Circulating CD4+CD161+CD196+ Th17 cells are not increased in immune thrombocytopenia

by Daria Sollazzo, Sara Trabanelli, Antonio Curti, Nicola Vianelli, Roberto Massimo Lemoli, and Lucia Catani

Haematologica 2010 [Epub ahead of print]

Citation: Sollazzo D, Trabanelli S, Curti A, Vianelli N, Lemoli RM, and Catani L. Circulating CD4+CD161+CD196+ Th17 cells are not increased in immune thrombocytopenia. Haematologica. 2010; 95:xxx
doi:10.3324/haematol.2010.038638

Publisher’s Disclaimer.
E-publishing ahead of print is increasingly important for the rapid dissemination of science. Haematologica is, therefore, E-publishing PDF files of an early version of manuscripts that have completed a regular peer review and have been accepted for publication. E-publishing of this PDF file has been approved by the authors. After having E-published Ahead of Print, manuscripts will then undergo technical and English editing, typesetting, proof correction and be presented for the authors’ final approval; the final version of the manuscript will then appear in print on a regular issue of the journal. All legal disclaimers that apply to the journal also pertain to this production process.

Haematologica (pISSN: 0390-6078, eISSN: 1592-8721, NLM ID: 0417435, www.haematologica.org) publishes peer-reviewed papers across all areas of experimental and clinical hematology. The journal is owned by the Ferrata Storti Foundation, a non-profit organization, and serves the scientific community with strict adherence to the principles of open access publishing (www.doaj.org). In addition, the journal makes every paper published immediately available in PubMed Central (PMC), the US National Institutes of Health (NIH) free digital archive of biomedical and life sciences journal literature.

Support Haematologica and Open Access Publishing by becoming a member of the European Hematology Association (EHA) and enjoying the benefits of this membership, which include free participation in the online CME program.
Circulating CD4⁺CD161⁺CD196⁺ Th17 cells are not increased in immune thrombocytopenia

Immune thrombocytopenia (ITP) is an autoimmune disorder in which, for still unknown reasons, platelet surface proteins become antigenic and stimulate the immune system to produce autoantibodies and self-reactive cytotoxic T lymphocytes. These findings result in immune-induced platelet destruction and suppression of platelet production (1,2).

Recently, a new subset of interleukin-17 (IL-17)-producing CD4⁺ effector T cells (Th17) has been discovered. Depending on the target cell population, IL-17 induces the release of colony stimulating factors, chemokines, metalloproteinases, Tumor Necrosis Factor-alpha, and IL-6. Moreover, IL-17 mobilizes and activate neutrophils. Since IL-17 has potent immunogenic properties, it is not surprising that a number of mechanisms contribute to the suppression of its production and function. For instance, both Th1 and Th2 cytokines suppress Th17 development. Several studies have suggested that Th17 T cells may be the major cell type involved in orchestrating tissue inflammation and autoimmunity. Specifically, Th17 cells have been shown to play a crucial role in the induction of rheumatoid arthritis, multiple sclerosis, systemic lupus erythematosus and psoriasis (3-5).

Previous studies investigated the role of Th17 cells in ITP patients, although contrasting results were reported. While some Authors demonstrated increased percentages of Th17 cells in the peripheral blood of ITP (6, 7), Guo et al. found comparable frequency of circulating Th17 cells (flow cytometry analysis) and comparable expression of IL-17 transcripts (RT-PCR evaluation) in patients and controls (8). Noteworthy, in these studies Th17 cells were enumerated after stimulation of mononuclear cells with various molecules (phorbol myristate acetate and ionomycin) and, therefore, not under physiological conditions. Moreover, these methods allowed the flow cytometry analysis of very low percentages of positive cells (around 2-3%) (6-8).

Recently, Cosmi et al (9), showed that human Th17 cells, expressing CCR6 (CD196), appear to originate exclusively from a small subset of CD161⁺CD4⁺ T-cell precursors detectable in the thymus and in umbilical cord blood, in response to the combined activity of IL-1beta and IL-23. Furthermore, IL-17-producing cells have been shown to be included in the CD161⁺ fraction of CD4⁺ T cells present in the circulation and purification of CD196⁺CD161⁺ circulating CD4⁺ T cells allows the enrichment of human IL-17-producing cells. These findings indicate CD161 as a novel surface marker for human Th17 cells.

In the present study, we evaluated Th17 cells in the peripheral blood of ITP patients and healthy subjects by using a panel of monoclonal antibodies according to Cosmi et al. (9). Specifically, we evaluated by flow cytometry the frequency of circulating Th17 cells, identified as CD161⁺CD196⁺.
cells in the CD4\(^+\) T-cell subpopulation. Fifteen patients with active ITP (7 men and 8 women; median age 42 years (range 21-70)) were studied. The diagnosis of ITP was made according to Provan et al. (2). Six patients were studied at diagnosis, 7 had persistent and 2 chronic ITP. At the time of sample collection, patients were off-treatment by at least three months and none of the patients had been previously splenectomized. The median platelet count was 50x10^9/L (range 8-99). Fifteen healthy subjects were also studied (6 men and 9 women; median age 40). All subjects provided written informed consent.

Mononuclear cells (MNCs), anticoagulated with ethylene diamine tetraacetic acid, were isolated from peripheral blood of healthy individuals and ITP patients via density gradient centrifugation using Ficoll-Hypaque (Cedarlane-Celbio, Milan, Italy). Immunofluorescence was performed using the following monoclonal antibodies: APC-conjugated anti-CD45, PerCP-conjugated anti-CD4, PE-conjugated anti-CD161, FITC-conjugated anti-CD196. All from BD Pharmingen (Milan, Italy). Immediately after washing with phosphate buffered saline (PBS), cells were analyzed by using a BD FACSCanto II equipment (Bekton Dickinson, Milan, Italy) and were gated for lymphocytes on the basis of their CD45 and side scatter profile (CD45 bright and SSC low). A minimum of 10,000 events of the MNC fraction was collected. Absolute cell counts were assessed using a “dual platform” technique where the flow cytometer provides the cell percentages and the hematology analyzer provides the absolute white blood cell count. Differences between groups were compared using the nonparametric Wilcoxon rank sum test. Spearman rank correlation test was used for correlation analysis. P values <0.05 were considered statistically significant.

As shown in Figure 1A and B, the mean percentage ± standard deviation and the mean absolute number ± standard deviation of circulating Th17 cells were comparable between ITP patients (12.22±4.82%; 107±72 cells/µL) and controls (11.88±4.95%; 93±39 cells/µL) (p=NS). In addition, according with Guo et al (8), there was no significant correlation between the number of Th17 cells and the platelet count in ITP patients. Thus, our results do not suggest that ITP is associated with a significant difference in the number of circulating Th17 cells as compared to healthy individuals. Consistently, previous studies demonstrated that plasma IL-17 levels were comparable between patients and controls (7, 10). Keeping in mind that Th1 cytokines suppress Th17 development (3), our findings might be explained, at least in part, by the previous documented elevated Th1 response in ITP patients (11). Furthermore, experimental evidence demonstrated quantitative/qualitative defects of regulatory T cells (Tregs) in ITP patients (11). In this view, the generation of Tregs and Th17 cells from naïve T cells was shown to be correlated and, depending on the availability of IL-6, the balance between Tregs and Th17 cells could be shifted (12). However, our results do not
indicate that the low number of circulating Tregs in ITP patients is due to an altered balance toward the Th17 pathway.

In summary, our study shows that in ITP, at variance with other autoimmune diseases, the number of circulating Th17 cells does not differ from normal individuals.

Daria Sollazzo, Sara Trabanelli, Antonio Curti, Nicola Vianelli, Roberto Massimo Lemoli, and Lucia Catani

Institute of Hematology “L. e A. Seràgnoli”, Department of Hematology and Oncological Sciences “L. e A. Seràgnoli”, University of Bologna, Bologna, Italy

Key words: Th17 cells, Immune Thrombocytopenia, CD4+CD161+CD196+

Correspondence

Daria Sollazzo, Institute of Hematology “L. e A. Seràgnoli”, Azienda Ospedaliero-Universitaria S. Orsola-Malpighi, Via Massarenti 9 40138 Bologna, Italy. E-mail: daria.sollazzo@fastwebnet.it
References

Figure 1. Flow cytometry analysis of circulating Th17 cells in ITP patients and controls. Th17 cells were identified as CD4⁺CD161⁺CD196⁺ cells and shown as (A) percentages of CD4⁺ cells or (B) absolute number. Results are expressed as mean ± SD.