Chapter 34

New White Blood Cell Adsorbent: Immunopure

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Abstract

Immunopure is a novel granulocyte/monocyte adsorption apheresis device (GMCAP) based on polyarylate resin (PAR) beads with a diameter of 1 mm for the treatment of patients with ulcerative colitis (UC). The device has been CE certified since 2011. In addition to other adsorbers for the treatment of UC, Immunopure offers a stronger platelet adsorption capacity while inducing only a low complement activation, making it attractive for UC patients with high platelet numbers and coagulation disorders. *In vitro*, after 60 min of circulation the module selectively reduced platelets by 76%, neutrophils by 42% and monocytes by 63%, while lymphocytes were minorly affected (only 3% reduction rate). Furthermore, the module showed a strong adsorption of inflammatory $CD14^{+}CD16^{+}$ monocytes and platelet leukocyte aggregates (PLAs). In animal studies with healthy premature pigs, the Immunopure device has been shown to be safe

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and tolerable. Two uncontrolled studies in active UC patients demonstrated that the Immunopure device was safe, well tolerated and clinically efficient with clinical remission rates of 80% and 67%, respectively. Laboratory data indicated a strong adsorption of platelets, PLAs, monocytes including $CD14^+CD16^+$ monocytes and granulocytes in these studies. However, like other adsorbers for the treatment of UC, cytapheresis using the Immunopure adsorber did not show any long-term influence on cell populations and seems to act by regulating cell populations and subsequently immune reactions. Additional studies are necessary to further elucidate the mode of action and the clinical benefit of Immunopure cytapheresis in controlled outcome studies.

1. Introduction

1.1. *Platelets as Therapeutic Targets in Inflammatory Bowel Disease*

Chronic inflammatory conditions such as inflammatory bowel disease (IBD) are associated with both inflammation and coagulation. Various cell populations are elevated in IBD, including circulating and/or tissue levels of macrophages, dendritic cells, neutrophils, platelets and plasma cells (Harries *et al.*, 1983; Sartor, 2006; Roberts-Thomson *et al.*, 2011), releasing a variety of inflammatory cytokines (Fig. 34.1). IBD is currently incurable. Standard medication like 5-aminosalicylic acid (5-ASA), corticosteroids, azathioprine and monoclonal antibodies to tumor necrosis factor alpha (TNFα) aims to suppress the symptoms of IBD by inducing anti-inflammatory effects (Burger and Travis, 2011). In spite of a good proportion of patients that improve or remit as a result of established therapies, a considerable number of cases are refractory to steroids and/or immunosuppressive drugs or steroid-dependent and develop complications due to drug toxicity as well as increased risk of colorectal cancer.

With the rationale to remove inflammatory mediators like granulocytes, monocytes and lymphocytes from the blood of patients with

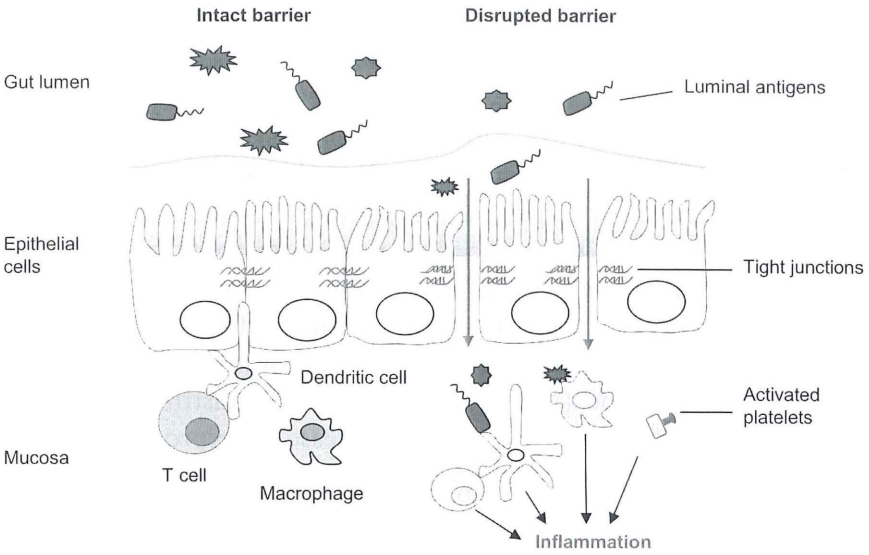

Fig. 34.1. Luminal antigens in genetically susceptible hosts are able to disrupt the epithelial barrier, triggering the activation of innate and adaptive immune responses. Multiple cell types are elevated in patients with IBD including macrophages, dendritic cells, neutrophils, platelets and plasma cells. Modified according to Xavier and Podolsky (2007).

IBD, adsorptive cytapheresis has been clinically applied for more than a decade (Shimoyama, 1999, 2001; Shibata *et al.*, 2003; Hanai *et al.*, 2008; Sigurbjörnsson and Bjarnason, 2008). However, aside from non-immune cells like endothelial, mesenchymal, nerve cells as well as platelets have become a focus of attention as key players in the IBD inflammatory cascade (Danese, 2008). Besides their traditional role in maintaining hemostasis, an increasing number of studies has focused on platelets as amplifiers of inflammation. Microvascular thrombosis and thromboembolism is frequent in patients with IBD (Irving *et al.*, 2005; Danese *et al.*, 2007; Yoshida and Granger, 2009). There is an increased incidence of thrombosis accompanied with abnormalities in platelets and platelet function (Voudoukis

et al., 2014). The platelet number is increased during active disease and correlates with disease severity. Therefore, thrombocytosis has been proposed as a biomarker of disease activity in IBD (Harries *et al.*, 1983; Nielsen *et al.*, 2000). However, platelet count increase is not due only to inflammation, but iron deficiency may also lead to the production of larger polyploid megakaryocytes, while iron replacement therapy was associated with normalization of platelet count (Kulnigg-Dabsch *et al.*, 2012). Other platelet abnormalities include morphological alterations (loss of discoid shape, acquisition of pseudopodia, increase in size, granular content augmentation, mean platelet volume (MPV) decrease, platelet distribution width (PDW) increase, plateletcrit (PCT) increase, overproduction and excretion of granular content products, increased incorporation of receptors, microparticles (MPs) release and aggregate formation. However, all of these changes are known for platelet activation, which is high in IBD patients (Voudoukis *et al.*, 2014).

The primary role of platelets, small nucleus-free fragments with 1–6 μm diameter and a lifespan of 5–9 d derived from bone marrow megakaryocytes, is to stop bleeding and to keep hemostasis via binding of von Willebrand factor to collagen from subendothelial layers at the site of injury. After adhesion, platelets become activated and clot the injured tissue. However, there is evidence that inflammation and coagulation are linked in a way that enables both to activate and propagate each other. Today, coagulation is regarded as an integral part of the innate immune response and inflammation as a potent prothrombotic stimulus that activates the coagulation cascade (Esmon *et al.*, 2011). Platelets are able to express toll-like receptors, internalize pathogens, and stimulate the formation of extracellular DNA nets by neutrophils (Youssefian *et al.*, 2002; Clark *et al.*, 2007; Cognasse *et al.*, 2007; Ma and Kubes, 2007). However, they may also act as mediators between innate and adaptive immune systems. In IBD, granulocytes and platelets are the first cells mobilized to the sites of inflammation. There they interact with lymphocytes to orchestrate the inflammatory response. Platelets can produce cytokines like TNFα (Limb *et al.*, 1999) and activate various cell types via contact with CD40L and secretion of soluble CD40L (Danese, 2007).

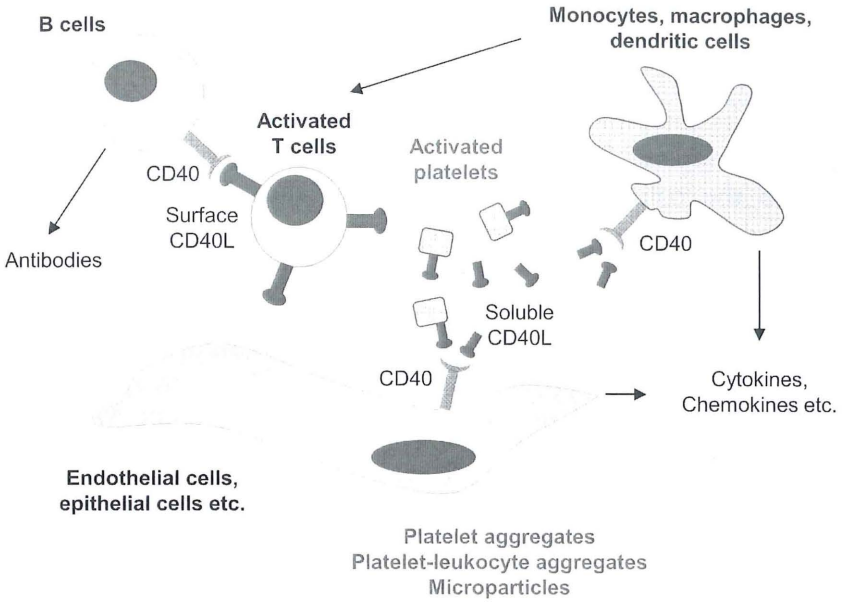

Fig. 34.2. Platelets and CD40/CD40L system. Pleiotropic effects result from the interaction of activated platelets expressing surface CD40L or soluble CD40L with immune and non-immune cell including immunoglobulin, cytokine and chemokine production. Modified according to Danese *et al.* (2004).

Engagement of CD40 on B cells leads to antibody production. Monocytes, macrophages, dendritic cells and non-immune cells activated through the CD40 pathway may produce a variety of cytokines, chemokines, reactive oxygen species, adhesion molecules etc. Furthermore, platelets do also adhere to other platelets, form complexes with leukocytes (PLA) and secrete microparticles (MPs), thereby aggravating the inflammatory process and the risk for thromboembolic events (Fig. 34.2).

Increased PLA and MP formation is observed in many chronic inflammatory disorders and may have an important role in IBD (Michelson *et al.*, 2001; Andoh *et al.*, 2005). Taking into account the high procoagulant and pro-inflammatory characteristics of platelets, PLAs and platelet-derived MPs, these particles could be ambitious targets for IBD therapies.

1.2. *Adsorptive Cytapheresis — Current Situation*

Adsorptive cytapheresis selectively removes cell populations taking part in intestinal inflammation. The effects of apheresis therapy are probably triggered by a dual mechanism, first by direct adsorption of cells to the column and second via immune regulation of non-adsorbed cells. The supposed underlying mode of action of adsorptive cytapheresis includes a reduction of activated leukocytes, downregulation of pro-inflammatory cytokines, modification of adhesion molecule expression, effects on the innate immune system and induction of regulatory processes (Kashiwagi *et al.*, 2002; Muratov *et al.*, 2006; Sigurbjörnsson and Bjarnason, 2008; Waitz *et al.*, 2008; Yokoyama *et al.*, 2007).

Currently, two blood perfusion systems are generally applied in Japan (as a standard therapy) and Europe (approved but without reimbursement). The commercially available cytapheresis systems preferentially adsorb granulocytes (46%), monocytes/macrophages (54%) and only a small number of lymphocytes (18.5%) and platelets (19%) (GMCAP) (Ramlow *et al.*, 2005) or lymphocytes (30–60%), platelets (35%) in addition to granulocytes and monocytes (almost 100% of cells in the blood line of the device) (leukocytapheresis, LCAP) (Shibata *et al.*, 2003). Both methods have been reported to be effective, however, success varied (Shimoyama, 1999, 2001; Shibata *et al.*, 2003; Hanai *et al.*, 2008; Sigurbjörnsson and Bjarnason, 2008). Remission rates deviated from nearly 100% (Suzuki *et al.*, 2006) to 17% being not superior compared to sham apheresis (Sands *et al.*, 2008). These results reflect the fact that it has to be considered that not every patient is suitable for apheresis, and that critical patient selection especially in large clinical trials is crucial. Best responders for adsorptive cytapheresis are patients early after initial diagnosis (Suzuki *et al.*, 2006), steroid-naïve patients (Hanai *et al.*, 2003; Suzuki *et al.*, 2004; Tanaka *et al.*, 2008, 2010), patients with markedly increased disease activity (Matsumoto *et al.*, 2008) shortly after clinical relapse (Yokoyama *et al.*, 2013). In contrast, patients with low disease activity, deep colonic lesions, a long medical history and refractory to existing drugs may possibly not benefit from apheresis (Saniabadi

et al., 2014). However, the big opportunity of cytapheresis treatment may be a slowdown of disease progression if the therapy is applied at an early stage of the disease, possibly preventing exacerbations and colectomy.

Predictive factors for the efficacy of adsorptive cytapheresis have rarely been reported. However, several reports have indicated that a decrease in platelets may be an early marker for a beneficial response in patients with severe UC (Fukunaga *et al.*, 2006; Takemoto *et al.*, 2009). This is in accordance with the observation that platelet parameters seem to display good predictive value regarding disease activity (Voudoukis *et al.*, 2014). Therefore, attempts have been made to develop a novel cytapheresis device with a stronger capacity to adsorb platelets.

1.3. *Immunopure Adsorber*

The basic idea was to develop an efficient adsorber using a material that is well proven for a long time in patients on maintenance hemodialysis with regard to both biocompatibility and suggested adsorptive properties. Synthetic polymer membranes are widely used as dialysis membranes. Polyester-polymer alloy (PEPA) membrane was developed and met the performance requirements for a hollow fiber in 1990 by Nikkiso Co., Japan. It is a synthetic polymer high-flux membrane with a good biocompatibility and has been shown to offer features like endotoxin and free light chain retentive capability (Igoshi *et al.*, 2011). PEPA consists of two polymer components: polyethersulfone (PES) and polyarylate (PAR). PAR is an aromatic polyester polymer (Fig. 34.3) which is hydrophobic with superior heat resistance, recovery performance and mechanical properties.

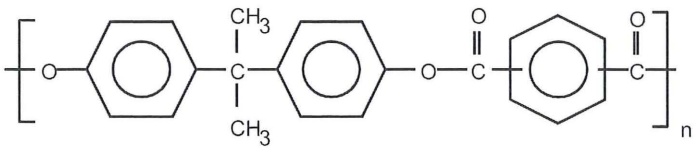

Fig. 34.3. Molecular structure of PAR (modified according to Igoshi *et al.*, 2011).

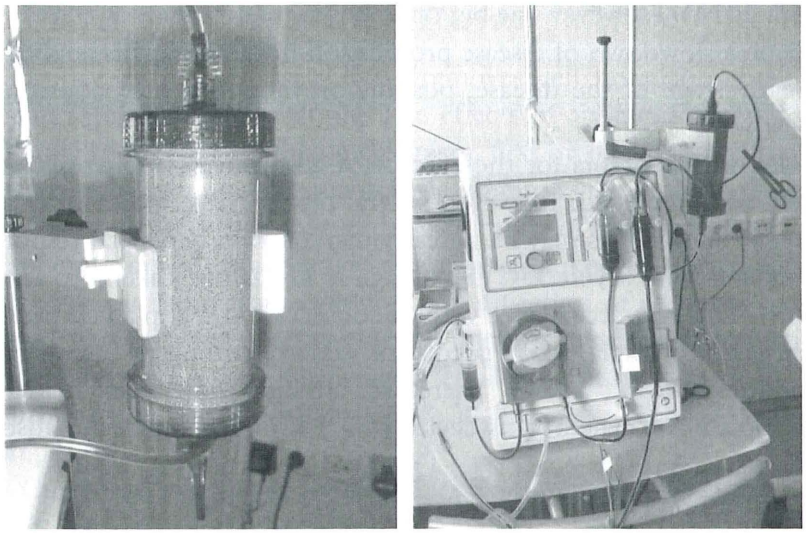

Fig. 34.4. Immunopure device (Nikkiso Co., Japan) (left) and blood monitor (LPM-01, Nikkiso Co., Japan) (right) during apheresis treatment.

Immunopure is a novel medical device based on PAR beads with a diameter of 1 mm to be used in a simple hemoperfusion setting provided by Nikkiso Co., Japan. The apheresis system comprises the Immunopure device, blood lines (ABT-002PX, Nikkiso Co., Japan) and the blood monitor (LPM-01, Nikkiso Co., Japan). The adsorption device has a length of 185 mm, a diameter of 59 mm, a total weight of 537 g, a total volume of 350 mL, and a priming volume of 139 mL (Fig. 34.4). The Immunopure device has been CE certified since 2011. In clinical application it is recommended to use standard heparin anticoagulation and the standard treatment protocol with a treatment time of 60 min once a week over five weeks. *In vitro* experiments as well as *in vivo* investigations in pigs (see Section 2) and humans (see Section 3) revealed that the module adsorbs granulocytes, monocytes/macrophages and platelets selectively. The results of the pilot study demonstrated a good safety profile and promising clinical remission rates (80% at week 10) (Ramlow *et al.*, 2013).

2. Basic Aspects

2.1. *In Vitro Circulation Experiments — Setup*

To analyze the adsorption behavior of the Immunopure adsorber beads, *in vitro* circulation experiments using mini-modules in downscaled circulation experiments and blood of healthy volunteers were performed. Investigations were accomplished with a special focus on the influence of the adsorber on platelets, PLAs and platelet-derived MPs. An adsorber mini-module consisting of PAR beads (diameter: 1 mm) placed in a syringe with a volume of 40 mL was designed (Fig. 34.5).

The *in vitro* experiments were performed utilizing a downscaled experimental setting with mini-module dimensions calculated from commercially available cytapheresis modules, which have a volume of about 300 mL, a cross section area of $A = 22.9$ cm^2, and are operated with a blood flow of $Q = 30$ mL/min. The contact time of blood to the adsorber beads depends on the blood speed v, which yields from the blood flow Q and the cross section area A ($v = Q/A = 1.3$ cm/min). The cross-section area of the 40 mL mini-module is

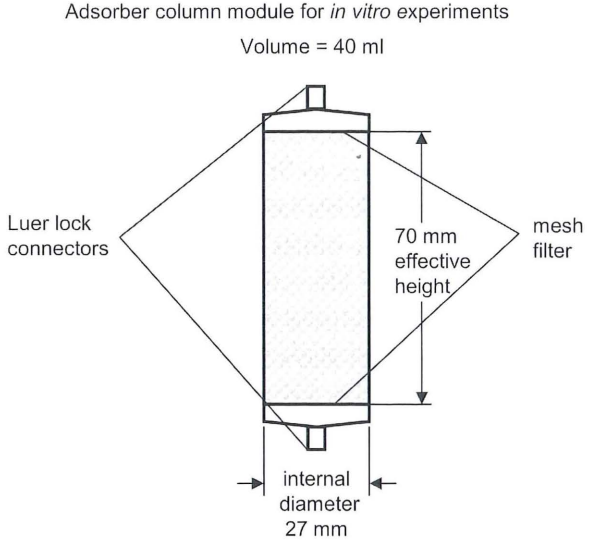

Fig. 34.5. Dimensions of the adsorber minimodule.

5.7 cm^2, therefore a blood flow of $Q = 7.4$ mL/min was necessary to achieve the same blood speed as in a clinical setting. As tubing system regular sets for infusion pumps were used (pump tube diameter about 1.5 mm).

Adsorber mini-modules filled with PAR beads and empty sham modules of same void volume were tested in parallel. After pre-rinsing with physiological saline, donor blood (500 mL divided into two parts of 250 mL each, 1.0 IU/mL Heparin, ratiopharm, Ulm, Germany) passed from the blood bag through the primed mini-modules and displaced the physiological saline. The circulation to the blood bag was closed when almost all of the physiological saline within the tubes was displaced by blood. Experiments were performed at a steady flow rate (7.4 mL/min) for a test period of 60 min. Collection of the baseline (0 min) blood samples was started after the column had been completely filled with blood. Collection of the influx and efflux samples was performed at the column entry and exit 15 min and 60 min after starting recirculation. Temperature was maintained at 37°C throughout the experiment by incubating the blood bag in a thermostatic water bath under gentle rotation (Fig. 34.6).

2.2. *In Vitro Circulation Experiments — Results*

The results of blood count analyses showed a significant reduction of platelets to 23.7%, leukocytes to 70.0%, neutrophils to 58.3%, monocytes to 36.8% and lymphocytes to 96.9% after 60 min of blood circulation through the mini-module containing beads from the Immunopure device compared to the 0-min-values (100%) (Fig. 34.7).

Clearly, platelets showed the strongest reduction, followed by monocytes and neutrophils with a maximum adsorption rate at 15 min. In contrast, lymphocytes showed minimal changes. The strong platelet adsorption capacity also became apparent in electron microscope photographs (Fig. 34.8).

The hemoglobin and hematocrit values remained constant throughout the observation period during all circulation experiments showing that there were no dilution or sedimentation effects. However, data were hematocrit corrected. There was a significant difference

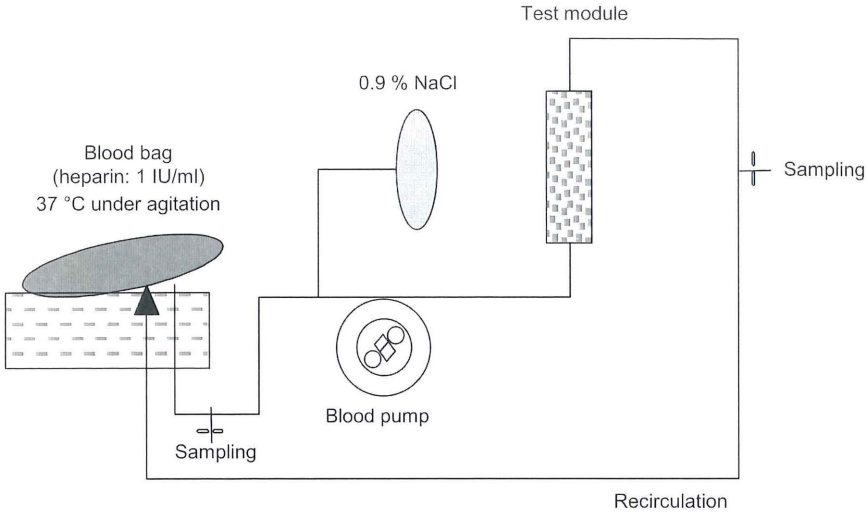

Fig. 34.6. A miniaturized module consisting of polyarylate resin beads (1 mm diameter) from Immunopure with a volume of 40 ml was designed. 250 ml of heparinized (1.0 IE/mL) blood from a healthy volunteer passed through the primed mini-module (7.5 mL/min, 37°C) for a test period of 60 min.

between filled modules and empty test modules with regard to platelet, leukocyte, monocyte, neutrophil and eosinophil adsorption. In this circulation experiments, adsorption decreased after 15 min of recirculation suggesting a saturation phenomenon of the mini-modules from that point on.

Furthermore, analyses with regard to adsorption of different cell populations were performed using flow cytometry. In detail, $CD3^+CD4^+$ T helper cells as well as $CD3^+CD8^+$ cytotoxic T cells did not show any adsorption to the Immunopure beads (not shown). On the contrary, there was a strong decrease of $CD10^+$ granulocytes to 63%, leukocytes expressing the cell adhesion proteins CD11b and L-selectin to 57%, $CD14^+$ monocytes/macrophages to 39% and inflammatory $CD14^+CD16^+$ monocytes to 41% after 15 min in the filled modules (Fig. 34.9). $CD14^+CD16^+$ monocytes exhibit a pro-inflammatory phenotype and have been shown to be reduced after GMCAP *in vivo* (Hanai *et al.*, 2008).

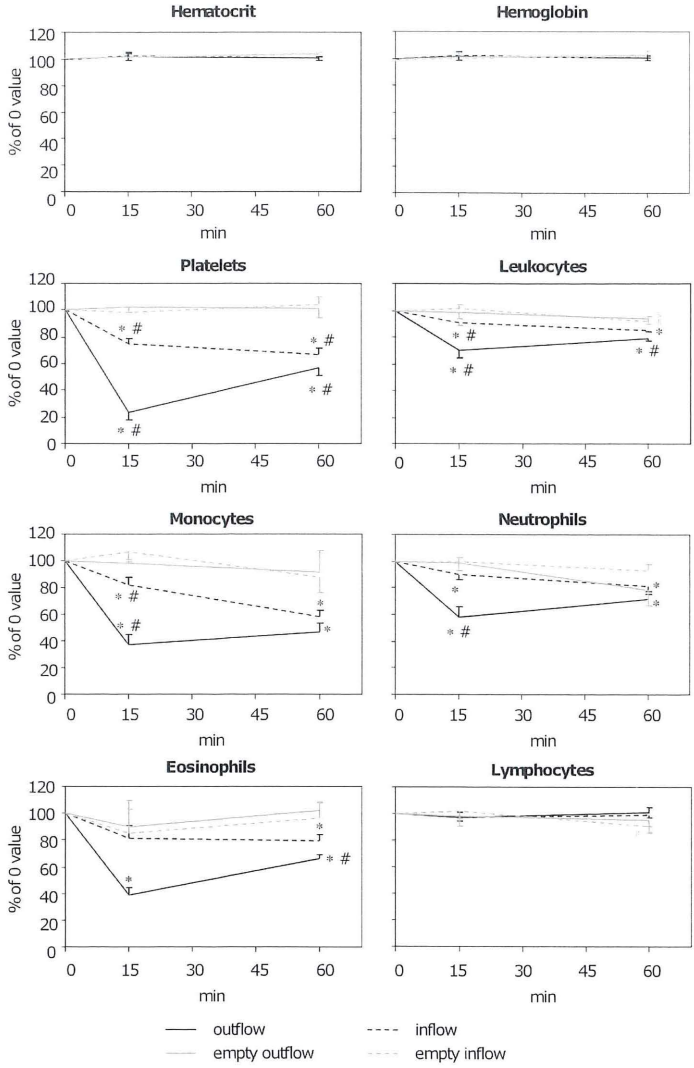

Fig. 34.7. Peripheral blood counts of platelets, leukocytes, monocytes, neutrophils, and eosinophils were significantly reduced by the Immunopure beads. Results (mean/SEM) were compared with baseline values at time point 0 min (100%) at the beginning of each experiment using the Wilcoxon test ($n = 6$) ($^*P < 0.05$) and with values of the empty modules ($n = 6$) using the U test ($\#P < 0.05$).

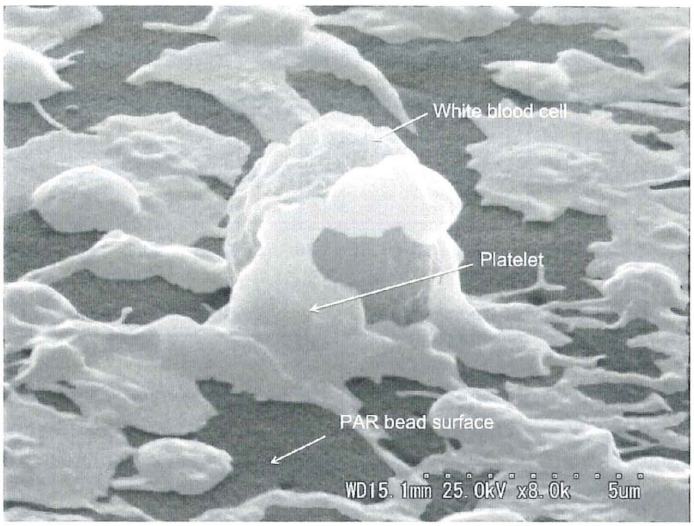

Fig. 34.8. Electron microscope photograph of the PAR bead surface with an attached white blood cell and numerous platelets (kindly provided by Nikkiso Co., Japan).

PLAs with $CD14^+$ monocytes, $CD11b^+$ cells, and $CD3^+$ T cells were strongly reduced by the Immunopure beads *in vitro,* too (Fig. 34.10). PLAs are formed during inflammation via binding of P-selectin on thrombin-stimulated platelets to its specific ligand on the leukocyte surface, P-selectin glycoprotein ligand-1. Then, PLAs are stabilized via binding of the leukocyte surface Mac-1 to platelet surface glycoprotein Ib (GPIb), integrin αIIbβ3, and/or junctional adhesion molecule 3. Leukocyte tethering by platelet P-selectin also triggers the expression of transcription factors such as nuclear factor kappa B (NF-kB), which activates gene transcription of pro-inflammatory molecules leading to an inflammatory status of the leukocytes (Cerletti *et al.*, 2012).

PLAs are also formed in healthy individuals, but an increased number has been described in several inflammatory and thrombotic conditions, including IBD (Michelson *et al.*, 2001; Pamuk *et al.*, 2006). Our own assessments were in accordance with these observations. Patients with UC had significant higher platelet numbers, $CD42b^+CD14^+$ and $CD42b^+CD11b^+$ PLAs (Fig. 34.11).

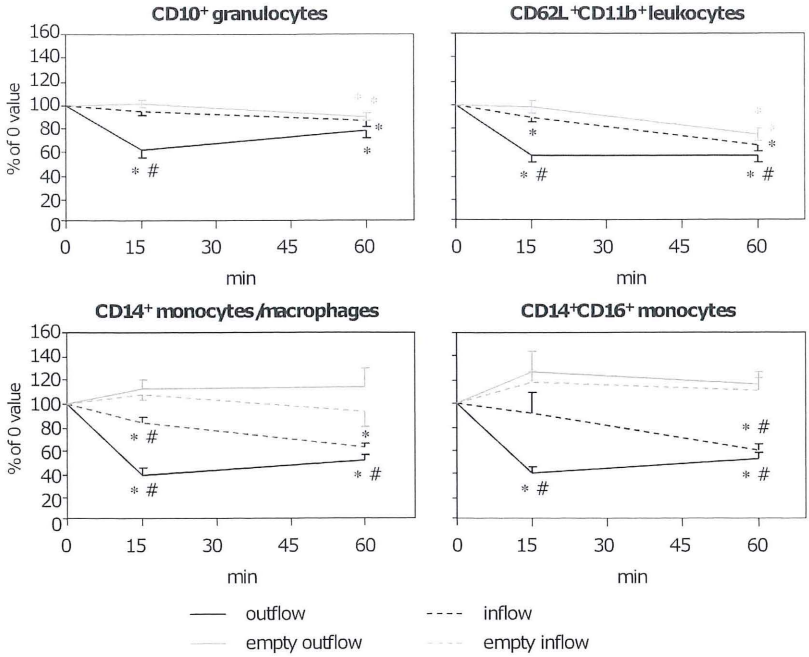

Fig. 34.9. Flow cytometry analyses of $CD10^+$ granulocytes, $CD62L^+CD11b^+$ leukocytes, $CD14^+$ monocytes and $CD14^+CD16^+$ monocytes after circulation of whole blood through Immunopure and sham mini-modules. Results (mean/SEM) were compared with baseline values at time point 0 min (100%) at the beginning of each experiment using the Wilcoxon test ($n = 6$) ($*P < 0.05$) and with values of the empty modules ($n = 6$) using the U test ($\#P < 0.05$).

Elevated PLA numbers in IBD may result from enhanced platelet and neutrophil activation and could contribute to the exacerbation of the inflammatory process. Increased numbers of PLAs were found in the mesenteric vasculature in patients with UC, but not in controls, which supports the hypothesis that the activated vascular endothelium stimulates PLA formation in UC. PLA formation seems to play a role in the pathogenesis of the mucosal lesion and contributes to the risk of systemic thrombosis in patients with UC (Irving *et al.*, 2008). Therefore, therapies that prevent or diminish PLA formation may be useful in patients with IBD. It has been shown that PLAs were reduced by antiplatelet agents like clopidogrel, prasugrel (reduction of P-selectin expression) and abciximab (blocking of glycoprotein

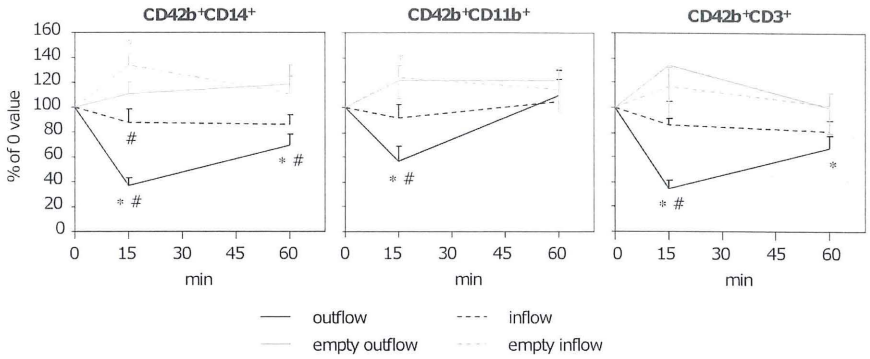

Fig. 34.10. Flow cytometry analyses of PLAs stained with the CD42b platelet marker combined with CD14, CD11b and CD3. Results (mean/SEM) were compared with baseline values at time point 0 min (100%) at the beginning of each experiment ($n = 6$) (*$P < 0.05$, Wilcoxon test) and with values of the empty modules ($n = 6$) (#$P < 0.05$, Mann–Whitney U test).

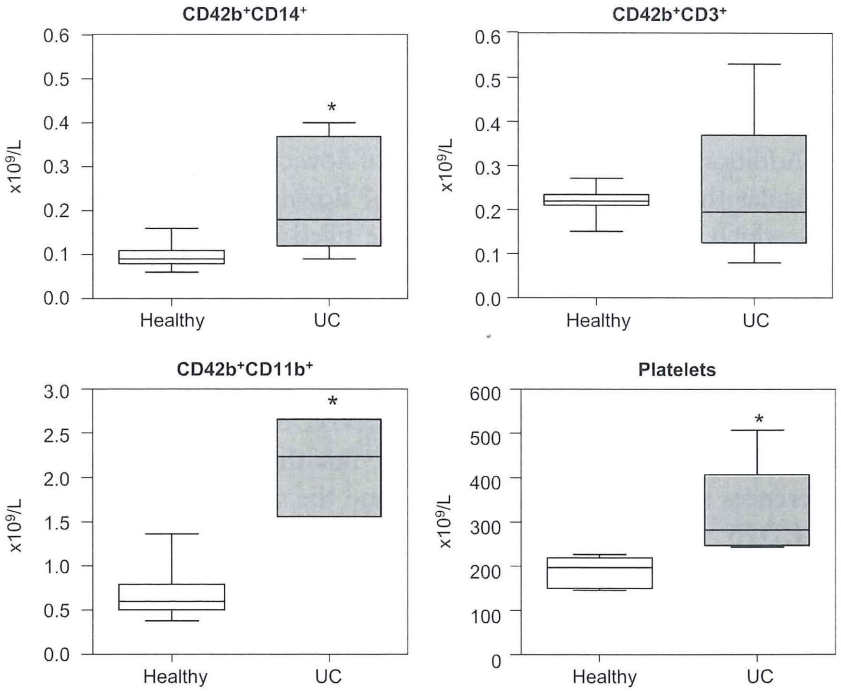

Fig. 34.11. Flow cytometry analyses of PLAs stained with the CD42b platelet marker combined with CD14, CD11b and CD3 and platelets (blood count): Median, healthy controls ($n = 8$) vs. patients with UC ($n = 6$) *$P < 0.05$ Mann–Whitney U test.

IIb/IIIa receptor) *in vivo*, and by epoprostenol (inhibition of intracellular signaling pathways) and low molecular weight heparins *in vitro* (Cerletti *et al.*, 2012). However, effective adsorption of PLAs by apheresis may be an interesting approach during active flare ups.

Platelet-derived MPs are very small vesicles (< 1 mm) generated from the plasma membrane upon platelet activation. To date, there are few studies about platelet-derived MPs in IBD, however, along with activated platelets they are thought to be involved in the pathogenesis of venous thromboembolism in IBD patients (Andoh *et al.*, 2005). For the analysis of platelet-derived MPs after *ex vivo* circulation of blood from healthy volunteers through the Immunopure and sham mini-modules, platelet-free plasma was obtained. Analyses were performed in a modified form according to Robert *et al.* (2009). Platelet-derived microparticles were defined according to their size as particles smaller than 0.9 µm, Annexin V^+/$CD42b^+$ and counted using Truecount tubes (BD, Heidelberg, Germany). Flow cytometry analyses revealed no influence of the Immunopure beads on platelet-derived MPs. Likewise, there were no significant differences utilizing filled mini-modules and empty sham modules.

Additionally, there were a number of flow cytometry parameters, in particular those which are expressed or down-regulated upon activation, which increased/decreased in the filled as well as in the empty sham modules (Table 34.1). The expression of CD62L, which is reflected by the mean channel fluorescence of these cells in flow cytometry, is known to be reduced on leukocytes after contact with artificial surfaces due to their activation. In contrast, the expression of CD11b is enhanced after leukocyte activation. CD62L expression was decreased and CD11b expression was increased, but there were no significant differences between the test modules and the sham modules.

CD25, the alpha chain of the IL-2 receptor, is present on activated T cells. A slightly enhanced expression of this T cell activation marker was observed after circulation through the filled Immunopure as well as through the empty sham mini-modules without any differences between the two module types. The same was true for the platelet activation markers CD63 and CD62, both of them were increased by each module type. These results clearly demonstrate the

Table 34.1. Flow Cytometry Analyses of CD62L (Mean Channel Fluorescence), CD11b (Mean Channel Fluorescence), $CD4^+CD25^+$ Cells, $CD42b^+CD62P^+$ and $CD42b^+CD62P^+$ Platelets after Circulation of Whole Blood through Immunopure and Sham Mini-modules. Results (Mean ± SD) were Compared with Baseline Values at Time Point 0 min (100%) at the Beginning of Each Experiment ($*P < 0.05$)

		Inflow	Outflow	Inflow	Outflow
Parameter	Module	15 min	15 min	60 min	60 min
Mean CD62L	Filled	82.6 ± 12.9*	77.6 ± 9.5*	54.5 ± 12.3*	57.2 ± 16.3*
	Sham	93.2 ± 13.8	89.7 ± 17.7	62.9 ± 15.0*	65.3 ± 13.9*
Mean CD11b	Filled	132.9 ± 53.6	153.5 ± 47.1*	185.7 ± 67.5*	181.7 ± 64.3*
	Sham	142.4 ± 39.8*	131.5 ± 30.8*	176.1 ± 36.2*	159.0 ± 38.7*
$CD4^+CD25^+$	Filled	135.2 ± 75.9	137.0 ± 73.0	125.0 ± 47.1	127.6 ± 41.6
	Sham	159.3 ± 146.6	128.0 ± 76.8	126.1 ± 65.5	97.2 ± 30.7
$CD42b^+CD63^+$	Filled	207.4 ± 79.1*	225.4 ± 117.2*	310.5 ± 249.8	276.9 ± 198.6
	Sham	158.4 ± 78.8	187.2 ± 80.6*	313.5 ± 248.4*	231.0 ± 106.6*
$CD42b^+CD62P^+$	Filled	584.4 ± 519.5*	625.7 ± 461.4*	535.9 ± 429.9*	509.9 ± 342.3*
	Sham	304.8 ± 305.3	308.7 ± 228.8*	481.6 ± 479.7	377.1 ± 237.5*

"sham" effect, which becomes apparent when blood gets into contact with artificial surfaces like tubing systems, housing material, daylight, or is influenced by shear stress due to the blood pump. Therefore, sham apheresis cannot be directly compared with placebos in drug trials. *Per se*, sham apheresis should be considered as a kind of treatment having an ever stronger influence than placebos. This should be taken into account when performing sham-controlled apheresis studies.

Despite the similar increase of $CD63^+$ and $CD62P^+$ platelets using both module types, plasma soluble CD40L concentrations were two to threefold higher in circulation experiments using the PAR bead filled mini-modules indicating enhanced platelet activation by the adsorber material. However, soluble P-selectin was not increased in the plasma by the Immunopure mini-modules compared to the sham modules (Fig. 34.12).

After contact of platelets with negatively charged surfaces like glass or adsorber materials, the intrinsic coagulation cascade (contact activation pathway) is activated. The intrinsic pathway starts with the formation of the primary complex by high-molecular-weight kininogen (HMWK), prekallikrein, and factor XII (Hageman factor).

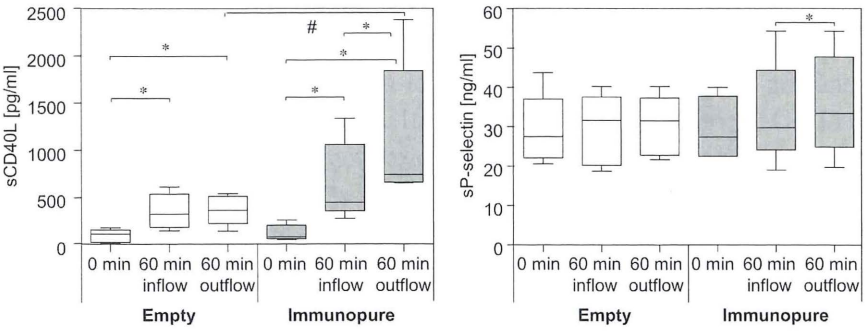

Fig. 34.12. Median plasma concentrations of soluble CD40L (left) and soluble P-selectin (right) as determined by enzyme-linked immunosorbent assay for each circulation experiment (empty modules $n = 6$, filled Immunopure modules $n = 6$). Results were compared with baseline values at time point 0 min ($*P < 0.05$, Wilcoxon test) and with values of the empty modules ($\#P < 0.05$, Mann–Whitney U test).

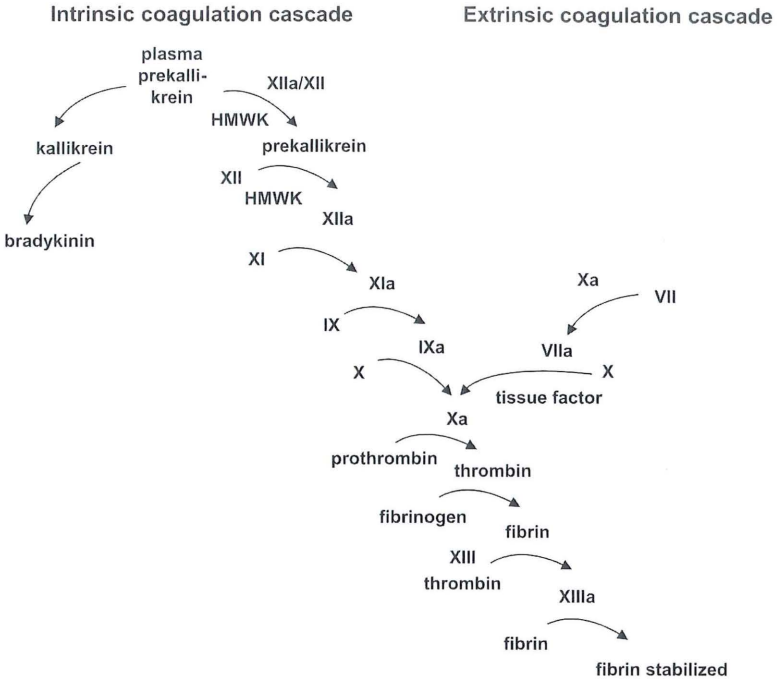

Fig. 34.13. Coagulation cascade modified according to Davie and Mac Farlane (High-molecular-weight kininogen (HMWK)) (Earl, 2003).

Prekallikrein is then converted to kallikrein. Activation of factor XII is followed by activation of factor XI and factor IX. The intrinsic and extrinsic pathways converge at the level of factor X activation. Factor Xa activates prothrombin to thrombin in the presence of the cofactor factor Va, and thrombin subsequently triggers conversion from fibrinogen to fibrin (Fig. 34.13).

Activated platelets express glycoprotein IIb/IIIa on their surface, which can bind fibrinogen. Subsequently, PLAs may be formed directly on the Immunopure adsorber surface, or preformed PLAs may be catched from the circulation. To prove whether leukocytes, platelets and fibrinogen are attached to the bead surface, several beads were removed from the Immunopure mini-modules after the circulation experiments and fixed in 3% paraformaldehyde.

Immunofluorescence was performed using the following primary antibodies: CD42b, fibrinogen gamma chain, CD14, CD11b, CD3 (BD Biosciences, San Diego, USA; abcam, Cambridge, GB; Immunotools, Friesoythe, Germany) and secondary antibodies: anti-mouse Alexa 546, anti-rabbit Alexa 488 (Life Technologies GmbH, Darmstadt, Germany). Beads were transferred on a coverglass-bottom dish and analyzed using a confocal laser microscope (Fluoview FV10i, Olympus America Inc., Center Valley, USA). Samples without primary antibody as well as beads without contact to blood served as controls (Fig. 34.14).

Indeed, confocal laser microscopy pictures showed an adsorption of fibrinogen, CD42b+ platelets, $CD11b^+$, $CD14^+$, and $CD3^+$ leukocytes to the bead surface. In summary, the suggested mechanisms of granulocyte, monocyte and platelet adhesion to the Immunopure device may be postulated as follows: Naïve platelets from the bloodstream get into contact with the artificial surface, are activated and release different mediators (factor XII, factor XI) which induce the conversion of fibrinogen to fibrin. Platelets adhered to fibrin form complexes with leukocytes. Moreover, the adsorber surface is enveloped with different proteins (complement, immune complexes) similar to processes as described for Adacolumn beads (Saniabadi, 2014) which may be recognized by Fc-gamma receptors of monocytes/macrophages and neutrophils (Fig. 34.15). However, this has to be further elucidated.

In addition to the described *in vitro* results, complement activation induced by the Immunopure mini-module was very low (plasma C5a mean ± standard deviation: from 0.55 ± 0.53 at 0 min to 2.40 ± 1.51 ng/mL at 30 min of circulation, $n = 5$), possibly due to the fast envelopment of the beads with fibrin. This suggests a very good biocompatibility which is a favorable characteristic for clinical application.

2.3. *Adsorptive-Type Cytapheresis in a Pig Model*

After *in vitro* analyses, the safety and technical feasibility of the Immunopure device was tested in large healthy pigs. The advantage

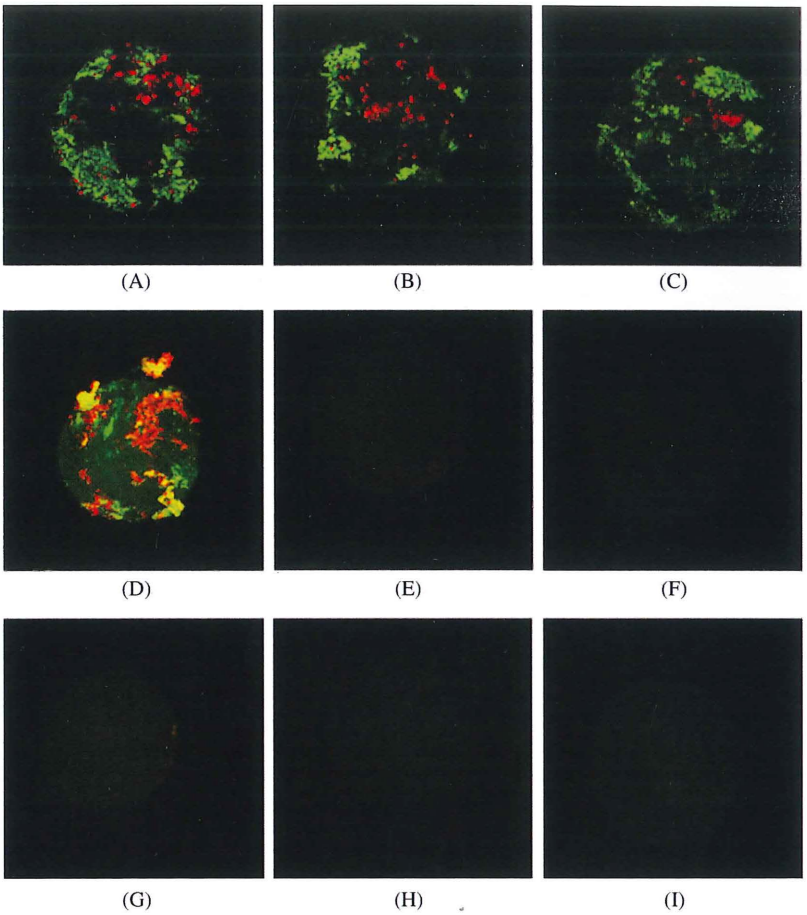

Fig. 34.14. Confocal laser microscopy pictures of Immunopure beads after circulation experiments. Staining: (A) CD42b (green)/CD11b (red); (B) CD42b (green)/CD14 (red); (C) CD42b (green)/CD3 (red); (D) fibrinogen (green)/CD42b (red); E-H: controls without blood; (E) CD42b (green)/CD11b (red); (F) CD42b (green)/CD14 (red); (G) CD42b (green)/CD3 (red); (H) fibrinogen (green)/CD42b (red), (I) control without primary antibody, after blood circulation.

of this pig model over other animal models is that it resembles humans by size and blood volume. This allowed the testing of the whole extracorporeal system setting in its original size as suggested for later human application.

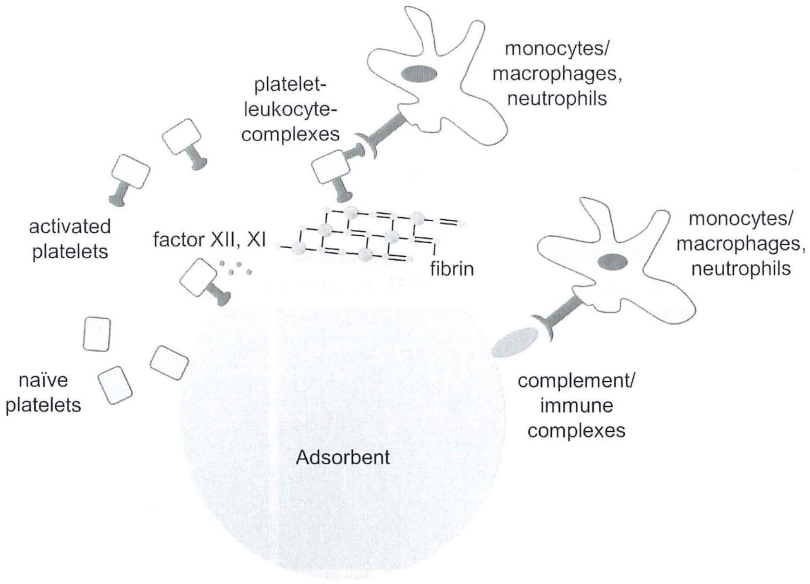

Fig. 34.15. The suggested adsorption mode of the Immunopure adsorber includes both fibrin formation on the bead surface with subsequent formation of PLAs and adsorption of proteins like complement/immune complexes which are recognized by Fc-gamma receptors on monocytes/macrophages and neutrophils.

A total of five large sized premature female pigs (German large white, 54–73 kg one week before first apheresis tested for malignant hyperthermia) were adapted to the standardized housing conditions and handling procedures of the institute (Core Unit for Biomedical Research, Medical University of Vienna) several weeks before the treatments. The animals had free access to standardized food and drinking water.

This study was carried out in strict accordance with the Austrian Federal Act on the protection of animals. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Medical University Vienna and the Austrian Ministry of Science and Research (permit number: 66.009/191-II/10b/2008). All interventions including cytapheresis treatments were performed under anesthesia (induced by i.v. thiopental, followed by ventilation with

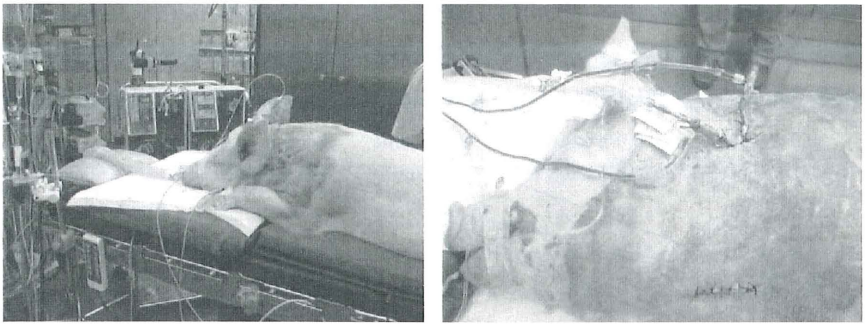

Fig. 34.16. Following arterial and venous catheter insertion, the pigs received a total of five apheresis treatments with the investigational device under anesthesia. The treatment time was 120 min per session with a blood flow of approximately 30 ml/min.

oxygen-room air mixture and 1.5–2.5 vol% isoflurane, piritramide 0.1 mg/kg/hr, i. v.) and all efforts were made to minimize suffering. Analgetic and antibiotic therapy were started during the surgical catheter insertion (fentanyl plasters 50 µg/hr, cefazoline for three days, 15 mg/kg i. v. every eight hours). The animals first received an arteriovenous catheter implantation followed by a total of five apheresis treatments with the novel PAR beads containing module under anesthesia (Fig. 34.16).

The first apheresis session was performed immediately after the catheter insertion. During the first two weeks the pigs received two apheresis treatments per week with a treatment free interval of three to four days. The last treatment session was performed in the third week. Blood samples were taken one week prior to surgical catheter insertion (week 0, W0), at final evaluation (seven days after the fifth treatment session, final) and at the end of the experiment (9–10 days after the fifth treatment session, follow up), as well as before (0 min), during and at the end of each apheresis treatment (15, 60 and 120 min of treatment time).

In spite of the catheter implantation as well as frequent anesthetic interventions during the apheresis treatments, the pigs developed normally and showed a steady increase in body weight over time (Fig. 34.17). After recovery from anesthesia the animals showed

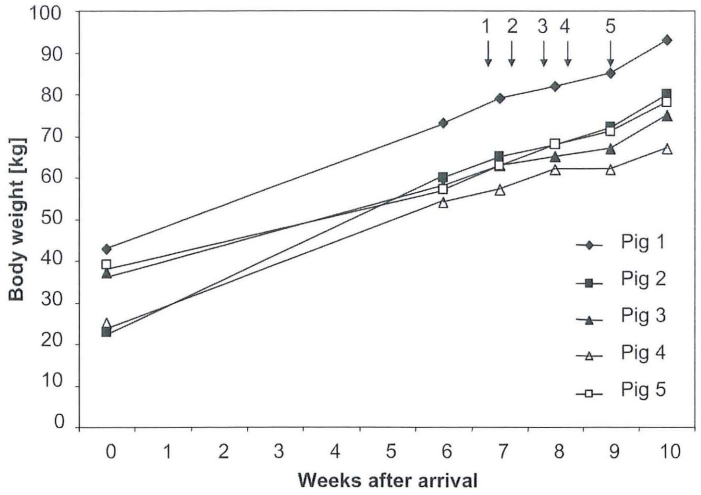

Fig. 34.17. The body weight of the growing pigs was not markedly influenced by the apheresis treatments. Apheresis treatments were performed during the seventh (first and second treatment), eighth (third and fourth treatment) and nineth (fifth treatment) week after arrival at the animal unit (marked with arrows).

a normal behavior, interest in companion animals, and normal food and water uptake during the whole observation period. Using the neurologic deficient score the animals showed a physiologic neural status during the whole observation period. All apheresis treatments with the investigational device were completed regularly according to the study protocol.

Concerning safety, no adverse events were observed during this animal trial. There were no serious long-term impacts of the apheresis treatments with the novel device on the chemical blood parameters apart from increased ACT and aPTT values before the first treatment session caused by earlier heparinization after catheter insertion. A special aspect in pigs is to achieve an optimal coagulation state to avoid thrombi formation and embolism over the time period of the apheresis procedure. Therefore, a relatively high dose of heparin was necessary, however, this was well tolerated by the animals without the occurrence of bleeding disorders. Altogether, the apheresis treatments with the novel device demonstrated a good safety profile. All measured safety parameters remained unchanged, or in case of minor

Table 34.2. Changes in Chemical Parameters during Apheresis Treatments of Pigs (Mean ± SD, $n = 5$, 25 Treatments)

Parameter	Start Values	15 Min Inflow	60 Min Inflow	120 Min Inflow
aPTT [s]	70.4 ± 123.5	400.0 ± 0.0*	400.0 ± 0.0*	400.0 ± 0.0*
ACT [s]	305.2 ± 378.4	812.0 ± 541.3*	1369.1 ± 364.5*	1276.0 ± 412.8*
Fibrinogen [mg/dL]	168.8 ± 68.0	148.7 ± 56.0*	146.1 ± 56.4*	145.8 ± 56.8*
Total protein [g/dL]	5.8 ± 0.4	5.2 ± 0.4*	5.1 ± 0.4*	5.0 ± 0.4*
Albumin [g/dL]	3.7 ± 0.5*	3.3 ± 0.5*	3.2 ± 0.5*	3.2 ± 0.5*
Bilirubin [mg/dL]	0.09 ± 0.06	0.12 ± 0.06*	0.12 ± 0.06*	0.11 ± 0.05
free Hb [g/dL]	3.3 ± 1.7	2.5 ± 1.2*	2.9 ± 1.6	2.6 ± 0.9
Glucose [mg/dL]	88.4 ± 12.3	78.9 ± 18.0*	89.4 ± 18.8	102.8 ± 9.0*
AST [U/L]	40.0 ± 37.6	35.8 ± 32.0*	33.0 ± 28.9*	31.4 ± 26.7*
ALT [U/L]	95.8 ± 67.5	84.9 ± 59.6*	83.4 ± 57.4*	81.8 ± 55.9*
LDH [U/L]	628.9 ± 281.4	543.8 ± 240.0*	524.6 ± 214.9*	504.4 ± 190.8*
AP [U/L]	84.6 ± 18.5	78.4 ± 19.5*	81.0 ± 20.2*	85.2 ± 19.1
γ-GT [U/L]	29.4 ± 4.3	25.9 ± 3.7*	25.8 ± 3.5*	25.7 ± 3.5*
CK [U/L]	3769.7 ± 6089.4	3232.6 ± 5086.4*	3070.4 ± 4924.8*	2833.6 ± 4484.3*
Crea [mg/dL]	1.1 ± 0.1	1.0 ± 0.2	1.1 ± 0.2*	1.2 ± 0.2*
BUN [mg/dL]	6.1 ± 1.0	6.2 ± 1.0	6.7 ± 1.3*	7.5 ± 1.5*
K^+ [mmol/L]	3.8 ± 0.2	3.8 ± 0.3	4.2 ± 0.3*	4.5 ± 0.4*
Na^+ [mmol/L]	139.0 ± 1.2	139.0 ± 1.1	138.7 ± 1.3	138.0 ± 1.7*
$Ca2^+$ [mmol/L]	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.0
Cl^- [mmol/L]	100.7 ± 2.0	101.7 ± 2.0*	101.2 ± 1.9	100.6 ± 2.2
Lactate [mmol/L]	0.8 ± 0.2	0.9 ± 0.3	1.1 ± 0.4*	0.9 ± 0.3

*$p < 0.05$ vs. start value, Wilcoxon-test.

changes, clinically irrelevant, especially in terms of inter-treatment comparisons (Tables 34.2 and 34.3).

Catheter-related problems occurred prior to six treatment sessions and were solved by switching to veno-venous apheresis (in four cases) or a new preparation of the arterial line (in two cases). However, this did not influence the technical feasibility of the novel device (defined as the ability to perform apheresis treatments with the module for 120 min at a blood flow rate of approximately 30 mL/min without replacement) which was 100%.

Table 34.3. Comparisons of Values One Week before Catheter Insertion (W0) with Pre-Treatment Data of First to Fifth Apheresis Treatment Session in the Pigs as well as with Final Values of the Follow-Up Examinations (Mean, $n = 5$). Tn: treatment number 1 to 5, final: one week after T5, follow-up: 9–10 days after T5

Parameter	W0	T1	T2	T3	T4	T5	Final	Follow up
aPTT [s]	13.2	284.1	18.7	15.6	17.4	16.0	23.8	16.8
ACT [s]	110.0	993.6*	137.0	136.0	160.0	99.2	128.8	113.8
Fibrinogen [mg/dL]	143.5	132.4	284.8	150.4	127.4	149.2	149.0	193.3
Total protein [g/dL]	5.7	5.3	6.2	5.8	5.6	6.0	6.4	6.3
Albumin [g/dL]	3.4	3.3	3.6	4.0	3.8	3.8	4.1	4.2
Bilirubin [mg/dL]	0.07	0.17	0.04	0.09	0.10	0.06	0.06	0.09
free Hb [g/dL]	3.8	2.1	2.6	4.2	5.1	2.4	5.0	3.2
Glucose [mg/dL]	103.5	93.6	91.6	79.2	89.0	88.6	98.2	104.8
AST [U/L]	18.5	20.6	68.6	53.0	38.0	19.8	20.8	18.8
ALT [U/L]	43.3	41.6	93.6	134.8	123.8	85.4	62.4	57.4
LDH [U/L]	583.0	507.2	787.6	674.0	648.4	527.4	521.6	471.4
AP [U/L]	110.5	111.4	84.6	70.6	71.8	84.8	97.4	96.2
γ-GT [U/L]	30.5	27.8	28.4	27.4	30.2	33.0	34.6	33.4
CK [U/L]	2162.3	2189.2	7461.4	6945.0	1773.4	479.4	813.0	773.2
Crea [mg/dL]	1.17	1.12	1.00	0.98	1.08	1.10	1.20	1.18
BUN [mg/dL]	6.8	6.6	5.8	5.4	6.4	6.2	6.8	5.6
K^+ [mmol/L]	3.46	4.06*	3.72	3.68	3.70*	3.66	3.74*	3.48
Na^+ [mmol/L]	140.0	140.2	138.6	139.4	138.4*	138.6	138.4*	140.0
$Ca2^+$ [mmol/L]	1.27	1.21	1.26	1.24	1.22	1.24*	1.31	1.26
Cl^- [mmol/L]	103.0	103.0	100.2	101.0	99.8	99.6	100.8	102.8
Lactate [mmol/L]	1.6	1.0	0.9	0.8*	0.8*	0.7*	0.7*	1.4
ESR [mm/h]	7.6	—	—	—	—	—	7.0	6.3

*$p < 0.05$ vs. W0, Wilcoxon-test.

Concerning the performance of the Immunopure device, a strong removal of platelets, monocytes and eosinophils to a maximum of 53, 69, and 44%, respectively, in the outflow, could be demonstrated. Neutrophil granulocytes and basophils were moderately reduced in

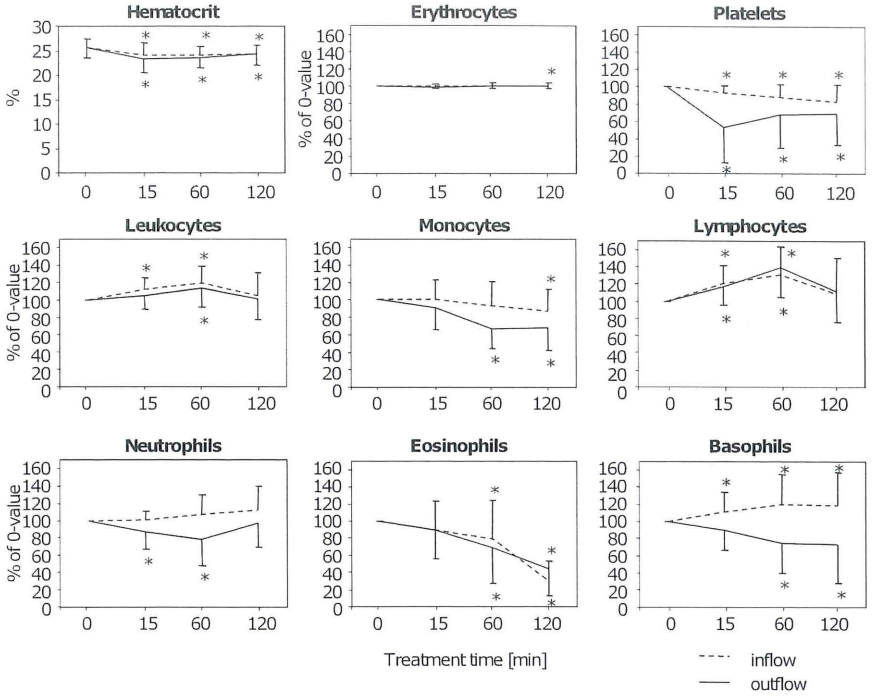

Fig. 34.18. Blood count graphs — intra-treatment impacts during and after the apheresis treatments of pigs. Comparison of baseline values (0 min) vs. 15, 60 and 120 min values (five animals, 25 treatments, mean ± SD, absolute counts are expressed as % of 0-min-value, $*P < 0.05$ vs. 0-min-value, Wilcoxon test).

the outflow sample, but increased after 120 min of treatment time (Fig. 34.18).

The reason for this may be an overshoot phenomenon, which has been observed previously leading to the delivery of cells from marginal or other pools (Yamaji *et al.*, 2002). The increase in leukocytes over time may also be ascribed to this phenomenon. There was also an increase of lymphocytes with a maximum at 60 min of treatment. These effects were not observed in humans (see Section 3) and seem to be characteristic for pigs or may be due to the longer treatment time. There was no long-term impact of the apheresis treatments on the blood cell counts.

In summary, this study demonstrated that a large animal model using the German large white pig is suitable for the safety evaluation of extracorporeal devices. The Immunopure device was shown to be safe and tolerable in this animal model.

3. Clinical Aspects

3.1. *Introduction*

Therapeutic strategies in chronic UC depend on localization, intensity of the illness, and response to steroids. Treatment goals include treatment of symptoms, induction of clinical remission, maintenance of steroid-free remission, prevention of hospital admission and surgery, mucosal healing, improvement of life quality, and avoidance of disability (Ordás *et al.*, 2012).

In general, medication for UC mainly includes 5-aminosalicylic acid (5-ASA), corticosteroids, immunosuppressive drugs, and monoclonal antibodies to TNF-α. 5-ASA is the first line treatment for mildly to moderately active UC. Proctitis is treated with topical 5-ASA. Combining topical 5-ASA with oral 5-ASA or topical steroid is considered for escalation of treatment. Systemic corticosteroids are appropriate if symptoms of active colitis do not respond to 5-ASA (Dignass *et al.*, 2010).

Patients with bloody diarrhea ≥ 6 per day and any signs of systemic toxicity suffer from severe colitis. They should be admitted to hospital for intensive treatment. Then, intravenous corticosteroids are the mainstay of conventional therapy. The response to intravenous steroids should be assessed around the third day. Second line therapy consists of either ciclosporin, infliximab or tacrolimus. If there is no improvement within 4–7 days of salvage therapy, colectomy is recommended. Third line medical therapy may be considered at a specialist center (Dignass *et al.*, 2010).

In clinical routine, about 70% of patients respond to the first course of corticosteroids, but 22% develop steroid dependency during the first year of treatment, and only half of them maintain steroid-free remission (Faubion *et al.*, 2001). Steroid-dependent patients are

treated with azathioprine/mercaptopurine. However, some patients exhibit UC that is refractory to corticosteroids or immunosuppressive drugs. Outpatients with steroid refractory disease should be treated with anti-TNF therapy or tacrolimus. Surgery used to be the ultimate therapeutic measure to cure, however, imposing other restrictions and complications. Thus, a treatment alternative, if possible with no or minor toxicity, is needed to handle refractory or steroid-dependent individuals, to save steroids and to treat high risk or other specific patient groups (pregnant women, children, toxic megacolon).

Cytapheresis has the potential to avoid drug-mediated side effects and to treat steroid-refractory patients while being not associated with safety concerns or serious side effects. However, the treatment is expensive, and its therapeutic success varied, also due to the fact that the patients' background and treatments are often very heterogeneous. Therefore, careful patient selection may improve efficacy and cost-benefit ratio of this treatment option. In addition, new developments like the Immunopure adsorber may be of special interest in UC patients with high platelet numbers and/or coagulation disorders.

3.2. *Uncontrolled Studies — Results*

In a pilot study including 10 patients with UC, the Immunopure device was shown to be safe, well tolerated and clinically efficient (80% remission rate). It offered very good biocompatibility and platelet elimination capacity (Ramlow *et al.*, 2013). In addition to the animal study (see Section 2.3), this uncontrolled human pilot study provided convincing data to achieve approval status of the Immunopure device in Europe (CE marking) in 2011. On average, the Clinical Activity Index [CAI according to Rachmilewitz (1989)] was reduced from 8.5 ± 1.5 (screening value) to 4.5 ± 4.1 at W6 and 2.2 ± 2.7 at W10 ($P < 0.05$). Clinical remission in UC is defined as a CAI score of ≤ 4. Clinical response is defined as CAI drop ≥ 3 or CAI ≤ 4. Endoscopic remission according to Rachmilewitz (1989) (EI < 4) was achieved in four of the nine patients (44%) who consented to endoscopic examination. The average EI of the nine eligible patients decreased from 7.4 ± 1.6 (screening value) to 5.2 ± 3.2 at W10

($p < 0.05$). There was a strong reduction of monocytes (outflow to 37.3% after 30 min), neutrophil granulocytes (outflow to 53.2% after 30 min), eosinophils (outflow to 51.5 % after 15 min) and platelets (outflow to 20.3% after 15 min) during the treatment.

The aim of a subsequent post-marketing surveillance study was to further evaluate the influence of the Immunopure module on platelets, platelet activation and PLAs in UC patients similar to the *in vitro* experiments (see Section 2.2). This non-interventional study according to the German law on medical devices §23b was conducted in accordance with the principle of ISO 14155 good clinical practice and the Declaration of Helsinki. Informed consent was received by all patients after the ethics committee of the University of Rostock had approved the study protocol. A total of six patients (three males, three females, mean age: 40.5 years, minimum age: 27 years, maximum age: 52 years) with moderately to severely active UC of 6–13, defined by the CAI according to Rachmilewitz, who had failed to achieve long-term remission with steroids and/or immunosuppessive agents or who had contraindications or were intolerant to high doses of steroids and/or immunosuppressive agents, were recruited. The mean disease duration was 7.8 years. Fifty percent of the patients suffering from active UC exhibited a pancolitis, 50% a left sided colitis (distal colitis). In general, the patients' medications were comprised of 5-ASA, tacrolimus and steroids (Table 34.4). Disease activity was

Table 34.4. Patient Data of Post-Marketing Surveillance Study

Patient No.	Sex	Age	Diagnosis[†]	Treatment	CAI Before	CAI W10
1	f	26	E3	Tacrolimus, 5-ASA	13	4
2[‡]	m	45	E2	5-ASA, Glucocorticoids, Tacrolimus	10	10
3	f	50	E2	5-ASA, Glucocorticoids	8	2
4[‡]	m	51	E3	5-ASA, Glucocorticoids, Tacrolimus	6	1
5	m	29	E2	5-ASA, Glucocorticoids	7	3
6	f	33	E3	5-ASA	13	5

[†]Montreal classification [54]: E2: left sided UC (distal UC), E3: extensive UC (pancolitis).
[‡]non-responder.

evaluated by assessing the CAI at baseline and 10 weeks after the first apheresis treatment.

The average CAI was significantly reduced from 9.5 ± 3.0 (screening value) to 4.2 ± 3.2 at W10 ($p < 0.05$). Remission of the disease was achieved in four of the six patients (67%), response in five out of the six patients (83%) at W10 (Table 34.4).

In accordance with the pilot study (Ramlow *et al.*, 2013), cytapheresis treatment with the Immunopure adsorber strongly decreased platelets, monocytes and granulocytes to a maximum of 33, 33 and 58%, respectively (Fig. 34.19). The hemoglobin and hematocrit values were slightly decreased during the treatment; values were hematocrit corrected. In accordance with the *in vitro* results (Fig. 34.7), there was a maximum of platelet adsorption after 15 min. After 15 min of treatment platelets increased in the outflow suggesting a saturation of the column with these cells. This behavior excludes safety problems with low platelet numbers; values had never fallen below 180×10^9/L in these patients. However, there was a different adsorption behavior for leukocytes, monocytes, neutrophils, and eosinophils which showed a continuous decrease over time. At the end of the treatment platelet and eosinophil numbers had a slightly, but significantly lowered level than before apheresis. Nevertheless, there were no significant differences of the single parameters between the different apheresis sessions.

Flow cytometry analyses confirmed these results showing a maximum decrease of $CD10^+$ granulocytes to 52%, leukocytes expressing the cell adhesion proteins CD11b and L-selectin to 50%, $CD14^+$ monocytes/macrophages to 31% and inflammatory $CD14^+CD16^+$ monocytes to 53% (Fig. 34.20).

In vivo, PLAs with $CD14^+$ monocytes, $CD3^+$ T cells, and $CD11b^+$ cells were strongly reduced by Immunopure apheresis too (Fig. 34.21). Values were reduced to a maximum of 29% ($CD42b^+CD14^+$, $CD42b^+CD11b^+$) and 30% ($CD42b^+CD3^+$). Similar to the adsorption of platelets, values increased again after 15 minutes towards the end of the treatment. After 60 minutes, inflow values were also significantly reduced to 84% ($CD42b^+CD14^+$) and 82% ($CD42b^+CD3^+$). However, there were no long-term effects on PLAs.

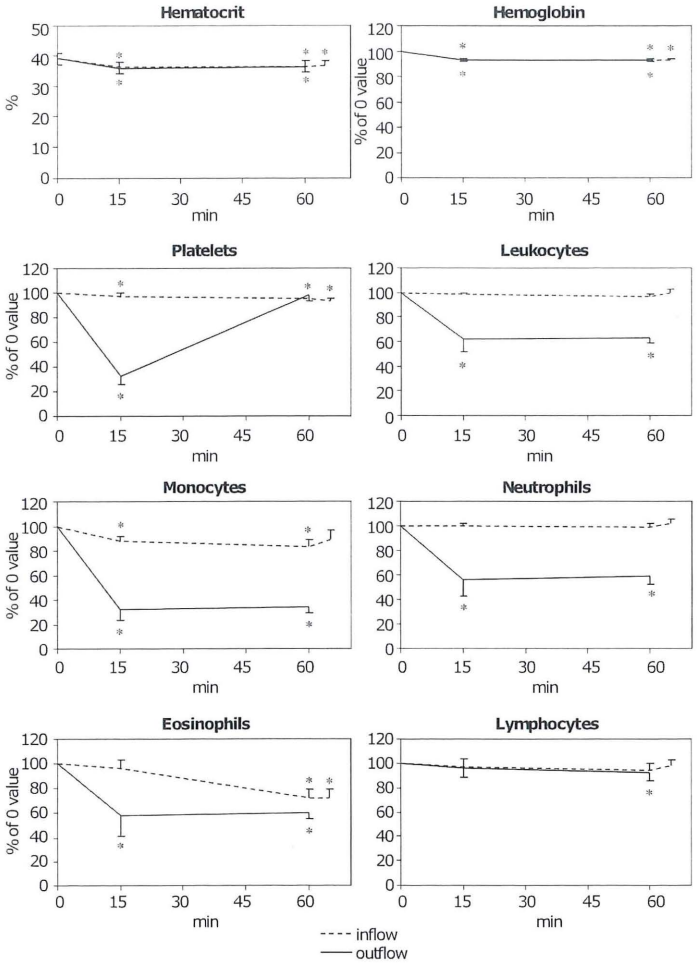

Fig. 34.19. Peripheral blood counts during and after treatment of patients with the Immunopure adsorber. Results were compared with baseline values at time point 0 min (100%) at the beginning of each treatment using the Wilcoxon test (six patients, five treatments each, mean/SEM out of 30 treatments) ($*P < 0.05$).

There was a minimal increase in platelet-derived MPs during the apheresis treatment, possibly due to an enhanced platelet activation. There was also a significant decrease in the inflow/outflow values after 15 minutes of treatment, indicating an MP adsorption at this

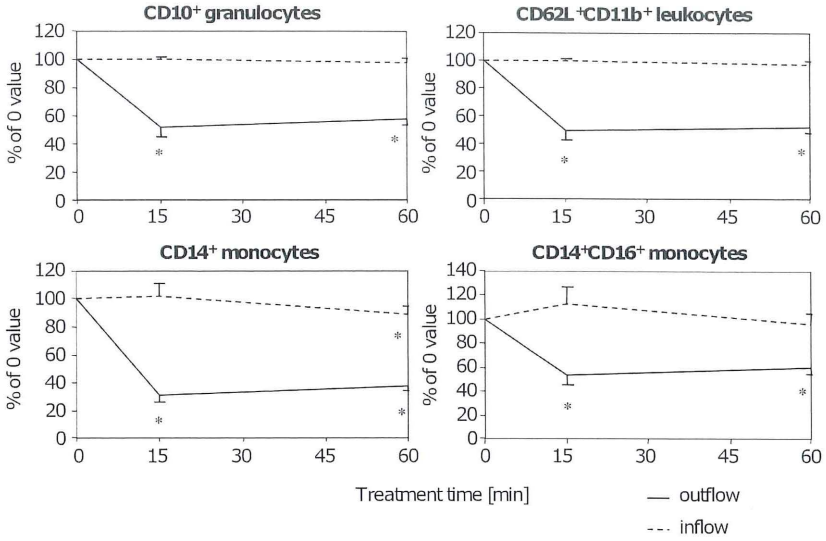

Fig. 34.20. Flow cytometry analyses of $CD10^+$ granulocytes, $CD62L^+CD11b^+$ leukocytes, $CD14^+$ monocytes and $CD14^+CD16^+$ monocytes during and after treatment of patients with the Immunopure adsorber. Results were compared with baseline values at time point 0 min (100%) at the beginning of the apheresis treatment using the Wilcoxon test (six patients, mean/SEM of first, third and fifth treatment session) ($*P < 0.05$).

time point (Fig. 34.22). However, differences were relatively small indicating that they were of minor relevance.

In accordance with the *in vitro* results (see Table 34.1), several activation parameters were elevated or down regulated due to activation of leukocytes or platelets. CD62L expression was slightly decreased and CD11b expression was moderately increased, suggesting a relatively low leukocyte activation. This also applied to T cell activation ($CD4^+CD25^+$). In contrast, platelet activation was relatively strong with large deviations (Table 34.5). However, as seen in the *in vitro* experiments, most of these markers were also changed using sham modules.

The high expression of the platelet activation markers CD63 and CD62P during Immunopure apheresis was accompanied by increased plasma soluble CD40L concentrations. Levels were six to sevenfold

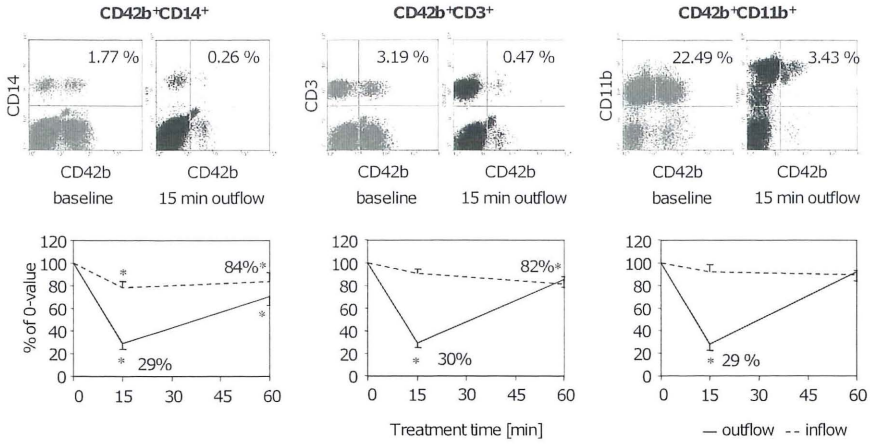

Fig. 34.21. Flow cytometry analyses of PLAs stained with the CD42b platelet marker combined with CD14, CD11b and CD3 during and after treatment of patients with the Immunopure adsorber. Results (mean/SEM) were compared with baseline values at time point 0 min (100%) at the beginning of the apheresis treatment (six patients, mean/SEM of first, third and fifth treatment session) (*$P < 0.05$, Wilcoxon test).

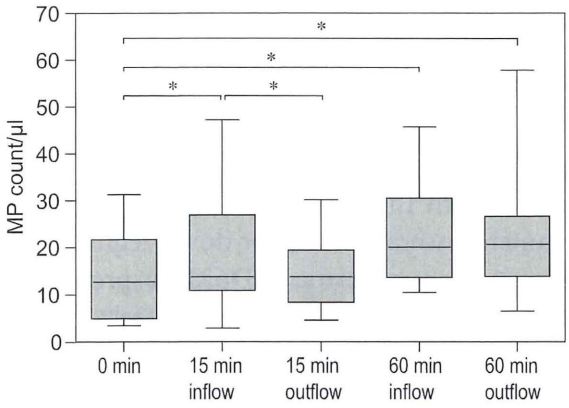

Fig. 34.22. Flow cytometry analyses of platelet-derived MPs (Annexin V+/CD42b+, <0.9 µm) during and after treatment of patients with the Immunopure apheresis (six patients, median of first, third and fifth treatment session) (*$P < 0.05$, Wilcoxon test).

Table 34.5. Flow Cytometry Analyses of CD62L (Mean Channel Fluorescence), CD11b (Mean Channel Fluorescence), $CD4^+CD25^+$ Cells, $CD42b^+CD62P^+$ and $CD42b^+CD62P^+$ Platelets after Treatment of Patients with Immunopure Apheresis. Results (Six Patients, mean ± SD of First, Third and Fifth Treatment Session) were Compared with Baseline Values at Time Point 0 min (100%) at the Beginning of Each Experiment ($*P < 0.05$)

	Inflow	Outflow	Inflow	Outflow
Parameter	**15 min**	**15 min**	**60 min**	**60 min**
Mean CD62L	100.1 ± 24.5	92.7 ± 26.5	97.1 ± 23.3	91.0 ± 23.1*
Mean CD11b	103.0 ± 28.2	145.7 ± 46.8*	93.3 ± 16.7	175.2 ± 56.0*
$CD4^+CD25^+$	112.2 ± 51.3	116.4 ± 56.9	111.5 ± 53.4	110.0 ± 51.1
$CD42b^+CD63^+$	102.5 ± 51.0	207.5 ± 112.9*	102.5 ± 43.0	285.2 ± 228.7*
$CD42b^+CD62P^+$	180.6 ± 205.8	901.7 ± 1159.1*	170.3 ± 150.0	1753.1 ± 2379.6*

higher after 60 min of treatment compared to baseline levels before treatment. Whether these increased soluble CD40L concentrations may have any consequences needs to be further elucidated. However, it seems to be only a temporary effect of the adsorber column as values return to basic levels before the next apheresis session starts. Soluble P-selectin concentrations were not significantly altered during Immunopure apheresis treatments. There was only a 1.3-fold increase during the third treatment session (Fig. 34.23).

In conclusion, these uncontrolled studies showed that Immunopure treatments were well tolerated and remarkably reduced disease activity. In addition to monocytes including proinflammatory $CD14^+CD16^+$ monocytes and granulocytes, Immunopure is able to adsorb platelets and platelet aggregates. Since UC is associated with abnormalities in platelet and leukocyte number and function, their temporary reduction by the Immunopure device seems to be related to clinical improvement. However, there was also a kind of inflammatory stimulus at the column, e.g. leading to leukocyte and platelet activation. Usually, the immune system has the capability to regulate immune responses, allowing robust responses against pathogens while avoiding autoimmunity. In UC, this capability is constrained, leading to an overshooting immune response. Possibly, adsorptive cytapheresis

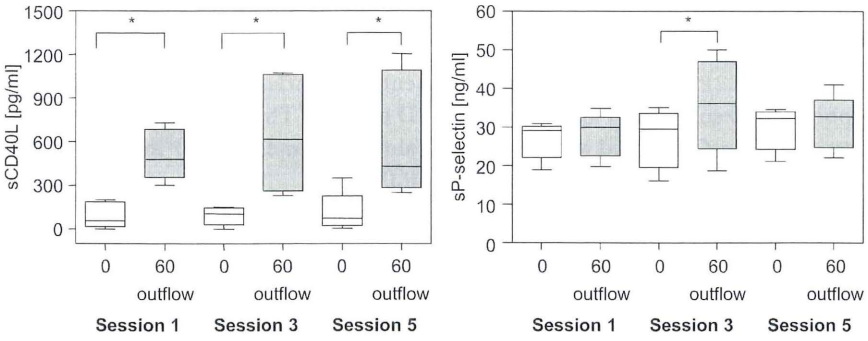

Fig. 34.23. Plasma concentrations of soluble CD40L (left) and soluble P-selectin (right) as determined by enzyme-linked immunosorbent assay before and after treatment of patients with the Immunopure adsorber (six patients, median of first, third and fifth treatment session) ($*P < 0.05$, Wilcoxon test).

is like a second stimulus which activates the immune system outside the body, finally leading to induction of regulatory processes that might also affect the course of UC. Generally, the following mechanisms are conceivable and have already partly been discussed for the mode of action of other cytapheresis adsorbers: a temporary removal of reactive cells including platelets and PLAs, a parallel activation of leukocytes and platelets that return to the patients' circulation and transfer signals to other cells, leading to mobilization of naïve and regulatory cells.

3.3. *Controlled Studies*

The clinical benefit of Immunopure on efficacy parameters merits further evaluation in controlled trials and studies with larger patient numbers. To date, there are no results of controlled studies investigating the Immunopure device. One multicenter trial is ongoing comparing Immunopure versus oral prednisolone in a small number of patients. However, further sufficiently powered, controlled studies are necessary, especially with regard to patients with active disease that are really supposed to respond to apheresis treatments. In particular, these are patients early after initial diagnosis with moderate to severe disease activity as well as steroid-naive patients only on 5-aminosalicylic acid

immediately after a clinical relapse. This should be the target group for initial cytapheresis trials, especially when apheresis treatment should be compared with sham-apheresis. In a second step, enhanced adsorptive cytapheresis protocols should be tested which possibly offer advantages for patients with a longer disease history and combined medication for UC. Moreover, international approved end points and disease indices should be applied.

Acknowledgements

This work was supported by NIKKISO Co. Ltd., Tokyo, Japan. We thank Udo Losert, Helga Bergmeister and Roberto Plasenzotti for performing the animal trial; Peter Ahrenholz for planning the *in vitro* experiments; and Matthias Schneider, Grynet Quintern, Anja Ramlow, Annett Bieber, Manuela Seidl and Katja Vedder for excellent technical support.

Abbreviations

azathioprine (Aza)
5-aminosalicylic acid (5-ASA)
clinical activity index (CAI)
endoscopic index (EI)
granulocyte/monocyte adsorption apheresis (GMCAP)
inflammatory bowel diseases (IBD)
leukocytapheresis (LCAP)
microparticle (MP)
mean platelet volume (MPV)
polyarylate resin (PAR)
plateletcrit (PCT)
platelet distribution width (PDW)
Polyester-polymer alloy (PEPA)
polyethersulfone (PES)
platelet leukocyte aggregate (PLA)
tumour necrosis factor alpha (TNFα)
ulcerative colitis (UC)

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