Mechanisms underlying autoimmunity in hematology

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Organ-specific autoimmune diseases are directed primarily at the autoantigens of particular tissues, for example, platelets in autoimmune thrombocytopenic purpura (AITP) and erythrocytes in autoimmune hemolytic anemia (AIHA). These particular diseases are the major autoimmune disorders in hematology. This review will outline the etiology of AITP and AIHA and discuss that although the two disorders are mediated by autoantibodies, T cell defects are the major driving force behind autoantibody production.

Introduction

The existence of autoimmune diseases in humans has been known for almost 100 years. Autoimmune diseases result from the failure of normal self-tolerance mechanisms and collectively they affect approximately 5–7% of the population, often with debilitating effects. The breakdown of self-tolerance might be the result of several 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. Both AIHA and AITP are mediated by autoantibodies that opsonize erythrocytes and platelets respectively leading to their enhanced destruction by Fc receptor-mediated phagocytosis by cells of the reticuloendothelial (RES) system primarily within the spleen (Fig. 1). Although the pathogenesis of these two diseases is mediated by autoantibodies, there is increasing evidence to support that their production is crucially dependent on abnormal T cell reactivities (Fig. 1). Understanding these T cell-mediated mechanisms is essential for the rational design of immunotherapies to specifically downregulate the autoantibody production.

Autoimmune thrombocytopenic purpura (AITP)

AITP is characterized by the production of autoreactive antibodies against one’s own platelets, resulting in increased platelet destruction by RES phagocytes. The majority of these autoantibodies are IgG, but IgM and IgA can also be identified in some patients with AITP [1]. Several excellent papers have recently been published, which review the autoantibody and T cell literature related to AITP [2–5]. Both acute and chronic forms of AITP can be distinguished. Acute AITP primarily affects children and often occurs after a viral or bacterial infection [6]. 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 × 109/L for more than 6 months) [6]. In contrast to the acute form, chronic AITP is predominant in adults, with more females
being affected than males [6]. Although both acute and chronic AITP are immune-mediated, it is becoming clear that the pathophysiological mechanisms are different.

**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 might be associated with the immune mechanisms stimulated by the preceding infection. For example, in children with acute AITP associated with Varicella Zoster virus (VZV) infection, Wright et al. [7] demonstrated that the patient’s serum IgM and IgG antiplatelet autoantibodies could be purified on affinity columns conjugated with VZV 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 [8]. At that the T cell level, however, 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 [7,9]. 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 (e.g. antigenic mimicry). This can explain why many children with acute AITP spontaneously go into remission without therapy, for example as the infectious agent is cleared and antibodies either disappear or affinity mature towards the infectious agent, the antiplatelet reactivity is lost. In fact, many hematologists do not treat acute AITP and simply monitor the patient’s platelet counts or for bleeding symptoms.

**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 [1,6]. The antibody specificities are directed against several platelet glycoprotein epitopes [2–5]. Since 1995, many investigators have demonstrated 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.

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 interleukin (IL)-2 upon stimulation with autologous platelets [10]. It suggested that chronic AITP might 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. [11] and since then, many reports have been published describing various T cell and cytokine abnormalities in patients with chronic AITP [5].

Filion et al. [12] reported that normal individuals contained anergic Th cells which could be activated in vitro following incubation with platelet glycoprotein (GP) IIbIIIa (fibrinogen receptor) and exogenous IL-2; once this tolerance was broken, however, the Th cells could secrete their own IL-2 upon stimulation with GPIIbIIIa. These results suggested that T cell tolerance against platelet autoantigens might involve the post-transcriptional regulation of IL-2 expression. Subsequently, Shimomura et al. [13] demonstrated 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 Th cells that might have expanded because of a breakdown in tolerance mechanisms.

Kuwana et al. [14] demonstrated that T cells from Japanese patients with chronic AITP could proliferate in vitro to disulfide-reduced GPIbIIIa or the molecule’s tryptic peptides. This suggested that autoreactive CD4+ Th cells in chronic AITP need to recognize a modified GPIbIIIa molecule, implying that antigen processing mechanisms within recipient APC might be required to present GPIbIIIa autoantigens in the context of self HLA-DR molecules. Subsequently, the same investigators mapped the antigen specificity of the GPIbIIIa-reactive Th cells by using six recombinant fragments encoding different portions of the GPIbα and GPIIia chains [15]. They demonstrated that the T cells frequently recognized the amino terminal portion of the two GP chains (GPIibα 18-259 and GPIIia22-262) and that these molecules also stimulated the production of antiplatelet autoantibodies [15]. More recent work has determined the fine specificity of Th cell clones from Caucasian patients with chronic AITP and revealed that at least one minimal peptide corresponding to amino acid residues 496–510 of the GPIIia molecule is sufficient to stimulate T cell IL-2 secretion [16]. These results support the concept that CD4+ Th cell activation is the primary defect that leads to autoantibody production in patients with chronic AITP.

**Autoimmune hemolytic anemia (AIHA)**

AIHA is characterized by the production of antibodies directed against self RBC. The etiology of most RBC autoantibodies is not well understood. Idiopathic or primary AIHA shows no apparent association with an underlying disorder. Given the frequent association between AIHA and other autoimmune disorders, however, generalized immune system dysfunction probably also plays a role, and the relationship between AIHA and lymphoproliferative disorders and other neoplasms supports the concept that a generalized dysfunction of immune surveillance is present in patients with AIHA. Recent studies on animal and human AIHA suggest that loss of immunological tolerance to red blood cell self-antigens might originate by different, nonmutually exclusive mechanisms that include antigenic mimicry, apoptosis and immunoregulatory disorders including cytokine network alteration.

In AIHA, IgM autoantibodies or cold agglutinins generally react with polysaccharide antigens on the red cell surface and are usually associated with neoplastic B cell populations [17,18]. By contrast, IgG warm autoantibodies generally react with protein antigens on the erythrocyte surface and are typically panagglutinins, reacting with all cells [19]. Recent studies employing immunoblotting have variably implicated Rh antigens, membrane protein band 4.1, protein band 3, and glycoporphin A as universal RBC targets. The association of warm-type AIHA with systemic autoimmune disorders has suggested that these antienhroncyte autoantibodies, in contrast to the clonal cold-reactive autoantibodies, might arise from polyclonal activation rather than from activation by specific (self) antigen [31].

Infectious agents have been shown to be associated with the development of antienhroncyte antibodies. For example, Coutelier et al. [20] showed that after intracerebral inoculation of lymphocytic choriomeningitis (LCM) virus, mice develop AIHA [20]; the immune hemolysis was strongly reduced by treatment with anti-CD4 antibody, suggesting that the virus-induced AIHA is a T helper-dependent autoimmune event.

Activation-induced programmed cell death is also believed to play an important role in regulating autoimmunity and several studies have shown various defects of apoptosis related to autoimmunity. This is also true for AIHA [21–25]. For example, defective apoptosis of autoaggressive T cells expressing IL-2 receptors can play a role in initiating AIHA pathogenesis. Thus, an ongoing autoimmune process can be viewed as a rather fine-tuned and fragile equilibrium of aggressive and regulatory components and the precise activation kinetics and survival times of all lymphocyte types implicated in the process will determine the outcome. In addition, genetic defects of Fas/Fasl mediated autoreactive lymphocyte apoptosis might be associated with AIHA [21].

Drugs might also be associated with the etiology of AIHA [26]. This can result from several types of interaction between drug, antibodies and red cell membrane components. Alpha methyl dopa is the prototypical drug operating by the induction of autoantibodies; these are typically panreactive, although documented specificities have been described. Other drugs that can induce autoantibodies include levodopa, mefenamic acid, procainamide and diclofenac. The antibody reacts with a normal membrane component and the epitope does not involve the presence of a drug, that is a true autoantibody [26]. The drugs can alter antigens on the red cell, resulting in production of antibody that cross-reacts with the unaltered antigen.

Interestingly, antienhroncyte autoantibody formation can also follow transfusions of allogeneic red blood cells particularly in multiply transfused individuals such as patients with sickle cell disease and thalassemia [27]. The phenomenon of RBC autoantibody formation in association with blood transfusion is not well understood. Alloantibodies might bind to transfused red cells and cause conformational changes in the red cell antigenic epitopes, leading to stimulation of autoantibody formation [28]. Alternatively, since the phenomenon has been observed particularly in multiply transfused patients, it has been suggested that some patients can simply have a predisposition to develop red cell autoantibodies, perhaps because of an overall dysfunction of their immune systems [28,29].

There is in vitro and in vivo experimental evidence to suggest that quiescent T and/or B cells specific for self-antigens might be activated in AIHA if adequate antigen presentation and co-stimulation occur [30]. Synthetic peptides
corresponding to the Rh polypeptide sequence, the most frequent target for autoantibodies in human warm-type AIHA, were tested in vitro for their ability to stimulate normal T cells [31,32]. Multiple peptides provoked T cell activation and their proliferation could be blocked by anti HLA-DR antibodies. It was suggested that autoreactive T cells in AIHA might not be deleted, but are anergic to autologous Rh polypeptides and thus immunologically ignorant against RBC self-antigen [31,32]. In addition, there is experimental evidence that autoantibody production in some cases of AIHA is caused by the activation of class-II-restricted helper T cells specific for cryptic Rh epitopes [33]; these autoreactive T cells seem to escape the clonal deletion and anergy during the induction of self-tolerance and remain quiescent even if the autoantigens they recognize are present.

Several mouse strains, for example NZB and NZB/NZW, which spontaneously develop a complex autoimmune syndrome including AIHA, have been extensively studied to identify the immunological factors contributing to the autoimmune onset of AIHA [34–40]. Studies in NZB mice have provided experimental evidence to support an antigen induction model of AIHA. In mice, red cell membrane band 3, a red cell anion exchange protein, appears to be the major antigen for RBC autoantibodies. Although not all autoantibodies binding to band 3 produce pathologic effects, in NZB mice with band 3-reactive CD4 T cells, pathogenic autoantibodies are found [36].

Therapeutics related to chronic AITP and AIHA
Currently, the major chemotherapeutic treatment modalities aimed at patients with AITP and AIHA are antigen non-specific immunosuppressive therapies (e.g. steroids, IVIg, cyclophosphamide, and so forth) (Table 1). 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. They include identification and modification of the offending autoantigen to suppress T cell activation (e.g. antigen-specific therapy targeting MHC-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. With respect to T cells, for example, several clinical trials were initiated in the 1990s using monoclonal antibodies specific for the CD40L on activated T cells (e.g. hu5c8, Antova). Initially, good results were obtained in patients with AITP. However, subsequently, in the fall of 1999, the clinical trials with Antova were cancelled after some patients developed life threatening thrombotic episodes. With respect to B cells, Rituxan, a monoclonal antibody specific for CD20, has been used to treat patients with AITP. It appears that this antibody can be beneficial in raising the platelet counts in patients with AITP. Taken together, therapies aimed at the cellular immune system are just beginning to take place and some 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

Table 1. Common therapies in patients with AITP (in general order of use)a

<table>
<thead>
<tr>
<th>Therapy</th>
<th>Target</th>
<th>Strategic approach to target</th>
<th>Expected outcome</th>
<th>Side effects</th>
</tr>
</thead>
<tbody>
<tr>
<td>Steroids (Prednisone)</td>
<td>Inhibition of Phospholipase A 2</td>
<td>Anti-inflammatory</td>
<td>Raises platelet counts after 2–3 weeks.</td>
<td>Substantial with overuse (weight gain, mood changes, elevated blood pressure, insomnia, blood sugar changes, calcium loss, muscle wasting, and increased susceptibility to infections).</td>
</tr>
<tr>
<td>Intravenous gammaglobulin (IVIg, e.g. Gammunex, Bayer)</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Raises platelet counts within 24 h</td>
<td>Allergic reactions, heart palpitations, headache</td>
</tr>
<tr>
<td>Anti-D (WhinRhoSDFTM, Cangene)</td>
<td>Rh antigen on RBC</td>
<td>Causes mild transient anemia to rescue platelet destruction</td>
<td>Raises platelet counts within 24 h.</td>
<td>Acute reactions (e.g. rigors, chills, headache, and so forth)</td>
</tr>
<tr>
<td>Splenectomy</td>
<td>spleen</td>
<td>Removal of the site of platelet destruction</td>
<td>Raises platelet counts immediately</td>
<td>Potential surgical complications</td>
</tr>
<tr>
<td>Chemotherapy (e.g. cyclophosphamide, azathioprine, vincristine, cyclosporine)</td>
<td>Various</td>
<td>Used when above therapies fail</td>
<td>Raise platelet counts</td>
<td>Several (immunosuppression, myelosuppression, and so forth)</td>
</tr>
</tbody>
</table>

*a These therapies are also used in patients with AIHA except for anti-D treatment.*
to be remembered that whereas these new therapies might be beneficial to patients with AITP, they are still considered antigen non-specific and might have the side effect of generalized immunosuppression. Because of this, more research will be required to develop newer and more antigen-specific T cell-directed immunotherapies for chronic AITP.

Conclusions
The immunopathogenic etiology of AITP and AIHA remains not well understood. It appears, however, that in both hematological disorders, a generalized dysfunction of the immune system might be involved or a generalized dysfunction of immune surveillance. Disruption of any of the processes or control points that maintain a balance between tolerance of platelet and RBC self-antigens and need to respond to foreign antigens might be a potential cause of the onset of AITP and AIHA. Superimposed on this, genetic and environmental factors are also likely to play a role in the production of the autoantibodies in both AITP and AIHA. It is clear that more research is warranted to clearly map the underlying immune defects in AITP and AIHA. This can lead to the development of more antigen-specific therapies for the disorders.

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