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The Cellular Immunology Associated with Autoimmune Thrombocytopenic Purpura: An Update

John W. Semple, PhD*†
Alan H. Lazarus, PhD*
John Freedman, MD*

■ Chronic autoimmune thrombocytopenic purpura (AITP) is an organ specific autoimmune bleeding disease in which autoantibodies are directed against the individual's own platelets, resulting in increased Fc-mediated platelet destruction by macrophages in the reticuloendothelial system. Although AITP is primarily mediated by IgG autoantibodies, their production is regulated by the influence of T lymphocytes and antigen presenting cells (APC). This review argues that enhanced T helper cell/antigen presenting cell interactions in patients with AITP may be responsible for IgG anti-platelet autoantibody production. Understanding these cellular immune responses in AITP may lead to the development of more immune specific therapies for the management of this disease.

*Division of Haematology, St Michael's Hospital, The Toronto Platelet Immunobiology Group and The Departments of Pharmacology and Medicine, University of Toronto, Toronto, Ontario, Canada.
†Author for correspondence at Division of Haematology, St Michael's Hospital, 30 Bond Street, Toronto, Ontario, M5B 1WB, Canada. Fax: +1-416-86405693; e-mail: semple@smh.toronto.on.ca

THE IMMUNE RESPONSE

The initiation of a humoral immune response to a foreign antigen is a complex biologic process involving the interaction of several cell types and their secreted products. The response occurs when the antigen e.g. a cell surface glycoprotein, first interacts with an antigen presenting cell (APC), which is a major histocompatibility complex (MHC) class II-positive dendritic cell or macrophage. The APC internalizes and ''processes'' the antigen by proteolysis into smaller antigenic peptides which associate with the antigen binding groove of MHC molecules. Subsequently, the MHC: peptide complexes are presented on the APC surface membrane for interaction with T cell receptors (TcR). With sufficient affinity, the TcR / MHC interaction, together with co-stimulation (e.g. B7/CD28 interaction), leads to T cell activation, proliferation and differentiation into mature cells which control immune responsiveness. One immune outcome is that the T cell/ APC events stimulate antigen-primed B cells to differentiate into plasma cells and secrete antigen specific IgG antibodies. The CD4+ T helper (Th) cells are critical in determining whether antibodies are produced against the foreign antigen and finely control the response. Thus, any defect in, or abnormal stimulation of, antigen-specific T helper cells

can significantly alter the course of an immune response.

Normally, a host does not overtly mount an immune response against it's own antigens; it behaves as if it is immunologically ''ignorant'' or tolerant. The mechanisms associated with this tolerance at the MHC level have been extensively studied and involve several processes such as central deletion of self-reactive T cells within the thymus and/or peripheral mechanisms such as T cell anergy induction or suppressive cytokine networks. Autoimmunity can develop if one or any of these tolerance mechanisms are breeched.

AUTOIMMUNE THROMBOCYTOPENIC PURPURA

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 which may cause thrombocytopenia. The presence of platelet-associated immunoglobulins (IgG in particular) and/or C3 are frequently demonstrated in AITP but are not necessarily required for a positive diagnosis.[1–4] 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) for greater than6 months.[5] On the other hand, AITP in adults is generally chronic and often requires treatment with immunosuppressive therapy or splenectomy. Although both acute and chronic AITP are immune-mediated,[6] it now appears that different pathogenetic mechanisms are responsible for the two forms of the disease. Several T cell abnormalities have been described in patients with AITP and have been extensively reviewed elsewhere.[7] Table 1 summarizes the known T cell defects in AITP.

HYPOTHESIS

The initial stimulation for the production of platelet autoantibodies is undoubtedly driven and regulated by T helper (Th) lymphocytes and antigen presenting cells (APC). To elucidate abnormalities in these stimulatory events in chronic AITP, we have developed a hypothesis focused on platelet-reactive Th cell activation within the constraints of two basic assumptions:

(1) the platelet is the primary source of the autoantigen(s) which stimulate Th cells and (2) 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 as platelets interact with and are normally destroyed by MHC class II positive macrophages within the spleen, some of their surface glycoprotein antigens are intracellularly shunted to cellular compartments rich in MHC class II molecules. The ''processed'' platelet glycoprotein peptides, presumably generated in phagolysosomes, are ultimately re-expressed on the APC surface in association with the MHC class II molecules. As Th cells constantly home and survey antigen-laden APC within lymph nodes and the spleen, some may have TcR with sufficient affinity for the platelet antigen-MHC complex and may, with proper costimulation, be activated to drive platelet-specific 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. The major question which arises from this hypothetical scenario is where the immune defect(s) responsible for abnormal autoantibody stimulation occurs; is it an abnormal

antigen presentation event or Th cell activation event?

This report will discuss evidence which supports the view that enhanced APC-Th cell interactions in patients with AITP are important factors which influence platelet autoantibody production. These interactions may ultimately be an important focus for immune specific therapies.

CELLULAR IMMUNITY IN AITP

In AITP, a consistent finding is increased numbers of CD3+HLA-DR+ T lymphocytes which suggests that T cells are being abnormally activated in vivo.[8] CD4+ Th cell responses can be generally distinguished by their secreted cytokine products.[9–17] A Th1 response is characterized primarily by the presence of IL-2, IFN-γ, GM-CSF and TNF-α and is associated with delayed type hypersensitivity reactions and the synthesis of complement-fixing IgG isotypes.[9,10] Th2 responses on the other hand produce IL-4, IL-5, IL-6 and IL-10 and are superior in mediating non-complement fixing IgG and particularly IgE synthesis.[9,10] 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.[11–15] With respect to these responses and AITP, peripheral blood mononuclear cells (PBMC) from patients with chronic AITP can be stim ulated to secrete IL-2 when incubated with either allogenic or autologous platelets *in vitro*.[18,19] *In vivo*, we have demonstrated the presence of Th0/Th1 serum cytokines (IL-2, IL-10 and/or IFN-γ) in the sera from children with chronic AITP but not acute AITP.[20] Similar results were found *in vitro* by Garcia-Suarez *et al.*[21] who showed that PHA-stimulated PBMC from patients with chronic AITP produced elevated levels of TNF-α and IFN-γ. On the other hand, Nugent *et al.*[22] has demonstrated that IL-4 production was significantly reduced in cultures of PHA-stimulated PBMC derived from children with acute AITP. We have also demonstrated that CD19+ B cells from patients with chronic AITP can be stimulated to produce antiplatelet autoantibodies in vitro when incubated with IL-2, IFN-γ and autologous platelets.[23,24] Thus, these data suggest that the abnormal 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 be come activated, APC, particularly macrophages are absolutely required to present processed antigen in association with MHC class II molecules.[25]

ROLE OF MACROPHAGES IN AITP

Macrophages are the primary cells of the reticuloendothelial system (RES) which mediate the normal destruction of senescent platelets within the spleen.[26,27] While it is clear that these cells play a critical role at the ''end'' destructive process in AITPi.e. phagocytosis of opsonized platelets,[28–30] little work has been devoted to the APC function of macrophages in AITP. For example, the bulk of phagocytosed platelet-derived proteins probably undergo extensive terminal degradation into constitutive amino acids within lysosomes.[25,31] However, a number of inflammatory events can induce APC to up-regulate MHC class II expression[32] and significantly alter intracellular protein traffic and processing mechanisms toward endocytic compartments.[33] It is possible then that during platelet destruction a macrophage can become activated and potentially divert platelet-derived peptides towards cellular compartments rich in MHC class II molecules. This could lead to peptide loading of MHC class II molecules for expression on the APC's plasma

Table 1. Consistent Abnormalities of Cellular Immunity in Patients With Chronic AITP

In vivo activated T cells	Increase
In vitro platelet-induced IL-2 production	Increase
I*n vitro* TNF-α and IFN-γ production	Increase
In vitro IL-4 production	Decrease
Serum IL-2, IFN-γ, IL-10	Increase
In vitro IL-2/IFN-γ-mediated immunoglobulin production	Increase
I*n vitro* macrophage inhibitory factor (MIF) production	Increase
Serum M-CSF and GM-CSF	Increase
In vitro GPIIbIIIa-stimulated IL-1β production	Increase
In vivo CD68+ microparticles	Increase

membrane and subsequent activation of antigen specific T cells. To support this possibility, we have found in a murine model of platelet immunity that platelet-pulsed adherent APC can activate platelet-immune T cells in vitro to secrete IL-2.[34] Thus, after phagocytosis, APC can divert platelet antigens to MHC class II molecules for presentation. There is a large body of evidence which suggests that activated macrophages and their secreted products are associated with AITP. For example, significantly elevated serum levels of macrophage-colony stimulating factor (M-CSF) have been found in patients with chronic AITP.[35] This cytokine specifically supports the differentiation of monocytic lineage cells[36,37] and is a potent activator of macrophages; it enhances both phagocytosis and IL-1β production.[38,39] 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.[35] It has also been shown that in patients with chronic AITP, a correlation between *in vivo* antiplatelet antibodies and *in vitro* GPIIbIIIa-stimulated PBMC IL-1β secretion exists.[40] Since macrophages are a rich source of IL-1, it may be that these cells contribute to the immunopathology in chronic AITP. Furthermore, increased circulating CD68 positive microparticles have also been identified in patients with chronic AITP.[41] CD68 is a 110 kD lysosomal glycoprotein thought to be involved in endocytosis and internal membrane trafficking and is restricted to macrophages;[42,43] its surface expression on macrophages is significantly enhanced by inflammatory events.[44] 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 Th cells which stimulate macrophage phagocytosis resulting in an increase of the CD68+ microparticles and platelet destruction.[41] Similar results of increased GM-CSF levels in patients with AITP have been reported by others.[45]

In order to study macrophage/Th cell interaction in AITP, a source of stable platelet reactive T cell lines would be desirable. Although T cell clones have been generated from the peripheral blood of patients with AITP, their long term propagation has been a formidible task (JWS, unpublished results). In an attempt to circumvent this problem, we have focused on the spleens from patients with AITP. Splenic mononuclear cells have the advantage in that they are probably the site of autoimmune eitiology and large amounts of autologous mononuclear feeder cells can be harvested for long term experimentation. In preliminary experiments, we have generated 16 platelet reactive T cell lines which cross react with purified GP IIibIIIa (JWS, unpublished). We are currently, analysing their specificity using peptides from GP IIbIIIa. These lines will enable us to identify

potential T cell reactive epitopes in the GP IIibIIIa molecule.

CONCLUSIONS

It appears therefore that in addition to Th1 activation in AITP, activated macrophages are present in patients with AITP which can have enhanced interactions with platelets to stimulate T cells. By analysing platelet-reactive T cell lines at the clonal level, it will be possible to elucidate the APC/T cell interactions and identify platelet derived epitopes with activate autoreactive T cells. Understanding these events in chronic AITP will further elucidate the immune pathogenesis of the disease and ultimately lead to the development of better immune specific therapies.

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