Updates In The Pathogenesis Of Alopecia Areata

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Abstract:
Alopecia areata is the most prevalent autoimmune disorder which causes non-scarring hair loss. It may increase the anxiety of patients and increase their chances of developing psychological and psychiatric disorders. There are two proposed theories for the pathogenesis of alopecia areata. The most evidence-based hypothesis is an autoimmune reaction caused by the collapse of hair follicle immune privilege, Immune privilege collapse is assumed to be either a primary event that triggers antigen presentation in a disturbed hair follicle environment or an event that occurs as a result of dysregulation of the central immune system that involves the follicles. Several gene loci have been identified with alopecia areata. The key immune effectors in the pathogenesis include autoreactive effector T cells, natural-killer group 2, member (NKG2D) + CD8+ cytotoxic T cells, natural killer (NK) cells, Janus kinase, signal transducers, and activators of transcription (JAK/STAT) pathway, MHC-I chain-related gene A (MICA), interferon-γ (IFN-γ) and interleukin-15 (IL-15). Alopecia areata has no accepted cure and has an unpredictable response to treatment. The recognition of the exact pathogenic mechanisms of alopecia areata is necessary to identify the potential therapeutic targets.

Keywords: alopecia areata, pathogenesis, immune privilege.

Introduction:
Alopecia areata (AA) is a prevalent autoimmune skin disease that manifests as non-scarring patchy hair loss on the scalp and other hair-bearing areas. It has a general of 2% and has a lifetime risk of 1.7% (1). It has no age, sex, or ethnic predilection (2). Skin lesions of AA display peribulbar lymphocytic infiltration (3), consisted of intra-follicular CD8+ T cells and peri-follicular CD4+ T forming "swarm of bees" (4,5), with elevated counts of telogen hairs in the acute and chronic stages, catagen hairs in the subacute stage and miniaturized hairs in the chronic stage (6).

The hair follicle (HF) immune privilege (IP):
The HF (IP) occurs during anagen. It's hypothesized to contribute to the de novo induction of peripheral tolerance, and is maintained through (7):
1-Downregulation of MHC I, MHC II (on antigen-presenting cells, APCs), and β2-microglobulin expression. This inhibits IFN-γ production by macrophages (8,9).
2-Local immunosuppressants: transforming growth factor β1 (TGF-β1), IL-10, α-Melanocyte stimulating hormone (α-MSH), Indoleamine 2,3 dioxygenase (IDO), protein red encoded by IK gene (red/IK), insulin-like gro-
with factor 1 (IGF-1), calcitonin gene-related peptide and somatostatin (8-12).

3- Extracellular matrix barriers that inhibit immune cell migration (8,9).

4- Expression of Fas ligand and programmed cell death ligand 1 (PD-L1) which target immune cells that penetrate the physical barriers (13,14).

Normal human anagen HFs decrease the liability of interaction with NKG-2D+ cells and suppress the NK cells via continuous downregulation of expression of NKG2D on local NK cells, and NKG2D ligands (MICA or UL16-binding protein 3) on the keratinocytes, together with the secretion of macrophage migration inhibitory factor (15-17).

The theories that explain alopecia areata development:

1- Immune privilege collapse:
   When the reactive oxygen species (ROS) can’t be overcome effectively, it accumulates in HF keratinocytes and promotes MICA expression (15). Upon MHC presentation, MICA+ cells become seen by NK cells through NKG2D receptors, activating the innate immunity (15, 18). MHC-I presentation exposes the secured antigens to the immune cells (9,15,19,20).
   Initially, HFs enter an anagen phase without the IP. The APCs recognize and present self-antigens via increased MHC I and II expression, too naïve T cells, which proliferate and differentiate into effector cells (5).
   Autoreactive lymphocytes, especially CD8+ cells and NKG2D+ cells, travel toward the HFs, destruct the anagen HFs and induce premature catagen (21) through Fas–FasL, perforin-granzyme pathways, IFN-γ, and IL-15. Antigen presentation activates CD4+ cells, especially T helper 1 (Th 1) subtypes, which secrete TNF-α, IFN-γ, and IL-2 (5).

Lymphocytic recruitment can induce the onset of AA, while the IFN-γ positive feedback cycle can explain its chronicity and progressiveness (22).

The infiltration of the anagen hair bulb by lymphocytes, dendritic cells, and NK cells (4), the lower levels of IP maintaining factors such as IDO, red/IK, TGF-β, and α-MSH in lesional or peri-lesional alopecic skin (11, 23), the strong positive MICA expression by HF cells (15), as well as the upregulated IFN-γ, chemokines (CXCLs) and intercellular adhesion molecules (ICAM2 and ICAM3) in AA lesions, prove the IP collapse (23).

2- The hair follicle as a target of an impaired immune system:
   According to the second theory of AA, the initial step is the activation of immune cells, which then invade HFs and promote MHC and MICA expression through IFN-γ, resulting in IP collapse in the HFs (24).

Evidence supporting this theory is based on several experimental studies.

The induction of AA just by transferring immune cells from AA affected mice like CD8+ cells, CD8+ NKG2D+ T cells, and cells co-cultured with IL-15, which upregulates NKG2D receptors (24-26). The CD8+ NKG2D+T cells depleted lymph node cells are unable to induce AA (27).

Mice with severe combined immune deficiency develop AA after exposure to IL2 rich peripheral blood mononuclear cell (PBMC) cultures from healthy populations. High doses of IL2 enhance the expression of NKG2D+ CD56+ cells (15,28).

The autoimmune regulator (AIRE) gene mutation, inducing autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy (APECED), in which AA is present in 40% of patients, supports this hypothesis (29). AIRE gene
polymorphisms were detected with non-APECED AA (30).
The first cytokines released around HFs are IFN-γ and TNF-α. IFN-γ stimulates the expression of MHC-I, MHC-II, NKG2D, and chemokines (CXCL9, CXCL10, CXCL11), which continuously attract immune cells (8, 9, 15, 27, 31,32). CXCL10, CCL3, and CCL5 were highly expressed, with chemokine receptor (CXCR3) + T cells around the HFs in AA (33).
IL-2 and IL-15 and their receptors on CD8+ T cells are abundant around HFs (22, 34). IL-15 restricts the T reg cells’ suppressive ability (35), activates JAK, transforms CD8+ T cells to a cytotoxic phenotype (36), and promotes NKG2D expression on NK cells transforming them into effector cells (37).

Candidate genes for AA:
* Human leucocyte antigens; HLA-DRB1* 1104 and DQB1* 03 loci (38).
* NKG2D gene and its ligands, UL16-binding proteins (ULBP3), MICA, and retinoic acid early transcript 1L protein (RAET1L=ULBP6) (16).
* The genes of protein tyrosine phosphatase N22 (PTPN22), cytotoxic T lymphocyte-associated protein 4 (CTLA-4), IL2 receptor subunit alpha (IL2RA) (39), IL-2/IL21 locus, and IKZF4 (Ikaros family zinc finger 4) (16).
* Duplications in melanin-concentrating hormone (MCH) receptor 2; MCHR2 and MCHR2 antisense RNA1, implicated in MCH signaling (40).
* Peroxiredoxin-5 (PRDX5) gene, its dysregulation allows survival of impaired cells (affected by ROS), and self-antigen and presentation (41).
* The autoimmune regulator (AIRE) gene (30).
* Syntaxin-17 (STX17) gene, its protein has a role in melanogenesis (42).

Epigenetics and alopecia areata:
Epigenetic mechanisms, such as increased methylation of genomic DNA and histone acetylation were found in PBM-Cs of AA patients (43, 44).

Targeted antigens in alopecia areata:
Melanogenesis-associated antigens (45), trichohyalin, and keratin 16 are possible targets for T cells (46) and autoantibodies (47).

Immune cells implicated in the pathogenesis of AA:
1- CD8+NKG2D+ T cells:
The dominant effectors infiltrating the HFs are CD8+NKG2D+ T cells. CXCR3+CD8+ T cells invade the HF in AA lesions, with upregulated CXCR3 ligands (CXCL9, CXCL10, CXCL11) (32). Th17 cells around the HFs can play a role in AA (48). Invariant NK cells, classic NK cells, type 1 innate lymphoid, cells, and gamma delta T cells produce IFN-γ (49).

2- T Helper 2 (Th2) CD4+ T cells:
In AA lesions, the counts of Th2 cells (50), their cytokines (IL-23 and IL-9) (34), and their serum markers of the immune response (IL-4, IL-5, IL-6, IgE, CCL17, IL-13, IL-31, CCL13, CCL22, and CCL26) are increased (51), this may explain the association between atopic dermatitis and AA (52).

3- Mast Cells (MCs):
The numbers, proliferation, and degranulation of perifollicular MCs were increased with significantly more physical MCs and CD8+ T cell contact in human AA skin compared to non-AA control skin. MCs may present autoantigens to CD8+ T cells and/or costimulatory signals (53).
4- Plasmacytoid dendritic cells (PDCs):
The PDCs represent a connection between innate and adaptive immunity. They were identified infiltrating around HFs of AA (54). Activated PDCs secrete large amounts of (IFN-α/β), which triggers CD4+ cells, CD8+ cells, and NK cells responses towards the HFs (55,56).

5- T-regulatory cells (Treg cells):
The numbers of Treg cells were significantly lower in AA skin compared to controls and other skin diseases. They preserve peripheral tolerance by suppressing auto-reactive T cells (57). Immune tolerance collapse and T cell-mediated autoimmunity may be induced by Treg cell deficiency (58).

Cytokines and alopecia areata:
TNF-α, IFN-γ, and IL-1α/IL-1b had aberrant expression in AA lesions (59) and peripheral blood (60). The serum level of IL-15 was elevated in AA patients (61). The Th1 markers (CXCL10, CCL3, CCL2) were increased in long-lasting AA (50). CCL17 serum level correlated with AA severity (62). IL-17, IL-22 in AA lesions, and IL-1, IL-17, TNF-α, and TGF-β in the serum, were significantly higher in AA patients than in controls. Lesional and serum IL-17 levels positively correlated with the severity of AA (63). TNF-α has an anti-proliferative effect on the keratinocytes. It disturbs the hair cycle and induces catagen in ex vivo HFs (64). TNF-α blockers induce AA (65) by allowing uncontrolled secretion of IFN-γ by PDCs (54).

Molecular profile in chronic, recurrent, and severe alopecia areata:
Long-lasting AA is associated with increased Th1 markers (CXCL10, CCL3, CCL2) (50). The severity of AA positively correlated with serum levels of CCL17 and IL-17, as well as lesional IL-17 levels (62,36).

Toll-like receptors (TLRs) in alopecia areata:
Intracellular TLRs 3,7,8,9 were significantly up-regulated with increased expression of their mRNA in PBMCs, lesional, and peri-lesional HF bulbs of patients with AA (66). Polymorphisms of TLR1 may be associated with AA susceptibility (67).

Immune checkpoints:
Immune checkpoints negatively regulate the immune response to avoid prevent autoimmunity. They include CTLA-4, PD-1 receptors, and PD-L1. CTLA-4 is an inhibitory receptor essentially expressed by Treg cells. It binds to B7 co-stimulatory molecules on APCs preventing CD28 costimulatory induced activation of effector T cells (68). PD-1 is an inhibitory receptor, which on binding to PD-L1 create a strong inhibitory signal, which suppresses pro-inflammatory T cell activation and maintains self-tolerance (68). PD-1 inhibitors block T-cell inactivation and facilitate the T-cell invasion of the hair bulbar area inducing AA (69).

Oxidative Stress (OS):
Exposure to ROS induces catagen and hair growth retardation in cultured HFs (70). Values of OS index (OSI), serum total oxidant capacity (TOC), and malondialdehyde (MDA) were significantly higher in AA cases than in controls. Serum total antioxidant capacity (TAC) was significantly lower in AA patients than in controls, with significantly higher MDA, TOC, and OSI and significantly lower TAC values in severe AA (19).

The JAK/STAT pathway and alopecia areata:
The JAK/STAT signaling is suppressed in the HFs during anagen (71), as it can inhibit hair stem cell proliferation and activation (72), and reduce angiogenesis (73). IFN-γ and IL-15 are JAK/STAT pathway-dependent (74).

Through JAK signaling, CD8+NK-G2D+T cells are believed to mediate AA (75). JAK3 was strongly expressed in skin lesions of AA (76). The JAK activation has been recognized by the existence of phosphorylated STAT proteins in HFs of AA, but not in normal HFs (27).

**Vitamin D and alopecia areata:**
Vitamin D suppresses IFN-γ secretion by in vitro activated human PBMCs and CD4+ T cells (77), CD8+ T cells, CD4+ T cells proliferation, and the JAK/STAT pathway. It down-regulates the abnormally up-regulated intracellular TLRs, interferes with the interaction between MCs and CD8+ T cells, maintains MCs stability, enhances Treg cells function, and up-regulates PD-1/PDL-1, all of which help maintain IP (78).

Vitamin D intake significantly increased serum levels of TAC and glutathione and decreased MDA concentration compared to placebo (79).

**Psychological stress as a trigger of alopecia areata:**
In response to acute emotional stress, the receptors of corticotropin and corticotropin-releasing hormone (CRH) are upregulated in AA lesions (83), also, acetylcholine is upregulated, which can modulate the immune cells and upregulate TNF-α, IFN-γ and IL-6 production (80).

There are anatomical anomalies of the nerves supplying AA-affected HFs (81). Substance P, CRH, and nerve growth factor (NGF) provoke degranulation of MCs with the secretion of histamine, TNF-α, IL-6, and IL-1. Substance P promotes the ectopic expression of MHC-I on the anagen HFs and upregulates NGF, MHC-I, and β2-microglobulin in ex vivo HFs leading to premature catagen induction and IP collapse (82).

**Microbiota and diet relation to alopecia areata:**
The gut bacteria may contribute to the modulation of the onset of AA (83). The diet has a geographical effect on the lifetime risk of acquiring AA. In the United States of America, the lifetime risk of AA is estimated to be around 1.7% as most of the population adhere to a western diet, whereas, in Japan, where persons adhere to soya-based eastern diets, the lifetime risk is no higher than 1%. A soy-rich diet can delay AA onset or decrease its susceptibility (84).

**Environmental factors linked to alopecia areata:**
The connection between Helicobacter pylori (H. pylori) and AA is debatable. Infection with H. pylori triggers AA by stimulating Th1 and Th17 cell response and IFN-γ secretion which (85). Alopecia areata was induced by swine flu virus infection (86), infectious mononucleosis (87), and shortly after vaccinations against hepatitis B virus (88), herpes zoster virus (89), Japanese encephalitis (90), clostridium tetani (91), and human papillomavirus (92). Interestingly, new cases of AA including alopecia totalis and Universalis, as well as, relapse of previous AA lesions were reported with COVID-19 infection, which may be related to the inflammatory storm of COVID-19 infection, and the psychological stress of the quarantine (93,94). The low serum levels of zinc and selenium are confirmed risk factors for AA (94).

**Conclusions:**
The collapse of IP, MHC presentation, IFN triggering of IP collapse, and disruption of the JAK/STST pathway are all essential and evidence-based pathogenic mechanisms of alopecia areata.
CD8+ NKG2D+ T cells are the most important effectors in the immune response to AA. Oxidative stress, infections, and psychologic stress are all risk factors for AA induction.

**Abbreviations:**

AA: Alopecia areata  
AIRE: Autoimmune regulator  
APCs: Antigen-presenting cells  
APECED: Autoimmune polyendocrinopathy, candidiasis, and ectodermal dystrophy  
CD: Cluster of differentiation  
COVID-19: Coronavirus Disease-2019  
CRH: Corticotropin-releasing hormone  
CTLA-4: Cytotoxic T lymphocyte-associated protein 4  
CXCL: Chemokine  
HF: Hair follicle  
HLA: Human leukocyte antigen  
H. pylori: Helicobacter pylori  
ICAM: intercellular adhesion molecules  
IDO: Indoleamine 2, 3 dioxygenase  
IFN-γ: Interferon-gamma  
IgE: Immunoglobulin E  
IGF-1: Insulin-like growth factor 1  
IL: interleukin  
IP: Immune privilege  
JAK: Janus kinase  
JAK/STAT: Janus kinase, and signal transducers and activators of transcription  
MCH: melanin-concentrating hormone  
MCs: Mast Cells  
MDA: Malondialdehyde  
MHC: Major histocompatibility antigen class  
MICA: MHC class I chain-related A α-MSH: Melanocyte stimulating hormone  
NGF: Nerve growth factor  
NK: Natural-killer  
NKG2D: Natural-killer group 2, member  
OS: Oxidative Stress  
OSI: Oxidative stress index  
PDCs: Plasmacytoid dendritic cells  
PD-L1: Programmed cell death 1 ligand  
PBMC: Peripheral blood mononuclear cell  
PTPN22: Protein tyrosine phosphatase N22  
RAET1L: Retinoic acid early transcript 1L protein  
ROS: Reactive oxygen species  
TAC: Total antioxidant capacity  
TOC: serum total oxidant capacity  
STX17: Syntaxin-17  
TGF-β1: Transforming growth factor β1  
TLRs: Toll-like receptors  
TNF-α: Tumor necrosis factor-alpha  
ULBP3: UL16-binding proteins

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