Supplementary Methods

Subjects

The characteristics of the patients are shown in Table 1. All patients fulfilled the diagnostic criteria for chronic ITP as evidenced by isolated thrombocytopenia lasting more than 6 months, presence of normal or increased numbers of megakaryocytes in bone marrow biopsies, normal spleen size and no other cause of thrombocytopenia. The patients were divided into two groups based on their platelet count (plc): (i) "active disease" < 50 x 10^9/L and (ii) "in remission" > 150 x 10^9/L. The controls consisted of 15 healthy adults (11 females and 4 males) with normal plc (range 184-340; mean 249±10). The study was approved by the Ethics Committee of Göteborg University, and all subjects gave their informed consent.

Table 1. Patient characteristics.

Patient	Gender	Age (years)	Plc (10^9/L)	Medication	Category	Included in:		
						Expression profiling	Flow cytometry	Lysis assay
1*	M	37	177	None	Remission	Yes	Yes	Yes
2*	M	56	299	None	Remission	Yes	Yes	Yes
3*	M	59	11	Prednisone 5 mg q.d.	Active	Yes	No	No
4*	F	53	45	Prednisone 5 mg q.d.	Active	Yes	Yes	Yes
5*	F	49	382	None	Remission	Yes	Yes	No
6	F	28	173	None	Remission	Yes	No	No
7	F	28	50	None	Active	Yes	No	No
8*	F	44	33	Prednisone 10 mg q.d.	Active	Yes	Yes	Yes
9	F	69	324	Prednisone 7.5 mg q.d.	Remission	Yes	No	No
10*	M	38	24	None	Active	Yes	Yes	Yes
11	M	49	163	None	Remission	No	No	Yes
12	M	37	35	None	Active	No	Yes	Yes
13*	F	26	20	None	Active	No	No	Yes
14*	F	25	165	None	Remission	No	No	Yes
15*	F	22	23	None	Active	No	Yes	Yes
16*	F	75	265	Prednisone 2.5 mg q.d.	Remission	No	Yes	Yes
17	M	30	212	None	Remission	No	Yes	No
18*	M	70	150	None	Remission	No	Yes	No
19	F	30	237	None	Remission	No	Yes	No
20*	M	70	159	Prednisone 10 mg q.d.	Remission	No	Yes	No
21	F	68	47	None	Active	No	Yes	Yes
22	F	68	30	Prednisone 7.5 mg q.d.	Active	No	No	Yes
23*	M	57	454	None	Remission	No	No	Yes

* indicates that the patient has been splenectomized

Preparation of peripheral blood mononuclear cells (PBMCs) and T-lymphocytes.

PBMCs were prepared from heparinised blood by density gradient centrifugation. After removal of CD14+ cells, T-lymphocytes were positively selected using CD3+ magnetic microbeads, according to the manufacturer's recommendations (MACS; Miltenyi Biotec, Surrey, UK). The purity of isolated cells was determined by flow cytometry; 93.6-96.2% of the cells were CD3+ T-cells, with no significant differences between the study groups.

Preparation of RNA and hybridization to DNA microarrays

RNA was isolated from the CD3+ T-cell preparations using the Chomczynski method (Chomczynski, P. & Sacchi, N. *Anal. Biochem.* **162**, 156-159 (1987)), followed by RNeasy clean up (Qiagen, Hilden, Germany). The RNA from 5 patients in each group was pooled in equal amounts. Each pool was analysed in duplicates as described (Benson, M. et al. *Clin. Exp. Allergy* **32**, 301-308 (2002)). Briefly, RNA was transcribed into cDNA (Life Technologies, Rockville, MD) and biotin-labeled cRNA (Enzo, Farmingdale, NY) was resynthesized. Hybridization to DNA microarrays (Human Genome U95A array version 2; Affymetrix, Santa Clara, CA) and detection of hybridized target cRNA was performed according to the Affymetrix Gene Chip Expression Analysis manual. Quality of the cDNA-synthesis and in vitro transcription was assessed by hybridization to Test2-arrays (Affymetrix).

Data analysis

Scanned output files were visually inspected for hybridization artifacts and then analyzed with MicroarraySuite 5.0 software (Affymetrix). The arrays were scaled to an average intensity of 500. Genes were clustered and selected using Genespring 5.0 (Silicon Genetics, Redwood City, CA).

Flow cytometry

PBMCs were incubated with anti-CD3-Peridine-Chlorophyll-Cy5.5, anti-CD158a-phycoerythrin (PE) (specific for KIR3DL2), anti-CD158b-PE (specific for KIR2DL3) and NKB1-PE (specific for KIR3DL1). Appropriate isotype matched control antibodies were used. All monoclonal antibodies (MoAbs) were from Becton Dickinson Bioscience (San Diego, CA,). A FACScan (Becton Dickinson, Mountain View, CA) was used, and 30 000 events were collected. Data were analyzed using the WinMDI software (trotter@scripps.edu). The fraction of CD3+ cells expressing CD158a, CD158b and NKB1 was determined by setting a quadrant gate in the fluorescence dotplot for CD3+ cells. All samples were analyzed in triplicates.

Analysis of cell-mediated cytotoxicity

PBMC were prepared as described above. CD14+ and CD19+ cells were removed from PBMCs by magnetic microbeads (MACS; Miltenyi Biotec). The mean percentages of T- (CD3+) and NK-cells (CD3-/CD16+/CD56+) in the cell preparations were 75±4% and 14±3%, respectively. The cell concentration was adjusted to 2 x 10^6 cells/mL and then diluted in RPMI-1640 (Life Technologies, USA) medium supplemented with 5% heat-inactivated human AB+ serum to achieve a final effector/target (ET) ratio of 0.625:1. Autologous platelets were radiolabeled with ^{111}In (Amersham, Buckinghamshire, UK) as described (Wadenvik, H. & Kutti, J.

Br. J. Haematol. **78**, 523-528 (1991)) and used as target cells. All cells were washed free from plasma. To stimulate cytolytic effector T-cells, anti-CD3-antibody (R&D Systems, Abingdon, UK) was added in five 4-fold dilutions, starting with 4 μg/ml. Effector cells, target cells and anti-CD3 MoAb were added to a 96-well plate in triplicates and incubated for 4 hours. Thereafter, the plates were centrifuged and the supernatants were counted in a gamma counter. Spontaneous lysis was determined in control wells only holding culture media and radiolabeled platelets. Maximal lysis was determined after addition of Triton X-100 (Sigma Chemical Co, Saint Louis, MI) to a final concentration of 1%. The specific lysis was calculated as follows: (observed lysis-spontaneous lysis)/(maximal lysis-spontaneous lysis), and expressed in percent.

The specificity of the cytotoxic cell lysis was addressed in another set of experiments in which radiolabeled HLA disparate platelets, obtained from blood group 0 donors, were used as target cells. The effector cells were CD14-/CD19- PBMCs obtained from active ITP patients (n=5) known to have a positive lysis test against autologous platelets.

Additionally, to address the relative role of NK- and CD8+ T-cell mediated platelet lysis, purified fractions of NK-cells and CD8+ T-cells were isolated, from active ITP patients known to have a positive platelet lysis test, by magnetic microbeads (MACS NK cell isolation kit and CD8+ T cell isolation kit, respectively; Miltenyi Biotec) and used as effector cells (n=5). The purity of the isolated CD3+/CD8+ cells was 88±0.9%; less than 0.2% of the cells were NK-cells (CD3-/CD16+/CD56+). Similarly, the purity of the isolated NK-cells was 75±5.8%; less than 0.8% was CD3+ T-cells. The target cells in this experiment were autologous radiolabeled platelets.

Statistics

Unless otherwise stated, the mean values ± SEM are reported. Differences between groups were evaluated using analysis of variance (ANOVA for repeated measurements). Differences in number of patients with a positive platelet lysis test between groups were evaluated using the Fisher`s Exact Test (2x2). A *P*-value < 0.05 was considered statistically significant.