Cellular Immune Mechanisms in Autoimmune Thrombocytopenic Purpura: An Update

Malini D. Coopamah, M. Bernadette Garvey, John Freedman, and John W. Semple

Autoimmune thrombocytopenic purpura (AITP) is a bleeding disorder in which autoantibodies are directed against an individual's own platelets, leading to enhanced clearance through Fc receptor (R)-mediated phagocytosis by macrophages residing in the reticuloendothelial system (RES), particularly in the spleen. The production of IgG autoantibodies is critically dependent on cellular immune mechanisms particularly relating to T cells. We review the recent literature of the cell-mediated immunology of AITP focusing on platelet phenotype, genetics, T-cell reactivities, and cytokine profiles in patients with AITP. Understanding the interaction between these cell-mediated mechanisms is vital for developing antigen specific immunotherapies to treat this autoimmune disease.

IT HAS BEEN 10 years since we first reviewed the literature relating to the cellular immune pathophysiology of AITP.[1] At that time, little work had been done to address the issue of how IgG autoantibodies are actually produced against platelet autoantigens. By 1995, data from several research groups began to suggest that T-cell abnormalities in chronic AITP are most likely responsible for the stimulation of autoantibodies.[2] Since then, an expanding body of research has shown that autoreactive T cells exist in patients with AITP and that these T cells direct B cells to differentiate and secrete IgG antiplatelet autoantibodies.

NORMAL IMMUNITY AND AUTOIMMUNITY

The initiation of a humoral immune response to a foreign antigen is a complex biological association of many cell types and their secreted products. The response occurs when an antigen, such as a cell surface glycoprotein, first interacts with an antigen-presenting cell (APC).[3] APCs are generally major histocompatibility complex (MHC) class II–positive macrophages or dendritic cells and, in certain instances, B cells.[3] After internalization by the APC, the antigen is processed into smaller antigenic fragments by proteolytic degradation.[3] The antigenic peptides are then transported to the cell membrane of the APC, where they are re-expressed in conjunction with MHC-encoded class II molecules for presentation to antigen-specific T-helper (Th) lymphocytes.[3] When the MHC-peptide complexes are recognized with sufficient affinity by T-cell receptors (TcR) on a CD4+ Th cell, antigen-specific signal 1 is met and initiates a set of coordinated molecular events in both the APC and T cell that culminate as signal 2 (costimulation) and full T-cell activation (eg, CD40 upregulation of B7 molecules on the APCs surface for interaction with CD28 molecules on the T cell).[4] These T-cell/APC events can stimulate antigen-primed B cells to differentiate into plasma cells and secrete antigen-specific IgG antibodies.[5] Thus, any defect in, or abnormal stimulation of, antigen-specific Th cells can significantly alter the course of an immune response. This underscores the vital importance of Th cells in determining whether antibodies will be generated against a foreign antigen and once generated, regulate the response by stimulating events such as antibody affinity maturation, regulatory T cells, and/or secretion of soluble cytokines.

The importance of the CD40L/CD40 costimulation pathway for T- and B-cell cooperation pathway has been clearly established in animal models and man.[6] This pathway is essential for human Th-cell function and immunoglobulin class switching.[6] Defects in this pathway lead to severe immunodeficiency, characterized by hypogammaglobulinemia.[6] In addition, there is compelling evidence suggesting that the CD40L/CD40 pathway also affects initial Th-cell activation and is important for the cellular regulation of immunity.[6] Perhaps,

From the Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital, Departments of Pharmacology, Medicine and Laboratory Medicine and Pathobiology, University of Toronto, Canadian Blood Services and the Toronto Platelet Immunobiology Group, Toronto, Ontario Canada.

Address reprint requests to John W. Semple, PhD, Department of Laboratory Medicine and Pathobiology, St. Michael's Hospital, 30 Bond St, Toronto, ON, Canada, M5B 1W8. E-mail: semplej@smh.toronto.on.ca.

0887-7963/03/1701-0005$35.00/0

doi:10.1053/tmrv.2003.50004

more importantly, it has been shown that blockade of CD40L/CD40 interactions by antibody can be toleragenic.[6] These observations have been the rationale for developing clinical trials using anti-CD40L antibodies in patients with different autoimmune diseases (eg, systemic lupus erythematosus, autoimmune thrombocytopenic purpura [AITP], and so on).

Once Th cells become activated, their responses can be generally distinguished by their secreted cytokine products.[7,8] A Th1 response is characterized primarily by the presence of interleukin (IL)-2, interferon γ (IFN-γ), granulocyte macrophage colony-stimulating factor (GM-CSF), and tumor necrosis factor α (TNF-α) and is associated with delayed type hypersensitivity reactions and the synthesis of primarily complement-fixing IgG isotypes.[7,8] Th2 responses on the other hand produce IL-4, IL-5, IL-6, IL-10, and IL-13 and are superior in mediating noncomplement fixing IgG and particularly IgE synthesis.[7,8] A third type of Th response, Th0, is thought to be generated by cells less differentiated than those mediating Th1 and Th2 responses because many or all of the Th1/Th2 cytokines are present.[9,10] These cytokine responses are thought to be the result of the coordinated efforts between APC and Th cells and ultimately responsible for the particular outcome of an immune response (eg, generating a particular isotype of antibody for effectively removing the initial antigenic insult).

Normally, a host does not mount an immune response against its own antigens; it behaves as if it is immunologically ignorant or tolerant. Immunological tolerance is the acquisition of unresponsiveness to self-antigens and is essential for the preservation of the host.[11] Two major theories postulate that T-cell tolerance can be induced by either clonal deletion of high-affinity, self-reactive T cells within the thymus (ie, central tolerance[12]) or by autoreactive T cells being deleted or rendered anergic by specific and nonspecific mechanisms in the extrathymic milieu (ie, peripheral tolerance) (Fig 1).[13] The function of central tolerance is to remove the majority of high-affinity, self-reactive T-cell clones by positive and negative selection mechanisms before allowing them to be released into the periphery. However, this process is not totally efficient, and some T cells released into the periphery still have the potential to induce autoimmunity and need to be constantly suppressed by peripherally based mechanisms.[14] Several peripheral tolerance mechanisms have been described that could potentially suppress autoreactive T and B cells from becoming activated. These include self-antigen sequestration, cytokine suppression, regulatory cells (eg, CD8+ T cells, veto cells, and so on) and/or antiidiotypic antibody suppression.[14-16]

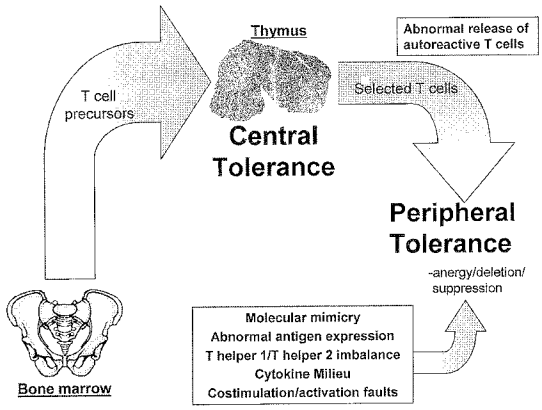

Fig 1. Central and peripheral tolerance mechanisms. Bone marrow precursor cells migrate to the thymus where they undergo a series of negative and positive selection events in order to be screened for anti-self MHC affinity. Only those T cells displaying low to moderate affinity for self-MHC and peptide will be released into the periphery. Because of incomplete selection in the thymus, some peripheral T cells can become autoreactive and thus need to be constantly suppressed by peripheral tolerance mechanisms such as anergy induction, deletion, and/or suppression (eg, by cytokines). Autoimmune disease can result when one or more of these tolerance mechanisms are broken by such events as those shown in the boxes. For example, central tolerance selection in the thymus may be faulty and allow the release of high affinity autoreactive T cells or an environmental agent (eg, a virus) can mimic a self antigen leading to the breakdown of peripheral tolerance mechanisms.

Autoimmune diseases result from the failure of normal self-tolerance mechanisms, and, collectively, they affect approximately 5% to 7% of the population, often with debilitating effects.[16] The breakdown of self-tolerance may be the result of a number of nonmutually exclusive mechanisms such as a failure of central tolerance leading to the abnormal accumulation of self-reactive T cells, environmental stimuli that can mimic self antigens (antigenic mimicry), aberrant self-antigen expression, cytokine secretion, and/or defects in costimulation (Fig 1). Organ-specific autoimmune diseases are directed primarily at particular tissues (eg, insulin producing β cells in type I diabetes, thyroid cells in Graves disease, or platelets in AITP).[17,18] The production of autoantibodies in organ-specific

autoimmunity is dependent on T-cell activation. For many autoimmune diseases, the precise specificity and cytokine profiles of autoreactive T cells have been described, although the actual disease-inducing autoantigens and etiology of the abnormal T-cell activation remain elusive.[18,19] Of interest, organ-specific autoimmune diseases are generally associated with skewed cytokine patterns. Most active organ-specific autoimmunity tends to be associated with proinflammatory Th1 responses, whereas Th2 immune responses are generally associated with autoimmune protection. Based on these observations, a potential immunotherapy for autoimmunity is to shift the cytokine patterns from Th1 to Th2. This approach has been successful in many experimental models of autoimmune diseases.[11,19]

AUTOIMMUNE THROMBOCYTOPENIC PURPURA

AITP is characterized by the production of autoreactive antibodies against one's own platelets, resulting in increased platelet destruction by RES phagocytes. Several excellent papers have been published that review the autoantibody literature related to AITP.[20-23] The majority of these autoantibodies are IgG, but IgM and IgA can also be identified in some patients with AITP.[23] The prevalence of AITP has been estimated at 1 per 10,000.[24]

Both acute and chronic forms of AITP can be distinguished. Acute AITP primarily affects children and often occurs after a viral or bacterial infection.[25] It usually spontaneously resolves within 6 months of diagnosis, but in approximately 20% of children, the disease progresses to the chronic form, defined as persistence of thrombocytopenia (platelet counts $< 150 \times 10^9$/L for more than 6 months).[25] In contrast to the acute form, chronic AITP is predominant in adults, with more women being affected than men.[25] Although both acute and chronic AITP are immune mediated, it is becoming clear that the immune pathophysiologic mechanisms are different in the 2 disorders.

Acute AITP

Little research has focused on the cellular immunology of acute AITP in children. However, because this form of the disease often follows an infectious event, it suggests that acute AITP may be associated with the immune mechanisms stimulated by the preceding infection. In 1997, Wright et al[26] showed that acute AITP might be the result of tolerance breakdown because of antigenic mimicry. Antigenic mimicry is perhaps one of the oldest and most prevalent theories of tolerance breakdown resulting in autoimmunity.[27] It is caused by molecular structures on infectious or environmental agents that have similarities to host antigenic structures and stimulate an immune response that then cross-reacts with host antigens.[27] In children with acute AITP associated with Varicella Zoster virus infection, Wright et al[26] showed that the patient's serum IgM and IgG anti-platelet autoantibodies could be purified on affinity columns conjugated with Varicella Zoster virus glycoproteins and that the eluted IgG molecules were cross-reactive with normal group O–positive platelets. Analogous findings were found with HIV glycoproteins and sera from patients with immune thrombocytopenia associated with HIV infection.[28] At the T-cell level, it was found that antiplatelet reactive T-cell activity in children with acute AITP was no different than what was observed in healthy individuals, which suggests that T cells in this disorder are not necessarily directed at platelet antigens.[29] Together, these results support the hypothesis that at least in some patients with acute AITP, antiplatelet antibodies are because of the antiviral antibodies cross-reacting with the patient's platelets. This may explain why many children with acute AITP spontaneously go into remission without therapy (eg, as the infectious agent is cleared and antibodies either disappear or affinity mature toward the infectious agent, the antiplatelet reactivity is lost). Still unresolved is why approximately 20% of children initially diagnosed with acute AITP go on to develop the chronic form of the disease and whether these patients can be identified early. Perhaps in those patients, immune dysregulation has taken place during the infection that allowed the cross-reactive IgG antiplatelet B cells to propagate and become chronically focused toward platelet autoantigens.

Chronic AITP

Chronic AITP appears to be a true organ-specific autoimmune disorder. It rarely remits spontaneously; there is usually no previous history of an infectious event and almost always requires some form of immunosuppressive therapy.[25] The anti-

body specificities are directed against several platelet glycoprotein epitopes.[20-23] Since 1995, many investigators have shown that the chronic form of the disorder is associated with T-cell–related and cytokine abnormalities, which appear to be responsible for the production of IgG antiplatelet autoantibodies.

ABNORMAL ANTIGENIC PHENOTYPE OF THE TARGETED TISSUE

One possible cause of autoimmunity is that the immune-targeted tissue abnormally expresses self-antigens that are then recognized by autoreactive Th cells. For example, the induced expression of some HLA class II glycoproteins in cells that do not normally express them is known to play a significant role in autoimmune diseases.[16] This defective expression of the HLA class II molecules at the cell surface may influence the presentation of self-antigens to normally quiescent autoreactive Th cells that, if activated, can affect autoantibody production. In 1992, Boshkov et al[30] reported the appearance of class II HLA-DR on a child's platelets during the acute thrombocytopenic period. On recovery, these molecules were no longer expressed, suggesting a link with HLA and the pathogenesis of AITP in this patient. We subsequently showed, using flow cytometric techniques, that in immune thrombocytopenia, HLA-DR antigens were expressed on platelets and microparticles and that the percentage of HLA-DR+ platelets was inversely proportional to the platelet count.[29] It was found that physical contact with macrophages could induce platelet HLA-DR surface expression, an effect further enhanced by preactivating the macrophages with IFN-γ.[31] It is possible that events that activate macrophages in vivo (eg, infections or IFN) and increase their HLA-DR expression may facilitate transfer of HLA-DR molecules to platelets during, for example, normal platelet senescence and destruction. This possibility is supported by the observation that the HLA-DR–positive platelets in AITP also expressed CD45, CD14, and CD80 markers.[29] The clinical significance of HLA-DR+ platelets in AITP is unknown; however, if HLA-DR–positive platelet-macrophage microparticles are released in vivo, they could influence a normally quiescent Th repertoire by providing an antigen-specific signal 1, which may be propagated by helper signals from an inflammatory event. On the other hand, if costimulation requirements are not met, HLA-DR+ platelet microparticles could potentially suppress Th cells.

In 1998, Henn et al[32] showed that activated platelets express the CD40 ligand (CD40L, CD154) and suggested that these platelets may initiate an inflammatory response of the vessel wall. CD40L is structurally related to TNF-α and was originally identified on stimulated CD4+ T cells; its interaction with CD40 on B cells is of critical importance for the development and function of the humoral immune system.[6] The potential role of CD40L on platelets in AITP is unknown; however, if this molecules were expressed with HLA-DR, autoreactive Th cell activity may be affected.

Increased circulating CD68-positive platelet microparticles have also been identified in patients with chronic AITP.[33] CD68 is a 110-kd lysosomal glycoprotein thought to be involved in endocytosis and internal membrane trafficking and is restricted to macrophages; its surface expression on macrophages is significantly enhanced by inflammatory events.[34] The expression of this molecule in patients with chronic AITP underscores the importance of inflammatory mechanisms linked to platelet phagocytosis and potential APC autoantigen presentation events in this disease.

HLA ASSOCIATIONS AND GENETIC STUDIES IN CHRONIC AITP

Expression of certain HLA molecules has been shown to be associated with autoimmune diseases and may predispose the host to autoimmunity.[16] This predisposition may be because, in part, of how polymorphic HLA molecules present antigens to autoreactive T-helper cells. For certain autoimmune diseases, it has been possible to identify short stretches of amino acids within the polymorphic regions of HLA molecules, which appear to play a major role in disease susceptibility and/or resistance.[35] Early studies in white patients showed that chronic AITP may be associated with HLA-DR2[36] and also class I HLA molecules (HLA A28, B8, and B12)[37-39]; others, however, showed little or no HLA association with AITP.[40] Furthermore, Gaiger et al[41] reported a lack of association between HLA expression and AITP. They did, however, note a slight increase in the HLADPB1*1501 allele in patients with detectable antiplatelet autoantibodies and HLADPB1*0402 allele in patients

who responded poorly to splenectomy. These inconsistencies may be because, in part, at least, of the fact that for a given population of patients with a diagnosis of chronic AITP, a differing autoimmune etiology may exist that will affect genetic marker studies. Further in-depth studies with large patient populations will be needed to ascertain which HLA molecules (if any) may be associated with chronic AITP in a white population.

Recently, 2 Japanese groups examining HLA serotypes and alleles have also generated somewhat controversial data. Nomura et al,[42] using HLA serotype and PCR-RFLP analysis, found that patients with chronic AITP had a high frequency of HLA-DR4.1 expression associated with the DRB1*0410 allele. Anti-GPIIbIIIa autoantibody production, however, was associated with only HLA-DR4.1 expression but not the HLADRB1*0410 allele.[42] The authors cautioned that the finding should be considered preliminary because of the possible racial differences between HLA status in the Japanese patients and other patients with AITP.[42] Conversely, Kuwana et al[43] recently showed a direct correlation between autoantibody production and HLA class II genes in Japanese patients with chronic AITP. For instance, the presence of HLADRB1*0405 and HLADQB1*0401 was associated with anti-GPIIbIIIa antibody formation. However, with the exception of a modest increase in the frequency of HLADPB1*201, there was no significant difference, relative to the control, with HLA-DRB and HLA-DQB1. The authors concluded that HLA class II genes affect autoantibody generation rather than development of the disease itself. Given the distinctive HLA diversity across the major ethnic groups,[44] differences in disease associations between HLA genes and phenotypes with AITP might be expected. For example, within the white population, the distribution of HLA alleles is more heterogeneous than in the Japanese population, and thus HLA association studies may be more difficult to show.

In other genetic studies of patients with AITP, a comparison of cytokine and low-affinity Fc gamma receptor polymorphisms revealed an association for the proinflammatory cytokines TNF-α and lymphotoxin A with FcγR3A and Fc$\gamma\gamma$R3B.[45] The authors suggested that variant genotypes of FcγRs and cytokines might contribute to the pathogenesis of chronic AITP. However, it remains to be determined whether these associations are important to the initiation of the disease or whether they may aggravate the established autoimmune response.

T-CELL REACTIVITIES IN CHRONIC AITP

In 1991, we reported the novel finding that chronic AITP was associated with a CD4+ T-helper cell defect in which peripheral blood T cells could secrete IL-2 on stimulation with autologous platelets.[46] It suggested that chronic AITP may be the result of an abnormal Th cell defect that could direct autoreactive B cells to differentiate and secrete IgG autoantibodies. These IL-2 results were subsequently confirmed by Ware et al.[47] In 1996, Filion et al[48] reported that normal individuals contained anergic Th cells that could be activated in vitro after incubation with glycoprotein (GP) IIbIIIa and exogenous; once this tolerance was broken; however, the Th cells could secrete their own IL-2 on stimulation with GPIIbIIIa. These results suggested that T-cell tolerance against platelet autoantigens may involve the posttranscriptional regulation of IL-2 expression. Subsequently, Shimomura et al[49] showed that, in the peripheral blood of patients with chronic AITP, there was an oligoclonal accumulation of CD4+ Th cells that frequently used Vβ3, 6, 10, and 13.1 to 14 genes for their T-cell receptors. The authors concluded that distinctive T-cell clones accumulated in patients with chronic AITP and were related to the disease pathogenesis. Taken together, these results suggest that patients with chronic AITP have CD4+ Th cells that may have expanded because of a breakdown in tolerance mechanisms. However, their fine antigen specificities or their ability to stimulate autoreactive immunoglobulin production were not yet determined.

In 1998, Kuwana et al[50] showed that T cells from Japanese patients with chronic AITP could proliferate in vitro to disulfide-reduced GPIIbIIIa or the molecule's tryptic peptides. This suggested, for the first time, that autoreactive CD4+ Th cells in chronic AITP need to recognize a modified GPIIbIIIa molecule, implying that antigen processing mechanisms within recipient APC may be required to present GPIIbIIIa autoantigens in the context of self HLA-DR molecules. More importantly, however, Kuwana et al[50] showed that the modified GPIIbIIIa proteins could also induce autoantibody formation in vitro, supporting a link between the Th-cell reactivity and B-cell stimulation. Subsequently, the same investigators mapped

the antigen specificity of the GPIIbIIIa-reactive Th cells by using 6 recombinant fragments encoding different portions of the GPIIbα and GPIIIa chains.[51] They showed that the T cells frequently recognized the amino terminal portion of the 2 GP chains (GPIIbα 18-259 and GPIIIa22-262) and that these molecules also stimulated the production of antiplatelet autoantibodies.[51] Because no Th-cell reactivity against other portions of the 2 GP molecules was observed, it was concluded that the amino terminal portion of GP IIbIIIa was primarily responsible for the stimulation of autoreactive Th cells and subsequent autoantibody production.[51] In contrast, recent work examining the fine specificity of Th-cell clones from white patients with chronic AITP has revealed that at least 1 minimal peptide corresponding to amino acid residues 496 to 510 of the GPIIIa molecule is sufficient to stimulate T-cell IL-2 secretion.[52] These apparent contrasting results may reflect the heterogeneity of T-cell responsiveness between racial populations but, perhaps more importantly, suggest that T-cell epitopes can be found throughout the GPIIbIIIa molecule. Furthermore, these results support the concept that CD4+ Th cell activation is the primary defect that leads to autoantibody production in patients with chronic AITP. This latter possibility is supported by the recent observations suggesting that the spleen is the primary site of activation of platelet-reactive T and B cells in patients with chronic AITP.[53] These new findings may set the foundations for designing antigen-specific therapies targeted at autoreactive T cells (eg, modified and suppressive MHC binding peptides for inhibition of Th cells).

THE CYTOKINE PROFILE OF CHRONIC AITP

Autoimmune diseases have often been shown to be associated with cytokine abnormalities.[8] Several cytokine abnormalities have been found to be associated with chronic AITP. With respect to Th1 and Th2 cytokines, it was originally showed that chronic AITP in children was associated with an early CD4+ Th0 cytokine pattern that tended toward a Th1-activation pattern.[29] Increased serum levels of IL-2, IFN-γ, and IL-10 could be found, but no IL-4 was detected in the patients with chronic AITP.[29] Several subsequent studies have confirmed that Th0- and particularly Th1-associated cytokines are abnormally distributed in chronic AITP. Garcia-Suarez et al[54] showed that CD2+ lymphocytes from patients with chronic AITP secreted elevated levels of IFN-γ and TNF-α on stimulation with the T-cell mitogen PHA. They concluded that patients with severe thrombocytopenia had a functional switch to Th1 reactivity.[54] Abboud et al[55] subsequently showed that 8 of 10 children with chronic AITP had significantly elevated levels of GM-CSF in their serum. GM-CSF is also marker of Th1 cells and may play a role in the response to severe thrombocytopenia in some patients with chronic AITP. Subsequently, Lazarus et al[56] found elevated levels of serum IL-15, a cytokine that has properties similar to IL-2. Yoshimura et al[57] also found increased levels of IL-2, IFN-γ, and M-CSF levels in patients with chronic AITP. Of interest, this group found higher levels of soluble Fas and FasL and suggested that these apoptosis-related molecules may have a role to play in disease pathogenesis.[57] The abnormal Fas and FasL levels in patients with chronic AITP were recently confirmed by Shenoy et al[58]; they concluded that altered Fas pathway signaling with or without defective IL-2 secretion should be considered in the etiology of hematologic autoimmunity. More recently, Mouzaki et al[59] performed a molecular Th-cytokine analysis in children with acute and chronic AITP and found that the patients with acute AITP presented with a Th0-cytokine profile, whereas those patients with chronic AITP had a skewed Th1-cytokine response. More interesting, those patients in stable remission or treated with intravenous immunoglobin presented with a polarized Th2-cytokine response.[59] Related to these results, Andersson et al,[60] using DNA microarrays and qRT-PCR (Taqman) technology, found that several T-cell genes were upregulated and that IFN-γ was significantly increased in patients with active AITP (H. Wadenvik, Sahlgrenska Hospital, Gothenburg, Sweden, oral communication, 2001).[60] These results suggest that patients with active disease may have a Th1-cytokine activation pattern and, when in remission, the cytokines are skewed to a Th2 pattern. It is therefore possible that manipulating Th-cytokine responses in patients with chronic AITP may be an effective form of immunotherapy.

In contrast to some of the previously mentioned results; however, 2 groups have found that active chronic AITP may be associated with a Th0- to Th2-cytokine pattern. Crossley et al[61] found that patients with chronic AITP had peripheral blood mononuclear cells (PBMCs) that on stimulation

with PHA in vitro, secreted low levels of IL-2 and IFN-γ, whereas some of the patient's PBMCs secreted high levels of IL-10. When the patients were treated with INF-α; however, their PHA-stimulated PBMC increased IL-2 and IFN-γ secretion and decreased the levels of IL-10.[61] The authors concluded that INF-α therapy increased Th1-cytokine levels and reduced autoantibody production.[61] Furthermore, Webber et al[62] showed that plasma levels of IL-4 were significantly higher in patients with chronic AITP, whereas IFN-γ levels were not different than controls. The authors suggested that IL-4 could be a potential target for therapy in chronic AITP.[63] However, it is difficult to determine whether the cytokine profiles in this study may have been related to therapy. On balance, however, the predominant overall view of Th-cytokine patterns in chronic AITP appears to be that the disease is associated with an early cytokine response (eg, Th0 profiles, whereas active severe disease is skewed toward Th1 activity and remission is associated with a Th2-cytokine pattern).

Other cytokines not classically associated with the Th1/Th2 paradigm have also been shown to be abnormal in chronic AITP and may have a role to play in the development of autoantibodies. During an immune response, the IL-2 receptor can be released from T cells, and its soluble form is thought to control the levels of free IL-2 and, ultimately, the immune response. Three reports have indicated that in patients with active chronic AITP, the levels of soluble IL-2 receptor were significantly elevated.[57,63,64] These results suggest that perhaps during active disease, increased IL-2 production leads to the release of the IL-2 receptor, which may play a role in controlling the autoimmune response by binding and inactivating free IL-2. Alternatively, Andersson et al[65] found that patients with chronic AITP in remission had significantly elevated levels of transforming growth factor β (TGF-β), and this was recently confirmed by Mouzaki et al.[59] TGF-$\beta\beta$ is a potent immunosuppressive cytokine and is thought to mark a T-cell subset now termed Th3 cells.[66] Andersson et al[65] suggested that this cytokine may be part of bystander immunosuppression in patients with AITP in remission. They further suggested that elevating the in vivo levels of TGF-β by oral tolerance induction may be a viable immunotherapeutic approach.[65] Interestingly, in a case report of a patient with Evan's syndrome, Karakantza et al[67] showed that this disorder was associated with a Th0/Th1-cytokine pattern and that splenectomy significantly reduced the Th-cytokine profile and significantly increased plasma levels of TGF-β. These 2 reports suggest that TGF-β may be a potential target cytokine to aid in the suppression of the autoimmune response in chronic AITP.

In an early study, the cytokine M-CSF was found to be increased in chronic AITP.[68] Subsequently, Nomura et al[69] showed that M-CSF levels were decreased in patients with chronic AITP treated with intravenous immunoglobin. Furthermore, Baker and Levin[70] showed that M-CSF could produce a dose-dependent thrombocytopenia when infused into mice; it was found that the M-CSF effects were not due to a reduction in thrombopoiesis but rather because of increased phagocytic activity of the monocyte/macrophage system. Perhaps abnormal M-CSF production in patients with chronic AITP enhances the uptake and presentation of platelet autoantigens by macrophages to Th cells, and this may aggravate the disease.

The mechanisms that lead to abnormal cytokine synthesis in chronic AITP are unknown. One possibility for the abnormal T-cell activation seen in patients with chronic AITP may be because of defects in transmembrane signaling subsequent to T-cell receptor engagement. Lazarus et al[71] showed that T cells from patients with chronic AITP may have a defect in their ability to phosphorylate various tyrosine residues on stimulation with the T-cell mitogen phytohemagglutinin. The authors suggested that defective T-cell signaling may be responsible for inducing altered T-cell function and cytokine production.[71] Alternatively, there is a growing body of evidence to suggest that activated platelets, either via P-selectin or β_3 integrin interactions, can stimulate the release of cytokines (eg, IL-1α and TNF-α) from mononuclear cells[72] and that platelets themselves can release IL-1β from their granules.[73] These types of interactions may promote an inflammatory response that could potentially support autoimmune mechanisms.

Table 1 summarizes the cellular immune defects in AITP, whereas Figure 2 outlines the potential stimulatory and suppressive immune pathways that may control the production of anti-platelet autoantibodies in chronic AITP.

Table 1. Summary of Defects Related to Cellular Immunity in AITP

Parameter	Response	Acute AITP (Ref No.)	Chronic AITP (Ref No.)
Evidence of antigenic mimicry		26, 28	
Platelet phenotype			
HLA-DR	Increased	29, 30	29, 30
CD68	Increased		33
CD40L	Unknown		
HLA associations			
With disease			
No association			40, 41
HLA-DR2			36
HLA-A28,B8,B12			37-39
HLADRB1*0410			42
With autoantibody formation			
HLA-DR4.1			42
HLADPB1*1501			41
HLADRB1*0401			43
HLADQB1*0405			43
T-cell reactivity/defects			
Platelet-stimulated IL-2 production	Increased		46, 47
T-cell GPIIbIIIa reactivity	Increased		48
Oligoclonal expansion	Increased		49
Reduced GPIIbIIIa and tryptic peptide reactivity	Increased		50
Help for autoantibodies	Increased		50, 53
GPIIbα18-259 reactivity	Increased		51
GPIIIa22-262 reactivity	Increased		51
GPIIIa496-510 reactivity	Increased		52
Fas/FasL levels	Increased		57, 58
Signaling defects	Increased		71
Cytokine defects			
Th0 profile	Increased	29, 59	29, 59
Th1 profile (active disease)	Increased		29, 52, 54-60
Th2 profile (active disease)	Increased		61, 62
Th2 profile (disease remission)	Increased		59
sIL2R	Increased		57, 63, 64
TGF-β (disease remission)	Increased		59, 65
M-CSF	Increased		68, 69

THERAPEUTICS RELATED TO THE CELLULAR IMMUNOLOGY OF AITP

Currently, the major chemotherapeutic treatment modalities aimed at increasing the platelet counts in patients with autoimmune disease are antigen nonspecific immunosuppressive therapies (eg, steroids, intravenous immunoglobin, and cyclophosphamide).[25] These therapies have their limitations because of side effects, incomplete responses, and generalized immunosuppression. With respect to autoimmunity, a therapeutic goal has been to develop antigen-specific therapies that target only the offending autoreactive T or B cells while leaving the rest of the immune system relatively intact. Several of these types of therapies have been examined, particularly within animal models and some clinical trials.[6,11,16,17] They include identification and modification of the offending autoantigen to suppress T-cell activation (eg, antigen-specific therapy targeting MHC–T-cell receptor (TcR) interactions), antibody-mediated disruption of MHC-TcR interactions, costimulatory blockade of activated autoreactive T cells, oral tolerance induction, and antibody-mediated blockade of costimulation or deletion of autoreactive B cells.

The recognition of cellular immune defects in AITP has lead to the use of therapies directed at T and B cells. George et al[74] tested a humanized monoclonal against CD40L (hu5c8, Antova Biogen, Cambridge, MA) to treat 23 patients with chronic AITP in a phase 1 dose escalation safety trial and found that 2 of 6 patients randomized to a 10 mg/kg dose had a substantial rise in their plate-

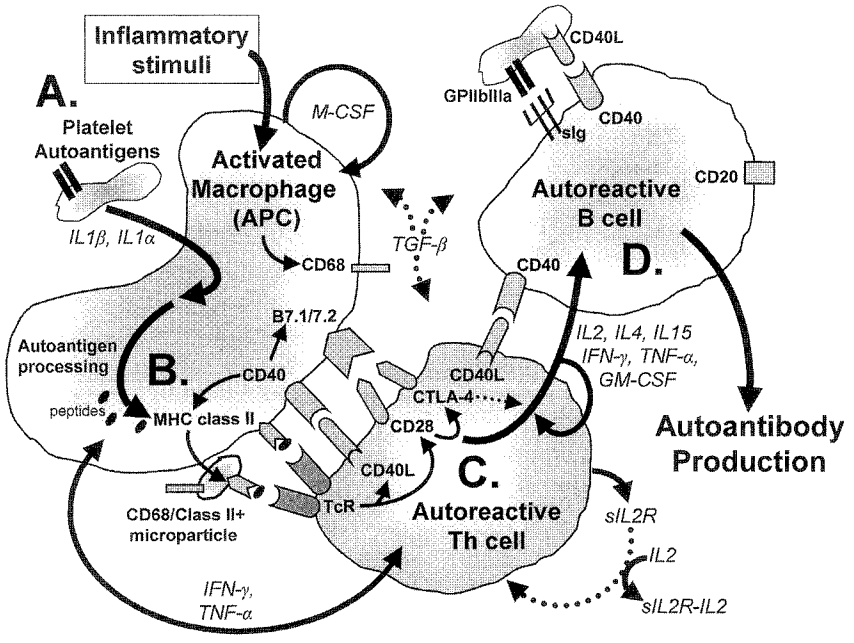

Fig 2. Potential stimulatory (solid arrows) and inhibitory (hatched arrows) pathways that may affect the pathogenesis of chronic AITP. This models assumes that a tolerance event has broken down, allowing autoreactive T and B cells to be present. (A) Platelets are normally taken up by macrophages during senescence and are presumably destroyed intracellularly within lysosomes. Inflammatory stimuli can activate macrophages that may alter the way they normally process platelet autoantigens or induce the expression of inflammatory cytokines (eg, IL1) that may support autoimmune responses. (B) In the activated APC, platelet autoantigenic peptides can be loaded onto MHC class II molecules for presentation to the TcR of autoreactive Th cells. C. Once the TcR has been occupied by the MHC class II molecules and platelet autoantigen, it can initiate a series of molecular costimulatory interactions within the T cell. First, CD40L upregulated on the T-cell surface interacts with CD40 on the APC that stimulates B7.1 and B7.2 expression. The TcR-MHC interaction also enhances CD28 expression on the Th cells that then interact with the B7.1, culminating in a strong costimulation response and activation of the Th cell. D. Th activation leads to the secretion of Th0/Th1 cytokines (eg, IL2, IL10, and IFN-γ) that effectively drive autoreactive B cells to divide and differentiate into plasma cells and secrete anti-platelet autoantibodies. Also shown are a number of potential regulatory events that could either enhance or suppress autoimmunity in AITP. For example M-CSF can activate macrophages within the spleen to enhance their phagocytosis of platelets. Additionally, IFN-γ and TNF-α produced by the autoreactive Th cells can feedback on macrophages enhancing their expression of MHC class II molecules and potentially aggravate the response. On the other hand, TGF-β (produced by either Th3 cells or platelets) together with the Th cell's soluble IL2 receptor can control the autoimmune response by inhibiting lymphocyte activation. This may also occur during costimulation via the expression of CTLA-4 on the Th cell. Also shown are the potential interactions of MHC class II+ and CD40L+ platelets with Th and B cells activation respectively. These events may be responsible for initiating and/or aggravating autoimmunity in patients with AITP.

let counts. They concluded that the antibody was safe at the doses used. Subsequently, Bussel et al[75] examined the same anti-CD40L antibody at a 20 mg/kg dose in 15 refractory patients with chronic AITP and found that 5 patients had a sustained platelet response. The authors suggested that this treatment may be an important and safe treatment for refractory patients with chronic AITP. Unfortunately, however, in the fall of 1999, the clinical trials with Antova were canceled after some patients developed life-threatening thrombotic episodes. Using a second humanized monoclonal against anti-CD40L (IDEC-131), Kuwana et al[76] recently followed 20 patients with chronic AITP after a single dose of the antibody. They found few side effects in the patients and that a dose of 10 mg/kg induced selective suppression of T- and B-cell responses to GPIIbIIIa resulting in blockade of autoantibody synthesis and an increase in platelet counts.[78] Studies with this newer antibody preparation are continuing. It appears that despite the initial failure of 1 anti-CD40L antibody, safer preparations may be available, and these may be effective treatments particularly for refractory patients with chronic AITP. With respect to B cells, Rituxan^R (Genentech, San Francisco, CA), a monoclonal antibody specific for CD20, has been used to treat patients with AITP.[77,78] For example,

Stasi et al[78] treated 25 patients with refractory chronic AITP with Rituxan^R at a weekly dose of 375 mg/m^2. Five of the patients had a complete response, whereas 5 had a partial response and there were only mild side effects.[78] They concluded that Rituxan^R therapy had a limited but valuable effect in patients with chronic AITP and in view of its mild toxicity and the lack of effective alternative treatments, its use in patients with chronic refractory ITP was warranted.[78]

Taken together, therapies aimed at the cellular immune system are just beginning to take place and most appear to have reasonable success in patients with refractory AITP. Potential future therapies could be aimed at other T-cell costimulatory molecules such as CTLA-4. However, it needs to be remembered that although these new therapies may be beneficial to patients with AITP, they are antigen nonspecific and may suppress any activated T cell leading to generalized immunosuppression. Because of this, more research will be required to develop newer and more antigen-specific T-cell–directed immunotherapies for chronic AITP. Of interest, oral tolerance induction with autologous platelets was recently suggested as a novel hypothetical approach to antigen-specific therapy in AITP.[79] Thus, based on the current state of cellular immune research in chronic AITP, there is an optimistic outlook that in the near future, newer and more antigen-specific therapies will be developed for this autoimmune disease.

SUMMARY AND FUTURE CONSIDERATIONS

In our last review,[2] we concluded by posing 2 questions for future consideration: (1) Are the T-cell defects observed in chronic AITP responsible for autoantibody production, and (2) What are the platelet autoantigens recognized by the autoreactive T cells? Currently, the first question appears to have been answered. It is now clear that abnormal Th-cell activation is taking place in chronic AITP, and these cells appear to be driving autoreactive B cells to differentiate and secrete antiplatelet autoantibodies. Although, the second question has been partially answered, in that GPIIIa peptides have been identified that are recognized by the autoreactive Th cells, it is still not clear whether these peptides are the initiators of the autoimmune response. Further research is required to elucidate the general initiation mechanism of autoimmunity in chronic AITP and to develop more antigen-specific therapies to combat this disorder. One other interesting aspect of chronic AITP is that in many patients, autoantibodies cannot be identified; is it possible that in these patients, cell mediated mechanisms are responsible for platelet destruction? Only more research efforts will be able to solve some of these unanswered questions.

ACKNOWLEDGMENT

We would like to thank Dr Alan H. Lazarus for critically reviewing this manuscript.

REFERENCES

1. Semple JW, Freedman J: Cellular immune mechanisms in chronic autoimmune thrombocytopenic purpura. Autoimmunity 13:311-319, 1992

2. Semple JW, Freedman J: Abnormal cellular immune mechanisms associated with autoimmune thrombocytopenia. Transfus Med Rev 9:327-338, 1995

3. Watts C: Capture and processing of exogenous antigens for presentation on MHC molecules. Annu Rev Immunol 15: 821-850, 1997

4. Chambers CA: The expanding world of co-stimulation: the two-signal model revisited. Trends Immunol 22:217-223, 2001

5. Elson,CJ, Barker RN: Helper T cells in antibody-mediated, organ-specific autoimmunity. Curr Opin Immunol 12:664-669, 2000

6. Chess L: Blockade of the CD40L/CD40 pathway, in Austen KF, Burakoff, S, Rosen, F & Strom, T (eds): Therapeutic Immunology (ed 2). New York, NY, Blackwell Sciences, 2001, pp 441-456

7. Mosmann RT, Sad D: The expanding universe of T-cell subsets: Th1, Th2 and more. Immunol Today 17:138-146, 1996

8. Romangnani S: Th1 and Th2 subsets in human diseases. Clin Immunol Immunopath 80:225-235, 1996

9. Paliard X, de Waal Malefyt R, Yssel H: simultaneous production of Il-2, Il-4 and INF-γ by activated human CD4+ and CD8+ T cell clones. J Immunol 141:849-855, 1988

10. Yssel H, de Vries J, de Waal Malefyt R: IL-10 is produced by subsets of human CD4+ T cell clones and peripheral blood T cells. J Immunol 149:2378-2384, 1992

11. Coutinho A, Haas W: In vivo models of dominant T-cell tolerance: Where do we stand today? Trends Immunol 22:350-351, 2001

12. Kappler JW, Staerz U, White J, et al: Self-tolerance eliminates T cells specific for MLS-modified products of the major histocompatibility complex. Nature 332:35-40, 1988

13. Mueller DL, Jenkins MK, Schwartz RH: Clonal expansion versus functional clonal inactivation: A costimulatory signaling pathway determines the outcome of T cell antigen receptor occupancy. Annu Rev Immunol 7:445-480, 1989

14. Goronzy JJ, Weyand M: Thymic function and peripheral T-cell homeostasis in rheumatoid arthritis. Trends Immunol 22:251-255, 2001

15. Jonuleit H, Schmitt E, Steinbrink K, et al: Dendritic cells as a tool to induce anergic and regulatory T cells. Trends Immunol 22:394-400, 2001

16. Sinha AA, Lopez T, McDevitt HO: Autoimmune diseases: the failure of self-tolerance. Science 248:1380-1388, 1990

17. Mackay IR: The "autoimmune diseases" 40th anniversary. Autoimmun Rev 1:5-11, 2002

18. Rosmalen JGM, van Ewijk W, Leenen PJM: T-cell education in autoimmune diabetes: Teachers and students. Trends Immunol 23:40-46, 2002

19. Doyle HA, Mamula MJ: Post-translational protein modifications in antigen recognition and autoimmunity. Trends Immunol 22443-449, 2001

20. Wadenvik H, Stockelberg D, Hou M. Platelet proteins as autoantibody targets in idiopathic thrombocytopenic purpura, Acta Paediatr Suppl 424:26-36, 1998

21. MehtaYS, Pathare AV, Badakere SS, et al: Influence of auto-antibody specificities on the clinical course in patients with chronic and acute ITP. Platelets 11:94-98, 2000

22. McMillan R: Autoantibodies and autoantigens in chronic immune thrombocytopenic purpura. Semin Hematol 37:239-248, 2000

23. Cines DB, Blanchette VS: Immune thrombocytopenic purpura. N Engl J Med 346:995-1008, 2002

24. Frederiksen H, Schmidt K: The incidence of idiopathic thrombocytopenic purpura in adults increases with age. Blood 94:909-913, 1999

25. Blanchette VS, Semple JW, Freedman J: Intravenous immunoglobulin and Rh immunoglobulin as immunomodulators of autoimmunity to blood elements in Silberstein LE (ed): Autoimmune Disorders of Blood. American Association of Blood Banks, Bethesda MD, pp 35-77, 1996

26. Wright JF, Blanchette VS, Wang H, et al: Characterization of platelet-reactive antibodies in children with varicella-associated acute immune thrombocytopenic purpura (ITP). Br J Haematol 95:145-152, 1996

27. Oldstone MBA: Molecular Mimicry and immune-mediated diseases. FASEB J 12:1255-1265, 1998

28. Chia WK, Blanchette VS, Mody M, et al: Isolation of HIV-1-specific and platelet-crossreactive antibodies from HIV-1-infected thrombocytopenic hemophiliacs. Blood 90:628, 1997 (suppl, abstr)

29. Semple JW, Milev Y, Cosgrave D, et al: Differences in serum cytokine levels in acute and chronic autoimmune thrombocytopenic purpura. Blood 87:4225-4254, 1996

30. Boshkov LK, Kelton JG, Halloran PF: HLA-DR expression by platelets in acute idiopathic thrombocytopenic purpura. Br J Haematol 81:552-557, 1992

31. Semple JW, Mitelman B, Allen D, et al: Platelet HLA-DR expression is mediated by physical interaction with adherent CD14+ macrophages: Possible role in the pathogenesis of autoimmune thrombocytopenia. Blood 86:2148, 1995 (suppl, abstr)

32. Henn V, Slupsky JR, Grafe M, et al: CD40 ligand on activated platelets triggers an inflammatory reactions of endothelial cells. Nature 391:591-594, 1998

33. Nomura S, Yanabu M, Kido H, et al: Significance of cytokines and CD68-positive microparticles in immune thrombocytopenic purpura. Eur J Haematol 55:49-56, 1995

34. Fukuda M: Lysosomal membrane glycoproteins. Structure, biosynthesis, and intracellular trafficking. J Biol Chem 266:21327-21330, 1991

35. Schreuder GMT, Tilanus MGJ, Bontrop RE, et al: HLA-DQ polymorphism associated with resistance to type I diabetes detected with monoclonal antibodies, isoelectric point differences, and restriction fragment length polymorphism. J Exp Med 164:938-943, 1986

36. Karpatkin S: Association of HLA-DRw2 with autoimmune thrombocytopenic purpura, J Clin Invest 63:1085-1088, 1979

37. El-Khateeb MS, Awidi AS, Tarawneh MS, et al: HLA antigens, blood groups and immunoglobulin levels in idiopathic thrombocytopenic purpura. Acta Haematol 76:110-114, 1986

38. Goebel KM, Hahn E, Havermann K: HLA matching in autoimmune thrombocytopenic purpura. Br J Haematol 35:341-342, 1977

39. Porges A, Bussel J, Kimberly R, et al: Elevation of platelet associated antibody levels in patients with chronic idiopathic thrombocytopenic purpura expressing the B8 and/or DR3 allotypes. Tissue Antigens 26:132-137, 1985

40. Gratama JW, D'Amaro J, deKoning, J, et al: The HLA system in immune thrombocytopenic purpura: Its relation to the outcome of therapy. Br J Haematol 56:287-293, 1984

41. Gaiger A, Neumeister A, Heinzl H, et al: HLA class I and II antigens in chronic idiopathic autoimmune thrombocytopenia. Ann Hematol 68:299-302, 1994

42. Nomura S, Matsuzaki T, Ozaki Y, et al: Clinical significance of HLA-DRB1*0410 in Japanese patients with idiopathic thrombocytopenic purpura. Blood 91:3616-3622, 1998

43. Kuwana M, Kaburaki J, Pandey JP, et al: HLA class II alleles in Japanese patients with immune thrombocytopenic purpura. Associations with anti-platelets glycoprotein antibodies and responses to splenectomy. Tissue Antigens 56:337-343, 2000

44. Cao K, Hollenbach J, Shi X, et al: Analysis of the frequencies of HLA-A, B, and C alleles and haplotypes in the five major ethnic groups of the United States reveals high levels of diversity in these loci and contrasting distribution patterns in these populations. Hum Imunol 62:1009-1030, 2001

45. Foster CB, Zhu S, Erichsen HC, et al: Polymorphisms in inflammatory cytokines and Fcgamma receptors in childhood chronic immune thrombocytopenic purpura: A pilot study. Br J Haematol 113:596-599, 2001

46. Semple JW, Freedman J: Enhanced anti-platelet lymphocyte reactivity in patients with autoimmune thrombocytopenia (AITP). Blood 78:2619-2625, 1991

47. Ware RR, Howard TA: Phenotypic and clonal analysis of T lymphocytes in childhood immune thrombocytopenic purpura. Blood 82:2137-2142, 1993

48. Filion MC, Bradley AJ, Devine DV, et al: Autoreactive T cells in healthy individuals show tolerance in vitro with characteristics similar to but distinct from clonal anergy. Eur J Immunol 25:3123-3127, 1995

49. Shimomura T, Fujimura K, Takafuta T, et al: Oligoclonal accumulation of T cells in peripheral blood from patients with idiopathic thrombocytopenic purpura. B J Haematol 95: 732-737, 1996

50. Kuwana M, Kaburaki J, Ikeda Y: Autoreactive T cells to

platelets GPIIbIIIa in immune thrombocytopenic purpura: A role in production of anti-platelet autoantibody. J Clin Invest 102:1393-1402, 1998

51. Kuwana M, Kaburaki J, Kitasato H, et al: Immunodominant epitopes on glycoprotein IIb-IIIa recognized by autoreactive T cells in patients with immune thrombocytopenic purpura. Blood 98:130-139, 2001

52. Semple JW, Speck ER, Kim M, et al: Characterization and fine specificity of platelet-reactive T cell lines from children with chronic autoimmune thrombocytopenic purpura (AITP). Blood 98:440a, 2001 (suppl, abstr)

53. Kuwana K, Okazaki Y, Kaburaki J, et al: Spleen is the primary site for activation of platelet-reactive T and B cells in patients with immune thrombocytopenic purpura. J Immunol 168:3675-3682, 2002

54. Garcia-Suarez J, Prieto A, Reyes E, et al: Abnormal γIFN and αTNF secretion in purified CD2+ cells from autoimmune thrombocytopenic purpura patients: Their implication in the clinical course of the disease. Am J Hematol 49:271-276, 1995

55. Abboud MR, Laver J, Xu F, et al: Serum levels of GM-CSF are elevated in patients with thrombocytopenia. Br J Haematol 92:486-488, 1996

56. Lazarus AH, Ellis J, Semple JW, et al: Comparison of platelet immunity in patients with SLE and ITP. Transfus Sci 22:19-27, 2000

57. Yoshimura C, Nomura S, Nagahama M, et al: Plasma-soluble Fas (APO-1, CD95) and soluble Fas ligand in immune thrombocytopenic purpura. Eur J Haematol 64:219-224, 2000

58. Shenoy S, Mohanakumar, T, Chatila, T, et al: Defective apoptosis in lymphocytes and the role of IL-2 in autoimmune hematologic cytopenias. Clin Immunol 99:266-275, 2001

59. Mouzaki A, Theodoropoulou M, Gianakopoulos I, et al: Expression patterns of Th1 and Th2 cytokine genes in childhood idiopathic thrombocytopenic purpura (ITP) at presentation and their modulation by intravenous immunoglobulin G (IVIg) treatment: their role in prognosis. Blood 100:1774-1779, 2002

60. Andersson PO, Olsson B, Carlsson B, et al: Gene expression profiling of T lymphocytes in chronic ITP. Proc Eur Haematol Assoc Annual Meeting (Italy), abstr 0612, 2002

61. Crossley AR, Dickinson AM, Proctor SJ, et al: Effects of interferon-alpha therapy on immune parameters in immune thrombocytopenic purpura. Autoimmunity 24:81-100, 1996

62. Webber NP, Mascarenhas JO, Crow MK, et al: Functional properties of lymphocytes in idiopathic thrombocytopenic purpura. Hum Immunol 62:1346-1355, 2001

63. Erduran E, Aslan Y, Aliyazicioglu Y, et al: Plasma soluble interleukin-2 receptor levels in patients with idiopathic thrombocytopenic purpura. Am J Hematol 57:119-123, 1998

64. Tan B, Zhao X, Yin C: Detection of IL-2 receptor in patients with idiopathic thrombocytopenic purpura. Hunan Yi Ke Da Xue Xue Bao 23:191-193, 1998

65. Andersson PO, Stockelberg D, Jacobsson S, et al: A transforming growth factor-beta 1- mediated bystander immune suppression could be associated with remission of chronic idiopathic thrombocytopenic purpura. Ann Hematol 79:507-513, 2000

66. Letterio JJ, Roberts AB: Regulation of immune responses by TGF-β. Annu Rev Immunol 16:137-161, 1998

67. Karakantza M, Mouzaki A, Theodoropoulos M, et al: Th1 and Th2 cytokines in a patient with Evan's syndrome and profound lymphopenia. Br J Haematol 110:968-970, 2000

68. Ziegler ZR, Rosenfield CS, Nemunatis JJ: Increased macrophage colony-stimulating factor levels in immune thrombocytopenic purpura. Blood 81:1251-1254, 1993

69. Nomura S, Yasunaga K, Fujimura K, et al: High-dose intravenous gamma globulin reduces macrophage colony-stimulating factor levels in idiopathic thrombocytopenic purpura. Int J Hematol 63:227-234, 1996

70. Baker GR, Levin J: Transient thrombocytopenia produced by administration of macrophage colony-stimulating factor: investigations of the mechanism. Blood 91:89-99, 1998

71. Lazarus AH, Joy T, Crow AR: Analysis of transmembrane signaling and T cell defects associated with idiopathic thrombocytopenic purpura (ITP). Acta Paediatr Suppl 424:21-25, 1998

72. Aiura K, Clark BD, Dinarello CA, et al: Interaction with autologous platelets multiplies interleukin-1 and tumor necrosis factor production in mononuclear cells. J Infect Dis 175:123-129, 1997

73. Lindemann S, Tolley ND, Dixon DA, et al: Activated platelets mediate inflammatory signaling by regulated IL-1β synthesis. J Cell Biol 154:485-490, 2001

74. George J, Raskob G, Lichtin A, et al: Safety and effect on platelets count of a single-dose monoclonal antibody to CD40 ligand (Antnova™) on patients with chronic ITP. Blood 92:707a, 1998 (suppl, abstr)

75. Bussel J, Wissert M, Oates B, et al: Humanized monoclonal anti-CD40 ligand antibody (hu5c8) rescue therapy of 15 patients with severe chronic refractory ITP. Blood 94:646a, 1999 (suppl, abstr)

76. Kuwana M, Nomura S, Fujimura K, et al: Immunomonitoring after a single injection of humanized monoclonal antibody to CD40 ligand in patients with chronic ITP. Blood 98:441a, 2001 (suppl, abstr)

77. Delgado J, Bustos JG, Jimenez-Yuste V, et al: Anti-CD20 monoclonal antibody therapy in refractory immune thrombocytopenic purpura. Haematologica 87:215-216, 2002

78. Stasi R, Pagano A, Stipa E, et al: Rituximab chimeric anti-CD20 monoclonal antibody treatment for adults with chronic idiopathic thrombocytopenic purpura. Blood 98:952-957, 2001

79. Hermida G, Manjón R, Casanova M, et al: Oral vaccination with autologous platelets in chronic autoimmune thrombocytopenic purpura. Med Hypotheses 57:612-615, 2001