bjh review

The pathogenesis of immune thrombocytopaenic purpura

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Summary

Immune thrombocytopaenic purpura (ITP) is an autoimmune bleeding disease that is rarely fatal. However, in many adults treatment is unsatisfactory, with as much morbidity from the immunosuppressive effects of treatment as from bleeding. Identifying the underlying disease process should help us to identify more targeted therapies and improve not only the treatment but also the quality of life of patients with this disorder.

Keywords: thrombocytopaenia, immune thrombocytopaenic purpura, pathology, T cells, cytokines.

Immune thrombocytopaenic purpura (ITP) is a heterogeneous disease characterised by increased platelet destruction and thrombocytopaenia. A number of features suggest this destruction is immune-mediated and that it may involve not only the destruction of the platelet, but also inhibition of platelet release by the megakaryocyte. The exact mechanism of the immune dysfunction, however, is generally not known. For example it is unclear whether ITP is initially caused by a B-cell abnormality, a T-cell disorder, an abnormality of thrombopoiesis, or even from increased mononuclear phagocyte activation. In addition, certain patients who apparently have ITP may have an indolent form of myelodysplasia that is not yet evident on bone marrow examination. Some of the difficulties in defining the pathology of ITP arise because it a heterogeneous disease with individual patients having different causes of thrombocytopaenia and other difficulties relate to the limited nature of assays, such as the antiplatelet antibody.

There are many issues connected to the pathology of ITP that need resolution or at least improved understanding. First, what initiates ITP; what is the underlying defect resulting in the accelerated platelet destruction? Secondly, what maintains the disease; why do some people recover while others have persistent thrombocytopaenia? Thirdly, what is the mechanism of the thrombocytopaenia in different patients? Fourthly, why do some patients have severe thrombocytopaenia and bleeding, even serious bleeding, while others with the same platelet count are relatively asymptomatic? Fifthly, why do some patients respond to certain therapies and others do not? The answers to the latter, more clinical questions presumably reflect the physiological processes in the first three questions. Finally, does 'ITP' associated with other diseases, such as systemic lupus erythematosus (SLE) and hypothyroidism have the same pathology as primary (idiopathic) ITP?

A better understanding of these questions will lead to improved management of ITP, including more appropriate choice of when to treat and which treatment to select.

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The role of the spleen

In 1916, Kaznelson, while a medical student in Vienna, prevailed upon the attending surgeon to perform a splenectomy in a patient with ITP. The splenectomy was successful in normalising the platelet count and, with other cases, first established the critical role of the spleen in ITP (Kaznelson, 1916). However, the cause of thrombocytopaenia remained unclear. Was the spleen destroying the platelets or did it secrete a suppressive substance that inhibited platelet production and/or release into the circulation? Doan *et al* (1960) examined a number of spleens from patients with ITP. They demonstrated sea blue (lipid laden) histiocytes in the spleen, suggesting it was the platelet 'destroyer'. What directed the spleen to prematurely destroy platelets, however, remained unclear.

The antiplatelet factor

The first direct evidence that ITP is caused by a plasma-derived antiplatelet factor was provided by Harrington *et al* (1951), when they showed that infusion of plasma from patients with ITP-induced thrombocytopaenia in normal recipients. Shulman *et al* (1965) demonstrated that this thrombocytopaenic effect of patient plasma was dose-dependent and that a larger dose was required to create equivalent thrombocytopaenia in recipients who had been splenectomised. They showed that the thrombocytopaenic factor was in the immunoglobulin (Ig)G-rich serum fraction that it could be adsorbed by platelets and that it reacted with autologous platelets. On this basis and because of transient neonatal thrombocytopaenia in infants of mothers with ITP, this factor was conjectured to be an antiplatelet antibody. Platelet-associated IgG was first quantified by Dixon and Rosse (1975). The initial studies utilised the

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whole platelet as the target and were very exciting because they demonstrated a high degree of sensitivity, >85–90%, in patients with ITP (reviewed by McMillan, 1981). However, it soon became clear that this high sensitivity was accompanied by a low specificity.

The specificity of the antiplatelet antibodies to individual platelet glycoproteins (GP), such as GPIIb/IIIa, was demonstrated by van Leeuwen *et al* (1982) using the platelet immunofluorescent test (PIFT). In 32 of 42 cases, platelet IgG eluated from patients with ITP bound to normal platelets but not to platelets from patients with Glanzmann's thrombasthenia, who lack platelet GPIIb/IIIa. Subsequently, both platelet-associated and plasma-derived antibodies have been identified with varying degrees of specificity and sensitivity. Current studies using platelet GP-specific assays, modelled on the monoclonal antibody immobilised platelet antigen (MAIPA; Kiefel *et al*, 1987), maintain a high specificity, 85–90%, at the expense of a lower sensitivity, 50–70%.

The coating of platelets with antibodies rarely seems to interfere with their function, as patients with ITP very infrequently suffer from major bleeding when their counts are $>50 \times 10^9$/l. Rather these antibodies target them for early destruction by the mononuclear phagocyte system (MPS), primarily in the spleen but also in the liver and bone marrow. The destruction of platelets by the MPS seems to result in the presentation of additional platelet antigens to the immune system by the antigen-presenting cells with epitope spreading. Hence, patients with chronic ITP often have antiplatelet antibody with specificity directed to multiple GP; i.e. anti-GPIIb/IIIa and anti-GPIb/IX and anti-GPIa/IIa (Cines & Blanchette, 2002).

What causes the initial development of the antiplatelet antibodies, however, is not clear. The V_H3-30 heavy chain has been found to be highly represented among platelet-reactive Fab fragments from patients with ITP when compared with its prevalence in the general library (Roark *et al*, 2002). This V_H3-30 heavy chain gene has also been implicated in the pathogenesis of diseases such as autoimmune haemolytic anaemia (AIHA), SLE, chronic lymphocytic leukaemia (CLL), common variable immunodeficiency (CVID) and human immunodeficiency virus (HIV) infection, which may explain why ITP often occurs with these diseases and is consistent with the finding that some patients with ITP have been shown to have oligoclonal B-cell populations (van der Harst *et al*, 1990). Quite separately, other studies have shown light chain restriction of antibodies in patients with ITP (Stockelberg *et al*, 1995; McMillan *et al*, 2001). These two studies suggest that antiplatelet autoantibodies may be clonally restricted and that antiplatelet antibodies are produced from a limited number of B-cell clones. As antigen-driven affinity selection and somatic mutation are involved, this indicates T cell-driven antibody production.

The above observations, suggesting B-cell abnormalities in some patients and T-cell abnormalities in others, expose one facet of the heterogeneity of the thrombocytopaenia occurring in patients with ITP.

Immune tolerance

The immune system has a number of mechanisms to establish and maintain self-tolerance. The primary mechanism is central thymic tolerance as a result of the deletion of differentiating T cells that express antigen-specific receptors with high binding affinity for intrathymic self-antigens. Only self-reactive T cells of low affinity and T cells with receptors specific for antigens that are not represented intrathymically mature and join the peripheral T-cell pool (Kruisbeek & Amsen, 1996).

However, a number of potentially pathogenic, self-reactive T cells survive and form part of the normal T-cell repertoire (Fowell & Mason, 1993; Hafler & Weiner, 1995; Sakaguchi *et al*, 1995). Control of these cells involves post-thymic or peripheral tolerance of which there are a number of mechanisms. For example, self-antigens may be sequestered from the circulation in tissues, such as the lens and the testes (Doherty, 1997). Secondly, under certain conditions, self-reactive T cells are deleted or rendered anergic because they do not receive the required co-stimulatory signals (Schwartz, 1996). Thirdly, tolerance to self-antigens can be maintained by T-regulatory cells, which keep self-reactive T cells in check (Le Douarin *et al*, 1996; Takahashi *et al*, 1998; Seddon & Mason, 1999). Finally, peripheral tolerance is also achieved by B-cell deletion in the bone marrow. Differentiating B cells that express surface immunoglobulin receptors with high binding affinity for membrane-bound self-antigens are progressively deleted as they mature in the bone marrow. The majority of antibodies expressed by early immature B cells are self-directed. Almost all of these cells are removed from the developing population at two checkpoints during B-cell development (Wardemann *et al*, 2003).

Autoimmunity

Despite these complex mechanisms to achieve and maintain tolerance, a number of self-reactive B and T cells survive. Usually these cells are not given the complex set of co-stimulatory signals required to proliferate and become activated, i.e. via CD40–CD40 ligand interactions or CD28–CD80/86; they therefore remain quiescent. Tolerance can be overcome in a variety of ways. Cross-reaction of antigens, such as those found on bacteria or viruses, may stimulate self-reacting B cells via molecular mimicry. *Helicobacter pylori* and varicella zoster virus may be examples of this. A number of antigens are able to provide T-independent signals to B cells. Polyclonal activation of B cells by stimuli as diverse as Epstein–Barr virus (EBV), malaria and graft-*versus*-host disease (GVHD) may result in autoantibodies being formed. Interruption of the checkpoints at which the immature B cells producing autoreactive antibodies are removed from the circulation will also result in an increase in the numbers of

autoantibodies (Wardemann *et al*, 2003). Finally, breakdown of the suppressor cell and also of the anti-idiotype regulatory networks can result in autoimmune reactions. This latter explanation is thought to be the one linking autoimmunity and immunodeficiency and therefore may be the one most pertinent to ITP.

An additional potential factor in the autoimmunity of ITP is that platelets can present themselves to the immune system by membrane major histocompatibility complex (MHC) class I molecules, enabling destruction by cell-mediated mechanisms. When activated, platelets increase their expression of CD40L, and potentially other immune molecules, which may contribute to their immunological recognition and therefore to the development of autoimmunity to platelets. Human leucocyte antigen (HLA) types have only been clearly linked to the development or clinical course of ITP in genetically homogeneous populations, such as the Japanese (Nomura *et al*, 1998). Nonetheless, immune recognition as a result of HLA may also be critical in development of autoimmunity in heterogeneous populations.

Th1 and Th2 responses

$CD4^+$ helper T (Th) cells are thought to be the most important regulatory component of the immune system. They regulate the response to infection and control the immune system to prevent autoimmunity. Th cells secrete cytokines and modulate the cellular response to antigens.

Two main 'types' of T-helper cells (Th1 and Th2) have been described. While an oversimplification, they nonetheless are useful in considering patterns of the immune responses. Generally, Th1 cytokines include interferon (IFN)-γ, tumour necrosis factor (TNF)-β and interleukin (IL)-2. Th1 cells are involved in cell-mediated inflammatory reactions and delayed hypersensitivity reactions. Several of the Th1 cytokines activate cytotoxic, inflammatory and delayed hypersensitivity reactions. In contrast, Th2 cells produce IL-4, IL-5, IL-6, IL-9, IL-10 and IL-13. These cells encourage the production of antibodies and are associated with regulation of strong antibody and allergic responses.

T-cell abnormalities in patients with ITP

A number of T-cell abnormalities have been demonstrated in patients with ITP (summarised in Fig 1) and it is likely that there are three main mechanisms by which T cells could be involved in the thrombocytopaenia in patients with ITP. First, a number of studies suggest a Th1 bias, compared with Th2, in adults with chronic ITP. For example, increased numbers of HLA-DR+ T cells, increased soluble IL-2 receptors, and a cytokine profile suggesting the activation of precursor helper T and type 1 helper T cells have been described (Semple *et al*, 1996, reviewed in Andersson & Wadenvik, 2004). Reduced levels of IL-10 have also been described in patients with active disease when compared with those in remission or healthy controls (Andersson *et al*, 2002) but conversely, raised IL-10 levels have been described in children with chronic ITP (Semple *et al*, 1996; Mouzaki *et al*, 2002). Further evidence of Th1 involvement in the pathology of ITP is illustrated by an increase in the Th1 cytokines, IL-2 and IFN-γ, in patients with ITP when compared with controls (Panitsas *et al*, 2004). Interestingly, this increase was more marked in patients in remission than in those with active disease. The same study also found suppression of expression of Th2 cytokines, IL-4 and IL-5, in patients with active disease relative to patients in remission and suppression of IL-10 expression, following

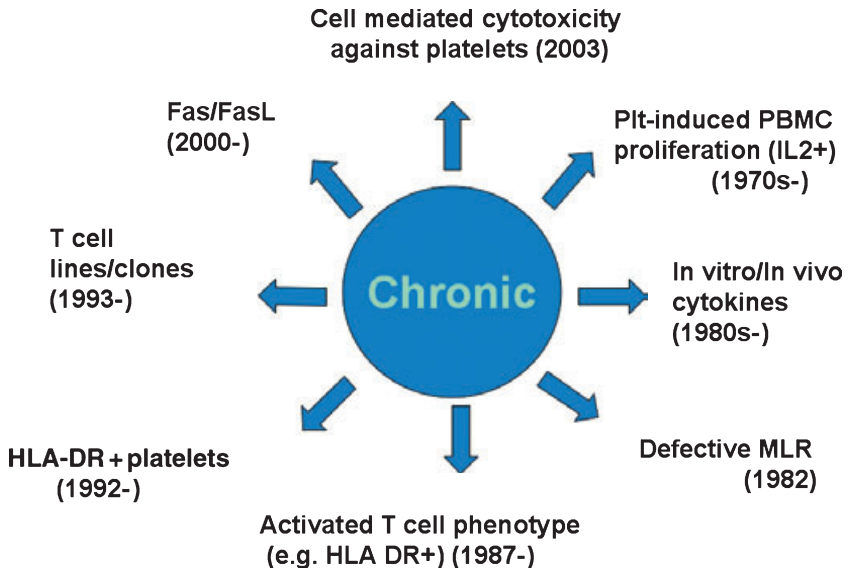

Fig 1. Summary of the variety of T-cell abnormalities found in adults with ITP. PBMC, peripheral blood mononuclear cells; MLR, mixed lymphocyte reaction; ITP, immune thrombocytopaenic purpura.

mitogenic stimulation in patients with active disease. Overall, they describe significantly increased Th1/Th2 ratios in patients with both active and quiescent disease when compared with controls. These findings may be related to ongoing immune activation as part of autoimmunity. The activity of regulatory T cells and the potential for *in vivo* T-cell exhaustion because of prolonged *in vivo* activation has not been well studied in ITP.

A second method of potential T-cell involvement is the release of cytokines that interfere with megakaryocyte maturation and/or platelet release. Transforming growth factor (TGF)-β1 level has been inversely correlated with disease activity (Yoshimura *et al*, 2000; Andersson *et al*, 2002). The role of TGF-β1 in ITP is thought to be as a potent inhibitor of megakaryocyte maturation. Two studies have shown increased granulocyte-macrophage colony-stimulating factor (GM-CSF) levels, and one increased macrophage (M) CSF levels, suggesting that monocyte-macrophage activation is associated with ITP (Abboud *et al*, 1996). Circulating cytokines may also alter the response of HLA class II presentation, and/or influence the interaction between B and T lymphocytes causing pre-existing B cells to proliferate and produce high-affinity autoantibodies (reviewed by Chanock, 2003).

Finally, there is evidence to suggests a direct cytotoxic effect of T cells, as illustrated in Fig 2. By DNA microarray screening, Olsson *et al* (2003) found increased expression of several cytotoxic genes, such as granzyme A, granzyme B and perforin, as well as increased expression of genes involved in the Th1 cell response, such as INFγ and IL-2 receptor-β in a small number of patients with ITP when compared with controls. As apparent compensation for this increased cytotoxicity, they also found increased expression of the killer cell immunoglobulin-like receptor (KIR) family on $CD3^+$ T cells in patients with ITP in remission when compared with controls and to those with active ITP. KIRs downregulate cytotoxic T lymphocytes (CTL) and natural killer cell (NK) responses by binding to MHC class 1 molecules, preventing lysis of target cells. These findings suggest that CTLs may be involved in ITP. In a direct assay similar to that measuring NK cell activity by using radiolabelled K562 targets, these investigators assessed platelet destruction *in vitro* by T cells. They found that six of eight patients with active ITP showed platelet lysis by T cells whereas none of the patients in remission did. The effector cells were found to be $CD3^+CD8^+$ T cells (Olsson *et al*, 2003).

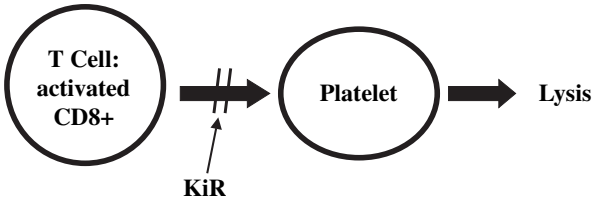

Fig 2. Hypothetical effect of T cells on platelets in immune thrombocytopaenic purpura (ITP).

This expanded role of cytotoxic T cells may explain why not all of the patients originally described by Harrington *et al* (1951) had a fall in their platelet count following the infusion of plasma from patients with ITP, and may also explain a percentage of patients without measurable antiplatelet antibodies, and again points to the heterogeneity of this disease.

Complement

The role of complement (C) and complement receptors in the pathogenesis of ITP has yet to be defined. Several studies have demonstrated increased platelet-associated C3 and C4 on ITP platelets but these are thought to be secondary in importance to platelet IgG and/or the result of antiplatelet IgM (Hed, 1998). Furthermore, there is an association of (especially) C4 deficiency and ITP that has not been well studied and is of additional interest because the C4 genes are in the midst of the HLA region on chromosome 6.

Megakaryocytes and platelet production

It has always been assumed that there is compensatory but inadequate increased platelet production in patients with ITP. The two initial studies of platelet survival suggested that platelet production was increased but only by one- to threefold and not in all patients (Harker, 1970; Branehog *et al*, 1974). A number of follow-up studies using chromium-labelled allogeneic platelets confirmed 'rapid platelet turnover'; several even showed a platelet survival time as short as several minutes (Mueller-Eckhardt, 1988). Calculations based on the apparent high rate of turnover suggested a substantially increased platelet production. However, in the early 1980s, the survival time of circulating autologous platelets in several studies using 111-indium showed that platelet survival was longer than expected (near normal). Therefore platelet turnover, and by inference platelet production, was found either to be decreased or at best normal in approximately two-thirds of patients with ITP (Heyns *et al*, 1982, 1986; Stoll *et al*, 1985; Ballem *et al*, 1987; Gernsheimer *et al*, 1989).

This finding is presumed to be due to a direct effect of antibody on megakaryocyte maturation or platelet release. An antibody effect on megakaryocytes is consistent with the fact that megakaryocytes are known to express GPIIb–IIIa and GPIb–IX on their surfaces (Vainchenker *et al*, 1982) that most ITP antiplatelet autoantibodies react with one or both of these GP complexes (McMillan *et al*, 1987; Kiefel *et al*, 1992) and also that platelet antibody has been demonstrated on megakaryocytes similar to the way that antibodies have been shown to be bound to platelets. However, direct megakaryocyte or intramedullary platelet destruction is unlikely because of the adequate numbers of megakaryocytes and the absence of lipid-laden macrophages in the bone marrow.

Two recent studies examined the effects of ITP plasma on control megakaryocytopoiesis *in vitro*. Chang *et al* (2003) assessed the effect of plasma from patients with childhood ITP

(44 acute and nine chronic) on thrombopoietin (TPO)-induced production of megakaryocytes from cord blood cells in liquid culture *in vitro*. They described suppression of *in vitro* production of megakaryocytes from cord blood cells by plasma from ITP patients with detectable antiplatelet antibodies. Plasma from control subjects or patients with ITP without detectable antibodies did not have the same effect (Chang *et al*, 2003). McMillan *et al* (2004) similarly studied the effect of plasma from adult patients with chronic ITP on *in vitro* megakaryocytopoiesis. $CD34^+$ cells from healthy donors were cultured in medium-containing pegylated recombinant human megakaryocyte growth and development factor (PEG-rHuM-GDF, a form of TPO) and 10% plasma from either ITP patients or healthy subjects. The cultures containing plasma from 12 of 18 ITP patients showed a significant decrease (26–95%) in megakaryocyte production when compared with control cultures. ITP plasma also inhibited megakaryocyte maturation, resulting in fewer 4N, 8N and 16N cells. A study of 205 patients with chronic ITP found only one case of anti-TPO antibody, demonstrating that this was not a factor in these findings (Aledort *et al*, 2004). Finally, a number of studies have shown contradictory results, describing either increased numbers of megakaryocyte colony-forming units (CFU-M; Bellucci *et al*, 1991; Houwerzijl *et al*, 2004), or decreased numbers of CFU-M in patients with ITP (Abgrall *et al*, 1993).

While antibodies appear to mediate this effect on platelet production, other mechanisms, i.e. T cell-mediated inhibition of platelet production, are possible and largely unexplored and could have an effect by altering the cytokine milieu of the bone marrow. In addition, as previously commented upon, rare cases of myelodysplasia, characterised as having poor platelet production, may resemble 'refractory' ITP especially because they may also have a component of autoimmunity.

Recent studies with a thrombopoietic agent in ITP have suggested that there is a dose-dependent increase in the platelet count following a single or multiple injections (Bussel *et al*, 2003; Kuter *et al*, 2004; Newland *et al*, 2004). This is in contrast to the lack of effect of rHuIL-11 (Bussel *et al*, 2001). *In vitro* studies in mice have explored the role of TPO and discovered that, although it stimulates proliferation of precursor cells (CFU-M), other agents, such as fibroblast growth factor (FGF) and stromal cell-derived factor (SDF1), may be required to facilitate platelet release into the circulation (Avecilla *et al*, 2004). This may in part explain the residual platelet level (15% of normal) seen in TPO or TPO receptor (TPO-R) knockout mice. Humans with mutations of the TPO-R, who have amegakaryocytic thrombocytopaenia; however, have platelet counts that are typically considerably <15% of normal, i.e. $<10 \times 10^9$/l suggesting that chemokines may have a different role in humans.

In addition, even early studies of the bone marrow in patients with ITP described normal or high numbers of megakaryocytes with an increase in younger forms lacking cytoplasmic granularity and platelet formation and displaying degenerative changes in the nucleus and cytoplasm (Dameshek & Miller, 1946; Diggs & Hewlett, 1948). Subsequent electron microscopic studies showed that 50–75% of ITP megakaryocytes had extensive damage, with abnormalities of the membrane system. In some cases, damaged cells showed attached monocytes, which appeared to phagocytose megakaryocyte fragments (Stahl *et al*, 1986). More recently, ultrastructural abnormalities compatible with apoptosis have been described in around 78% of ITP megakaryocytes (Houwerzijl *et al*, 2004). These features were reversed by *in vivo* treatment with prednisone and intravenous immunoglobulin. The same group also described extensive apoptosis and an increased proportion of megakaryocytes with activated caspase-3 ($28 \pm 4\%$ vs. 0%) in bone marrow biopsies of ITP patients. This may just represent the normal process by which megakaryocytes release platelets, which is increased in patients with ITP, or could suggest a direct effect of autoacting B or T cells on megakaryocytes.

In summary, it appears that antiplatelet GP antibodies and, possibly, antiplatelet T cells have effects on megakaryocytes as well as on platelets, probably contributing to thrombocytopaenia in a substantial number of patients. Studies have shown both that TPO levels are inappropriately low (essentially the same as in normal controls) in thrombocytopaenic patients with ITP and that treatment with thrombopoietic agents is capable of dramatically increasing the platelet count in the majority of patients. These findings are consistent with the hypothesis that decreased platelet production is more central to ITP than had been previously appreciated.

Fc receptors

Platelets coated by antibody are thought to be targeted for destruction by the MPS. While complement receptors may contribute to this process, this is thought to be primarily mediated by the Fc receptor (FcR) system. The net FcR effect is based on the balance of interaction of antibody-coated particles between activating (FcγRIIA and FcγRIIIA) receptors and inhibitory receptors (FcγRIIB). The role of FcγRI is uncertain although infusion of blocking antibody to it increased the platelet count in a small number of patients with ITP (Terjanian *et al*, 2000). How this balance is determined remains unclear. Studies of single nucleotide polymorphisms (SNPs) and clinical responses preliminarily suggest that individual FcγR may be particularly important for response to given treatments. Specifically, cytokine responses to IV anti-D appear to depend upon FcγRIIA (Cooper *et al*, 2004a) and rituximab on FcγRIIIA (at least for lymphomas). In mice, blocking studies show that response to IVIG depends upon FcγRIIB (Samuelsson *et al*, 2001). Exploration of FcγRN in mouse models suggest that it is important in recycling antiplatelet antibody (reviewed in Bussel, 2000).

Precipitating events

The studies summarised above describe some of the abnormalities seen in patients with ITP, including B cell, T cell and megakaryocyte abnormalities. How these interruptions in the normal functioning of the immune system (i.e. loss of tolerance) occur is not clear. It is likely that a combination of genetic susceptibility and environmental factors are involved. The next section will attempt to address some of these issues.

Genetic susceptibility

Unlike other autoimmune diseases, a series of studies have demonstrated only a weak association with HLAB8DR3 in patients with ITP (Stanworth *et al*, 2002). HLA types have only been clearly linked to the development or clinical course of ITP in genetically homogeneous populations, such as the Japanese (Nomura *et al*, 1998). Nonetheless, immune recognition as a result of HLA may be critical in development of autoimmunity in heterogeneous populations as well. ITP is infrequently found in close family members, although a handful of families in which a number of autoimmune cytopenias, such as ITP, autoimmune neutropenia and AIHA occur within different siblings have been described.

Immune thrombocytopaenic purpura is, however, found in increased incidence in patients with immunodeficiency diseases. It is most common in B-cell disorders, including CVID (Cunningham-Rundles & Bodian, 1999), secondary hypogammaglobulinaemia, selective IgA deficiency (Khalifa *et al*, 1976), autoimmune lymphoproliferative syndrome (ALPS) and CD40 ligand deficiency (hyper-IgM syndrome). However, the association between ITP and immunodeficiency is also likely to be related to T-cell dysregulation (Arkwright *et al*, 2002). Of note, patients with Bruton's X-linked agammaglobulinaemia, who have no B cells but normal T cells, are more profoundly hypogammaglobulinaemic than CVID patients yet do not seem to have ITP or other autoimmune diseases. This could relate to the presence of normal T cells or to the absence of abnormal B cells.

Recent genomic advances have enabled the assessment of genetic factors influencing the pattern of ITP. In a pilot study of childhood ITP, common genetic polymorphisms in TNF lymphotoxin (LTA), as well as the FcγRIIa and FcγRIIIb, have all been identified as possible factors contributing to development of chronic disease (Foster *et al*, 2001). In a separate study of children with ITP, these variants were associated with the development of ITP but did not differentiate who would develop chronic ITP (Carcao *et al*, 2003). FcγRIIa variants could predict for response to immunoglobulin therapy (Lehrnbecher *et al*, 1999; Fujimoto *et al*, 2001). Alterations in immune complex (antibody bound to antigen) clearance and/or processing are thought to explain the role of FcγRs in ITP.

In a study of SNPs performed in Japanese subjects, an increase was found in the frequency of the TNF-β (+252) G/G phenotype in patients with ITP when compared with control (21% vs. 7%, $P = 0{\cdot}04$). Furthermore, the frequency of circulating anti-GPIIb/IIIa antibody-producing B cells was significantly higher in ITP patients with the TNF-β (+252) G/G phenotype than those with the G/A or A/A phenotype, suggesting that the SNP located at TNF-β (+252) contributes to susceptibility to chronic ITP by controlling the B-cell response to platelet membrane GPs (Satoh *et al*, 2004). These findings are presumably just scratching the surface of the SNPs that play a role in development of or natural history of ITP.

Environmental effects

Virus-associated ITP

Childhood ITP often occurs following a viral illness. This viral infection is cleared normally but initiates ITP probably either via molecular mimicry or B-cell stimulation, although the exact mechanism has not been explored. ITP is often associated with HIV (Bettaieb *et al*, 1992), hepatitis C virus (HCV) infection (Pockros *et al*, 2002; Zhang *et al*, 2003) and EBV infection. Treatment of especially not only HIV but also, to a lesser extent, HCV with antiviral medication frequently results in a platelet increment in this setting in which the infection is persistent and this persistence directly contributes to the thrombocytopaenia.

It is likely that these viruses act in different ways to induce secondary ITP with loss of tolerance. HIV causes T-cell immunodeficiency, with an eventual profound decrease in T-helper CD4 cells. These cells are vital for immune regulation. The autoimmune diseases that predominate in AIDS are generally CD8-driven. There is also evidence for B-cell stimulation, possibly via loss of T-cell regulation, and a wide variety of autoantibodies are reported in HIV patients (Zandman-Goddard & Shoenfeld, 2002). Interestingly ITP often occurs shortly before the fall in CD4 counts and occurrence of AIDS defining diseases. The relationship between HCV and ITP is less clear, but this virus appears to generally increase the production of a number of autoantibodies (Pivetti *et al*, 1996). EBV has a direct effect on B cells, inducing lymphoproliferation and increased antibody formation. ITP following EBV is typically transient in immunocompetent patients.

It is also likely that viral infections have other effects in the immune system. For example, Musaji *et al* (2004) showed a more dramatic fall in the platelet count in mice with ITP induced by infusion of antiplatelet antibodies when mouse lactate dehydrogenase-elevating (LDH) virus was added before the antiplatelet antibody, when compared with antiplatelet antibody alone. These findings suggest that macrophage activation is an important component in disease severity (see studies cited above regarding GM-CSF and M-CSF). The increased antibody-coated platelet destruction seen with macrophage activation could explain viral-induced exacerbation of ITP and cyclical thrombocytopaenia.

Bacteria-associated ITP: H. pylori

A number of studies have suggested an association between *H. pylori* and ITP. Gasbarrini *et al* (1998) reported a significant platelet increase in all eight *H. pylori*-positive patients with ITP in whom *H. pylori* was eradicated. A large number of studies from Italy and Japan have subsequently suggested an increased prevalence of *H. pylori* in patients with ITP and shown a response rate (either partial or complete platelet increment) of between 38% and 73% in patients in whom *H. pylori* is eradicated. However, studies from France, Spain and the USA have not replicated these results, suggesting that *H. pylori* may have different pathogenicity depending on the area studied (Michel *et al*, 2003; Franchini & Veneri, 2004). Patients with more newly diagnosed ITP and those with milder thrombocytopaenia may be more likely to increase their platelet counts following eradication of *H. pylori* (Stasi *et al*, 2005).

The associations between pathogens, such as *H. pylori*, and ITP may be related to molecular mimicry, where the production of antibodies to the pathogen causes the production of antibodies to self. Platelet eluates from patients who were both *H. pylori*-negative and *H. pylori*-positive recognise *H. pylori* cytotoxin-associated gene A (CagA) protein (Takahashi *et al*, 2004). The same study also found that in some patients whose platelet count responded to *H. pylori* eradication, levels of anti-CagA antibody in platelet eluates declined, thus suggesting that molecular mimicry may occur in the pathogenesis of at least some patients with ITP. Alternatively, chronic infection may change the cytokine milieu, encourage loss of tolerance and stimulate B cells. *Helicobacter pylori* has been described to both causes a Th1-type of response, a response that has been described in a number of patients with ITP, and to directly stimulate B cells (D'Elios *et al*, 2005; Goll *et al*, 2005; Yun *et al*, 2005).

Conclusion

Some of the difficulties in describing the pathology of ITP are the apparently large number of different underlying causes of and contributing factors to thrombocytopaenia (Fig 3). What causes the loss of tolerance to one's own platelets remains unclear and is likely to be a result of a number of different co-operating factors including genetics, environment and acute precipitating events. As has been discussed above, most people have B cells directed to make autoantibody as well as detectable peripheral blood T cells reactive to GPIIb/IIIa. Therefore, the immunological machinery does not need to be created *de novo* but merely turned on.

The limited reports illustrating genomic associations suggest that patients with ITP may have a more general genetic susceptibility towards autoantiplatelet antibody production. The associations with other diseases including autoimmune thyroid disease and SLE support this hypothesis, even though a strong direct association with an HLA-type has not been found.

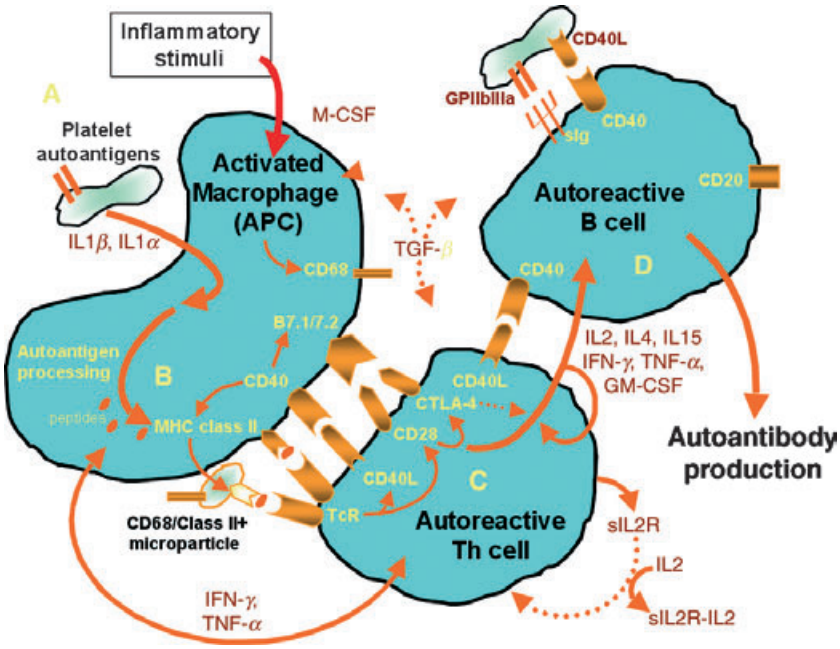

Fig 3. Summary of the complex mechanism of antiplatelet activity in patients with immune thrombocytopaenic purpura (ITP) including: platelet antigen presentation to macrophages, activation of macrophages by inflammatory cytokines, for example, following viral infection, interaction of macrophages with T cells, stimulation of T cells and interactions between T and B cells. This figure shows the complex interactions between the immune cells. It is likely that different parts of this network are abnormal in different patients. The heterogeneity of patients with ITP explains the difficulties in diagnosis and the often variable response to treatment (adapted from Coopamah *et al* (2003), with permission from Elsevier).

The earliest studies and many since have suggested that in the majority of patients, ITP is caused by an antiplatelet antibody. Why only these patients produce antibodies to self that result in clinically significant thrombocytopaenia remains unexplained. There is evidence of a B-cell disorder in some patients, yet on the other hand, a variety of T cell and cytokine abnormalities have been reported, suggesting that T cells are likely to be involved in the majority of cases of ITP. This could occur either by a direct cytotoxic effect as recently suggested by Olsson *et al* (2003) or perhaps more commonly by inappropriate stimulation of autoimmune B cells by over activated T cells. In addition, T cells could mediate damage of the platelet that subsequently results in antiplatelet antibodies being formed.

The genetic susceptibility is only well documented thus far in regard to FcR polymorphisms, which may result in abnormal handling of pathogens specifically related to their processing by antigen-presenting cells. In the context of the appropriate genetic setting modulated by environmental influences, such as diet, drug or toxin effect, the final incident resulting in immune thrombocytopaenia may be triggered by an external event. For example, inadequate clearance of *H. pylori* may result in continued immune stimulation and molecular mimicry. Alternatively, interference with the regulation of T cells by HIV infection results in inadequate regulation of tolerance. While genetic factors are clearly important, the absence of any true tendency to familial ITP argues for the importance of environmental factors and precipitating events. Many different SNPs may affect the response to different environmental factors and precipitating events.

As previously mentioned, autoreactive T and B cells escape central deletion (central tolerance) and are usually kept quiescent by lack of stimulation: antigen must be presented and simultaneous co-stimulation is required. Alternatively, T-regulatory cells ($CD4^+CD25^+$) may mediate peripheral control of cytotoxic T cells. The presentation of platelet antigens will stimulate the proliferation of these cells. Why should platelets suddenly be presented? Perhaps there is an abnormal response to infection, viral or bacterial. Even with this, antibody-coated platelets may continuously circulate, but stimulation of the phagocytic cells by viruses, with co-stimulation via platelet expression of CD40 ligand, may be needed for MPS activation with enhanced phagocytosis and resulting thrombocytopaenia. In turn, this process may recruit additional autoreactive T and B cells while epitope spreading may occur concomitantly. Finally, macrophage activation and enhanced phagocytosis may take a mild, subclinical immune thrombocytopaenia and convert it into serious chronic ITP.

In conclusion, ITP is likely to be due to different immune defects in individual patients. This causes problems in identifying the underlying pathology and almost certainly contributes to the differing response rates to certain treatments, such as the 60–70% complete response (CR) to splenectomy (Pawelski *et al*, 1981; Coon, 1987; Fabris *et al*, 1989) and the 30% CR to rituximab (Cooper *et al*, 2004b). Many additional studies will be required before testing to determine clinical outcomes will readily be available.

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