypes and phenotypes?**

These 2 images from this great article: Czarnowicki et al. J Allergy Clin Immunol 2019: 143

We have not identified distinct phenotypes or endotypes for dogs; however, it is clear that we recognize breed differences in clinical manifestations and in changes found in genomic analyses.

I would like to suggest some resources for you that make it easier to keep up with how our understanding of the immunopathogenesis of allergic diseases changes over time.

1. Revolutionizing Atopic Dermatitis. This is a wonderful organization that has offered vritual CE sessions during the pandemic. They will go back to live meetings December 2021. I would encourage you to go to the website and watch these meetings. Registration for the April 2020 meeting was $25! And for the December 2020 version $37. These meetings combine updated pathogenesis with summaries of new approaches to treatment, discussions of comorbidities, and information about topical therapy. I find it quite useful. https://revolutionizingad.com/

2. Journal of Allergy Clinical Immunology. This journal has well written and well referenced original and review articles on all allergic diseases. From the point of view of pathogenesis, these are great.

3. Veterinary allergic disease. Continue to watch Veterinary Dermatology, but the best way to keep on top of our profession is to do a Pub Med search frequently, using the term canine atopic dermatitis. We know less about cats and horses, but the PubMed search is the best option for those species as well, in my view. Many cool papers are published in journals you might not ordinarily think to read. I do recommend this paper: Nuttall et al. Update on pathogenesis, diagnosis, and treatment of atopic dermatitis in dogs. J Amer Vet Med Assoc 2019; 254:1219. It summarizes what we know about the canine disease and contains many references of interest. See also the series published in Veterinary Dermatology in 2015 for summaries of our collective knowledge at that time.

4. Do you have questions? Please feel free to email me at fadokv@gmail.com or valerie.fadok@zoetis.com. If I don't know the answer, I am happy to find some help!

Current model for the pathogenesis of atopic dermatitis

Key concepts

1. Skin barrier, microbiota (skin and gut), immunologic dysregulation are tightly related and influence each other. Integrating these inputs becomes critical for proper diagnosis and individualized treatment.
2. There are genetic and epigenetic mechanisms that contribute to the disease.
3. There are several environmental inputs (not only allergens, but pollutants, temperature, changes in humidity, psychosocial factors, etc.)
4. Multiple T helper subsets are involved, but T helper 2 cytokines remain important from acute to chronic disease.
5. Phenotypes and endotypes in humans likely occur in dogs (breed differences?); increased knowledge will lead to precision medicine
6. There is no one biomarker for this disease.

We are going to talk about the human disease in addition to the canine disease. I have 3 reasons for this.

1. There is so much more information about the disease in humans and mouse models.
2. The canine disease is considered to be similar to the human disease.
3. Our literature is quite small,and understanding what is important in human disease will help generate further research for dogs and other species.

An important precept for you to take to heart: Negative research is never as powerful as positive research. It would be foolish to discount mechanisms or molecules in our patients based on one paper and/or small groups of animals. Repeat, repeat, repeat and publish!

Atopic dermatitis: how does it start? Why does it progress?

1. Important questions we need to ask
    a. What is the Impact of genetics? This table provides a list of the genes currently believed important in the human disease. If atopic dogs have this level of complexity, it is easy to see why recommendations for selective breeding may not be practical (see Nuttall et al. JAVMA 2019; 254:1291)

This table illustrates the genes involved in the human disease.

Table 1. Main groups of genes associated with atopic dermatitis (AD) pathogenesis [5–7,9,10,12,23–32].

Pathological Process in AD	Example of Genes Involved
Epidermal barrier genes	*Filaggrin, filaggrin 2, hornerin* Corneodesmosomal genes (*desmoglein, desmocollin*) and tight junction genes (*claudins, ocludins*) Epidermal protease genes (*kallikreins, cathepsins, caspase* 14), and their inhibitors (*SPINK5, Cystatin A)* *OVOL1* (ovo like transcriptional repressor)—transcription factor that regulates FLG expression
Genes of the innate immune mechanisms	*TLR1, TLR2, TLR4, TLR6, TLR9, TLR10, CD14, NOD1* and defensins (*DEFB1*) Genes of receptor subunits for IgE (*FcεRI α i FCεRI-¥)*
Genes of the adaptive immune mechanism	Genes of Th2 response: *IL-4, IL-5, IL-13, IL2RA, IL-13RA IL-5RA, TSLPR, IL-4R, IL-18, IL-31* Other genes of Th bias *IL17A, TNFα, IL-22* Treg genes: *STAT-6, FOXP3, LRRC32*
Genes encoding alarmins produced by keratinocytes	*IL-25, TSLP, IL-33*
Genes regulating DNA methylation	*KIF3A*
Genes regulating vitamin D pathways	*CYP27A1, CYP2R1, VDR*

Int. J. Mol. Sci. **2020**, *21*, 6484; doi:10.3390/ijms21186484

Table from: Nedoszytko B et al. Genetic and Epigenetic Aspects of Atopic Dermatitis. Int J Mol Sci 2020

b. The most well-known mutations in human atopic dermatitis occurs in the gene for filaggrin. Not all humans with atopic dermatitis have these mutations, and there are plenty of other epidermal barrier genes that can be affected.
    i. The filaggrin situation in dogs is still unclear. But whether certain breeds of dogs have mutations or not, keep in mind that expression and function of filaggrin can be affected by Type 2 cytokines. A number of studies have looked at canine filaggrin expression. These studies have been reviewed recently (Combarros D et al. Update on canine filaggrin: a review. Vet Q 2020: 40:162
    ii. A number of genomic analyses have been performed from samples comparing heatlhy and atopic dogs. Many of these studies have been reviewed in Nuttall T et al. J Amer Vet Med Assoc 2019: 254:1291. You can start there to see what is available and then pull the papers in which you are interested.

c. What is the Impact of epigenetics?
    i. Epigenetics is the study of how the environmental (pollutants, chemicals, drugs, diet) and behaviorial factors (e.g. stress, anxiety) affect gene expression without changing the sequence of the DNA. These changes are known to be passed from pregnant women to their offspring, affecting prevalence of atopic diseases in the children.
    ii. DNA methylation is one epigenetic mechanism; when the promoter of a gene is methylated, repression of transcription occurs.
    iii. Other mechanisms include histone acetylation and methylation, chromatin structural changes, as well as expression of microRNAs that are noncoding, but can affect gene expression by targeting it for cleavage and degradation or by repressing translation. This is a hot topic in human atopic dermatitis right now; there are a number of open-access papers in PubMed if you would like to learn more.
    iv. These changes are reviewed (Nedoszytko B et al. Genetic and epigenetic aspects of atopic dermatitis. Int J Mol Sci 2020; 21: 6484. Bau L, Leung DYM. Genetic and epigenetic studies of atopic dermatitis. Allergy Asthma Clin Immunol 2016; 12:52
    v. There is one paper about microRNA's identified in dogs with atopic dermatitis. (Santoro D et al. Identification of differentially expressed microRNAs in the skin of experimentally sensitized naturally affected atopic beagles by next-generation sequencing. Immunogenetics 2020; 72:241-250)

d. What is the Impact of environmental stimuli?

    i. The traditional environmental factors we have considered include pollens, molds, dusts, danders, mites, foods, and microbes.

The influence of other environmental factors are becoming more clear. There is good evidence that pollutants in the air induce production of reactive oxygen species (ROS) and free radicals in the skin. The pollutants enter the skin directly through epidermal cells, and also through hair follicles and sweat glands. The cell's normal antioxidant mechanisms can be overwhelmed, so inflammation is made worse. What do the reactive oxygen species and radicals do? They oxidize lipids in the cell membranes, activate proteases, thereby making the barrier defect worse. They cause release of proinflammatory mediators from neutrophis and other phagocytes, which amplify the production of free radicals. The exact role of air pollutants in atopic dermatitis is still under investigation, but accumulating evidence suggests that these pollutants may increase the likelihood of developing clinically apparent atopic dermatitis, and atopic flares have been associated with increased pollutants. (Albolhasani R et al. The impact of air pollution on skin and related disorders: a comprehensive review. Dermatol Ther 2021; Feb 1. doi: 10.1111/dth.14840. Online ahead of print.)

ii. What about canine atopic dermatitis? There is one paper that reported a significant association between exposure to passive tobacco smoke and atopic dermatitis in dogs (Ka D et al. Association between passive smoking and atopic dermatitis in dogs. Food Chem Toxicol 2014; 66:329.)
iii. A hot topic is the role of the aryl hydrocarbon receptor (AHR) in atopic dermatitis. AHR is a member of the steroid receptor superfamily and is a transcription factor; it can bind a wide variety of exogenous and endogenous chemicals. Depending on its binding partner, it can either activate or repress gene transcription. Ligand-bound AHR in keratinocytes induces transcription and transduction of a neurotrophic factor called artemin, causing hyperinnervation of the epidermis. (Hidaka T et al. The aryl hydrocarbon receptor AhR links atopic dermatitis and air pollution via induction of the neurotrophic factor artemin. Nat Immunol 2017; 18:64)
iv. The AHR story is complicated, but there are polymorphisms in this gene that appear to be associated with severe dry skin in human atopic patients. (Li ZZ et al. Aryl hydrocarbon receptor polymorphisms are associated with dry skin phenotypes in Chinese patients with atopic dermatitis. Clin Exp Dermatol 2019; 44:613

v. I mention AHR for 2 reasons:
    1. A new topical therapeutic, tapinarof, is in phase 2b trials as a treatment for human atopic dermatitis. It is called an AHR modulator and is believed to activate the AHR in a way that

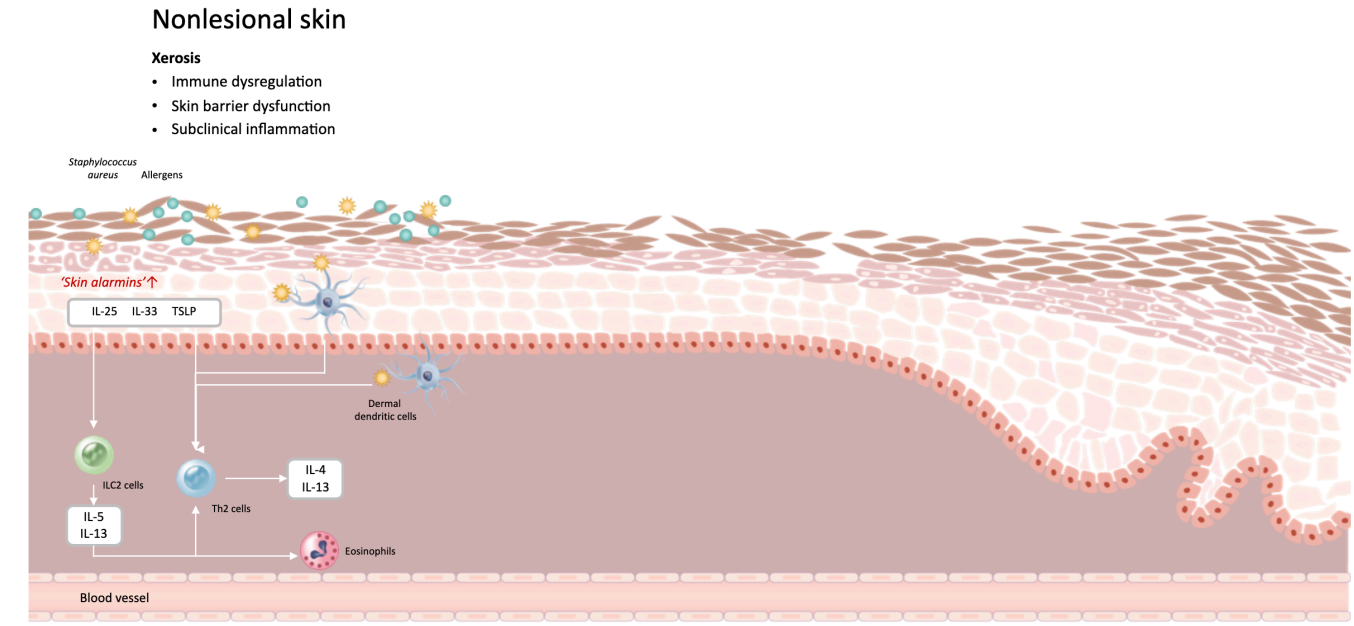
reduces Type 2 cytokines and oxidative damage. (Bissonnette R et al. J Am Acad Dermatol 2021: 4: S0190-9622(21)00485-0. doi: 10.1016/j.jaad.2021.03.005. Online)

2. The old topical therapeutic coal tar binds the AHR. (Smits JPH et al. Targetting the cutaneous microbiota in atopic dermatitis by coal tar via AHR-dependent induction of antimicrobial peptides. J Invest Dermatol 2020; 140:415)
3. Dogs have AHR but I have found no papers yet that allude to study of it in canine atopic dermatitis. (e.g. Giantin M et al. Expression of the aryl hydrocarbon receptor pathway and cyclooxygenase-2 in dog tumors. Res Vet Sci 2013; 94:90)

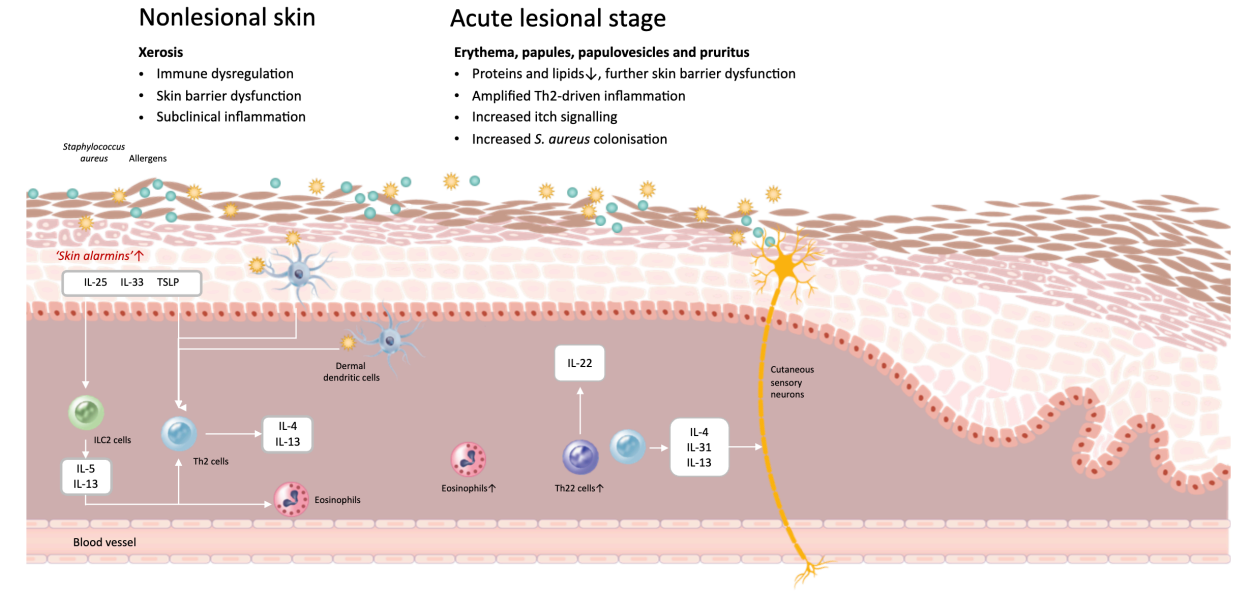
2. Why are the atopic diseases increasing in prevalence?
    a. The hygiene hypothesis and increased prevalence in Western urban environments
        i. A few studies in dogs confirm that living in an urban environment appears to be a risk factor, and that living in a rural environment is protective. (Harvey N et al. Vet Dermatol 2019; 30:396. Meury et al. Vet Dermatol 2011; 22:327)
        Also see https://pub.epsilon.slu.se/1440/1/ANfin0.pdf. This is a very interesting thesis by Dr. Ane Nodvedt, Uppsala, entitled Epidemiology of Canine Atopic Dermatitis.
    b. Climate change (see Ng GH et al. Climate change and atopic dermatitis: is there a link? Int J Dermatol 2019; 58:279)
        i. Changes in duration and severity of pollination
        ii. Changes in temperature and humidity (these are documented flare factors in humans with atopic dermatitis)
        iii. Changes in UV light
    c. Change in the microbiome of the gut and skin (de Pessemier B et al. Gut-skin axis: current knowledge of the interrelationhip between microbial dysbiosis and skin conditions. Microorganisms 2021: 9:352. Craig JM. Atopic dermatitis and the intestinal microbiota in humans and dogs. Vet Med Sci 2016: 2:95)
    d. Diet.
        i. It has been suggested that the highly processed Western diet that may be a predisposing factor. (Smith PK. Do advanced glycosylation end-products cause food allergy? Curr Opin Allergy Clin Immunol 17:325. Frei R et al. Microbiota and dietary interactions: an update to the hygiene hypothesis? Allergy 2012; 67:451. Lambrecht BN, Hammad H. The immunology of the allergy epidemic and the hygiene hypothesis. Nat Immunol 2017; 18:1076.)
        ii. Fun fact that won't be on the exam: apparently the ingestion of instant noodles in Korea is associated with increased prevalence of atopic dermatitis ☺ (Park S et al. Instant noodles, processed food intake, and dietary pattern are associated with atopic dermatitis in an adult population. Asia Pac J Clin Nutr 2016; 25:602)

iii. There is some evidence that diet can impact the development of atopic dermatitis in young dogs and that diet can support epidermal barrier function. (van Beeck FL et al. The effect of long-term feeding of skin barrier-fortified diets on the owner-assessed incidence of atopic dermatitis symptoms in Labrador retrievers. J Nutr Sci 2015; 4:1. Watson AL et al. Dietary constituents are able to play a beneficial role in canine epidermal barrier function. Exp Dermatol 2006:15:74)

Here are some diagrams that illustrate the current state of the art on the immune dysregulation associated with atopic dermatitis. These were presented by Dr. Alan D Irvine, a pediatric dermatologist at Trinity College, Dublin, at the recent AAAAI meeting; he has given me permission to share them with you. I like these for their clarity and simplicity.



AD, atopic dermatitis; IL, interleukin; ILC2, type 2 innate lymphoid cell; Th, helper T cell; TSLP, thymic stromal lymphopoietin.
Adapted from Weidinger S et al. Nat Rev Dis Primers 2018;4:1, with permission from Springer Nature Publishing AG.



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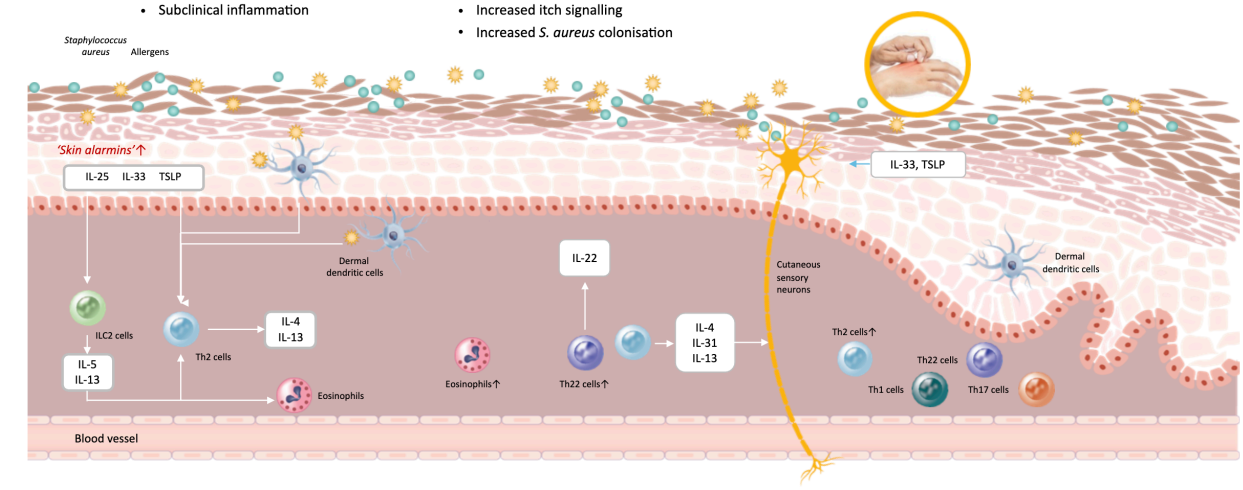

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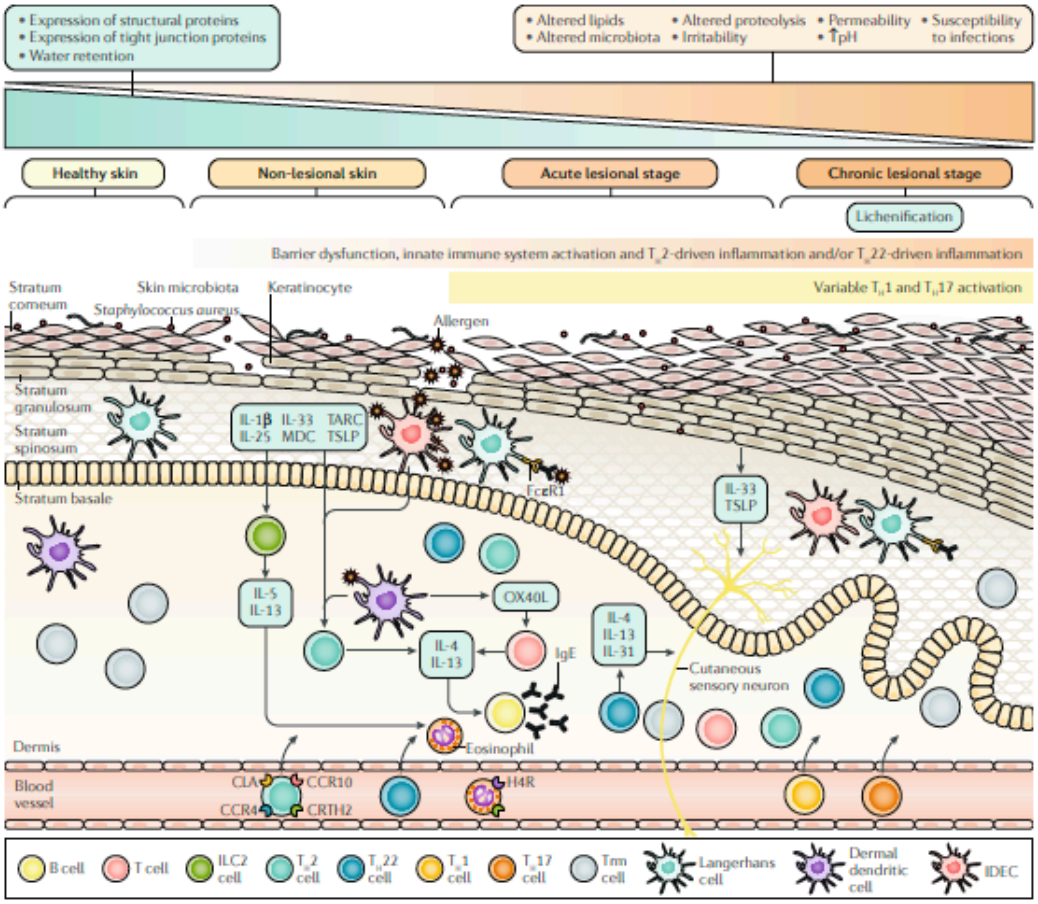
These figures were adapted from the following review article. I highly recommend it.
Weidinger S et al. Atopic dermatitis. Nat Rev Dis Primers 2018 Jun 21; 4 (1):_1.
doi: 10.1038/s41572-018-0001-z.

Current hypothesis on how the disease starts and progresses

1. The barrier dysfunction is associated with activation of keratinocytes.
2. There are changes in the skin microbiota; many patients (human and canine) are colonized with pathogenic Staphylococci, which release a number of toxins. The superantigens in particular are known to drive the Type 2 response.
3. Keratinocytes release proinflammatory cytokines (e.g. IL-1, the alarmins IL-25, IL-33 and TSLP) and chemokines (TARC/CCL17 [thymus and activation-regulated chemokine]and MDC/CCL 22 [macrophage-derived chemokine). Both these chemokines bind to CCR4, expressed on Th2 especially those expressing CLA (cutaneous lymphocyte antigen), Treg cells, and dendritic cells.
4. These mediators expand and activate basophils to produce IL-4 and skin-resident group 2 innate lymphoid cells (ILC-2) which produce IL-5 and IL-13.
5. These mediators also activate dendritic cells to promote the Type 2 response. A costimulatory molecule called OX40L is upregulated. This molecule is important in inducing T cells to make IL-4, IL-5, and IL-13. Current terminology suggests we call them DC2. Epidermal Langerhans cells are the classic instigators of the allergic response, but it is likely other DC contribute also. Upregulation of the chemokine receptor CCR7 is associated with dendritic cell maturation and promotes migration to the lymph nodes. CCR7 is expressed on B and T lymphocytes also, including T regulatory cells. The ligands for CCR7 are CCL19 and CCL21.
6. The dendritic cells (DC2) migrate to the lymph node, where they educate naïve T helper cells to become skin-homing T helper 2 cells. These Th2 also provide help to B lymphocytes to make allergen-specific IgE, aided by the IL-4 producing T follicular helper cells (Tfh2).
7. Skin homing T (cutaneous lymphocyte antigen/CLA, CCR4, PgD2R2/CRTH2) and B cells head to the skin, where they secrete the Type 2 cytokines you all know and love: IL-4, IL-5, IL-13, IL-31. Itch and inflammation!
8. There are also Type 2 CD8+ cells that infiltrate AD skin. They can produce Type 2 cytokines and IFN-γ. The latter is believed to make keratinocytes more sensitive to Fas-mediated apoptosis. Apoptosis is believed to further contribute to the barrier defect.
9. Eosinophils are also recruited; they express CRTH2 (chemoattractant receptor homologous)/PgD2R2 and H4R. CRTH2/PgD2R2 is a receptor for Prostaglandin D2, stimulating chemotaxis in Th2, basophils, and eosinophils. Antagonists for this receptor are currently being studied for the treatment of several allergic disease.
10. As the disease progresses, epidermal hyperplasia results, likely associated with IL-22 from the Th22 lymphocytes.
11. Depending on the individual, there are varying inputs of Type 1, Type 17, and Type 22 cells and cytokines.



Summary image from Weidinger S et al. Atopic dermatitis. Nat Rev Dis Primers 2018 Jun 21; 4 (1):_1.doi: 10.1038/s41572-018-0001-z.

How do we know what cytokines are most important in this disease when there are so many of them? Monoclonal antibodies and targeted medications are leading the way.

1. There are no head to head comparisons of mAb in the treatment of human atopic dermatitis.
2. A recent paper performed an indirect analysis, comparing data between studies. Here is a link to the open access PDF. Alexander H et al. Novel systemic therapies in atopic dermatitis: what do we need to fulfil the promise of a treatment revolution? F1000 Res 2019; 8 F1000 Faculty Rev-132. (https://www.ncbi.nlm.nih.gov/pmc/articles/PMC6357995/).
    a. Dupilumab (Anti-IL-4Rα) was the most effective when looking at the SCORAD (SCORing Atopic Dermatitis, which uses percentage of affected areas, intensity of clinical signs, and subjective signs, itch and insomnia) and EASI (Eczema Area and Severity Index). By blocking the common chain of the receptor for IL-4 and IL-13, signalling through these cytokines
    b. Baricitinib (JAK1/JAK2 inhibitor) was also very effective in reducing SCORAD and EASI. Not reported in this paper, JAK-1 selective inhibitors

c. Anti-IL-13 monoclonal antibodies (tralokinumab, lebrikizumab) appeared to be similar to baricitinib.
d. Nemolizumab (anti-IL-31R) had some activity, reducing SCORAD by 40% and EASI by 40-50%. The most recent study, performed in Japanese adolescents and adults, suggested less efficacy.
e. Much less effective were ustekinumab (binds p40 subunit of IL-12 and IL-23) and fezakinumab (anti-IL-22).
f. Omalizumab (anti-IgE) had no efficacy.
g. Not reported in this paper: some efficacy for Anti-OX40 (costimulatory molecule on T cell)
h. Nemolizumab reduced itch by 80% and dupilumab by 50%.
i. Earlly work not reported in this paper suggests lack of efficacy for
    i. Tezepelumab (anti-TSLP), although it works for asthma
    ii. Etokimab (anti-IL-33)
    iii. Anti-IL-17C? it is not being moved forward
j. JAK-1 selective kinases looking promising for human atopic dermatitis include upadacitinib and abrocitinib.

3. What about the veterinary disease? We only have one mAb (Cytopoint) and one JAK1 selective inhibitor (Apoquel), with which you are familiar. Please review these papers to remind yourself of the data.
    a. Cosgrove S et al. Efficacy ad safety of oclacitinib for the control of pruritus and associated skin lesions in dogs with canine allergic dermatitis. Vet Dermatol 2013; 24:587
    b. Cosgrove S et al. A blinded, randomized, placebo=controlled trial of the efficacy and safety of the Janus kinase inhibitor oclacinitinib in client-owned dogs. Vet Dermatol 2015: 26:23
    c. Cosgrove SB et al. Long-term compassionate use of oclacitinib in dogs with atopic and allergic skin disease: safety, efficacy, and quality of life. Vet Dermatol 2015: 26:171
    d. Little PR et al. A blinded randomized clinical trial comparing the efficacy and safety of oclacinitib and ciclosporin for the control of atopic dermatitis in client-owned dogs. Vet Dermatol 2015; 26:23
    e. Gadeyne C et al. Efficacy of oclacitinib compared with prednisolone for the control of pruritus and clinical signs associated with allergic dermatitis in client-owned dogs in Australia. Vet Dermatol 2014; 25:512.
    f. Michel GM et al. A blinded, randomized, placebo-controlled, dose determination trial of lokivetmab, a canonized, anti-canine IL-31 monoclonal antibody in client owned dogs with atopic dermatitis. Vet Dermatol 2016: 27:478.
    g. Michel GM et al. A blinded, randomized, placebo-controlled trial of the safety of lokivetmab, a canonized anti-canine IL-31 monoclonal antibody in client-owned dogs with atopic dermatitis. Vet Dermatol 2016; 27:505

What can we conclude from the mAb and JAK inhibitor data about the pathogenesis of this disease?

1. Type 2 cytokines remain important in this disease; blocking IL-13 and IL-4 appears to have the most impact on skin inflammation and blocking IL-31 appears to be best for itch control.
2. The data generated for Cytopoint and Apoquel are quite similar to those generated for people.

Itch is mediated by numerous cytokines produced during the disease process.

1. Atopic dermatitis is associated with hyperinnervation. IL-31 and NGF are both implicated in this. So is artemin, produced when the AHR is bound to air pollutants
2. Patients with moderate to severe atopic dermatitis have skin pain in addition to itch.
3. Cytokines whose receptors are known to be present on neurons.
    a. IL-31
    b. IL-4
    c. IL-13
    d. TSLP
    e. IL-33

How allergen immunotherapy works: rebalancing the immune system

The goal of allergen immunotherapy is do induce long term tolerance and prevent progression of the disease. The 2 best papers I can find that discuss how it works are these:

1. Burks WA et al. Update on allergy immunotherapy. J Allergy Clin Immunol 2013; 131:1288. It is older but does a great job of discussing mechanisms. Some of the comments on oral immunotherapy and SLIT are no longer correct.
2. Sozener ZC et al. Tolerance mechanisms in allergy immunotherapy. Curr Opin Allergy Clin Immunol 2020; 20:591

Key concepts

1. Desensitization of mast cells, basophils, eosinophils occurs very quickly once AIT is started. Two potential mechanisms for why
    a. Increased expression and function of inhibitory Fc receptors (FcγRIIa and FcγRIIb) which helps suppress activation through the FcεRI receptor
    b. Increased expression of histamine receptor (HR2); when histamine binds the HR2, which is believed to downmodulate the responses to histamine.
2. Tolerance
    a. Generation of regulatory cells
        i. Regulatory dendritic cells (DCreg) producing IL-10, IL-27, and TGFβ to polarize T cells into FoxP3+ Treg
        ii. IL-10+ innate lymphoid cells (ILCreg)
        iii. Regulatory B cells
            1. Make IL-10, TGFβ, IL-35
            2. Expression of allergen-specific IgD
        iv. Regulatory T follicular cells (Tf reg) express FoxP3+

v. Regulatory T cells (Treg)
    1. How they work
        a. Produce inhibitory cytokines (IL-10, TGFβ)
        b. Suppress antigen presenting cells and ILCs by PD-1, CTLA-4, ICOSL (inhibitory surface molecules)
        c. Inhibit target cell metabolism
        d. Induce cytolysis with Granzyme A and B
        e. Impair B cell production of IgE
    2. Types of Tregs
        a. Natural nTreg(FoxP3+, Helio+, CD25+)-tend to work by
        b. Inducible iTreg (FoxP3+)
        c. Inducible Tr1 which produce IL-10
        d. Inducible Tr3 which produce TGFβ
        e. Inducible IL-35 producing Treg (iTR35)

b. Reduction of T helper 2 to T helper 1 ratio (probably associated with Treg function)

3. Decrease of IgE and increase in IgG1,IgG2, IgG4 and IgA
    a. Compete for allergen binding
    b. Associated with conversion of macrophage from M2A proinflammatory phenotype to the M2B anti-inflammatory phenotype
4. Decreased numbers of mast cells, basophils, and eosinophils in tissue

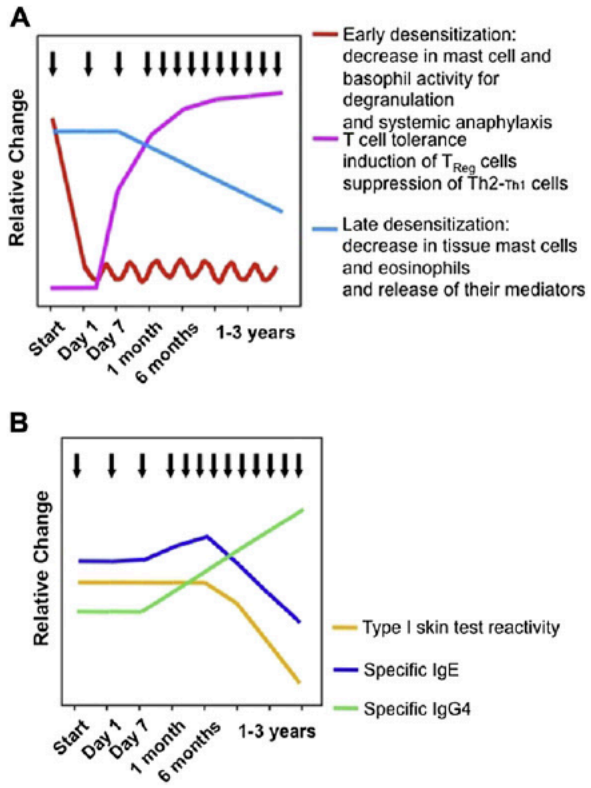


FIG 1. Immunologic changes during the course of AIT. **A,** Although there is significant variation between subjects and protocols, an early decrease in mast cell and basophil degranulation and decreased tendency for systemic anaphylaxis is observed immediately after the first administration of allergens with a native-like structure. This is followed by generation of allergen-specific Treg cells and suppression of allergen-specific T_H1 and T_H2 cells and possibly other effector cells. **B,** An early increase and a very late decrease in specific IgE levels are observed. IgG_4 levels show a relatively early increase that is dose dependent. In some studies allergen-specific IgG_1 and IgA levels also increase. A significant decrease in the allergen-specific IgE/IgG_4 ratio occurs after several months. A significant decrease in type I skin test reactivity is also observed relatively late in the course of specific immunotherapy. After a few months, a decrease in tissue mast cell and eosinophil numbers and release of their mediators is observed, as well as a decrease in the late-phase response. These effects are partially demonstrated in SLIT and are rather weak compared with those seen in SCIT. Novel AIT approaches might or might not show these effects, although they still can be effective.

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Are the mechanisms among the different delivery systems the same or different?

1. For our purposes, assume that the same mechanisms mediating AIT-induced tolerance are the same, particularly for SCIT and SLIT.
2. There are some subtle differences; e.g. peptide immunotherapy does not seem to shift the Th2:Th1, but these are not germane to what we do.

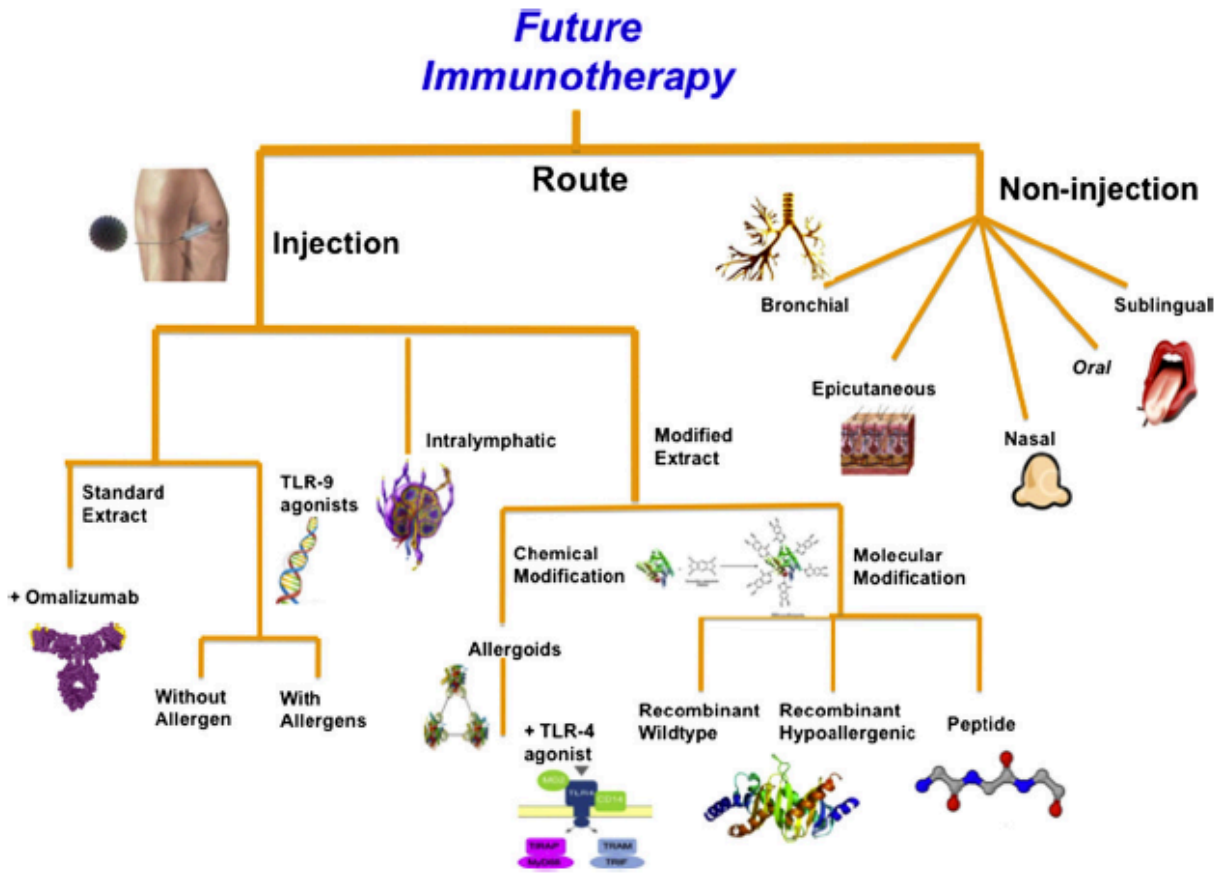

FIG 2. Novel approaches to AIT.

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