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Inflammation Research

Expression of platelet P-selectin and detection of soluble P-selectin, NPY and RANTES in patients with inflammatory bowel disease

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Abstract. *Objective and Design:* P-selectin, a membrane glycoprotein which is expressed on activated platelets and endothelial cells, plays a crucial role in the inflammatory response. The main action is adhesion of leukocytes, facilitation of diapedesis and induction of cytokine production from monocytes (MCP-1 and IL-8), mediated via RANTES released from activated platelets. An abnormal platelet activity has been reported in patients with ulcerative colitis (UC) and Crohn's disease (CD), jointly referred to as inflammatory bowel disease (IBD), which could have an aggravating influence on the inflammatory response. In addition, an up-regulation of platelet IL-8 receptors among patients with IBD has been reported. To reveal a presumptuous platelet dysfunction we analysed the expression of platelet surface P-selectin at resting state and after stimulation with thrombin, collagen, epinephrine and interleukin 8 (IL-8), and plasma levels of soluble P-selectin, neuropeptide Y (NPY) and RANTES in patients with IBD.
Subjects: Blood from twelve healthy subjects (control group) and twenty-one patients with IBD who had not taken any anti-platelet drugs or steroids were analysed.
Methods: Patients were sub-grouped according to disease entity, disease activity and 5ASA medication. Surface P-selectin expression on isolated human platelets and plasma P-selectin, NPY and RANTES were analysed with ELISA. All values are presented as mean ± standard error of the mean (SEM). Mann-Whitney U test and Wilcoxon matched rank test were used for statistical analyses.
Results: Patients with IBD in remission (n = 9) had higher basal P-selectin expression, 0.38 ± 0.04, compared to the control group (n = 12), 0.22 ± 0.03, $p < 0.01$. UC patients (n = 16) showed down-regulation of P-selectin expression after stimulation with IL-8, 0.26 ± 0.03 to 0.22 ± 0.02, $p < 0.05$. No significant differences could be observed concerning soluble P-selectin and NPY in plasma. Patients with 5ASA (n = 12) had lower levels of plasma RANTES, 2.39 ± 0.06 µg/l, compared to the control group (n = 12), 3.29 ± 0.19 µg/l, $p < 0.01$, and patients without 5ASA (n = 9), 2.90 ± 0.17 µg/l, $p < 0.05$.
Conclusions: Patients with IBD in remission have higher basal platelet surface P-selectin expression. An exaggerated platelet activity with increased expression of platelet P-selectin and release of inflammatory mediators such as RANTES, which is chemotactic and induce chemokine production, could have a reinforcing and aggravating influence on the inflammatory response and increase the susceptibility to IBD. In addition IL-8 has a down-regulating effect on platelet surface P-selectin expression and 5ASA medication seems to lower plasma RANTES. If 5ASA is responsible for lowering the concentration of RANTES this could be one of the beneficial outcomes of 5ASA medication.

Key words: Platelet – P-selectin – IBD – IL-8 – RANTES

Introduction

Inflammatory bowel disease (IBD) is, despite intense research, a gastrointestinal disorder of unknown aetiology. Virus, bacteria and auto immunity amongst others have been suggested as causative agents but no conclusive results have been presented [1–3]. Current research has drawn the attention towards a platelet dysfunction as a possible contributor to the disease [4] and the expected prevalence of IBD seems to be lower among patients with inherited disorders of coagulation [5].

Platelets play a crucial role in hemostasis and inflammatory response. Normally, platelets circulate in a quiescent state but upon activation a major event is the expression of P-selectin (CD62P; previously GMP-140 or PADGEM) and release of inflammatory mediators and factors promoting hemostasis, including RANTES (acronym for regulated upon activation, normal T cell expressed and secreted) and neuropeptide Y (NPY).

P-selectin, a membrane glycoprotein belonging to the selectin family together with E-selectin and L-selectin, acts

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as a cell-adhesion molecule in activated platelets and endothelial cells. In quiescent platelets P-selectin is stored in α-granules and is mobilised to the surface within minutes after stimulation with agonists such as thrombin, adenosine-*di*-phosphate (ADP) and epinephrine [6]. The mobilisation is agonist and dose dependent [7] and the main action is adhesion of neutrophils and monocytes [8] and facilitation of diapedesis. Thrombin-activated platelets also induce secretion of the chemokines, monocyte chemotactic protein-1 (MCP-1) and IL-8, from monocytes via RANTES [9]. Diacovo et al. suggests that platelets parallel the neutrophil/endothelial interaction and may function as a surrogate for endothelial cells at sites of vascular damage [10]. It is known that rolling of leukocytes on endothelial cells is absent in P-selectin knockout (KO) mice, but normal in L-selectin KO mice, and completely blocked in wild-type animals after addition of P-selectin monoclonal antibody [11]. Further, there is no extravasation of neutrophils, at early stages of inflammation, among P-selectin KO mice because of the endothelium and/or platelet P-selectin deficiency [12]. At later stages of inflammation and in preparations treated with TNF-α, leukocyte rolling is normal and seems to be L-selectin dependent [11]. After activation circulating platelets lose surface P-selectin, but continue to have an aggregating function [13].

NPY, a relatively new substance discovered in human platelets [14], is a linear peptide containing 36 amino acid residues [15] closely related to peptide YY and pancreatic polypeptide (PP). NPY has a wide distribution in the organism and besides in platelets it exists in heart, brain, spleen, peripheral noradrenergic neurones innervating lymphoid tissues, adrenal medulla and bone marrow. It has been shown that NPY from rat platelets, identical to human NPY, is released upon activation with collagen and thrombin. The vasoconstrictor effect of NPY is well-known and other effects are; enhancement of neutrophil adhesion to endothelial cells, induction of histamine release from mastcells and stimulation of macrophage adhesion, chemotaxis, phagocytosis and superoxide anion production [16, 17].

The chemokine RANTES, member of the human chemokine β family [18], is a potent inflammatory mediator secreted by activated T-lymphocytes and, as mentioned above, platelets. Besides the induction of chemokines in monocytes it also facilitates the migration of monocytes and memory T-lymphocytes [19, 20], modulates histamine release from basophils and acts as a chemotactic and activating factor for eosinophils [21]. In addition, expression of RANTES mRNA has been discovered in synovial lining cells, synovial fibroblasts, renal tubular epithelium and vascular endothelium. Increased transcripts have been shown in intestinal mucosa of patients with IBD [22].

Multifocal microvascular infarction has earlier been proposed as a possible pathological mechanism in Crohn's disease [23] and an increased risk of thromboembolic disease as well as abnormal platelet activity has been reported in patients with IBD [24]. Schürmann et al. have reported an increased expression of P-selectin in veins and capillaries among IBD patients [25] and Collins et al. have shown, with flow cytometry, significantly increased expression of P-selectin in circulating platelets in patients with IBD [26]. In addition, an up-regulation of platelet IL-8 receptors among patients with IBD, independently of disease activity, has also been reported [27] and to our knowledge has the effect of IL-8 stimulation of platelets never been evaluated.

In conclusion, the central role of platelets in hemostasis and formation of thrombi, and the important function of activated platelets and expression of P-selectin in the inflammatory response, has led us to the question if activation of platelets and expression of platelet surface P-selectin in patients with IBD differ compared to healthy subjects, and if there is a difference between patients in remission and patients in relapse. Hypothetically, an increased platelet activity, with increased expression of P-selectin and release of mediators promoting inflammation such as NPY and RANTES, could have an aggravating influence on the inflammatory process.

To reveal a presumptuous platelet dysfunction in patients with IBD we analysed the expression of platelet surface P-selectin at resting state and after stimulation with thrombin, collagen, epinephrine and IL-8. We also detected plasma levels of soluble P-selectin, NPY and RANTES in the same patients. Healthy blood-donors served as a control group.

Materials and methods

Ethical consideration

The study protocol was approved by the Ethics Committee of the Faculty of Health Sciences, Linköping University, Sweden, and was conducted according to the Declaration of Helsinki.

Patients and controls

Controls and patients with intake of anti-platelet drugs or steroids two weeks prior to sample collection were excluded to minimise the influence on the aracidonic acid pathway.

Twelve healthy blood-donors served as a control group and patient samples consisted of blood from twenty-four IBD patients. Diagnosis was verified with histological examination; seventeen patients had ulcerative colitis (UC) and seven patients had Crohn's disease (CD), three patients were excluded due to steroid intake, one in the UC group and two in the CD group, (Table 1a). Further, patients were sub-grouped according to disease entity and 5ASA medication, (Table 1b).

The analyst had no information concerning the patients disease entities until all measurements had taken place. The only information given prior analysis was the patients/blood-donors age and sex.

Collection of blood samples

Blood used for analysis of platelets was collected in silicone-coated vacutainer tubes containing 1:6 volumes of ACD (71 mM citric acid,

Table 1a. Control group and patient groups.

Group	male:female	age	excluded (n)
Control (n = 12)	6:6	48 (25–65)	none
UC relapse (n = 13)	7:5	47 (19–78)	1
UC remission (n = 4)	3:1	47 (32–59)	none
CD remission (n = 7)	3:2	49 (22–56)	2

UC; ulcerative colitis, CD; Crohn's disease.

Table 1 b. Patient groups divided according to 5ASA medication.

Group	male:female	age
UC relapse + 5ASA (n = 6)	3:3	53 (24–78)
UC relapse – 5ASA (n = 6)	4:2	42 (19–65)
UC remission + 5ASA (n = 3)	2:1	42 (32–59)
UC remission – 5ASA (n = 1)	1:0	52
CD remission + 5ASA (n = 3)	1:2	49 (22–56)
CD remission – 5ASA (n = 2)	2:0	50 (46–54)
All patients + 5ASA (n = 12)	6:6	58 (22–78)
All patients – 5ASA (n = 9)	7:2	46 (19–65)

UC; ulcerative colitis, CD; Crohn's disease.

85 mM sodium citrate and 111 mM dextrose) just before preparation and analysis.

Blood used for analysis of plasma was collected in vacutainer tubes containing 1:10 volumes of CTAD (0.11 M citric acid, 15 mM theophyllamine, 3.7 mM adenosine and 0.198 mM dipyrimadole) and immediately put into ice-cooled water preceding centrifugation at 1920 × *g* for 30 min at 4°C. Aliquots of 125 µl were frozen at –70°C for later assay of P-selectin, RANTES and NPY.

All tubes were purchased from Becton Dickinson, England.

Samples were collected after written agreement.

Preparation of isolated platelets (IP)

Blood from tubes containing ACD was centrifuged at 220 × *g* for 20 min at room temperature (RT) to obtain platelet rich plasma (PRP). Acetylsalicylic acid (100 µM) and apyrase (Grade III, 1.0 U/ml), both Sigma Chemical Co., St. Louis, MO, USA, were added to PRP prior to a second centrifugation at 480 × *g* for 20 min. After removal of the supernatant (platelet poor plasma) the platelet pellet was gently resuspended in a HEPES (N-2-hydroxyethyl-piperazine-N′-2-ethanesulfonic acid) buffered solution (145 mM NaCl, 5 mM KCl, 1 mM $MgSO_4$, 10 mM D-glucose and 10 mM HEPES, pH 7.4 at RT) also containing apyrase (1.0 U/ml). HEPES was from Sigma Chemical Co., St. Louis, MO, USA. IP were adjusted to a constant count (1×10^8/ml) in the same buffer, (pH 7.4).

Measurement of surface P-selectin in IP by ELISA

10 µl aliquots of solvent, thrombin (1.0 U/ml), epinephrine (0.1 mM), Sigma Chemical Co., St. Louis, MO, USA, collagen (0.1 mg/ml), Chrono-Log Corp. Havertown, PA, USA, and IL-8 (10 µg/ml), Biosource International, Camarillo, CA, USA, were added to microtiter plates prior to the addition of 90 µl of IP. The reaction was terminated with 0.2% paraformaldehyde, Sigma Chemical Co., St. Louis, MO, USA, in PBS (137 mM NaCl, 2.7 mM KCl, 8 mM Na_2HPO_4, 1.5 mM KH_2PO_4) after 1 min (solvent, thrombin, collagen, epinephrine) and 10 min (IL-8). The plates were centrifuged at 640 × *g* for 15 min. After centrifugation the supernatants were discarded and each well was incubated with 5% bovine serum albumin in PBS for one hour and thereafter washed with PBS. IP were then incubated with mouse monoclonal antibodies (mAbs) against human P-selectin (Anti-GMP-140, clone AC1.2, IgG1, dilution 1/2000) and against CD61 (Anti-gpIIIa, clone RUU-PL7F12, dilution 1/50) for 50 min. Both antibodies purchased from Becton Dickinson, San Jose, CA, USA. After incubation IP were washed twice with NaCl-Tween (0.5 ml Polyoxyethylene-Sorbitan-monolaurate and 9 g NaCl/l destilled H_2O, Sigma Chemical Co, St.Louis, MO, USA) and incubated for 50 min with alkaline phosphatase conjugated rabbit anti-mouse IgG (D314), (dilution 1/1000), Dakopatts, Glostrup, Denmark. All incubations were made at RT with a gentle rocking motion. Following a final wash, with NaCl-Tween, 100 µl of *p*-nitrophenylphosphate was added, Sigma Chemical Co., St. Louis, MO, USA, (1 tablet phosphate substrate/5 ml 1 M dietanolamin buffer, pH 9.8, Aldrich-Chemical Co, Gillingham-Dorset, UK). Substrate hydrolysis by phosphatase was measured at 405 nm, using a Spectramax kinetic microplate reader (Molecular Devices, Sunnyvale, CA, USA) after 10 min incubation [6]. A subtract prereading at 405 nm was also performed.

Assay for soluble P-selectin, NPY and RANTES

Levels of soluble P-selectin were estimated using a monoclonal antibody based ELISA kit from R&D Systems, Abingdon, Oxfordshire, UK. Measurement of RANTES was done using an ELISA kit from Endogen, Woburn, MA, USA and levels of NPY were detected with a competitive enzyme immunoassay kit from Peninsula Laboratories Europe, Merseyside, UK. All analyses were made according to standard procedures.

Statistical analysis

All values in this study are presented as mean ± standard error of the mean (SEM). Unpaired statistical analyses were calculated by the Mann-Whitney U test and Wilcoxon's matched rank test was used for paired data. Paired statistical analyses were used upon comparisons within groups and unpaired analyses when comparisons were made between groups. Two-tailed *p* values were used throughout. Differences were considered to be significant when $p < 0.05$.

Results

Surface expression of P-selectin on IP

As a measurement of basal P-selectin expression IP were assayed after addition of solvent and showed the following results (significance in comparison with the control group); control group 0.22 ± 0.03 (n = 12), CD in remission 0.39 ± 0.09 (n = 5), $p < 0.05$, UC in remission 0.37 ± 0.12 (n = 4), $p = 0.052$, and UC in relapse 0.23 ± 0.02 (n = 12), $p > 0.05$, (Fig. 1a). All patients in remission, analysed as one group, 0.38 ± 0.04 (n = 9), $p < 0.01$.

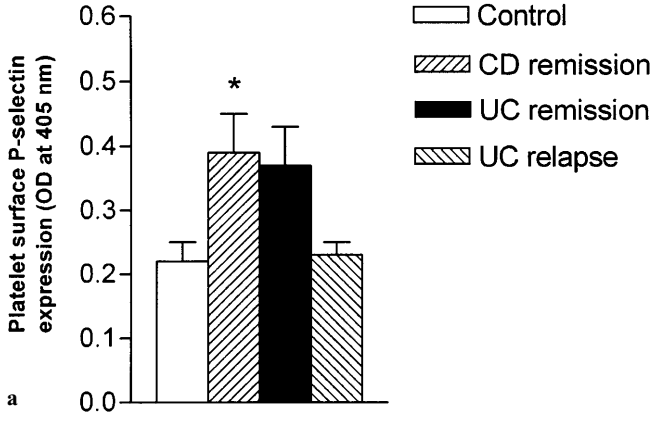

Fig. 1 a, b. a Basal expression of surface platelet P-selectin on isolated washed human platelets. Significant differences compared to the control group, calculated by the Mann-Whitney U test, are indicated by *, corresponding to $p < 0.05$ (Mean ± SEM, control group, n = 12, Crohn's disease in remission, n = 5, ulcerative colitis in remission, n = 4, ulcerative colitis in relapse, n = 12).

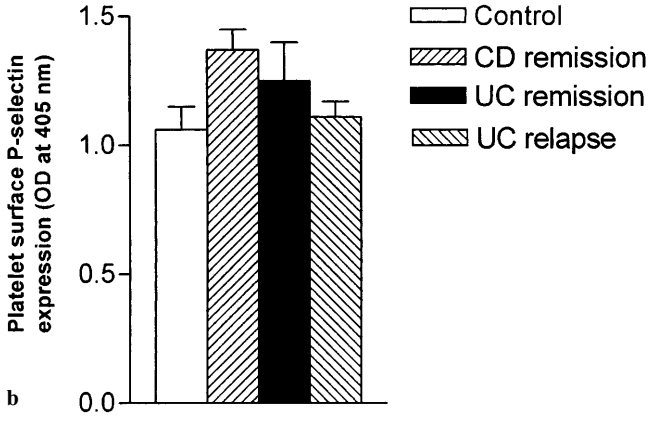

Fig. 1 *(continued)* **b** Expression of surface platelet P-selectin on isolated washed human platelets after incubation with thrombin (0.1 U/ml) for one minute (Mean ± SEM, control, n = 12, Crohn's disease in remission, n = 5, ulcerative colitis in remission, n = 4, ulcerative colitis in relapse, n = 12).

UC in relapse sub-grouped according to 5ASA treatment showed similar values of basal P-selectin expression; 0.22 ± 0.04 with 5ASA (n = 6), and 0.23 ± 0.03 without 5ASA medication (n = 6). All patients, irrespectively of disease entity, with 5ASA 0.29 ± 0.03 (n = 12), and without 5ASA 0.29 ± 0.05 (n = 9). None of the groups showed significant differences in comparison with the control group, 0.22 ± 0.03 (n = 12).

All groups showed significant increase in P-selectin expression after stimulation with thrombin compared to solvent, $p < 0.001$. No significant differences could be seen between the groups, (Fig. 1b).

A significant increase of P-selectin expression after incubation with collagen could be seen in the control group, 0.22 ± 0.03 to 0.28 ± 0.04, $p < 0.01$, (n = 12), and UC in relapse, 0.23 ± 0.02 to 0.27 ± 0.02, $p < 0.05$, (n = 12). None of the groups showed significant differences in comparison with the control group.

Concerning incubation with epinephrine an increase in P-selectin expression could be seen only in the control group, 0.22 ± 0.03 to 0.27 ± 0.03, $p < 0.01$, (n = 12), and UC in relapse, 0.23 ± 0.02 to 0.26 ± 0.02, $p < 0.05$, (n = 12). None of the groups showed significant differences in comparison with the control group.

All groups showed a decrease in P-selectin expression after exposure to IL-8, with a significant decrease in UC (relapse and remission taken together as one group), 0.26 ± 0.03 to 0.22 ± 0.02, $p < 0.05$, (n = 16), but no differences could be seen in comparison with the control group, (Table 2).

The surface membrane molecule CD61, which normally does not show any quantitative increase of surface expression after stimulation with agonists such as thrombin, epinephrine and collagen, was used as a control of stimulation. A P-selectin/CD61 ratio analysis was made to see if there was higher entrapment of platelets in some of the wells. Patient groups in relapse had significant increase in CD61 expression in comparison with solvent, but no significant differences compared with the control group. The only group that showed significant increase of CD61 expression, compared to the control group, was UC in remission, but there was no divergence in the results after P-selectin/CD61 ratio analysis (data not shown).

Table 2. Expression of platelet surface P-selectin after incubation with IL-8.

Group	Solvent	IL-8
Control (n = 12)	0.22 ± 0.03	0.18 ± 0.02
UC all (n = 16)	0.26 ± 0.03	0.22 ± 0.02*
UC relapse (n = 12)	0.23 ± 0.02	0.21 ± 0.02
UC remission (n = 4)	0.37 ± 0.12	0.24 ± 0.10
UC relapse + 5ASA (n = 6)	0.24 ± 0.04	0.22 ± 0.03
UC relapse – 5ASA (n = 6)	0.23 ± 0.03	0.21 ± 0.03
CD remission (n = 5)	0.39 ± 0.13	0.31 ± 0.11
All patients + 5ASA (n = 12)	0.29 ± 0.03	0.25 ± 0.03
All patients – 5ASA (n = 9)	0.27 ± 0.05	0.22 ± 0.04

UC; ulcerative colitis, CD; Crohn's disease. * corresponding to $p < 0.05$ compared to solvent.

Soluble P-selectin, NPY and RANTES in plasma

All patient groups had lower levels of NPY compared with the control group, although insignificantly. No significant differences were detected concerning plasma P-selectin, (Table 3).

Table 3. Plasma levels of P-selectin (ng/ml) and NPY (pg/ml).

Group	P-selectin	NPY
Control (n = 12)	47.6 ± 5.5	514 ± 41
UC all (n = 16)	50.9 ± 4.1	455 ± 23
UC relapse (n = 12)	50.0 ± 5.3	477 ± 25
UC remission (n = 4)	53.5 ± 4.7	387 ± 40
UC relapse + 5ASA (n = 6)	44.5 ± 5.0	502 ± 15
UC relapse – 5ASA (n = 6)	55.6 ± 8.2	453 ± 43
CD remission (n = 5)	46.2 ± 3.0	414 ± 31
All with 5ASA (n = 12)	48.8 ± 5.5	444 ± 24
All without 5ASA (n = 9)	51.1 ± 5.4	447 ± 32

UC; ulcerative colitis, CD; Crohn's disease.

Table 4. Plasma levels of RANTES (µg/l) in patient groups compared to the control.

Group	RANTES
Control (n = 12)	3.29 ± 0.19
UC relapse and remission (n = 16)	2.57 ± 0.10**
UC relapse + 5ASA (n = 6)	2.33 ± 0.08*
UC relapse – 5ASA (n = 6)	2.95 ± 0.11
UC all + 5ASA (n = 9)	2.40 ± 0.08**
UC all – 5ASA (n = 7)	2.79 ± 0.18*
CD remission (n = 5)	2.72 ± 0.28
All patients + 5ASA (n = 12)	2.39 ± 0.06**
All patients – 5ASA (n = 9)	2.90 ± 0.17

* and ** corresponding to $p < 0.05$ respectively $p < 0.01$ compared to the control. UC; ulcerative colitis, CD; Crohn's disease.

Assay of plasma RANTES showed results as presented in Table 4, (significance in comparison with the control group). UC in relapse and remission, taken together as one group, had significantly lower level of RANTES compared to the control group, and UC in relapse and UC in remission (three with 5ASA treatment) had significantly lower levels of plasma RANTES compared to the control group, 2.64 ± 0.11 µg/l (n = 12), respectively 2.38 ± 0.21 µg/l (n = 4), both groups $p < 0.05$.

When patients with UC in relapse were sub-grouped according to 5ASA medication, the patients with 5ASA treatment showed significantly lower levels of plasma RANTES compared to the control group and no significant difference could be seen concerning the UC group without 5ASA treatment.

All patients with 5ASA, irrespectively of disease entity, had a significant lower level of plasma RANTES compared to the control group and no significant difference could be seen between the control group and patients without 5ASA. There was a significant difference between patients with 5ASA versus patients with no 5ASA treatment, $p < 0.05$.

Analysis of plasma total protein was made for the eventuality of high protein content in some of the subjects and hence higher risk of disturbances in the analyses. No differences in protein content could be seen between the groups and there were no deviating results after ratio analysis with plasma total protein (data not shown).

Discussion

The results in our study show that patients with IBD in remission have an increased basal expression of platelet surface P-selectin compared to healthy subjects. A result partly in accordance with earlier findings made by Collins et al., although they reported a higher expression among patients with active disease as well [26]. The results from our study could indicate i); higher platelet content of P-selectin, ii); higher P-selectin expression rate or iii); normal response to inflammation.

It is not plausible that the source of P-selectin could be another than from platelets because the measurements were made on isolated washed platelets in suspension, devoid of other intravascular components. One possible source, in vivo though, is endocytosis from the environment. Studies have shown an active endocytosis of 5-hydroxytryptamine in platelets [28] and re-uptake of P-selectin in endothelial cells [29]. If platelets have a re-uptake ability of P-selectin through endocytosis, an up-regulation of this mechanism could result in higher content of intracellular P-selectin, and as a consequence higher access of P-selectin available for expression upon activation. Another possible source of high P-selectin content could, of course, be an increased synthesis of P-selectin in megacaryocytes.

Considering an increased platelet P-selectin expression as a normal inflammatory response, Collins et al. could not find a basal up-regulation of platelet surface P-selectin in patients with rheumatoid arthritis (these patients were comparable with healthy subjects), which was the case among patients with IBD [26].

We could observe an increased expression of P-selectin upon stimulation with thrombin after one minute among patients in remission compared to the control group, although not significantly, which seems to reflect the higher basal expression. It is possible that patients in relapse have depletion of intracellular stores, because of activation, and therefore too low levels to reveal a presumptuous difference in P-selectin expression. The increase of CD61 surface expression among patients in relapse, after stimulation with thrombin, could be due to exposure of receptors from a pool within the open canalicular system and/or α-granule membranes [30].

An increase in P-selectin expression upon stimulation with collagen and epinephrine could be observed only among healthy subjects and UC in relapse, and this could reflect that these agonists are too weak to induce further P-selectin expression among patients in remission due to the high basal expression.

Stimulation of platelets with IL-8, to our knowledge never previously done, yielded a decrease in expression of surface P-selectin in all groups, and the levels were significantly lower in UC. Previous studies have shown that the expression of P-selectin after incubation with solvent is stable over time for up to at least ten minutes [6, 7], so the results could not be explained by a time-dependent factor. IL-8 is an inflammatory mediator, acts as a chemoattractant for neutrophils, and is secreted by neutrophils, macrophages [31] and endothelial cells [32]. The secretion of IL-8 from leukocytes [33], and from endothelial cells, via an IL-1-mediated event [32], is induced by activated platelets. An up-regulation of platelet IL-8 receptors among patients with IBD, independently of disease activity, has been reported [27]. In monocytes the secretion is platelet/P-selectin dependent and antibodies directed against P-selectin block secretion [9]. In addition, different studies have pointed out an increased mucosal production of IL-8 in patients with IBD [34, 35]. Noteworthy is that the groups in remission, with the highest basal expression of P-selectin, have the highest proportional decrease of surface P-selectin after IL-8 stimulation, and one could speculate that besides the chemoattractant effect on leukocytes, IL-8 has a modulating/negative feedback effect on platelet P-selectin expression, and/or stimulates release of surface P-selectin from activated platelets.

Göke et al. found higher levels of soluble P-selectin in both UC and CD compared to healthy subjects [36], a result we can not confirm. A major difference though, is that Göke et al. measured P-selectin in serum, with activation of the clotting cascade and degradation of cellular components as a consequence, compared to measurements of P-selectin in plasma, collected in CTAD-tubes which gives minimal activation of platelets, in this study. A release of platelet P-selectin after activation [13] among patients in relapse could explain the abundance of high basal levels among these patients. In such a case, it would be possible to detect an elevation in plasma. It seems, however, that the half-life of soluble P-selectin in plasma is relatively short, approximately two hours [13], and the concentrations may have been normalised at the time of analysis.

The analysis of plasma RANTES showed significantly lower levels among patients with 5ASA, compared both to healthy and patients without 5ASA. In the CD group, with three patients out of five taking 5ASA, the highest levels were found in the two patients with no pharmacological

treatment. In the group with all UC patients without 5ASA, consisting of six patients in relapse and one in remission, the significant difference could be explained by a very low value for the patient in remission. It is known that glucocorticoids mediate inhibition of RANTES expression in T-lymphocytes [37] and epithelial cells of the lung [38] and our results could indicate a similar action of 5ASA. To our knowledge this has never previously been reported.

In summary, the aim of this study was to reveal a presumptuous platelet dysfunction in patients with IBD using the membrane surface molecule P-selectin as an indicator. We found higher basal expression of platelet surface P-selectin among patients with IBD in remission. In addition we found that patients with 5ASA medication have lower levels of RANTES and that IL-8 has down-regulating effect on platelet P-selectin expression. A platelet dysfunction with an increased expression of platelet P-selectin and release of inflammatory mediators such as RANTES, which is chemotactic and induce chemokine production, hypothetically could have a reinforcing and aggravating influence on the inflammatory response and increase the susceptibility to IBD. The intention of future studies is to clarify if IBD patients differ in intra-platelet content of P-selectin and RANTES, and further evaluate the down-regulating effect of IL-8 on platelet P-selectin expression. If 5ASA is responsible for lowering the concentration of RANTES this could be one of the beneficial outcomes of 5ASA medication.

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