IgG immunoadsorption in experimental allergic neuritis: effect on antibody levels and clinical course

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SUMMARY The effect of IgG immunoadsorption upon the course of chronic experimental allergic neuritis (EAN) is described. Miniature membrane plasma separators coupled with a Protein A (PA)-Sephadex immunoadsorbent column were used to perform upon conscious rabbits 5 IgG immunoadsorption treatments over 6 days. Quantitation of anti-myelin IgG and IgM by ELISA revealed that 55-65% of plasma IgG was removed per treatment. Rapid post-treatment antibody rebound was observed for anti-myelin IgG although no antibody overshoot above control levels could be observed. Anti-myelin IgM levels remained relatively unaffected by PA immunoadsorption. Comparisons of clinical scores between control and treatment animals showed that IgG immunoadsorption was significantly beneficial (day 1 post-treatment $p < 0.001$; day 2 post-treatment $p < 0.05$). However, rapid relapse was observed in all treatment animals such that by day 3 post-treatment no significant clinical difference between control and treatment groups could be observed. IgG immunoadsorption suppresses the clinical progression of chronic EAN in a manner similar to that seen with plasma exchange. This finding suggests that antibody modulates early disease pathogenesis.

The results from large controlled clinical trials have established that plasma exchange (PE) is an effective therapy for certain cases of Guillain-Barré syndrome (GBS).¹⁻³ However, such trials of PE do not elucidate the mechanisms of disease nor the exact means by which benefit is derived. They do, however, suggest that humoral factors play a role in at least the early disease phase of GBS and leave open the possibility of specific targeting of suspected pathogenic factors such as antibodies.

Protein A immunoadsorption is attracting increasing scrutiny as a technique applicable in a therapeutic setting in humans. The main advantage of IgG immunoadsorption using protein A compared with PE is that it is a closed loop system which removes a suspected pathogenic factor without the necessity of wasteful discard and replacement of a large proportion of a patient's plasma protein, the majority of which may be normal and fully functional.

In both acute experimental allergic neuritis (EAN)^{4,5} and chronic EAN⁶ PE has been shown to be of clinical

benefit and this further confirms the usefulness of EAN as an experimental model of human peripheral demyelinating neuropathies such as GBS and chronic inflammatory demyelinating polyradiculoneuropathy (CIDP). Because of the potential use for protein A immunoadsorption in a variety of neuromuscular disorders including GBS and CIDP involving suspected pathogenic immunoglobulins, the effects of IgG removal using protein A immunoadsorption were examined in animals with chronic EAN.

Materials and methods

Induction of EAN

Female outbred NZW rabbits (mean body weight 2.8 ± 0.2 kg) were housed in individual cages and given food and water *ad libitum*. To produce EAN the animals were inoculated with peripheral nervous system myelin prepared from fresh bovine cauda equina using a discontinuous sucrose density gradient centrifugation method similar to that of Norton and Poduslo.^{7,8} The inoculum consisted of bovine myelin, sterile saline and Freund's complete adjuvant containing 0.5 mg/ml *Mycobacterium tuberculosis* (Commonwealth Serum Laboratories, Melbourne, Australia) mixed in the ratio of 1 g:1 ml:2 ml respectively and homogenised (Polytron, Kinematica). Under halothane/oxygen anaesthesia each animal received a single subcutaneous multiportal inoculation consisting of 0.25 ml to each paw and 0.25 ml to two separate sites on the back.

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Table 1 Clinical assessment of EAN

Grade	Clinical disability
0	Normal
1	Slight postural abnormality, less lively
2	Mild but definite paraparesis
3	Moderate paraparesis, possible front leg weakness
4	Tetraparesis
5	Severe tetraparesis, still ambulatory
6	Severe tetraparesis, non-ambulatory, respiratory difficulty, possible incontinence
7	Moribund or dead

Clinical assessment and blood sampling

Animals were periodically weighed and examined for signs of EAN and a clinical disability score assigned according to the classifications in table 1. Animals were scored independently by two observers during each clinical examination.

Two ml blood samples were taken by venepuncture from peripheral ear veins into sequestrene tubes and centrifuged at 500 g for 10 minutes. Plasma samples were stored at -70°C.

Blood access

Blood access was obtained via an externalised carotid-jugular arteriovenous shunt inserted under halothane/oxygen anaesthesia as described previously.⁶ Control animals underwent a sham operation under halothane/oxygen anaesthesia in which the right carotid artery and jugular vein were isolated and ligated.

Extracorporeal circuit

The extracorporeal circuit is depicted in fig 1. The membrane plasma separators were of the hollow fibre type made of polypropylene (Enka AG, Wuppertal, FRG). The filters had an effective surface area of 200 cm² with a maximum pore size of 0.5 µm. The blood side filter priming volume was approximately 2 ml. The immunoadsorption column was constructed using PA-Sepharose CL-4B (Pharmacia, Uppsala, Sweden) swollen and packed between 30 µm

polyethylene supports in a 25 ml glass column with a length/diameter ratio of 6:1. Based upon the known IgG binding capacity sufficient PA-Sepharose was packed to enable a total binding capacity of approximately 500 mg representing at least 50% of the circulating plasma IgG in the rabbit. This column capacity for IgG has been confirmed previously.⁹ The column void volume was approximately 15 ml. Blood and plasma flow rates were regulated by a multi-channel peristaltic pump (Gilson Medical, Paris, France). Blood flow through the filter was maintained at 12 ml/min while plasma flow through the column was maintained at 2.5 ml/min enabling a plasma residence time within the gel of approximately 6 minutes. Total extracorporeal blood circuit volume was approximately 15 ml.

Immunoadsorption procedures

As animals reached a clinical grade of 2-3 they were paired and randomly allocated into control and treatment groups. Immediately after allocation the treatment animals received an externalised arteriovenous shunt and control animals underwent a sham operation.

Before each immunoadsorption procedure the extracorporeal circuit was primed with 0.15 M sterile saline. Animals were lightly restrained by wrapping in a surgical drape and each remained conscious and calm throughout the procedure. Just prior to connection to the circuit, rabbits received 1500 U heparin intravenously and this was allowed to distribute within the intravascular space. A pre-treatment blood sample was taken and the arterial extracorporeal line leading to the filter was then connected to the shunt and the blood circuit primed under arterial pressure to expel residual saline. When the blood circuit was complete the venous extracorporeal line was connected to the shunt and the pump started. The first 20 ml of saline effluent from the column were discarded after which time the post-column plasma line was reconnected to allow treated plasma to be returned to the animal. Column flow rate was measured prior to reconnection and the time necessary to process 1 plasma volume (30 ml/kg) calculated. After processing 1 plasma volume the peristaltic pump was stopped and the arterial line disconnected from the shunt. The extracorporeal circuit was then flushed with sterile saline to return the contents to the animal. Similarly, the column was flushed with 25 ml of sterile saline returning unbound column contents to the animal. The venous line was then disconnected from the animal and arterial and venous shunt cannulae were reconnected behind the ear. After a brief period (approximately 5 minutes) to allow vascular mixing, a post-treatment blood sample was taken.

The PA-Sepharose column was regenerated between immunoadsorption treatments by desorption with at least 3 column volumes of 0.1 M glycine-HCl (pH 2.8) and then equilibrated with sterile saline.

Treatment animals underwent immunoadsorption procedures five times within 6 days. Each was treated daily for the first 3 days followed by a day with no treatment and then the final two treatments were conducted on the 5th and 6th days.

Measurement of anti-myelin IgG and IgM

Anti-myelin IgG and IgM were quantitated using an ELISA assay which has been previously described in detail.⁸

Fig 1 Schematic diagram of extracorporeal immunoadsorption system.

Measurement of biochemical and haematological parameters
Biochemical analyses were conducted on an American Monitor Parallel autoanalyser and haematological analyses were conducted using a Technicon H1 blood analyser.

Results

Clinical features

Out of a total of 20 animals inoculated with myelin, 17 animals were allocated into either control or treatment groups. In these animals onset of neurological signs of EAN occurred between days 12 and 16 post-inoculation. Three animals showed an EAN disease of late onset, one on day 23 and two on day 40 post-inoculation and were not included in the study. After pairing and random allocation, eight animals were assigned to the treatment group and nine to the control group. Mean clinical score at allocation for the control group was 2.56, SD 0.53 and for the treatment group 2.57, SD 0.53. One treatment animal died between the first and second immunoadsorption procedures from catastrophic shunt uncoupling and was not included in the final study.

Clinical data are depicted in fig 2. Data are displayed as arithmetic changes in clinical scores from the scores at allocation. Control animals showed a severe progressive neuropathy with one animal becoming moribund on day 44 post inoculation and two showing severe tetraparesis with respiratory difficulty on days 47 and 55 post-inoculation. Animals becoming moribund were immediately killed. Animals attaining a score of 6, that is, severe quadriparesis and respiratory difficulty (with possible incontinence) that did not show improvement after 2 days were killed and included in the data as attaining a score of 7.

In contrast to the progressive disease seen in the control group, treatment animals showed significant

Table 2 Changes in blood components after a single immunoadsorption

Component	% Change (Mean SD, n = 5)
Na, K, Cl, Ca, PO ₄	no change —
Albumin	-13.79, 6.42 p < 0.05
Total protein	-19.08, 7.45 p < 0.01
Haematocrit	no change —
Red cell count	no change —
White cell count	-47.58, 11.11 p < 0.01

clinical remission as a result of IgG immunoadsorption. Statistical analysis of data taken directly after the course of treatments (days 7 and 8 post-allocation) using 2-tailed Mann-Whitney U tests showed significant differences between the two groups (p < 0.001; p < 0.05 respectively). Remission was transient and most animals showed a relapse by days 9 to 11 post-allocation.

Blood biochemistry and haematology

Changes in blood composition from a single immunoadsorption are shown in table 2. Percentage changes in haematological parameters were calculated using blood samples taken from five animals before and directly after the first immunoadsorption procedure. There was no change in plasma solutes, haematocrits or erythrocyte counts. The lack of effect upon haematocrits reflected minimal alterations in plasma volume. However, immunoadsorption did result in significant decreases in albumin and total protein concentrations and white cell counts. Changes in white cell counts have been previously reported in other forms of extracorporeal therapy such as haemodialysis.¹⁰ Total protein concentration was reduced by approximately 20% due mainly to IgG removal. The slight but significant decrease in albumin concentration associated with the extracorporeal procedures was due to haemodilution resulting from the return of blood in the venous line with saline and to a lesser extent nonspecific protein binding within the gel matrix.

Plasma anti-myelin IgG and IgM

Anti-myelin IgG and IgM levels were assessed in both groups and are depicted in figure 3. Antibody levels are displayed normalised to a pre-treatment level of 100%. Anti-myelin IgG levels rose steadily in the control group throughout the period of observation. In the treatment group, each IgG immunoadsorption decreased circulating anti-myelin IgG concentrations by 55–65%. Rapid post-treatment antibody rebound was observed after each procedure so that levels were re-established within 24 hours. However, the course of immunoadsorption treatments held anti-myelin IgG concentrations at pre-treatment levels in the treatment group so that at day seven post-allocation there were

Fig 2 Mean changes in clinical score after allocation for the control group (open circles, n = 9) and treatment group (closed circles, n = 7). Bar indicates period of treatment. Significant differences were determined by two-sided Mann-Whitney U-tests; p < 0.001***; p < 0.05*.

Fig 3 Plasma anti-myelin IgG and IgM concentrations (arbitrary mass units) determined from samples obtained up to 21 days post allocation. Data are normalised to a pre-treatment level of 100%. Mean, SD is displayed for the control group (open circles, $n = 9$) and treatment group (closed circles, $n = 7$). Bar indicates period of treatment.

significant differences in anti-myelin IgG concentrations between control and treatment groups (2-tailed Student's t test, $p < 0.001$). Anti-myelin IgG levels quickly increased after cessation of treatment so that two days later, that is, day nine post-allocation there was no significant difference in levels between groups.

Although rapid antibody rebound was observed, there was no evidence of antibody overshoot above control levels as a result of transient removal. The level in the treatment group was still identical to the control level when last measured at day 20 post-allocation.

Anti-myelin IgM levels are also shown in figure 3 normalised to a pre-treatment level of 100%. In the control group, levels were constant throughout the period of observation. In the treatment group, anti-myelin IgM levels appeared to decrease slowly during treatment although comparisons of pre- and post-immunoabsorption concentrations showed no differences and suggest no significant removal via specific binding to protein A. The slight decreases in anti-myelin IgM concentrations could be due to some entrainment in the PA-Sepharose column. Although

after the course of immunoabsorption procedures anti-myelin IgM levels appeared to increase above control levels, statistical analysis using 2-tailed Student's t test showed no significant differences.

Discussion

The results of this study demonstrate that a short intensive course of IgG immunoabsorption removing 55–65% of circulating IgG is sufficient to induce clinical remission during the early disease phase of EAN. The observed benefit in this study complements that previously seen in EAN with the use of membrane PE.⁶ The significant but temporary nature of remission is undoubtedly due to the severe chronic progressive type of disease of which the clinical, electrophysiological and histological features have been well documented.¹¹ In both therapeutic studies assessment of clinical disability directly after the course of treatments showed significant clinical benefit. This was followed by rapid relapse within one week so that animals receiving a single course of treatment were in the long term clinically indistinguishable from controls. The situation is therefore similar to that in CIPD in man where the need for repeated exchanges to maintain benefit is well recognised,¹² although in most cases the period between exchanges is somewhat longer.¹³

The early disease phase in EAN in these animals is characterised by weight loss and mild to moderate paraparesis. At this stage detectable levels of anti-myelin IgG are present and show gradual increases during disease progression. High levels of anti-myelin IgM can also be detected in some but not all animals.⁸ The concomitant increases in clinical disability with increases in anti-myelin IgG combined with the ability of IgG removal alone to mimic the clinical remission observed in EAN with membrane PE⁶ and centrifugation PE^{4,5} suggest a pathogenic role for IgG antibodies. Intraneural transfer experiments involving whole EAN serum have demonstrated humoral factors which cause demyelination and conduction failure¹⁴ but although these experiments may detect the presence of pathogenic humoral factors, they fail to determine their nature. Anti-myelin activity of whole rabbit EAN serum has been demonstrated in organotypic PNS cultures to be complement dependent¹⁵ suggesting antibody involvement. Rat intraneural injection studies have clearly shown advanced myelin and Schwann cell damage with rabbit EAN serum well before a macrophage presence¹⁶.

Intraneural injection of GBS serum into rat sciatic nerves also induces demyelination^{17–20} although the severity of effect is often less than with EAN serum. Recent detailed sequential morphometric studies confirm the ability of GBS serum to demyelinate peri-

pheral nerves in vivo in the absence of cells.²¹ The presence of anti-nerve antibodies in GBS serum has been described²²⁻²⁵ and correlates with response to PE.²⁴ Levels appear to correlate with disease severity and are at a peak during the early disease phase.^{26,27} These observations along with results from large controlled trials showing that therapeutic benefit in GBS is derived from PE only if used early in the disease course¹⁻³ strongly suggest a causative role for anti-nerve immunoglobulins.

This study demonstrates the ability of IgG immunoadsorption to produce clinical remission during the early disease phase of EAN. Although PE has been used extensively in the treatment of human as well as experimental diseases, therapeutic immunoadsorption is still in its infancy. Immunochemical adsorbents have long been utilised for routine in vitro separation but Terman *et al*²⁸ and Bansal *et al*²⁹ were the first to incorporate affinity chromatographic techniques and plasma separation technology to achieve ex vivo on line adsorption of specific plasma proteins.

Bansal *et al*³⁰ first described the successful treatment of colon carcinoma using a protein A-carrying immunoadsorbent perfused with patient's plasma. This study utilised the discovery of the known capacity of protein A to bind IgG.³¹ Since then, with more widespread use of extracorporeal systems, specific haemodynamic effects have been attributed to the use of extracorporeal therapy. The use of membranes for plasma separation generally induces transient decreases in white cell counts.³² This effect has been clearly measured in our study. Such decreases are thought to be the result of complement activation and leukocyte sequestration in the pulmonary vascular bed.^{10,33} The binding of IgG to protein A forms an immune complex which may activate the classical complement pathway³⁴ although this effect was not reported in more recent studies.³⁵

In our study the level of complement activation and the metabolic fate of activated cascade proteins have not been measured. McLeod *et al*³² reported a C3 conversion of 8-15% upon plasma contact with polypropylene membranes. Charlton³⁶ measured transient decreases in rabbit C3 concentrations of approximately 20% with the use of polypropylene membrane plasma separators. In this study there were no further decreases in C3 levels as a result of exposure to protein A-Sepharose suggesting minimal complement activation from the immune complexing of IgG to protein A. The extent to which complement activation contributed to or attenuated the clinical remission observed during early phase EAN is not known. It is highly improbable that transient complement activation alone of the magnitude likely in this present study could account for the marked abatement of disease progression observed in treatment animals. Such animals showed no apparent signs

of lung congestion or bronchospasms. Although such symptoms were noted by Bansal *et al*³⁰ and Terman³⁷ in patients treated with protein A-carrying staphylococcus immunoadsorbents, these were probably attributable to the use of a bacterial product in place of the purified protein A used in our study.

Of more concern is the possible clinical effect of endogenous steroid synthesis in the treated animals. In the present study, control animals underwent a sham operation but no sham immunoadsorption treatments. There exists the possibility that steroid production in treated animals may have had a positive clinical effect over untreated control animals. Studies have shown both prophylactic and therapeutic effects of steroids upon EAN³⁸⁻⁴⁰ although earlier experiments were unable to detect significant effects.⁴¹ In a previous study examining the effects of membrane PE upon chronic EAN,⁶ plasma cortisol levels were determined in all control and treated animals. The results showed no significant lasting effects of sham operations and shunt insertions upon plasma cortisol concentrations. Moreover, analysis of plasma cortisol levels before and after PE in the treated animals showed a decrease attributable to plasma removal exactly as predicted from a steady state single pool model and no significant increases in endogenous cortisol synthesis during treatment. The lack of effect of membrane PE upon plasma steroid concentration would strongly suggest a typical lack of effect in the similarly treated but selectively IgG depleted animals of the present study.

A major drawback of therapeutic PE is the need to use substitute fluids such as albumin or fresh frozen plasma with the concomitant expense and accompanying side effects. Extracorporeal immunoadsorption promises to be a viable alternative in diseases with identified circulating pathogenic material and as a valuable experimental tool for elucidating speculative roles for plasma components such as antibodies. A trial of IgG immunoadsorption in acute phase GBS seems appropriate. The results of experimental trials of PE and IgG immunoadsorption in EAN together with accumulating evidence for a role for antibodies in GBS and CIDP in initiating or augmenting the early disease process suggest that the specific targeting of possible effectual agents such as antibodies remains a rational and relevant therapeutic approach.

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