Peripheral blood lymphocyte phenotype analysis in immune thrombocytopenic purpura

OBJECTIVE Differing dominant T-cell and B-cell pathophysiological mechanisms may be involved in immune thrombocytopenic purpura (ITP). In this study phenotype analysis was made of peripheral blood lymphocyte abnormalities and correlation explored between circulating lymphocyte subtypes and age, sex, serological findings and disease course. METHOD A retrospective study was conducted of 20 adults and 3 children with ITP whose lymphocyte phenotype analysis data were compared with those of 20 age- and sex-matched healthy volunteers. RESULTS No differences were observed between patients with well-responding and relapsing ITP. Negative correlation was demonstrated between CD20+/CD23+ B-cell levels and the number of relapses per year and positive correlation between high CD19+ and CD22+ levels and the need for splenectomy. CD5+ and CD7+ T-cell levels were inversely related with the detection of ANA and IgG anti-cardiolipin autoantibodies. Patients aged above 60 years had significantly lower levels of CD2+ and CD3+ T-cells and higher levels of CD5+/CD19+ co-expression. Finally, CD19+, CD20+, CD22+ B-cell levels, CD19+/CD79b+, CD19+/CD25+ and CD20+/CD23+ markers, and Fmc7+/CD11c+ co-expression were all significantly raised in patients with ITP. CONCLUSIONS B-lymphocyte abnormalities and age-related T-cell defects may be implicated in the pathogenesis and outcome of ITP.

Key words
Immune thrombocytopenic purpura
Immunophenotype
ITP
Thrombocytopenia

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reactive T-cell and B-cell proliferation,\textsuperscript{11,12} upregulation of CD86+ macrophages\textsuperscript{10} and changes in the immune system with advancing age.\textsuperscript{7} Overall, ITP appears to be a heterogeneous disorder in which differing T-cell dominant and B-cell dominant pathophysiological mechanisms may be operating.\textsuperscript{11} The aim of this study was to determine, using immunophenotype analysis, specific lymphocyte subsets in the peripheral blood of patients with ITP in comparison with normal subjects, and explore their possible correlation with age, gender, serological findings and outcome of the disease.

**MATERIAL AND METHOD**

**Patients**

Twenty adults with ITP, 15 females and 5 males, with a median age of 32.5 years (range 18–69 years), and 3 children, 2 boys and 1 girl, with a median age of 14 years (range 10–16 years) were studied retrospectively. All of the patients had been treated in the same department between 2002 and 2008 and their median follow-up was 38.3 months (range 2–144 months). Twenty healthy adult age- and gender-matched volunteers, 13 females, 7 males, median age 27 years (range 22–68 years), were enrolled as control group. All the patients with ITP met the standard diagnostic criteria: Platelet count of less than 80×10\textsuperscript{9}/L, normal or increased number of megakaryocytes on bone marrow aspiration and no other clinically apparent cause of thrombocytopenia. Patients with positive antinuclear and anti-cardiolipin autoantibodies, but with no evident underlying disorder were also enrolled in the study.

**Assays**

All the serological tests had been conducted at diagnosis to eliminate acute viral infections or other autoimmune disorders as the cause of thrombocytopenia. In addition, all the patients had undergone peripheral blood flow cytometric immunophenotype analysis in the same hospital laboratory to exclude other hematological disorders as the cause of thrombocytopenia, with the exception of one of the children who had first been admitted to a pediatric hospital, from which his medical records were obtained. The phenotype analysis had been conducted either at diagnosis or during relapse after a minimum of 3 months without administration of any drug.

All results expressed as percentages were converted into absolute counts using the absolute lymphocyte count (ALC) on the day of the examination (this does not apply for co-expressions CD5+/CD19+, CD20+/CD23+, Fmc7+/CD11c+, CD19+/CD79b+, CD10+/CD22+, CD19+/CD25+; CD43+/CD19+). Percentages of circulating lymphocyte subsets and natural killer (NK) cells were measured in 20 healthy volunteers by flow cytometric analysis using a panel of six fluorescence monoclonal antibodies (FACSfanta, BD Biosciences, San Jose, California) and converted to absolute counts using the ALC.

**Statistical analysis**

All continuous variables were expressed as mean±2SD (standard deviation) of the controls and classified on an ordinal scale. Differences in the frequency between groups were compared using the Chi-squared test, supported by the Monte Carlo procedure, and Fisher’s exact test, as well as the Mann-Whitney U-test, when applicable. The data were analyzed using the Statistical Package for Social Sciences (SPSS), version 15.0 and Minitab 12 software. Probability values of p<0.05 were considered to be statistically significant.

**RESULTS**

**Case control study**

The levels of CD19\textsuperscript{+}, CD20\textsuperscript{+} and CD22\textsuperscript{+} B-cells were significantly higher in patients with ITP than in healthy subjects. CD19\textsuperscript{+}/CD79b\textsuperscript{+}, CD19\textsuperscript{+}/CD25\textsuperscript{+} and CD20\textsuperscript{+}/CD23\textsuperscript{+} (activated and mature B-cell markers) were also significantly higher in the patients, as was the co-expression of Fmc7\textsuperscript{+}/CD11c\textsuperscript{+} (p=0.01). No other statistically significant differences were demonstrated. No differences between the two groups were observed in NK cells and suppressor T-cells (tab. 1, figures 1, 2).

**Correlations**

Concerning the course of the disease, no differences were observed between patients with well-responding and relapsing ITP. The number of relapses per year was lower in patients with a raised CD20\textsuperscript{+}/CD23\textsuperscript{+} rate (p=0.024). A significant number of those with high CD19\textsuperscript{+} and CD22\textsuperscript{+} levels were eventually splenectomized (p<0.05). Comparing the patients' immunophenotype analysis findings with

**Table 1.** Statistically significant differences in lymphocyte immunophenotype findings between patients with immune thrombocytopenic purpura and control subjects.

<table>
<thead>
<tr>
<th>Markers</th>
<th>p value</th>
</tr>
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<tbody>
<tr>
<td>CD19\textsuperscript{+}</td>
<td>0.014</td>
</tr>
<tr>
<td>CD20\textsuperscript{+}</td>
<td>0.001</td>
</tr>
<tr>
<td>CD22\textsuperscript{+}</td>
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<tr>
<td>CD20\textsuperscript{+}/CD23\textsuperscript{+}</td>
<td>0.001</td>
</tr>
<tr>
<td>CD19\textsuperscript{+}/CD79b\textsuperscript{+}</td>
<td>0.000</td>
</tr>
<tr>
<td>FMC7\textsuperscript{+}/CD11c\textsuperscript{+}</td>
<td>0.001</td>
</tr>
<tr>
<td>CD19\textsuperscript{+}/CD25\textsuperscript{+}</td>
<td>0.028</td>
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their serological tests at diagnosis, negative correlation was established between CD5\(^+\) and CD7\(^+\) cell levels and positive anti-nuclear antibodies (ANA) and IgG anti-cardiolipin (p<0.05). Patients aged above 60 years had significantly lower levels of CD2\(^+\) and CD3\(^+\) and higher rates of CD5\(^+\)/CD19\(^+\) co-expression. The mean age of those with a high rate of CD5\(^+\)/CD19\(^+\) was 66.5 years. No differences were observed between male and female patients (figures 3, 4).

**DISCUSSION**

These findings suggest that patients with ITP present an increase in the mature and activated B-cell population compared with healthy control subjects. This is consistent with the findings of a study which showed that CD19\(^+\)/CD40\(^+\) and CD19\(^+\)/CD40\(^+\)/CD19\(^+\) B-cells were significantly higher in patients with ITP.\(^{11}\) Elsewhere CD19\(^+\) B-cells have also been found to be upregulated in ITP.\(^{8}\) The above studies and recent works from China\(^{5}\) and Canada\(^{9}\) support the conclusion that T-cells, especially CD4\(^+\) and activated CD3\(^+\) cells, play a major role in the pathogenesis of the disease, especially its chronic form. The idea that T-cell activation is the critical event which determines the production of autoantibodies against platelets is widely accepted,\(^{10,12}\) although B-cell specific therapeutic agents (rituximab) are very effective for many patients with ITP, leading to a rapid and long-lasting response.\(^{13}\) Although recent studies suggest that NK cells also play a role in the biology of ITP,\(^{14-16}\) in the present study no significant differences in NK cells were found between patients with ITP and healthy control subjects.

The significantly higher expression of Fmc7\(^+\)/Cd11c\(^+\) (a marker of B-lymphoproliferative disorders) in patients with ITP with no clinically or otherwise apparent underlying hematological disorder remains to be investigated, although it suggests that ITP may occur when an underlying B-cell disorder is present but not yet recognizable.

Patients with refractory ITP who had raised rates of CD20\(^+\)/CD23\(^+\) (the mature B-cell phenotype) had a significantly lower number of relapses per year, while almost 60% of those with raised CD19\(^+\) and CD22\(^+\) levels were eventually splenectomized because of the resistance of the disease to conventional drug therapy. There were no post-operative differences in the course of the disease.

The inverse correlation found between CD5\(^+\) and CD7\(^+\) cells and positive antinuclear antibodies or anti-cardiolipin IgG is consistent with the overall changes in the immune system.
ΠΕΡΙΛΗΨΗ

Ανάλυση του φαινοτύπου λεμφοκυττάρων του περιφερικού αίματος στην αυτοάνοια θρομβοπενική πορφύρα

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Αρχεία Ελληνικής Ιατρικής 2016, 33(4):527–531

ΣΚΟΠΟΣ

Ανάλυση του φαινοτύπου των λεμφοκυττάρων του περιφερικού αίματος σε ασθενείς με αυτοάνοια ιδιοπαθή θρομβοπενική πορφύρα (ΙΘΠ) και διερεύνηση των πιθανών συσχέτισεων των υποτύπων των λεμφοκυττάρων ανάλογα με την ηλικία, το φύλο, τα ορολογικά ευρήματα και την πορεία της νόσου.

ΥΛΙΚΟ-ΜΕΘΟΔΟΣ

Είκοσι έννοικοι και 3 παιδιά μελετήθηκαν αναδρομικά. Τα δεδομένα της ανάλυσης του φαινοτύπου συγκρίθηκαν με τα δεδομένα υγιών εθελοντών, ηλικίας 20 ετών και ως προς το φύλο.

ΑΠΟΤΕΛΕΣΜΑΤΑ

Δεν βρέθηκαν διαφορές μεταξύ ατόμων με καλή ανταπόκριση και υποτροπή της νόσου. Διαπιστώθηκε αρνητική συσχέτιση μεταξύ των επιπέδων των CD20+/CD23+ B-λεμφοκυττάρων και του αριθμού των υποτροπών ανά έτος, καθώς και θετική συσχέτιση μεταξύ των αυξημένων επιπέδων CD19+ και CD22+ λεμφοκυττάρων και της συχνότητας της σπληνεκτομής. Υπήρχε αντίστροφη συσχέτιση των επιπέδων των CD5+ και CD7+ T-λεμφοκυττάρων με τη συχνότητα θετικών ANA και IgG αυτοαντισώματων κατά της καρδιολιπίνης. Οι ασθενείς ηλικίας >60 ετών είχαν σημαντικά χαμηλότερα επίπεδα CD2+ και CD3+ T-λεμφοκυττάρων, καθώς και υψηλότερα επίπεδα λεμφοκυττάρων με συνέκφραση CD5+/CD19+. Τελικά, τα επίπεδα των CD19+, CD20+, CD22+ B-λεμφοκυττάρων, καθώς και η συνέκφραση των δεικτών CD19+/CD79b+, CD19+/CD25+ και CD20+/CD23+ αλλά και η συνέκφραση Fmc7+/CD11c+ βρέθηκαν σημαντικά αυξημένα στους ασθενείς με ΙΘΠ.

ΣΥΜΠΕΡΑΣΜΑΤΑ

Τα ευρήματα υποθέτουν ότι οι διαταραχές των Β-λεμφοκυττάρων, καθώς και οι ελλείμματα των Τ-λεμφοκυττάρων που σχετίζονται με την ηλικία, μπορεί να ευθύνονται για την παθογένεια και την πορεία της νόσου.

Λέξεις ευρετηρίου: Ανοσοφαινότυπος, Θρομβοπενία, Ιδιοπαθής θρομβοπενική πορφύρα, ΙΘΠ

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