Autoimmune Thrombocytopenia: Flow Cytometric Determination of Platelet-Associated CD154/CD40L and CD40 on Peripheral Blood T and B Lymphocytes: Their Role in Immunological Aspects of the Disease

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Abstract:

The CD40-CD40L system has pleiotropic effects in a variety of cells and biological processes including immune response. Within the immune system, these molecules represent a critical link between its humoral and cellular arms. Immune or idiopathic thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by antibody-induced platelet destruction and clearance because of anti-platelet autoantibodies, which bind to circulating platelets resulting in their destruction by the reticuloendothelial system. Despite its clinical importance, the diagnosis of ITP is one of exclusion, thus, inevitably associated with potential difficulties. CD40 is a cell surface receptor that belongs to the tumor necrosis factor-receptor (TNF-R) 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 the T cell-dependent activation of B lymphocytes. Therefore it is now thought that CD40-CD40L interactions play a more important role in ITP immune regulation.

The expressions of CD154 and CD40 on peripheral blood (PB) T and B lymphocytes, respectively, were measured using the technique of flow cytometry. An antigen-specific assay for platelet-associated antibody CD154 (CD40L) on CD4+ T lymphocytes and for CD40 on CD19+ B lymphocytes was tested in 30 children patients with acute ITP, 30 adult patients with chronic ITP, and in 20 age- and sex-matched healthy controls.

The results of this showed that the expressions of CD4+CD154+ and of CD4+CD154+/CD4+ on PB T lymphocytes, and of CD19+CD40+ and of CD19+CD40+/CD19+ on PB B lymphocytes were significantly higher in acute and chronic ITP patients compared to controls, and in acute patients compared to chronics (p<0.001).

Conclusions: CD40-CD40L interaction plays an important role in the pathology of certain autoimmune diseases. ITP is an autoimmune disease characterized by increased platelet destruction caused by anti-platelet autoantibodies, which mainly target a platelet surface antigen. It is speculated that platelet-associated CD154 is competent to induce the CD40-dependent proliferation of B lymphocytes. Therefore, platelet-associated CD154 expression is increased in ITP patients and is able to drive the activation of autoreactive B lymphocytes in this disease. These findings are particularly useful for clarifying the pathogenic process in ITP patients and for developing a therapeutic approach that blocks pathogenic anti-platelet antibody production. Blockade of the CD40/CD154 signal is a potential immunomodulatory strategy for T-cell-mediated diseases, and many findings suggest that CD40/CD154 blockade therapy is potentially effective for ITP through selective suppression of autoreactive T and B lymphocytes to platelet antigens. Key Items: CD154 (CD40L)/CD4, CD40/CD19, ITP.

Introduction:

Idiopathic or immune thrombocytopenic purpura (ITP) is characterized by antibody-mediated destruction of platelets. The etiology is unknown. Studies postulated that increased autoantibody production in ITP might be attributable to either increased or prolonged expression of CD40 ligand (CD40L/CD154) in T lymphocytes. Some studies indicated that overexpression of CD154 in T lymphocytes is likely to be a primary pathophysiological defect in most patients with ITP.
The data support that cell membrane antigens such as CD154 should be considered as potential targets for therapy in this disease.\(^1\) Idiopathic thrombocytopenic purpura (ITP) is an autoimmune disease characterized by increased platelet destruction caused by anti-platelet autoantibodies, which mainly target a platelet surface antigen, GP Ib/IIa. CD4+ T cells reactive with GP Ib/IIa play a primary role in the disease process, since these autoreactive T cells are involved in the production of pathogenic anti-platelet autoantibodies. These findings are particularly useful for clarifying the pathogenic process in ITP patients and for developing a therapeutic approach that blocks pathogenic anti-platelet antibody production.\(^2\)

Immune thrombocytopenic purpura (ITP) is an autoantibody-mediated disease. Platelet-reactive T cells have been found in the blood of patients with this disorder, with the major target antigen being platelet membrane glycoprotein Ib/IIa (GPIb/IIa); autoreactive CD4+ T cells are found in lesser numbers in the blood of healthy controls. Many workers in a series of studies have demonstrated that B-cell production of antplatelet antibody requires antigen-specific CD4+ T-cell help. Thus, T cells are related to the pathogenic process in ITP.\(^3\)

CD40 is a cell surface receptor that belongs to the TNF-R family, and that was first identified and functionally characterized on B lymphocytes. CD40L (CD154, gp39), a transmembrane protein and a member of the TNF family, is expressed on activated CD4+ T cells, mast cells, basophils, eosinophils, natural killer cells, and activated platelets. CD40L is important for T-cell-dependent B-cell responses. The interaction of CD40L-CD40 is essential for T-cell priming and the T-cell-dependent humoral immune response. Therefore, interruption of the CD40-CD40L interaction with an anti-CD40L monoclonal antibody (mAb) has been considered to be a possible therapeutic strategy in human autoimmune diseases.\(^4\)

It is speculated that platelet-associated CD154 is competent to induce the CD40-dependent proliferation of B lymphocytes. Therefore, platelet-associated CD154 expression is increased in ITP and is able to drive the activation of autoreactive B lymphocytes in this disease. Studies stated that flow cytometric assay is clinically useful for ITP patients; they noted that their findings are particularly useful for clarifying the pathogenic process in ITP patients and for developing a therapeutic approach that blocks pathogenic anti-platelet antibody production. Blockade of the CD40/CD154 signal is a potential immunomodulatory strategy for T-cell-mediated diseases, and many findings suggest that CD40/CD154 blockade therapy is potentially effective for ITP through selective suppression of autoreactive T and B cells to platelet antigens.\(^5,6\)

We here, using flow cytometry, aimed at measuring expressions of CD154/CD40L on CD4+ T cells and of CD40 on CD19+ B cells to clarify and correlate their role in the mechanism and therapy of the disease.

**Subjects and Methods:**

We here describe a feasible method using the commonly available technique of flow cytometry. The expression of CD154 and CD40 on T and B cells, respectively, was measured. An antigen-specific assay for platelet-associated antibody CD154 (CD40L) on CD4+ T lymphocytes and for CD40 on CD19+ B lymphocytes was tested in 30 children patients with acute ITP, 30 adult patients with chronic ITP, and in 20 age- and sex-matched healthy controls. Diagnosis of ITP was based on history, physical examination, CBC thrombocytopenia and peripheral blood (PB) smear examination, and bone marrow (BM) aspiration.

CD154 on T lymphocytes and CD40 on B lymphocytes were assessed in 60 peripheral blood samples from 30 children with acute ITP (17 males and 13 females, with male to female ratio: 1.3) with a mean age of 6.45 +/- 3.5 years, 30 patients with chronic ITP (12 males and 18 females, with male to female ratio: 0.7) with a mean age of 14.3 +/- 3.9 years, and 20 age- and sex-matched controls. All cases with acute ITP were de novo cases not receiving treatments yet, on the contrary to all cases with chronic ITP which were all receiving treatments. Treatment of chronic ITP include Prednisone 2mg/kg/d for 2-4 weeks, IV Anti-D 50ug/kg/d every 4 weeks, IVIG 0.8gm/kg/d every 4 weeks and splenectomy was very occasionally done, the same as the supposed treatment for acute ITP except that IV Anti-D and IVIG have to be given as a single dose.

All patients and controls were subjected to the following:

- Thorough history taking.
- Clinical examination both general and local.
- Routine laboratory investigations including:
  - Complete hemogram.
  - Bone marrow aspiration.
- Specific laboratory investigations:
  - Study of the surface expressions of CD154 and CD40 by flow cytometry on T and B lymphocytes respectively, on peripheral blood lymphocytes. T lymphocytes were stained with fluoresceinisothiocyanate (FITC)-conjugated McAb CD4 (CD4-FITC) and phycoerythrin (PE)-conjugated McAb CD154 (CD154-PE). B lymphocytes were stained...
with fluorescein-isothiocyanate (FITC)-conjugated McAb CD19 (CD19-FITC) and phycoerythrin (PE)-conjugated McAb CD40 (CD40-PE). CD154/CD40L/gp39 recognizes a 39-kDa type II membrane glycoprotein antigen that is a member of the TNF receptor family. CD40L is transiently expressed on activated CD4 T lymphocytes. The expression of CD40L by activated T-helper cells triggers B-cell cycling through binding to CD40. This co-receptor interaction is required for B-lymphocyte maturation, response to T-dependent ligands, and isotype switching. CD40 reacts with a 45-48 kDa type I integral membrane glycoprotein present on peripheral blood B lymphocytes, but not expressed on terminally differentiated B cells, not monocytes, nor granulocytes, with the reactivity being restricted to CD19+/CD20+ lymphocytes. CD19 reacts with the 95 kDa type I transmembrane glycoprotein expressed during all stages of B-cell differentiation and maturation, except on plasma cells. It is not found on T cells or on normal granulocytes. CD4 recognizes an antigen 56-59 kDa transmembrane glycoprotein, present on T-helper/inducer subset of peripheral blood lymphocytes.

Results:
This study included 30 children patients with acute ITP, 30 adult patients with chronic ITP, and 20 age- and sex-matched controls for the study on their PB T and B lymphocytes, respectively, the expressions of CD154+(CD40L+)/CD4+ and CD40+/CD19+ by flow cytometry for the possible immunological role of these surface molecules in the etiopathogenic mechanisms in ITP. Patients were collected from the Outpatients Clinics and the Internal Departments of Pediatrics and Internal Medicine of Kasr El-Aini and Bani-Swaif University Hospitals. Patients were diagnosed as ITP on the basis of history, physical examination, CBC thrombocytopenia and PB smear examination, and BM aspiration.

CD154/CD40L on PB T lymphocytes and CD40 on PB B lymphocytes were assessed in 60 peripheral blood samples from 30 children with acute ITP (17 males and 13 females; with male to female ratio: 1.3, and with a mean age of 6.45 +/- 3.5 years), 30 patients with chronic ITP (12 males and 18 females; with male to female ratio: 0.7, and with a mean age of 14.3 +/- 3.9 years), and 20 age- and sex-matched healthy subjects serving as controls. All cases with acute ITP were de novo cases not receiving treatments yet, on the contrary to all cases with chronic ITP which were all receiving treatments. Highly statistically significant increases were demonstrated in percentages of CD4+CD154+, CD19+CD40+, CD4+CD154+/CD4+, and CD19+CD40+/CD19+ in acute and chronic ITP patients compared to controls (p<0.001), and in acute ITP patients compared to chronic ITP patients (p<0.001). Tables I-IV and figures 1-4 show the results of acute (pediatric) and chronic (adult) ITP patients included in this study.

Table I shows characteristics of acute and chronic ITP patients included in this study.

Table II shows clinical findings of acute and chronic ITP patients.
Table III shows results of expressions of CD154/CD4 and of CD40/CD19 on PB T and B lymphocytes in ITP patients and in normal controls.
Table IV shows comparisons of expressions of CD154/CD4 and of CD40/CD19 on PB T and B lymphocytes in ITP patients and in normal controls.

Figure 1 shows positive expression of CD154/CD4 on PB T lymphocytes.
Figure 2 shows negative expression of CD154/CD4 on PB T lymphocytes.
Figure 3 shows positive expression of CD40/CD19 on PB B lymphocytes.
Figure 4 shows negative expression of CD40/CD19 on PB B lymphocytes.
Table II: Clinical findings of acute and chronic ITP patients included in this study

<table>
<thead>
<tr>
<th>Clinical Finding</th>
<th>Number</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acute ITP Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Petechial rash</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>- Epistaxis</td>
<td>5</td>
<td>16.7%</td>
</tr>
<tr>
<td>- Pallor</td>
<td>10</td>
<td>33.3%</td>
</tr>
<tr>
<td>- Splenomegaly</td>
<td>7</td>
<td>23.3%</td>
</tr>
<tr>
<td>- Hypertension</td>
<td>2</td>
<td>6.7%</td>
</tr>
<tr>
<td>Chronic ITP Patients</td>
<td></td>
<td></td>
</tr>
<tr>
<td>- Petechial rash</td>
<td>30</td>
<td>100%</td>
</tr>
<tr>
<td>- Epistaxis</td>
<td>5</td>
<td>6.7%</td>
</tr>
<tr>
<td>- Pallor</td>
<td>16</td>
<td>53.3%</td>
</tr>
<tr>
<td>- Splenomegaly</td>
<td>21</td>
<td>70%</td>
</tr>
<tr>
<td>- Hypertension</td>
<td>12</td>
<td>40%</td>
</tr>
</tbody>
</table>

Table III: Results of expressions of CD154/CD4 and of CD40/CD19 on PB T and B lymphocytes in ITP patients and in normal controls

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Acute ITP (n=30)</th>
<th>Chronic ITP (n=30)</th>
<th>Controls (n=20)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD154+CD4+</td>
<td>29/30</td>
<td>20/30</td>
<td>0/20</td>
</tr>
<tr>
<td>% of +ve patients</td>
<td>96.7%</td>
<td>66.7%</td>
<td>0%</td>
</tr>
<tr>
<td>% of +ve cells</td>
<td>(69+/-5.2%)</td>
<td>(41+/-6.5%)</td>
<td>(11+/-3%)</td>
</tr>
<tr>
<td>CD40+CD19+</td>
<td>27/30</td>
<td>24/30</td>
<td>0/20</td>
</tr>
<tr>
<td>% of +ve patients</td>
<td>90%</td>
<td>80%</td>
<td>0%</td>
</tr>
<tr>
<td>% of +ve cells</td>
<td>(23+/-4.1%)</td>
<td>(13+/-3.4%)</td>
<td>(5+/-2%)</td>
</tr>
</tbody>
</table>

Table IV: Comparisons of expressions of CD154/CD4 and of CD40/CD19 on PB T and B lymphocytes in ITP patients and in normal controls

<table>
<thead>
<tr>
<th>Category</th>
<th>Mean % of +ve cells</th>
<th>Significance</th>
<th>P Value</th>
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<tbody>
<tr>
<td>CD154/CD4 on PB T lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P1</td>
<td>Acute ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P2</td>
<td>Chronic ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P3</td>
<td>Acute vs. Chronic ITP patients</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>CD40/CD19 on PB B lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P4</td>
<td>Acute ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P5</td>
<td>Chronic ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P6</td>
<td>Acute vs. Chronic ITP patients</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>CD154/CD4/CD4 on PB T lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P7</td>
<td>Acute ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P8</td>
<td>Chronic ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P9</td>
<td>Acute vs. Chronic ITP patients</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>CD40/CD19 on PB B lymphocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>P10</td>
<td>Acute ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P11</td>
<td>Chronic ITP patients vs. controls</td>
<td>HS</td>
<td>p&lt;0.001</td>
</tr>
<tr>
<td>P12</td>
<td>Acute vs. Chronic ITP patients</td>
<td>HS</td>
<td>p&lt;0.001</td>
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</table>
Discussion:

Idiopathic or autoimmune thrombocytopenic purpura (ITP) is an autoimmune disorder characterized by platelet destruction due to an antiplatelet autoantibody which coats platelets and leads to their elimination by the reticuloendothelial system (RES). Acute ITP is a disorder found mainly in children, usually preceded by a viral infection. Chronic ITP occurs mostly in adults. The platelet immunofluorescence test detects platelet-specific autoantibodies; the antibodies are usually bound to platelets and are detectable as free circulating antibodies. While in childhood ITP is more usually an acute and self-limiting problem which needs no drug treatment, adult ITP is a relatively common chronic hematological disease. Treatment aims at inhibition of antibody-production and binding to platelets and platelet phagocytosis by the RES. Therapy should result in a platelet count of >100,000/ul or at least in stabilization of the platelet count without bleeding. Therapeutic approaches were divided into emergency and long-term treatment. In patients who require non-emergency treatment conventional-dose corticoids; 1-2 mg/kg/d prednisone are recommended as initial treatment, whereas pulsed high-dose dexamethason is recently reported to be effective in refractory ITP. After unsuccessful splenectomy or if treatment with gamma-globulins fails, alternative and partly experimental therapies may have to be used. Treatment of adult ITP includes established medical, immunological and surgical measurements. Their application depends on diseases progression as well as imminent or manifest complications. Remission is achieved in up to 75% of all patients. Alternative treatments remain for refractory cases.7,8

Most research in ITP has focused on characterization of the antiplatelet autoantibodies, while little has been developed to the cellular immune mechanisms leading to autoantibody production. T cells reactive with autoantigens targeted by autoantibodies were identified in patients with various systemic or organ-specific autoimmune diseases, as well as in ITP. T lymphocytes require a number of costimulatory pathways for activation. If activating or costimulatory signals were aberrantly provided to platelet-reactive T cells, ITP may occur.9-13

The aim of this work was to study the surface expressions of the activating molecules CD154 on CD4+ T lymphocytes, and CD40 on CD19+ B lymphocytes, of the peripheral blood of patients with ITP, to clarify their role in the mechanism and treatment of the disease.

This study included 30 patients with acute ITP (17 males and 13 females; their age mean +/- SD = 6.45 +/- 3.5 years), 30 patients with chronic ITP (12 males and 18 females; their age mean +/- SD = 14.3 +/- 3.9 years), and 20 age- and sex-matched healthy subjects as controls. Highly statistically significant increases were demonstrated in percentages of CD4+CD154+, CD19+CD40+, CD4+CD154+/CD4+, and CD19+CD40+/CD19+ in all ITP patients (acute and chronic) compared to the controls (p<0.001), and also highly statistically significant increases in CD4+CD154+, CD19+CD40+, CD4+CD154+/CD4+...
and CD19+CD40+/CD19+ in acute ITP patients
compared to chronic ITP patients (p<0.001).
Solanilla et al.,6 in 2005, stated that CD40L/CD154 is
expressed on activated CD4+ T lymphocytes and is
essential for the T cell-dependent activation of B
lymphocytes. They showed that platelet-associated
CD154 is increased in ITP, a disease characterized
by an autoimmune response against proteins of the
platelet membrane. CD154 and its messenger RNA
were also present in increased amounts in the
megakaryocytes of patients with ITP. They found that
platelet-associated CD154 is competent to induce the
CD40-dependent proliferation of B lymphocytes,
and they observed an in vitro CD154-dependent
production of antibodies to the GPIIb/IIIa complex
when platelets and peripheral blood B lymphocytes
from ITP patients with circulating anti-GPIIb/IIIa
antibody were cultured together. Therefore, platelet-
associated CD154 expression is increased in ITP
and is able to drive the activation of autoreactive B
lymphocytes in this disease.

Tomer et al.,14 in 2005, noted that ITP is an
autoimmune disorder characterized by antibody-
induced platelet destruction. Despite its clinical
importance, the diagnosis of ITP is one of exclusion,
thus, inevitably associated with potential difficulties.
They described a feasible diagnostic method using
the commonly available technique of flow cytometry
(FCM). An antigen-specific assay for platelet-
associated antibody was developed and tested in 62
adult patients with chronic ITP, 14 patients with
thrombocytopenia of decreased production and 60
healthy controls. The difference between the ITP
patients and both groups was highly significant
(p<0.001). A comparison of the FCM assay with the
radioactive immunobead assay previously reported
on the same cohort of patients showed significant
correlation. They concluded that the present FCM
assay is clinically useful for routine diagnosis and
follow-up of ITP patients.

Cines and McMillan,15 in 2005, stated that ITP is a
common hematomal disorder manifested by
immune-mediated thrombocytopenia. The diagnosis
remains one of exclusion, after other thrombocytopenic
disorders are ruled out based on history, physical
examination, and laboratory evaluation. The goal of
treatment is to raise the platelet count into a
hemostatically safe range. The disorder is usually chronic, although there is
considerable variation in the clinical course and most
patients eventually attain safe platelet counts after

treatment. However, a subset of patients has severe
disease refractory to all treatment modalities, which
is associated with considerable morbidity and
mortality. Many articles focus on the management of
primary ITP. They discussed criteria for treatment,
the roles of splenectomy and other treatment options
along with their side effects, and the management of
ITP on immunological basis and immunomodulatory
treatments.

Shouxi et al.,3 in 2004, denoted that ITP is an
autoantibody-mediated disease. Platelet-reactive T
cells have been found in the blood of patients with
this disorder, with the major target antigen being
platelet membrane glycoprotein IIb/IIIa; autoreactive
CD4+ T cells are found in lesser numbers in the
blood of healthy controls. Workers in a series of
studies have demonstrated that B-cell production of
antiplatelet antibody requires antigen-specific CD4+
T-cell help. Thus, T cells are related to the
pathogenic process in ITP. CD40L/CD154 expressed
on activated CD4+ T cells, and on activated platelets,
is important for T-cell-dependent B-cell responses.
The interaction of CD40L-CD40 is essential for T-cell
priming and the T-cell-dependent humoral immune
response. Therefore, interruption of the CD40-CD40L
interaction with an anti-CD40L mAb has been
considered to be a possible therapeutic strategy in
human autoimmune diseases.

Fan et al.,16 in 2004, stated that CD40L, a 33-kDa
cell membrane molecule, a member of the TNF
superfamily, is an important costimulatory molecule
during immune response. Sidiroopoulos and
Boumpas,17 in 2004, noted that the CD40-CD40L
system has pleiotropic effects in a variety of cells and
biological processes including immune response.
Within the immune system, these molecules
represent a critical link between its humoral and

cellular arms. Kuwana et al.,5 in 2004, denoted that
blockade of the CD40/CD154 signal is a potential
immunomodulatory strategy for T-cell-mediated
diseases. They suggested that CD40/CD154
blockade therapy is potentially effective for refractory
ITP, through selective suppression of autoreactive T
and B cells to platelet antigens.

Sandler,18 in 2004, denoted that ITP is an acquired
disease in which autoantibodies to platelets cause
their sequestration and destruction by mononuclear
macrophages, principally in the spleen. If increased
production of platelets by megakaryocytes does not
compensate for platelet destruction, the number of
circulating platelets decreases (thrombocytopenia),
resulting in a characteristic bleeding tendency
(purpura). While most children with the disease
experience a relatively short and benign clinical
course, ITP in adults often lasts more than 6 months
(chronic ITP) and is resistant to conventional
treatment (corticosteroids, intravenous immune
globulin, or splenectomy). The goal of medical
management is to increase the platelet count to a
safe level, without the risks of bacterial infections associated with splenectomy or toxicity from prolonged corticosteroid therapy. Splenectomy increases platelet counts in hours to days in most patients with acute ITP, but nearly 50 percent experience recurrent thrombocytopenia by 5 years postsplenectomy.

Sandler and Tutuncuoglu,19 in 2004, noted that the treatment of patients with ITP is changing rapidly, as new agents demonstrate the capability of improving outcomes and decreasing toxicity. Prior to 1981, the only effective treatment options available to increase platelet counts in persons with ITP were corticosteroids and splenectomy. In recent years, intravenous immunoglobulin (IVIg) and intravenous Rh immunoglobulin (IV RhIg) have demonstrated efficacy comparable to that of corticosteroids for increasing platelet counts in ITP. In addition, IVIg and IV RhIg have demonstrated efficacy for maintaining corticosteroid-induced increased platelet counts by periodic infusion, causing a transient impairment of reticuloendothelial clearance function (medical splenectomy). Thus, the time-proven efficacy of corticosteroids for initial treatment of ITP (induction) may now be supplemented with IVIg or IV RhIg infusions for patients requiring ongoing treatment to support a timely and complete steroid taper, while sustaining the increased platelet count (maintenance) with less toxicity. Several investigators have reported that rituximab (anti-CD20) induced sustained remissions with minimal toxicity, in patients with chronic ITP. These reports are promising and, if confirmed, will provide another effective (spleen-sparing) option for managing acute ITP and a long-awaited option for patients who have had a splenectomy and are refractory to conventional agents. Other treatments, including danazol, azathioprine, cyclophosphamide, vinca alkaloids and cyclosporin A, have advocates, but evidence of their efficacy is limited to relatively small and mostly uncontrolled clinical trials. In our opinion, these agents should be reserved for symptomatic thrombocytopenia after refractoriness to corticosteroids, IVIg, IV RhIg, splenectomy and rituximab has been clearly established.

Pusiol et al.,20 in 2004, stated that rituximab is a chimeric McAb directed against normal and malignant mature B-lymphocytes and results in prolonged and severe B-cell depletion. Recently, rituximab has been successfully used in adult and pediatric disorders of B-lymphocytes such as autoimmune hemolytic anaemia. They reported on two children with chronic ITP refractory to steroids and immunoglobulins who achieved complete normalisation of their platelet counts after treatment with rituximab, 375 mg/m2 given weekly in four doses. In both cases the B-lymphocyte count dropped to zero after the second dose of rituximab and an unsupported platelet count >100,000/ul was achieved during treatment. Six and twelve months after treatment, both patients remain well with normal platelet counts. They concluded that their report supports the concept that rituximab may also be a valuable therapeutic option in children with chronic ITP refractory to standard treatment. Controlled clinical trials are needed to evaluate the efficacy and long-term side effects of rituximab in this group of patients.

Nomura et al.,21 in 2003, stated that normal B cells can be induced to express immune costimulatory molecules by activated T cells, and activated CD4+ T cells can express CD40L, a molecule that can engage CD40 on the B-cell surface. CD40-CD40L interaction plays an important role in the pathology of certain autoimmune diseases such as ITP. Kuwana et al.,22 in 2003, denoted that the potential immunosuppressive effect of an anti-CD154 mAb on the pathogenic autoreactive T-cell response was evaluated using an in vitro culture system with glycoprotein IIb/IIIa-reactive T cells from patients with ITP. They indicated that blockade of the CD40/CD154 interaction induces generation of autoantigen-specific anergic CD4+ T cells with regulatory function and could be a therapeutic option for suppressing pathogenic autoimmune responses in patients with ITP.

Kuwana,2 in 2003, Ogawara et al.,23 in 2003 and Zhao et al.,24 in 2003, noted that ITP is an autoimmune disease characterized by increased platelet destruction caused by anti-platelet autoantibodies, which mainly target a platelet surface antigen, GP IIb-IIIa. CD4+ T cells reactive with GP IIb-IIIa play a primary role in the disease process, since these autoreactive T cells are involved in the production of pathogenic anti-platelet autoantibodies. These findings are particularly useful for clarifying the pathogenic process in ITP patients and for developing a therapeutic approach that blocks pathogenic anti-platelet antibody production.

Nagahama et al.,25 in 2002, stated that they investigated levels of soluble CD40L (sCD40L) in ITP patients, in order to determine the influence of CD40-CD40L interaction on the pathogenesis of ITP. Thirty-eight of the 65 ITP patients (58.5%) had elevated levels of sCD40L. They found significant decreases in platelet counts in sCD40L+ ITP patients. Although the sCD40L level did not differ significantly between the control and non-immune thrombocytopenia groups, but among ITP patients, sCD40L level was significantly higher in those with untreated ITP than...
in those with treated ITP. Their findings suggested that there are two groups of ITP patients, one with elevated levels of sCD40L and one with normal levels of sCD40L. ITP cases in which sCD40L was increased appeared to involve changes in platelet counts. The pathogenesis of ITP may in some patients include alterations of the CD40/CD40L pathway.

Nishimura et al., 26 in 2002, denoted that McAb therapies have conducted to not only hematological malignancies but also disorders of hemostasis and coagulation. Their article described the recent advances of McAb therapy for bleeding disorders such as ITP. Rituximab, chimeric anti-CD20 McAb treatment has a valuable effect in the patients with ITP, and clinical trials using anti-CD40 ligand McAb for ITP are underway.

Webber et al., 1 in 2001, noted that ITP is characterized by antibody-mediated destruction of platelets. The etiology is unknown. They postulated that increased autoantibody production in ITP might be attributable to either increased or prolonged expression of CD40L/CD154 in T lymphocytes. Cell surface CD154 expression was measured in freshly isolated and in vitro-activated peripheral blood lymphocytes of sixteen ITP patients and eight healthy volunteers. They observed that CD154 expressions in un-stimulated and in vitro-activated lymphocytes differ between ITP patients and healthy controls as they were significantly higher in the ITP patients. These studies indicated that overexpression of CD154 in lymphocytes is likely to be a primary pathophysiological defect in most patients with ITP. These data support that cell membrane antigens such as CD154 should be considered as potential targets for therapy in this disease.

Kuwana et al., 27 in 2001, stated that ITP is an autoimmune disease characterized by increased platelet clearance caused by anti-platelet autoantibodies, which bind to circulating platelets resulting in destruction by the reticuloendothelial system. They have recently developed enzyme-linked immunospot assay to detect circulating B cells secreting anti-platelet antibody. An increase in anti-platelet antibody-producing B cells in peripheral blood was specifically detected in ITP patients, but in none of thrombocytopenic patients without ITP or healthy donors. While earlier studies reported the presence of platelet-reactive T cells in ITP patients, they have recently found that GPIIb/IIIa is one of major target antigens recognized by platelet-reactive CD4+ T cells. Since GPIIb/IIIa-reactive CD4+ T cells had helper activity promoting production of anti-platelet antibody, these autoreactive T cells are involved in production of pathogenic anti-platelet autoantibodies in ITP patients. Suppression of GPIIb/IIIa-reactive CD4+ T cells may be of therapeutic use in treating refractory patients.

Kuwana et al., 28 in 2001, stated that it was recently reported that autoreactive CD4+ T cells to GPIIb/IIIa mediated antiplatelet autoantibody production in patients with ITP. To further examine the antigenic specificity of the GPIIb/IIIa-reactive T cells, 6 recombinant fragments encoding different portions of GPIIb or GPIIIa were generated and tested for their ability to stimulate antigen-specific T-cell proliferation and anti-GPIIb/IIIa antibody production in vitro. T cells from the PB of 25 patients with ITP and 10 healthy donors proliferated in response to recombinant GPIIb/IIIa fragments in various combinations. The amino-terminal portions of both GPIIbα and GPIIIa were frequently recognized (60% and 64%, respectively) compared with other fragments (4%-28%) in patients with ITP, but this tendency was not detected in healthy donors. These results indicate that the immunodominant epitopes recognized by pathogenic CD4+ T cells in patients with ITP are located within the amino-terminal portions of both GPIIbα and GPIIIa.

References: