The Evolving Pathogenesis of Alopecia Areata and Biomarkers of Disease

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Conflicts of interest: „Plenty“
Consultant for competing companies interested in hair loss
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Relevant: None
Alopecia areata (AA) = autoimmune hair loss disorder → always?

Gilhar/Paus *N Engl J Med* 2012

Pathognomonic skin & hair phenotype
- focal/total alopecia in “normal” skin
- “exclamation mark hair”, “cadaver hairs”
- regrowth of white hair (poliosis)

→ indicates immune privilege collapse

→ Regrowth of white hair = diagnostic!
Immunocompetent HF epithelium

Immunoprivileged HF epithelium

Invisible "Immunological watershed"

Human HF immunology
Follicular repigmentation in vitiligo

Paus  Nat Med 2013
Strong immune privilege: BULB pigmentary unit well-shielded from immune attack.

Vitiligo:
Epidermal repigmentation from HF reservoir.

Conrad et al. BJD 2000

Ito et al. Am J Pathol 2004
Human HF melanocytes „live a life of (immune) privilege“

→ Key reason why
HF melanocytes & their progenitors are least likely to get attacked

Seat of HF melanocyte stem cells
Harries et al. *J Pathol* 2013

Strong Immune privilege: BULB
Christoph et al. *BJD* 2000

Vitiligo: Epidermal repigmentation from HF reservoir

→ HF pigmentary unit well-shielded from immune attack
Immunocompetent HF epithelium

Immunoprivileged HF epithelium

HF immune privilege (IP)

- MHC class I, β2 mg, TAPs
- LCs: MHC class II+
- CD4+ or CD8+ TCs

"Immunological watershed"

- No MHC class I, β2 mg, TAPs
- LCs: MHC class II-negative
- Very few (functionally inhibited?) CD4+ or CD8+ TCs and NK cells
- Almost no γδ TCs
- Strong CD200 (bulge)
- IDO (bulge)
- α-MSH, MC-1R
- TGFβ1/2 & TGFβRI/II
- VIP-R
- IGF-1
- Cortisol
- Perifollicular Tregs
- Immunoinhibitory mast cells

→ Creation of immunoinhibitory, tolerance-promoting milieu
Indicators if HF IP collapse

- **Immunocompetent**
  - HF epithelium
- **Immunoprivileged**
  - HF epithelium

**Indicators**

- High MHC class I+II, β2 mg
- Numerous CD4+ or CD8+ TCs, NK cells & γδTCs
- Low CD200 (bulge)
- Low α-MSH, TGFβ1/2, IGF-1
- Low VIP-R
- Low cortisol
- Proinflammatory mast cells
- Insufficient Treg activity?

**Inducers** → IFNγ, subst P

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Harries et al. *J Pathol 2013 & TMM 2018*

LPP = bulge IP collapse

SCARRING

Paus et al *Yale J Biol Med 1993*

AA = bulb IP collapse

REVERSIBLE

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"Immunological watershed"
AA = HF cycling disorder

Perifollicular inflammatory infiltrate

1. Attack only anagen hair bulb (anagen III-VI)

2. Catapult anagen HFs prematurely into catagen

3. MHC class I-based HF IP collapses, CD8+ T & other NKG2D+ cells attack anagen hair matrix

→ Key role of IFNγ
AA = HF cycling disorder

Perifollicular inflammatory infiltrate

1. Attack only anagen hair bulb (anagen III-VI)

2. Catapult anagen HF's prematurely into catagen

3. MHC class I-based
   HF IP collapses,
   CD8+ T & other NKG2D+ cells
   attack anagen hair matrix
   → Key role of IFN\(\gamma\)

→ JAK inhibitors
   e.g. ruxolitinib, tofacitinib etc.
   Antagonize IFN\(\gamma\); may suppress catagen
No AA without
1) HF IP collapse
2) Perifollicular inflammatory cell infiltrate
3) Premature catagen induction in anagen HFs
4) HF dystrophy


**AA is an autoimmune-response against melanogenesis-related autoantigens, exposed by abnormal MHC class I expression in an anagen hair bulb with collapsed HF IP**

Experimental confirmation:

→ Alli et al. J Immunol 2012 (mouse AA)
“Swarm of bees” infiltrate

How does it get there?

NKG2D+ Cells:
CD8+ TC
NK & gdTCs

Permanent alopecia

Reversible alopecia

Mast cells

Ito et al. JID 2008
Bertolini et al. PLoS ONE 2014
"Swarm of bees" infiltrate

How does it get there?

1. **CD8+ TCs:**
   - MHC class I-presented (auto-)antigens
   - *Melanogenesis!*

2. **MICA**
   - "stress/danger" signal

**What do they recognize?**

- CD4+
- CD8+ TC
- NKG2D+ cells (NK & γδTCs)

Permanent alopecia

Reversible alopecia


*Not for Distribution*
**Excessive MICA**

"stress/danger“ signal expression

**What attracts them?**

"Swarm of bees" infiltrate

CD8+ T
NKG2D+ cells
(NK & \(\gamma\delta\)TCs)
CD4 TCs follow

Chemo-
kines
CXCL10

Permanent alopecia

Reversible alopecia

Excessive MICA

"stress/danger“ signal expression

What attracts them?

"Swarm of bees" infiltrate
Bulb IP continuously threatened:

1. **HF „tissue stress“**
   e.g. trauma, hypoxia, excessive ROS production
   Excessive MICA expr & chemokine release
   → Activates NK & gdTCs
   → IFNg release → further IP collapse

2. **Psychoemotional stress**
   → Perifollicular neurogenic inflammation
   SP, mast cells, NGF, CRH
   → SP induces IP collapse ! Peters et al. *AJP* 2007

3. **HF dysbiosis ?**
   → Excessive chemokine release
   → Attracts IFNg-secreting infiltrate (CD8+, gdTCs !)

4. **Ectopic expression of HF autoantigens**
   e.g. melanogenesis- & hair shaft-associated
   → Stimulation of pre-existing autoreactive CD8+TCs

5. **Loss of peripheral tolerance / weak HF IP**
   e.g. insufficient Treg activity & HF IP guardian production
1. HF "tissue stress"
2. Psychoemotional stress
3. HF dysbiosis
4. Ectopic HF autoantigen expression
5. Loss of peripheral tolerance

- Acquired & inherited **interindividual differences in IP robustness**, e.g.
  - Constitutive levels of MHC I, HF guardian & VIP-R expression; levels of Treg activity;
  - atopy (proinflamm. MCs, eos), AIRE defective
  - accelerate or slow down IP collapse
**AA variants**

Focal/multifocal AA

AA totalis

AA universalis

AA incognita/diffuse variant

„overnight graying“

= diffuse AA
attacking only pigmented HFs,
demasking pre-existent white hair

**AA clinical trials should be standardized to homogeneous AA populations, also in terms of prognosis**
Biomarkers?

AA subtype?

Disease activity?

Guidance for optimal personalized therapy?

Prognosis?
Prognosis

„Rules of thumb clinical biomarkers“

Poor

• First AA attack *before* puberty
• Rapid progression to AT or AU
• Atopy + (especially AD !)
• Ophiasis
• Associated autoimmune diseases
• Down syndrome

Not so good

• Positive family history for AA
• Nail pitting
• Multiple affected sites

Good

• 1st episode & none of the above
**Prognosis: „Rules of thumb“**

**Poor**
- First AA attack *before* puberty
- Rapid progression to AT or AU
- Atopy + (especially AD !)
- Ophiasis
- Associated autoimmune diseases
- Down syndrome

**Not so good**
- Positive family history for AA
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**Good**
- 1st episode & none of the above

**JAK inhibitors?**
- Adjust aggressiveness of therapeutic approach
  - „Pragmatic Personalized Medicine“
  - Topical clobetasol
  - Triamcinolone injection
Best "biomarkers" for AA subtype, Disease activity
guidance for optimal personalized therapy & prognosis
are still:

→ Personal & family history
   → Age, Atopy
   → Associated diseases
   → Clinical signs

→ Presentation at onset, speed of progression

IFNg; Number & activation status of NKG2D+ cells in peripheral blood?
Pertinent pathogenesis questions

• Is AA really always (auto-)antigen-specific?

• Do we always face a CD8+ T cell-dependent autoimmune disease when we see an AA hair loss phenotype?

• Is a specific genetic predisposition required to develop AA?
• AA: always (auto-)antigen-specific?  
  **No**

• Is AA always CD8+ T cell-dependent?  
  **No**

• Is a genetic predisposition required to develop AA lesions?  
  **No**

2 main lines of evidence  ➔ NK cells, γδTCs
Why is there no NK cell attack on MHC class Ia-negative normal anagen HFs?

Ito T et al  *J Invest Dermatol* 2008
• **MICA expression**
  (=NK cell stimulating NKG2D ligand)
  is **low** in normal anagen HF
  (except proximal anagen hair matrix ?)

• **massively up-regulated**
  in AA lesions!

• **MIF & KIR** (=inhibit NK functions)
  High in normal, **low in AA**

• **NKG2D expression** on peripheral blood NK cells **increased** in AA

→ **NKG2D+** cell activity is kept „low key“ around healthy HFs

→ **Non-antigen specific** NK cells, NKG2D
  & its ligands important in AA pathobiology

Constitutive „Achilles‘ heal“ of HF ?

Ito T et al, *JID* 2008
AA strongly associated with **NKG2D activating ligands MICA, ULPB3**

Sinclair Lab *JID* 2016:
**MICA, but not ULPB3 overexpressed in lesional AA HFs**

**ULBP3 and NKG2D expression and immune cell infiltration of AA hair follicles**
NKG2D+ cells induce AA in healthy human skin in vivo


**Not for Distribution**

**→ Specific autoantigen & genetic predisposition DISPENSABLE for developing AA!**
**Vδ1**+T-cells infiltrate in /around AA hair bulbs

... also seen in experimentally induced AA in human skin xenotransplants *in vivo*

(Gilhar's humanized AA mouse model)

→ Do **Vδ1**+T cells operate as stress sentinels around human HFs?

+ increased NKG2D & IFNγ expression!

Youhei Uchida et al *In prep.*

<table>
<thead>
<tr>
<th>Number of <strong>Vδ1</strong>+T-cells in &amp; around HF</th>
<th>HS</th>
<th>NL</th>
<th>AA</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>HS</strong></td>
<td>5</td>
<td>10</td>
<td>30</td>
</tr>
<tr>
<td><strong>NL</strong></td>
<td>20</td>
<td>15</td>
<td>25</td>
</tr>
<tr>
<td><strong>AA</strong></td>
<td>30</td>
<td>35</td>
<td>40</td>
</tr>
</tbody>
</table>

**HS** = healthy skin

**NL** = non-lesional AA skin

**AA** = lesional AA skin
Several different pathobiology pathways lead to AA, which all coalesce in a stereotypic HF damage response pattern to IFN-γ- or SP-driven immunological damage. This creates the clinical AA phenotype. This response pattern also occurs in healthy HFs.
• AA is not a single disease entity, but just a stereotypic response pattern to inflammatory HF damage

• that even the healthiest of HFs will show,

• irrespective of genetic predisposition (but more easily so, if genetically predisposed, e.g. by constitutively weak IP or constitutive overexpression of „danger“ signals [MICA])

\textbf{if and only if}

• HF IP collapse \textit{coincides} with anagen and attracts an inflammatory cell infiltrate (not necessarily only CD8+ T cells but also NK cells [Ito et al. \textit{JID} 2008], \(\gamma\delta\)TCs [Uchida et al., \textit{under prep.}])

• that secretes so much IFN\(\gamma\) that major HF dystrophy & premature catagen are induced
Alopecia areata is not one disease

→ Ikeda 1965

and some forms of AA may not even be a „disease“ at all!

Paus et al.  *JID Symp Proc* 2018
HYPOTHESIS

→ Only in some AA patients, this response pattern occurs because they have autoreactive, IFNγ-secreting CD8+ T cells that recognize an anagen HF-associated autoantigen = „AA disease“

→ Autoimmune alopecia areata (AAA)


→ Frequently relapsing episodes, associated AID: AAA?

→ Ikeda AA Type IV, 1965

→ In these patients, causal & curative therapy only possible if autoantigen identified, peripheral tolerance restored and/or autoreactive T cells eliminated
HYPOTHESIS

In other AA patients (majority?)
  Ikeda Type 1?

antigen-specific HF autoimmunity may be missing
  e.g.

  A: „IFNγ storm“: massive IFNγ release by NK and/or γδ T cells
     → AA after trauma/stress, infection, HF dysbiosis ??

  B: stress-induced, substance P-dependent neurogenic inflammation

  → Symptomatic therapy suffices here!

„You are treating a mere HF response pattern, not a disease“
Both AA forms display the "AA pathobiology quartet": perifollicular infiltrate, HF IP collapse, HF dystophy, premature catagen

\[\text{Targeting the AA trio therapeutically always makes sense no matter how the AA response pattern was triggered!}\]

\[\text{restore IP + inhibit catagen + repair HF damage}\]

We must get much more effective in this! What else, besides JAK inhibitors...?

\[\text{Re-explore known immune privilege guardians}\]

\begin{itemize}
  \item e.g. FK506, apremilast (PDE4 inh.), aprepitant (SP antagonists),
  \item melanotan & other stable aMSH, VIP receptor agonists
\end{itemize}
Take home messages

- **AA** = “a stereotypic HF response pattern” to mainly IFN-γ-driven immunological HF damage, not necessarily a disease

- **Distinguish antigen-specific autoimmune AA (AAA) from non-antigen-specific AA**
  - causal therapy only needed, possible & useful for AAA
  - symptomatic therapy suffices for all other AA variants

- **Personalized medicine approach to AA management needed**

- **However:** Protection from IP collapse & HF dystrophy works & is useful in all AA forms

- **Biomarkers:** Stick with prognosis classics, for now