Microbiome

Rosacea

Atopic Dermatitis

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A few facts :

Human body: 10^{13} cells

Microbiota: 10^{14} cells, $8x10^{6}$ genes (vs 20.000 !)

Weight: 1,5 kg (!)

CUTANEOUS MICROBIOTA

Whole of saprophytic and pathogenic microorganisms that colonize our body

Resident flora stratum corneum

proteobacterias
firmicutes
bacteriadetes
actinobacterias

Form microbial imprint
25% in hair follicles and sweat glands

Transitory flora

streptococcus
neisseria
pseudomona
E coli
C albicans
S aureus

Takes advantage of habitat modification to proliferate

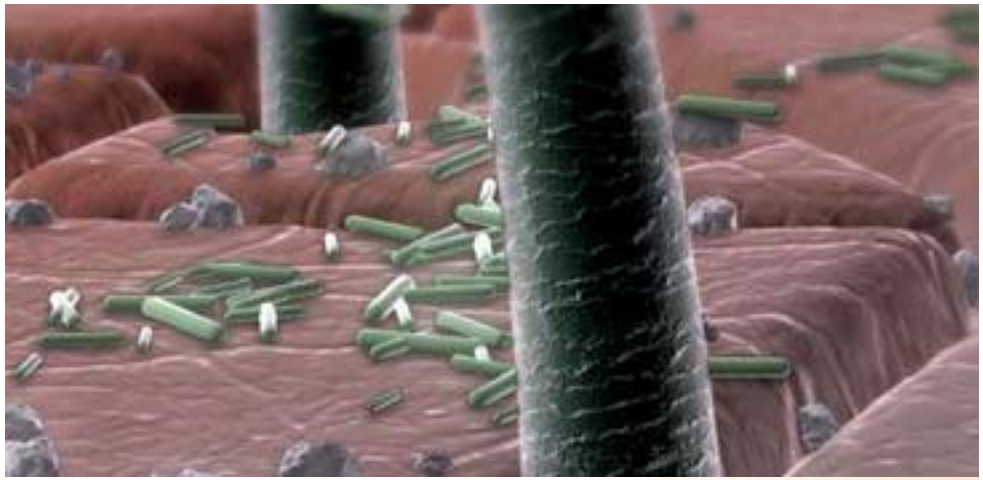
An average healthy human body possesses 10 [14]microorganisms – 10-fold the number of human cells.

These bacterial, viral, fungal and eukaryotic microorganisms are absolutely essential to everyday life

The gut microbiome is by far the most extensively studied. The human gut contains nearly 10 trillion bacterial cells and over 2000 different species

The bacterial communities in other parts of the body are quite distinct from those in the gut, and differ significantly depending on body site differences and are influenced by pH, temperature and oxygen content.

MICROBIOTA



- A single square centimetre of the human skin can contain up to one billion microorganisms. A single square centimetre of the human skin can contain up to one billion microorganisms
- Distinct skin microbial communities arise, becoming increasingly diverse over time.
- These microbial niches and their inhabitants continue to transform over time with puberty, aging and environmental exposure

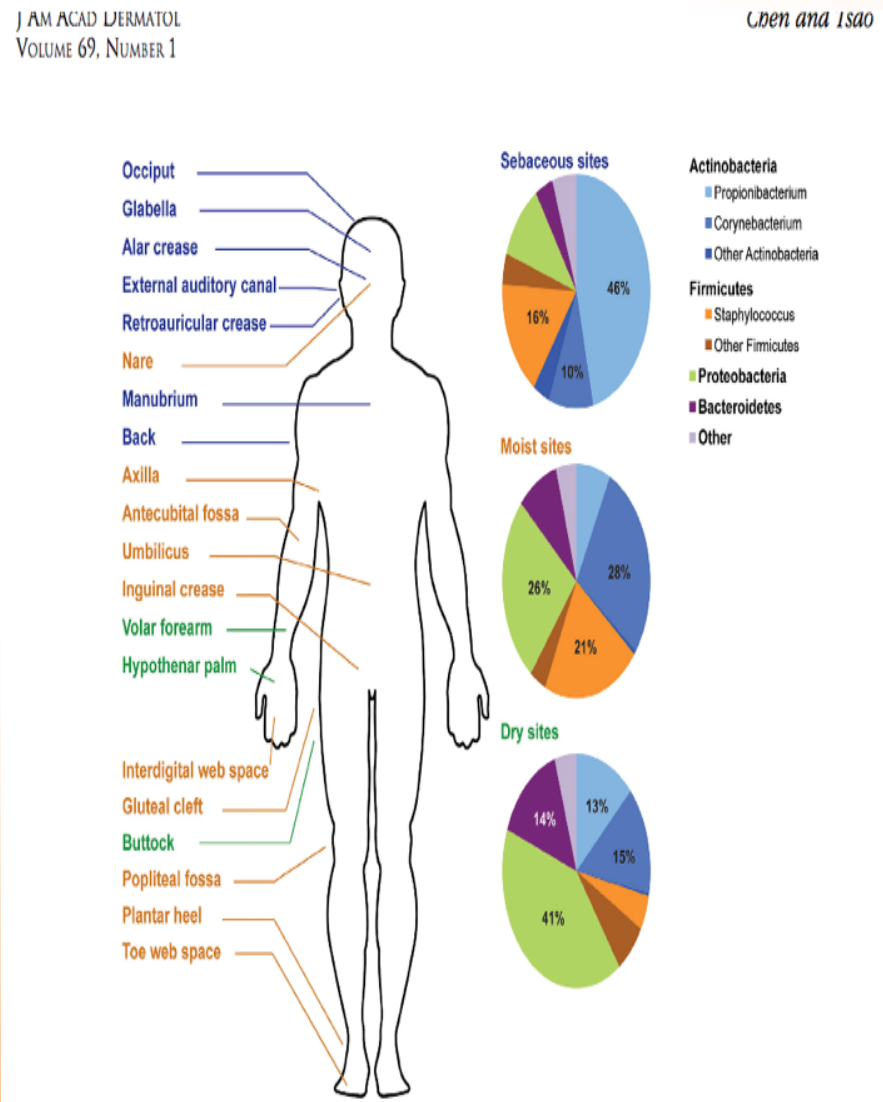
Leyden JJ, McGinley KJ, Mills OH *et al*. Age-related changes in the resident bacterial flora of the human face. *J. Invest. Dermatol.* 1975; **65**: 379–81.

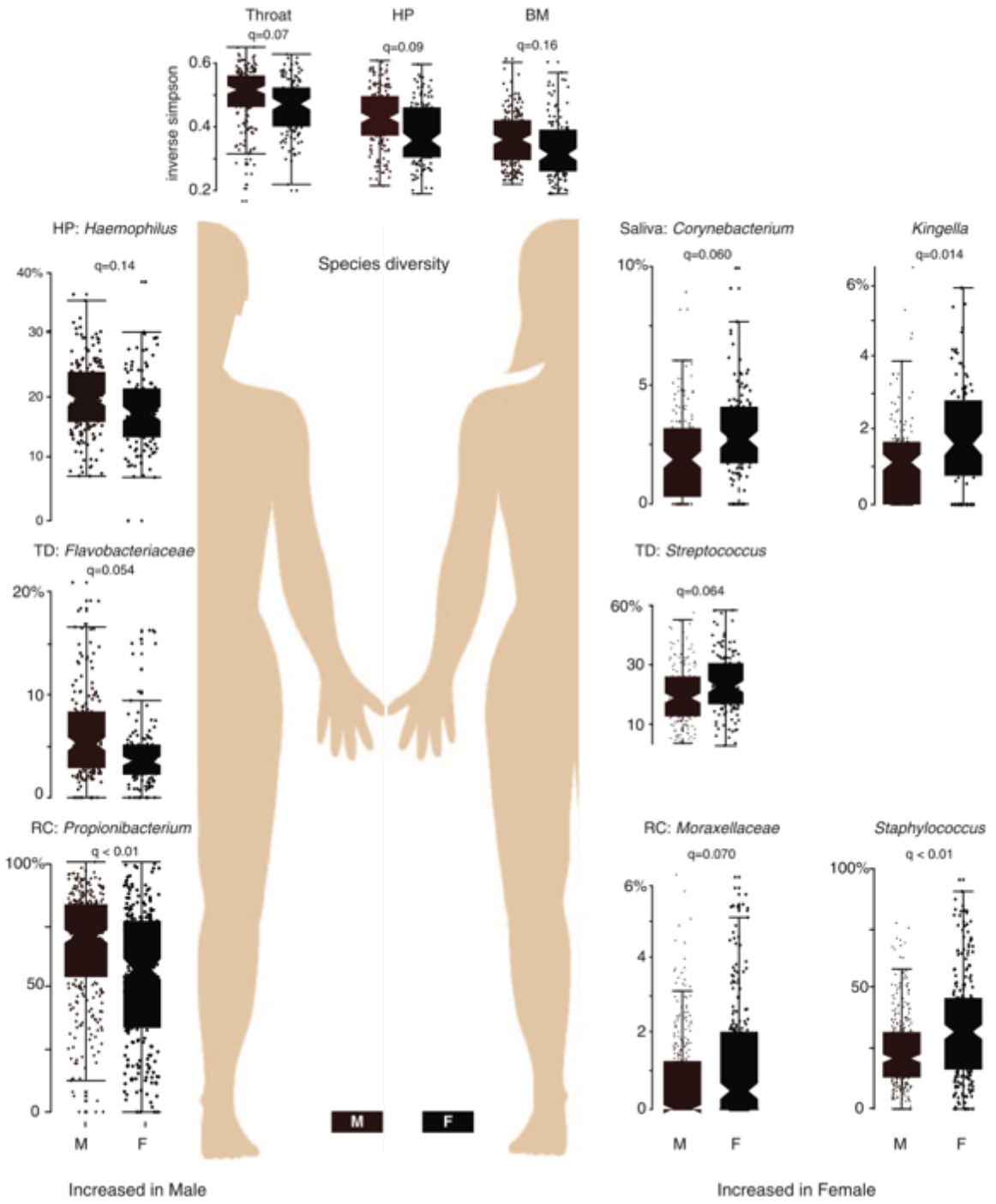
- .

2008 The human microbiome project

Has helped to characterise the skin microbiome

Studied healthy volunteers and discern how it varies across different niches, individuals and time





Small variations from one person to another

The microbioma of the same places of different people is more similar than the one of another place

Our bacterial signature is common at the family level but specific at the level of genera and species

- There is a specific relationship between the immune system of each tissue and the naturally colonizing bacteria

- There are skin diseases where there is a modification of the local microbiota
  - ACNE / **ROSACEA** (sebaceous area)
  - PSORIASIS (dry area)
  - **ATOPIC dermatitis** (wet area)

There is a break in balance between inflammatory versus anti-inflammatory flora

Resident bacteria are not passive to protect against infection but are a necessary element for the development of immunity

ROSACEA

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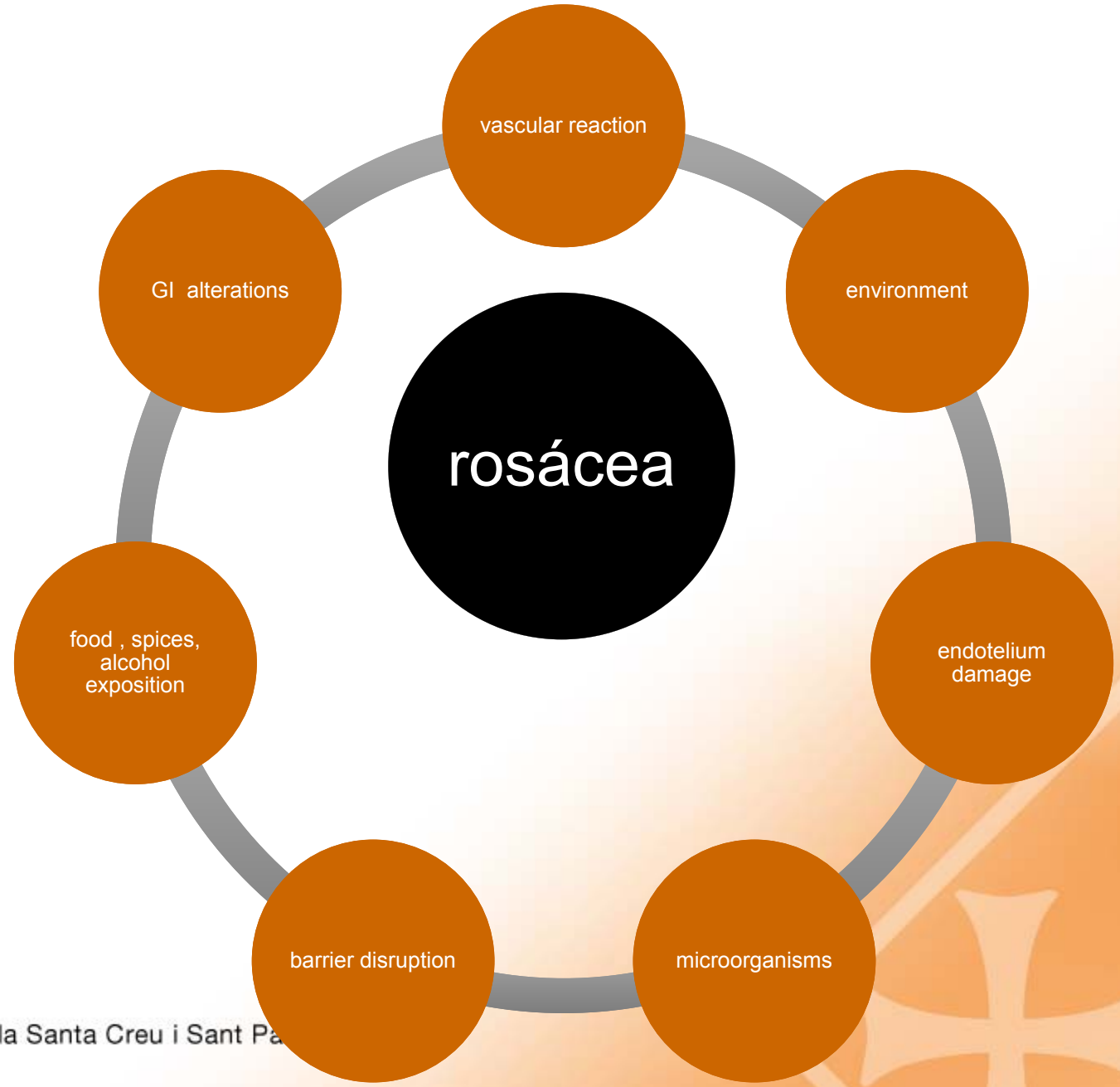
- Rosacea is a chronic disease, with evolution in outbreaks, characterized by an inflammatory process and vasculopathy.

- usually rosacea is primarily characterized by erythema of several and persistent degree, which worsens suddenly (flushing), with or without papular inflammatory or even pustular lesions, similar to acne, and, in more chronic forms, of telangiectasias.


vascular reaction
environment
endotelium damage
microorganisms
barrier disruption
food , spices, alcohol exposition
GI alterations
rosácea

- National Rosacea Society (NRS) developed a classification according to the clinical findings, regardless of evolutionary or pathogenic aspects (erythematotelangiectatic, papulo-pustular, phymatous and ocular) and only one variant, the granulomatous .

Jansen T, Plewig G. Rosacea: classification and treatment.
J R Soc Med. 1997;90:144-50.

- Rosacea may occur in patients with dry skin or skin with important seborrheic levels, not being necessarily linked to a skin type.

- A recent study evaluated 135 patients with rosacea and without treatment, and showed that the erythematotelangiectatic form of the disease is more common in dry skin than in seborrheic skin.

Tan J, Blume-Peytavi U, Ortonne JP, Wilhelm K, Marticou L, Baltas E, et al. An observational cross-sectional survey of rosacea: clinical associations and progression between subtypes. Br J Dermatol. 2013;169:555-62.

- Papulopustular form
  - sebaceous levels were normal, but with a significant reduction in hydration levels.
  - imbalance in the fatty acid concentration in sebum
    - myristic acid was present in higher concentration in sebum of these patients compared with a control group without the dermatosis
    - saturated long-chain fatty acids (behenic, tricosanoic and lignoceric), as well as monounsaturated cis-11-eicosanoic, were in lower concentrations

Ní Raghallaigh S, Bender K, Lacey N, Brennan L, Powell FC. The fatty acid profile of the skin surface lipid layer in papulopustular rosacea. Br J Dermatol. 2012;166:279-87

- these patients exhibit a modified innate immune response to environmental stimuli, favoring inflammation. Over time, these inflammatory outbreaks lead to dermal fibrotic conditions

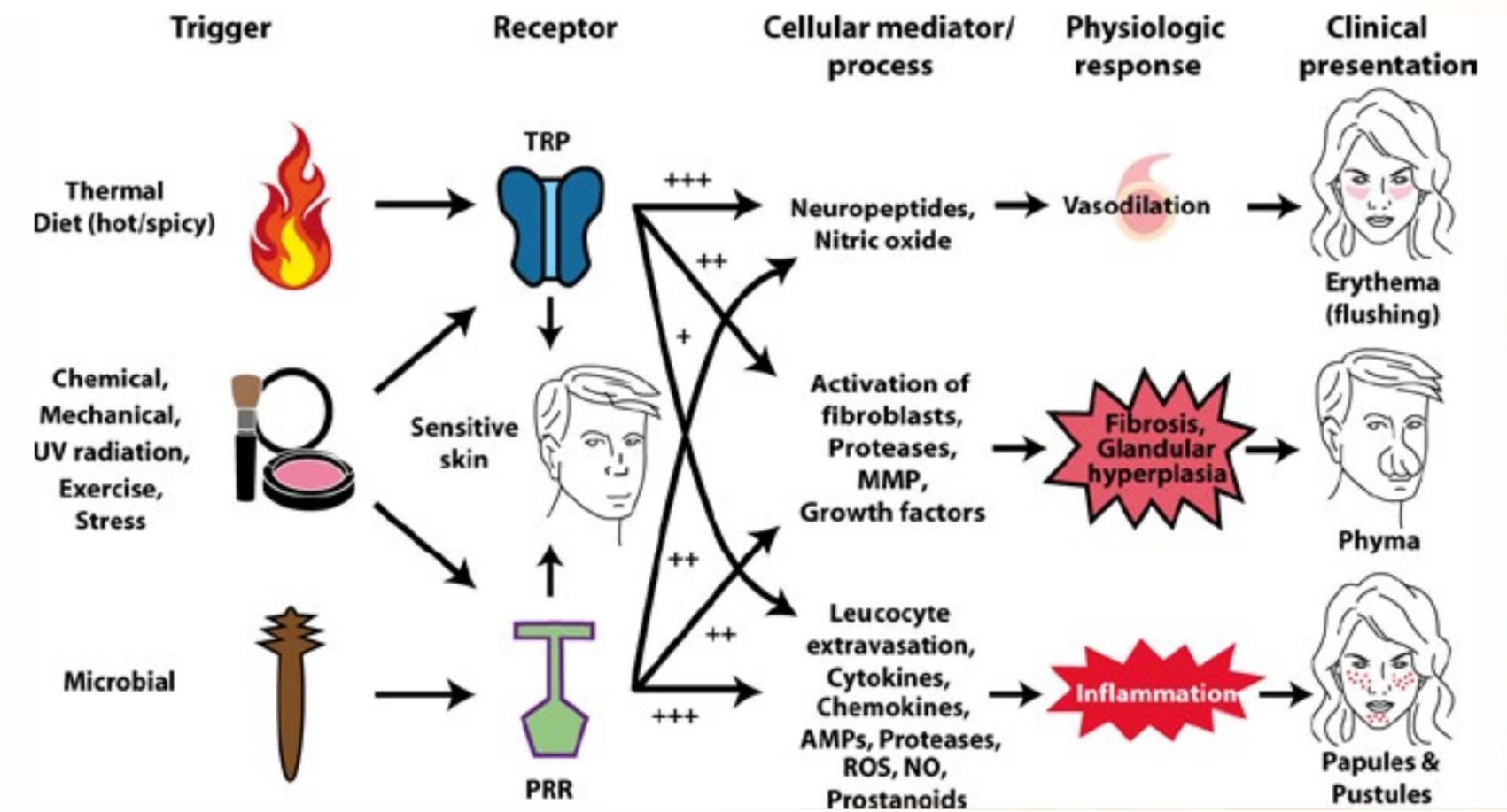
- However, not all patients with erythematotelangiectatic form necessarily progress to phymatous or papulopustular form.

- The innate response involves the secretion of antimicrobial peptides (AMP), from the keratinocytes, sebocytes and mast cells. Expression of these peptides is strictly regulated, because although it is an important line of defense, it can also cause tissue damage.

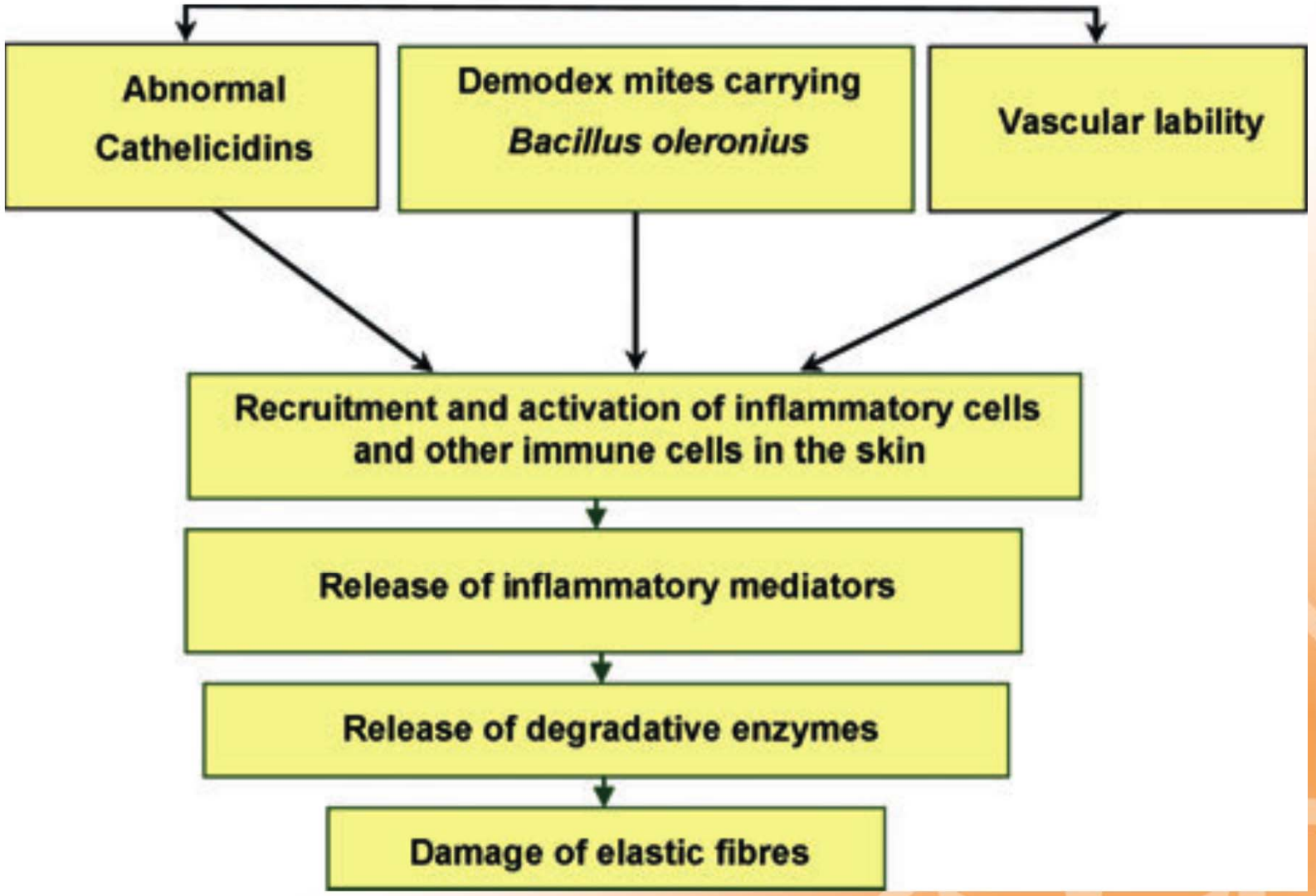
- Among AMPs, the group of cathelicidins has received special attention in the physiopathology of rosacea.

- Cathelicidin LL-37 is an important molecule in the innate metabolism, and in ammatory diseases such as rosacea, there is a disturbance in its processing, which results in peptide fragments that cause inflammation

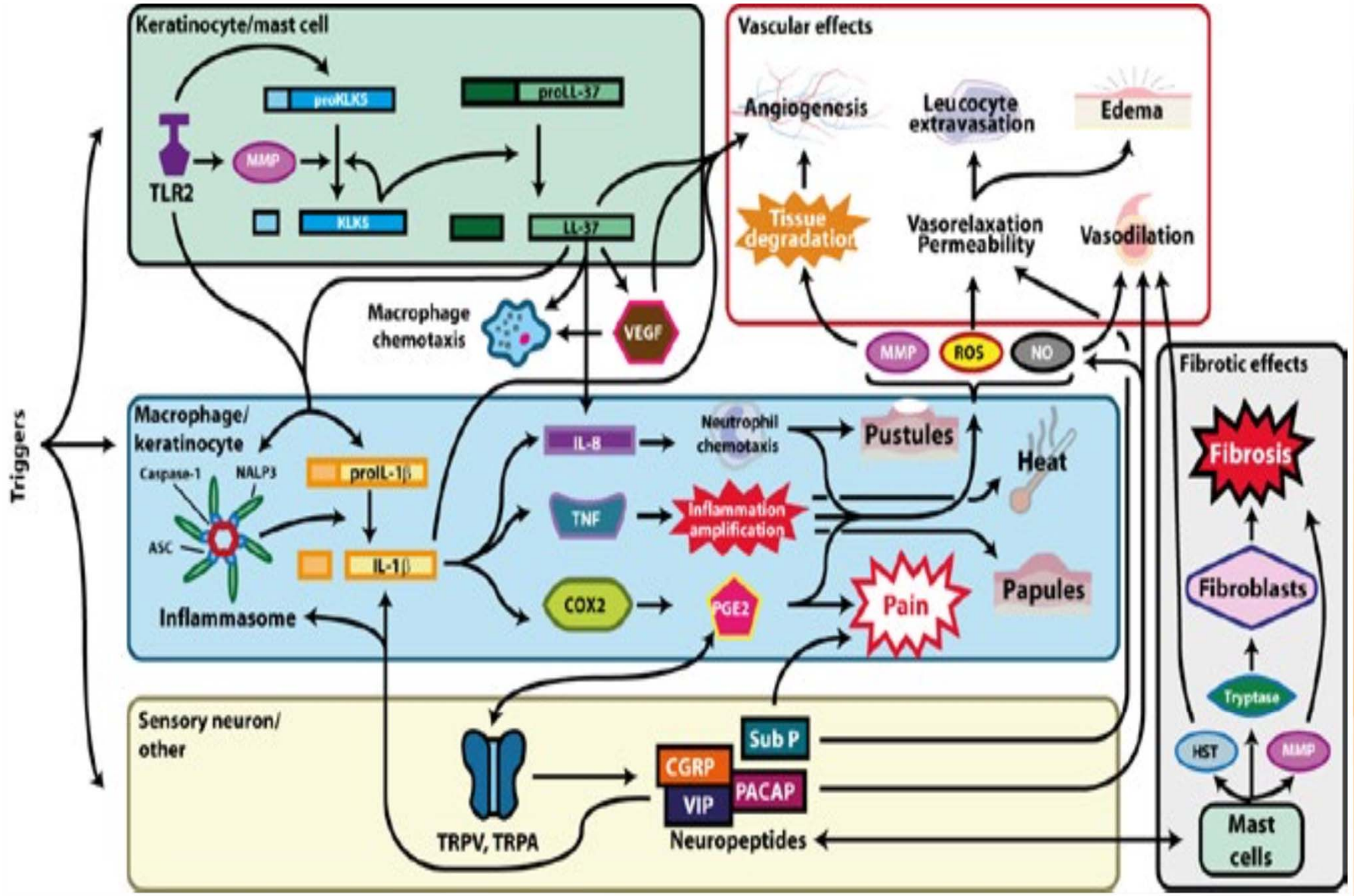
An Bras Dermatol. 2016;91(1):59-63.



Experimental Dermatology 2016; 1–9



JEADV 2011, 25, 188–200


Keratinocyte/mast cell
proKLK5
proLL-37
TLR2
MMP
KLK5
LL-37
Vascular effects
Angiogenesis
Leucocyte extravasation
Edema
Tissue degradation
Vasorelaxation Permeability
Vasodilation
Macrophage chemotaxis
VEGF
MMP
ROS
NO
Fibrotic effects
Fibrosis
Fibroblasts
Tryptase
HST
MMP
Mast cells
Triggers
Macrophage/ keratinocyte
Caspase-1
NALP3
ASC
Inflammasome
proIL-1β
IL-1β
IL-8
Neutrophil chemotaxis
Pustules
Heat
TNF
Inflammation amplification
COX2
PGE2
Pain
Papules
Sensory neuron/ other
TRPV, TRPA
Sub P
CGRP
PACAP
VIP
Neuropeptides

Epidemiology

5% USA (16 million)

Germany 2.2%

Sweden 10%

Estonia 22%

Prevalence increase from south to north

Phototypes I and II

Older adults . Women early onset.

Fimas in menPoor QoL stigmatization, loss of self-esteem and anxiety

Berg M, Lidén S: An epidemiological study of rosacea. Acta Derm Venereol 1989, 69:419-23.

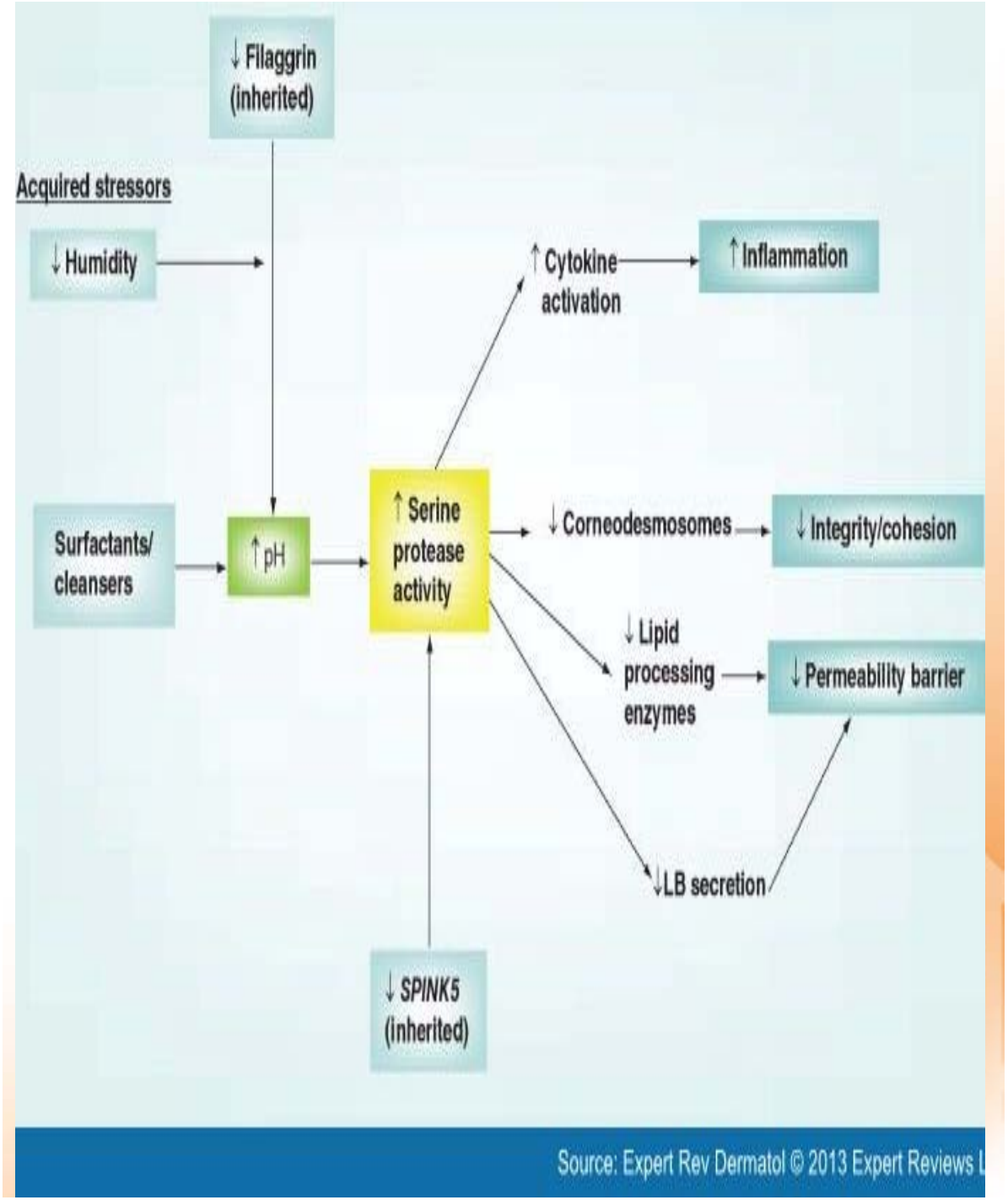
Abram K, Silm H, Oona M: Prevalence of rosacea in an Estonian working population using a standard classification. Acta Derm Venereol 2010, 90:269-73.

Böhm D, Schwanitz P, Stock Gissendanner S, Schmid-Ott G, Schulz W: Symptom severity and psychological sequelae in rosacea: Results of a survey. Psychol Health Med 2013; in press.

Pathophysiology

- Innate immunity altered
- Neurogenic inflammation
- Vascular changes
- Environmental factors
- Genetics: 30-40% patients have a relative with rosacea

- High association of rosacea with sensitive skin
- Concept of alteration of the barrier function in this entity
- Restricted to facial area /// a DA
- Increased TEWL activates epidermal proteases (serine proteases stratum corneum)



Innate immunity– microbiome

expression PRR modified

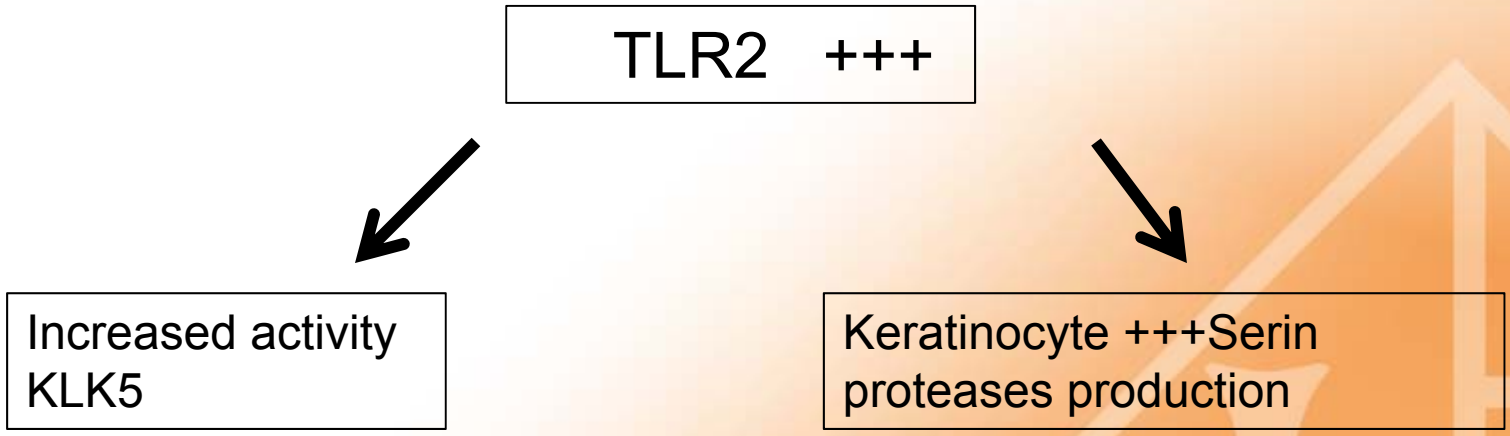
- Are innate immune receptors expressed by keratinocytes, which release cytokines, chemokines and AMP
- TLR2 Recognize Bacterial Wall Constituents

PRRs

- **TLR** Toll like receptors----TLR2…..IL-10
- NOD nucleotide oligomerization domain
- NLR nucleotide like receptors
- CLR c-type lectin receptors
- GRR G-protein coupled receptors
- RIG retinoid acid inducible gene
- RLR retinoid I like receptors

PRR = pattern recognition receptors
PAMP = pathogen associated molecular patterns

In rosacea there is increased expression TLR 2
Hence greater susceptibility to stimulate innate immune system
LL37 (AMP) increased levels in rosacea (it is angiogenic, antimicrobial and proinflammatory)



Meyer-Hoffert U, Schröder J: Epidermal proteases in the pathogenesis of rosacea. J Investig Dermatol Symp Proc 2011, 15:16-23.
Larrick JW, Hirata M, Zhong J, Wright SC: Anti-microbial activity of human CAP18 peptides. Immunotechnology 1995, 1:65-72.

LIVING AGENTS

Demodex Prevalence 60% approx.

It would be an aggravating factor, not causal

Hypersensitivity to Demodex Folliculorum

Distribution in the sebaceous follicle

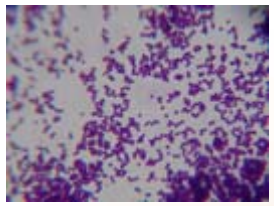
Demodex folliculorum

- Upper density 5 mites / cm2
- Can activate NOD-like receptors in vitro
- NALP3 activates the 1ß inflamasome activating the immune response

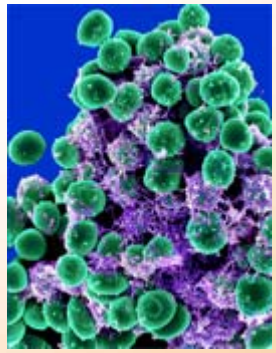
Bacillus oleronius

- Endoespora in the mobile gram-negative
- Its pathogenic potential unknown
- Their proteins trigger proliferative response in mononuclear cells in peripheral blood

Staphylococcus epidermidis



- Most prevalent commons of healthy skin
- Presence associated with skin protection against pathogenic species
- Potential pathogen only in wounds and medical devices inserted
- Abundant presence in pustular lesions
- Altered secretion profile

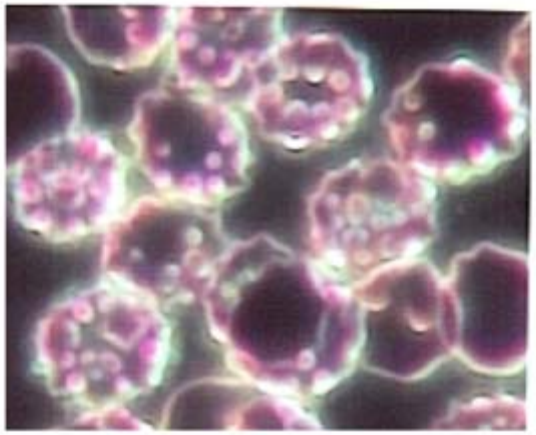


Helicobacter pylori

- Controversial literature on its role
- CagA (virulence factor) inconclusive

Chlamydophila pneumoniae

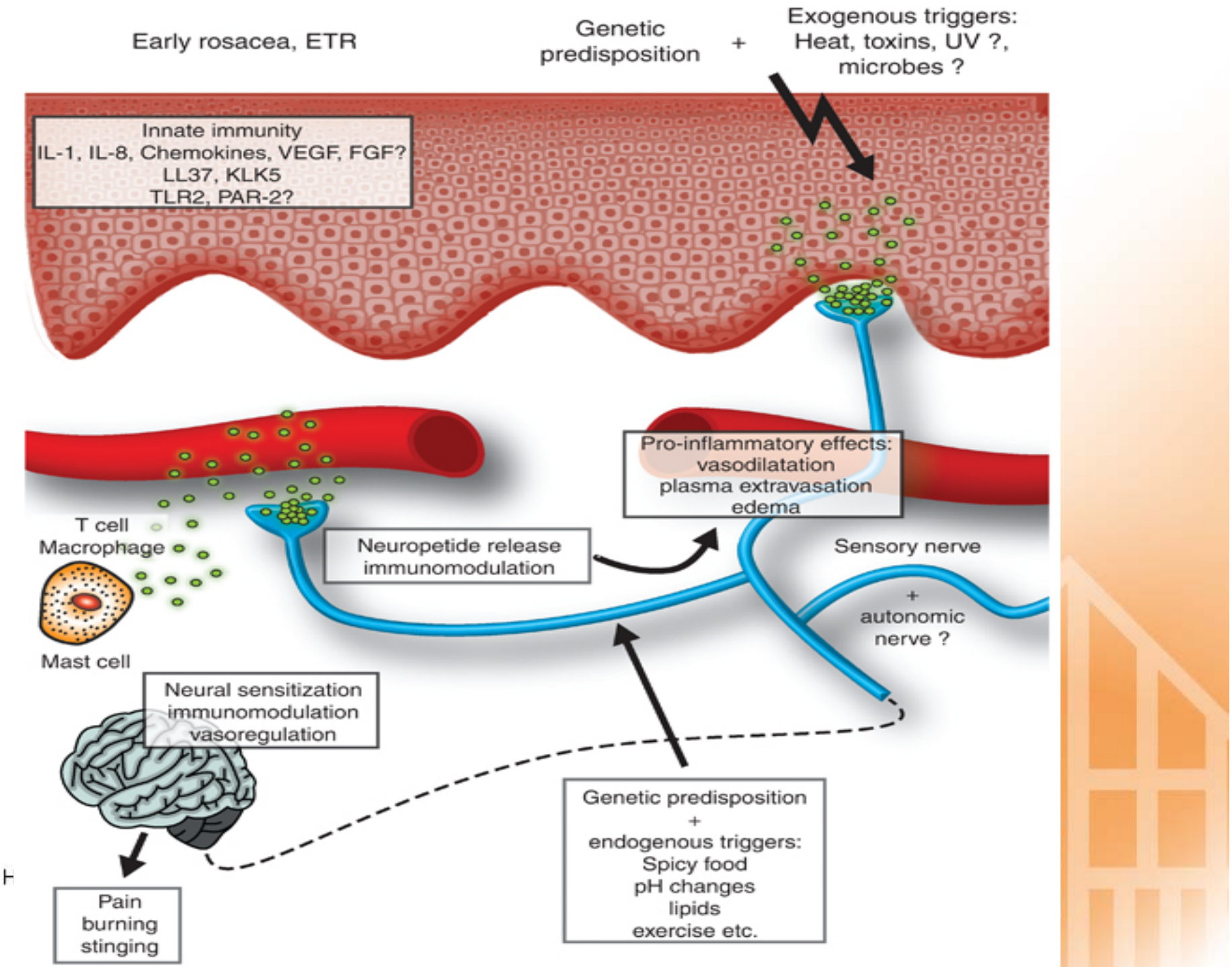
- Intracellular pathogen
- Study single uncontrolled detection Ag C pneumoniae in 40% samples malarial biopsies and Ac in 80% of sera



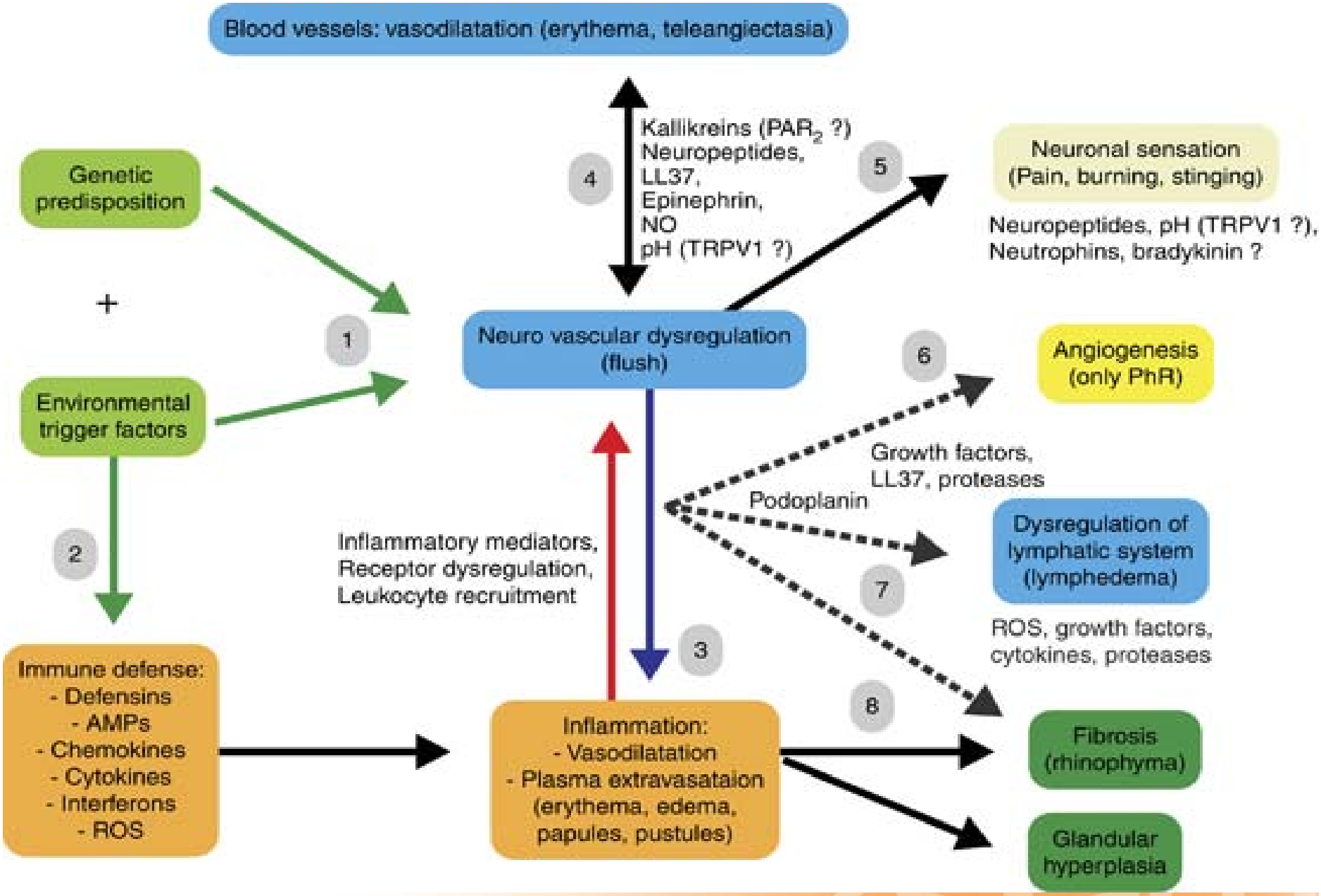
Fdez-Obregon A, Patton DI. The role of Chlamydia pneumoniae in the etiology of acne-rosacea: response to the use of oral azithromycin. Cutis 2007; 79: 163-7

Neurogenic inflammation
Vascular changes

- Vascular endoltelial growth factor (VEGF) is present is the epidermis and is expressed by infiltrating cells
- VEGF receptor-ligand binding contributes to vascular changes and cellular infiltrate
- The intestinal vasoactive peptide (VIP) is increased in the serum and in the cutaneous blood vessels of the phymomatous R
- LL37 induces cell-endothelial changes promoting vascular effects on rosacea


Early rosacea, ETR
Genetic predisposition
+
Exogenous triggers: Heat, toxins, UV ?, microbes ?
Innate immunity
IL-1, IL-8, Chemokines, VEGF, FGF?
LL37, KLK5
TLR2, PAR-2?
Pro-inflammatory effects:
vasodilatation
plasma extravasation
edema
T cell
Macrophage
Mast cell
Neuropetide release
immunomodulation
Sensory nerve
+
autonomic nerve ?
Neural sensitization
immunomodulation
vasoregulation
Pain
burning
stinging
Genetic predisposition
+
endogenous triggers:
Spicy food
pH changes
lipids
exercise etc.

- Sust P has also been linked to rosacea
- Regulates local blood flow
- Induces mast cell degranulation
- Increased proinflammatory cytokines and chemokines CXCL8, TNF-α, IL-3



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Journal of Investigative Dermatology Symposium Proceedings **15**, 2–11

- The affected skin of rosacea has a lower heat sensitivity threshold than normal skin.
- Possible participation of transient potential receptors of vanilloide ions TRPV1 and ankirin 1 (TRPA1) ions as heat and cold cellular sensors
- Its activation increases the release of sust P
- The expression of TRPV1 genes is increased in type 1 rosacea
- The immunoreactivity of TRPV2, TRPV4 and TRPV2 gene expression is increased in type 2 of rosacea
- TRPV3 and TRPV4 increased in type 3

Environmental factors

- Food: food temperature, alcohol
- Stress as a trigger for seborrhea
- Climate: temperature variations.
- Solar exposure as a triggering factorUV induces ROS formation (reactive oxygen species)TLR2-ROS interaction increases levels CXCL8
- Myeloid differentiation factor (MyD88) overexpressed

Table 1. Questionnaire scores in the two rosacea categories and in controls

	Controls ($n = 86$)	Mean ± SD		Statistical significance	
		Mild rosacea ($n = 19$)	Severe rosacea ($n = 12$)	F ratio	Partial η^2
Blushing propensity	35.5 ± 8.6	41.7 ± 10.3	49.6 ± 7.6†	11.15***	0.165
Stress	10.7 ± 6.8	11.2 ± 4.3	17.8 ± 10.9†	4.82**	0.079
Anxiety	4.9 ± 5.2	4.8 ± 4.7	5.1 ± 3.2	0.06	0.001
Depression	4.6 ± 6.2	3.7 ± 4.4	7.1 ± 8.4	1.18	0.021
Fear of negative evaluation	12.6 ± 7.4	15.6 ± 7.7	15.2 ± 8.3	1.78	0.030
Social interaction anxiety	18.0 ± 9.1	19.5 ± 13.5	24.9 ± 17.4	1.40	0.024
Social phobia	10.8 ± 7.1	12.6 ± 10.3	18.7 ± 12.8†	3.89*	0.064

SD = standard deviation.
†Greater than the control and mild rosacea group ($p < 0.05$ in tests of simple main effects).
*$p < 0.05$.
**$p < 0.01$.
***$p < 0.001$.

Acta Derm Venereol 2015; 95: 395–400

SPECIAL REPORT

Health-related Quality of Life in Patients with Cutaneous Rosacea: A Systematic Review

Mireille M. D. VAN DER LINDEN[1], Dominique C. VAN RAPPARD[1], Joost G. DAAMS[2], Mirjam A. G. SPRANGERS[3], Phyllis I. SPULS[1] and John DE KORTE[1]

[1]Department of Dermatology, [2]Medical Library, [3]Department of Medical Psychology, Academic Medical Centre, Amsterdam, The Netherlands

Out of 984 references, 12 studies were included. Three HRQoL instruments were used: Short Form-36 (SF-36), Dermatology Life Questionnaire Index (DLQI) and Rosacea Quality of Life Index (RosaQoL). Because of the heterogeneity of the included studies, data synthesis was hardly feasible. All studies reported a negative impact on HRQoL, which appeared to be associated with disease severity and age. Results regarding the association with sex and subtype were mixed. With regard to the clinical relevance of HRQoL scores of rosacea patients, it seems that rosacea has a small to moderate effect on HRQoL.

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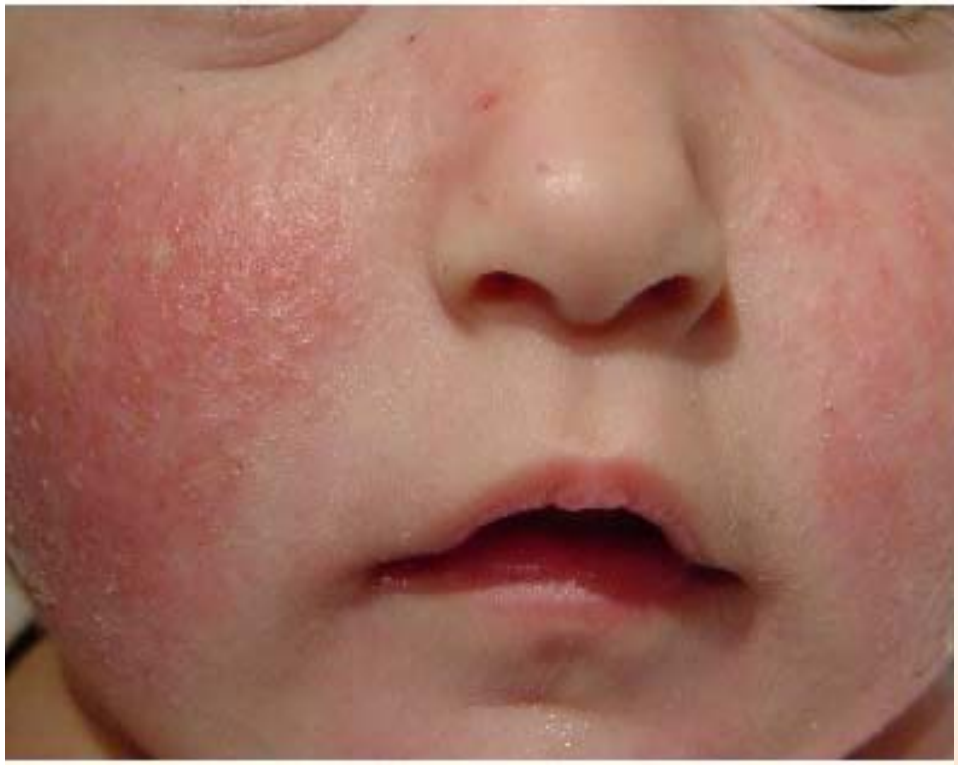
Atopic dermatitis



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- Atopic dermatitis (AD) is characterized by intense itch, disruption of the skin barrier, and upregulation of type 2 (including type 2 helper T cell [Th2]) immune responses[1,2]
- AD significantly impairs quality of life in both adults and children[3–5]
- There is relatively little qualitative information on patient perception

of AD and impact of the disease on their life

1. Brandt EB, Sivaprasad U. J Clin Cell Immunol. 2011:2 :pii 110. 2. Gittler JK, et al. J Allergy Clin Immunol. 2012;130:1344-54; 3. Sánchez-Pérez J, et al. Actas Dermosifiliogr. 2013;104:44-52. 4. Silverberg JI, et al. J Invest Derm. 2015;135:56-66. 5. Simpson EL, et al. J Am Acad Dermatol. 2016a;74:491-8.

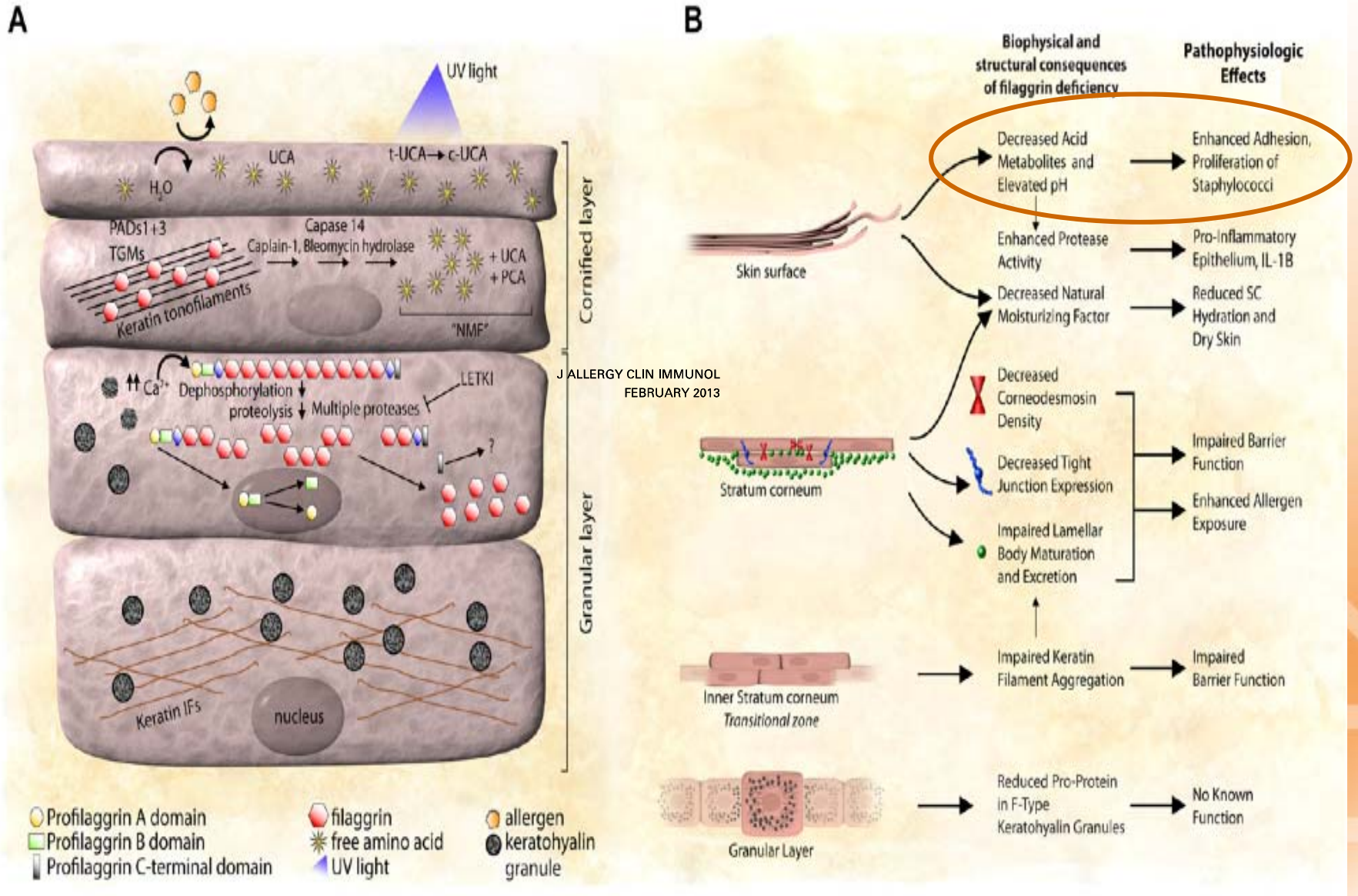
- The most annoying symptom is a recalcitrant pruritus
- There is an 'itch-scratch cycle' response

- Epidermal epithelial terminal differentiation anomalies
- Contains a defective stratum corneum
- Increased penetration of allergens and IgE sensitization

- Usual onset at early age apr.20%
- Prevalence in adults 2-5%
- Chronic and relapsing course
- Requires safe and long-term treatment strategies

J ALLERGY CLIN IMMUNOL
FEBRUARY 2013

Cutaneous barrier



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FEBRUARY 2013

Clinical phenotype AD

Childhood onset, disappearance adolescence

Childhood onset, persistent severe eczema

Adolescent onset / adult mild eczema

Adolescent onset / adult, severe eczema

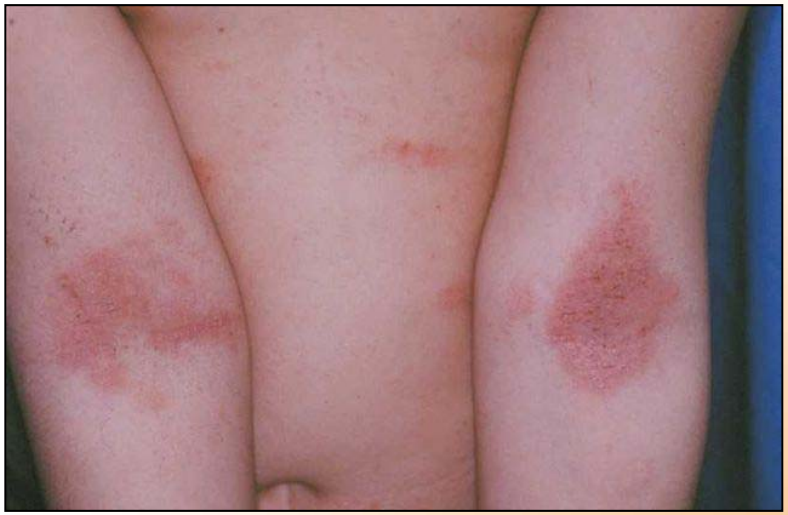
Intrinsic (IgE N) 20%

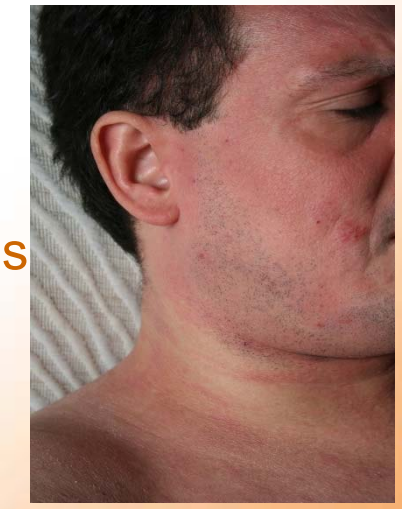
Extrinsic (elevated IgE),

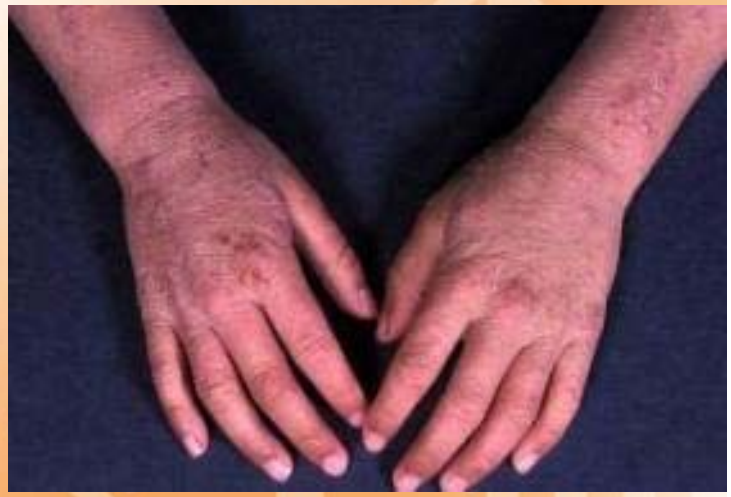
Food / sensitization, aeroallergens

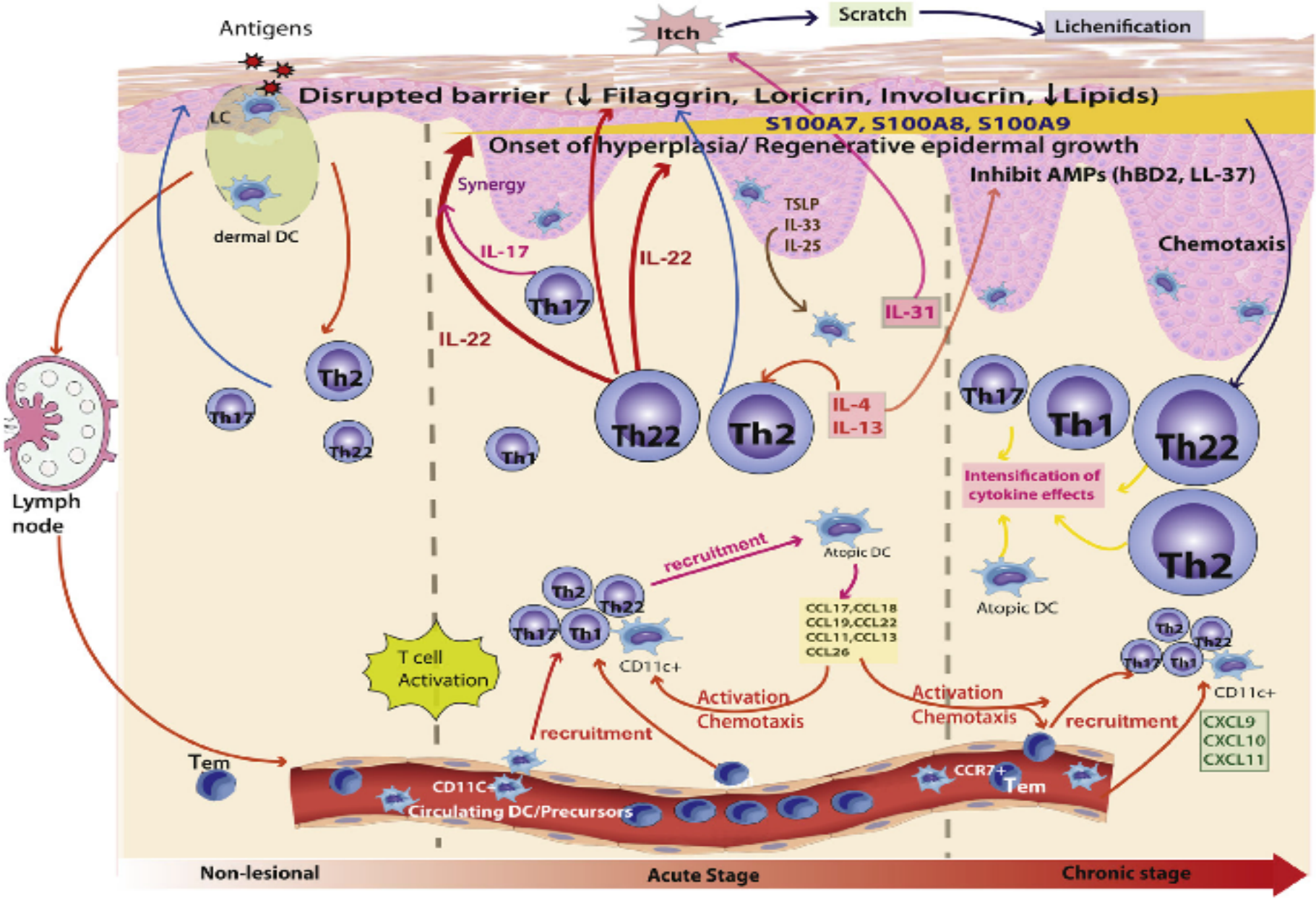
Infection / colonization S aureus

Disseminated infection (viral)









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VOLUME 134, NUMBER 4

- Biomarkers

to assess polarized immune end-points in patients with AD

Defines patients who are TH2 polarized vs. non-TH2

IgE ⬆

Eosinophilia

TSLP serum ⬆

FUTURE:

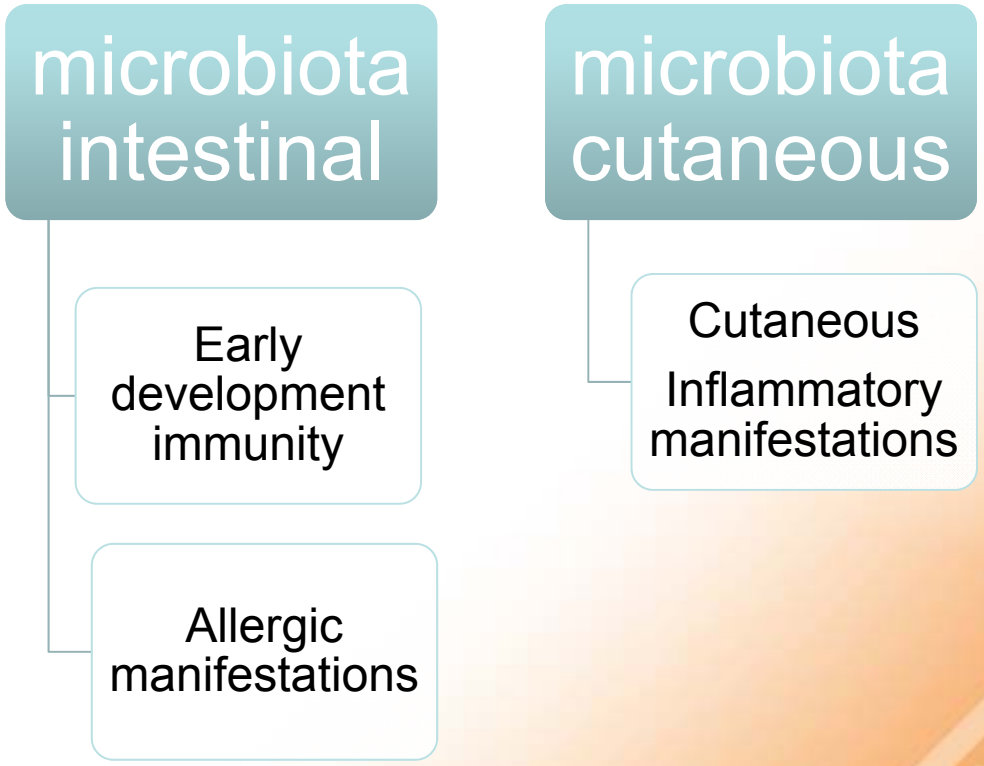
epidermal proteomics

Genomic

Transcriptosomes gene

Blood biomarkers

Microbiota –Levels influence DA



S aureus are present in 90% of patients with AD in healthy or affected skin

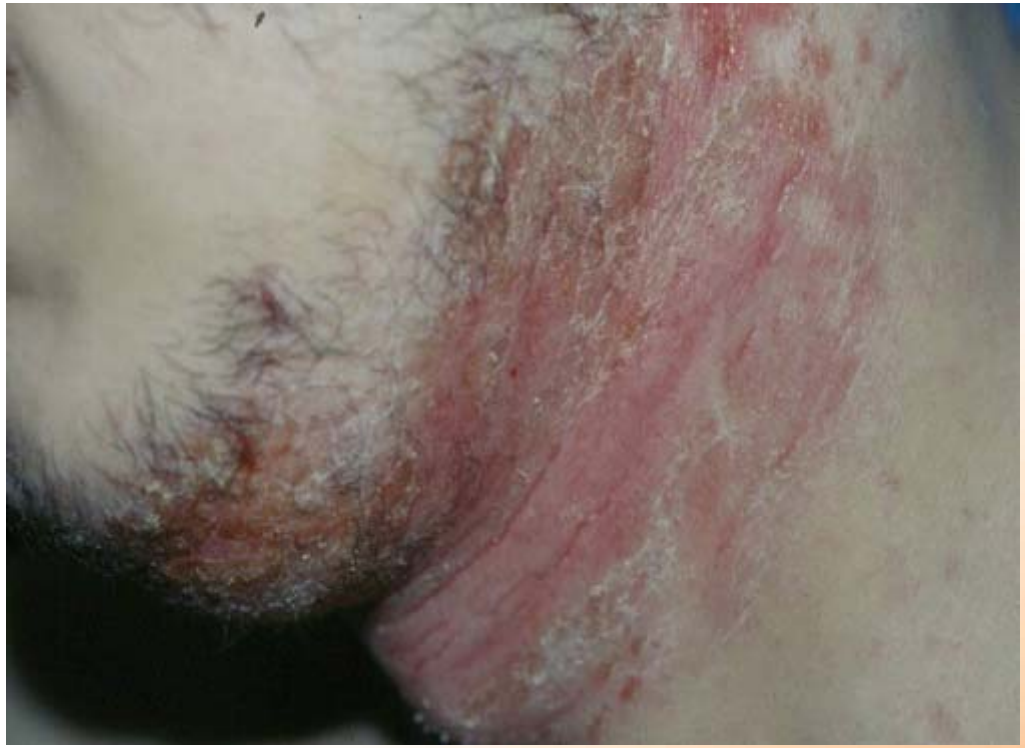
Partly because of AMP deficit

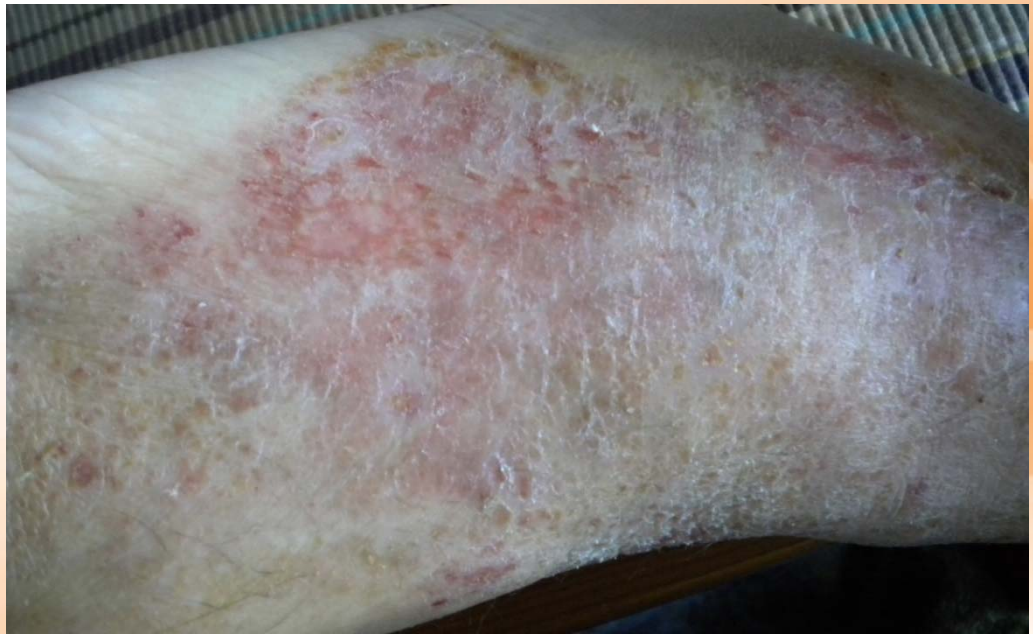
The IL4 and. IL13 inhibit expressionHBD-2 gene and LL-37

S aureus toxins act as super Ags triggering or aggravating the immune and inflammatory response

Also would be factors of resistance to corticosteroids

In addition, α-toxin induces keratinocyte death





The Role of the Skin Microbiome in Atopic Dermatitis: A Systematic Review

R. Dybboe[1,*], J. Bandier[2], L. Skov[2], L. Engstrand[3] andJ. D. Johansen[1]

- A systematic literature search was conducted October 21 2016 in PubMed, Embase, Scopus and ClinicalTrials.gov using search terms from the categories: Skin, microbiome and AD – without language and date limitations

- In this systematic review demonstrated that AD skin in humans is characterised by low bacterial diversity and high non-Malassezia fungal diversity. On involved skin the bacterial diversity was even lower.

- The data is not sufficiently robust for good characterisation; however, dysbiosis in atopic dermatitis does not only implicate Staphylococcus species, but also microbes such as Propionibacterium and Malassezia.

- A causal role of dysbiosis in eczema in mice encourages future studies to investigate if this applies in humans too.

- Other important aspects are temporal dynamics and the influence of methodology on microbiome data.

Skin microbiome before development of atopic dermatitis: Early colonization with commensal staphylococci at 2 months is associated with a lower risk of atopic dermatitis at 1 year

Elizabeth A. Kennedy, BS,[a] Jennifer Connolly, MSc,[b] Jonathan O'B. Hourihane, DM,[b] Padraic G. Fallon, PhD,[c,d] W. H. Irwin McLean, DSc, FRS,[e] Deirdre Murray, PhD,[b] Jay-Hyun Jo, PhD,[a] Julia A. Segre, PhD,[f] Heidi H. Kong, MD, MHSc,[a]* and Alan D. Irvine, MD, DSc[c,d,g]*

Bethesda, Md, Cork and Dublin, Ireland, and Dundee, United Kingdom

Background: Disease flares of established atopic dermatitis (AD) are generally associated with a low-diversity skin microbiota and *Staphylococcus aureus* dominance. The temporal transition of the skin microbiome between early infancy and the dysbiosis of established AD is unknown.

microbiome sampling at 3 points in the first 6 months of life at 4 skin sites relevant to AD:
the antecubital and popliteal fossae, nasal tip, and cheek. They identified 10 infants with AD and compared them with 10 randomly selected control infants with no AD.
They performed bacterial 16S ribosomal RNA sequencing and analysis directly from clinical sample

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Results: Bacterial community structures and diversity shifted over time, suggesting that age strongly affects the skin microbiome in infants. Unlike established AD, these patients with infantile AD did not have noticeably dysbiotic communities before or with disease and were not colonized by *S aureus*. In comparing patients and control subjects, infants who had affected skin at month 12 had statistically significant differences in bacterial communities on the antecubital fossa at month 2 compared with infants who were unaffected at month 12. In particular, commensal staphylococci were significantly less abundant in infants affected at month 12, suggesting that this genus might protect against the later development of AD.
Conclusions: This study suggests that 12-month-old infants with AD were not colonized with *S aureus* before having AD. Additional studies are needed to confirm whether colonization with commensal staphylococci modulates skin immunity and attenuates development of AD. (J Allergy Clin Immunol 2017;139:166-72.)

Dermatology

Australasian Journal of Dermatology (2017) 58, 18–24 doi: 10.1111/ajd.12435

REVIEW ARTICLE

The microbiome and atopic eczema: More than skin deep

Charlotte L Thomas and Pablo Fernández-Peñas

An early interaction between the microbiome and immune system via multiple routes (skin–gut–lung) could feasibly affect the risk of a subsequent development of atopic diseases.

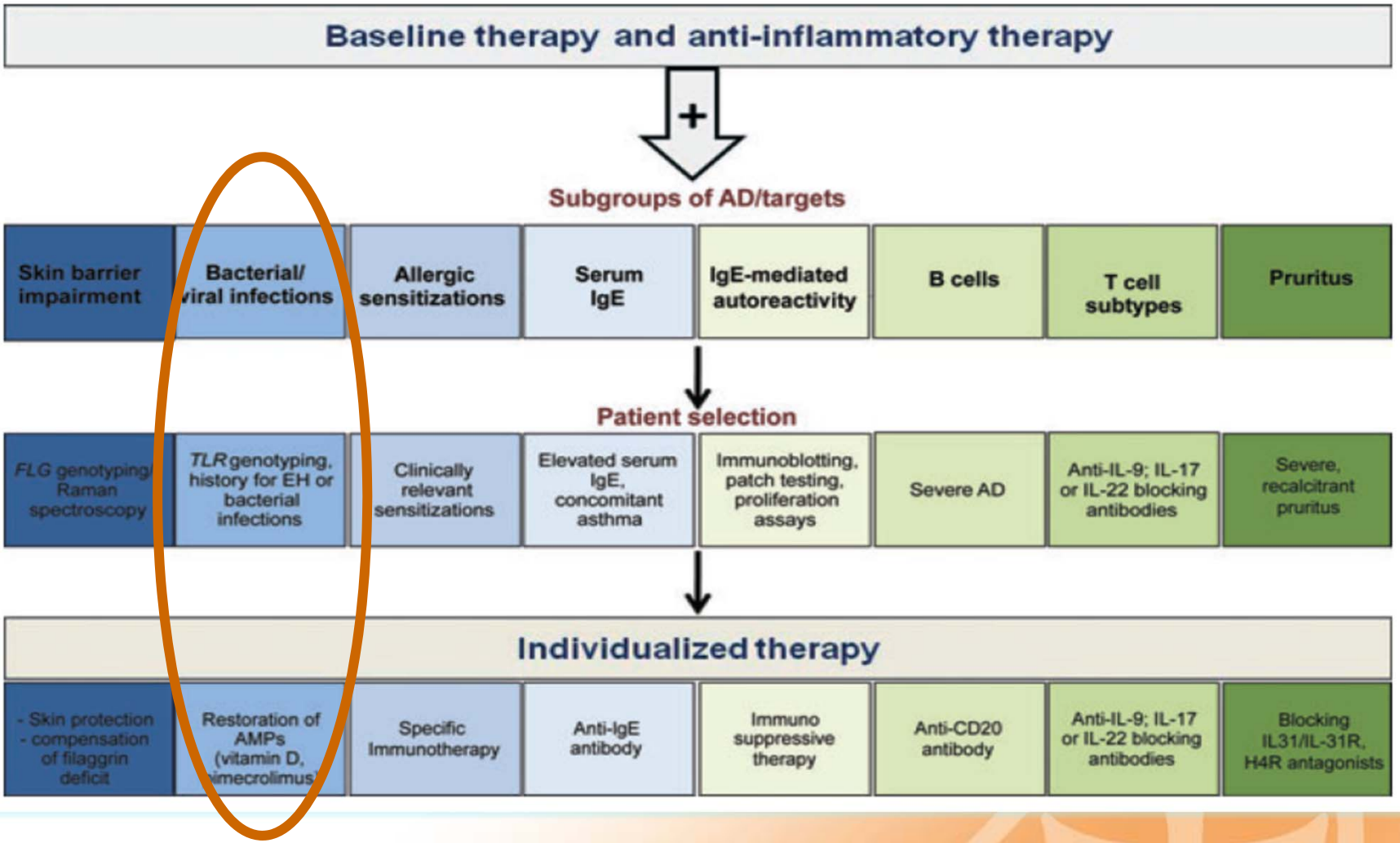
We understand that the composition of the fungal community may be important in AD, yet we do not know how this pattern changes in flares. Understanding the complex interactions of the human microbiome will aid the development of novel and more targeted treatments to modulate the microbiome and influence AD outcomes. Principles gleaned from the success of faecal transplantation in the treatment of C. difficile infections may be translated to AD with organism transplantation used to normalise the skin or gut of those with, or at high risk of developing AD.

Correcting dysbiosis to a symbiotic state is very important

It can be achieved with non-pathogenic microorganisms

Lysat of Vitreoscilla filiformis + TLR2 with production of β defensins

Individualized treatment



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Novak and Simon Allergy 2011; 66: 830–839

Thank you very much

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