COMBINATION OF PROSORBA® IMMUNOADSORPTION FOLLOWED BY B-CELL DEPLETION WITH RITUXIMAB IN THE TREATMENT OF RHEUMATOID ARTHRITIS
AN INDEPENDENT OPEN CLINCIAL TRIAL

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I. INTRODUCTION:

Rheumatoid Arthritis (RA) is a chronic, systemic inflammatory disease of unknown etiology affecting approximately 1% of the world's population. It is associated with long-term morbidity and early mortality despite treatment with anti-rheumatic medications. There has been a general agreement that the pathogenesis of RA includes a significant contribution by T-cells[1, 2], though over the past several years, efforts directed at targeting T-cells in this disease have been disappointing[3-6]. Macrophages have also been considered to play a prominent role in RA disease pathogenesis, and treatments designed to inhibit proinflammatory cytokines predominately produced by these cells, have achieved greater success[7-10]. However, 20-40% of patients do not respond to these biologic response modifiers. In a large clinical practice, a considerable number of RA patient's disease activity remains poorly controlled[11].

II. BACKGROUND:

A. B-Cell Depletion Therapy for Rheumatoid Arthritis:

The B-cell's role in the pathogenesis of RA has not been so well established, though abnormalities in the humoral immune response were some of the first observations described in RA[12]. In fact, the production of the autoantibody, rheumatoid factor (RF), is the most constant immunologic perturbation found in RA patients [13]. Also, in RA, the subsynovial lining area is typically heavily infiltrated with lymphocytes, including B-cells, plasma cells, and dendritic cells, and often contain germinal center-like structures[14-16]. The RA joint produces and contains huge quantities of immunoglobulin (Ig) as immunoaggregates[17-20], most of which consist of RF-Ig or RF-RF complexes[21-23]. These aggregates were once the focus of interest in mainstream theories concerning the pathogenesis of RA[21, 24-27].

Resurgent interest in the importance of the B-cell in RA pathogenesis has been driven by new information concerning the genetics of RF[28-31], and the mechanism of survival of autonomous RF-secreting B-cells[32]. This joint centric, B-cell centric, theory has been well summarized by Edwards[33]. In brief, pathologic, self-sustaining autoantibodies are felt to develop spontaneously[28-31]. These deleterious events are made possible because the process of affinity maturation cannot be purposefully directed by the B-cell[28-30]. Rather, somatic mutations occur randomly, and, under normal circumstances, mutations that lead to antibodies with reduced affinity for the non-self antigen, or antibodies that then react with self-antigen will lead to apoptosis of the responsible B-cells. There is both a T-cell and a B-cell effector mechanism for accomplishing this role.

However, in RA, an unlucky chance event transpires and a B-cell begins producing an autoantibody directed with high affinity to the Fc portion of IgG, i.e. a RF. This B-cell is able to fool T-cells into providing the necessary T-cell help. The B-cell first binds to its antigen, the IgG molecule, which in turn is complexed to a non-self antigen. The non-self antigen is internalized, processed, and presented by the B-cell with MHC class II proteins to the T-cell via the T-cell receptor, in the presence of the appropriate accessory molecules, resulting in activation of the T-cell[34, 35]. The end result of this antigen presentation is production by the T-cell of the necessary helper factors. The B-cell lives on.

The IgG RF which is produced forms self aggregates[13, 22, 23, 36-40] and aggregates with IgM of all sizes[13, 21, 41, 42]. Many of these aggregates are immunostimulatory and promote macrophages to produce proinflammatory cytokines after interaction with cell surface FcγRIIIa receptors[43].

Likewise, these aggregates are ideal for further contributing to the autonomy of the autoreactive B-cell by interacting with the B-cell receptor, the membrane bound IgG, and simultaneously contacting CR2 receptors via C3d fragments.[33]. The intracellular signals that result promote survival of the B-cell, and the vicious circle is closed. Autoimmunity is perpetuated indefinitely.

These hypotheses lead to the prediction that by sufficiently depleting autoreactive B-cells, this self-generating cycle could be broken and long-term remission could be achieved in RA. Therefore, treatment of RA utilizing a specific B-cell depleting agent, Rituximab, has been undertaken[44, 45], and was found to be both efficacious and safe. Rituximab[46] is a monoclonal murine/human chimeric antibody directed against the B-cell antigen, CD20 approved for the treatment of B-cell lymphoma. Serious infusion reactions occur when Rituximab is used to treat lymphoma, in part due to tumor cell lysis. The absence of such serious infusion reactions in RA to date is most likely due to the far fewer cells lysed in this patient population.

B. PROSORBA® Immunoadsorption and the Treatment of Rheumatoid Arthritis:

The PROSORBA® column is a 3 by 6 inch polycarbonate cylinder, which contains about 125 grams of inert silica matrix to which 200 mg of Staph Protein A (SPA) is covalently bound. SPA binds certain human immunoglobulins and circulating immune complexes[47-50]. It is a single use device. The treatments employ the use of a cell separator to separate plasma from blood cells, after which the plasma is perfused through the column, reunited with the blood cells, and then given back to the patient. Treatments are typically given weekly for twelve weeks at a recommended flow rate of 20 mL/min for a total of 1250 mL plasma treated. At any given time, the total extracorporeal volume is about 400 ml. Immunoadsorption is thus distinct from plasmapheresis where the patient's plasma is discarded. The duration of treatment is about 2 hours. The FDA approved the PROSORBA® column in 1987 for the treatment of idiopathic thrombocytopenic purpura (ITP) (US approval application PMA No. P850020).

In the early 1990s we treated eleven severely refractory RA patients with immunoadsorption with PROSORBA® columns[51] in an uncontrolled trial. Patients were treated twice a week for three weeks, and then once a week for an additional 9 weeks, for a total of fifteen treatments. Each treatment consisted of 2000 mls perfused through the column. Results were promising, and there were two complete remissions. Several years after our trial, a second pilot trial, this time a multi-center trial, was performed using a different treatment regimen[52]. To make treatments more convenient for all involved, the frequency of treatment with the PROSORBA columns was reduced to once weekly for 12 weeks, and the amount of plasma treated was reduced to 1250 mls. A marked response rate was found in this open label trial with 9 of 15 patients demonstrating a > 50% Paulus criteria response 4 months after starting treatments.

These two successful pilot trials lead to the ambitious undertaking of a prospective, multi-center, randomized, double-blind, sham-controlled trial of the PROSORBA® column in the treatment of RA, now know as the PROSORBA® Pivotal Study. The results of this trial were reported in 1999[53] and confirmed

both the efficacy and safety of PROSORBA® immunoadsorption in the treatment of RA. The PMA approval was expanded on March 15 1999 “for use in the therapeutic reduction of the signs and symptoms of moderate to severe rheumatoid arthritis (RA) in adult patients with long standing disease who have failed or are intolerant to disease-modifying anti-rheumatic drugs (DMARDs).” The Canadian Therapeutic Products Program (PTT) approved the device for both ITP and RA in February of 2000.

III. MODE OF ACTION OF PROSORBA® IMMUNOADSORPTION IN THE TREATMENT OF RHEUMATOID ARTHRITIS:

The mode of action of PROSORBA® immunoadsorption in the treatment of RA remains speculative. This technology was originally developed as a method to remove “blocking factors” in the treatment of cancer [54, 55]. But few studies show reduction in CIC with treatments, and in some cases CIC have been shown to increase[56]. Recent work demonstrates that the column removes only about 500 mg to 700 mg of immunoglobulin[57]. Since the plasma volume contains greater than 30 grams of immunoglobulin, bulk removal of material is not likely to explain the mode of action of the column. In addition, it has been shown that SPA has distinct sites that bind to the heavy chain variable region of immunoglobulin encoded by genes of the V_H 3 family[58, 59]. SPA can therefore bind up to half of human IgM. Also, several studies have shown that complement breakdown products C3a and C5a are present at high levels in the plasma [57, 60, 61] during treatments and presumably reenter the patients circulation in high concentrations. Also, small quantities of SPA, 100 ug to 200 ug, are released from the column and enter the circulation and have been raised as a possible mechanism of action for the column as well as a the cause of side effects[62, 63], although these amounts seem trivial. Lastly there are a number of idiotype anti idiotype systems that have been described associated with the column and treatments responses which are alluring explanations, but difficult to pin down[64-69].

Recently, data was presented demonstrating that massive amounts of Type III cryoglobulins entered the circulation either during, and/or after column treatments in all three consecutively tested patients with RA during a majority of treatments[70]. The most likely source of this material is the RA joint. Cryoglobulins are abundant in the synovial fluid[71, 72], and the above mentioned immunoglobulin aggregates represent polyclonal IgM and IgG Rfs, the components of Type III cryoglobulins[73].

A unifying theory for the mode of action of PROSORBA® immunoadsorption in the treatment of RA, which would explain the majority of clinical observations, is that the column and apheresis tubing emits large quantities of complement fragments which reenter the circulation. There are several factors favoring ingress of these fragments into the rheumatoid joint, such as their small size[74-76], and the excessive permeability of the rheumatoid joint vasculature[77, 78]. Once in the joint, these fragments can readily solubilize the aggregates[79-81],

and there are several factors which would aid in the aggregates egress via the synovial lymphatics[82-84]. Given this mode of action, it would suggest that treatments with higher volumes, and perhaps more frequent treatments, as used prior to the pivotal trial, would be more efficacious.

One interesting characteristic of RA patients who respond to PROSORBA® immunoadsorption is the long duration of action. In view of the new thoughts about the mechanism of B-cell depletion in RA, as described above, it would be predicted that removal of joint aggregates would have a long-lasting down-regulating affect on the proinflammatory milieu in the rheumatoid joint. The long-term responses and remissions that occur in some patients treated with PROSORBA® columns suggest that such an approach can, at least temporary, recapture deranged immunologic control mechanisms.

IV. THE RATIONALE FOR COMBINING PROSORBA® IMMUNOADSORPTION AND B-CELL DEPLETION IN THE TREATMENT OF RHEUMATOID ARTHRITIS:

In this light, it is of interest that the B-cell depletion studies were contrived to eliminate the source of these autoantibodies, in a stated effort to reset the immune system. The results to date, though impressive and preliminary, suggest that complete and permanent remissions are not achieved. The authors felt that the failure of B-cell depletion to induce cures in these patients is because of inadequate B-cell killing obtained with the current methods.

These results therefore suggest the possibility that the retention of immune aggregates within the joint during and following treatment would allow even a small number of surviving autonomous B-cells to subsequently flourish, reestablishing the disease state. Also, there is a definite possibility that the aggregates, present in such quantities, could sterically, or by molecular interaction, restrict or even completely block access of the drug, which is a chimeric IgG monoclonal antibody, to its target CD20 antigen on the B-cell surface.

The side effect profiles of these two treatments are complimentary, allowing for the use of PROSORBA® immunoadsorption prior to infusions with Rituximab. Pretreating with PROSORBA® columns could greatly potentiate Rituximab's effect, possibly in a synergistic fashion, by removing these aggregates and reestablishing access of Rituximab to its target.

V. PATIENT ELIGIBILITY:

A. Inclusion Criteria

1. Patients who will be asked to participate in this treatment protocol will be chosen from my clinic.

2. They will have refractory RA as defined as having failed >=4 DMARDs. The majority of the patients selected will have failed all standard DMARDs available to them.

3. They will have active RA defined as >10 painful and >10 swollen joints

4. Patients must be diagnosed as having definite or classic rheumatoid arthritis, according to American Rheumatism Association (ARA) criteria (13), of more than 12 months duration with onset after age 16.

5. Patients entering into this protocol may be male or female 18 years or older.

6. Patients must have adequate peripheral venous access.

7. Patients must have the ability to give informed consent.

B. Exclusion Criteria

1. Patients having any medical condition which makes extracorporeal immunoadsorption with a protein A column medically contraindicated (i.e., intolerance of therapeutic apheresis, demonstration of a prior hypersensitivity associated with therapeutic apheresis, inability to obtain adequate anticoagulation, pre-existing abnormalities of the coagulation system in which activation of the coagulation system may cause a thrombotic event, concurrent use of angiotensin converting enzyme [ACE] inhibitor medications).

2. Patients with myocardial infarction within the past 6 months, active cardiac disease (American Heart Association Class 3 or 4), congestive heart failure, or prosthetic heart valve disease.

3. Patients with life-threatening pulmonary dysfunction (as defined by a value of less than 40% of the ratio of the forced expiratory volume in the first second of expiration [FEV1] divided by the forced vital capacity [FVC]) or chronic obstructive pulmonary disease with carbon dioxide partial pressure > 50 mm Hg.

4. Patients with evidence of active liver disease (levels of SGOT [AST] and alkaline phosphates >2x the upper limit of the normal range for the laboratory performing the test).

5. Patients with renal impairment (creatinine > 130% of the upper limit of the normal range for the laboratory performing the test).

6. Patients with hematocrit <27.

7. Patients with systemic infection.

8. Patients having recent major surgery (within 6 weeks).

9. Patients undergoing concomitant anticoagulant therapy.

10. Patients with active peptic ulcer or inflammatory bowel disease.

11. Patients with known sensitivity to Staphylococcal products.

12. History of severe allergic or anaphylactic reactions to humanized or murine monoclonal antibodies

13. Known active bacterial, viral, fungal, mycobacterial, or atypical mycobacterial disease

14. History of significant recurrent infections.

15. Lymphopenia, defined as total lymphocyte count of < 1,000 for more than three months duration immediately prior to entry.

16. Intolerance or contraindication to corticosteroids.

17. Serum creatinine >1.6 mg/dl

18. Platelet count < 100,000

19. Positive Hepatitis B or C

VI. GOAL OF TREATMENT PROTOCOL:

This is an open label independent trial, which will be conducted under the guidelines of good clinical practice, to assess safety and efficacy of a novel combination therapy in refractory RA in patients with few other therapeutic options available to them.

VII. PLAN OF TREATMENT:

Patients who are interested in considering participating in this treatment protocol will undergo an extensive consent process including standard written consent form review with the clinic's staff and independent investigator, as well as an additional viewing session with the clinic staff of a DVD which will thoroughly delineate the benefit risk issues of these complicated therapies. The DVD will present this information in a narrated 3D format, aiding the patient in the concepts necessary to make an informed decision.

A. Immunoadsorption with PROSORBA® Columns:

As discussed in section III, larger volume and more frequent immunoadsorption therapy could offer greater efficacy in the treatment of RA. Twice weekly 2000 ml treatment regimens are employed during the first three weeks in the treatment of ITP[85, 86] and were utilized in the first pilot trial for RA [51]. This method was found to be safe (see safety issues section). Therefore, patients in this trial will receive PROSORBA® immunoadsorption column treatments twice a week for six weeks, which consist of 2000 mls perfused through the column each treatment. These treatments will be performed at the Kootanai Medical Center Short Stay Unit by the staff of the Inland Empire Blood Bank (Medical Director, Diane Eklund, M.D.).

The independent investigator will discuss the possibility of utilizing a central line catheter with the patient prior to entry into the study since in an occasional patient apheresis cannot be repeatedly continued through the antecubital venous access. Use of a central line is strongly discouraged, but has been used in patients who have exhausted all other treatment options, and still have severe active disease.

- Patients must be fully cognizant of adverse events associated with central line catheters;
- Patient or family member must be able to perform all necessary maintenance involved with a central line placement; and
- The independent investigator will monitor patient on an on-going basis to assess that proper care and attention is being administered while catheter is in place.

B. B-Cell Depletion with Rituximab:

Rituximab infusions will be administered at an infusion center (Coeur d'Alene Arthritis Clinic) with crash cart capability including a cardiac monitor, cardioverter, and standard cardiac resuscitation medications. Several clinic staff are certified

in advance life support, including the independent investigator and his Rheumatology partner, and a medical doctor will be present throughout the infusion period. This center has had extensive experience with the infusion of experiment monoclonal agents in the treatment of RA, and is currently treating over 70 patients with Remicade, a murine chimeric monoclonal antibody.

The first dose of Rituximab will follow the last Immunoadsorption treatment by from 2 to seven days. The shorter duration between treatment would be preferred. Patients will be premedicated with Tylenol 650 mg p.o.and Diphenhydramine 25 mg p.o. Rituximab infusions will be preceded by Solumedrol 125 mg given as an I.V. drip over 15 minutes. Rituximab 500 mg will then be given I.V. over three hours.

The second and final dose of Rituximab will be given in an identical fashion one week later.

(The dose of Rituximab chosen is at the lower end of doses used to treat RA. The lower dose is chosen because these two treatments have not been employed together in the past and there may an unpredictable interaction. Also, as discussed above, there is the possibility that immunoadsorption will potentiate the effects of Rituximab, negating the need for larger doses or more frequent administration).

VIII. CONCOMITANT THERAPIES:

All biologic response modifiers (etanercept, remicade, D2E7, Anakinra) will be discontinued prior to initiation of this treatment regimen. Decisions about other DMARDs to be taken by each patient thoughout the treatment period will be individualized for the patient. In general, it is likely that DMARDs will be continued. In my practice these DMARDs will most likely include methotrexate, leflunimide, methotrexate/lefunimide combinations, Azulfidine and hydroxychloraquine, roughly in order of frequency and with the last two agents generally in combination with other DMARDs.

An effort will be made to not increase DMARDs or daily steroid dose during the treatment period, but steroids and DMARDs will be reduced or discontinued as clinically indicated.

IX. STUDY PARAMETERS:

A. Screening and Baseline Determinations

Patients eligible for this study will undergo a pre-study evaluation prior to the first treatment, which will consist of the following:

1. A comprehensive evaluation: medical history, inclusion/exclusion criteria.

2. A physical examination, including detailed accounting of previous and current therapies.

3. Complete blood count (CBC): red blood cell count, white blood cell count, hemoglobin, and hematocrit, including platelet count and white blood cell differential counts;

4. Blood chemistries: sodium, potassium, chloride, carbon dioxide, glucose, blood urea nitrogen (BUN), creatinine, bilirubin, SGOT (AST), alkaline phosphatase , lactate dehydrogenase (LDH), total protein, and album

5. Prothrombin time (PT), activated partial thromboplastin time (APTT), and fibrinogen;

6. Total rheumatoid factor (RF) titer;

7. determination of C-reactive protein level (CRP) and erythrocyte sedimentation rate (ESR)

B. Treatment Evaluation

Rheumatoid Arthritis Disease Activity Rating Report (ACR)

The disease activity rating report used to establish effectiveness will include the following assessment parameters:

1. the number among 68 joints with tenderness on pressure or pain on passive motion (or both); the joints to be examined are: temporomandibular (n=2), sternoclavicular (n=2), acromioclavicular (n=2), shoulder (n=2), elbow (n=2), wrist (n=2), metacarpophalangeal (n=10), interphalangeal of thumb (n=2), distal interphalangeal (n=8), proximal interphalangeal (n=8), hip (n=2), knee (n=2), ankle mortise (n=2), ankle tarsus (n=2), metatarsophalangeal (n=10), interphalangeal of great toe (n=2), and proximal/distal interphalangeal of the toes (n=8)

2. the number among 66 joints with swelling: the same as those examined for tenderness in the 68-joint count, except the hip joints

3. the patient’s assessment of pain as measured with a visual analog scale;

4. the patient’s global assessment of disease activity as measured with a visual analog scale;

5. the physician’s global assessment of disease activity as measured with a visual analog scale;

6. he patient’s assessment of physical function with several validated instruments; and

7. determination of acute phase reactant (C-reactive protein level (CRP) and erythrocyte sedimentation rate (ESR))

These assessments will be obtained during the week prior to or on the day of initiating the treatment (first immunoadsorption), on the day of and prior to the first infusion of Rituximab, and 3 months, 6 months, 1 year, and 2 years following the last infusion of Rituximab.

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