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Immunobiology of T helper cells and antigen-presenting cells in autoimmune thrombocytopenic purpura (ITP)

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Autoimmune thrombocytopenic purpura (AITP) is a bleeding disease in which autoantibodies are directed against the individual's own platelets, resulting in enhanced Fc-mediated platelet destruction by macrophages in the reticuloendothelial system. Most research in AITP has focused on characterization of the autoantibodies, while little has been devoted to the cellular immune mechanisms leading to autoantibody production. This report summarizes the current state of the literature and argues that enhanced T helper cell/antigen-presenting cell interactions in patients with AITP are the primary stimulus for the development of antiplatelet autoantibody production. Understanding these events is important for eventually identifying disease-initiating platelet autoantigens and ultimately developing specific immunotherapies for AITP. □ *Antigen-presenting cells, chronic autoimmune thrombocytopenia, platelets, T helper cells*

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Autoimmune thombocytopenic purpura (AITP) is characterized by thrombocytopenia due to increased platelet destruction, normal or increased megakaryocyte numbers in the bone marrow and the absence of both splenomegaly and any other clinical condition that may cause thrombocytopenia. The presence of platelet-associated immunoglobulins (IgG in particular) and/or C3 is frequently demonstrated in AITP but is not necessarily required for a positive diagnosis (1–3). Both acute and chronic forms of disease can be distinguished. In children, acute AITP is often associated with a viral or bacterial infection and generally resolves spontaneously within 6 weeks. Approximately 20% of children with acute AITP progress to the chronic form, defined as persistence of thrombocytopenia (platelet counts $<150\times10^{9}\ l^{-1}$) for greater than 6 months (4). In contrast, AITP in adults is generally chronic and often requires treatment with immunosuppressive therapy or splenectomy. Although both acute and chronic AITP are immune mediated (5), it now appears that different pathogenetic mechanisms are responsible for the two forms of the disease.

The initial stimulation for the production of platelet autoantibodies is unknown but undoubtedly is driven and regulated by complex cellular and soluble mechanisms, primarily involving T helper (Th) lymphocytes and antigen-presenting cells (APC). To elucidate these stimulatory events in chronic AITP, we have developed a working hypothesis focused on platelet-reactive Th cell activation within the constraints of two basic assumptions: (i) the platelet is the primary source of the autoantigen(s) which stimulate Th cells; and (ii) macrophages, which are responsible for the normal destruction of senescent platelets *in vivo*, are the initial APC which stimulate platelet-reactive Th cells. Thus, this hypothesis suggests that the platelet first interacts with a major histocompatibility complex (MHC) class II-positive macrophage which subsequently processes platelet glycoprotein antigen(s) into smaller antigenic peptides. These peptides, presumably generated in phagolysosomes, are translocated to endosomal compartments and ultimately re-expressed on the APC surface in association with MHC class II molecules. If the Th cell receptor (TcR) has a sufficient affinity for the antigen–MHC complex and appropriate co-stimulatory events are met, the Th cell would be activated and would subsequently drive antigen-primed B lymphocytes to produce autoantibodies. In this view, Th cell activation in AITP is the critical event which determines whether autoantibodies are produced against the platelet. This report will discuss evidence to support the view that enhanced APC–Th cell interactions in patients with AITP are potentially important factors which influence platelet autoantibody production. These interactions may ultimately be an important focus for immune specific therapies.

Several abnormalities within T cell populations have been described in patients with AITP and these have been recently reviewed in detail (6). For example, one consistent abnormality described by several laboratories is an increased number of activated $CD3^{+}HLA\text{-}DR^{+}$ T lymphocytes in patients with chronic AITP (7–9). This observation may have importance in autoimmune pathology since activated $HLA\text{-}DR^{+}$ T cells can significantly influence resting $CD4^+$ Th cells and may modulate autoreactive recognition *in vivo* (10). $CD4^+$ Th cell responses can be generally distinguished by their secreted cytokine products (11–19). A Th1 response is characterized primarily by the presence of interleukin (IL)-2, interferon (IFN)-γ, granulocyte macrophage-colony-stimulating factor (GM-CSF) and tumour necrosis factor (TNF)-α and is associated with delayed type hypersensitivity reactions and the synthesis of complement-fixing IgG isotypes (11, 12). Th2 responses produce IL-4, IL-5, IL-6 and IL-10 and are superior in mediating non-complement fixing IgG and particularly IgE synthesis (11, 12). A third type of Th response, Th0, is thought to be generated by cells less differentiated than those mediating Th1 and Th2 responses, since many or all of the Th1/Th2 cytokines are present (13–17). With respect to these responses and AITP, it was initially demonstrated that peripheral blood mononuclear cells (PBMC) from approximately 60% of patients with chronic AITP could be stimulated to secrete IL-2 when incubated with either allogeneic or autologous platelets *in vitro* (Fig. 1) (7), and this was subsequently confirmed by others (20). Consistent with these *in vitro* data, a recent blinded clinical study demonstrated the presence of *in vivo* Th0/Th1 serum cytokines (IL-2, IL-10 and/or IFN-γ) in children with chronic AITP (21). These cytokine patterns were not seen in children with acute AITP. Furthermore, Garcia-Suarez et al. (22) showed that PHA-stimulated PBMC from patients with chronic AITP produced elevated levels of TNF-α and IFN-γ, whereas Nugent et al. (23) demonstrated that IL-4 production was significantly reduced in cultures of PHA-stimulated PBMC derived from children with acute AITP. Of perhaps greater interest, was the demonstration that negatively enriched $CD19^+$ B cells from patients with chronic AITP could be stimulated to produce antiplatelet autoantibodies when incubated with IL-2, IFN-γ and autologous platelets (24, 25; Fig. 2). Taken together, these data suggest that the T cell responses in chronic AITP are associated with Th0 and Th1 activation patterns and are probably responsible for stimulating autoantibody production. What triggers and regulates these T cell responses in patients with AITP still remains unknown. However, in order for Th1 cells to become activated, APC, particularly macrophages, are required to present processed antigen in association with MHC class II molecules (26).

Macrophages, as scavengers, are the primary cells which mediate the normal destruction of senescent platelets within the spleen (27, 28). While it is clear that these cells play a critical role in the ‘‘end’’ destructive process in AITP, i.e. phagocytosis of opsonized platelets (29–31), little work has been devoted to the APC function of macrophages in AITP. For example, the bulk of phagocytosed platelet-derived proteins probably undergoes extensive or terminal degradation into constitutive amino acids within lysosomes (26, 32). During the course of platelet destruction, however, the macrophage may become activated or altered and divert incompletely digested MHC binding peptides towards cellular compartments rich in MHC class II molecules, such as endosomes. This may lead to the peptides being re-expressed in association with MHC class II molecules on the macrophage plasma membrane for potential presentation to autoreactive Th cells. Using

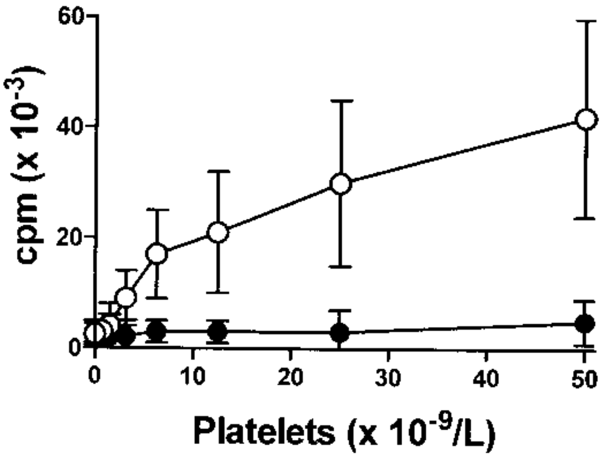

Fig. 1. IL-2 production by PBMC from patients with AITP (○, $n = 10$) and healthy individuals (●, $n = 10$) stimulated with the indicated concentrations of autologous platelets for 7 d. Supernatants were harvested, diluted 1:3, and tested for their ability to stimulate the proliferation of the IL-2-dependent cell line CTLL. Results are expressed as [^{3}H]thymidine incorporation (cpm) ± SD. Background cpm was subtracted from each point.

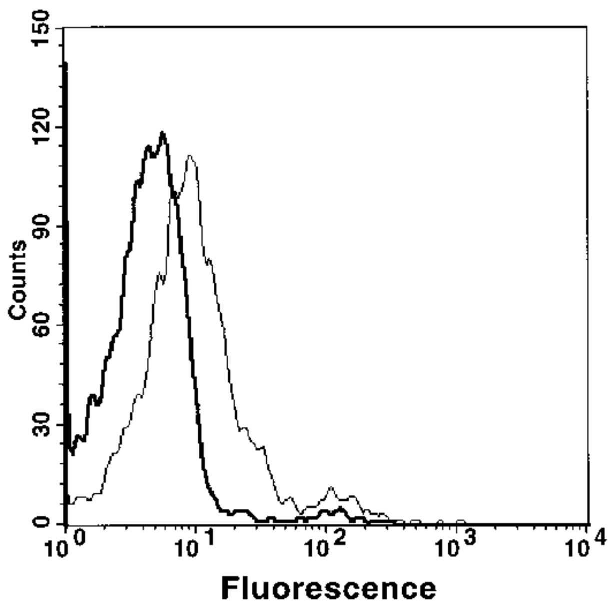

Fig. 2. Histogram analysis of IgG antiplatelet reactivity of immunoglobulins produced by $CD19^+$ B cells from a healthy individual (heavy line) or a patient with chronic AITP (light line). $CD19^+$ B cells were negatively enriched by a magnetic activated cell sorter and incubated with 100 U IFN-γ, 20 U IL-2 and 10×10^9 l^{-1} normal platelets for 10 d at 37°C. The supernatants were harvested and tested for IgG reactivity against group O^+ platelets using a fluorescein isothiocyanate (FITC)-conjugated goat anti-human IgG.

platelet-pulsed adherent cells to activate autologous platelet-reactive Th cell clones, preliminary evidence has be obtained which confirms this processing mechanism (JWS, unpublished results). In addition, it has been demonstrated that inflammatory processes, and particularly IFN-γ, can induce macrophages to upregulate MHC class II expression (33) and significantly alter intracellular protein traffic and processing mechanisms toward endocytic compartments (34). Alternatively, activated macrophages and their soluble factors can induce platelet structural changes and activation (35) that may also cause altered presentation of platelet-derived antigens to Th cells.

There is a growing body of evidence to suggest that activated macrophages and their secreted products are associated with AITP. Zeigler et al. (36) demonstrated significantly elevated serum levels of macrophage-colony stimulating factor (M-CSF) in patients with chronic AITP. This cytokine specifically supports the differentiation of cells within the monocytic lineage (37, 38) and is a potent activator of mature monocytes and macrophages; it enhances both phagocytosis and IL-1 production (39, 40). It was suggested that the high M-CSF levels in patients with AITP may contribute to or initiate enhanced platelet destruction by affecting macrophage function (36), but no evidence was provided. Interestingly, Nugent et al. (41) showed that in 21 of 24 patients with chronic AITP, a correlation between *in vivo* antiplatelet antibodies and *in vitro* GPIIbIIIa-stimulated PBMC IL-1 secretion was found. Since macrophages are a rich source of IL-1, it may be that these cells contribute to the immunopathology in chronic AITP. Further support for this has come from data demonstrating increased circulating CD68$^+$ microparticles in patients with chronic AITP (42). CD68 is a 110 000 mol. wt lysosomal glycoprotein thought to be involved in endocytosis and internal membrane trafficking. It is restricted to macrophages (43, 44) and its surface expression on macrophages is significantly enhanced by inflammatory events (45). In conjunction with the enhanced CD68 expression, elevated serum levels of GM-CSF were also found in the patients with AITP and it was concluded that GM-CSF was released from activated Th1 cells which stimulate macrophage phagocytosis, resulting in an increase in the CD68$^+$ microparticles and platelet destruction (42). Similar results of increased GM-CSF levels in patients with AITP were also reported by Abboud et al (46).

Many autoimmune diseases are associated with abnormal HLA-DR expression on the target tissues. This expression may modulate CD4$^+$ T cell responses and may be a cause of autoimmune pathology. In 1992, Boshkov et al. (47) detected HLA-DR$^+$ platelets in a child with acute AITP and, in a blinded study, the present authors recently reported that circulating GPIb$^+$ platelets and microparticles from patients with chronic AITP coexpress CD45, CD14, CD80 and HLA-DR molecules (21; Fig. 3). Of potential importance was that the platelet HLA-DR expression was inversely correlated to platelet count. It was subsequently shown that the platelet HLA-DR

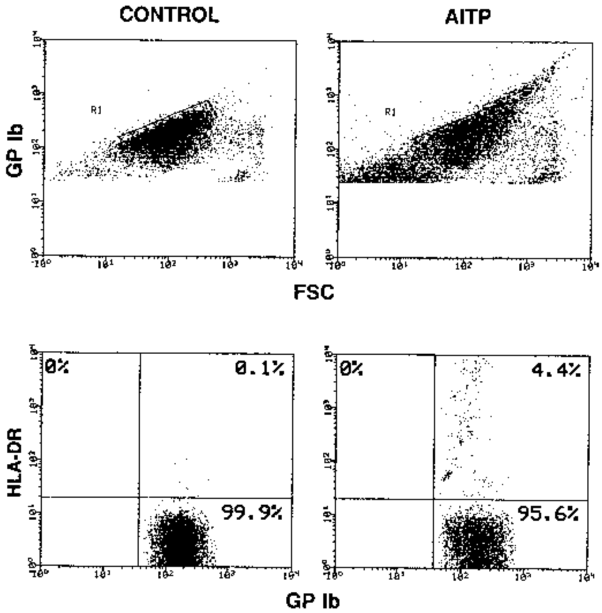

Fig. 3. Flow cytometric analysis of HLA-DR expression on GPIb$^+$ platelet populations prepared from the whole blood of a healthy individual (left panels) and a child with chronic AITP (right panels). The top panels are dot plots of cells acquired through a forward scatter versus FITC-GPIb live gate. The bottom panels are fluorescent analysis using FITC-anti GPIb and phycoerythrin (PE)-anti HLA DR of the above gated events; 20 000 events were acquired. The numbers indicate the percentage of cells in each quadrant.

expression in patients with AITP was mediated by physical contact with adherent macrophages and that *in vitro* pre-activation of macrophages with IFN-γ significantly enhanced the platelet HLA-DR expression (48). Although these macrophage markers on platelets probably reflect *in vivo* macrophage activation, they may play a role in Th cell regulation. A platelet or microparticle expressing macrophage-derived MHC class II molecules may be able to interact directly with CD4$^+$ T cells and, in the presence of inflammatory-like stimulating factors (e.g. help), modulate Th cell activation.

It therefore appears that, in addition to Th1 activation in AITP: (i) activated macrophages are present in patients with AITP; (ii) macrophages have enhanced interactions with platelets in AITP; and (iii) activated macrophages induce platelet abnormalities such as HLA-DR expression. These data, together with the fact that macrophages are a primary APC which induce Th1 cell activation, present a tempting argument that enhanced macrophage/Th cell interactions play a critical role in the development of autoantibody production in patients with chronic AITP. We are currently studying how macrophages process and present platelet autoantigens and stimulate platelet-reactive CD4$^+$ Th cell lines. Understanding these events in chronic AITP may further elucidate the immune pathogenesis of the disease and ultimately lead to the development of better immune specific therapies.

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