Immune Tolerance to Commensal Skin Bacteria: Timing is Everything

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No conflicts to disclose
Immune tolerance to skin commensals: how we keep the peace

1. Immune sensing of commensal antigens
2. No inflammation

1 + 2 = Immune tolerance to commensals

Why is immune tolerance to skin commensals important?

Atopic dermatitis  Acne vulgaris  Hidradenitis suppurativa

Failure of immune tolerance → Inflammatory skin disease
Immune tolerance to skin commensals: fundamental questions

What cell populations are critical for establishing tolerance?

Do commensal microbes play an active role in facilitating tolerance?

Is there a critical window for establishing tolerance to commensals?

Does structural organization of skin promote tolerance?
A new model to study the commensal-specific immune response

Need an *in vivo* model with:

1. Defined commensal antigen
   
   Engineered *S. epidermidis* to express 2W antigen (Epi-2W)

2. Tool to identify T cells specific for that antigen
   
   2W-tetramer to isolate
   
   *S. epi*-specific CD4+ T cells
Is this a model of bacterial commensalism?

Epi-2W integrates into skin flora

No skin inflammation

T cells respond to S. epi antigen
Hypothesis #1: Establishing tolerance to commensal skin microbes requires exposure during neonatal life
Epi-2W Challenge + barrier breach

Week 5

Examine skin inflammation & *S.epi*-specific response
Neonatal colonization limits skin inflammation
Neonatal colonization promotes *S. epi*-specific tolerance
Does this happen in adults?
ADULT

No pre-colonization

Epi-2W Pre-colonization
Week 6

Epi-2W Challenge + barrier breach
Week 10

Examine skin inflammation & 2W-specific response
Adult colonization does not establish tolerance

Skin Neutrophils  S. epi-specific Teffs  S. epi-specific Tregs

# Neutrophils in skin  

# 2W+ CD4+ Teff cells in LN

% 2W Treg cells in skin

ns

ns

ns
What is unique about neonatal skin?
Abrupt influx of Tregs into neonatal skin
Neonatal skin Tregs are highly activated
Hypothesis #2: Neonatal skin Tregs mediate establishment of immune tolerance to commensal bacteria
(1) **Untreated** (Treg wave intact)

(2) **FTY720** (Treg wave blocked)

**Expected results...**

*Increased* inflammation in FTY720 group when Tregs cannot enter neonatal skin
Neonatal skin Tregs are required to establish tolerance to *S. epi*
Summary 1: First impressions matter

Tolerance to skin commensals is preferentially established in early life

Neonatal skin Tregs are required to establish this tolerance

*Scharschmidt et al. Immunity (2015)*
Many more questions than answers...

Sequelae of early life antibiotics?

Tolerance to skin pathogens?

Role of skin barrier integrity?

How is tolerance maintained/lost?

What signals direct Tregs into skin?
What drives migration of Tregs into neonatal skin?
Neonatal window is a busy time in skin...

Hair follicle morphogenesis

Day 6

Day 13

Microbial Colonization

Neonatal Tregs localize to hair follicles

Belkaid & Naik. Nat Imm (2013)
Hypothesis #3: (a) Developing hair follicles produce chemokines that direct Tregs into skin & (b) commensals augment this process
Do hair follicles and/or skin microbes drive accumulation of Tregs in neonatal skin?
Neonatal Tregs are reduced without hair follicles or commensals.

Other skin T cells unchanged in germ-free pups.
What are the molecular mechanisms that mediate accumulation of Tregs in neonatal skin?
Combined discovery approach:

Which chemokines are expressed in skin during Treg wave?

Which of these chemokines are augmented by commensals?

Are the corresponding receptors expressed by neonatal skin Tregs?

- Ccr6/Ccl20
- qPCR array D6 v. D13 skin
- qPCR array SPF v. GF D13 skin
- RNAseq on Tregs from D13 skin + SDLN
Commensals augment expression of Ccl20 in developing HFs

Ccl20 is expressed by HF keratinocytes

Various commensals can augment Ccl20

![Image showing expression of Ccl20 in HF keratinocytes](image.png)

![Graph showing FC Ccl20 expression](graph.png)
Ccr6 on Tregs promotes their migration into neonatal skin

Transfer into T cell deficient neonatal mouse

Examine skin & lymphoid organs

![Graph showing Treg ratio skin:spleen](image)
Summary 2: Commensals and hair follicles coordinately direct Tregs into neonatal skin

Scharschmidt et al. Cell Host & Microbe (2017)
So what does this mean for human infants?
There is an early window for tolerance

Tregs are enriched in pediatric tissues

Early exposure reduced peanut allergy in atopic cohort

Cordoro et al. JAAD (2017)

Du Toit et al. NEJM (2015)
Initial Treg accumulation in human skin coincides with fetal hair follicle development
Immune tolerance to skin commensals: some first-level answers

- Neonatal skin Tregs are critical
- Commensal microbes are *not* just bystanders
- Neonatal life represents an important window
- Hair follicle chemokines facilitate Treg accumulation
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