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The use of protein A columns in the treatment of cancer and allied diseases

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Summary. The staphylococcal cell-wall protein known as protein A has been explored as a therapeutic modality in the treatment of cancer and allied diseases. Protein A binds the Fc fragment of IgG 1, 2 and 4, and preferentially binds to IgG incorporated into immune complexes. Early investigators focused on the immune-suppressive effects of immune complexes in cancer and, based on in vitro experiments, postulated that clearance of immune complexes in vivo would permit effective immune clearance of cancer cells. A large clinical trial of the perfusion of cancer patient plasma over protein A was subsequently undertaken. Results were generally disappointing, with no complete remissions and overall response rates of 22%. Response rates for Kaposi's sarcoma (39%) and breast adenocarcinoma (26%) were somewhat encouraging, and further clinical trials in these disorders are ongoing. More impressive have been the responses to protein A perfusion in immune thrombocytopenia and hemolytic-uremic syndrome. Using a protein A-silica device, Snyder et al. reported responses in 42% of immune thrombocytopenia patients, with mean increases in platelet count from 27×10^9/l to 120×10^9/l. On the basis of these results, the protein A-silica column was approved by the United States Food and Drug Administration for treatment of immune thrombocytopenia. Equally encouraging are reports of an overall 59% response rate in cancer chemotherapy-related hemolytic-uremic syndrome. Reported toxicities include fever, chills, hypotension, dyspnea and musculoskeletal pain. With rare exceptions, these reactions are easily treated and do not result in cessation of therapy. Unfortunately, the mechanism of action of plasma perfusion over protein A is very unclear. The best available evidence would point to an immunomodulatory role, manifested by stimulation of an anti-idiotype response in immune thrombocytopenia. A better understanding of how protein A immunoadsorption alters the immune response will be necessary to permit optimum use of this therapy.

Key words: Protein A – Immunoadsorption – Immunomodulation

Introduction

Immunomodulation as a primary or adjunct therapy in the treatment of cancer and allied diseases has been an active area of research for nearly 30 years. During that period, the complex interactions of the immune response, normal and abnormal, have been increasingly elucidated; unfortunately, the translation of that basic knowledge into useful therapeutic agents has lagged behind. In this review, I will discuss the use of an innovative and poorly understood technique – plasma perfusion over protein A – as an immunomodulatory agent in these diseases.

Background on the use of protein A

The staphylococcal cell-wall protein called protein A has been well described, and is recognized most widely for its utility as a laboratory reagent. It binds the Fc portion of IgG 1, 2, and 4, with diminished binding activity for IgG 3, IgA, and IgM, via Ig C_H2 and C_H3 binding sites on the N-terminus portion of the protein A [2, 4]. Perhaps more importantly, protein A binds preferentially to IgG present in immune complexes [7]. In an experimental system [12], cat plasma containing an antigen (an envelope glycoprotein, gp70, from feline leukemia virus), IgG, and immune complexes (gp70/anti-gp70) was perfused over a finite amount of protein A in a continuous fashion for several hours (Fig. 1). The concentration of IgG was in the milligram per milliliter range, while that of the immune complexes was in the nanogram per milliliter range. As shown in Fig. 1, immune complexes displaced IgG over time, and bound preferentially to the protein A, despite the marked differences in the concentrations of the two forms of IgG.

Investigators in the 1960s and 1970s demonstrated the immune-suppressive effect of immune complexes in vitro, and tumor antigens were discovered in the circulation of patients with a wide variety of tumors [6, 11]. The serum of cancer patients was often shown to contain immune inhibitors, which prevented the killing of autologous tu-

Table 1. Binding of IgG and immune complexes to protein A-silica (adapted from [8])

No. of perfusions	Protein A per column (mg)	Volume of plasma perfused (ml)	CIC as % of IgG (mean)		CIC concentration factor (mean)
			Plasma	Column eluates	
85	200	250	0.20	12.8	63×
34	200	2000	0.25	17.5	70×

CIC, Circulating immune complexes

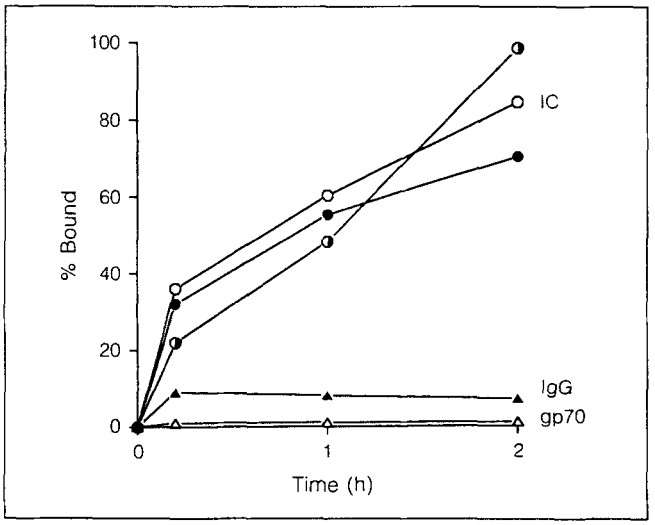

Fig. 1. Binding of perfused antigen, (gp to, △) IgG (▲), and antigen-IgG complexes (*IC*) to protein A. Differing molar ratios of gp70/IgG were utilized (◐ [Ag/Ab equivalence] > ● > ○)

mor cells by a patient's cytotoxic lymphocytes. After it was demonstrated that this in vitro inhibitory property of serum could be removed by adsorption with protein A, animal and human trials were reported [5, 17].

The largest trial of protein A immunoadsorption therapy has been performed using the Prosorba column (Imré, Seattle, Wash.), a protein A-silica device consisting of either 50 mg or 200 mg purified staphylococcal protein A bound to 125 g silica. The Prosorba column has a maximum IgG-binding capacity of about 1 g/200 mg column, and has been shown to retain the ability to preferentially bind immune complexes (Table 1). Immune complexes were concentrated up to 70 times in these studies of human cancer patients [9].

Protein A immunoadsorption in human cancer

A trial using Prosorba in cancer patients was first reported in 1988 [8], and an update has now been published [9]. One hundred and fifty-five patients with a variety of neoplasms were entered into a multicenter trial, and evaluable results are available from 101 patients. All patients had histologically demonstrable cancer which had failed to respond to conventional therapies. Treatment protocols were randomized to plasma perfusion over the 50-mg or 200-mg column, with a perfusion volume per treatment of either 250 ml or 2000 ml plasma. Patients received 12 treatments, usually over 4 weeks.

Overall response rates were disappointing, with partial responses (defined as >25% decrease in tumor size) observed in 22 of 101 patients. No complete responses were observed. Different histological types had differing response rates, with the highest responses in human immunodeficiency virus (HIV) related Kaposi's sarcoma (39%) and breast adenocarcinoma (26%).

The 50-mg columns removed a mean of 381 mg IgG and 40 mg immune complexes/treatment, while the 200-mg columns removed a mean of 958 mg IgG and 76 mg immune complexes/treatment. Pre- and post-therapy IgG and immune complex levels were not changed. Total complement levels did not change either, although treatments were associated with a 20-fold increase in C3a and C5a, with a peak at 2–3 h and a return to baseline by 12 h.

Despite the overall poor clinical results, evidence of treatment-induced immunomodulation was observed in selected patients [13]. Extensive B and T cell infiltration and IgG deposition were noted histologically, and blood CD4+ lymphocytes and natural killer activity were transiently increased in responding patients. Most interesting was the change in the immune response to a tumor marker, Le^x, in selected adenocarcinoma patients. This antigen was also detected in column eluates after treatment. In normal tissues, Le^x is a monofucosylated glycolipid antigen with a sparse tissue distribution. It is variably expressed in breast, liver, and colon adenocarcinomas, with an altered bi- and trifucosylated structure. In six patients with responses to Prosorba treatments, a shrinkage in the Le^x-bearing tumor burden was associated with an increase in plasma levels of anti-Le^x and of immune complexes containing Le^x/anti-Le^x. These very interesting findings provide insight into the mechanism of immunomodulation after protein A immunoadsorption, but before discussing this further, the side effects of the treatment will be discussed.

Toxicity

An update on the toxicity of Prosorba immunoadsorption in cancer patients has been recently published [15]. One hundred and forty-two cancer patients received a total of 1306 treatments. Side effects were reported at least once by 112 of 142 patients (79%) and during or after 786 of 1306 treatments (60%). Fever, chills, and musculoskeletal discomfort were most common, while

Table 2. Protein A immunoadsorption at New York Medical College 1988–1989 observed side effects

Side effect	No. of patients	No. of episodes
Fever/chill	8	12
Hypotension	6	8
Thrombosis	4	4
Musculokeletal pain	2	15
Chest pain	1	1
Total	14[a]	40
Percentage of total	14/32 (44%)	40/196 (20%)

[a] Some patients had more than one side effect

nausea, rash, and respiratory complaints (usually dyspnea) were also not infrequent. Hypotension, respiratory compromise, and severe musculoskeletal or constitutional complaints occasionally necessitated interruption or cessation of the treatment course. No deaths or chronic morbidities were reported.

Anecdotal but unpublished reports of more serious complications have appeared since the original publication of the relative safety of Prosorba immunoadsorption, prompting us to review our personal experience (Table 2) [18]. Thirty-two consecutive patients treated over 2 years with 196 immunoadsorption procedures were studied. Side effects were noted in 14 of 32 patients (44%) and during or after a total of 40 of 196 procedures (20%). Interestingly, 4 patients developed serious pathological thrombosis associated with treatment, although all 4 patients had factors predisposing them to thrombosis (thrombocytosis, vasculitis, metastatic carcinoma). It is of interest that we reported a possible bleeding tendency due to a diminution in platelet function following Prosorba therapy in patients participating in the early cancer trials [1]. To our knowledge, no other published reports have documented either bleeding or thrombotic risk following immunoadsorption.

Overall then, minor side effects are frequent, but they do not necessitate interruption of the treatment course. Antipyretics are usually the only treatment required, although meperidine has been successfully utilized for severe rigor and musculoskeletal pain. Reactions occurred equally as often during and after (1–4 h) treatment, and may occur as a single episode in the middle of a treatment course or consistently in a given patient. Tailoring antipyretic or other drug therapy according to the patient's pattern of side effects can then be performed. Serious side effects are very unusual, and therapy is usually carried out on an outpatient basis.

Protein A immunoadsorption in allied diseases

The clearest indication to date for the use of protein A immunoadsorption is immune thrombocytopenia, including classic idiopathic thrombocytopenic purpura (ITP), HIV-related ITP, and hemolytic-uremic syndrome

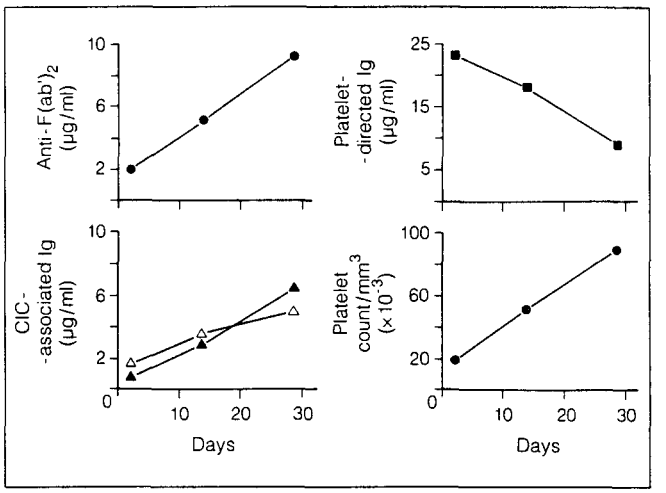

Fig. 2. Laboratory changes during Prosorba therapy in idiopathic thrombocytopenic purpura (adapted from [16]). ▲, Anti-F(ab')$_2$ Ig; △, platelet-derived Ig

[3]. The Prosorba column has been approved for use in immune thrombocytopenia by the United States Food and Drug Administration (the only protein A column so approved) [3].

Snyder et al. [14] reported 143 patients with chronic ITP refractory to multiple therapies treated with Prosorba as part of a multi-institutional study. Patients received an average of six treatments over 2–3 weeks. Responses were defined as a 100% increase over baseline for initial platelet counts below 50×10^9/l and a 50% or greater increase if the initial count was above 50×10^9/l. Responses were seen in 60 of 143 patients (42%) with an increase in mean platelet count from 27×10^9/l to 120×10^9/l. The mean response exceeded 5 months, and responses were seen equally as frequently in patients presenting with platelet counts above as below 20×10^9/l.

Mittelman et al. [10] utilized protein A immunoadsorption in HIV-related ITP. Responses were seen in 26 of 29 patients, with increases in 16 of 29 patients to a mean of 140×10^9/l, a median 300% of pretreatment values. Toxicities reported for both classical and HIV-related ITP were similar to cancer patients.

Interestingly, clear evidence of immunomodulation was observed in responding patients (Fig. 2). Rises in the platelet count were associated with a decrease in platelet-directed IgG (antiplatelet antibodies). Evidence for stimulation of an anti-idiotype response to the platelet-directed IgG was noted, as anti-F(ab')$_2$ antibodies rose, both free and incorporated into immune complexes. These immune complexes also contained increasing amounts of platelet-directed IgG, suggesting that Prosorba column treatment stimulated a clinically significant down-regulation of autoantibody production in these patients.

Recently, protein A immunoadsorption has been shown to be useful in the treatment of the hemolytic-uremic syndrome associated with chemotherapeutic agents [10, 16]. Thirty-seven patients with microangiopathic hemolytic anemia, thrombocytopenia, and renal dysfunction following chemotherapy were treated with

plasma perfusion over the 200-mg Prosorba column. Seventy percent of patients received 6 or fewer treatments (1–3 times/week), with most patients receiving a maximum of 12 treatments. Overall, 22 of 37 patients (59%) responded to therapy, with patients without residual tumor responding more frequently than those with residual tumor (46% versus 91%, $P=0.03$, chi-squared test). These results compared favorably with historical, published reports of no or other therapies, including standard plasma exchange, antiplatelet drugs, corticosteroids, and cytotoxic drugs.

In summary, protein A immunoadsorption has been established as a first or second line therapy in immune thrombocytopenias. Interest in the use of protein A columns in other autoimmune diseases is also growing. Although response rates in cancer have been disappointing, an understanding of the immunomodulation induced by protein A immunoadsorption therapy may lead to better tailoring of this therapy to selected patient populations.

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