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Annika Borde & Annika Åstrand

To cite this article: Annika Borde & Annika Åstrand (2018): Alopecia areata and the gut –the link opens up for novel therapeutic interventions, Expert Opinion on Therapeutic Targets, DOI: [10.1080/14728222.2018.1481504](https://doi.org/10.1080/14728222.2018.1481504)

To link to this article: <https://doi.org/10.1080/14728222.2018.1481504>



Accepted author version posted online: 29 May 2018.



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Publisher: Taylor & Francis

Journal: *Expert Opinion on Therapeutic Targets*

DOI: 10.1080/14728222.2018.1481504

Alopecia areata and the gut –the link opens up for novel therapeutic interventions

Annika Borde & Annika Åstrand

Respiratory, Inflammation and Autoimmunity IMED Biotech Unit, AstraZeneca, Gothenburg, Sweden

Corresponding author:

Dr Annika Åstrand
AstraZeneca Gothenburg
SE-43183 Mölndal
Sweden
Annika.astrand@astrazeneca.com
+46 708 467525

Accepted Manuscript

Keywords: Alopecia areata, intestinal permeability, chronic inflammation, autoimmunity

Abstract

Introduction: This review aims to raise the potential of the modern society's impact on gut integrity often leading to increased intestinal permeability, as a cause or driver of Alopecia Areata (AA) in genetically susceptible people. With the increasing rate of T cell-driven autoimmunity, we hypothesize that there is a common root cause of these diseases that originates from chronic inflammation, and that the gut is the most commonly exposed area with our modern lifestyle.

Areas covered: We will discuss the complexity in the induction of AA and its potential link to increased intestinal permeability. Our main focus will be on the gut microbiome and mechanisms involved in the interplay with the immune system that may lead to local and/or peripheral inflammation and finally, tissue destruction.

Expert opinion: We have seen a link between AA and a dysfunctional gastrointestinal system which raised the hypothesis that an underlying intestinal inflammation drives the priming and dysregulation of immune cells that lead to hair follicle destruction. While it is still important to resolve local inflammation and restore the IP around the hair follicles, we believe that the root cause needs to be eradicated by long-term interventions to extinguish the fire driving the disease.

Article highlights

- Chronic inflammation as an underlying driver of Alopecia areata (AA) has not previously been discussed. We have seen a link between disease manifestation and IBS-like symptoms, indicating that inflammation in the gut – causing a leaky epithelium and increased stress on the immune system – may be a driver of AA as well as in other autoimmune conditions where this is more commonly described.
- The gut microbiota is important in the function of the immune system and also for oral tolerance, which is of importance particularly in genetically susceptible people. Having a balanced microbiome is thus essential for a tight epithelial barrier and a functional and regulatory immune system.
- Modern society with its processed Western diet has greatly impacted on global health and dramatically increased allergies and autoimmune diseases.
- A high intake of fibers affects the make up of the intestinal microbiota, primarily increasing short-chain fatty acid concentrations that have beneficial immunomodulatory effects (eg increasing Treg numbers and function).
- Several concomitant triggers are needed to induce AA, however, an underlying chronic inflammation and its effect on the immune system may undermine its regulatory function which then permits the maintenance of the ongoing tissue destruction.
- We believe that addressing the root cause and driver of disease is essential for a successful outcome. Thus, we recommend future therapies and interventions in AA to start with eradication of any ongoing inflammation – today commonly located in the gut – before also dealing with the local attack on the hair follicles.

1. Introduction

The determinants of the development of an autoimmune disease have previously been allocated to genetics, environmental triggers and chance [[1],[2],[3]]. However, there is now growing evidence that an increased intestinal permeability plays a major role in the pathophysiology of autoimmune induction [[4],[5],[6],[7]] and that degradation of the intestinal epithelial barrier function induces inflammation that can lead to autoimmunity, either locally or remotely [[8],[9],[10]].

Autoimmunity is today considered a growing epidemic in industrialized countries [[11],[12]], although this might not be true for all autoimmune disorders (AIDs) such as *e.g.* rheumatoid arthritis (RA) [[13]]. Dietary and modern lifestyle changes are factors that affect the intestinal barrier and make it penetrable which initiates or perpetuates inflammatory responses [[14]]. Intestinal inflammation *per se* leads to increased epithelial permeability and hence increased exposure of foreign proteins to the immune system. This can eventually lead to a breakdown in immune competence, whereby the immune system mistakenly attacks self-tissues. Genetically susceptible people with a decreased ability to regulate immune responses and/or ability to handle an increased pressure on the immune system would thus be more prone to develop autoimmunity upon a trigger.

The immune system is tightly coupled to the gut microbiome which develops from the flora inherited at birth, and matures through environmental exposures and in response to diet [[15],[16]]. An imbalance of the gut microbiome, as in *e.g.* small intestinal bacterial overgrowth (SIBO), can also cause inflammation and increased intestinal permeability [[17],[18]]. SIBO is increasingly common and tightly coupled to 50+ diseases, out of many regarded as autoimmune [[19],[20]]. Additionally, constant exposure to toxic agents, from ingestion or produced by bacteria or parasites, is known to cause increased epithelial barrier dysfunction and thereby impacts our immune system [[21]]. Psychological stress is also connected to the initiation and maintenance of autoimmunity [[22],[23]], directly impacting on

the epithelial barrier or via the gut-brain-axis. It has further been shown that corticotropin-releasing factor from the hypothalamic-pituitary-adrenal (HPA) axis has a potent effect on the gut through modulation of inflammation, increased gut permeability, contribution to visceral hypersensitivity, increased perception of pain and modulation of gut motility [[14]]. Thus, modern lifestyle, gut homeostasis and autoimmunity are becoming increasingly connected and it is most likely that the now more common combination of these different “triggers” contributes to the increase in the development of AIDs.

Alopecia areata (AA) is an autoimmune condition in which immune cells attack self-tissue. AA is common with a life-time risk of 1.7-2% and a prevalence of 0.2% (reviewed by Gilhar et al [24]) but these numbers have not been updated since 1989. There is to date no FDA-approved therapeutics for AA, but a couple of interesting approaches are currently in clinical development (eg more selective JAK inhibitors and PDE4 inhibitors). Systemic corticosteroids (continuous or as pulse therapy), cyclosporine A, mycophenolate mofetil, methotrexate (monotherapy or with corticosteroids), and azathioprine are all used with off label prescription, but improved approaches for AA with better safety profiles are needed and nicely reviewed by Wang & Christiano [[25]] and Renert-Yuval & Guttman-Yassky [[26]] in 2017. Hitting only one cytokine in this complex disease is likely not as efficacious as the more general immunosuppressants. IFN γ blockade was less efficacious in a clinical trial despite good efficacy in preclinical animal studies [[27]]. TNF α -blockers have been found to trigger AA [[28]], which is likely due to the shifted TNF α /IFN γ ratio, where IFN γ is the main driving cytokine of AA. Blockade of IL-13 (Tralokinumab) is also currently under investigation for AA/AD.

Recently, Sobolewska-Wlodarczyk et al. [[29]] published an overview on comorbidities between AA and IBD (inflammatory bowel disease, such as ulcerative colitis, UC, and Crohn's disease, CD) and the common pathways that might be drivers of the different manifestations. There is to date no scientific basis for the hypothesis of a leaky gut as a cause - or driver - of AA. Rather the opposite, as a significant association was found between IBD and inflammatory skin diseases but not specifically with vitiligo or AA in a Korean population [[30]]. As most AIDs, AA is a polygenic disease, which may explain the vast variability of severity ranging from patchy alopecia areata to complete hair loss as in alopecia universalis, as well as whether it establishes into a stable or relapsing/remitting status. Interestingly, the genetic clustering of AA with other T-cell driven autoimmune diseases (rheumatoid arthritis (RA), type-1 diabetes (T1D), celiac disease (CeD), systemic lupus erythematosus (SLE), multiple sclerosis (MS) and psoriasis (Ps) as in Petukhova et al. [[31]] is very similar to the clustering of diseases with an increased intestinal permeability such as T1D, CD, CeD, RA, irritable bowel syndrome (IBS), atopic dermatitis, ankylosing spondylitis and rosacea [[29]]. Collectively, our hypothesis is based on the idea that compromised

tolerance induction in the gut contributes to, or even drives, these diseases and that they might be treated locally where the disease is manifested rather than remotely where it is symptomatically observed.

It has previously been stated that less than 10% of people with a genetic predisposition to develop AIDs actually do develop clinical disease [[4], or the risk can be expressed as two- to five-fold increased *versus* people that do not have the predisposition (source: American Autoimmune Related Diseases Association). Countering this statement, it is now more than 10% of the population (50 million in the US) that are diagnosed with an autoimmune disease, making it the third largest disease after cardiovascular diseases and cancer. Most importantly, the cost of treatments and research are greatest in autoimmunity and this is an important issue of modern times which requires understanding of the root cause/-s in order to be adequately and successfully addressed.

2. Complexity in the induction of AA

AA is completely unpredictable and there is today no diagnostic marker that can be used to predict an individual's prognosis, except that early onset and increased severity of disease are associated with poor outcomes. The onset of AA is commonly associated with immunological responses to triggers such as viral infections, trauma, hormones and emotional/psychological stress [[24]]. We are now proposing that an increased pressure on the immune system is simultaneously required for disease manifestation and that this could originate from any chronic inflammation, but where intestinal inflammation is increasingly common. The stated triggers result in an increased release of cytokines and chemokines that cause the now well-established collapse of the immune privilege (IP) of the hair follicle (HF) that starts expressing MCH class I and II peptides and becomes vulnerable to attack by inflammatory cells [[32],[33],[34],[35],[36]]. To date it is not clear what is the chicken or the egg in the process of the undesirable immune cell attack [[36],[37]] but the fact that AA is a common disease might be just because the HF is a sensitive organ. It seems that people who carry a deficiency in resolving ongoing inflammation, and thus the ability to restore IP, are the ones that develop alopecia areata, totalis or universalis. It is also likely that the same individuals have an inability to regulate the generation or suppression of autoreactive T cells and NK cells by *e.g.* adequate function of Tregs [[31]] or tolerising dendritic cells (DCs). The seemingly serendipity-dependent specificity of tissue destruction in different autoimmune diseases could be driven by at least three specifics: the MHC I expressed epitope (mimicry), differences in tissue homing markers on DCs/antigen presenting cells (APCs), and/or the concentration gradient of different cytokines/chemokines in and around the HFs. The search for efficacious treatment options for AA has focused on the inhibition of cytokines and

chemokines to diminish their recruitment of inflammatory cells to the HF, and also direct inhibition of the inflammatory cells. The hypothesis that autoreactive T cells are primed either in the gut mucosa or in peripheral lymphoid tissues by gut-primed DCs/APCs has as yet not much evidence in the literature, but there are a couple of emerging examples. For example; α -gliadin, a gluten by-product, contains similar epitopes to trichohyalin (THH) [[38]], which is the most likely autoantigen in the HF [[39],[40]]. Gluten by-products have also been associated to the HF peptide peroxiredoxin 5 (PRDX5) [[41]] which is one of the genes in which polymorphisms have been associated with AA in genome-wide association studies [[31]]. Moreover, Nair et al. [[42]] demonstrated the necessity for *Lactobacillus* in the gut microbiome for the induction of AA. Understanding the origin of the priming of autoreactive T cells might open up new therapeutic angles, providing a more preventive way treat autoimmune diseases, or treatments to maintain patients in remission.

Our hypothesis is that the priming of autoreactive T cells ($CD8^+NKG2D^+$ cells in the case of AA) is likely initiated by DCs or other APCs close to the site of inflammation (skin draining lymph nodes) or in the gut mucosa where loss of tolerance along with the mechanism of molecular mimicry/identity initiates T cell-driven AIDs, and that a leaky gut significantly contributes to the initiation of the process [[43],[44],[45]]. We will next discuss the homeostatic mechanisms in the intestine that support maintenance of immune competence.

3. Gut homeostasis and the immune system –importance in health and consequences in disease

3.1 Gut Microbiota and diet

Our gut microbiota is established early in life and the diversity and composition of the intestinal microbiome in a newborn child largely derives from its mother during birth [[9],[46]]. In infants and in early childhood, differences in the gut flora have a great influence on the development and priming of the immune system [[47],[48]]. In the same time frame, factors such as antibiotic intake, environmental factors, breast feeding and diet affect the gut microbiota development and composition. Besides the above-mentioned genetic aspects in developing AA and other AIDs, it is known that diet modulates and regulates the microbiota of the gut, and that the increasing prevalence of these diseases are considered to be an effect of the modern Western lifestyle [[11]]. Several studies, both in animals and humans, have shown a clear relationship between diet and the gut microbiota composition [[49],[50],[51],[52],[53],[54],[46],[55],[56]]. As such, alterations of the gut microbiome have been suggested in many studies to affect host physiology [[57]] and changes from the healthy gut microbiota have been observed in obesity [[58]] and in inflammatory disorders such as IBD, eczema and allergy [[59],[60],[48]].

The gut microbiome outnumbers our own somatic and germ cells ten times and mainly populates the large intestine [[61],[62]]. Bengmark has described the dependence of the immune system on the gut microbiota [[63]] where lack of proper nutrition for bacteria is a major contributor not only to a dysfunctional microbiota and dysbiosis, but also to chronic inflammation with its production and leakage of endotoxins through various tissue barriers. In recent years, whole genome shotgun (WGS) and 16S rRNA gene sequencing have enabled us to explore the identity of “our other genome” and its inhabitants in health and disease (The human microbiome project consortium Nature 2012 [64]). Large scale sequencing studies have enabled researchers to explore the association between gut microbiota, diet and AID [[65],[66],[67],[68]] which will most likely lead to a better understanding of the connecting points between our diet and the development of AIDs and thus open up for better ways to treat and/or even prevent them.

Bischoff and Volynets [[50]] demonstrated that the intestinal microbiota distinctly differed between wild and laboratory mice and noted the increased intake of sugars and the cleaner environment of the laboratory animals. It was suggested that diet may be an explanation for the differences [[50]]. Given that diet affects the microbiota, and that the intestinal microbiota seems to be involved in driving host physiology, the diet fed to research animals might have a great impact on the outcome of a study, especially if related to immunity.

The growing interest in functional food, functional medicine and anti-inflammatory diets speak in favor of the benefits associated with reducing inflammatory triggers via the gut, and that it seems to work both with regards to restoring intestinal and general homeostasis but also by dampening inflammatory diseases, including autoimmunity [[67],[69],[70]]. Although our diet largely impacts the microbiome diversity and consequently our health, other factors such as hygiene also impact the microbiota and the development of our immune system. According to many studies, a high level of hygiene increases the risk for developing allergies, asthma and autoimmune diseases, including during pregnancy [[71],[72],[73]]. However, when comparing Japan to the USA, the number of asthma patients is clearly higher in the US and the hygiene level much higher in Japan [[55]]. This indicates that diet, and possibly other lifestyle factors, out rules the hygiene hypothesis in the significance of a well-regulated immune system.

3.2 SIBO – a cause of increased intestinal permeability

Small intestinal bacterial overgrowth (SIBO) is a bacterial infection of the small intestine caused by too much bacteria, or the wrong type of bacteria in the wrong place [[74],[18],[20]]. Most often, this occurs when the large intestinal microbiota has moved into, or resided in, the small intestine. Less commonly, SIBO results from an increase of the otherwise normal bacteria of the small intestine. SIBO causes excess gas production, abdominal pain, diarrhea

and/or constipation, but most importantly intestinal permeability [[17],[75]] and thereby an increased pressure on the immune system. SIBO is linked to 50+ diseases [[20]] whereby IBS, CD, celiac disease (CeD) and histamine intolerance are particularly associated [[18],[20]].

Normally, relatively few bacteria live in the small intestine (less than 10,000 bacteria per milliliter of fluid) as compared with the colon (at least 1,000,000,000 bacteria per milliliter of fluid) and the types of bacteria in the small intestine are not the same as those in the colon [[18]]. They play an important role in digesting food and absorbing nutrients but are also important regulators of the immune system with its impressive network of lymphoid cells in the intestinal submucosa. Moreover, the bacteria help maintain the normal muscular activity of the small bowel (Migrating Motor Complex, MMC). The MMC is responsible for moving the intestinal content through the gut, but most importantly, to clean the small intestine in between meals so that bacteria cannot overgrow. The MMC seems to be the most frequent dysfunction in people with SIBO [[74]] but also proper gastric acid secretion, biliary and pancreatic secretions, immunoglobulins in the intestinal fluid and the ileocecal valve (which allows the flow of bowel contents into the large intestine but prevents them from refluxing back into the small intestine) are important for an adequate small intestinal function [[76]]. The exact prevalence of SIBO is difficult to predict as this condition is often underdiagnosed due to unsatisfactory diagnostic tests as well as knowledge and experience of patients and health care providers. Different studies have estimated the prevalence of SIBO in clinically healthy individuals to 2.5-22.5% [[18]] and 5.9-15.6% [[74]], where the higher prevalence was found in older people. In comparison, the prevalence of SIBO in disease is going as high as 90% (eg 30-40% in chronic pancreatitis, 56% in CF, 59% of acute diverticulitis, 30-85% in IBS, and 90% for small intestinal motility disorders).

SIBO has been shown to negatively affect both the structure and function of the small intestine primarily by damaging the cells lining the mucosa. It thus significantly interferes with digestion of food and absorption of nutrients and allows large protein molecules to escape into the bloodstream. This is known to have a number of potential complications including immune reactions that cause food allergies or sensitivities, generalized inflammation and autoimmune diseases [[77]]. Collectively, AA is a common autoimmune disease and our observation that AA is often associated with a dysfunctional gastrointestinal system make us speculate that SIBO could be a prevalent underlying stressor of the immune system in genetically susceptible people which leads to a poorly regulated immune self-attack of the hair follicles.

3.3 SCFAs – roles & functions

Gut bacteria can thus be seen as a link between what we eat and what happens physiologically via their metabolites. The modern “Western” diet is generally characterized by low fiber, less vegetables and high sugar and fat content, even though there are differences between countries and cultures which affect the metabolites produced and consumed in the gut.

Our enteric microbiota has throughout history co-evolved with humans, making us able to access nutrients and to synthesize vitamins and essential amino acids. The most abundant microbial metabolites in the intestine are short chain fatty acids (SCFAs) resulting from bacterial fermentation of soluble fibers and oligosaccharides that have reached the colon [[78]]. SCFAs are free fatty acids with an aliphatic tail of 2-6 carbon atoms (making formic, acetic, propionic, butyric and valeric acids, respectively). They are water soluble and can easily be absorbed into the gut epithelial cells where they can be metabolized.

SCFAs have various physiological effects in the gut and are important for the maintenance of intestinal function. They regulate ion absorption and gut motility, and favor the production of mucins and gastrointestinal peptides. Butyrate, in particular, is the primary energy source for colonocytes [[79]]. Thus, it is very likely that SCFAs have an effect in protecting our intestinal barrier. SCFAs, and mainly butyrate, have also been shown to regulate the number and function of colonic Tregs [[80], [81], [82]], thus are important regulators of the immune system and peripheral (oral) tolerance.

Taking into account all these roles that SCFAs play in the colon and the fact that fibers is a main substrate for bacterial SCFA production, one would expect profound effects of our Western low-fiber diet on the immune system and our health. The maintenance of a functional intestinal barrier and immune regulation thus depends on what you feed the bacteria, and the expression “Feed your Tregs more fiber” might be worth keeping in mind [[83]]. The significant difference of the microbiota composition and an almost 3-fold increase in total fecal SCFA concentration in Burkina Faso children (where the main intake are natural fibers) as compared to Europeans, support this statement [[52]].

3.4 Receptors for SCFAs and their potential role in autoimmune diseases

The SCFAs discussed above bind to different G protein-coupled receptors (GPCRs) on immune and gut epithelial cells [[65],[84],[85]]. The best characterized GPCRs that respond to SCFAs are GPR43 (also known as FFAR2), GPR41 (FFAR3) and GPR109 (NIACR1, HM74) [[70]]. Their physiological roles and actions are extremely complex and FFAR2 signaling in leukocytes needs further dissection and analysis [[79]] but it has been shown that propionate triggers FFAR2-dependent release of the anti-inflammatory cytokine IL-10 in Tregs [[80]]. The potential for drug targeting of GPR43/41 in the treatment of immune disorders is still unclear, both with regards to if one aims to activate or inhibit of the receptors [[86]]. In

addition, the generation of agonists that are functional *in vivo* has been technically difficult [[79]]. There is, however, a useful tool for GRP43 inhibition that works in human systems (GLPG0634), but unfortunately not in mouse models. It seems most likely that SCFAs, via GPR43 interaction or in other ways, affect inflammatory responses in a positive matter [[79],[87]]. Trompette *et al* showed both a positive correlation of fiber content in the diet and circulating SCFA levels in mice as well as an effect of a fiber-rich diet in protection against allergic lung inflammation [[88]]. Moreover, exacerbated or unresolved inflammation has been observed in GPR43-deficient (*Gpr43*^{-/-}) mouse models of colitis, arthritis and asthma [[89]], indicating the influence of this receptor in aggravating inflammatory disease. In order to investigate the potentials for SCFAs in Alopecia Areata, we used the C3H/HeJ model of AA where the disease was induced by transplantation of skin grafts from a diseased donor C3H/HeJ mouse to recipient mice from the same strain [[90]]. These mice have spontaneous genetic mutations that lead to several dysfunctions and spontaneous development of AA in approximately 20% of animals within 6-12 months of age [[91],[92]] and with grafting the prevalence becomes almost 100% within 6-8 weeks. It has been proposed that these mice have a compromised immune system by means of dysfunctional Tregs [[93],[39]] however, we demonstrated that C3H/HeJ Treg suppressive function on T-effector cell proliferation *in vitro* is similar to Tregs from other strains, both in disease and health [[94]]. *In vivo* it may however be different. We hypothesized that propionate in the gut would through stimulation of GPR43 (GPR41 or GPR109) receptors induce more tolerogenic Tregs that could protect the hair follicles from immunological attack, as an increased abundance but unknown role for Tregs within the HF shafts had been recently demonstrated [[95],[96]]. In a pilot study, we observed regrowth of fur in 5 out of 5 mice after 11 weeks of propionate treatment (*ad libitum* access to 200 mM propionate via the drinking water) vs none in the vehicle-treated mice (n=3) and we assessed skin cell differentials after an additional four weeks on treatment (unpublished data). We observed a 2.3-fold increase of Treg count in the propionate-treated mice (no statistically significant difference, $p=0.16$) but importantly an increased Treg/CD4⁺ ratio ($p=0.08$) *versus* vehicle controls. We repeated the study (n=6 per group) but were unable to reproduce the positive effects on hair growth. Moreover, we failed to prevent disease manifestation by prophylactic treatment (starting 3-4 days before grafting). In another study, we investigated the effect of probiotics (4 bacteria used in Synbiotic 2000) in order to generate all SCFAs, and in particular butyrate *in vivo*. Treatment over 16 weeks did not reverse chronic AA but slightly increased the Tregs/CD4⁺ ratio (15 vs 12%, $p<0.01$, n=6) in skin draining lymph nodes. Hence, both propionate and probiotics (4 selected strains) affect T-cell ratios, specifically with regards to Tregs, but does not seem to be a strong enough mechanism to reproducibly reverse or prevent disease.

Despite our inconclusive/negative results, we suggest that an adequate intake of fibers (complex plant polysaccharides) affects the makeup of the intestinal microbiota which in turn leads to a higher production of immunomodulatory products, in particular SCFAs that affect the function of the gut epithelium and the immune system. In the case of AA, this could enhance the regulation of the immune system (peripheral tolerance) to resolve local inflammation around the hair follicles –especially on the basis that Tregs have recently been found prominent in HF and important for hair growth [[95],[96]]. In support of this notion, 2 cases of fecal microbiota transplants (FMT) in people with alopecia areata/universalis, demonstrated long term hair growth post FMT [[97]].

4. Potential for therapeutic interventions aiming at improving intestinal function in AA & autoimmunity in general

We have described intriguing links between disease and intestinal dysbiosis in autoimmune diseases and hypothesize that restoring a healthy gut will boost the inherent capacity of the immune system to resolve autoimmune inflammation. Due to the vast inter-individual variability of gut microbiome, life styles and diet, it is not an easy task to scientifically prove clinical benefit by restoring a healthy microbiome in people with inflammatory disease, but it is clear that many diseases are linked to a less rich flora and increased intestinal permeability.

The fastest therapeutic intervention to test this hypothesis would be to transplant a healthy microbiome (fecal transplant/FMT) to individuals with autoimmune diseases and keep them on a fiber-rich/low sugar diet for a few months up to years for follow up. Alopecia areata could be a good model system of T cell-driven AID to prove the concept, as hair growth is a simple readout. By only improving the diet and read out on efficacy (change of microbiome and resolution of inflammation) would not be optimal since it is harder to control these clinical studies and it may also not be possible to restore a functional microbiome if it has never been functional and diverse in a susceptible individual. Another shortcut could be to use biosynthetic gene therapy, *i.e.* replacing the ligands that should have been synthesized by the “good” commensal bacteria of the gut [[98], [99]] but it would be questionable whether it is possible to get these right in identity and concentration.

Besides diet and microbiome interventions, future therapeutics could aim to tighten the epithelial barrier of the intestine [[100]], *e.g.* by means of Zonulin inhibitors [[77]], other tight junction regulators like PAR-2 [[101],[102]] or affecting the integral transmembrane proteins; claudins, occludin, tricellulin and junctional adhesion molecules, like β -catenin [[100]].

Moreover, zinc supplementation has demonstrated increased barrier function [[103],[104]] as

well as L-glutamine [[105]]. A more non-scientific postulation is that bone broth heals the gut barrier by its main ingredient gelatin which enhances gastric acid secretion, restores the mucosal lining and promotes adequate intestinal transit and bowel movements [[62]].

An immunological angle would be to pharmaceutically interact locally with the gut mucosal immune system, as the largest collection of intestinal lymphocytes reside in the gut-associated lymphoid tissue (GALT). Induction of Treg function or increasing the tolerogenicity of dendritic or other antigen-presenting cells could be a way to increase the regulatory capacity of the immune system in susceptible people [[43]].

Most importantly, any ongoing chronic inflammation in the gut mucosa (eg SIBO, IBD) or elsewhere (e.g. periodontitis that drives systemic inflammation) is essential to eliminate as these may limit the effectiveness of any therapeutic intervention.

5. Conclusion

We have seen a compelling link between the induction and progression of AA and gastrointestinal disorders (IBS-like symptoms). With this review, we would like to raise the awareness that an increased intestinal permeability may be one of the major drivers and underlying causes of the increased prevalence of autoimmunity, including AA, in today's modern society. There is still not much scientific support for our hypothesis in AA, but there is indeed growing evidence between intestinal inflammation and a leaky GI epithelium in other autoimmune conditions. Chronic inflammation results in an increased burden on the immune system and suppresses its regulatory effect which makes susceptible people more prone to develop inflammatory and autoimmune conditions. Whether the stress on the immune system comes from increased intestinal permeability due to inflammation and/or dysbiosis, or from other chronic inflammations, high exposure to toxic agents or psychological stress, may be irrelevant – as long as the root cause is identified and eradicated.

6. Expert Opinion

Restoring the intestinal integrity (microbiome, epithelial barrier and immune tolerance) may not completely eradicate autoimmune tissue damage *per se*, but the evidence listed in this paper clearly indicates that it is necessary to extinguish an underlying chronic inflammation before attempting to locally inhibit the cytotoxic cell destruction. Inevitably, directly targeting the cytotoxic activity of NK cells and CD8⁺NKG2D⁺ cells in diseases such as celiac, type-1 diabetes or AA is important to interrupt tissue destruction and preserve organ function. But in our opinion, the root cause and driver of disease needs to be eliminated first in order to achieve longstanding and successful treatment of this type of autoimmune conditions, such as AA.

We would like to propose a new treatment regimen in newly diagnosed AA patients whereby the general dermatology practitioner should explore if the patient has an ongoing chronic inflammation and start adequate therapy to resolve it. Worth to highlight here, is that an increased intestinal permeability is not an easy and straight forward dysfunction to measure, and may take additional questions and tests that evaluates food intolerances, acid secretion, bowel movements and digestion capability disorders in order to be correctly diagnosed. It is known to be easier to reverse patchy AA versus complete AT/AU, and as with other AIDs it is better to prevent and treat early, to reduce the number of cytotoxic cells and/or antibodies to decrease the extent of tissue destruction. Luckily, AA and the HF function are reversible, which also highlights the potential role for this disease as a good model system in research whereby longterm studies can be performed with rather simple and cheap readouts. As the knowledge of the disease drivers in AA is increasing, clinical endpoints such as biomarkers of efficacy and disease signatures are becoming available. Here, also dosing regimens (eg induction *versus* maintenance therapies) can be evaluated as biomarkers can be studied in blood, lymph node biopsies and/or skin biopsies. The prevalence of AA is high with a vast unmet medical need and patients are more than willing to participate in clinical studies with the potential of future therapeutic options. Once autoimmune diseases can be viewed and treated as a whole integrated system, we will be able to find an effective treatment, or even a cure. With the hypothesis that an underlying inflammation of the gut is driving autoimmune disorders, including AA, we suggest to add the dimension of a healthy gut – either by medicine or by diet/lifestyle changes – to current and future therapeutic interventions.

Funding

This paper was not funded.

Declaration of interest

The authors are both employed by AstraZeneca (Respiratory, Inflammation and Autoimmunity IMED Biotech unit). The authors have no relevant affiliations or financial involvement with any organization or entity with a financial interest in or financial conflict with the subject matter or materials discussed in the manuscript. This includes employment, consultancies, honoraria, stock ownership or options, expert testimony, grants or patents received or pending, or royalties. Peer reviewers on this manuscript have no relevant financial relationships or otherwise to disclose.

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