Gut inflammation in the pathogenesis of acquired aplastic anemia

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Introduction

Acquired aplastic anemia (AAA) is an auto-immune disease (AID) resulting from aberrant T-cell-mediated and antigen-driven immune responses, with preferential insult, to hematopoietic stem and progenitor cells (HSPCs) in genetically susceptible individuals. Significantly enhanced suppression and apoptosis of HSPCs result in markedly hypocellular bone marrow (BM) and varying degrees of pancytopenia. Active systemic and local inflammation is responsible for the overall pathophysiology. The dysregulated auto-immunity is characterized by the abnormally increased number and function of T-helper type 1 (Th1) cells, cytotoxic T lymphocytes (CTLs), effector memory CTL (mCTLeff) cells, Th17 cells, and myeloid dendritic cells, by the abnormally decreased number and function of peripherally induced regulatory T cells (Tregs), T-helper type 2 (Th2) cells, B cells, and plasmacytoid dendritic cells, and with the enhanced expression and secretion of pro-inflammatory mediators and the reduced production of immunoregulatory cytokines. Natural killer (NK) cells skew their differentiation into the pro-inflammatory NK2 phenotype, and natural killer T (NKT) cells skew their differentiation into the NKT2 phenotype. Significantly up-regulated expression of human leukocyte antigen (HLA) and Fas molecules on CD34+ HSPCs induces enhanced antigen-presenting activities and accelerated apoptosis. The high efficiency of rapamycin in ameliorating hematopoietic suppression clearly indicates the abnormally activated mTOR (mammalian target of rapamycin) pathway and its contribution to AAA pathogenesis.[²]

Currently, allogeneic hematopoietic stem-cell transplantation and immunosuppressive therapy (IST) are the main treatments. Standard first-line IST, based on a combination of anti-thymocyte globulin and cyclosporine (CsA), produces approximately two-thirds of a response; the response rate is much lower in severe aplastic anemia (SAA). Patients who responded to first-line IST generally remain dependent on sustained CsA treatments, and complete responses can be achieved in approximately 10% of these treated patients. In recent years, the addition of eltrombopag to IST has given rise to a tremendous increase in hematological responses.[³]

However, how to initiate and sustain the chronic inflammation remain unknown. Diverse infectious and genotoxic agents have been implicated,[¹] and it is well known that the exacerbation and amelioration of disease severity fluctuates frequently in parallel with the waxes and wanes of certain physical and mental stresses, which seem to be driven by chronic and recurrent episodes of agnogenic infections. Recently, the driver of deranged auto-immunity has been proposed to come from altered gut microbiota and compromised intestinal epithelium.[⁴]

This manuscript is based on superficial knowledge in the treatment of a patient with SAA[⁵] and in a preliminary investigation by referencing the researches in other AIDs, with a focus on the possible role of gut inflammatory conditions (GICs) in the pathogenesis of AAA and some open questions in the treatment with gut cleansing preparations (GCPs).

Shaping the Host Immune System by Gut Microbiota

Human gastrointestinal (GI) tract not only provides the largest and most vulnerable interface by linking the host psycho-neuro-endocrino-immune system with environmental exposures but also harbors the most enriched gut-associated lymphatic tissue and the most complex microbial community.[⁶] In germ-free-reared mice, their gut microbiota and compromised intestinal epithelium.[⁴] auto-immunity has been proposed to come from altered gut microbiota and compromised intestinal epithelium.[⁴] This is an open access article distributed under the terms of the Creative Commons Attribution-Non Commercial-No Derivatives License 4.0 (CCBY-NC-ND), where it is permissible to download and share the work provided it is properly cited. The work cannot be changed in any way or used commercially without permission from the journal.
evolve with the host have an in-dispensable and obligatory role in the development, education, and maturation of host lymphatic tissues, ensuring appropriate responses to potential pathogens and tolerance to commensal microbes and self-antigens.[3] Given that the intestinal epithelium is constantly exposed to antigens derived from microorganisms and ingested foods, a finely tuned and well-maintained gut environment is in-dispensable and essential for host immune homeostasis and normal metabolism. The intricate communication among the intestinal epithelium, immune cells and commensal microbes has definite impacts on host health and disease. In genetically predisposed individuals, a disturbance in any of these elements may lead to active and chronic inflammation, impaired integrity and increased permeability of the intestinal mucosa. While well-balanced composition and abundant diversity in the microbial community are beneficial to health, perturbation of the gut microbiota may prompt inflammation by pattern recognition receptors (PRRs) on the intestinal epithelium and innate immune cells sensing pathogen-associated molecular patterns (PAMPs) on commensal microbes, favoring the development of local and systemic auto-immune reactions.

**Definite Impacts of Gut Inflammatory Conditions on Auto-Immunity**

Compromised intestinal epithelium allows antigens from commensal microbes and ingested foods in the intestinal lumen to translocate into the lamina propria, blood, and BM (leaky gut), resulting in an increased opportunity for host immune cells to intimately come in contact with exogenous antigens.[4] Th17 cells activated by gut commensal microbes could act on remote organs, and thereby provoking autoimmune.[5] Changes in the composition and diversity of the gut microbiome resulting from changes in dietary protein sources, could lead to changes in mTOR activity, fueling inflammatory reactions.[6] The reduced production of short chain fatty acids (SCFAs) due to the decreased proliferation of anaerobic bacteria and an insufficient supply of indigestible polysaccharides could induce Treg dysfunction, CTL activation, and impaired epithelial repair, which preferentially promotes type 1 immune responses.[7] Dysbiosis and GICs may serve as intensifiers, linking host immunogenetics with environmental challenges, to amplify which preferentially promotes type 1 immune responses.[8] CTL activation, and impaired epithelial repair, of host immune cells sensing an altered environmental or damaged intestinal epithelium.

**Reported AAA-Associated Gut Inflammatory Conditions**

The association between GICs and AAA has been documented in few reports (reported AAA-associated GICs include inflammatory bowel disease [IBD], celiac disease, and neutropenic colitis, and these are listed in Supplementary Table 1 http://links.lww.com/CM9/A254). In most cases, complicated by IBD, the authors attributed AAA development directly to the adverse effects of antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) and immuno-suppressants (items 1–13 in Supplementary Table 1 http://links.lww.com/CM9/A254). Successful treatment of “drug-induced AAA” by IST strongly indicates a commonly shared mechanism for both AAA and IBD; hematological improvements were frequently accompanied by the alleviation of GI symptoms (items 4–5 in Supplementary Table 1 http://links.lww.com/CM9/A254). In some patients, the occurrence of AAA preceded drug use or the drugs had not been administered (items 14–17 in Supplementary Table 1 http://links.lww.com/CM9/A254). These drugs may not exert direct toxicity on HSPCs but rather disturb the gut ecological environment or damage intestinal epithelium.

In total, 12 cases in six publications documented the association between AAA and celiac disease, of which celiac disease was diagnosed concurrently with AAA in eight patients (items 18–23 in Supplementary Table 1 http://links.lww.com/CM9/A254). Improvements in autologous hematopoiesis and GI symptoms were recorded in three patients following a gluten-free diet, clearly indicating the pathogenic role of GICs in AAA development (items 18, 21, and 22 in Supplementary Table 1 http://links.lww.com/CM9/A254). In AAA patients complicated by neutropenic colitis, the authors attributed colitis to severe neutropenia, and these patients commonly presented with rapidly progressive hematopoietic suppression (items 24–28 in Supplementary Table 1 http://links.lww.com/CM9/A254). However, the presence of bleeding polyloid lesions in the colon strongly indicates chronic gut inflammation proceeding the onset of severe leukopenia and colitis (item 28 in Supplementary Table 1 http://links.lww.com/CM9/A254). Apart from AAA characterized by pancytopenia and trilineage hypoplasia, pure red cell aplasia, amegakaryocytic thrombocytopenia, and agranulocytotic neutropenia have also been reported to be associated with GICs (items 29–34 in Supplementary Table 1 http://links.lww.com/CM9/A254). Similar to IBD-associated AAA, successful treatment by IST indicates an immune-mediated mechanism (item 30 in Supplementary Table 1 http://links.lww.com/CM9/A254).

**Convincing Evidence Underpinning the Role of Gut Inflammation in Hematopoietic Suppression**

We have reported an SAA patient who gained an unexpected hematological response to treatment of gut inflammation.[5] This patient was refractory to CsA, stanozolol, recombinant human granulocyte colony stimulating factor (rhG-CSF), and eltrombopag, which worsened and accelerated the hematopoietic injury. He experienced a 3-month-long episode of agnogenic febrile disease without response to intensive treatment with multiple kinds of broad-spectrum antibiotics. When presenting with abdominal cramps, he was prescribed oral administration of mannitol and gentamycin to eliminate the gut infection. This treatment not only resulted in a quick resolution to the fever but also produced an excellent hematological response. He had undergone three episodes of recurrence within one year of treatment. However, subsequent treatments were able to induce subsequent remissions, and consecutive treatments were successful in producing prolonged hematological improvements. Enlightened by these findings, we conducted a preliminary investigation on five other patients with SAA and 27 patients with non-SAA, and the reproducible and
efficacious therapeutic outcomes support the idea that GICs drive deranged auto-immunity. Unfortunately, the patient eventually developed refractory adynamic ileus and an erythroid proliferative disease, and eventually died of septic shock. Nevertheless, an increase in the absolute reticulocyte count and a cellular BM on admission indicated the absence of BM suppression. This investigation had to be terminated for our inability to identify patients who were highly predisposed to developing malignant proliferation after resolution of hematopoietic suppression. This provides direct evidence to support the indispensible role of GICs in BM suppression, whether "self-reactive CTL cells" target antigens of infectious agents or transformed malignant cells.

Possible Role of Gut Inflammation in Hematopoietic Suppression

Although the precise mechanism underlying GICs in the development of AIDs remains unclear, AAA may be driven, at least in a significant proportion of cases, by altered gut microbiota and compromised epithelium. Similar to other AIDs, the possible role of GICs in the development of AAA may serve as follows:

(a) Providing a sufficient supply of exogenous antigens and facilitating the generation of self-reactive mCTLeff cells. Because of the complex and elaborate immuno-regulatory and self-limiting mechanisms in immune competent individuals, the activated immune system in response to pathogenic antigens would rapidly return to homeostasis after eliminating antigens. Chronically progressive hematopoietic suppression suggests the existence of a constant source to continuously supply exogenous antigens or the host's inability to cleanse pathogenic antigens in immune-compromised subjects. Structurally and functionally integrated intestinal epithelium plays a crucial role in keeping gut microbes away from host immune cells, which is the most important factor for the host to maintain immune homeostasis and normal metabolism. In the setting of GICs, the compromised epithelium allows antigens to intimate contact with host immune cells, thereby rendering persistent autoimmune responses. Intimate contact of host immune cells with high-dose exogenous antigens cross-reactive with self-antigens on HSPCs may breakdown host immune homeostasis, leading to the activation of self-reactive CTL cells that have low affinities in healthy conditions and eliciting abnormal responses to HSPCs (molecular mimicry).

Endotoxins and other microbial metabolites can also transfer from the intestinal lumen into the blood and BM (leaky gut), thereby inducing persistent autoimmune responses. Intimate contact of host immune cells with high-dose exogenous antigens cross-reactive with self-antigens on HSPCs may breakdown host immune homeostasis, leading to the activation of self-reactive CTL cells that have low affinities in healthy conditions and eliciting abnormal responses to HSPCs (molecular mimicry).

(b) Amplifying the pro-inflammatory reactions. Immuno-genetics, either constitutional polymorphism or acquired gene mutations, actively shapes the composition and abundance of gut microbiota and thus influences gut homeostasis and immune responses to commensal microbes. In the setting of GICs, dysbiosis and compromised epithelium allow exogenous antigens to intimately come in contact with host immune cells, priming inflammatory reactions by PPRs sensing PAMPs. The bidirectional interplay between host immunogenetics and gut microbiota could act as intensifiers to amplify the auto-immune reactions and provide extended inflammatory environments in genetically predisposed individuals.

(c) Linking host immunogenetics to environmental challenges. Although host immunogenetics is the major determinant for susceptible individuals to develop AIDs, engagement of environmental challenges is required and indispensible. Since hosts have to confront various environmental exposures, the GI tract, being the largest and most vulnerable interface as well as harboring the most complex lymphatic and microbial architecture in human body, has become a common spot to bridge host immunogenetics and environmental challenges. The significantly increased frequency of AIDs including AAA, in recent decades may be attributed largely to changes in lifestyle and diet, the accommodation of those has gone through more than thousands of years.

Promising Pathway to Investigate AAA Pathogenesis and Treat Patients With AAA

As discussed earlier, dysbiosis and gut inflammation play indispensible roles in driving immune-mediated pathophysiology. Similar to other AIDs, modulation of gut microbiota and treatment of GICs may open a novel avenue in etiological research and treatment options for AAA patients. Rifamycin may be a promising drug due to its eubiotic properties and indirect mTOR inhibiting activity and this is the case for berberine and other Chinese medications. Before these treatments used in practice, several open questions have been raised and merit extensive investigation:

(1) How to evaluate the possible protective role of hematopoietic suppression in limiting GICs, in that long-term remission of IBD could be achieved by the pancytopenia state in azathioprine treatment (items 10...
(2) How to evaluate the possible protective role of hematopoietic suppression in limiting the over-proliferation of various pathogens that infect and proliferate in hematopoietic and immune cells[1];

(3) How to evaluate the possible protective role of hematopoietic suppression in the repression of malignant proliferation caused by genotoxic agents and intracellularly proliferated viruses,[1] as indicated by approximately 10% of AAA patients that develop malignant hematopoietic diseases after recovery of autologous hematopoiesis following effective IST,[3] similar immunological profile in hypocellular myelodysplastic syndromes[4] and the development of malignant proliferation in our reported patient[1];

(4) How to evaluate the presence of low-dose lipopolysaccharide in blood and BM,[8] and PPRs and HLA-DR expression on HSPCs in presenting exogenous and endogenous antigens;

(5) How to evaluate the long-term effects of the indiscriminate deletion of gut microbiota on the host immune system and metabolism, since gut microbiota is indispensable and crucial for educating and shaping the host immune system and for normal physical metabolism[6,7];

(6) How to evaluate and treat the genetic predisposition that influences the susceptibility to developing AAA and relapse;

(7) How to treat the mCTLeff cells to prevent rapid relapse[1,3];

(8) Which microbes and mechanisms drive the pathophysiology;

(9) How to select the optimal gut-modifying regimen and dietary.[18]

Conflicts of interest
None.

References

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