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Gut inflammation in the pathogenesis of acquired aplastic anemia

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Acquired aplastic anemia (AAA) is an auto-immune disease (AID) resulting from aberrant T-cell-mediated and antigen-driven immune responses to hematopoietic stem and progenitor cells (HSPCs) in genetically susceptible individuals, leading to the significantly enhanced suppression and apoptosis of HSPCs. 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 proinflammatory cells and the abnormally decreased immunoregulatory cells. Significantly up-regulated expression of human leukocyte antigen (HLA) and Fas molecules on $CD34^+$ HSPCs induces enhanced antigen-presenting activities and accelerated apoptosis.[1] The high efficiency of rapamycin in ameliorating bone marrow suppression (BMS) clearly indicates the abnormally activated mTOR (mammalian target of rapamycin) pathway and its contribution.

Currently, standard first-line immunosuppressive therapy (IST) is the main treatment. IST, based on a combination of anti-thymocyte globulin and cyclosporine A (CsA), produces approximately two-thirds of a response; the response rate is much lower in severe aplastic anemia (SAA). Patients who responded to this IST generally remain are dependent on sustained CsA treatments.[2]

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.[3] This manuscript is based on superficial knowledge in the treatment of an SAA patient[4] 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).

Human gastrointestinal (GI) tract not only provides the largest and most vulnerable interface that links 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.[5] In germ-free-reared mice, their universal reduction in the size of lymph nodes, number of macrophages, and immune competent cells strongly indicates an undeveloped immune system. Apart from providing additional nutrients, symbiotic microbes that co-evolve with the host have an in-dispensable and obligatory role in the development, and maturation of host lymphatic tissues, ensuring appropriate responses to potential pathogens and tolerance to commensal microbes and self-antigens.[6] Given that gut mucosa constantly exposes to the intestinally derived antigens, 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 favoring the development of local and systemic auto-immune reactions.

Compromised intestinal epithelium allows for the intestinally derived antigens to translocate into the lamina propria and blood (leaky gut), resulting in an increased opportunity for host immune cells to intimately come in

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contact with exogenous antigens.[7] Th17 cells activated by gut commensal microbes could act on remote organs, and thereby provoking auto-immunity.[8] Changes in the microbial composition and diversity due to changes in dietary protein sources could lead to changes in mTOR activity, fueling inflammatory reactions.[9] 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 regulatory T cell (Treg) dysfunction, cytotoxic T lymphocyte (CTL) activation, and impaired epithelial repair, which preferentially promotes type 1 immune responses.[10] Dysbiosis and GICs may serve as intensifiers, linking host immunogenetics with environmental challenges, to amplify the dysregulated auto-immune responses.[11] Given that genetically driven mice under germ-free conditions have gained their ability to protect themselves from developing AIDs, the gut microbiota definitely influences the auto-immune pathophysiology.[12] Dysbiosis and GICs are closely associated with almost all known AIDs.

The association between GICs and AAA has been documented in few reports. These GICs include inflammatory bowel disease (IBD), celiac disease, and neutropenic colitis (listed in Supplementary Table 1, http://links.lww.com/CM9/A254). In most IBD-associated AAA cases, the authors attributed AAA development directly to the adverse events of antibiotics, nonsteroidal anti-inflammatory drugs (NSAIDs) and immunosuppressants (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, as evidenced by the concomitant 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[13] 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 BMS (items 24–28 in Supplementary Table 1 http://links.lww.com/CM9/A254). However, the presence of bleeding polypoid lesions in the colon strongly indicates chronic gut inflammation proceeding the onset of severe leukocytopenia 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).

We have reported an SAA patient who gained an unexpected hematological response to treatment of gut inflammation.[4] 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 recurrences 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 this finding, 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, a patient with SAA 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 BMS. This investigation had to be terminated for our inability to identify patients who were highly predisposed to developing malignant proliferation after resolution of BMS. It provides direct evidence to support the in-dispensable role of GICs in BMS, whether "self-reactive CTL cells" target antigens of infectious agents or transformed malignant cells.

Although the precise mechanism underlying GICs in the development of AIDs remains unclear, AAA may be driven, at least in a significant proposition 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 CTLs*. In immune competent individuals, the activated immune system in response to pathogenic antigens would rapidly return to homeostasis after eliminating antigens. Chronically progressive BMS suggests a continuous supply of 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.[5,6] In the setting of GICs, the compromised epithelium allows for high-dose exogenous antigens that are cross-reactive with

self-antigens on HSPCs to intimately come in contact with host immune cells, which may breakdown the host immune homeostasis and lead to the activation of self-reactive CTLs that have low affinities in healthy conditions (molecular mimicry).[14] Endotoxins and other microbial metabolites can also enter into the blood and then be located in the bone marrow (BM), which, even at very low doses, may trigger active inflammation via pattern recognition receptors (PPRs) on antigen-presenting cells sensing endotoxins and other exogenous antigens.[7] This, in turn, activates CTLs and perpetuates inflammatory cycles, and thus suppresses autologous hematopoiesis (bystander activation).[15] Both mechanisms facilitate the creation of a "fertile field" to generate effector memory CTLs (mCTLeffs) cells in the chronic inflammatory circumstances.

(b) *Skewing host immune system toward type 1 responses*. Reduced production of SCFAs due to dysbiosis and insufficient indigestible polysaccharide supply leads to the skewed differentiation of CD4+Foxp3+ T cells into Th17 cells.[10] A decreased ratio of Treg/Th17 cells tip the balance from immunoregulatory to pro-inflammatory state, ultimately resulting in the over-activation of CTLs and the overproduction of type 1 cytokines, which is the characteristic immunological profile of the type 1 response in T-cell-mediated AIDs, including IBD, celiac disease, AAA, rheumatoid arthritis, multiple sclerosis, auto-immune hepatitis, and diabetes mellitus.

(c) *Amplifying the pro-inflammatory reactions*. Immunogenetics 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 pathogen-associated molecular patterns (PAMPs).[7] 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.[11]

(d) *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 in-dispensable. 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.[5] 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.

As discussed earlier, dysbiosis and GICs play in-dispensable roles in driving immune-mediated pathophysiology. Similar to other AIDs, modulation of gut microbiota and treatment of the GICs may open a novel avenue in etiological research and treatment options for AAA patients.[16] Before these treatments used in practice, several open questions have been raised and merit extensive investigation:

(1) How to evaluate the possible role of BMS in limiting GICs (items 10 and 11 in Supplementary Table 1), in inhibiting the pathogens that are able to infect and proliferate in BM and immune cells,[1] and in repressing malignant proliferation caused by genotoxic agents and intracellularly proliferated viruses[1,2,4];
(2) How to evaluate the presence of low-dose lipopolysaccharide in blood and BM,[7] and PPRs and HLA-DR expression on HSPCs in presenting exogenous and endogenous antigens;
(3) How to evaluate the long-term effects of the indiscriminate deletion of gut microbiota on the host immune system and metabolism[5,6];
(4) How to evaluate and treat the genetic predisposition that influences the susceptibility to developing AAA and relapse;
(5) How to treat the mCTLeff cells to prevent rapid relapse[1,2];
(6) Which microbes and mechanisms drive the pathophysiology;
(7) How to select the optimal gut-modifying regimen.[16]

Conflicts of interest

None.

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