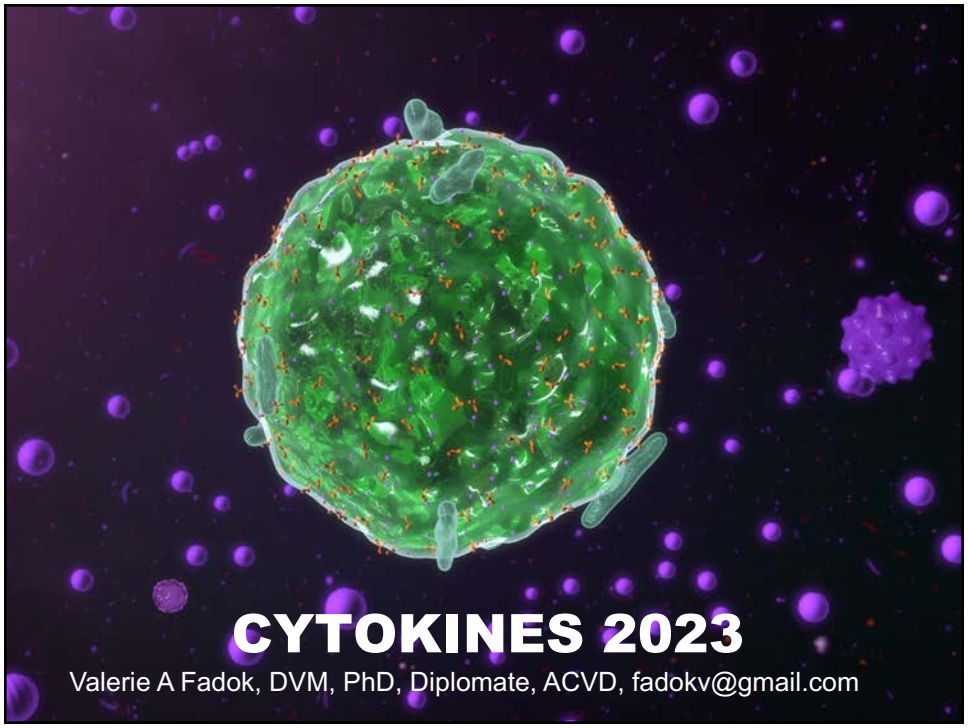


1

So many molecules
So little time

- How do we cope?
- Patterns
- Subsets
- Practical application to disease when possible
  - **What information do we have for our veterinary patients?**
  - **Can we extrapolate info from human disease?**

Fundamentals of allergy and immunology

Interleukins (from IL-1 to IL-38), interferons, transforming growth factor β, and TNF-α: Receptors, functions, and roles in diseases

Mübeccel Akdis, MD, PhD, Alar Aab, MSc, Can Altunbulakli, MSc, Kursat Azkur, DVM, Rita A. Costa, MSc, Reto Crameri, PhD, Su Duan, MD, Thomas Eiwegger, MD, Andrzej Eljaszewicz, PhD, Ruth Ferstl, PhD, Remo Frei, PhD, Mattia Garbani, PhD, Anna Globinska, MSc, Lena Hess, MD, Carly Huitema, PhD, Terufumi Kubo, MD, Zsolt Komlosi, MD, Patricia Konieczna, PhD, Nora Kovacs, MD, Umut C. Kucuksezer, PhD, Norbert Meyer, MD, Hideaki Morita, MD, Judith Olzhausen, PhD, Liam O'Mahony, PhD, Marija Pezer, PhD, Moira Prati, MSc, Ana Rebane, PhD, Claudio Rhyner, PhD, Arturo Rinaldi, MSc, Milena Sokolowska, MD, PhD, Barbara Stanic, PhD, Kazunari Sugita, MD, Angela Treis, PhD, Willem van de Veen, PhD, Kerstin Wanke, PhD, Marcin Wawrzyniak, PhD, Paulina Wawrzyniak, MSc, Oliver F. Wirz, MSc, Josefina Sierra Zakzuk, MD, and Cezmi A. Akdis, MD *Davos, Switzerland*

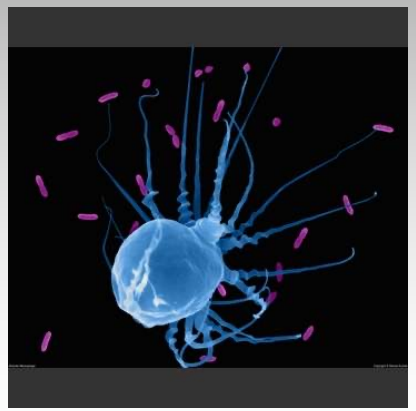
J ALLERGY CLIN IMMUNOL
VOLUME 138, NUMBER 4

J Allergy Clin Immunol 138:984-1010, October 2016

3

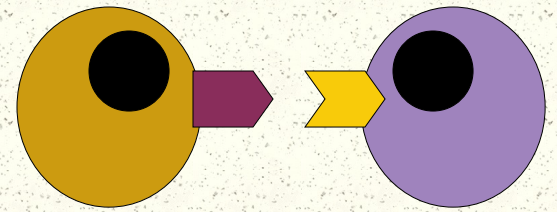
Why should veterinary dermatologists know so much about immunology in general and cytokines in particular?

- Skin is one of the largest immune organs
- Keratinocytes are second only to macrophages in cytokine-producing abilities
- We deal with a large number of inflammatory and/or immunologic disorders mediated by cytokines
- Understanding cytokines leads to improved therapy
- WE ARE USING therapeutics that target them!



How do cells communicate with each other?

Surface molecules



5

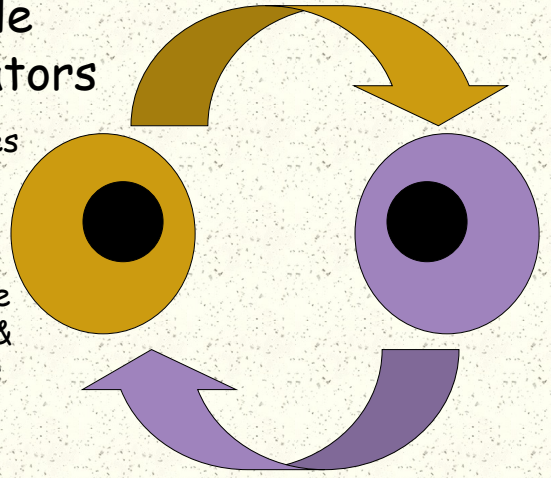
How do cells communicate with each other?

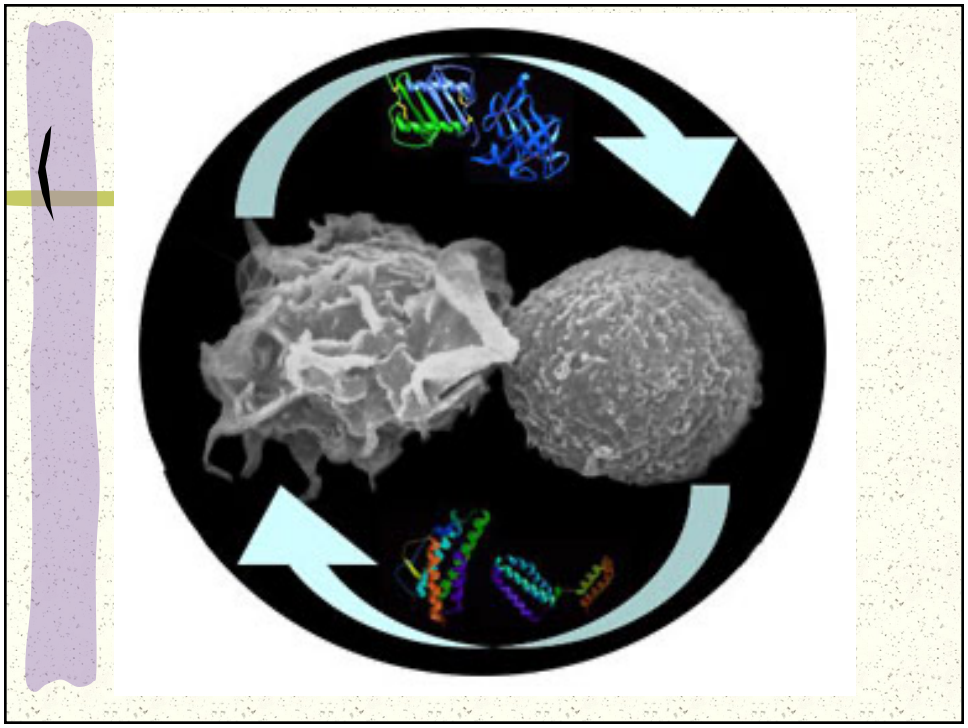
Soluble Mediators

Cytokines

Lipids

Bioactive Amines & Peptides





7

If you remember nothing else . . .

- No single cytokine can regulate a vital process like an immune response
- Rather, it takes a constellation of cytokines, tuned in concert, that produce complex phenotypes like “tissue damage” or “recovery from tissue damage.”
- Lawrence Steinman

What are cytokines?

Small molecular weight regulatory proteins

Growth

Differentiation

Survival (or death!)

Effector function

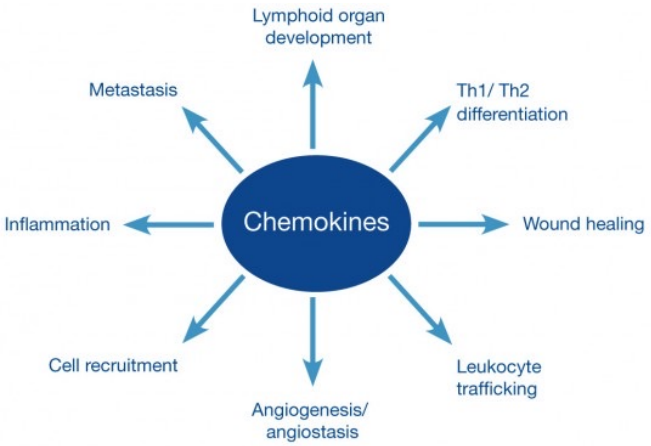
Chemokines

Cytokine subset

Lower molecular weight (8-10 kd)

Mediate chemotaxis (cellular migration along gradient)--this recruits inflammatory cells to infection

immune homeostasis
host defense

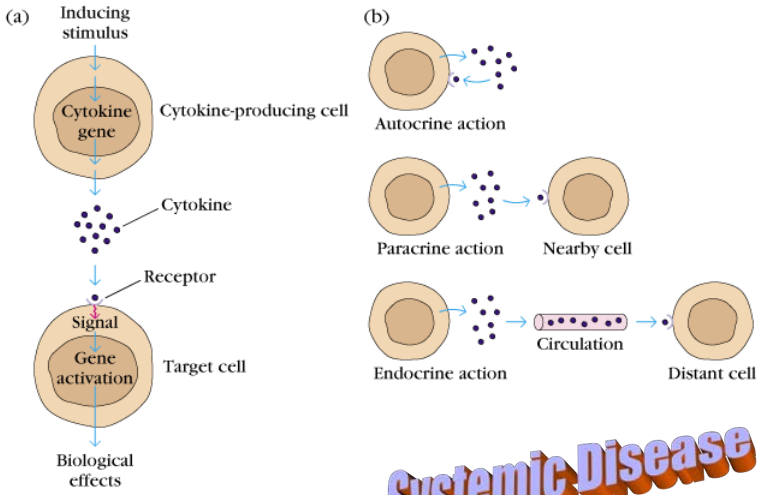


11

Cytokines vs. Hormones

Properties	Hormones	Cytokines
Producers	Few	Many
Targets	Many	Few
Biological Role	Homeostasis	Homeostasis
Redundancy	Low	High
Pleotropy	Low	High
Circulation	Yes	Rarely
Influence	Widespread	Local
Inducers	Physiologic variation	External insults

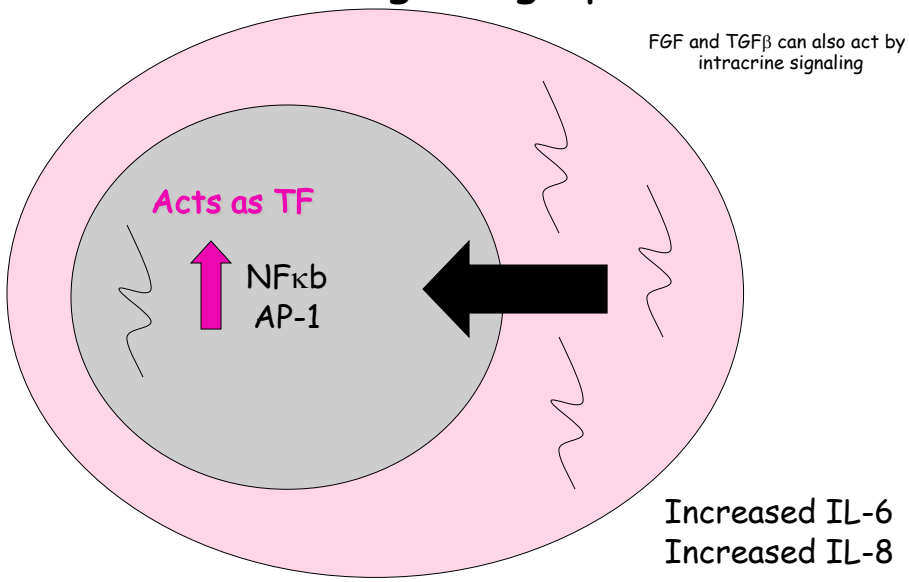
Effective at very low concentrations



Systemic Disease

Tend to work locally

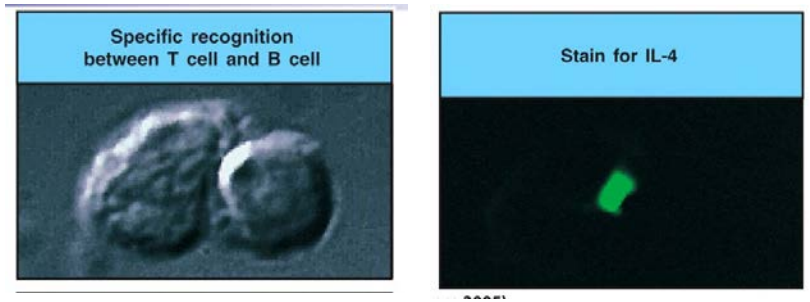
Intracrine Signaling: pro IL-1α



16

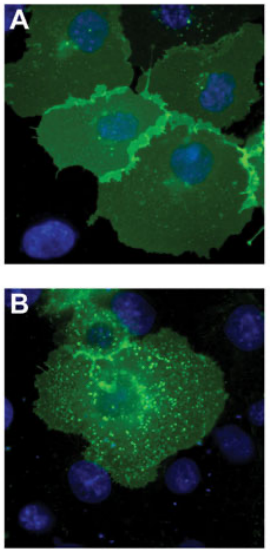
Restriction to Local Sites Is Critical

- Producer cells close to target cells
- Small amounts secreted
- Directional release



Restriction to Local Sites Is Critical

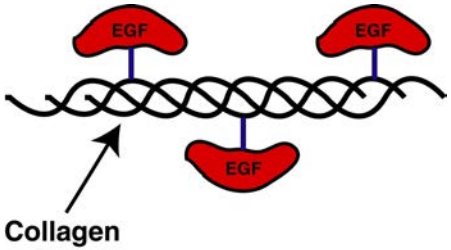
- Producer cells close to target cells
- Small amounts secreted
- Directional release
- Receptor endocytosis



18

Restriction to Local Sites Is Critical

- Producer cells close to target cells
- Small amounts secreted
- Directional release
- Receptor endocytosis
- Bind to extracellular matrix



Restriction to Local Sites Is Critical

- Producer cells close to target cells
- Small amounts secreted
- Directional release
- Receptor endocytosis
- Bind to extracellular matrix
- **Quenching in circulation by soluble receptors, other binding proteins**
  - Basis by which Etanercept (Enbrel) works
- **Natural antagonists**
  - IL-1 RA, TNFsRp55 (soluble TNF receptor)

Restriction to Local Sites Is Critical

- High levels of circulating proinflammatory cytokines = "cytokine storm"
- The killer in toxic shock, severe influenza
- Severe malaise of food poisoning
- Post-viral encephalopathies

Additional Controls On Activity

Secretion brief and limited

- not stored pre-formed
- synthesis initiated by gene transcription
- mRNA short-lived
- cytokines produced as needed

Not all cytokines active after synthesis--must be processed

IL-1, TGF-β

Cytokine Concepts

Pleotropy: one cytokine has multiple activities, e.g.

- IL-1
- IL-2
- IL-6
- TNF-α
- IFNγ

Cytokine Concepts

Pleotropy: one cytokine has multiple activities

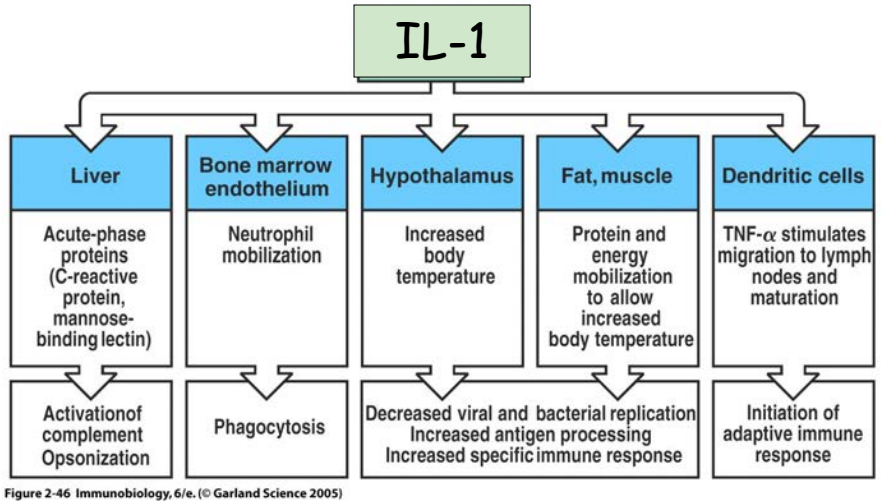
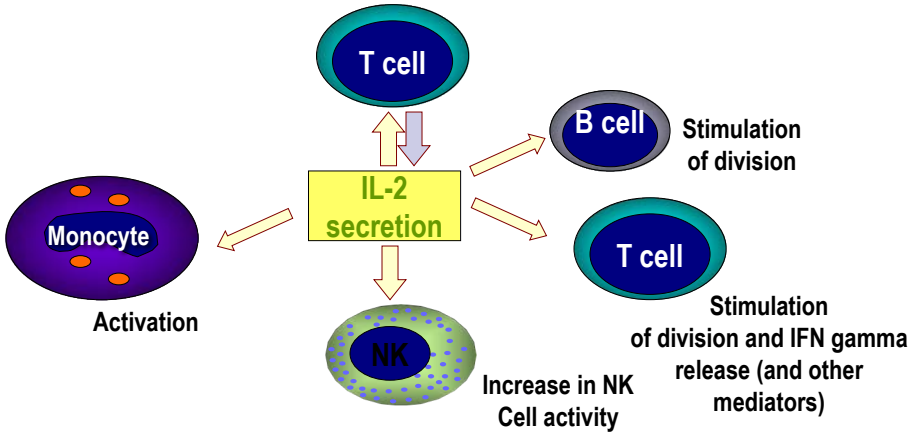


Figure 2-46 Immunobiology, 6/e. (© Garland Science 2005)

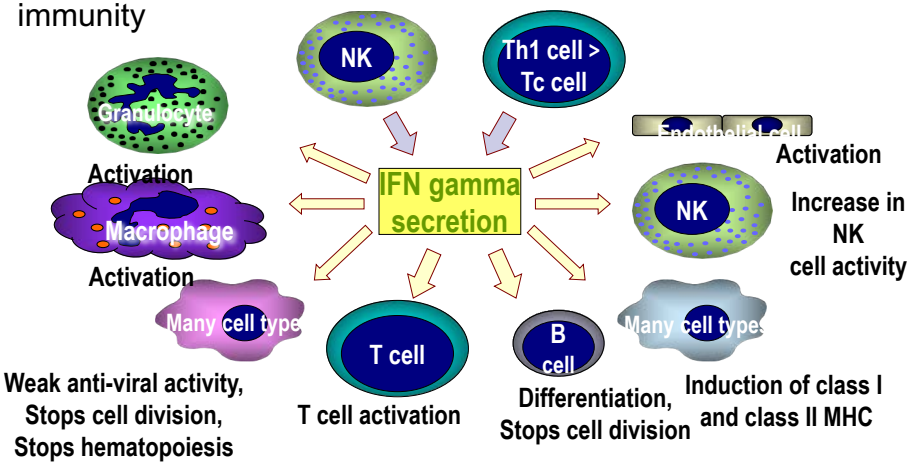
Interleukin-2 (IL-2)

- Produced by Th>>Tc, NK, NKT cells
- Main growth factor for T cells
- Affects function of many cells



Interferon-gamma (IFN-γ)

- Produced by Th cells **>>** Tc and NK cells
- Also produced by macrophages
- Numerous functions in both **natural** and **specific** immunity



Cytokine Concepts

Redundancy: multiple cytokines have same or similar activities

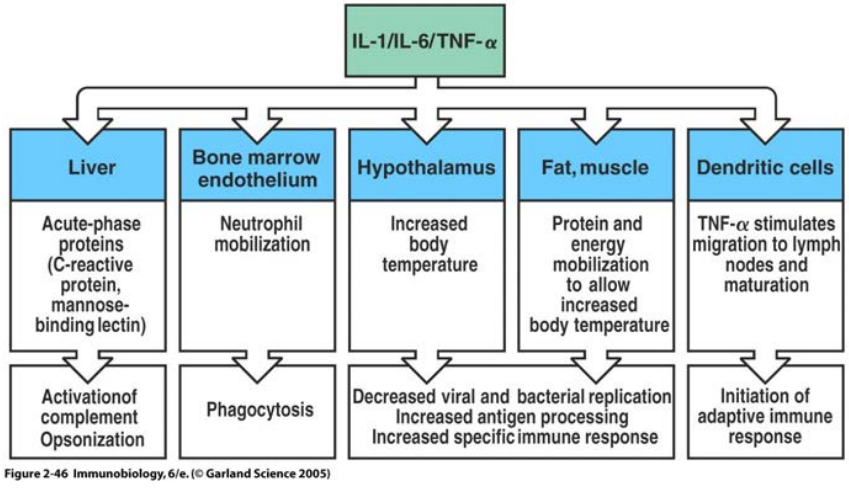


Figure 2-46 Immunobiology, 6/e. (© Garland Science 2005)

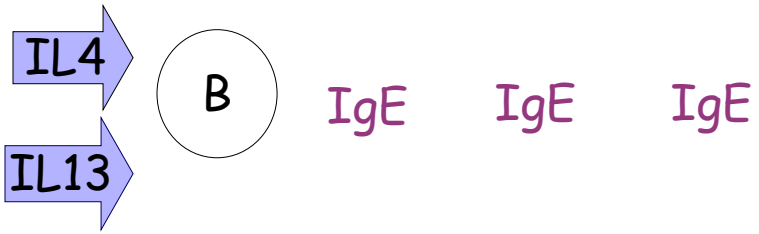
Cytokine Concepts

Redundancy: multiple cytokines have same or similar activities (may come from different sources)

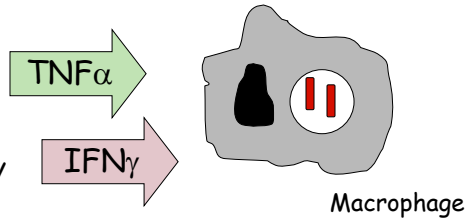
IL-1α and IL-1β
IL-2 and IL-15
IL-4 and IL-13
TNFα and TNFβ
IL-31 and TSLP

Cytokine Concepts

Synergism: Two cytokines acting together to enhance a biologic effect



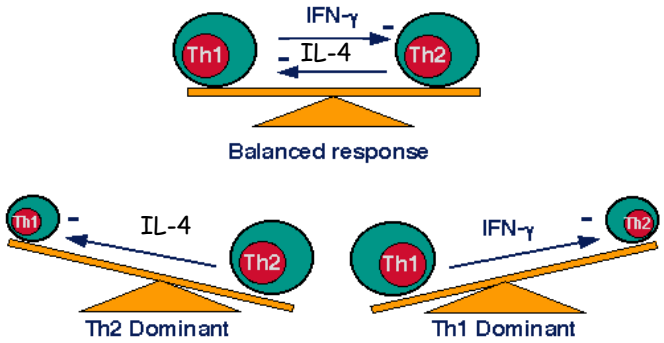
Why we have Innate and Adaptive Immunity



Cytokine Concepts

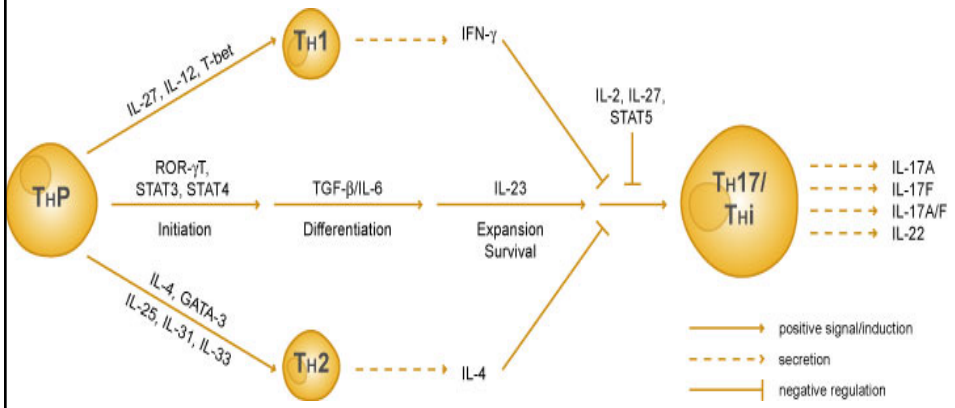
Antagonism: Two cytokines oppose each other's effects

Counter regulation of Th cell subsets



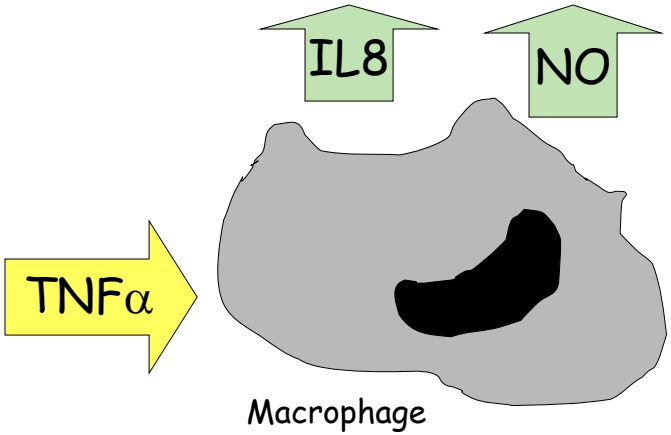
30

Development of Th-17 cells inhibited by IFN-γ or IL-4



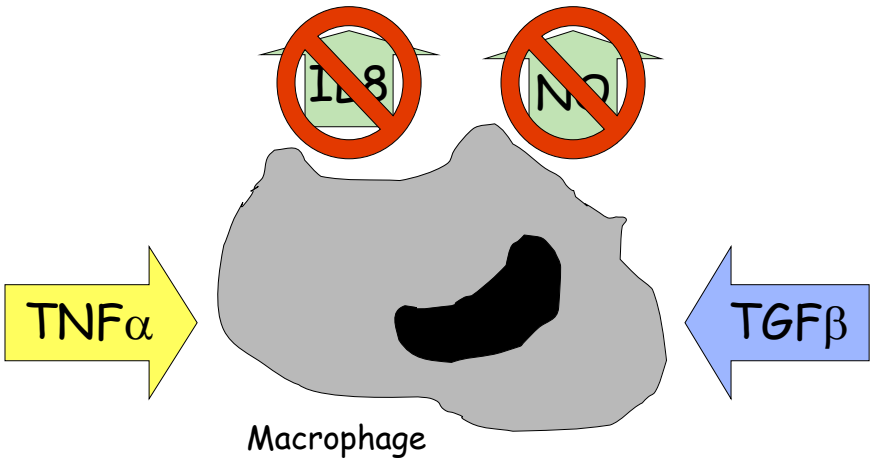
Cytokine Concepts

Antagonism: Two cytokines oppose each other's effects



Cytokine Concepts

Antagonism: Two cytokines oppose each other's effects



Cytokine Concepts

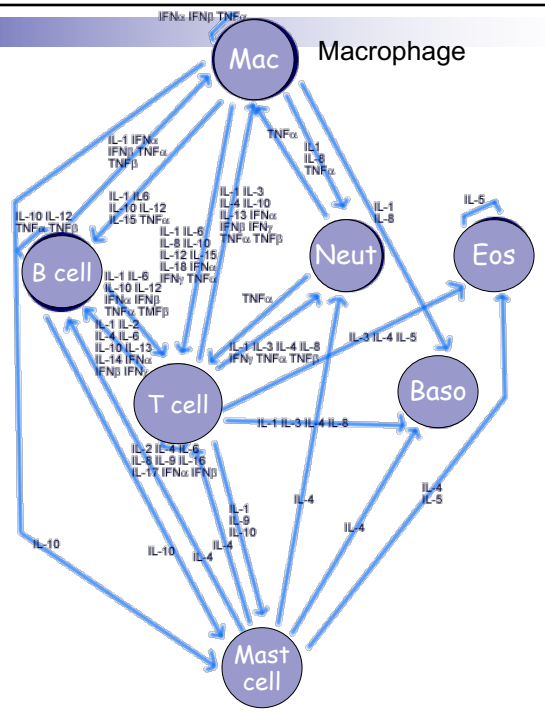
Cytokines act in context

Effects of a cytokine are determined by
target cell's state of activation or differentiation
or the milieu into which it is secreted

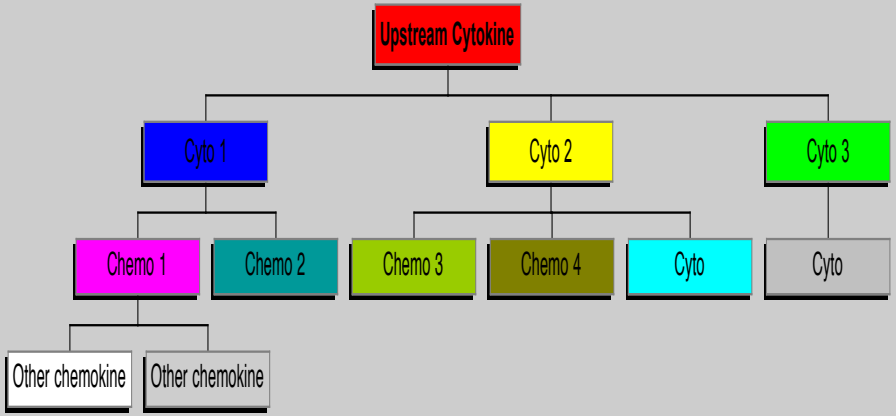
TGFβ

1. activates monocytes but deactivates macrophages
2. anti-inflammatory at low concentrations but profibrotic at high concentrations
3. In the absence of proinflammatory cytokines, induces Treg; in the presence of proinflammatory cytokines induces Th17

Cytokine Network

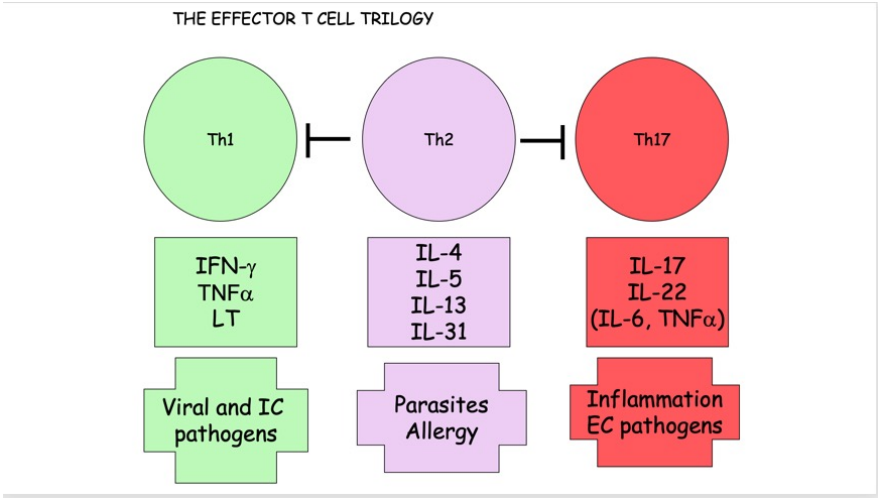


Cytokine Cascades



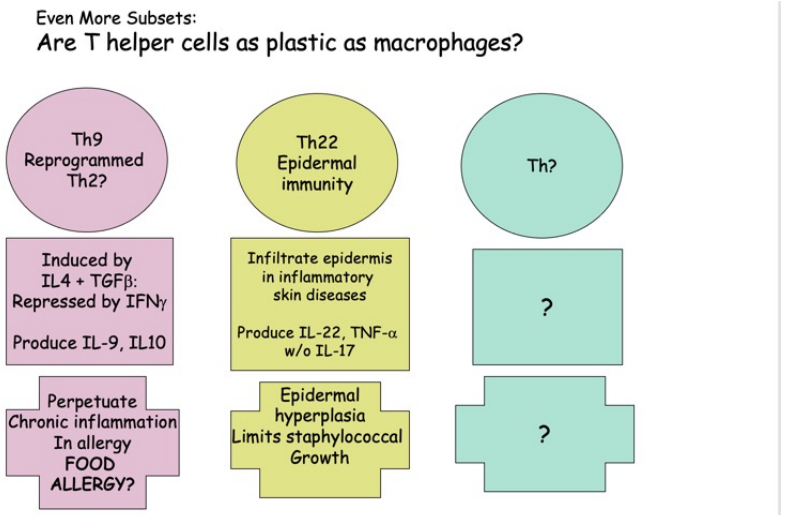
36

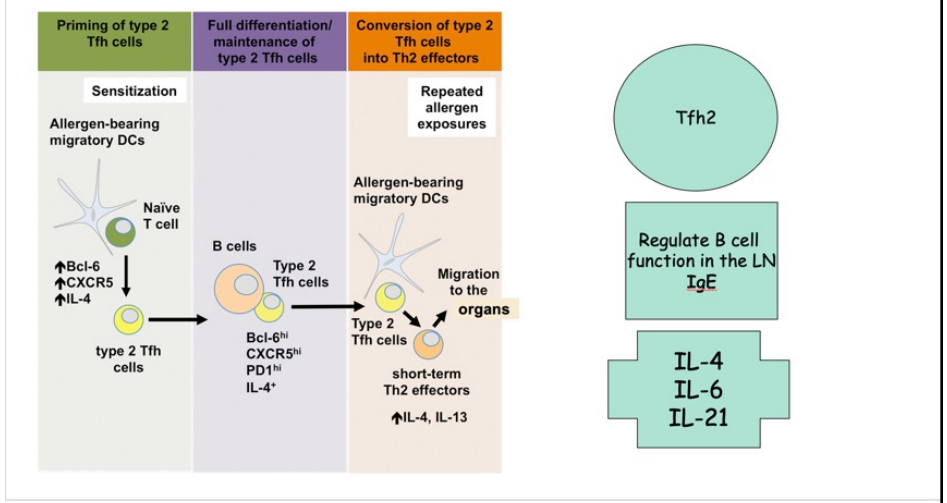
Let's focus on disease-related cytokines that we work with DAILY



38

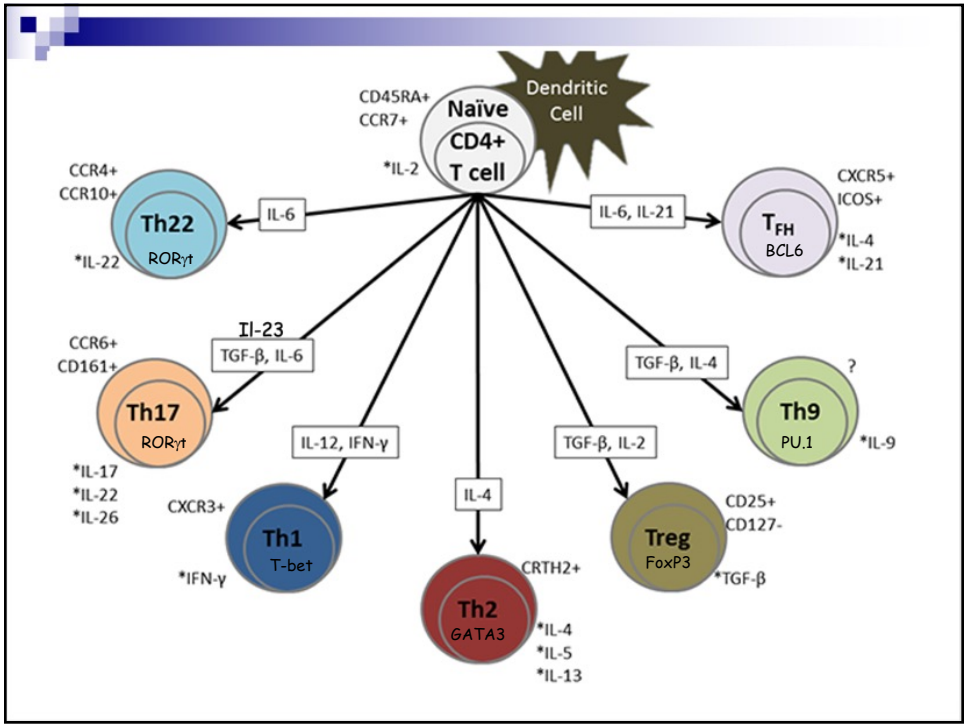
Oh no! Are there more?



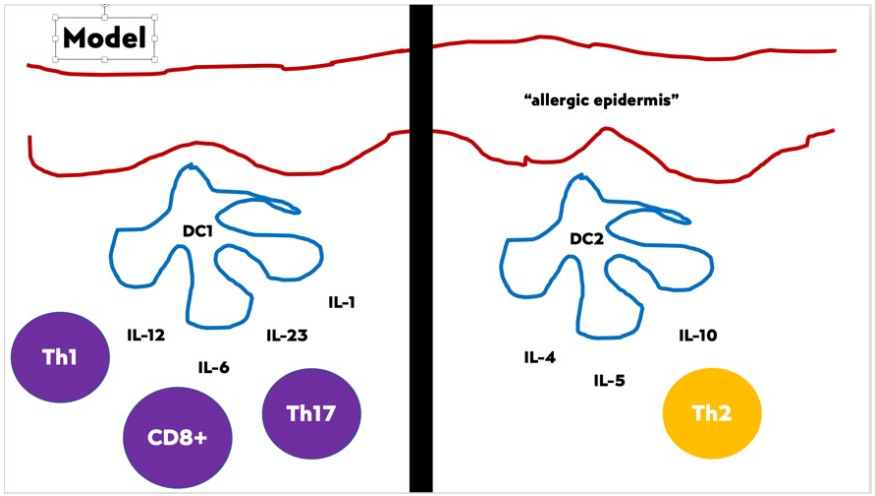


Long term humoral immunity

40

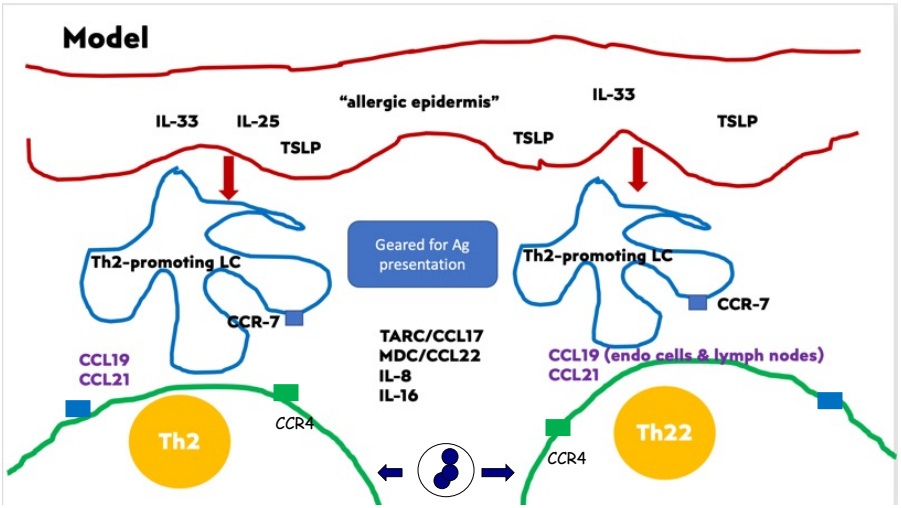


Dendritic cell activity: DC1 vs DC2

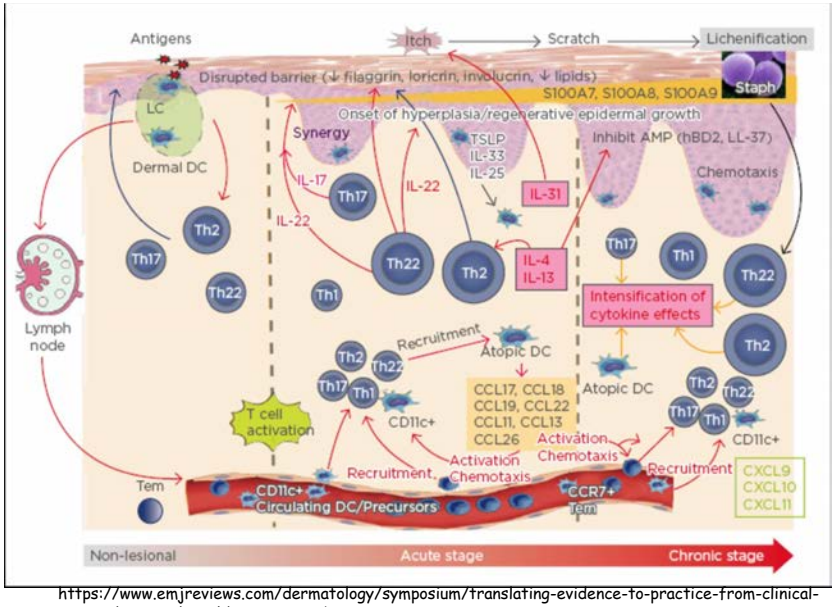


42

Langerhans cells are special

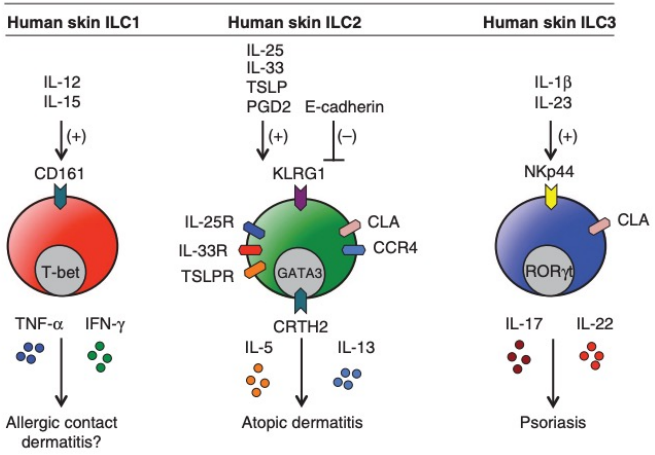


THE COMPLEXITY OF CYTOKINES IN ATOPIC DERMATITIS

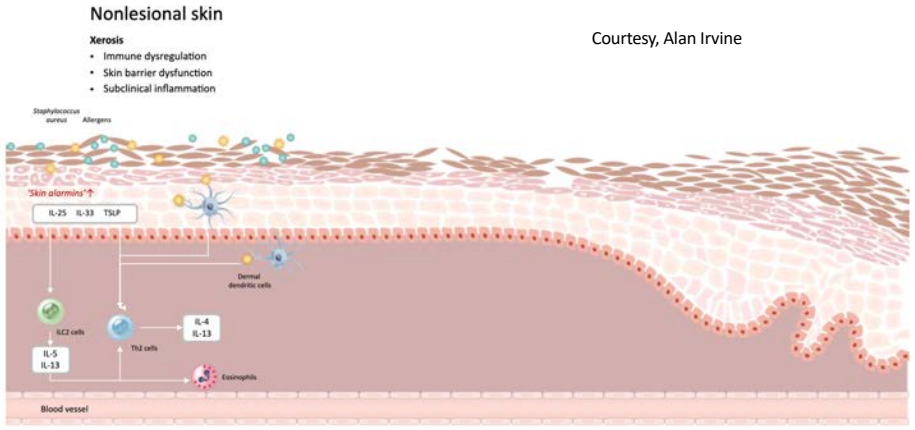


https://www.emjreviews.com/dermatology/symposium/translating-evidence-to-practice-from-clinical-research-to-real-world-experience/

Innate lymphoid cells in AD



JID 2015; 135:673



Courtesy, Alan Irvine

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.

Courtesy, Alan Irvine

Skin alarmins: innate cytokines that stimulate Type 2

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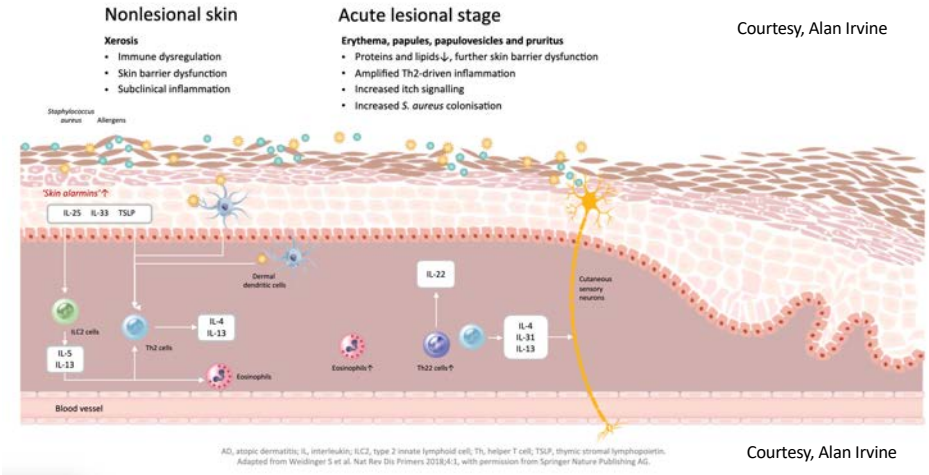

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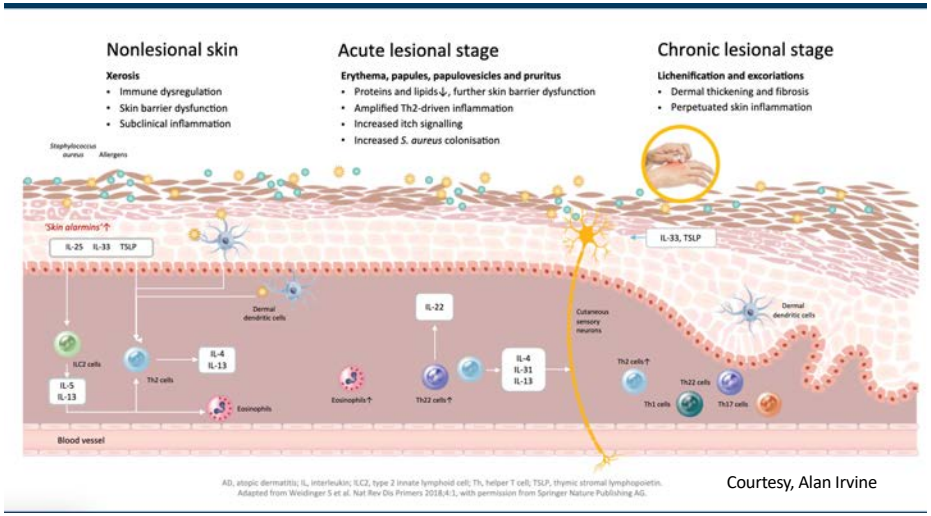
AD, atopic dermatitis; IL, interleukin; ILC2, type 2 innate lymphoid cell; Th, helper T cell; TSLP, thymic stromal lymphopoietin.
Adapted from Weidinger S et al. Nat Rev Dis Primers 2018;4:1, with permission from Springer Nature Publishing AG.

Courtesy, Alan Irvine



Courtesy, Alan Irvine

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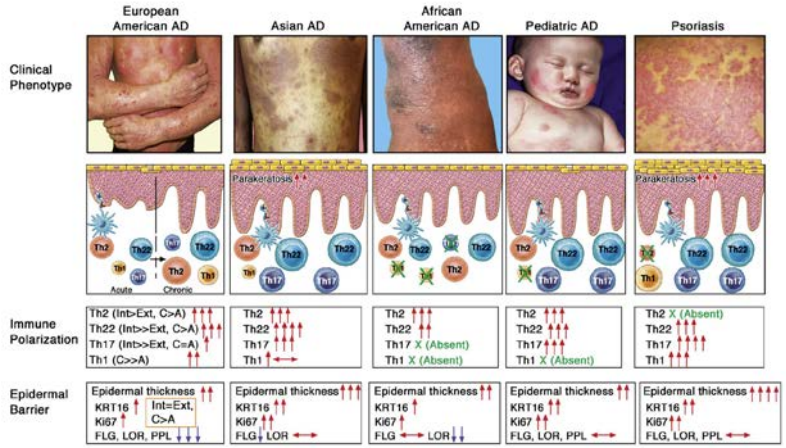
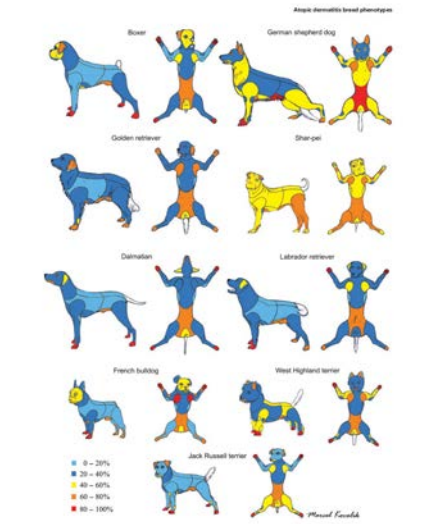
Phenotypes and endotypes



Czarnowicki et al. J Allergy Clin Immunol 2019; 143:1-11



Breed differences in distribution of atopic dermatitis

Will we learn that these have endotypes?

Veterinary Dermatology

Breed-associated phenotypes in canine atopic dermatitis

22:143-149, 2010

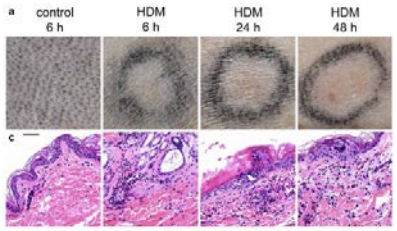
50

Skin biopsy microarray
Epicutaneous challenge with HDM

Early Activation of Th2/Th22 Inflammatory and Pruritogenic Pathways in Acute Canine Atopic Dermatitis Skin Lesions

JID Open

Thierry Olivry[1,2], David Mayhew[3], Judy S. Paps[1], Keith E. Linder[2,4], Carlos Peredo[5], Deepak Rajpal[6], Hans Hofland[5,7] and Javier Cote-Sierra[5,8]



6 h

ALOX5 NGF
ALOX5AP

24 h

NMB
CMA1 CTSS
MASTIN CTSC
IL31 ALOX5AP
ALOX5
IL13RA2 IL13
TSLP NTRK1

48 h

NMB
HDC HNMT
NGF NTRK1 CTSC
MASTIN CTSS TPS1
CMA1
IL31 IL6 IL33
TSLP IL4
IL13RA2 IL13
ALOX5AP CYSLTR1
ALOX5 LTA4H

proteases
cytokines
bombesins
histamine
neurotrophins
leukotrienes

> 1.5 fold-change
HDM vs control, P < 0.05
underlined = downregulated
size proportional to fold-change

4 PHENOTYPIC SYNDROMES
ARE THESE FELINE ENDOTYPES?

- Self induced alopecia
- Miliary dermatitis
- Eosinophilic dermatitis (eosinophilic granuloma complex)
- Head and neck pruritus




Andrea Cecilia Wolberg DVM and Alejandro Blanco, DVM - 08/05/2015

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TRANSCRIPTOME OF EOSINOPHILIC PLAQUES

Received: 21 April 2022 | Accepted: 21 July 2022
DOI: 10.1111/vde.13125

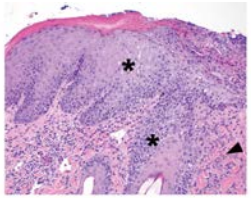
2023; 34:40

Veterinary Dermatology

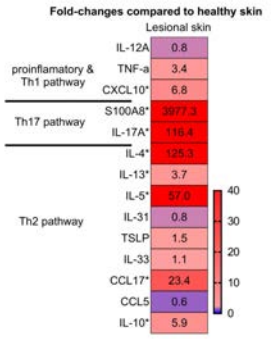
ORIGINAL ARTICLE

Transcriptome analysis of selected cytokine and chemokines in the eosinophilic plaques of cats with atopic skin syndrome

Cheryl Vargo[1] | Elizabeth W. Howerth[2] | Frane Banovic[1]



4 affected, 5 healthy



PLOS ONE **HORSES**

2020

RESEARCH ARTICLE

Investigating the epithelial barrier and immune signatures in the pathogenesis of equine insect bite hypersensitivity

Iva Cvitas[1,2,3]*, Simone Oberhänsli[4], Tosso Leeb[3,5], Martina Dettwiler[3,6], Eliane Müller[3,6,7,8], Remy Bruggman[4], Eliane Isabelle Marti[1,3]

Epidermal barrier

- ✓ Downregulation mRNA tight junction
- ✓ Alterations in keratins

Immune signatures for Th2 and Th1

- ✓ IL-13R
- ✓ IL-5R
- ✓ IL-4R

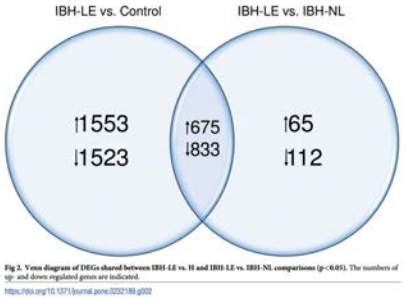


Fig 2. Venn diagram of DEGs shared between IBH-LE vs. H and IBH-LE vs. IBH-NL comparisons ($p<0.05$). The numbers of up- and down regulated genes are indicated.

https://doi.org/10.1371/journal.pone.0232188.g002

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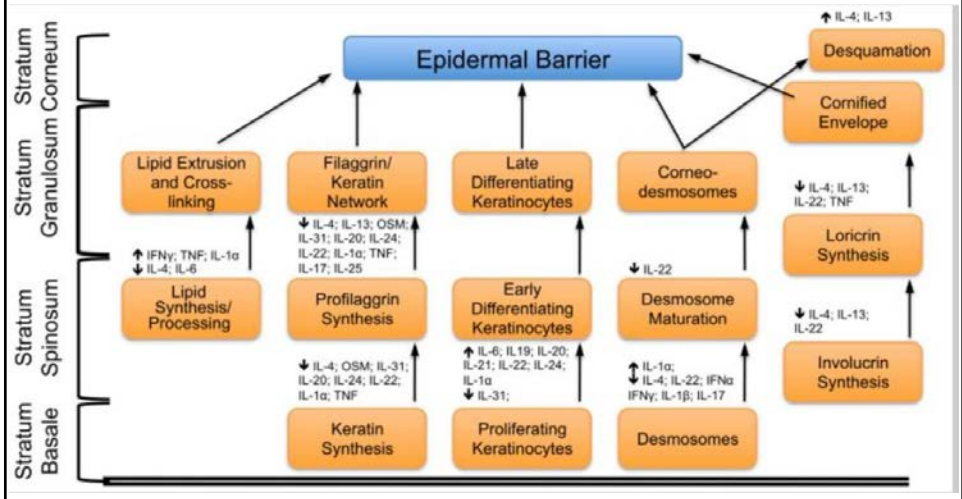
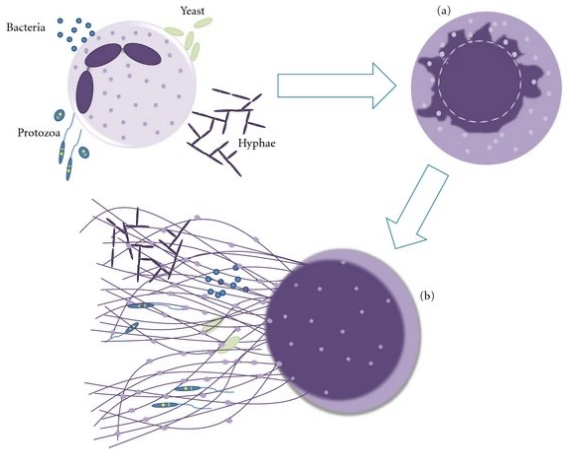
Impact of Type 2 cytokines (and others) on the skin barrier



Hanel et al. Int J Mol Sci 2013; 14:6720

Additional impact of IL-4 and IL-13 revealed by dupilumab in atopic humans

- Inhibition of neutrophil chemotaxis and phagocytosis
- Inhibition of neutrophil killing
- Inhibition of neutrophil NETS *(neutrophil extracellular traps)*
- Neutrophil function restored in patients treated with dupilumab



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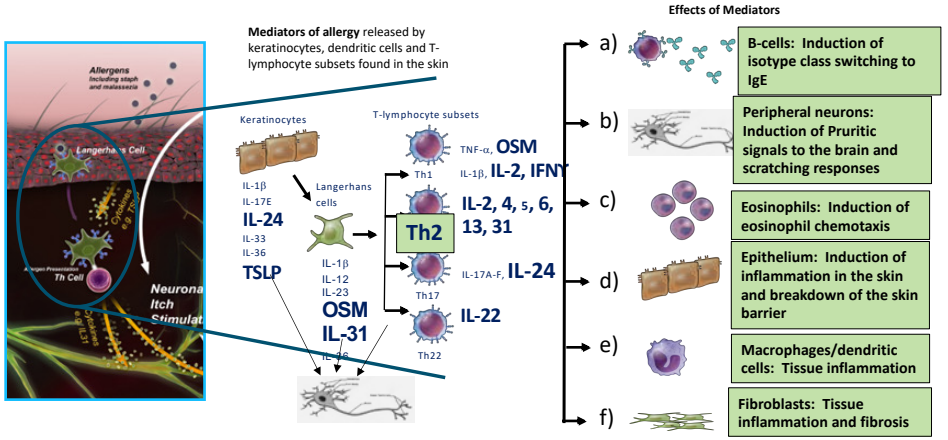
What we are learning now – new potential targets



Courtesy, Dr. Andrea Gonzales, Zoetis

THE COMPLEXITY OF ATOPIC ITCH

- Interleukin 31
- TSLP
- IL24
- IL-4
- IL-13
- Proteases and PARs
- Histamine via H4R
- Serotonin
- Neuropeptides
  - Substance P
  - CGRP (calcitonin gene-related peptide)
  - GRPR (gastrin-releasing peptide receptor)
  - VIP

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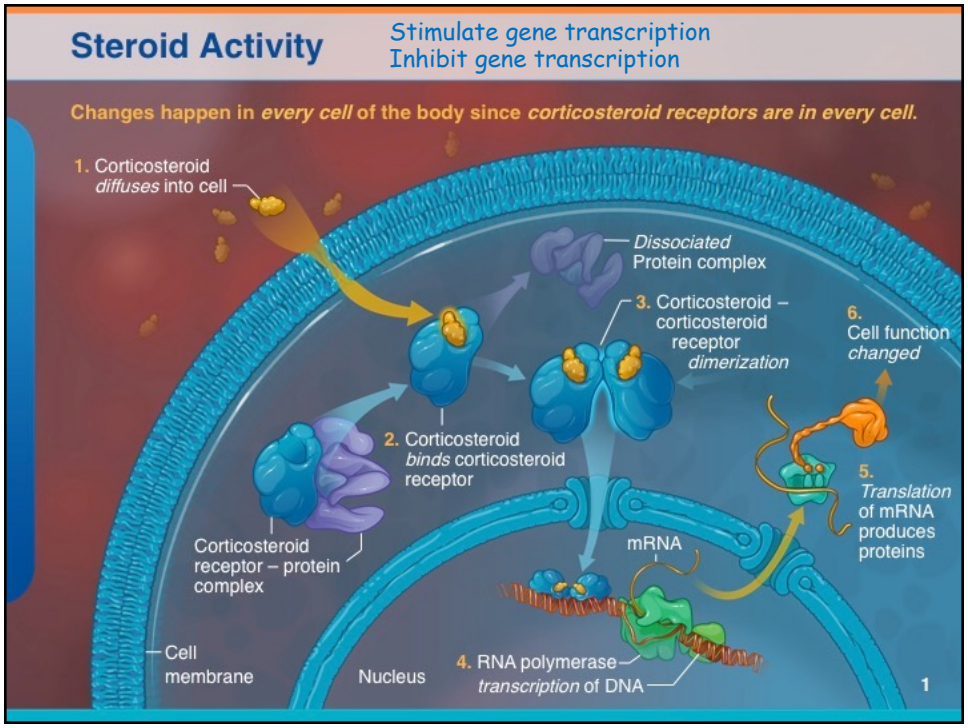
Other things you want to know

- How do steroids work in atopic dermatitis?
  - **What is the GR? How does it work?**
- How does cyclosporine work?
  - **What molecule does it bind? How does that work?**
  - **Why does it take several weeks to provide efficacy?**
- How does oclacitinib work?
  - **What does oclacitinib bind? How does it work?**
  - **What is JAK? What is STAT?**
  - **Be able to describe the selectivity of oclacitinib with regard to mechanism of action at recommended dosage and frequency**
- How does Lokivetmab (Cytopoint) work?
  - **What does it bind?**
  - **Why is the target important?**
- How does allergy immunotherapy work?
  - **Why does it take so long for this approach to provide help?**



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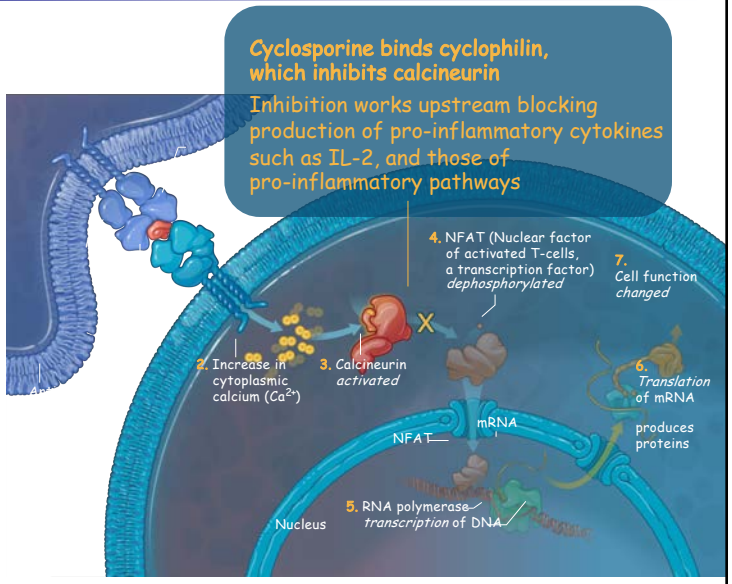
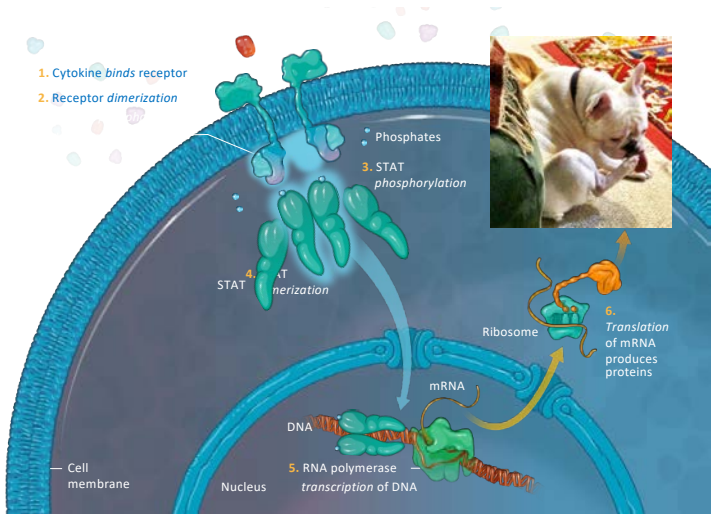
Cyclosporine Mechanism of Action: decreases production of cytokines



CsA inhibits more than IL-2 production

- Calcineurin inhibitors like CsA directly affect function of the innate immune system as well as the acquired immune system
- Directly affect keratinocytes
- Cyclophilins are present in most cells
- We don't know all the effects of calcineurin inhbitors

JANUS KINASE (JAK) SIGNALING SUMMARY



JANUS KINASE (JAK) INHIBITION

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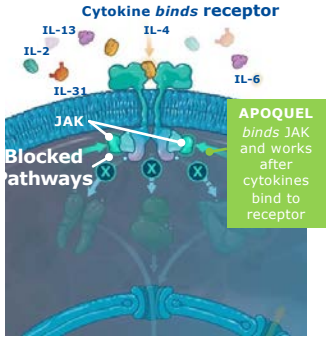
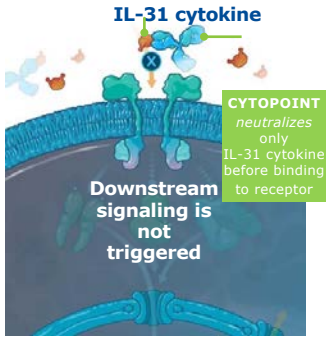
apoquel®



CYTOPOINT™



Post Grooming
Folliculitis/Furunculosis

+ Culture for
Pseudomonas aeruginosa

Let's hypothesize
the cytokine response

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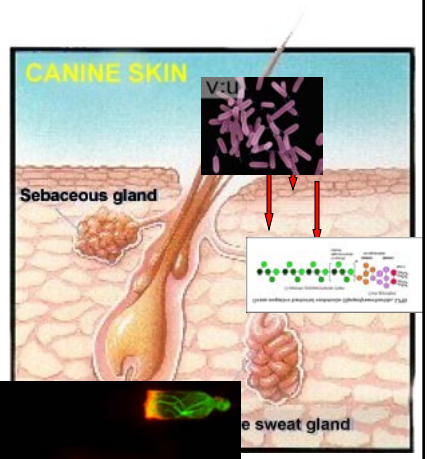
Endotoxin binds TLR on keratinocytes
and Langerhans cells
(TLR2, 4 on KC, TLR4 on LDC)

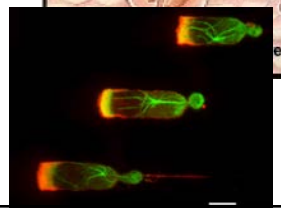
KC and LDC secrete proinflammatory
Cytokines (IL1, TNFa) and chemokines
(IL8, others-CCL2/MCP-1, CCL20/MIP3a),
upregulation of defensins, MHC Class II,
ICAM-1

Endotoxin binds TLR 4 on dermal DC
and macrophages (tissue ones AND the
ones called in by the chemokines!)

Secretion of IL-1, TNFa, IL-6,
IL-8, others), upregulation of killing
power in macrophages

Meanwhile, the neutrophils are coming!





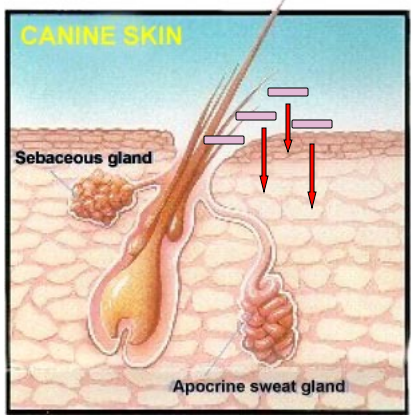
Neutrophils phagocytose and kill when they can (reactive oxygen species)

Neutrophils also die and release NETS (neutrophil extracellular traps) which are chromatin decorated with antimicrobial peptides from granules

Bacteria and toxins mopped up

Why are these dogs so painful?

Endotoxins stimulate prostaglandins which sensitize nerve fibers to lesser stimuli; other factors e.g. substance P, histamines, etc.



abcam