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SPECIFIC IMMUNOABSORPTION IN A RAT MODEL OF GOODPASTURE'S SYNDROME

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Summary

Extracorporeal immunoabsorption was studied in a model of anti-glomerular basement membrane (anti-GBM) disease in the Brown Norway rat, produced by a single intramuscular injection of homologous collagenase-solubilized GBM (1.0mg/kg). Procedures were performed in conscious unrestrained rats using a system in which blood was pumped into a membrane plasma separator, and the plasma then passed over an absorbent column before rejoining cellular elements on return to the animal. Single 60ml/kg perfusions were performed in groups of three animals producing anti-GBM antibody. Those treated with GBM-coated devices showed a prompt fall (33%) in antibody levels, without evidence of early antibody rebound; whereas controls (albumin-coated devices) showed no significant change.

Introduction

The introduction of plasma exchange has led to an improvement in the prognosis of Goodpasture's syndrome (anti-GBM disease) [1], and its use has now been extended to many other immunologically-mediated diseases. However, the cost of plasma substitutes and the risks inherent in their administration [2], make the development of specific immunoabsorption an important goal. We report initial studies of extracorporeal autoantibody removal in a model of anti-GBM disease in the Brown Norway rat.

Methods

Disease model Male Brown Norway rats (300g) were given a single intramuscular injection of 1mg/kg collagenase-solubilized rat GBM [3], without adjuvant. Six animals were immunized for plasma perfusion studies, and five to confirm the previously described kinetics of autoantibody synthesis [4]. Serial blood samples

were taken by tail artery puncture, and anti-GBM antibodies were measured by solid phase radioimmunoassay [3].

Plasma perfusion system Outbred Sprague-Dawley rats (300g) were used in experiments designed to evaluate the system. Vascular access was achieved by surgically implanted cannulas in the carotid artery and jugular vein, as previously described [5]. The cannulas were led in a harness device to the top of a specially designed cage, such that animals could be subjected to repeated extracorporeal circulation without anaesthesia or restraint. Blood was pumped from the arterial cannula into a membrane plasma separator (polyamide, pore size 0.45μm), and the plasma then pumped through an empty absorption column before rejoining the cellular elements on return to the animal via the jugular vein. The total extracorporeal volume was approximately 3.5ml, and anticoagulation was achieved with heparin. Further details of this system are to be described elsewhere (Ryan CJ et al, unpublished observations).

A group of five animals received single 40ml/kg (>1 plasma volume) perfusions through an empty column, and measurements were made of blood flow, plasma flow and transmembrane pressure. Total IgG levels in blood and filtered plasma were measured by radial immunodiffusion, and haematological changes assessed by standard techniques. Cannulas were left in situ for seven days and then surgically removed.

Specific immunoabsorption Groups of three Brown Norway rats, which had received an injection of rat GBM three months previously, were used. The experimental group received single 60ml/kg (2 x plasma volume) perfusions over GBM-coated absorption devices, and the control group were treated similarly using albumin-coated devices. The immunoabsorption column consisted of stacked discs of polyvinylidine fluoride based membrane ('Immobilon', Millipore, pore size 0.65μm) to which collagenase-solubilized human GBM (cross-reactive with rat anti-GBM antibody) was covalently coupled (DiLeo AJ, personal communication). Optimal conditions for the production of functional columns were predetermined by in vitro experiments. Blood samples were drawn from the arterial cannula, and anti-GBM antibody levels in blood and filtered plasma were measured by solid phase radioimmunoassay [3]. Cannulas were left in place for seven days and then surgically removed.

Results

Disease model The kinetics of anti-GBM antibody synthesis in a group of five rats are shown in Figure 1. Antibody levels reached a peak by eight weeks and were maintained for six months before falling towards background.

Plasma perfusion system Blood flow in the extracorporeal circuit could be maintained at 0.7ml/min in all five animals used, and at this rate the mean plasma flow was 0.19ml/min, giving a filtration fraction of 27 per cent. Mean transmembrane pressure was 17mmHg, and at this level there was no demonstrable

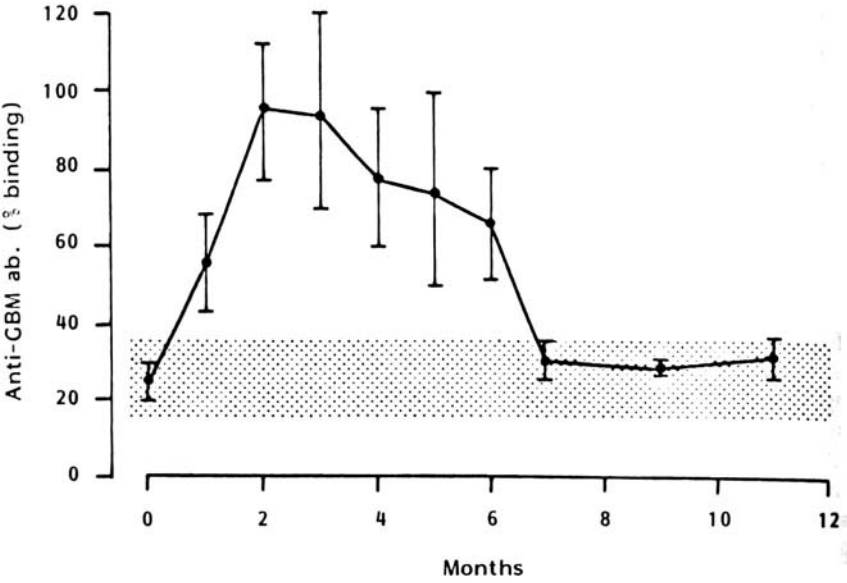

Figure 1. Serial anti-GBM antibody levels (mean ± SD) in a group of Brown Norway rats (n=5) given a single intramuscular injection of collagenase-solubilized rat GBM. The normal range is indicated by the stippled area

haemolysis. Total IgG levels in blood and filtered plasma did not change significantly during the procedure. The mean sieving coefficient for IgG was 1.0 at 15 minutes, and this was maintained at 0.98 at one hour. Haematological analysis revealed no significant change in erythrocyte, leucocyte or platelet counts during the procedure. All animals survived long term following removal of cannulas.

Specific immunoabsorption Anti-GBM antibody levels in blood and filtrate in one treated animal are shown as an example in Figure 2. The mean percentage fall in circulating antibody levels in the two groups of rats is shown in Figure 3. Antibody levels fell by a mean of 33 per cent in animals treated with the GBM-coated device, as opposed to five per cent in controls. After completion of perfusion, anti-GBM antibody levels remained below pre-treatment values for up to seven days, although there was a gradual rise from the first day. All animals survived long term following removal of cannulas.

Discussion

Evaluation of the role of plasma exchange in the management of Goodpasture's syndrome has been facilitated by easy measurement of the pathogenic autoantibody and of the target organ injury produced. The relationship between antibody levels, tissue damage, and therapeutic modalities has been retrospectively analysed [1]. Even so, there remain areas of uncertainty, for example the

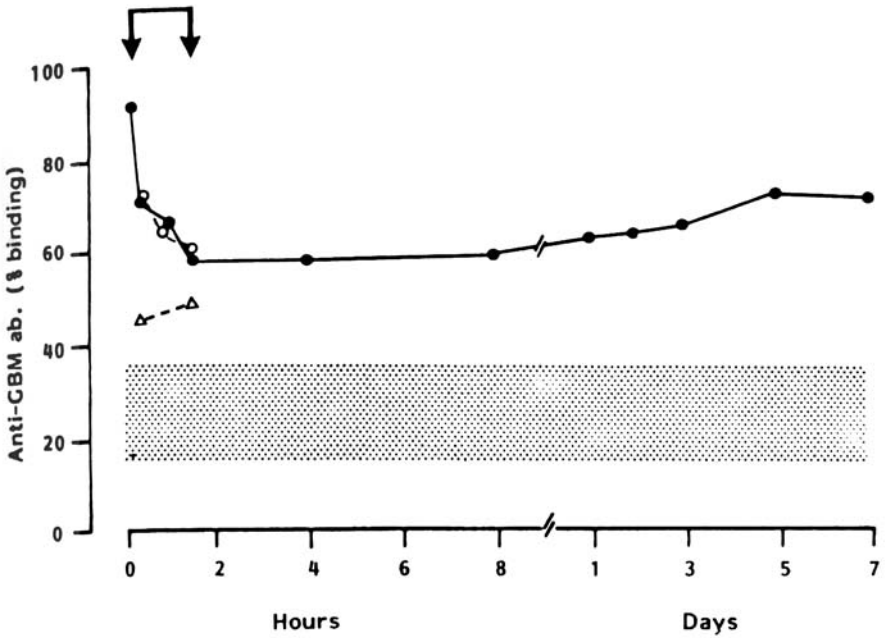

Figure 2. Anti-GBM antibody levels in blood (●–●), pre-column plasma (○- - -○) and post-column plasma (△- - - △) in a rat undergoing one 18ml plasma perfusion over a GBM-coated device. Perfusion is indicated by the arrows. The normal range is indicated by the stippled area

optimal concomitant drug regimen and the interaction between treatment and autoregulatory mechanisms. The next major development in apheresis is likely to be the use of selective or specific absorption techniques; this approach raises even more questions, the most important of which is whether the removal of a particular molecular species will have the same effects as complete replacement of a patient's plasma.

In order to investigate these problems, a new model of anti-GBM antibody synthesis was established in the Brown Norway rat [4]. This model has the advantage that it does not involve the use of immunological adjuvants or polyclonal activators, and the kinetics and specificity of the autoimmune response are therefore similar to that seen in man. Anti-GBM antibody synthesis in these rats lasts around six months and then ceases spontaneously, as usually occurs in untreated patients after one to two years. The long plateau phase allows adequate time for the investigation of therapy in an ongoing autoimmune response. Anti-GBM antibody production is unaccompanied by features of polyclonal activation, such as rise in total IgG or development of anti-DNA antibodies, and resembles the human disease more closely in this respect than do other models such as that induced by mercuric chloride in the Brown Norway rat [6].

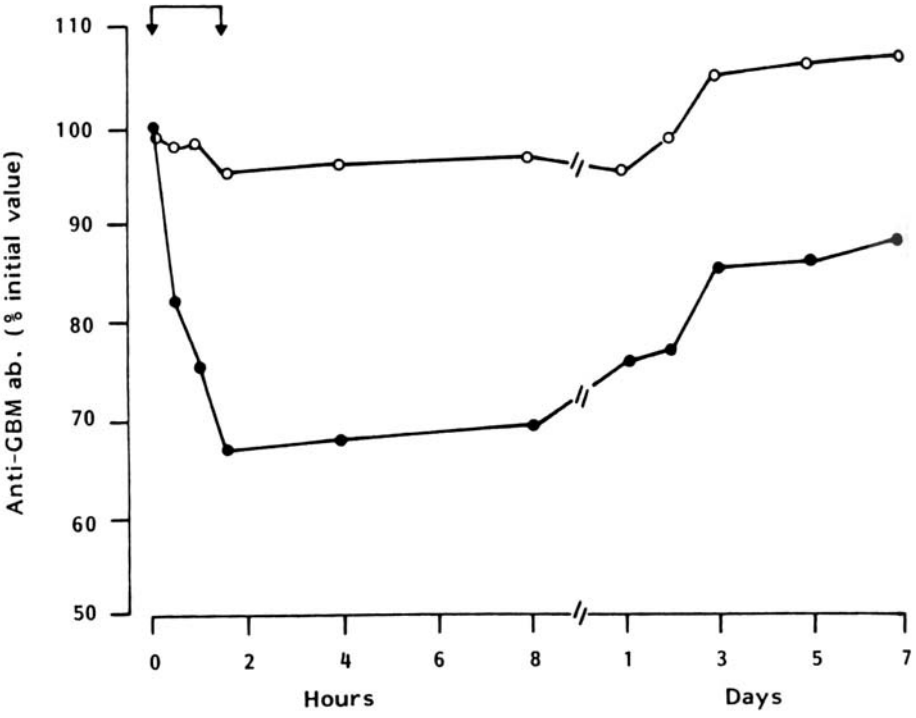

Figure 3. Circulating anti-GBM antibody levels in groups of three Brown Norway rats undergoing 18ml plasma perfusions over GBM-coated devices (●–●) or albumin-coated devices (○–○). Perfusion is indicated by the arrows. Results are expressed as a percentage of the initial antibody level in each group

The plasma perfusion circuit was designed to perform as a scale model of one that could be used clinically. An important feature of the system is that it allows repeated perfusions to be performed in the unrestrained rat (Ryan CJ et al, unpublished observations), eliminating as far as possible the effect of anaesthesia and stress. All of the operating characteristics, including blood and plasma flow rates, transmembrane pressures and sieving coefficients are compatible with clinical use and indicate that it should provide data of value for the development of specific immunoabsorption in man. A limiting factor for clinical application could be the availability of suitable antigenic material, but rapid advances in biochemical and molecular engineering techniques may soon overcome the problem.

The results of our preliminary experiments indicate that specific immunoabsorption is feasible in this model of autoimmunity. Although numbers are too small for formal analysis, all three animals treated with GBM-coated devices showed a rapid fall in anti-GBM antibody levels, and there was also a marked difference in the antibody content of pre- and post-column plasma. It was of great interest that there was no early antibody rebound or overshoot, as has been

observed in models examining the effects of removal of antibody to heterologous antigens [7,8]. Control mechanisms may be different in an autoimmune response, and when specific absorption rather than total exchange is used. Further studies using this model system on a repeated basis, and in combination with other forms of therapy, should provide a scientific basis for the introduction of specific immunoabsorption into the management of Goodpasture's syndrome and perhaps other autoimmune diseases.

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