

RECENT ADVANCES IN CHILDHOOD ITP

Manas Kalra, S P Yadav, Anupam Sachdeva

Pediatric Hematology, Oncology and Bone Marrow Transplant Unit,
Sir Ganga Ram Hospital, Rajinder Nagar, New Delhi-110060, India

Abstract : Immune thrombocytopenic purpura (ITP) is a disorder mediated by antiplatelet antibodies and characterized by accelerated destruction of platelets and impaired platelet production. The result is thrombocytopenia of varying degrees. Clinically recognized cases are typically associated with marked, isolated thrombocytopenia, mucocutaneous bleeding, and, rarely, more severe hemorrhage (ie, intracranial hemorrhage). Recent advances in our understanding of the specific pathways involved in immune-mediated platelet destruction and the significance of suboptimal thrombopoiesis have led to the development and investigation of new therapeutic agents. This article discusses the current knowledge on pathophysiology and new information on pharmacologic approaches, tolerability, toxicity, and efficacy data for established and novel investigational therapies for patients with this relatively common disorder of childhood.

The clinical signs and symptoms of idiopathic immune thrombocytopenic purpura (ITP) are caused by an increased rate of premature platelet destruction which occurs preferentially in the spleen, liver, bone marrow and lung. It was long suspected that immune thrombocytopenic purpura is mediated by autoantibodies, since transient thrombocytopenia occurs in neonates born to affected women, and this suspicion was confirmed on the basis of the development of transient thrombocytopenia in healthy recipients after the passive transfer of plasma, including IgG-rich fractions, from patients with immune thrombocytopenic purpura. The thrombocytopenia of ITP is mainly attributed to the early destruction of platelets by the activated reticuloendothelial system, following their sensitization by antiplatelet glycoprotein autoantibodies^{1,2}. Other mechanisms such as complement mediated lysis³, ineffective thrombopoiesis⁴, or direct T-cell cytotoxicity also contribute⁵. The severity of thrombocytopenia thus reflects the balance between platelet production by megakaryocytes and the accelerated clearance of sensitized platelets. This article focuses on the recent insights in the pathophysiology and the management of ITP in children .

PLATELET ANTIGEN AND AUTOANTIBODY

Platelets coated with IgG autoantibodies undergo accelerated clearance through Fc receptors that are expressed by tissue macrophages, predominantly in the spleen and liver. A compensatory increase in platelet production occurs in most patients. In others, platelet production appears to be impaired, as a result of either intramedullary destruction of antibody-coated platelets by macrophages or the inhibition of megakaryopoiesis⁶. The level of thrombopoietin is not increased⁷, reflecting the presence of the normal megakaryocyte mass. The first antigen to be identified was recognized on the basis of the failure of immune thrombocytopenic purpura antibodies to bind to platelets that were genetically deficient in the glycoprotein IIb/IIIa complex⁶. Antibodies that react with glycoproteins Ib/IX, Ia/IIa, IV, V and diverse other platelet determinants have since been identified⁸, and the presence of antibodies against multiple antigens is typical⁹. The destruction of platelets within antigen-presenting cells — presumably, although not necessarily, initiated by antibody — may generate a succession of neoantigens, resulting in sufficient antibody production to cause thrombocytopenia.

MECHANISM OF IMMUNE DESTRUCTION

The rapid destruction of platelets is due either to autoantibodies that bind via the antigenic site or immune complexes via Fc receptors on platelets. These opsonized cells are rapidly removed by cells of the mononuclear phagocytic system. The quantity of antibodies correlates with the severity of thrombocytopenia. Phagocytosis of platelets has been demonstrated by in vivo studies using reticuloendothelial blockade with monoclonal anti-Fc receptor antibodies. The factors that initiate autoantibody production are unknown. Most patients have antibodies against several platelet-surface glycoproteins at the time the disease becomes clinically evident. Here, glycoprotein IIb/IIIa is recognized by autoantibody (orange, inset), whereas antibodies that recognize the glycoprotein Ib/IX complex have not been generated at this stage. Antibody-coated platelets bind to antigen-presenting cells (macrophages or dendritic cells) through Fc receptors and are then internalized and degraded. Antigen-presenting cells not only degrade glycoprotein IIb/IIIa, thereby amplifying the initial immune response, but also may generate cryptic epitopes from other platelet glycoproteins. Activated antigen-presenting cells express these novel peptides on the cell surface along with co-stimulatory help (represented in part by the interaction between CD40 on the activated macrophages and CD154 on the T cell) and the relevant cytokines that facilitate the proliferation of the initiating CD4-positive T-cell clones. B-cell immunoglobulin receptors that recognize additional platelet antigens are thereby also induced to proliferate and synthesize anti-glycoprotein Ib/IX antibodies in addition to amplifying the production of anti-glycoprotein IIb/IIIa antibodies by B-cell clone 1. Naturally occurring antibodies against glycoprotein IIb/IIIa show clonal restriction in light-chain use^{8,9}, and antibodies derived from phage-display libraries show highly constrained VH gene use. Sequencing of the antigen-combining regions of these antibodies suggests that they originate from a limited number of B-cell clones by antigen-driven affinity selection and somatic mutation. Some patients with immune thrombocytopenic purpura often have increased numbers of HLA-DR+ T cells, increased numbers of soluble interleukin-2 receptors, and a cytokine profile suggesting the activation of precursor helper T and type 1 helper T cells. In these patients, T cells stimulate the synthesis of antibody after exposure to fragments of glycoprotein IIb/IIIa but not after exposure to native proteins. The derivation of these cryptic epitopes

in vivo and the reason for sustained T-cell activation are unknown. *Dysfunctional cellular immunity* is considered important in ITP pathophysiology⁵. Several studies have found evidence supporting a T helper 0 (Th0)/Th1 polarization of the immune response in ITP¹⁰, whereas others have yielded inconsistent or opposing results. Other studies¹¹ have shown the presence of activated platelet-specific auto reactive T cells that recognize and respond to autologous platelet antigens and drive the generation of platelet reactive autoantibodies by B cells in the peripheral blood of ITP patients. The autoimmune process is believed to be seated in the spleen; memory platelet-specific T cells are released into the peripheral circulation¹².

Eradication of Helicobacter pylori infection has been variably associated with a platelet response in patients with ITP. Responses occur in approximately half of ITP patients infected with this bacterium, more frequently in Japan and Italy than in other countries. For those with severe ITP (platelet count $< 30 \times 10^9/L$) and a long duration of disease, eradication therapy seems to be less effective. Despite extensive efforts, distinctive clinical features and factors predicting the response to eradication therapy have not been consistently identified. There is no established mechanism to explain how H pylori could be implicated in the pathogenesis of an immune-mediated platelet destruction. Several theories have been proposed to explain the platelet response to anti-H pylori therapy, including molecular mimicry, platelet aggregation, and the induction of a Th1 phenotype that favours the onset and/or persistence of ITP. The role of bacterium-related factors, such as the CagA (cytotoxin-associated gene A) protein, are still under investigation. Eradication therapy is simple and inexpensive, with limited toxicity and the advantage of avoiding long-term immunosuppressive treatment for those who respond. Although the evidence and follow-up are limited, it appears reasonable to routinely screen patients with ITP for H pylori, particularly in those populations with a high background prevalence of H pylori infection.

TREATMENT OF ITP

Many new therapies are now in early stages of development for the treatment of ITP. These new treatments have diverse mechanisms of action, aimed at amelioration of platelet destruction and enhancing platelet production. This progress has resulted from a sustained commitment to improving our understanding of megakaryopoiesis, thrombopoiesis, and autoimmunity, and a further elucidation of the mechanism of action of traditional therapies. Additional work is required to optimize efficacy and response rates, although given the natural heterogeneity in diseased pathophysiology among patients who have a diagnosis of ITP, it is unlikely that any single treatment

will be universally applicable. Further detailing of the pathologic factors underlying the variation among patients in clinical course and treatment response is required to enable further progress in drug development and will improve selection of the most appropriate management strategies for patients who have ITP.

“Observation Only” Approach

One of the most significant developments in management of acute and chronic ITP over last two decades has been wide acceptance of “Observation Only Approach.” Factors which have led to increasing acceptance of this approach include:

- Recognition that intracranial hemorrhage (ICH) - the most feared clinical feature (or complication) of ITP is extremely rare.
- Better understanding of natural history of ITP.
- Absence of significant bleeding even in patients with severe thrombocytopenia (platelet count $< 20 \times 10^3/mm^3$),
- Publication (and acceptance) of guidelines for treatment of ITP in children and adults.

Most of the recent treatment guidelines suggest taking into account the bleeding symptoms rather than low platelet counts alone for making decisions regarding drug therapy in acute ITP. Children with mild clinical disease can be managed with watchful waiting. Drug treatment should be reserved for children with moderate bleeding symptoms and platelet count $< 20 \times 10^3/mm^3$ or children with severe bleeds. The fear of ICH and high morbidity and mortality associated with it prompts most physicians to treat ITP. The risk of ICH was 0.9% of 1693 children included in various series reviewed by George and co-workers¹³. Even this figure is considered an overestimate as most series reviewed by them originated from academic centres

which are likely to have referred cases. Lilleyman estimated a much lower incidence of 0.2% based on UK data. A similar incidence of 0.17% has recently been reported in a series of 1742 newly diagnosed children with ITP¹⁴. Over the last decade, two surveys conducted in UK included 425 and 304 children respectively. In both these surveys, severe thrombocytopenia (platelet count $< 20 \times 10^3/mm^3$) was observed in 84% and 89% children. However, severe bleeding occurred in 3% and 5% cases only. Even moderate bleeding symptoms were observed in 21% and 22 % cases^{15,16}. Chandra et al have also reported similar observations in children with acute or chronic ITP during periods of severe thrombocytopenia. Most of the cases in their series also had no or only skin bleeds during severe thrombocytopenia¹⁷. These data suggest that most children with ITP have minor or moderate bleeding manifestations even when they have severe thrombocytopenia. Natural history of acute ITP in children is more clearly defined now. George and co-workers reviewed 12 series including 1597 cases. Complete remission rate of 74% was observed by them¹³. Kuhne and co-workers reported remission rates of 68%, 73 % and 66% in children with acute ITP receiving no treatment, intravenous immunoglobulins (IVIg) and corticosteroids respectively. These observations highlight excellent outcome of ITP in children irrespective of initial treatment¹⁸. The predictors of early response are identified and scoring system to that effect has recently been reported using abrupt onset, preceding viral infection, wet purpura, platelet count $< 5 \times 10^3/mm^3$, age under 10 years and male gender as predictors¹⁹. Development of scoring system for various bleeding manifestations and severity of ITP has made assigning the severity to a given case more objective which helps in decision making regarding need for therapeutic intervention^{15,20}. Over the years guidelines for management of ITP have been brought out by American Society of Hematology, British Society of Hematology and British Committee on Standards in Hematology^{13,15,21}. As a result of these new developments, there is wider acceptance of Observation Only Approach for management of ITP in children. UK surveys clearly recorded this change as 60% cases were

treated in the first survey while in the survey performed after wider circulation of guidelines showed the decline in number of cases receiving treatment to 37% only^{15,16}. A survey from Nordic countries has shown that only 57% cases were given platelet – enhancing therapy²².

ADVANCES IN DRUG THERAPY

IVIG has been used for treating ITP since 1981²³. Transient blockade of Fc receptor of macrophages in the reticuloendothelial system, particularly in spleen, is believed to play a major role in prompt increase in platelet count after IVIG infusion. Other mechanisms through which IVIG acts include inhibition of antibody binding to

platelets due to presence of anti-idiotypic antibodies in IVIG preparations and decreased antibody production through suppression of B lymphocytes. In addition, clearance of red cells has been demonstrated after IVIG infusion which is supposed to have at least a minor role in Fc blockade^{24,26}. The latter mechanism forms the basis of use of anti-D in treatment of ITP. In a series by Blanchette and co-workers, the rate of platelet response was faster in children treated with IVIG compared to those who were not treated with drugs²⁷. In their second study, they compared two IVIG treatment regimens (1 g/kg on 2 consecutive days and 0.8 g/kg once), oral prednisone (4 mg/kg per day for 7 days with tapering and discontinuation by day 21), and for the subset of children who were

blood group rhesus (D) positive, IV anti-D (25mg/kg on 2 consecutive days). The key findings from this second randomized trial in children who had newly diagnosed ITP and platelet counts less than $20 \times 10^9/L$ were (1) a single dose of IVIG (0.8 g/kg) was as effective as the larger dose of IVIG 1 g/kg for 2 days in raising the platelet count and (2) both IVIG regimens were superior to IV anti-D administered as 25 mg/kg for 2 days for the clinically important endpoint of time (number of days) to achieve a platelet count greater than or equal to $20 \times 10^9/L$ ²⁸. Currently this dose is recommended as higher doses do not offer advantage and are more often associated with side effects²⁹. Rapidity of rise of platelet count and thus shortening of duration of severe thrombocytopenia (with its favourable effect on patient, parents and physicians) with use of IVIG is most important factor for its wide use as initial management of ITP. A recent meta-analysis comparing rapidity of platelet rise with IVIG and corticosteroids has demonstrated that patients are more likely to have platelet count $>20 \times 10^3/mm^3$ with IVIG use than with corticosteroids at 24, 48 and 72 hours. This difference may hold clinical importance³⁰. Rapidity of platelet rise is similar when anti-D is used in higher doses²⁸.

Intravenous Anti- D (i.v anti-D): Salama and co workers demonstrated reversal of thrombocytopenia in patients who had ITP and were rhesus (D) positive with administration of i.v anti-D³¹. The mechanism of action of anti-D involves anti-D coating of red cells of rhesus (D) positive patients. These antibody coated red cells are preferentially removed by RES, thus sparing the platelets^{26,32,33}. Studies to look for other possible mechanisms through which anti-D might act have failed to demonstrate any effect of anti-D on humoral and cellular arms of immune system³³. After anti-D administration, patients become Coomb's positive

and have laboratory evidence of hemolysis. Study by Scaradavou and co-workers highlighted that platelet responses are better in children than in adults and responders tended to respond to re-treatment with iv anti-D. They also noted that conventional doses were ineffective in splenectomized patients³². A recent study on children with ITP compared the efficacy of IVIG and two different doses of anti-D. A clear superiority of 75mg/kg dose of anti-D was observed compared to 50mg/kg dose with respect to number of cases with platelet count $>20 \times 10^3/mm^3$ at 24 hours of therapy. While 50mg/kg of anti-D was found to be inferior to 0.8 g/kg of IVIG, efficacy of 75mg/kg dose of anti-D was equivalent to 0.8 g/kg of IVIG³⁴. Short term side effects of anti-D include fever, chills, nausea and vomiting and are observed to be more frequent with 75mg/kg dose. These side effects are probably related to pro-inflammatory cytokine release after anti-D administration. As no clinically significant increase in fall of Hb is noted with 50 or 75mg/kg anti-D, the latter (single) dose can now be recommended as standard dosing for treatment of ITP in children who are rhesus (D) positive²⁹. It should be remembered that this form of therapy is ineffective in D negative patients. In patients with significant anemia, use of anti-D should be avoided. Abrupt severe hemolysis has been reported after i.v. therapy, majority of these cases were in adults³⁵. It is important to advise the patients to be watchful for any change in the colour of urine. Rapidity of platelet rise and ease of administration (slow i.v push in contrast to infusion of IVIG) are important appealing factors for increasing clinical use of anti-D, especially as OPD administration. Survey conducted in USA, four years apart have observed increased use of anti-D from 10% to 33%³⁶. Another factor which would promote the increasing use of anti-D as a first line therapy is the rising cost of IVIG both at national and international level which would decrease the usage of IVIG in the developing countries.

Corticosteroids: Prednisolone at conventional dose of 2mg/kg ($60mg/m^2$) increases the platelet count slowly and hence cannot be usually recommended in children with very low platelet count or clinically significant bleeding. For this reason, high dose oral or parenteral corticosteroids have been used. Using oral high dose methyl-prednisolone (M-PDN), Ozsoylu and co-workers demonstrated efficacy equivalent to IVIG³⁷. Intravenous M-PDN was used by Van Hoff and Ritchey at 30mg/kg for three days. With this dose, median time to achieve platelet count $>20 \times 10^3/mm^3$ was 24 hours, an observation similar to IVIG or anti-D use³⁸. This schedule is currently more commonly used. Coraco et al's experience with short-course oral prednisone (4 mg/kg per day x 4 days without tapering) is complementary. Eighty-three percent of children who had acute ITP and platelet counts less than $20 \times 10^9/L$ achieved a platelet count above $20 \times 10^9/L$ within 48 hours of starting corticosteroid therapy. Reviewing all the studies using high dose oral steroids for shorter duration it can be concluded that a clinically significant increment in platelet count can be achieved rapidly in the majority of children who have acute ITP after the administration of high-doses of corticosteroids (approximately 4 mg/kg per day of prednisone or an equivalent corticosteroid preparation) administered orally or parenterally. It seems wise to use high-dose corticosteroid regimens for as short a period of time as is necessary to achieve a clinically meaningful endpoint (eg, cessation of bleeding or achievement of a platelet count $> 20 \times 10^9/L$). This approach

minimizes the predictable, and sometimes serious, adverse effects of long-term corticosteroid therapy. Use of corticosteroids other than prednisolone or M-PDN deserves mention. Oral pulsed high dose dexamethasone (HDD) was initially used for chronic ITP in adults by Anderson³⁹. He demonstrated 100% response rate. Subsequent studies in adults and children showed a moderate success only^{40,41}. More recently, this form of therapy has been used in newly diagnosed children and adults with ITP. This Italian study by Mazzucconi and co-workers⁴² modified the initial dosing schedule used by Anderson. Instead of giving HDD at monthly interval, they used four courses of HDD (each course using dexamethasone 20mg/m² for 4 consecutive days) at two weeks interval, thus completing the therapy in 2 months time. A complete response rate of 64.5% and overall response rate of more than 85% was recorded. Response was similar in adults and children. 87% responders enjoyed long term response with a median of 8 months (range 4-24 months) without relapse or need for any therapy. Therapy with HDD was described as well tolerated. Compared to IVIG and i.v anti-D, therapy with dexamethasone is very economical.

Rituximab: Use of rituximab for ITP and other immune cytopenias is relatively new. Rituximab is human murine (chimeric) monoclonal antibody that depletes B cells from the blood, lymph node and bone marrow by targeting CD20 which is expressed on the surface of premature and mature B lymphocytes. It was particularly developed for treatment of Non-Hodgkin lymphoma. Central role of B cells in autoimmunity and selective depletion of B cells by rituximab provided a case for exploring its use in the treatment of autoimmune diseases^{43,44}. Initial success with rituximab has led to its wider use in immune cytopenias including ITP, immune hemolytic anemia and Evan's syndrome. Mechanism of action of rituximab in ITP involves much more than mere B cell depletion. In fact, levels of autoantibodies are not always significantly affected by rituximab. Taylor and Lindorfer have recently suggested another mechanism of action of rituximab in autoimmune diseases- the immune complex decoy theory. They hypothesized that as rituximab-opsonized B cells will be recognized by monocytes and macrophages and these effector cells will be diverted away from interaction with autoimmune antibody complexes⁴⁵. Another theory was put forward by Stasi and co-workers to explain a rapid response in ITP. The auto-antibodies in ITP are driven by T cell dependent mechanisms and the normalization of these autoreactive T cell responses may be underlying mechanism of action of rituximab in

ITP⁴⁶. Over the past few years, rituximab has emerged as an important alternative treatment for ITP. The treatment regimen used most frequently was 375 mg/m² administered weekly for 4 weeks. A pediatric series by Bennet et al reported data including 36 patients, ages 2.6 to 18.3 years, six of whom had Evans's syndrome. Responses, defined as a platelet count greater than 50 x 10⁹/L during 4 consecutive weeks starting in weeks 9 to 12 after 4 weekly doses of rituximab (375 mg/m² per dose), were observed in 31% of cases (CI, 16% to 48%). Data on over 100 children treated for ITP with rituximab has been reviewed by Garvey⁴³. In various series complete response rate has varied between 32-68%. Additional 8-27% cases have enjoyed partial remission. The responses are described as durable with longest disease free interval of 3.2 years (ongoing). No differences have been observed between adults and children⁴⁷⁻⁴⁹.

NEWER APPROACHES TO TREATMENT OF ITP

The advances in understanding the pathogenesis of ITP have been in two fields: (a.) Demonstration that platelet production is impaired in some patients with ITP. (b.) Better elucidation of mechanism of production of antiplatelet antibodies.

It is believed that platelet production is increased in patients with ITP, the platelet count being determined by a balance between platelet production and rate of platelet destruction. At least in some patients with ITP, thrombopoiesis is inadequate to offset rapid destruction, actually platelet production may be decreased in some of them. Circulating or tissue bound thrombopoietin (TPO) levels are normal or slightly increased in ITP in contrast to the increased TPO levels observed in cases with thrombocytopenia due to impaired platelet production^{36,50}. Therapy with corticosteroids and IVIG also does not increase TPO levels⁵¹. Studies by Chang and co-workers demonstrated that in-vitro proliferation of megakaryocytes is impaired when they are incubated with plasma from patients with ITP containing antiplatelet antibodies, particularly anti-GPIIb antibodies⁵². Observations of Chang and co-workers and other studies have led to use of TPO and other drugs in ITP which increase platelet production. On the other hand studies in last decade have improved our understanding of how antiplatelet autoantibodies are produced and lead to platelet destruction. It is now believed that B lymphocyte activation in ITP and other autoimmune disorders is T cell-dependent. CD40 is a cell surface receptor that belongs to the tumor necrosis factor-receptor family, and that was first identified and functionally characterized on B lymphocytes. CD40-ligand (CD40L/CD154), a member of the TNF superfamily, is a cell membrane molecule expressed on activated CD4⁺ T lymphocytes and is essential for activation of B lymphocytes. It is speculated that platelet-associated CD154 is competent to induce the CD40-dependent proliferation of B lymphocytes. Studies have shown increased expression of platelet-associated CD40L/CD154 in ITP patients^{53,54}. These observations have led to development of antibodies interfering with this arm of immune dysregulation.

Drugs Increasing Platelet Production

Megakaryopoiesis is controlled by signalling through the TPO receptor (c-Mpl) present on megakaryocyte and platelet surface. Studies have evaluated polyethylene glycol-conjugated form of TPO (PEG-megakaryocyte growth and development factor (PEG-MGDF), a recombinant TPO. This led to increased platelet counts in four out of five patients with ITP^{55,56}. However, PEG-MGDF was found to be immunogenic and induced production of anti-TPO antibodies leading to severe thrombocytopenia in some recipients⁵⁷. Following these reports, further research on this compound was stopped. The second generation agents include TPO peptide mimetics, TPO non-peptide mimetics and TPO agonist antibodies⁵⁸.

Studies with two of these compounds i.e.: AMG 531 (Romiplostim) and Eltrombopag are in late stages of a positive signal for clinical usage. These are c-Mpl peptide agonists that share no sequence homology with native TPO.

AMG 531: In a study by Bussel and co-workers, escalating doses of 0.2 to 10mg/kg weekly subcutaneously for 6 weeks were administered to 41 patients. In phase I of the study, platelet count above 50 x 10³/mm³ was achieved in 7 out of 12 patients. Increase in platelet count

was dose dependent. In phase II, 10 of 16 patients receiving 10mg/kg weekly for six weeks showed platelet count above $50 \times 10^3/\text{mm}^3$. No major adverse events were noticed⁵⁹. Newland and co-workers have also reported similar observations. Eight of their 11 patients receiving doses >1.0 mg/kg showed increase in platelet count⁶⁰. Reversible marrow fibrosis has been documented in some patients receiving AMG 531²⁹.

Eltrombopag: Eltrombopag (SB-497115) is orally administered agent. It is a small-molecule, non-peptide thrombopoietin-receptor agonist. This drug initiates thrombopoietin-receptor signalling by interaction with trans-membrane domain of receptor, thereby inducing proliferation and differentiation of cells in megakaryocytic lineage. In a study by Bussel and co-workers, patients with chronic ITP were administered 30, 50 and 75 mg of eltrombopag daily. By day 15, more than 80 % patients receiving 50 or 75 mg of eltrombopag had an increased platelet count. TPO levels remained within normal range⁶¹.

IL-11: IL-11 stimulates megakaryopoiesis in vitro. A recombinant human interleukin-11 (rhu IL-11, oprelvekin), has been used in cancer patients having thrombocytopenia. Wilde and Faulds have reviewed its clinical use. The drug increases megakaryocyte size and ploidy. The recommended adult dosage of subcutaneous oprelvekin is 50 mg/kg once daily, administered until the platelet count is $\geq 50,000/\text{mm}^3$. Three placebo-controlled trials involving patients with cancer (mostly breast cancer) undergoing dose-intensive cancer chemotherapy, with or without autologous bone marrow transplantation (n=75 to 82), have been conducted. Compared with placebo, oprelvekin 50 mg/kg/day was associated with significantly fewer patients requiring platelet transfusions and a trend towards a lower median number of platelet transfusions⁶². Bussel and co-workers used rhuIL-11 in adults with refractory ITP. At 50 $\mu\text{g}/\text{kg}$ dose, rhuIL-11 failed to increase the platelet counts in these patients⁶³. There are other thrombopoietic agents under development that seem promising in trials in normal volunteers, such as AKR-501. Further study will reveal the potential of these agents for long-term maintenance therapy, and the usefulness of these agents in children who have ITP.

OTHER IMMUNOMODULATORY DRUGS

T cell mediated B lymphocyte activation in patients with ITP forms the basis of use of these compounds. Kuwana and co-workers conducted a dose escalating trial of *humanized monoclonal antibody to CD 154* (IDEC-131/E6040) in patients with refractory ITP. No rise in platelet count was observed with 1, 2 or 5 mg/kg dose. Three patients treated with 10 mg/kg dose showed an increase in platelet count⁶⁴. Another study by Patel and co-workers has demonstrated 24% overall response rate using 5-20 mg/kg dose of this monoclonal antibody⁶⁵. *Specific Inhibitors of Phagocyte-Mediated Consumption of Platelets:* the thrombocytopenia in ITP results at least in part from an interaction between platelet surface-bound immunoglobulin and Fc γ RIII receptors on macrophages. Specific antibodies of Fc γ RIII have therefore been designed as potential therapy to prevent platelet clearance. Early trials with a murine anti-human Fc γ RIII antibody demonstrated the feasibility of this approach, but treatment could not be repeated as a result of universal development of human antimouse antibodies. A humanized antibody, GMA-161, has been developed and used in low dose in four adults who had chronic refractory ITP. In these

initial studies, responses were fairly short-lived. Further study is required to demonstrate the applicability of specific Fc γ RIII targeting in the treatment of ITP and other autoimmune disorders. An inhibitor of syk kinase (R788) that targets the Fc γ RIII signaling pathway is also in early trial with promising results⁶⁶.

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ETHICAL GUIDELINES FOR BIOMEDICAL RESEARCH

The need for uniform ethical guidelines for research on human subjects is universally recognised. It has acquired a new sense of urgency as the critical issues in the area of biogenetic research involving human subjects have become acute. Apart from the mandatory clinical trails on new drugs, a number of diagnostic procedures, therapeutic interventions and prevention measures including the use of vaccines, are being introduced which involve human subjects. Further the advent of new medical devices and radio-active materials and therapeutic benefits of recombinant DNA products have added a new dimension to the ethical issues that need to be considered before evaluating these for their efficacy, utility and safety.

Any research using the human beings as subjects shall bear in

mind the following principles of : i) essentiality, (ii) voluntariness, informed consent, (iii) non exploitation, (iv) privacy and confidentiality, (v) precaution and risk minimisation, (vi) professional competence, (vii) accountability & transparency, (viii) maximisation of public interest and distributive justice (ix) institutional arrangements (x) public domain (xi) totality of responsibility and (xii) compliance.

Recent advances in the field of Assisted Reproductive technologies, organ transplantation, Human genome analysis, and gene therapy promise unquestionable benefits to mankind. At the same time, they raise many questions of law and ethics, stimulating public interest and concern.

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