REMOVAL OF CIRCULATING ANTIGEN AND IMMUNE COMPLEXES WITH IMMUNOREACTIVE COLLODION MEMBRANES

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1. Introduction

Considerable evidence has accumulated to substantiate the role of circulating antigens and antigen–antibody complexes in the pathogenesis of many experimental and human diseases [1]. Soluble circulating antigens may combine with antibodies to form pathogenic immune complexes which deposit in tissues causing inflammation. Circulating soluble antigens may also block cell mediated cytotoxic activity directed to neoplastic cells [2].

In previous studies, we have demonstrated the feasibility of specifically removing circulating antibodies with extracorporeal immunoadsorbents [3,4]. The selective removal of antigens and immune complexes from the circulation would be another desirable goal [5].

We herein describe a novel immunoadsorbent in which antibodies or Clq have been immobilized in collodion membranes. When placed in an extracorporeal circulation system, immobilized antibodies have shown a capacity to specifically withdraw circulating antigens and entrapped Clq has demonstrated an ability to extract circulating antigen–antibody complexes. There was no evidence of release of the immobilized immune reactants and no demonstrable toxicity to the host's hematologic and biochemical status.

In order to increase the efficiency of the immunoadsorbent, a plasma-cell separator was employed for in vivo studies in which only circulating plasma was circulated over the immunoadsorbent surface. We herein demonstrate the feasibility of utilizing this device as an adjunct to removing immune reactants from the circulation with extracorporeal immunoadsorbent.

2. Materials and methods

BSA antibodies were isolated from 50 ml of immune rabbit serum by the method of Ishizaka, et al. [6]. After concentration by ultra-filtration and dialysis against 0.1 M Tris buffer for 24 h at 4°C, the final solution demonstrated an antigen binding capacity of 33 μg BSA bound/ml of undiluted solution [7]. Clq was isolated from human serum by the method of Heusser et al. [8]. Approximately 10 mg of Clq was isolated from 1500 ml of human serum which demonstrated a single precipitin band against a monospecific rabbit antiserum to Clq (Behring Diagnostics, Somerville, N.J.).

For preparation of BSA–anti-BSA complexes, quantitative precipitin curve was plotted utilizing ^{125}I-BSA and anti-BSA. The point in antigen excess at which no spontaneous precipitation occurred was determined. For the anti-BSA used, this occurred at 1000 μg of BSA/ml serum. The isolation of soluble ^{125}I-BSA–anti-BSA complexes was completed according to our previously described procedure [10].

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2.1. *Preparation of collodion membranes containing anti-BSA antibodies or Clq*

The membrane casting equipment consisted of a bell jar mandrel 16 cm in length and 16 cm in diameter, which was rotated horizontally at 200 rev/min a mechanical pump. A solution containing 50 ml of collodion (Mallinckrodt, Inc., St. Louis, Mo.), 10 ml of absolute alcohol and 30 ml of anhydrous ether (Mallinckrodt, Inc., St. Louis, Mo.) was prepared. This solution was poured horizontally from a narrow mouth beaker over the rotating mandrel. A second solution consisting of 1 ml of collodion, 1.3 ml of absolute alcohol, 20 ml of ether was prepared in a 50 ml syringe with a stop cock attached to the outlet. A mototool (Dremel Co., Racine Wisconsin, Model 270) with a wire mixer at the end facilitated stirring of the solution. While the collodion–ether–alcohol solution was being stirred, 1 ml of anti-BSA or Clq plus ^{131}I anti-BSA [9] or ^{131}I-Clq [9] in 0.1 M Tris buffer was added drop-wise. This second solution was then poured slowly and evenly through the open stop cock with constant stirring over the semi-dry collodion base layer which was rotating at 200 ml/min on the mandrel. The entire membrane was then rotated for an additional 15 min until it was semi-dry. The membrane was then detached, washed in 0.1 M Tris buffer for 10 min at 27°C, wrapped in spiral fashion over an inert backing material and then placed in glass chambers 8 cm in length and 2.5 cm in diameter with 40 mesh stainless steel screens at the ends. The chambers were then filled with 0.1 M Tris buffer and held at 4°C for 1 h after which phosphate buffered saline, pH 7.4, was substituted and incubated for an additional 18 h at 4°C. Schematic representation of this preparation is shown in fig.1.

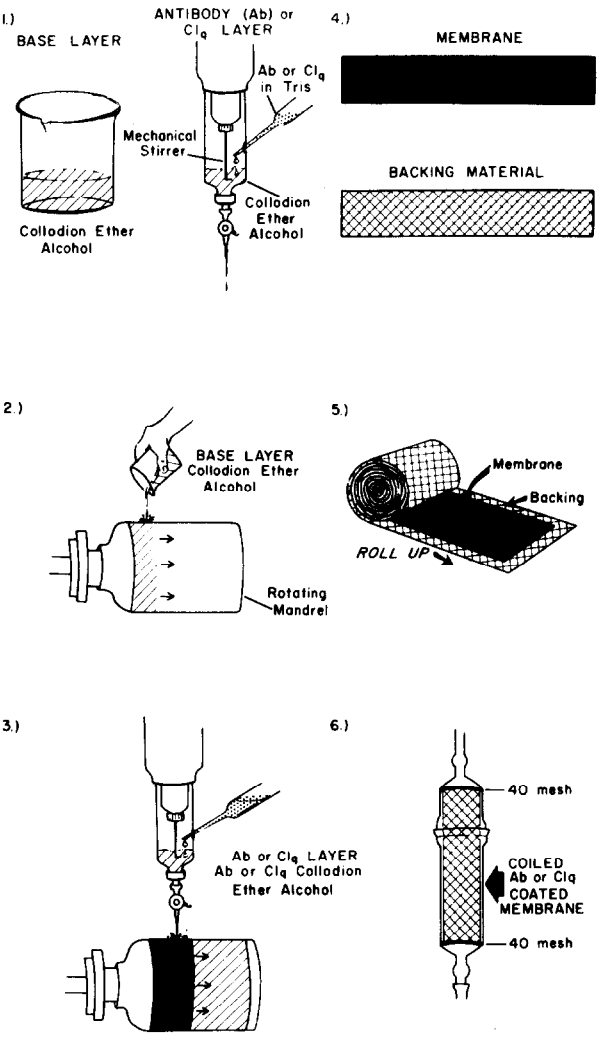

Fig.1. Schematic representation of the preparation of immunoreactive collodion membranes is depicted.

2.2. *In vivo extracorporeal system*

Dogs were anesthetized with sodium pentabarbitol and the femoral artery and vein cannulated with wide bore polyethylene tubing. Sodium heparin 3 mg/kg was injected intravenously into the femoral artery and the venous catheters were connected to a continuous flow celltrifuge (American Instruments Co., Silver Springs, Maryland). Arterial blood entering the celltrifuge was separated into plasma and formed elements by centrifugation. The plasma was them pumped at 40 ml/min through glass chambers containing collodion membranes. Each chamber was siliconized before use (Clay-Adams, Parsippany, N.J.). Formed elements of the blood, separated in the celltrifuge, were pumped at 40 ml/min to a site where they rejoined the plasma coming from the glass chambers. The recombined whole blood passed through a drip chamber and bubble trap and then to the femoral vein. Heparinized blood was circulated through the extracorporeal system for 20 min before intravenous injection of immune reactants. Perfusion of the collodion membranes then proceeded for an additional 120 min. Schematic representation of this in vivo system is shown in fig.2.

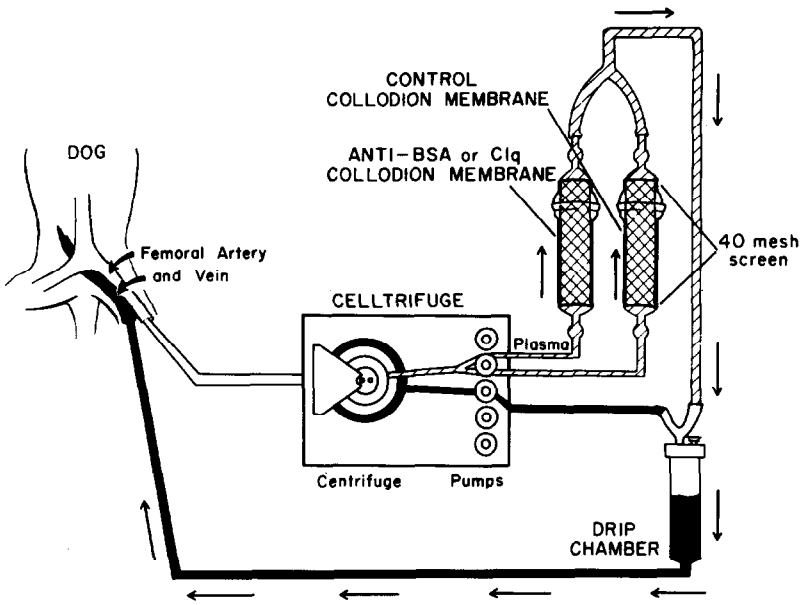

Fig.2. Schematic representation of the extracorporeal immunoadsorbent system is depicted.

3. Results

Anti-BSA antibodies 3.20 or 5.38 mg plus ^{131}I anti-BSA (1 μg) as a marker was added to collodion and membranes prepared as described in Methods. After completion of the entrapment procedure, the anti-BSA membranes were placed in glass cylinder chambers and at the conclusion of in vitro or in vivo circulation studies, they were removed and counted in a gamma scintillation counter. The quantity of anti-BSA retained was calculated by determining the percentage of total ^{131}I anti-BSA added which was entrapped in the collodion membranes at the end of the experiments. The results shown in table 1

Table 1
Retention of anti-BSA in collodion membranes

Experiment	Quantity of anti-BSA added (mg)	^{131}I anti-BSA added (cpm)	^{131}I anti-BSA recovered in membranes (cpm)	Anti-BSA retained in membranes (%)	Quantity of anti-BSA retained in membranes (mg)
In vitro	3.20	52 728	21 538	40.8	1.30
In vivo	5.38	17 749	13 849	78.0	4.19

Retention of Clq in collodion membranes

Experiment	Quantity of Clq added (mg)	^{131}I Clq added (cpm)	^{131}I Clq recovered in membranes (cpm)	Clq retained in membranes (%)	Quantity of Clq retained in membranes (mg)
In vitro	2.1	104 827	100 606	95.9	2.01
In vivo	4.5	107 266	89 421	83.3	3.75

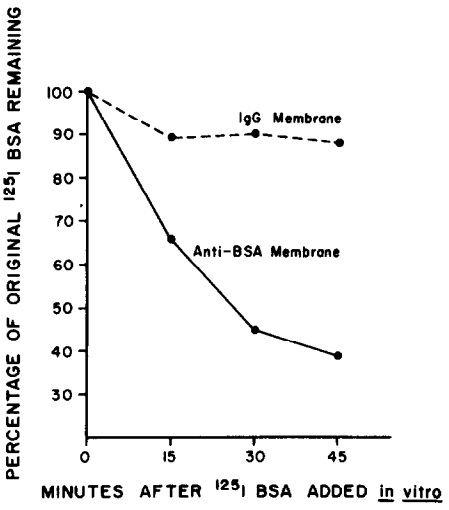

Fig.3. The kinetics of ^{125}I-BSA removal after in vitro circulation over anti-BSA or RGG collodion membranes is presented.

demonstrate that 1.36 and 4.19 mg of anti-BSA were retained in collodion membranes. Clq membranes were similarly prepared and counted with 2.01 and 3.75 mg entrapped in the membranes.

One hundred ml of PBS, pH 7.4, containing 1.2×10^5 cpm of ^{125}I-BSA [9] (Miles Laboratory, Indianapolis, Indiana) was circulated by a dual channel roller pump at 70 ml/min through each of 2 chambers containing anti-BSA or rabbit gamma globulin (RGG) (Miles Laboratory, Indianapolis, Indiana) entrapped in collodion membranes. Aliquots of buffer were extracted from each circuit at various intervals and counted for ^{125}I-BSA (fig.3). In addition, at the conclusion of the experiments, control and experimental membranes were removed from chambers washed with 400 ml 0.15 M NaCl and then counted in a gamma scintillation counter. Results shown in table 2 demonstrate a nearly 600% increase in the retention of ^{125}I-BSA on the membrane in which anti-BSA was immobilized compared to the RGG membrane. No ^{131}I anti-BSA which was incorporated in the membrane as a marker was recoverable from the final buffer sample (5 ml) above background cpm.

In a similar in vitro circulation system 1.4×10^5 cpm of ^{125}I-BSA–anti-BSA was circulated in each of 2 circuits over Clq-collodion and control collodion membranes. The kinetics of immune complex removal is shown in fig.4. Clq and control membranes were counted at the conclusion of experiments and showed nearly 300% increase in binding of immune complexes to Clq collodion compared to the control membrane. No ^{131}I-Clq [9] incorporated in the membrane as a marker was recoverable in the final buffer sample (5 ml) above background cpm.

For in vivo studies, ^{125}I-BSA was infused into

Table 2
Uptake of ^{125}I BSA on anti-BSA collodion membranes

Experiment	Anti-BSA collodion membranes		Normal gamma globulin collodion membranes	
	(cpm)	(μg)	(cpm)	(μg)
In vitro	73 616	1.84	11 484	0.28
In vivo	177 783	4.44	74 459	1.86

Uptake of ^{125}I BSA–anti-BSA on Clq collodion membranes

	Clq collodion membranes		Collodion membranes	
	(cpm)	(μg)	(cpm)	(μg)
In vitro	81 796	1.40	34 559	0.59
In vivo	20 033	0.34	7 167	0.12

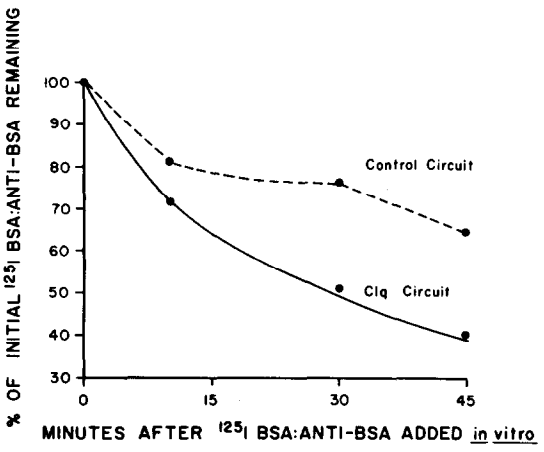

Fig.4. The pattern of removal of ^{125}I-BSA–anti-BSA after circulation across Clq or control collodion membranes in vitro is shown.

anesthetized dogs and plasma was then circulated over anti-BSA and RGG collodion membranes. Results shown in table 2 demonstrates that the membrane containing anti-BSA antibodies extracted nearly 250% more ^{125}I-BSA than the corresponding RGG membrane. In a second in vivo experiment, ^{125}I-BSA–anti-BSA complexes were circulated over Clq and control collodion membranes. There was nearly 300% uptake of complexes on the Clq membrane compared to the control (table 2).

To determine whether anti-BSA or Clq was released from the immunoadsorbent during the in vivo circulation studies, the vital organs, serum and urine of the dogs were counted for the presence of ^{131}I anti-BSA or ^{131}I-Clq at the conclusion of the experiments. No significant ^{131}I anti-BSA was discovered in the organs, serum and urine of the dogs above background cpm. In addition, sample areas of the experimental collodion membranes were counted at the conclusion of the experiments and showed no significant change in ^{131}I cpm from pre-perfusion values. Thus no significant ^{131}I anti-BSA was released from the membrane during the experiments.

Studies of hematocrit and leukocyte counts were performed on dogs before and after extracorporeal circulation revealed no significant alterations and there were no changes in serum sodium, potassium, calcium, magnesium and creatinine.

4. Discussion

These studies demonstrate that significant quantities of anti-BSA and Clq may be entrapped in collodion membranes. Once immobilized, anti-BSA and Clq appear to retain sufficient function to specifically withdraw circulating BSA and immune complexes respectively both in vitro and in vivo. In addition, there was no significant release of ^{131}I anti-BSA from the membranes since (a) there was no change in ^{131}I anti-BSA or ^{131}I-Clq on the membranes before and after the in vivo procedures, (b) no significant ^{131}I could be identified in a buffer sample taken at the conclusion of the in vitro circulation study, and (c) no significant uptake ^{131}I was observed in the thyroid gland, urine or vital organs at the conclusion of in vivo experiments. Collodion has been employed to coat small charcoal particles which have been utilized for the treatment of a variety of intoxications as well as in the extra-corporeal therapy of chronic renal failure [11,12]. The collodion membranes may possess many features of the optimal immunoadsorbent for in vivo use which include (a) large surface area capable of binding large quantities of functional protein, (b) large surface area with minimal extracorporeal volume requirements, (c) firm adherence of the material entrapped in it without release into the host, and (d) minimal toxicity to the host's hematologic and biochemical status.

The continuous flow celltrifuge employed in these studies has been utilized in the past for leukocyte and platelet collection from blood donors [13,14]. In the foregoing studies, we demonstrate the feasibility of using the celltrifuge to assist in withdrawal of circulating immune reactants. The celltrifuge might maximize the efficiency of specific antibody clearance by (a) increasing the contact of circulating antibodies with the immunoadsorbent surface and (b) preventing cellular deposition that might coat combining sites immobilized in the immunoadsorbent. Celltrifuge might also be of value in circumstances where it would be desirable to reutilize the immunoadsorbent by minimizing cellular deposits and therefore facilitating cleansing of the column.

The foregoing study represents a step in the development of technology toward the use of an effective immunoadsorbent to remove circulating pathogenic antigens and immune complexes. Indeed, many

antigens have been identified in disease processes which appear to play an important pathogenic role, namely, native and single stranded DNA in systemic lupus erythematosus, which, when combined with specific antibody form immune complexes which deposit in tissues causing tissue inflammation and destruction [15]. In addition, circulating tumor antigens have been shown to block vital lymphocyte functions in vitro [16,17]. Indeed, numerous diseases have been shown to be mediated by antigen–antibody complexes [1] and sensitive assays to monitor their quantity in serum have been described. Removal of pathogenic immune complexes from the circulation would be a desireable therapeutic objective and immobilized Clq might serve as an instrument for this purpose.

In the system herein described, one collodion membrane consisted of approximately 1000 cm^2 with a fluid volume requirement per chamber of only 20 ml. Recently, we have shown that these collodion membranes may incorporate up to 50 mg of protein [18]. Therefore, this membrane system may be markedly scaled up to incorporate larger quantities of antibody or Clq. Present studies are examining this possibility of removing larger quantities of antigen and immune complexes from the circulation of animals with naturally occurring autoimmune disease.

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