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REVIEW ARTICLE

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The low-throughput protein A adsorber: an immune modulatory device. Hypothesis for the mechanism of action in the treatment of rheumatoid arthritis

Abstract To achieve specific removal of pathogenic antibodies (Ab) or immune complexes (IC), several adsorbers have been developed. We discuss the mode of action of low-throughput staphylococcal protein A (SPA) immunoadsorption. The SPA-based Prosorba apheresis is likely to modify some of the autoantibodies (autoAb) or IC. The low-throughput adsorber showed very limited adsorption capacity of circulating autoAb and/or circulating IC. Besides changes of humoral diagnostic parameters, cellular changes could be observed in the Prosorba-treated patients. These changes were rather similar to those that have been observed in a patient successfully treated with Ab against tumor necrosis factor α. We propose an adsorber-catalyzed conversion of small, tissue-penetrating, scarcely detectable, non-complement-binding, proinflammatory IgG-rheumatoid factor (RF)-based IC into the more readily phagocytosed species of IC: intermediate-sized, partially cryoprecipitable, non-tissue penetrating IC that are opsonized with complement. These IC are rather short-lived and could quickly be cleared by the body's scavenging system.

Key words Apheresis · Immunmodulation · Immune complex (IC) · Rheumatoid arthritis (RA) · Staphylococcal protein A (SPA)

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Introduction

Inflammation induces destruction if it perpetuates and becomes chronic. This situation can either be caused by the failure of physiological mechanisms for the resolution of inflammation or by the persistence of the stimuli. Chronic inflammation can be stimulated by many factors and might develop self-perpetuating properties. Pharmacological suppression of ongoing inflammatory processes or eradication of the initiating stimulus may interrupt this vicious circle. Apheresis is thought to be one possibility for the latter.

History of Prosorba apheresis in patients with rheumatoid arthritis

Two pharmacologists at the Johns Hopkins University, Baltimore, first created a device for "plasma removal with return of corpuscles" in 1914.[1] They coined the name "plasmapheresis" from the Greek term for separating by force (aphairein). Plasmapheresis was an individual therapy between 1920 and 1940 in polycythemia and congestive heart failure and since 1959 in patients with Waldenstrom's disease. Jaffe was the first to successfully treat a patient with rheumatoid arthritis (RA) using apheresis at the New York Medical College Metropolitan Center in Manhattan in 1962.[2] Subsequently, he observed that some patients did not respond to this treatment modality.[3] The first running protocols for plasmapheresis were evolved in 1977. The results of treating 82 patients with different rheumatic diseases indicated improvement of the disease for months; again, however, casual relapses of RA were reported.[3] Some work with membrane filtration (Cryomax) in patients with RA was done in the early 1980s[4,5] followed by quite a number of further devices developed by various companies.[6–10]

The initial devices unselectively removed all noncorpuscular constituents of the patients' blood including essential plasma proteins, irrespective of their involvement in the

pathogenesis. Therefore, the patients had to be substituted with various serum fractions or albumin solutions in addition. This procedure did not effectively prevent relapses of the pathogenic conditions. Dysbalance of coagulation factors due to their bulk removal and different rates of synthesis turned out to be the major problem, causing thromboses as well as bleedings. These side effects could be treated with substitution of fresh frozen plasma. However, this was a rather expensive approach and, additionally, bore the risk of infection, especially with hepatitis viruses or human immunodeficiency virus.

The initial goal of the first apheresis attempts was to completely eradicate the putative noxious compounds. When the pathogenic molecules were better defined, adsorption procedures with ligands were initiated. The strategy was to selectively remove pathogenic plasma molecules such as immune complexes (IC), antibodies (Ab), or even more specifically certain autoantibodies (autoAb),[11] avoiding the need of replacing the whole plasma. The diversity of treatment modalities including patient-specific adsorbers became rather perplexing, while the small cohorts treated with defined protocols greatly limited the evaluation and comparison of the different methods. The number of nonresponders was high, partially since the pathogenic mechanisms of the diseases were still insufficiently clarified. Again, most approaches treated as much plasma as possible and tried to completely expunge their specific targets.

Two approaches, however, differ in this respect from all the others: extracorporeal photochemotherapy (ECP) and Prosorba treatment. These techniques deal with only a minor proportion of the circulating cells or plasma, respectively, and do not quantitatively remove putative pathogenic cells or plasma molecules. Instead, they modify them and return them into the circulation. In the case of ECP the treated cells orchestrate a downregulation or control of the pathogenic T cells in the body. The mechanism of action of ECP may include the induction of apoptosis and the generation of an anti-inflammatory cytokine profile,[12] the staphylococcal protein A (SPA)-based Prosorba apheresis is likely to modify some of the autoAb or IC. After reinfusion, the metamorphosed plasma constituents may then modulate the inflammatory responses by interacting with the immune and scavenging system. Therefore, this treatment modality can be considered as an immunomodulatory intervention. The method, in addition, usually does not require any substitutions. The trials by Wiesenhutter et al. initiated the renaissance of the treatment of severe RA with this SPA-based immune modulatory device.[13]

Basic concept of SPA-based extracorporeal immunomodulation-versatility

Staphylococcal protein A (SPA): the core of Prosorba

The Prosorba column is a medical device that consists of immobilized SPA, a virulence factor of *Staphylococci*. Staphylococcal protein A was discovered in the late 1950s by the Danish scientist Jensen[14] and has been shown to bind to the Fc portions of various immunoglobulins (Ig). Staphylococcal protein A is a single polypeptide chain with five extracellular domains (C, B, A, D, E; each 7 kDa).[15–18] Staphylococcal protein A preferentially binds to Fcγ, the constant region of IgG for effector functions. In addition, it is able to recognize the Fab fragments of approximately 50% of human IgM, 22% of human IgA, and 15% of human IgG.[19]

The overall shape of the Fc part of Ig resembles a horseshoe with two CH3 domains packed tightly at the base and two CH2 domains forming the opening of the horseshoe.[20] The Fc binding site of SPA is located at the CH2–CH3 interface of IgG1, IgG2, and IgG4 molecules.[21] In addition, SPA has distinct sites with affinity for the Ig heavy chain variable region encoded by genes of the VH3 family.[19,21] This Fab site is less well defined.[22] Staphylococcal protein A is known as a B-cell superantigen which can stimulate 30%–60% of the peripheral B cells carrying VH3-encoded B-cell receptors (BCR).[21] This is due to the interaction between the heavy chain of the Fab fragment and the helices II and III of domain D of SPA, which bind the Fab in the framework outside their complementarity-determining regions (CDR). The residues within the Fab part that are in contact with SPA are located in a network of four β-strands of the VH region and in the interstrand loops remote from the antigen-binding site. The residues within the domain D of SPA are highly conserved in other Ig-binding sites of SPA.[21,23]

Binding of SPA to peripheral B cells has been shown to cause T-cell-independent B1 cell depletion.[24] There is evidence that an interaction of SPA with VH3 IgG leads to an IC-mediated inflammatory response in vivo.[25]

Microbial pathogens frequently interfere with the immune response by superantigens (SAg). It is well known that Fab-mediated binding of SPA and the T-cell receptor-mediated recognition of T-cell SAg operate with similar mechanisms. SAg like SPA stimulate a large subset of mature lymphocytes. In vivo exposure to SPA has been shown to induce large-scale effects on murine B-cell clonal selection by mechanisms that include deletion of supraclonal sets. However, the requirements for murine Fab-binding of SPA remain incompletely defined.[26] Staphylococcal protein A interacts with the conserved V-region framework subdomains encoded by the VH clan III genes of the BCR rather than the CDR, binding conventional Ag.[27] Crystallographic analyses of the SPA–BCR interactions showed many similarities with the interactions of T cells with its T-cell SAg. Antibody-expressing VH3/S107 genes display the highest SPA binding affinity.[26] Therefore, B cells expressing these genes are most susceptible for the SPA-induced supraclonal deletion.[27] Genes from the largest clan VH III family, 7183, commonly encode for Fab-mediated binding of SPA, while Ab from five other VH families, J558, Q52, Sm7, VH11, and VH12, do not display Fab-mediated SPA-binding activity.[26] The SAg properties of SPA and those of pFv, an endogenous sialoprotein, involve binding interactions with concerted sites in the VH domain, indicating that

self and foreign proteins can employ highly conserved strategies to create SAg for the BCR.[28]

Treatment with SPA of neonatal or adult mice induced a T-cell-independent deletion of a large supraclonal set of susceptible B cells including clan III/VH S107 family-expressing cells.[24] After infusion of SPA (one infusion with 500μg SPA per mouse) in mice, splenic B cells showed an activation phenotype, downregulated BCR, CD19, and CD21, and underwent limited rounds of proliferation followed by apoptosis. Apoptotic bodies were deposited in the spleen, lymph nodes, and Peyer's patches.[27] Also, intraperitoneal instillation of SPA (100μg SPA in phosphate buffered saline 8 times every other day) induced a persistent T-cell independent loss of a large supraclonal set of susceptible lymphocytes, which includes clan III/VH S107 family-expressing type 1 B cells and their Ab. These long-term in vivo effects were not due to residual deposited Sag, since mice that received cells from SPA-treated donors exhibited the same induced supraclonal hole in the repertoire of natural IgM-secreting cells due to supraclonal deletion. The magnitude of the deletion directly correlated with the VH-specific binding avidity of different SPA forms.[29] B-cell apoptosis did not require Fas. B cells were protected from apoptosis by interleukin (IL)-4, CD40L, or Bcl-2. Upon cessation of SPA exposure, the representation of splenic type 2 B cells normalized. However, the VH family-restricted deficit of peritoneal type 1 B cells persisted. SPA also induced a persistent loss of certain natural Ab and resulted in a specific tolerance to phosphatidylcholine-containing immunogens that usually are targets of immune responses dominated by the S107-expressing type 1 B cells. Silverman and colleagues conclude that B-cell SAg exploit a primordial "Achilles heel" of type 1 B cells, which are important sources of natural Ab.[24]

Staphylococcal protein A can elevate proinflammatory cytokines (IL-1, IL-2, interferon [IFN] γ, and tumor necrosis factor [TNF] α) and downregulate the secretion of IL-4, IL-6, and IL-10. This will cause a bias for a Th1-driven immune response. Staphylococcal protein A may also initiate limited proliferative responses[30] and act as a VH-targeted B-cell superantigen. Furthermore, SPA confers interaction with soluble and immobilized von Willebrand factor and possibly plays a role in the pathogenesis of endovascular staphylococcal disease.[31]

Adsorption with *Staphylococcus aureus*, Cowan I had a reversing beneficial effect on the immunosuppression in patients with cancer through the binding of SPA to IgG and IC.[32] In the United States immobilized SPA was approved for the treatment of idiopathic thrombocytopenic purpura (ITP) by the Food and Drug Administration (FDA) in 1987. To treat patients' plasma a primary plasma separation device, i.e., a cell separator, is necessary. During the procedure the SPA column is integrated in an ex vivo plasma circuit. Prosorba treatment differs from plasma exchange by only removing a minor amount of the total plasma proteins and, therefore, saves essential plasma components. Thus no replacement fluids, proteins, or crystalloids are required.[33] When perfused with normal human plasma in vitro, Prosorba bound predominantly IgG with a maximal capacity of approximately 500 mg of Ig per column, equal to approximately 1.5% of circulating IgG.[19]

Immune complexes: the main target of Prosorba

Immune complexes are composed of Ig bound to their corresponding antigens and may contain varying amounts of complement or other opsonizing serum components. Immune complexes are pleomorphic in size, composition, solubility, and their ability to initiate inflammation. They can directly be generated in the fluid phase or on immobilized antigens. Circulating IC (CIC) are usually transported to the liver and spleen and cleared there by phagocytes. Immune complexes containing C3 or C4 fragments are often attached to CR1 and thereby are shuttled by erythrocytes to sites where they are removed. If the scavenging capacity is insufficient or overloaded, deposition of IC can lead to inflammation.[34] There is evidence that autoAb or IC are involved in various kinds of autoimmune diseases. Removing the pathogenic IC from patient's plasma is supposed to be an efficient way for the treatment of IC-based chronic inflammatory diseases such as RA and systemic lupus erythematosus (SLE).[34] In contrast to conventional apheresis, selective adsorption of IC or specific adsorption of pathogenic autoAb without depleting other plasma proteins including regular Ig would be advantageous. Immune adsorption employs physical (e.g., electrostatic) or chemical avidity to capture pathogenic molecules.

Clinical relevance

The players of immunoadsorption

To achieve specific removal of pathogenic Ab or IC several adsorbers have been developed. Some of them have already been approved by the FDA for the core indications RA, ITP, or hemophilia with inhibitors.[8,9,13,35,36] Whereas most immunoadsorption therapies are aimed to adsorb as much as possible of the pathogenic Ab, the mode of action of the low-throughput adsorber Prosorba must obviously be different.

The latter also bind IgG and IC due to the avidity of SPA to various Ig. However, the capacity is rather limited (as little as 1.5 % of the circulating Ig can be bound to the adsorber during a treatment session). The bulk removal of pathogenic autoAb by the adsorber is very unlikely, since there is no rationale for why the pathogenic autoAb should preferentially bind to the column. The separation is achieved by a continuous flow centrifugation or plasma filtration. The device is configured for the treatment of maximally 2 liters of plasma in a 2-hour session.[22,35] Neither the processed plasma volume nor the frequency of treatments permit substantial removal of Ig or CIC by binding to the column material.[18,19] Instead, the procedure is likely to provoke its therapeutic action in a more indirect manner, involving the patient's own scavenging systems for the clearance of pathogenic Ab and/or CIC. Thus

the Prosorba should be considered an immunomodulatory device.

Low-throughput SPA adsorption is a therapeutic option for patients with autoimmune hemolytic anemia,[37] hemophilia with inhibitors,[18], ITP,[18] and severe RA.[13,18,38] Off-label use may apply for forms of SLE, thrombocytopenic microangiopathy (TMA), vasculitis,[18] myasthenia gravis, the Guillain-Barré syndrome,[18] pemphigus vulgaris,[39,40] and various hepatopathies.[36] It was proposed that IC can cause malignancy-associated immunosuppression and disease progression. Therefore, in the therapy of certain cancers and in paraneoplastic neurological syndromes, low-throughput adsorber treatment might be considered.[18] However, the data are controversial in this field.[41,42] Diverse effects of the scavenging system following Prosorba treatment of various diseases imply an immunomodulatory impact.[19]

Side effects

In general, immunoadsorption is well tolerated. Rarely, the treatment has to be interrupted due to a decrease of renal function.[43] The most common adverse effects were "flu-like" symptoms with chills, mild fever, nausea, and joint/muscle pain. Sometimes arthritic flares of short duration, anemia, and fatigue have been described that are usually manageable and temporary.[13,33,38,44] Hypotension, abdominal pain, headache, diarrhea, respiratory symptoms, dizziness, weakness, and edema as well as hypersensitivity-type reactions have been reported in few patients.[13,38,42,45,46] Leukocytoclastic vasculitis of the skin is a rare involvement of the kidneys and is a very rare complication of immunoadsorption. Immune complexes in the glomeruli influence the course of the renal disease.[47] Mild to moderate side effects following SPA treatment of metastatic breast adenocarcinoma became manifest in transient fever, chills, rigors, and infrequent nausea, vomiting, diarrhea, episodic hyper- and/or hypotension, bronchospasm, venospasm, headache, and joint and tumor pain.[48] In the 1980s few fatalities were attributed to immunoadsorption.[49] A postmarketing survey of Prosorba treatment in RA revealed a number of thrombotic blood vessel occlusions. In an internal analysis most of these thrombotic events had occurred in combination with other risk factors that are declared to be exclusion criteria for Prosorba therapy, with about 0.5% of thrombotic events remaining associated with Prosorba. Contraindications for Prosorba are medications with converting enzyme inhibitors, a history of embolism, hypercoagulability, thrombocytosis, or vasculitic hypersensitivity.[33]

Rheumatoid factors (RF)

Structure of RF

RF are not unique for RA and also occur in healthy immunized individuals, in the elderly, and in patients with other autoimmune diseases or chronic infections. RF recognize epitopes in the Fc region of IgG.[50] IgM and IgG RF are produced abundantly by plasma cells, and form complexes with the autologous IgG in circulation and in the synovial fluid of patients with RA. IgG RF usually circulate as oligomers, predominantly dimers[51] which poorly fix complement and, therefore, escape regular erythrocyte-based clearance mechanisms. Due to their small size, dimeric IgG RF are able to leave the vessels and to enter the tissues including the synovial tissue. Dimers formed by IgG1 RF but not those formed by IgG3 RF display the structural prerequisites to engage FcRγIII (CD16) on macrophages. Cross-linking of CD16 induces the activation of the macrophages and the secretion of TNFα with all the consequences thereof.[52] In the synovial fluid, IgG RF tend to aggregate and accumulate in the synovial tissue. Aggregated RF are able to activate complement. RF titers, IC, and C1q concentrations in RA patients have been established as indicators of disease activity.[53,54] However, the relevance of circulating IC and RF for the pathogenesis of RA is still under debate for several reasons. (i) The correlation of RF and CIC with disease is rather low. (ii) There are only a few Ag-specific IC assays. (iii) The high-affinity IgG–RF dimers, which do not activate complement, are not detected by the CIC assays used in routine laboratories. These CIC can be also predicted to escape regular clearance mechanisms that are based on opsonization by complement. (iv) RF are not felt to be the primary cause of RA and small RF IC may only be relevant for a subset of patients with RA. Interestingly, the patients with circulating high affinity IgG-RF IC may be the candidates to respond to low-throughput SPA apheresis. Due to multiple molecular interactions, dimeric RF IC can be predicted to display a much higher avidity for the immobilized SPA than monomeric IgG. We propose the following hypothesis for the mode of action of low-throughput SPA immunoadsorption. During the course of immune adsorption the IC tend to accumulate on the matrix of the adsorber and increasingly displace the initially bound monomeric IgG. Due to the close vicinity of the small-sized non-complement activating IC, specific aggregation leads to an increased size and to an altered composition of the IC.

Although the avidity of the IC increases with the number of Fc portions, some of the aggregated IC may be dislocated from the column and re-enter the bloodstream and can therefore be detected as short term circulating cryoprecipitable material (Figs. 1 and 2). In vivo, the concentration of the complement-inhibitory citrate used for anticoagulation in the extracorporeal circuit immediately decreases. Thus the remodeled large IC can activate complement and are thereby opsonized, greatly facilitating phagocytic removal in the liver and spleen. This material may also contain some trace contaminants of SPA that have been cleaved off from the matrix of the adsorber by the action of proteolytic enzymes. However, in the human studies the leached and reinfused SPA is of much lower concentration than in the mouse experiments, in which approximately 26 mg SPA per kg bodyweight were reinfused. In contrast, Sato and colleagues report that in humans an average 257 μg of SPA was reinfused per treatment, corresponding to

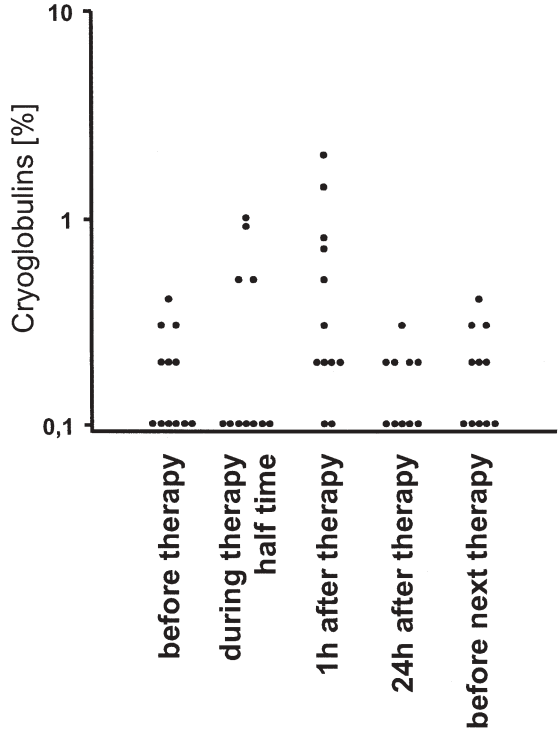

Fig. 1. Transient increase of circulating cryoglobulins observed after low-throughput staphylococcal protein A (SPA) apheresis of a patient with rheumatoid arthritis indicates physicochemical alterations of the circulating immune complexes. The concentrations of circulating cryoglobulins were measured in 12 consecutive therapeutic procedures in a patient with rheumatoid arthritis (RA). For the time frame see Fig. 2, patient 1. Cryoglobulins were measured before and during the apheresis, 1 and 24 h after treatment, as well as before the next therapeutic cycle. Cryoglobulin increases precede therapeutic responses and herald clinical improvement

approximately 5 μg SPA/kg body weight. A maximum amount of 414 μg of SPA, corresponding to approximately 8 μg SPA/kg body weight, was reinfused per treatment.[55] Balint and Jones measured approximately 80 μg SPA (approximately 1.3 μg/kg body weight) that was reinfused per treatment.[56]

In a crystallized complex formed by a low-affinity intermediate-avidity IgM RF and a monoclonal IgG4 myeloma protein, the RF did not bind its antigen (Fc portion of the IgG4) with the conventional binding site. Instead, the Fc portion was bound with the edge of the antigen recognition area engaging only four of the six CDR.[54] The structure of the complex evokes the hypothesis for molecular (anti-idiotypic) mimicry of bacterial Ig-binding proteins as the origin of RF, since there is only partial identity with the epitope recognized by RF. The authors suppose a novel form of cross-reactivity with the simultaneous binding of two antigens.[54] Some RF concomitantly bind antigen (Ag) and the IgG Fc using the conventional combining site and an adjacent site, respectively. Such RF may arise as a consequence from responses to any antigens. The V-gene somatic mutations are able to shape the classical and the alternative binding site independently. The RF BCR, the conventional Ag, and the IgG Fc form a ternary complex. This complex activates B cells leading to high level RF production. Furthermore, in the presence of the classical antigen, the complement-activating ternary complexes display an increased affinity for IgG. This hypothesis is based on the structural data of a IgM-RF–Autoantigen (AAg) complex.[54]

Another potential explanation for the mode of action of Prosorba includes the leakage of SPA.[19] However, the concentrations of SPA that get infused with every treatment procedure are rather low (see above). Staphylococcal protein A released from the column has been significantly reduced by better absorber devices. This new technology

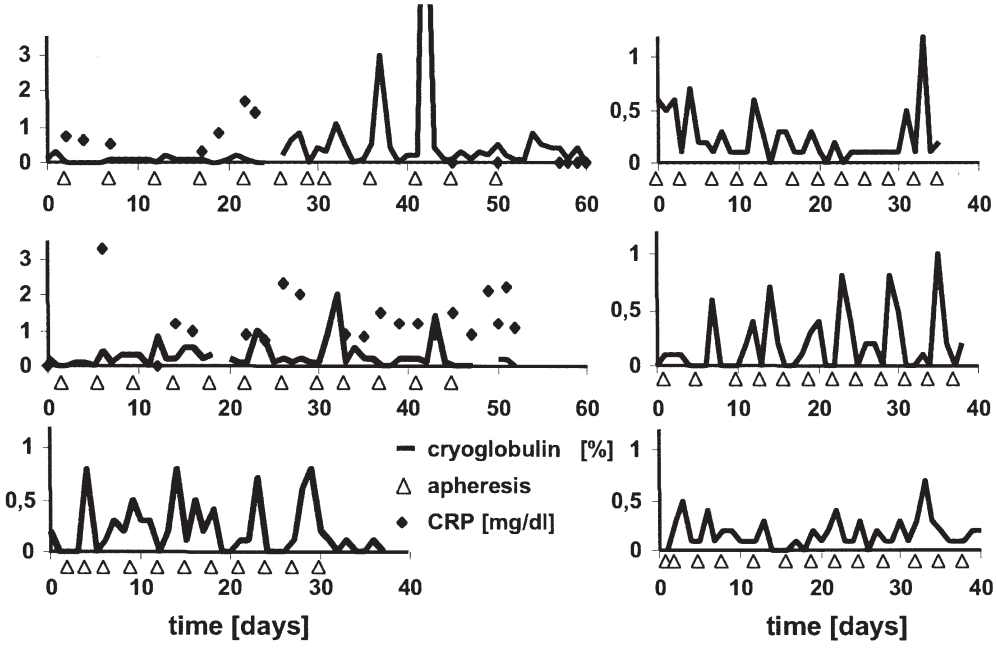

Fig. 2. A transient increase of circulating *cryoglobulins* was observed after low-throughput SPA *apheresis* of various patients with rheumatoid arthritis. Four RA patients with a 40-day observation period and two RA patients with a 60-day observation period are shown. The patients had received 11 and up to 13 therapeutic cycles. All of them had failed to clinically respond to conventional therapy. In two patients the correlation with C-reactive protein (*CRP*) plasma levels (mg/dl) is shown. All patients responded clinically to the apheresis treatment

produces SPA columns with a lower leakage of SPA. These new devices were approved by the FDA. Nevertheless, even a low leach may contribute to the supraclonal B-cell depletion. The small amount of SPA leakage has been proposed to be the cause of side effects as well as the mechanism of action of immunoadsorption by contributing to a supraclonal B-cell depletion.[57]

Rheumatoid factors are immunostimulatory – teamwork with FcγR, IC, and complement

The pleomorphic Ig Fc receptors (FcR) belong to the Ig superfamily and are expressed on multiple hematopoietic cells. FcR are cell surface proteins recognizing the C-terminal constant regions of Ig molecules. Furthermore, FcR do bind IC and play a central role in the immune response. Binding of IC to FcR activates or inactivates effector cells leading to phagocytosis, endocytosis, the release of inflammatory mediators, activation of NK cells and antibody dependent cellular cytotoxicity (ADCC).[20] FcR specific for all classes of Ig have been described: FcγRI, FcγRII, FcγRIII (CD64, CD32, and CD16), and neonatal FcR (FcRn) for IgG, FcεR (CD23) for IgE, FcαR (CD89) for IgA, FcδR for IgD, and FcμR for IgM.[58–62] FcγRII (CD32) and FcγRIII (CD16) are low-affinity receptors which need polyvalent interactions with IC for binding.[20] FcγR display different affinities for different IgG subclasses.[63] All FcγR are type I transmembrane glycoproteins with a ligand-binding subunit. Glycosyl-phosphatidylinositol (GPI) linked FcγRIIIA requires the association of the immunoreceptor tyrosine-based activation motif (ITAM) containing FcγR common γ-chain or T-cell receptor ζ chain for signaling.[20] Activating and inhibitory FcγR are coexpressed on macrophages, monocytes, neutrophils, and eosonophils, and consist of two Ig-like domains (D1 and D2).[64] FcγRIIIB is a GPI-linked receptor and is exclusively expressed on neutrophils. It does not contain its own signaling component and acts synergistically with other receptors. Contacts to Fc are organized through the D2 domain of the receptor.[20] A soluble form of FcγIIIR-B (sCD16) corresponding to the extracellular region of the receptor circulates in the plasma. It binds to CR3 (CDllb/CD18)- and CR4 (CDllc/CD18)-positive leukocytes and activates the CR3 complement receptor-dependent inflammatory process with upregulation of IL-6 and IL-8.[65] The structure of Fcγ describes a horseshoe with two CH3 domains bedded narrowly against each other at the base of the horseshoe and with two CH2 domains forming the open part. The receptor binds to the horseshoe opening of the Fcγ making contacts to the lower hinge regions of both chains of the Fcγ.[20] Interactions with the FcγR created on the Fcγ side of the complex are dominated by residues Leu 234–Pro 238 of the lower hinge γ.[20,63] RF are present in approximately 80% of the patients with advanced RA. Activation of TNFα secretion by cross-linking of the FcγRIII on macrophages or granulocytes by IgG RF may contribute to the pathology of RA.[66] Therefore, interfering with the receptor activation may inhibit the Ab-mediated autoinflammatory response. It has been shown in vitro that peptides derived from the lower hinge region of Ig can bind to FcγR and are supposed to inhibit IgG binding.[20]

	TNF	PROSORBA
CD62L+ monocytes	↓	↓
CD95+ PMN	↓	↓
CD54+/CD19- non B lymphocytes	↓	↓
Mn CD27 non B lymphocytes	↓	↓
CD40+/CD19+ B cells	↓	↓
HLA-DR+/CD19+ B cells	↓	↓
Mn CD45 on CD19+ B cells	↓	↓
Mn CD27 on CD19+ B cells	↓	↓
CD26+ lymphocytes	↓	↓
CD27+ lymphocytes	↓	↓
CD27+IgD- lymphocytes	↓	↓
CD123+ [count]	↑	↓
CD26-/CD19- non B lymphocytes	↑	↑

Fig. 3. Similar alterations of cellular parameters in patients with rheumatoid arthritis treated with low-throughput SPA apheresis or anti-tumor necrosis factor (*TNF*) α (responder). The data indicate activation of monocytes (reduced *CD62L* expression) and mitigation of the lymphocytes (reduced numbers of *CD26*, *CD27*, *CD40*, *CD54*, or histocompatibility leukocyte antigen (*HLA*) class II-positive lymphocytes). Note that the interleukin-3 receptor α-chain (*CD123*) was reduced during apheresis and elevated after anti-TNFα therapy

Dimeric IgG RF (and IgM RF) IC are able to activate macrophages via cross-linking of FcRγIII (CD16).[52] Activation of macrophages by tissue-penetrating IgG1 RF IC or by locally produced RF IC has been considered to be a decisive event in the affected synovial tissue in RA. Certain RF oligomers can stimulate PMN and macrophages and consequently induce upregulation of proinflammatory cytokines (e.g., TNFα). Upregulation of proinflammatory cytokines has been prominently considered the cause of chronic inflammation, cartilage destruction, and the formation of germinal center-like structures in the synovial tissue[52] seen in patients with RA. This concept is further supported by the proven efficacy of anti-TNF and anti-IL-1 biologic response modifiers currently being in clinical use.

Besides changes of humoral diagnostic parameters, cellular changes could be observed in the Prosorba-treated patients. These changes were rather similar to those that have been observed in a patient successfully treated with Ab against TNFα (Fig. 3). A reduced CD62L expression was observed, suggesting an activation of monocytes. Furthermore, the downregulation of characteristic markers of lymphocytes (Fig. 3) suggests a mitigation of the latter. Interestingly, the IL-3 receptor α-chain (CD123) was reduced during apheresis and elevated after anti-TNFα therapy. Additionally, a comparison of RA patients treated with low-throughput SPA apheresis or anti-TNFα therapy with cohorts of SLE patients and normal healthy donors was made. These data also indicate an activation of monocytes (reduced CD62L expression) and a mitigation of the lymphocytes, namely, reduced numbers of CD26-, CD27-, CD40-, CD54-, or CD45-positive lymphocytes after immune adsorption (Fig. 4).

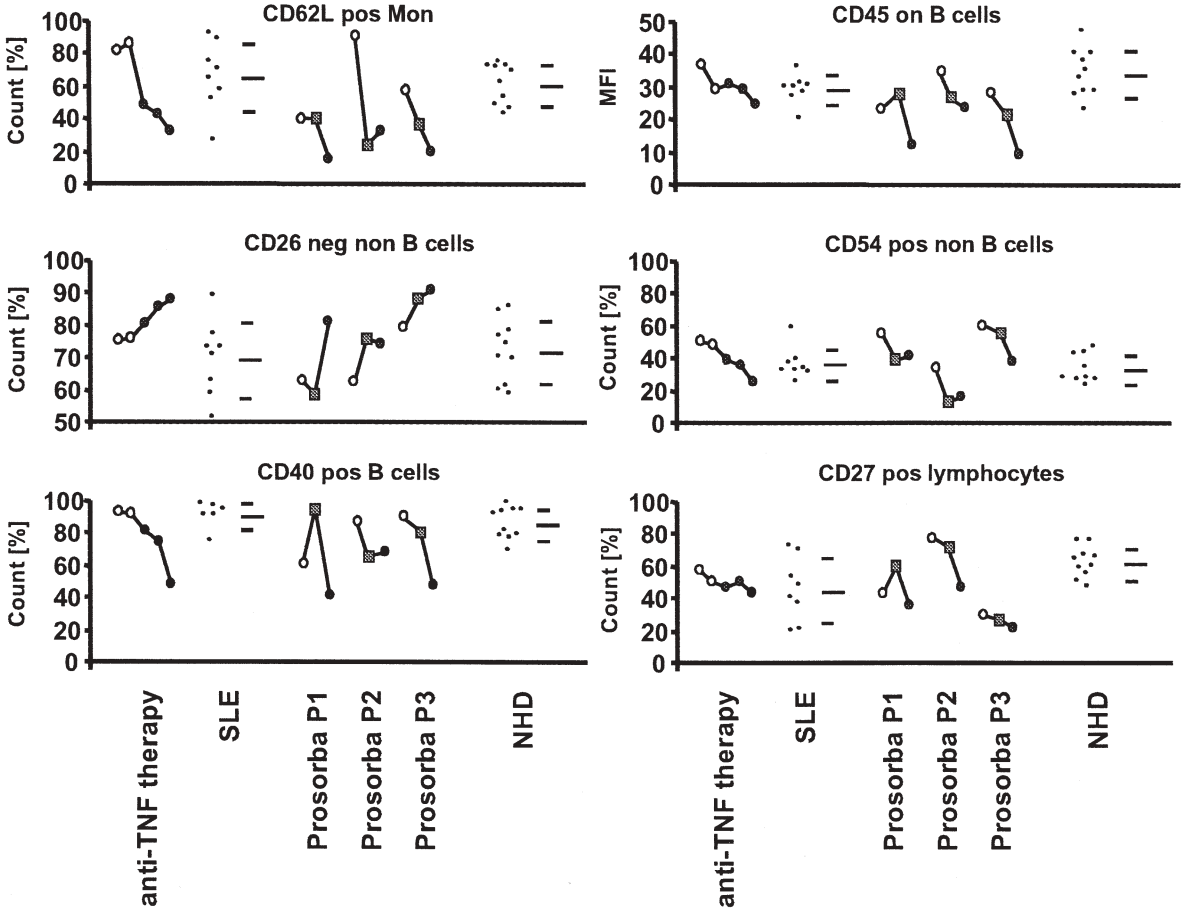

Fig. 4. Similar alterations of cellular parameters in patients with rheumatoid arthritis (RA) treated with low-throughput SPA apheresis or anti-tumor necrosis factor (*TNF*) α (responder). A comparison of RA patients treated with low-throughput SPA apheresis or *anti-TNFα* therapy with cohorts of systemic lupus erythematosus (*SLE*) patients and normal healthy donors (*NHD*) are shown. The reading points for the anti-TNFα patient are: 2 days before, 10 min before, 5 days after, 14 days after, and 28 days after treatment. The data indicate activation of monocytes (reduced *CD62L* expression) and mitigation of the lymphocytes. *Open circles*, before first immune adsorption; *squares*, after first immune adsorption; *closed circles*, after four cycles (=last immune adsorption). *Dots* indicate the values for SLE patients and NHD, respectively. *MFI*, mean fluorescence intensity; *Mon*, monocytes; *neg*, negative; *pos*, positive; *P*, patient

Edwards and colleagues have suggested a mechanistic course for the induction and maintenance of RF in RA. RF specificity was proposed to be generated from certain (e.g., pathogen reactive) Ab by somatic mutation in the germinal centers. If these randomly engendered autoAb fulfill certain requirements (e.g., IgG1 subclass), they are supposed to be able to self-perpetuate by delivering both Ag and survival signals to the autoreactive B cells that have produced them (reviewed in Edwards et al.[52]). Sato and colleagues have shown that IgM RF IgG3 interaction in synovial fluid initiates more complement-dependent inflammation than IgM RF reacting with other IgG subclasses.[67] In an RA model in monkeys, Kraan and colleagues observed a migration of macrophages into the synovial tissue before the occurrence of arthritis.[68]

The synovial tissue of clinically uninvolved joints of RA patients was characterized by infiltration with macrophages and by the expression of macrophage-derived cytokines.[68] IgG-RF IC have been suggested to play a major pathogenic role in the synovial tissue in RA.[69] Asymptomatic synovitis is the overture of RA, orchestrated by B cells, T cells, and macrophages. A failure of PMN to remove IC from the circulation allows their deposition in tissues, provoking chronic inflammation and release of autoAb. The vicious circle of RF production and of RF-induced survival of the autoreactive B cells is then responsible for the chronicity of inflammation and the generation and maintenance of RA.

Complement may play an ambivalent part in IC-mediated diseases by either suppressing or exacerbating the disorders.[70] Activation of the classical pathway may prevent the formation and tissue deposition of large IC at the time of the Ag–Ab reaction.[71,72] If IC deposition is not prevented, activation of the alternative pathway may lead to solubilization of previously deposited IC facilitated by the classical pathway and dependent on the composition of the IC.[73–75] Prevention of IC aggregation and precipitation results in opsonization and elimination of the IC without inflammation, whereas solubilization of aggregates can result in inflammation.[76] One to three hours after Prosorba treatment peak levels of C3a, C4a, and C5a were to be observed,

indicating the engagement of the classical pathway of complement activation. An in situ activation within the column appears highly unlikely, since there is a high concentration of complement- inhibiting citrate (a Ca^{2+} chelator) in the extracorporeal circuit. Therefore, the complement activation is presumably induced when modified IC re-enter the patient's circulation with the citrate being immediately metabolized. This suggests that the molecular composition of the IC has changed to a more complement-activating type during the passage through the column. This is most likely brought about by an increased size or state of aggregation. The IC now meet the requirement for efficient clearance by the phagocytic system of the liver and the spleen. The column-catalyzed conversion of the complexes might expand the capacity of the column: more IC may be cleared in a physiological way than the adsorber can bind at a time.[77]

Conclusion

A model for the accidental induction of RF and their chronic maintenance in a self-perpetuating system of autoreactive B cells and soluble IgG RF has recently been reviewed.[52] For the mode of action of low-throughput SPA apheresis we propose an adsorber-catalyzed conversion of small, tissue-penetrating, scarcely detectable, non-complement-binding, proinflammatory IgG-RF-based IC into the more readily phagocytosed species of IC: intermediate-sized, non-tissue-penetrating IC that are opsonized with complement. These IC are rather short-lived and are quickly cleared by the body's scavenging system. This hypothesis combines the following observations:

1. The Prosorba is a low-throughput adsorber with very limited adsorption capacity of circulating autoAb and/or CIC.
2. Following Prosorba treatment, circulating C3a, C4a, and C5a witnessing the activation of the classical complement pathway could be detected in the patients' plasma.
3. A short-term increase in cryoglobulins was to be observed in several patients immediately after the treatment.
4. The long-term clinical improvement was frequently preceded by a short interval of increased disease activity.
5. Besides changes of humoral diagnostic parameters, cellular changes could be observed in the treated patients, which are rather similar to those that have been observed in a patient successfully treated with Ab against TNFα (Figs. 3 and 4).
6. The cost-effectiveness of Prosorba column therapy is dependent on the patient population in which it is used. It is a therapeutic option for treatment-refractory patients. Indeed, Prosorba can be an alternative to other expensive new agents such as etanercept.[78]

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