val 3-5 months).

Among the 176 patients with MK we first considered the number of normal cells and, then separately, the number of abnormal cells. A univariate proportional hazards model indicated that survival improved as the number of normal cells increased (hazard ratio 0.95, 95% CI 0.9-0.99, *P*=0.02). The presence of 5 or more normal cells was associated with an HR of 0.76. The number of normal cells remained prognostic after accounting for age, gender, and treatment. In the multivariate model, older age was the only other factor associated with shorter survival. In contrast, the number of abnormal cells was not associated with survival in either univariate or multivariate analyses.

To jointly examine the number of normal cells and the number of abnormal cells we calculated the percentage of normal cells (which equals 1- percentage of abnormal cells). A higher percent of normal cells was associated with longer survival (hazard ratio 0.99, 95% CI 0.98-1.0, *P*=0.02) in both a univariate model and a multivariate model controlling for therapy, age, gender, WBC count, and blood blast percentage (Table 1).

How residual normal metaphases translate into longer survival is unclear. Despite the statistically significant findings, the survival of even those MK patients with residual normal cells is very poor (Figure 1). While the medical significance of our findings in MK patients is thus limited, our observations suggest that it might be worthwhile to examine the prognostic effect of residual normal metaphases in patients with better prognosis karyotypes. More generally, our results provide further evidence for the heterogeneity of even well defined cytogenetic groups.

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References

1. Byrd JC, Mrozek K, Dodge RK, Carroll AJ, Edwards CG, Arthur DC, et al. Pretreatment cytogenetic abnormalities are predictive of induction success, cumulative incidence of relapse, and overall survival in adult patients with de novo acute myeloid leukemia: results from Cancer and Leukemia Group B (CALGB 8461). Blood. 2002;100(13):4325-36.
2. Grimwade D, Walker H, Oliver F, Wheatley K, Harrison C, Harrison G, et al. The importance of diagnostic cytogenetics on outcome in AML: analysis of 1,612 patients entered into the MRC AML 10 trial. The Medical Research Council Adult and Children's Leukaemia Working Parties. Blood. 1998;92(7):2322-33.
3. Mrozek K, Bloomfield CD. Chromosome aberrations, gene mutations and expression changes, and prognosis in adult acute myeloid leukemia. Hematology Am Soc Hematol Educ Program. 2006;169-77.
4. Mrozek K, Heerema NA, Bloomfield CD. Cytogenetics in acute leukemia. Blood Rev. 2004;18(2):115-36.
5. Breems DA, Van Putten WL, De Greef GE, Van Zelderen-Bhola SL, Gerssen-Schoorl KB, Mellink CH, et al. Monosomal karyotype in acute myeloid leukemia: a better indicator of poor prognosis than a complex karyotype. J Clin Oncol. 2008;26(29):4791-7.
6. Medeiros BC, Othus M, Fang M, Roulston D, Appelbaum FR. Prognostic impact of monosomal karyotype in young adult and elderly acute myeloid leukemia: the Southwest Oncology Group experience. Blood. 2010;116(13):2224-8.
7. Estey EH, Pierce S, Keating MJ. Identification of a group of AML/MDS patients with a relatively favorable prognosis who have chromosome 5 and/or 7 abnormalities. Haematologica. 2000;85(3):246-9.

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 activates 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 used flow cytometry to evaluate 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 at least three months off-treatment and none of the patients had been previously splenectomized. The median platelet count was 50×10^9/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 non-parametric Wilcoxon's rank sum test. Spearman's rank correlation test was used for correlation analysis. *P* values below 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, in accordance 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. Previous studies consistently 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 previously 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.

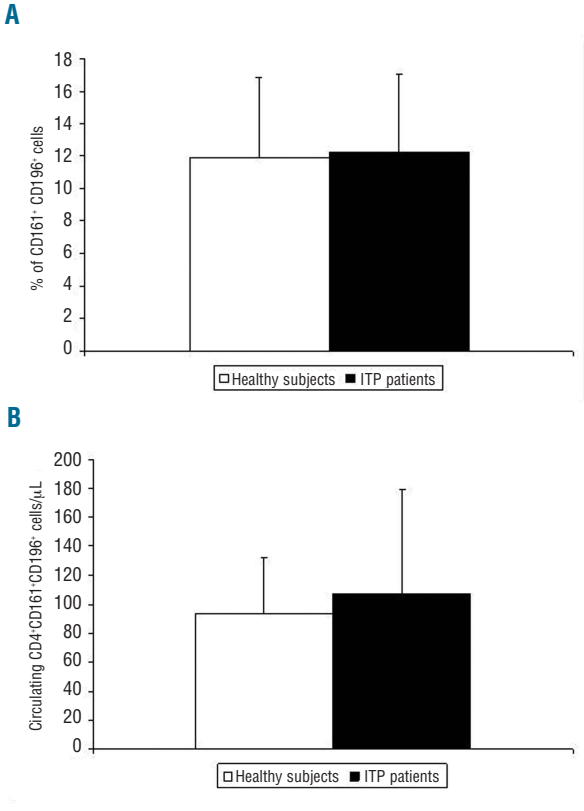

Figure 1. Flow cytometry analysis of circulating Th17 cells in ITP patients and controls. Th17 cells were identified as $CD4^+CD161^+CD196^+$ cells and shown as (A) percentages of $CD4^+$ cells or (B) absolute number. Results are expressed as mean ± SD.

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References

1. Stasi R, Evangelista ML, Stipa E, Buccisano F, Venditti A, Amadori S. Idiopathic thrombocytopenic purpura: current concepts in pathophysiology and management. Thromb Haemost. 2008; 99(1):4-13.
2. Provan D, Stasi R, Newland AC, Blanchette VS, Bolton-Maggs P, Bussel JB, et al. International consensus report on the investigation and management of primary immune thrombocytopenia. Blood. 2010;115(2):168-86.
3. Harrington LE, Hatton RD, Mangan PR, Turner H, Murphy TL, Murphy KM, et al. IL17-producing CD4+ effector T cells develop via a lineage distinct from the T helper type1 and 2 lineages. Nat Immunol. 2005;6(11):1123-32.
4. Romagnani S, Maggi E, Liotta F, Cosmi L, Annunziato S. Properties and origin of human Th17 cells. Mol Immunol. 2009;47(1):3-7.
5. Afzali B, Lombardi G, Lechler RI, Lord GM. The role of T helper 17 (Th17) and regulatory T cells (Treg) in human organ transplantation and autoimmune disease. Clin Exp Immunol. 2007; 148(1):32-46.
6. Zhang J, Ma D, Zhu X, Qu X, Ji C, Hou M. Elevated profile of Th17, Th1 and Tc1 cells in patients with immune thrombocytopenic purpura. Haematologica. 2009;94(9):1326-9.
7. Zhu X, Ma D, Zhang J, Peng J, Qu X, Ji C, et al. Elevated interleukin-21 correlated to Th17 and Th1 cells in patients with immune thrombocytopenia. J Clin Immunol. 2010;30(2):253-9.
8. Guo ZX, Chen ZP, Zheng CL, Jia HR, Ge J, Gu DS, et al. The role of Th17 cells in adult patients with chronic idiopathic thrombocytopenic purpura. Eur J Haematol. 2009;82(6):488-9.
9. Cosmi L, De Palma R, Santarlasci V, Maggi L, Capone M, Frosali F, et al. Human interleukin 17-producing cells originate from a CD161+CD4+ T cell precursor. J Exp Med. 2008;205(8):1903-16.
10. Ma D, Zhu X, Zhao P, Zhao C, Li X, Zhu Y, et al. Profile of Th17 cytokines (IL17, TGF-β, IL-6) and Th1 cytokine (IFN-γ) in patients with immune thrombocytopenic purpura. Ann Hematol. 2008;87(11):899-904.
11. Semple JW, Provan D, Garvey MB, Freedman J. Recent progress in understanding the pathogenesis of immune thrombocytopenia. Cur Opin Hematol. 2010;17(6):590-5.
12. Jager A, Kuchroo VK. Effector and regulatory T-cell subsets in autoimmunity and tissue inflammation. Scan J Immunol. 2010; 72(3):173-84.