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Citation: Zenhäusern R, Simcock M, Gratwohl A, Hess U, Bargetzi M, Tobler A on behalf of the Swiss Group for Clinical Cancer Research (SAKK). Rituximab in patients with hairy cell leukemia relapsing after treatment with 2-chlorodeoxyadenosine (SAKK 31/98) Haematologica 2008; 93:1426-1428. doi: 10.3324/haematol.11564

References

1. Cawley JC. Hairy-cell leukaemia: Biology and management. Br J Hematol 1999;106:2-8.
2. Mey U, Strehl J, Gorschlüter M, Ziske C, Glasmacher A, Pralle H, et al. Advances in the treatment of hairy-cell Leukaemia. Lancet Oncol 2003;4:86-94.
3. Hoffman MA, Janson D, Rose E, Rai KR. Treatment of hairy-cell leukemia with cladribine: Response, toxicity, and long-term follow-up. J Clin Oncol 1997;15:1138-42.
4. Saven A, Burian C, Koziol A, Piro LD. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood 1998;92:1918-26.
5. Goodman GR, Burian C, Koziol JA, Saven A. Extended follow-up of patients with hairy cell leukemia after treatment with cladribine. J Clin Oncol 2003;21:891-6.
6. Ginaldi L, De Martinis M, Matutes E, Farahat N, Morilla R, Catovsky D. Levels of expression of CD19 and CD20 in chronic B cell leukemias. J Clin Pathol 1998;51:364-9.
7. Hagberg H, Lundholm L. Rituximab, a chimaeric anti-CD20 monoclonal antibody, in the treatment of hairy cell leukaemia. Br J Haematol 2001;115:609-11.
8. Lauria F, Lenoci M, Annino L, Raspadori D, Marotta G, Bocchia M, et al. Efficacy of anti-CD20 monoclonal antibody, in the treatment of hairy cell leukemia. Haematologica 2001;86:1046-50.
9. Nieva J, Bethel K, Saven A. Phase 2 study of rituximab in the treatment of cladribine-failed patients with hairy cell leukemia. Blood 2003;102:810-3.
10. Thomas DA, O`Brien S, Bueso-Ramos C, Faderl S, Keating MJ, Giles FJ, et al. Rituximab in relapsed or refractory hairy cell leukaemia. Blood 2003;102:3906-11.

Increased cytotoxic T-lymphocyte-mediated cytotoxicity predominant in patients with idiopathic thrombocytopenic purpura without platelet autoantibodies

Idiopathic thrombocytopenic purpura (ITP) is a common hematologic disorder manifested by autoantibody-mediated platelet destruction. In the majority of ITP patients, autoantibodies were found to be specific to GPIIb/IIIa or GPIb/IX.[1] It may not only damage platelets via the reticuloendothelial system,[2] but may also inhibit platelet production.[3,4] However, platelet autoantibodies can be detected in only 50-70% of ITP patients,[5] indicating other mechanisms could be involved. Recently, *in vitro* studies suggested that $CD8^+$ cytotoxic T-lymphocyte (CTL) mediated lysis of autologous platelets may contribute to platelet destruction in ITP.[6,7] In the present study, we prospectively measured CTL-mediated cytotoxicity toward autologous platelets between ITP patients with and without autoantibodies, and evaluated the effect of high dose dexamethasone (HD-DXM) on this action.

Forty-eight previously-untreated ITP patients were enrolled by diagnostic criteria for ITP.[8] Blood sampling was performed before and two weeks after treatment with HD-DXM.[9] The control group consisted of 17 healthy adult volunteers with no history of blood transfusion or pregnancies (Table 1). The study was approved by the Medical Ethical Committee of Qilu Hospital. Informed consent was obtained from all subjects.

All plasma samples for modified monoclonal antibody specific immobilization of platelet antigen (MAIPA) assay were obtained from ethylenediaminetetraacetic acid (EDTA) anticoagulated blood by centrifugation and stored at -80°C prior to use. Platelets were prepared by differential centrifugation from EDTA-anticoagulated blood and used as target cells. $CD8^+$ T lymphocytes were isolated by magnetic microbeads (MACS; Miltenyi Biotec, Bergisch Gladbach, Germany) from peripheral blood mononuclear cells by density gradient centrifugation and used as effector cells. The purity of $CD8^+$ T cells was > 92%. The concentration of effector and target cells was adjusted to 10^5/mL and 10^6/mL respectively, and then diluted to achieve a final effector/target (E/T) ratio of 1:10. All cells were washed free from plasma.

Detection of specific anti-platelet GPIIb/IIIa and/or GPIb autoantibodies was performed as described by Hou *et al.*[10] Measurement of CTL-mediated cytotoxicity was carried out according to the protocol developed by Goldberg *et al.*[11] based on two-color flow cytometry to identify different populations of live targets ($CD41a^+$ Annexin V-) and apoptotic targets ($CD41a^+$ Annexin V^+) using antibodies from BD Biosciences (San Jose, CA, USA). Anti-CD3 antibody (BD Biosciences, San Jose, CA, USA) was added at a final concentration of 0.32 μg/mL to stimulate cytolytic effector T cells. Spontaneous lysis was determined in control tubes holding only culture media and platelets. A minimum of 10,000 events were acquired and data were analyzed using CellQuest Software on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA). Percent lysis and specific lysis were calculated as follows: percent lysis=total ($CD41a^+$, Annexin V^+)=Total ($CD41a^+$); specific lysis=percent induced lysis=percent spontaneous lysis, and expressed as a percentage.

In the plasma of 48 patients with ITP, antibodies against GPIIb/IIIa and/or GPIb/IX were detected in 22 samples (group I). Negative reactions to both glycoproteins were displayed in the remainders (group II). Before HD-DXM treatment, positive platelet lysis was seen in 11 of group I and 21 of group II (group I vs. group II, $p<0.05$) and in 4 of group I and 7 of group II after treatment (group I *vs.* group II, not significant (NS); pre-treatment *vs.* post-treatment in group I, $p<0.05$, and in group II, $p<0.01$) (Figure 1A-F). On the other hand, before treatment, both group I and group II had increased platelet lysis compared with controls (group I *vs.* controls, $p<0.05$; group II *vs.* controls, $p<0.01$; group I *vs.* group II, $p<0.01$), whereas platelet lysis was substantially decreased in both groups after treatment (pre-treatment *vs.* post-treatment in group I, $p<0.01$, and in group II, $p<0.01$) (Figure 1G). Interestingly, positive

Table 1. Subjects' characteristics.

Characteristics	*ITP patients*	*Controls*
Age (median, range)	29 (11-73)	27 (18-40)
Sex (number, %)		
female	30 (62.5)	10 (58.8)
male	18 (37.5)	7 (41.2)
Platelet count ($\times10^9$/L) (median, range)		
pre-treatment	10.5 (1-55)	239 (156-350)
post-treatment	78.5 (10-305)–	

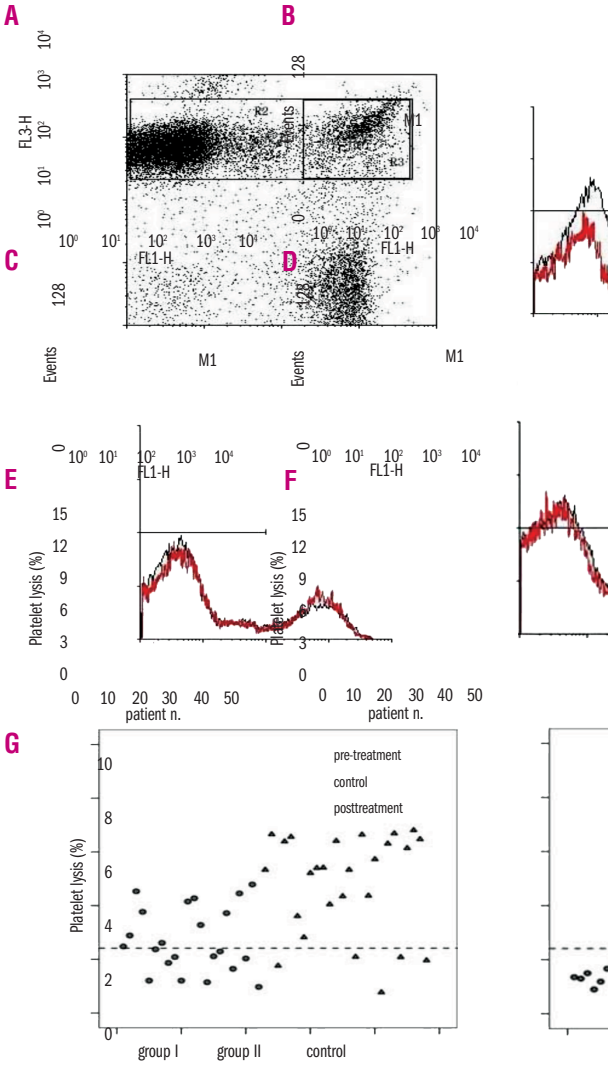

Figure 1. (A-D) One representative for CD41a-PEcy5 and Annexin V-FITC dual (A) and single (B-D) parameter analysis of CTL-mediated cytotoxity. In panels B-D, black lines designate the platelet incubated alone for estimation of spontaneous lysis, while the platelets and CD8+ cells mixed together for evaluation of induced lysis is indicated by red lines. The specific lysis is 10.03% (B), 4.33% (C), and 2.35% (D) respectively in one representative for group II and group I before treatment, and group II after treatment. (E, F) The number of patients with positive platelet lysis, defined as mean+2SD (dotted line) recorded for the controls, before (E) and after (F) treatment. Circles represent group I and triangles group II. (G) Analysis of cell-mediated cytotoxicity against autologous platelets in groups before and after treatment (n=48).

platelet lysis was also observed in one control subject (*data not shown*). Additionally, the platelet lysis was found to be negatively correlated with the platelet count in group II (r=-0.439, $p<0.05$) (Figure 2), but not in group I (r=-0.322, NS). Despite the use of the most sophisticated assays, platelet autoantibody cannot be detected in all patients with ITP.[5] Thus, autoantibody-mediated platelet destruction cannot account for all the observations in ITP. Recently, Olsson *et al.*[6] discovered increased expression of several cytotoxic genes in T cells from ITP patients and 6 out of 8 patients with active ITP showed platelet lysis by T cells, but none in remission. Subsequently, Zhang *et al.*[7]

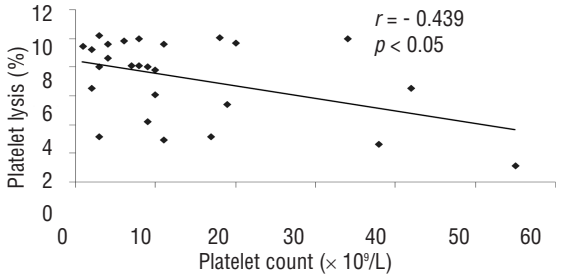

Figure 2. Negative correlation between platelet lysis and platelet count in group II.

found that platelet lysis was seen when using purified CD8+ T cells as effector cells and that CTLs were abnormally activated in ITP. In the present study, we found a significant difference in CTL-mediated platelet lysis between MAIPA positive and negative ITP patients, as well as a negative correlation between platelet lysis and platelet count. This evidence strongly supports the view that CTL-mediated cytotoxicity toward autologous platelets is dominant in ITP patients without autoantibodies, and protection against this action might be an effective therapeutic approach for this subgroup of ITP patients. As a promising alternative to prednisone for the first- or second-line treatment in ITP patients,[9] HD-DXM was demonstrated to correct the Th1 cytokine dominance, shown to promote cell-mediated cytotoxicity and IgG production by Guo *et al.*[12] In this study, platelet lysis was found to be significantly lower after HD-DXM treatment, indicating that HD-DXM could be an effective protection against both autoantibody-mediated and CTL-mediated destruction of platelets in ITP patients. Finally, the limitation of all currently available platelet antibody tests, including MAIPA, must be noted. Furthermore, autoantibodies against other glycoproteins such as GPIa/IIa, GPIV, GPV, and GPVI do exist, although their incidence is scarce in ITP.

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Funding: this work was supported by grants from National Natural Science Foundation of China (No. 30570779, No. 30470742, No. 30600259, No. 30770922, and No. 30300312), 973 Program (No. 2006 CB 503803), Foundation for the Author of National Excellent Doctoral Dissertation of PR China (No. 200561), Key Clinical Research Project of Public Health Ministry of China 2007-2009, the 1020 Program from Health Department of Shandong Province, and Commonweal Trade for Scientific Research (No. 200802031).

Key words: idiopathic thrombocytopenic purpura, apoptosis, cytotoxic T-lymphocyte, dexamethasone.

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Citation: Zhao C, Li X, Zhang F, Wang L. Peng J, Hou M. Increased cytotoxic T-lymphocyte-mediated cytotoxicity predominant in patients with idiopathic thrombocytopenic purpura without platelet autoantibodies. Haematologica 2008. 93:1428-1430.

doi: 10.3324/haematol.12889.

References

1. McMillan R, Wang L, Tani P. Prospective evaluation of the immunobead assay for the diagnosis of adult chronic immune thrombocytopenic purpura (ITP). J Thromb Haemost 2003;1:485-91.
2. Cines DB, Blanchette VS. Immune thrombocytopenic purpura. N Engl J Med 2002;346:995-1008.
3. Chang M, Nakagawa PA, Williams SA, Schwartz MR, Imfeld KL, Buzby JS, et al. Immune thrombocytopenic purpura (ITP) plasma and purified ITP monoclonal autoantibodies inhibit megakaryocytopoiesis in vitro. Blood 2003;102:887-95.
4. McMillan R, Wang L, Tomer A, Nichol J, Pistillo J. Suppression of in vitro megakaryocyte production by antiplatelet autoantibodies from adult chronic ITP patients. Blood 2004;103:1364-9.
5. Brighton TA, Evans S, Castaldi PA, Chesterman CN, Chong BH. Prospective evaluation of the clinical usefulness of an antigen specific assay (MAIPA) in idiopathic thrombocytopenic purpura and other immune thrombocytopenias. Blood 1996;88:194-201.
6. Olsson B, Andersson PO, Jernas M, Jacobsson S, Carlsson B, Carlsson LM, et al. T-cell-mediated cytotoxicity toward platelets in chronic idiopathic thrombocytopenic purpura. Nat Med. 2003;9:1123-4.
7. Zhang F, Chu X, Wang L, Zhu Y, Li L, Ma D, et al. Cell-mediated lysis of autologous platelets in chronic idiopathic thrombocytopenic purpura. Eur J Haematol 2006; 76: 427-31.
8. British Committee for Standards in Haematology General Haematology Task Force. Guidelines for the investigation and management of idiopathic thrombocytopenic purpura in adults, children and in pregnancy. Br J Haematol 2003;120:574-96.
9. Cheng Y, Wong RS, Soo YO, Chui CH, Lau FY, Chan NP, et al. Initial treatment of immune thrombocytopenic purpura with high-dose dexamethasone. N Engl J Med 2003; 349:831-6.
10. Hou M, Peng J, Shi Y, Zhang CQ, Qin P, Zhao CL, et al. Mycophenolate mofetil (MMF) for the treatment of steroid-resistant idiopathic thrombocytopenic purpura. Eur J Haematol 2003;70:353-7.
11. Goldberg JE, Sherwood SW, Clayberger C. A novel method for measuring CTL and NK cell-mediated cytotoxicity using annexin V and two-color flow cytometry. J Immunol Methods 1999;224:1-9.
12. Guo C, Chu X, Shi Y, He W, Li L, Wang L, et al. Correction of Th1-dominant cytokine profiles by high-dose dexamethasone in patients with chronic idiopathic thrombocytopenic purpura. J Clin Immunol 2007;27:557-62.

Elevated factor XIII level and the risk of peripheral artery disease

The prothrombotic state resulting from the elevation of certain clotting factors and suppression of fibrinolysis contribute to the risk of atherothrombotic diseases. Peripheral artery disease (PAD) has been associated with elevated plasma fibrinogen concentration, increased plasminogen activator inhibitor (PAI-1) level and decreased tissue plasminogen activator (tPA) activity.[1-3] Coagulation factor XIII (FXIII) circulates in association with fibrinogen and plays a central role in fibrin stabilization and fibrinolysis.[4] We have recently shown that in women with coronary stenosis and history of myocardial infarction adjusted plasma FXIII levels are elevated as compared to clinical controls, and in women, but not in men, elevated FXIII level represents a 2.5-3.0-fold increased risk of myocardial infarction.[5] In this study we investigated FXIII levels in patients with PAD and determined the risk of PAD conferred to patients by elevated FXIII level.

The study included 302 consecutive patients with PAD recruited over a 2-year period from the 3rd Department of Medicine, University of Debrecen. Patients with history of myocardial infarction, stroke, symptoms of angina and renal insufficiency were excluded. The patients were exempt of acute inflammation during the last two months before blood drawing and did not have any chronic inflammatory state, other than PAD. Finally, 278 patients (173 males and 105 females) with ankle brachial pressure index (ABPI) ≤0.9 remained in the study group (173 with Fontaine classification II and 105 with Fontaine classification III). Patients were compared to sex-matched clinical controls (n=278) who also presented at the hospital, but no significant health problem, other than diabetes mellitus in some of the patients, was diagnosed and ABPI was in the range of 0.91-1.3. The mean age±SD was 59.0±9.5 in the control and 64.3±12.2 in the patient group. The occurrence of diabetes mellitus was 20% and 25% among controls and patients respectively. Thirty percent of controls and 36% of patients were smokers. The differences were not statistically significant. Two hundred and seventeen PAD patients received aspirin and 33 patients received statins; since in the FXIII levels there was only a non-significant difference (<1%) between PAD patients on treatment and the rest of the patients, these groups were not analyzed separately. Eighty-eight percent of women were menopausal; none of them on hormonal replacement therapy. Ethical approval was obtained from the Ethics Committee of the University of Debrecen, and subjects gave their informed consent.

Plasma FXIII activity and antigen were measured by established methods[6,7] using commercially available reagent kits (REA-chrom FXIII assay and R-ELISA FXIII, Reanal-ker, Budapest, Hungary). In the measuring range, the CV for both assays was below 3%. Serum total cholesterol, LDL-cholesterol, HDL-cholesterol, triglyceride, apo AI, apo B, lipoprotein (a) and high sensitivity C-reactive protein (hsCRP), plasma fibrinogen, homocysteine, folic acid and vitamin B12 were determined by routine laboratory methods and used for the determination of parameters independently associated with FXIII levels.

Neither FXIII activity nor FXIII antigen levels of clinical controls differed significantly from the reference interval established in our laboratory.[6,7] Mean FXIII activity and antigen levels, both non-adjusted and adjusted for the respective independently associated parameters, were moderately, but significantly higher in patients than in the clinical control group (Table 1). In a small (n=50) early study elevation of non-adjusted FXIII levels has also been observed in PAD patients.[8] However, in this case differences according to gender and severity of the disease were not analyzed. In our study PAD was associated with a statistically significant elevation of FXIII activity and antigen levels in women. In men with PAD, elevations were somewhat more moderate and in the case of FXIII antigen concentrations were not statistically significant. No difference in adjusted FXIII levels were found in PAD patients with Fontaine II and Fontaine III stage disease (*data not shown*). As determined by Pearson's method, ABPI values showed no significant correlation with FXIII activity or antigen level. One may conclude from these results that the existence of PAD and not its severity is associated with the elevation in FXIII levels. As opposed to controls, in the patient group there was a statistically significant correlation between fibrinogen and FXIII activity (r=0.273, $p<0.001$) or FXIII antigen (r=0.148, $p=0.014$) level. However, the calculated coefficients of determination (r^2) indicate that changes in fibrinogen level contributed to