Immune pathogenesis of autoimmune thrombocytopenic purpura

J. W. Semple
St. Michael’s Hospital and the University of Toronto, Toronto, Ontario

Abstract Chronic autoimmune thrombocytopenic purpura (AITP) is an immune-mediated disorder in which platelets are opsonized by autoantibodies and prematurely destroyed by phagocytic cells in the reticuloendothelial system. It is classed as an organ-specific autoimmune disease primarily mediated by immunoglobulin G (IgG) autoantibodies and its etiology appears to be similar to that observed for other organ-specific autoimmune diseases. Th1 cells are important in the process, and the costimulation of Th1 cells and B cells takes place in a cytokine milieu that is reminiscent of a proinflammatory process. Chronic AITP has classically been treated with nonspecific, immunosuppressive regimens (e.g., steroids). One of the most significant developments in the treatment of AITP in the last 20 years has been the use of intravenous immunoglobulin (IVIg) and anti-D preparations. These treatments confer benefit to patients with AITP by significantly raising platelet counts. Despite this, their exact mechanisms of action remain elusive. This review focuses on cell-mediated and cytokine abnormalities within AITP, and presents data related to the mechanism of action of anti-D. © 2002 Harcourt Publishers Ltd

KEY WORDS:

INTRODUCTION

Autoimmune thrombocytopenic purpura (AITP) is an immune-mediated disorder in which platelets are opsonized by autoantibodies and prematurely destroyed by phagocytic cells in the reticuloendothelial system. The thrombocytopenia seen in AITP is primarily the result of increased platelet clearance by the spleen and liver. While humoral abnormalities in AITP are well defined, it is increasingly apparent that T cells play a major role in the onset of AITP. Acute and chronic forms of the disease differ in that acute AITP is often preceded by an infectious illness and generally resolves spontaneously within a few weeks of initial presentation. The chronic form of the disorder, defined as persistence of thrombocytopenia for greater than 6 months, generally occurs in adults and is classed as an organ-specific autoimmune disease that is primarily mediated by IgG autoantibodies. The following review will focus on the etiology, pathophysiology, and therapies for chronic AITP.

AITP AND ORGAN-SPECIFIC AUTOIMMUNITY HAVE COMMON ETIOLOGIES

Since AITP and organ-specific autoimmune have common etiologies, AITP is discussed here in the general framework of organ-specific autoimmune. In principle, autoimmune disorders arise because of the failure to eliminate or deactivate self-reactive lymphocytes, which is reflected in a deficiency of central and/or peripheral tolerance induction mechanisms. The etiologic theories can be explained on the basis of molecular mimicry, determinant spreading, Th1/Th2 balance, and the cytokine milieu that triggers the onset of organ-specific autoimmune diseases and AITP, in particular. In all likelihood, it is a combination of these theories that will explain the pathophysiology of AITP.

MOLECULAR MIMICRY

The basis of molecular mimicry lies in the fact that host proteins may have similar antigenic determinants to those exhibited by microbial pathogens or viral agents. Consequently, in the course of infection, an immune response mounted against the invading pathogen produces antibodies that have the ability to cross-react with host tissues, leading to inflammation, tissue destruction, and autoimmunity. Often, it is difficult to establish the correlation between the triggering event such as a viral infection and the onset of autoimmunity because of the unusually long lag period that is required for the development of autoimmunity. Thus, viruses causing latent and persistent infections can permit chronic stimulation of autoimmune reactions. In this context, a biochemical analysis on blood samples of seven children with acute ITP following a Varicella Zoster (VZV) viral infection showed that the patients’ sera had anti-VZV antibodies that were reactive with normal blood-group O platelets. These antibodies did not cross-react with antibodies from other patients whose ITP progression was not preceded by a Varicella Zoster viral infection. Platelet clearance was also associated with the cross-reacting antibodies, which suggests that molecular mimicry mechanisms may be a likely reason for the onset of acute ITP.

DETERMINANT SPREADING

In the evolution of organ-specific autoimmune disease, there is an acquired recognition of new self-determinants, a process called determinant spreading. The induction of T-cell tolerance by negative selection in the thymus presumes that clonal deletion occurs when the antigenic determinants are present at a certain critical threshold. Self-determinants present at suboptimal concentrations escape from being detected and hence are not eliminated. These determinants often share cross-reactivity with cryptic epitopes present on foreign antigens. Thus, when cryptic epitopes are exposed in an immune response, T-cell activation sets up an inflammatory cascade that can lead to tissue damage in the target organ. Phagocytic events perpetuate and exacerbate the inflammatory response in mechanisms that include upregulation of costimulatory molecules, modulation of the antigenic processing pathways, and alteration of the cytokine profile in the target tissue.

Th1–Th2 BALANCE

The differentiation of naïve or primordial T cells (Th0) into Th1 or Th2 is dependent on the cytokine milieu and the antigen presentation pathways, which are present. The
Th1 and Th2 subgroups characteristically have a discrete pattern of cytokine secretion. For example, Th1 cells secrete interleukin-2 (IL-2) and interferon-gamma (IFN-γ), and can elicit delayed hypersensitive reactions, cell-mediated immune responses and the production of complement-fixing IgG isotypes. Th2 cells on the other hand produce IL-4, IL-5, IL-10, and IL-13, and are important in suppressing cell-mediated immune reactions and eliciting anaphylaxis reactions via the production of IgE. In general, the Th1 phenotype tends to promote the pathogenesis of organ-specific autoimmune diseases, while the Th2 phenotype may play a protective role in this event. As expected, the Th1 phenotype is prevalent in patients with AITP and any clinical decision that counteracts the Th1/Th2 balance could result in a reduced incidence or severity of disease. Thus, in patients with chronic AITP, CD4+ T-helper cells are stimulated to secrete IL-2 by normal platelet antigens, probably modulating their enhanced antiplatelet autoantibody response.

THE CYTOKINE MILIEU

When immune reactivity undergoes dysregulation, predisposed individuals develop a Th1/Th2 balance that favors the induction of organ-specific autoimmunity. As stated previously, the cytokine milieu dictates the differentiation of T cells into the Th1 or the Th2 phenotype. As expected, patients with AITP and other hematologic disorders have cytokine profiles that favor the Th1 phenotype. For example, before treatment, patients with hematologic disorders showed elevated levels of IL-2, IL-6, and IL-8. While levels of IL-10 did not change significantly after treatment, levels of IL-6 and IL-8 were significantly lower. Soluble IL-2 receptor (sIL-2R) and soluble vascular cell adhesion molecule-1 (sVCAM-1) levels were also significantly higher in patients with hematologic disorders as compared to platelet activation markers, CD62P, CD63, and PMP.

In a separate study, low platelet count was correlated to high sIL-2R levels in patients with ITP. Alterations in the Fas/FasL pathway may also be involved in the pathogenesis of ITP. When soluble Fas was present in patients, it correlated to an increase in IL-2 and sIL-2R levels. Additionally, the activation of Th cells (CD4+ and HLA-DR+ cells) and natural killer (NK) cells (CD16+ and CD56+ cells) were significantly higher in ITP patients who were soluble Fas-positive. IL-2, IL-6, IFN-γ, and macrophage colony-stimulating factor were also higher in ITP individuals compared to those in healthy individuals.

PATHOPHYSIOLOGY OF AITP

Chronic AITP manifests as persistence of thrombocytopenia for greater than 6 months and, as stated earlier, appears to be an organ-specific autoimmune disease, with autoantibodies enhancing platelet destruction. Chronic AITP is more truly an organ-specific autoimmune disease, with autoantibodies targeted against platelet glycoproteins (GP). The effector phase of chronic AITP occurs with the IgG opsonization of platelets and their Fc receptor-mediated destruction. The IgG antibodies are primarily IgG1 and IgG3, though IgM and IgA may also be present. Most of the major autoantibodies target platelet GP Ibb/Illa, Ib/Ix, and X, with antigen-specific assays identifying autoantibodies against either platelet GPIb/Illa or GPIb/IX in about 75% of patients with ITP. Anti-GPIb/Illa and anti-GPIb/IX antibodies are prevalent in equal frequency in patients with AITP and, thus, their presence may be indicative of active disease. These results were recently reviewed by McMillan.

The role of T cells in AITP has been under investigation for the past 30 years. The involvement of T cell-derived cytokines in the induction of platelet autoantibodies was first demonstrated by McMillan’s group. Platelet-induced, peripheral blood cell mononuclear cell proliferation has also been shown in patients with AITP. Moreover, lymphocytes of patients with AITP secrete significantly higher levels of IL-2 and other proinflammatory cytokines. Phenotypic analysis indicates that this response is due to CD4+ T cells. The cytokine pattern seen in patients with chronic ITP suggests an early CD4+ Th0 and Th1 cell activation. Taken together, these results indicate that in patients with AITP, the Th1 subpopulation that is activated secretes proinflammatory cytokines that modulate the enhanced antiplatelet autoantibody response.

The autologous mixed lymphocyte reaction is also markedly impaired in patients with chronic AITP. Addition-ally, peripheral blood cells of patients with chronic AITP show a high expression of HLA-DR, and platelets recovered from the spleen of patients with chronic AITP are HLA-DR+. Characterization of T-cell responses induced by platelet GP also indicates that the response is restricted by HLA-DR, the corresponding T cells having the CD4+ phenotype. It is conceivable, then, that the activated macrophages in the spleen undergo improper phagocytosis, and in the process transfer the DR molecules onto the platelet surface. Elevated levels of T-cell receptor CD3+ T cells have been observed in children with chronic AITP and the levels correlated to the degree of thrombocytopenia in each patient. Thus, T-cell receptor CD3+ T cells may be important in the pathogenesis of AITP. The first platelet reactive T cell clones from children with chronic ITP were isolated in 1993. These types of clones have been shown to cross-react with purified GPIbα (JWS, unpublished). The GPIbIIa T cell lines were found to be predominantly CD4+ and secreted high levels of IL-2, IL-15 and IFN-γ. Moreover, these GP reactive T cells had a strict requirement for adherent macrophages in order to be stimulated. Recently, Kurwana et al. have identified epitopes within the GPIbα and GPIIbα molecules for T cells derived from patients with AITP. Although the minimal HLA-DR binding motifs were not identified, these results suggest the exciting possibility that peptide-specific therapies for chronic AITP may be feasible.

Platelet autoantigens from either the platelets or an infectious agent are processed by antigen-presenting cells within the spleen in the context of major histocompatibility complex class II molecules on T-helper cells. Co-signaling pathways are established such that the T-helper cells besides becoming activated also activate autoreactive B cells that differentiate into plasma cells secreting autoantibodies. In the context of these cellular interactions, what is of paramount importance is the
cytokine profile within this milieu, which is expected to be rich in proinflammatory cytokines such as IL-2, IL-10, IFN-γ, GM-CSF, TNF-α, and TGF-β that promote the antigen-presenting cell/T cell and T helper cell/B cell interactions.

THERAPEUTIC APPROACHES IN AITP

Since autoantibodies in AITP are responsible for enhanced Fc-mediated platelet destruction by macrophages in the reticuloendothelial system, most of the therapeutic approaches in the past have concentrated on abrogating this effect. However, it is increasingly evident that enhanced interactions between T helper cells and antigen-presenting cells are the primary stimuli for the development of antiplatelet antibodies. Hence, current approaches target the cellular interactions that are responsible for the generation of the antiplatelet antibodies. The use of general immunosuppressants, such as corticosteroids, vinca alkaloids, danazol, cyclophosphamide, and cyclosporin, gave way to the use of IVIg and anti-D therapy in the 1980s. Recent developments in the treatment of chronic AITP are directed specifically to B cells or T cells. The use of anti-CD20 antibody targets B cells while T cell-directed approaches include the use of anti-CD40L, the induction of oral tolerance, antibody targets B cells while T cell-directed approaches include the use of anti-CD40L, the induction of oral tolerance, and antigen-specific therapy.

Infusion of large amounts of these IVIg and anti-D reverses platelet counts in patients with AITP within hours of treatment. The exact mechanisms to explain the benefits of these therapeutics remain to be elucidated. IVIg and anti-D exert short-term effects and long-term effects in increasing platelet counts. With respect to IVIg, the short-term effects are largely believed to occur via an FcR blockade or inhibition while the long-term effects may be explained by the presence of anti-idiotypic antibodies in IVIg preparations that are directed against idiotypes located on GPIIb/IIIa autoantibodies. While FcR blockade has been invoked to explain the short-term effects, the mechanism by which anti-D exerts a long-term effect is yet to be elucidated. To address this we treated seven children with chronic AITP with anti-D. Post-treatment blood samples were obtained at various times. The FcR blockade mechanism was evident in that there was a general reduction in phagocytosis in terms of a per cell basis and in the number of cells that are recruited for the process. In terms of serum cytokine levels, however, as little as 3 hours after anti-D infusion there is a massive burst of proinflammatory and anti-inflammatory cytokines, which lasted for at least 24 hours. The levels declined to baseline levels by day 8 post anti-D infusion. This effect was correlated with a reduction in phagocytosis and an increase in platelet count.

In summary, chronic AITP is associated with T cell activation patterns resembling a proinflammatory Th0/Th1 cytokine phenotype. Platelet-reactive T cells targeted against platelet GPIIb/IIIa can be generated from the peripheral blood and spleen, suggesting that costimulation of T helper cells and autoreactive B cells is involved in the generation of platelet autoantibodies. While IVIg and anti-D preparations have been a mainstay of therapy, newer approaches involve modulating B cells or T cells via cytokines or altered autoantigens. It thus appears that we are at the beginning of a new era of treatment for this often difficult-to-treat autoimmune disease.

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Author Queries

Pp 5, 6 - insert missing Greek letters (boxes on MS and in electronic file). Total of 11 instances.

Keywords to be supplied.