

In Vitro Evaluation of Biospecific and Pseudobiospecific Ligands Aimed at Extracorporeal Treatment for Immunoglobulin E Removal

*Isa S. Duarte, †Ricardo L. Zollner, and *Sonia M.A. Bueno

*School of Chemical Engineering; †School of Medical Sciences, State University of Campinas, Campinas, São Paulo, Brazil

Abstract: This work investigated the potential use of an alternative adsorbent to anti-immunoglobulin E (IgE)-agarose for IgE selective adsorption therapy. A screening of several commercially available adsorbents (Concanavalin A, *Lens culinaris* [Lc], D-tryptophan, poly-L-lysine, and aminohexyl immobilized on agarose) was done through batch system assays, considering some criteria, such as adsorption capacity, selectivity, and biocompatibility. In the Lc-agarose adsorbent, total IgE, and specific IgE—for the airborne allergens *Dermatophagoides pteronyssinus* and *Blomia tropicalis*—were significantly better removed

(63, 58, and 59%, respectively) than immunoglobulin G (19%), immunoglobulin A (33%), immunoglobulin M (9%), and albumin (18%). This adsorbent was packed into a column and the effect of superficial velocity, ratio of plasma volume to bed volume, number of perfusions, and temperature on IgE adsorption were evaluated. In vitro simulation of therapeutic adsorption (single perfusion) indicated that about 50% of total IgE could be eliminated. **Key Words:** Immunoglobulin E—Removal—Affinity—Lectins—Allergy.

Allergy is an immunologic system disorder, affecting approximately 20–30% of the general population and brings about disability and productivity loss throughout the world (1). Among allergic reactions, the immediate hypersensitivity is mediated by immunoglobulin E (IgE). Individuals presenting strong response to immediate hypersensitivity generally have higher serum levels of IgE antibodies than the nonallergic ones. Current therapy aims at inhibiting mast cell mediator production and blocking or counteracting the effects of released mediators, through the administration of drugs such as corticosteroids, sodium cromolyn, and antihistamines. Unfortunately, these drugs have a few side effects (2). The other therapy, called desensitization, tries to prevent or reduce T_H2 cell responses to specific allergens and the production of IgE. This approach was successful in preventing acute anaphylactic responses to protein

antigens (e.g., insect venom) (3,4). Nevertheless, its efficacy is variable for chronic atopic conditions, such as allergic rhinitis and asthma (5). In acute anaphylactic responses and allergic emergency cases, alternative therapies were investigated as adjuvant, such as the extracorporeal treatment for selective removal of IgE. In this approach, the patient's plasma is loaded to a column containing an agarose immobilized ligand in order to remove IgE returning the nonadsorbed proteins to the patient. Lebedin et al. (6) used monoclonal antibodies anti-IgE immobilized on Sepharose CL 4B in 17 clinical IgE apheresis treatments of five atopic asthma patients, and they effectively removed IgE from plasma (83% to 98%). In spite of good results, the ligands employed in this system are anti-IgE antibodies, which are expensive and difficult to purify (7,8). Due to these disadvantages, the employment of anti-IgE antibodies as affinity ligand has been limited, motivating the search for simpler and less expensive ligands than anti-IgE antibodies.

Aiming a different purpose of the extracorporeal treatment, biospecific ligands (with broad specificity, e.g., lectins) and pseudobiospecific ones (polyamines

Received October 2005; revised April 2006.

Address correspondence and reprint requests to Dr. Sonia Maria Alves Bueno, School of Chemical Engineering, State University of Campinas, CP 6066, 13083-970 Campinas, SP, Brazil. E-mail: sonia@feq.unicamp.br

and tryptophan), both less expensive than anti-IgE, were employed in purification, interaction, and structure–function studies involving IgE molecules.

Shibasaki et al. (9) investigated the interaction of 12 types of lectins with human IgE from allergic individuals. They got high affinity for Concanavalin A (Con A), *Lens culinaris* (Lc) hemagglutinin (LcH), *Pisum sativum* agglutinin (PSA), *Ricinus communis* agglutinin I (RCA-I), and phytohemagglutinin E (PHA-E). In routine diagnostic tests for detection of IgE, Zhao et al. (10) verified that in some sera from allergic patients, the IgE presented interaction for poly-L-lysine (PLL). In addition, these researchers carried out structure–function studies, evaluating the structural recognition of several polyamines for human IgE antibodies. The interaction between IgE and tryptophan was investigated by Ikononov et al. (11) through comparative adsorption studies of commercially available adsorbents. They verified that high amounts of IgE were adsorbed on tryptophan immobilized on a polyvinyl alcohol gel.

The present work evaluated the feasibility (in terms of capacity and selectivity) of using either biospecific ligands with broad specificity (Con A and Lc) or pseudobiospecific ones (D-tryptophan [D-trp], PLL, and aminoethyl [Ah]) for the in vitro removal of IgE from human serum. Initial experiments (screening) were designed in a batch system to determine the most appropriate ligand among Con A, Lc, D-trp, PLL, and Ah. Aside from adsorption of total IgE, adsorptions of specific IgE, such as *Dermatophagoides pteronyssinus* (IgE_{D1}) and *Blomia tropicalis* (IgE_{D201}), were also taken into account. The allergic sensitization to the mites *B. tropicalis* and *D. pteronyssinus* is related to allergic respiratory diseases development in many countries, mostly tropical ones (12). The effects of operating variables (superficial velocity [v], number of perfusions [np], ratio of plasma volume [pv] to bed volume [bv] [pv/bv], and temperature [T]) on the capacity and selectivity of

the system were investigated. The alternative adsorbent was tested by in vitro chromatographic assays in order to simulate an extracorporeal circulation.

EXPERIMENT

Materials

Sephacrose CL-4B gel (Seph) was provided by Amersham Biosciences (Uppsala, Sweden). Con A, Lc agglutinin, PLL, D-trp, and Ah immobilized on agarose, crystalline bovine serum albumin (BSA), methyl- α -D-glucopyranoside, and melibiose were supplied by Sigma-Aldrich (St. Louis, MO, U.S.A.). Jacalin-agarose was provided by Pierce (Rockford, IL, U.S.A.). All other chemicals were of analytical grade. Ultrapure water was obtained using a Milli-Q System (Millipore, Billerica, MA, U.S.A.).

Human sera and plasma samples

Sera and plasma samples, provided by the Serum Bank of Laboratório de Imunologia & Alergia Experimental (LIAE, FCM, UNICAMP, Brazil), were obtained from blood collected either with heparin (to obtain plasma) or without anticoagulant (to obtain serum). Donors were either nonallergic or allergic individuals to the airborne allergens *D. pteronyssinus* and *B. tropicalis*. The serum samples were used in the batch system assays, while the plasma sample was used in the chromatographic experiments. These samples were arranged in four types: (i) sera pool with high levels of total IgE, IgE_{D1}, and IgE_{D201}, from allergic individuals; (ii) control A serum with moderate levels of total IgE and undetectable levels of IgE_{D1} and IgE_{D201}, from nonallergic individuals; (iii) control B serum with undetectable levels of total IgE, IgE_{D1}, and IgE_{D201}, from nonallergic individuals; and (iv) plasma with moderate levels of total IgE, IgE_{D1}, and IgE_{D201} from allergic individuals. Table 1 summarizes the content of these samples, concerning total IgE, IgE_{D1}, IgE_{D201},

TABLE 1. Ig and albumin content of serum and plasma samples

Protein	Serum samples			Plasma sample
	Pool	Control A	Control B	
Total IgE (kU/L)	1437	133	<2.00	174
IgE _{D1} (kUA/L)	253	<0.35	<0.35	34
IgE _{D201} (kUA/L)	70	<0.35	<0.35	12.2
IgA (mg/mL)	1.73	2.77	0.983	0.75
IgG (mg/mL)	12.90	8.32	12.40	13.8
IgM (mg/mL)	1.62	0.673	1.98	1.07
Albumin (mg/mL)	45.80	44.00	40.30	43.8

The maximum coefficient of variation [$C_v = (SD/mean) \times 100$] for FEIA method was lower than 15%; C_v for IgA, IgG, IgM, and albumin detection was lower than 3.0%.

immunoglobulin A (IgA), immunoglobulin G (IgG), immunoglobulin M (IgM), and albumin.

Quantitative assays

Concentrations of total and specific IgE for the airborne allergens *D. pteronyssinus* (IgE_{D1}) and *B. tropicalis* (IgE_{D201}) were determined by fluoro-enzymeimmuno assay (FEIA) using UniCAP 100 (Pharmacia Diagnostics AB, Uppsala, Sweden). Detection of IgA, IgG, IgM, albumin, C3, and C4 was performed by nephelometry (Array Protein System 360, Beckman Coulter, Fullerton, CA, U.S.A.). Protein concentrations in human serum and plasma were determined by the Bradford method (13). Crystalline BSA was used as a reference protein.

Batch system adsorption assays for screening of adsorbents

Batch adsorption assays were carried out according to Ikononov et al. (11), using 1.5-mL polypropylene tubes. The employed samples were the sera pool and controls A and B. Initially, 250 µL of the adsorbent was placed in the tube and, after sedimentation and removal of excessive buffer, the wet mass of the adsorbent was measured. The sample was applied at the ratio 1:2 (adsorbent mass per sample mass) and then incubated for 120 min at 37°C in a shaking water bath. Thereafter, the supernatant was recovered by centrifugation (10 min; 1500 × g). IgG, IgA, IgM, and albumin concentrations were determined by nephelometry and total IgE, IgE_{D1}, and IgE_{D201} by FEIA. Each adsorbent was tested in triplicate. The removal percentage (R%) of the various proteins was expressed as mean values ± SD and was calculated by Eq. 1.

$$R\% = \frac{(A_b - A_a)}{A_b} \times 100 \quad (1)$$

where A_b is the amount of protein (U or mg) before incubation and A_a is the amount of protein (U or mg) after 120 min of incubation (supernatant). Results were compared using the Kruskal–Wallis test (non-parametric analysis of variance). Differences were considered significant if P was ≤ 0.05.

In order to verify if the interaction of Con A and Lc with IgE molecule is not a common feature to all lectins, another immobilized lectin adsorbent was tested in the adsorption assays (like a control). The jacalin was chosen because it does not present affinity to IgE (14), and when immobilized on agarose, it is used to purify human serum or secretory IgA₁ (15). This lectin has been isolated from the *Artocarpus integrifolia* crude seed extract (14,16) and presents affinity to D-galactose and N-acetyl-galactosamine

(Gal/GalNAc binding lectin) (17). Then, the same batch adsorption experiments were carried out with jacalin-agarose, using pool and control A samples.

A similar procedure was carried out at 4°C for C3 and C4 consumption studies, in which fresh frozen serum was incubated with the adsorbent for 60 min. C3 and C4 were quantified by nephelometry. The consumption of C3 and C4 was expressed as the amount of C3 and C4 in supernatant consumed over the control serum sample (incubated with an equal volume of phosphate-buffered saline [PBS] instead of adsorbent).

Chromatographic assays

Chromatography was carried out with an automated chromatographer (Econo Liquid Chromatography System, Bio-Rad, Richmond, CA, U.S.A.) employing the Lc-agarose gel packed into columns A (10 × 5-mm inner diameter [i.d.]) and B (10 × 10-mm i.d.) (Amersham Biosciences) to give bvs of 0.8 and 1.6 mL, respectively. A control assay was carried out using the Sepharose (without a ligand) packed into column A. The assays consisted of three steps: (i) priming; (ii) perfusion; and (iii) elution.

In the priming step, the packed column was equilibrated with 10 mM PBS at pH 7.4 (equilibration buffer), followed by washing with 3.0 mL of heparinized 0.9% saline solution according to recommendations for patient treatment. In the perfusion step, plasma samples were continuously circulated once, twice, and thrice through the column, corresponding to single, double, and triple perfusions. The column was then extensively washed in open loop in order to remove the unbound plasma components. The washing solution (equilibration buffer) contained 18 mM sodium citrate to prevent column-bound fibrinogen from being polymerized during the rinsing procedure (18). The elution step was carried out with 20 mM Tris–HCl at pH 7.4, containing 0.5 M sodium chloride, 0.5 M methyl-α-D-glucopyranoside, and 18 mM sodium citrate. Finally, the column was washed thrice alternatively with 20 mM Tris–HCl at pH 8.5 and 20 mM sodium acetate at pH 4.5, both containing 0.5 M sodium chloride. One-milliliter fractions were collected during chromatographic experiments, and concentrations of total IgE, IgE_{D1}, IgA, IgG, IgM, and albumin present in them were determined by FEIA and nephelometry.

Two different chromatographic procedures are described hereafter. The first aimed to investigate the effects of some operating variables on IgE adsorption; the second aimed to study the performance of the Lc-agarose adsorbent in conditions likely to be encountered in a clinical setting.

Effects of operating variables on IgE adsorption

The aim of these experiments was to analyze the effects of operating variables on the performance of the Lc-agarose concerning adsorption of total IgE, IgE_{D1}, and immunoglobulins (Igs) A, G, and M through chromatographic assays using plasma samples.

Six experiments were carried out varying the v , defined as the ratio of flow rate to cross-sectional area (cm/min), the np , pv/bv (mL/mL), and T (°C). The values used were 0.26 cm/min (0.05 mL/min/0.196 cm²) and 0.78 cm/min (0.15 mL/min/0.196 cm²) for v ; single, double, and triple perfusions for np ; 5.7 and 2.85 for pv/bv ; and 25 and 37°C for T . The plasma samples (4.56 or 2.28 mL) were perfused through the column A.

The protein adsorption (total IgE, IgE_{D1}, IgA, IgG, IgM, and albumin) was expressed as percentage adsorption (A%) and was calculated according to Eq. 2.

$$A\% = \frac{E_p}{I_p} \times 100 \quad (2)$$

where E_p is the eluted protein (U or mg) and I_p is the protein (U or mg) present in the initial sample.

The control assay was carried out loading 4.56 mL of plasma through the Sepharose-column A (v , 0.78 cm/min; np , single perfusion; pv/bv , 5.7; T , 25°C).

In vitro simulation of therapeutic immunoadsorption

In these assays, the experimental conditions remained constant (v , 0.78 cm/min; np , single perfusion; pv/bv , 2.85; T , 25°C). The perfusion step consisted of circulation of 4.56 mL of plasma through the column B for 7.6 min, corresponding to one passage of plasma through the column. Then, the amount of proteins in the tank (total IgE, IgE_{D1}, IgE_{D201}, IgA, IgG, IgM, and albumin) was measured and the R% was calculated using Eq. 1, where A_b was the amount of protein before circulation and A_a was the amount of protein after circulation. The column was then extensively washed in open loop in order to remove unbound proteins and afterward, the elution step was performed as previously described. The protein contents in the washing and elution fractions were determined to calculate the mass balance.

RESULTS

Screening of adsorbents

The screening of adsorbents was based on adsorption capacities and selectivities for IgE and C3 and C4 consumption studies, through batch assays.

Adsorption capacity of adsorbents

Adsorptions of IgE, IgE_{D1}, and IgE_{D201} were accomplished by all adsorbents studied (Fig. 1). The Kruskal–Wallis test indicated that R% values for Con A–Sepharose were significantly higher than for Seph, D-trp-agarose, PLL-agarose, and Ah-agarose, in assays using the pool sample ($P < 0.05$, Fig. 1a,c,d). In assays using the control A sample, the R% values for Lc-agarose were significantly higher than those for Seph, D-trp-agarose, PLL-agarose, and Ah-agarose ($P < 0.05$, Fig. 1b). There were no significant differences between Con A–Sepharose and Lc-agarose. Thus, among the assessed adsorbents, Con A–Sepharose and Lc-agarose presented the best adsorption capacities for total and specific IgE. Considering the results for IgE_{D1} and IgE_{D201}, it is observed that the adsorbents were not selective, that is, none of them had higher affinity for either one. A significant difference was observed among the R% values for total IgE and specific IgE when the D-trp-agarose was used. Probably, this ligand has more affinity to IgE_{D1} and IgE_{D201} than total IgE.

For jacalin-agarose (lectin adsorbent control), the R% values for total IgE, IgE_{D1}, and IgE_{D201} were lower than the UNICAP 100 detection range, indicating that Con A–Sepharose and Lc-agarose really have affinity to IgE.

Selectivity of adsorbents

The Seph gel adsorbed considerable amounts of all Igs and albumin, ranging from 20 to 30% (Fig. 2). Comparing the Con A–Sepharose and Lc-agarose, both of them adsorbed IgA, IgG, IgM, and albumin in assays using the pool sample. However, the Con A–Sepharose gel presented a very higher IgM R% value than Lc-agarose ($P < 0.05$, Fig. 2c). This behavior also occurred in assays using the control samples A and B: the Con A–Sepharose gel presented a very higher IgM R% value than Lc-agarose ($P < 0.05$, data not shown).

Then, among the studied affinity supports, the Lc-agarose gel is the most selective concerning IgE removal, as the prevailing protein adsorbed was the targeted compound (R%, 63%; Fig. 1a).

C3 and C4 consumption studies

Among the assessed adsorbents, Seph and Lc-agarose showed the lowest decrease in C3 and C4 concentrations, whose values were not significantly different from control serum values ($P > 0.05$, Table 2). This indicated that complement activation

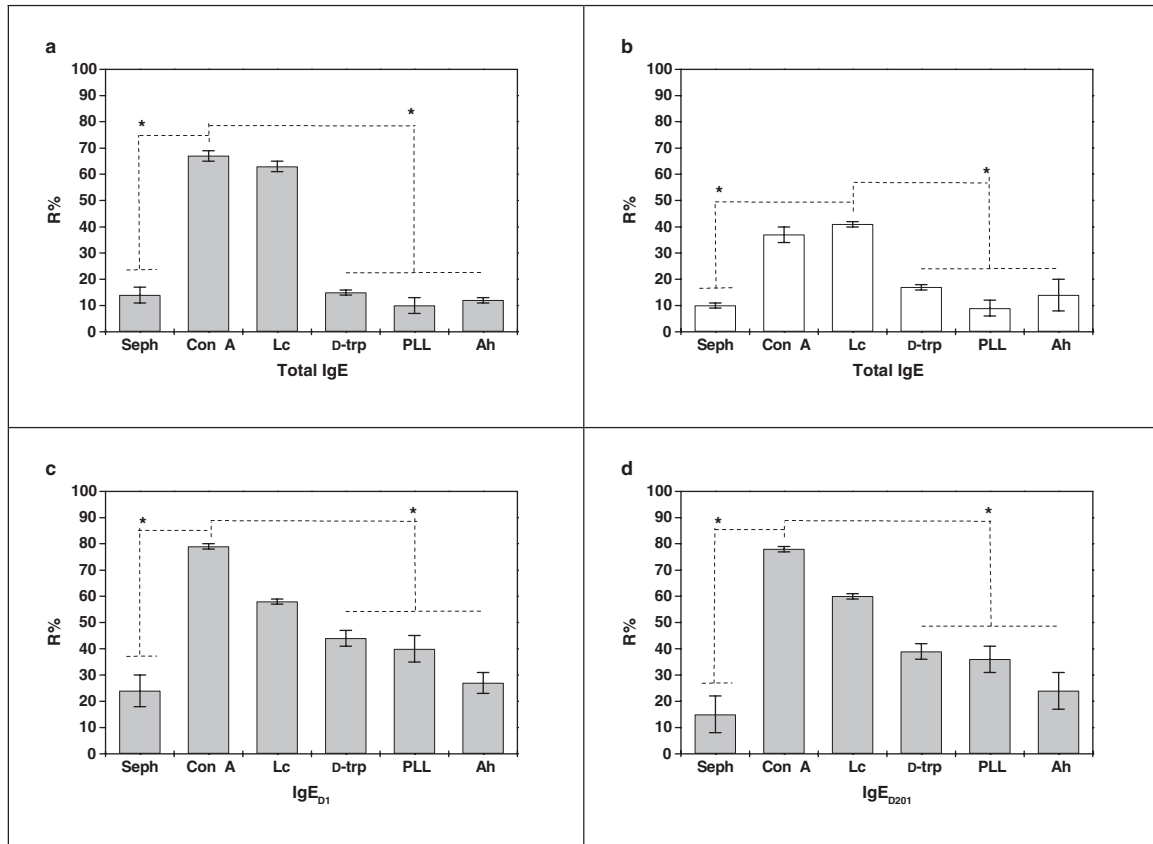


FIG. 1. Removal percentage (R%) for total IgE (a, b), IgE_{D1} (c), and IgE_{D201} (d), tested by in vitro batch system, using sera pool (filled bars) and control A (open bars). Figures are mean $\pm$ SD of triplicate samples. * $P \leq 0.05$ (Kruskal–Wallis test).

did not occur. In contrast, Con A-Sepharose and PLL-agarose showed the highest consumptions of C3 and C4, suggesting complement activation. As a result of the screening, Lc-agarose was selected

TABLE 2. Percentage consumption (c%)* of C3 and C4 for the studied adsorbents, tested in in vitro batch system, using fresh frozen serum samples[†]

Adsorbent	C3		C4	
	C _{sup} (mg/dL) [‡]	c%	C _{sup} (mg/dL) [‡]	c%
Control serum	82.2 $\pm$ 0.8	00	14.7 $\pm$ 0.1	00
Sepharose	69.8 $\pm$ 3.5	15	13.6 $\pm$ 0.6	08
Con A-Sepharose	17.5 $\pm$ 0.8	78	4.9 $\pm$ 0.4	67
Lc-agarose	66.6 $\pm$ 0.3	19	12.0 $\pm$ 0.1	18
D-trp-agarose	50.0 $\pm$ 1.1	39	6.9 $\pm$ 0.2	53
PLL-agarose	32.9 $\pm$ 2.7	60	2.1 $\pm$ 0.1	86
Ah-agarose	59.2 $\pm$ 1.1	28	11.6 $\pm$ 0.5	21

*Percentage consumption $c\% = [(C_{CS} - C_{Ads}) / (C_{CS})] \times 100$. C_{CS} is the concentration of C3 or C4 in the control serum (after incubation) and C_{Ads} is the concentration of C3 or C4 in the supernatant after incubation of each adsorbent.

[†]C3 and C4 contents of fresh frozen sera were 83.7 and 15.6 mg/dL, respectively.

[‡] C_{sup} = concentrations of C3 and C4 in the supernatant (after incubation) of each tube. Mean values $\pm$ SD of triplicate samples.

among all adsorbents studied, as the most promising for IgE selective adsorption therapy.

Effect of operating variables on IgE adsorption

Adsorption of Igs (Table 3, assays 1 and 2) did not depend on the v (ranging from 0.26 to 0.78 cm/min). The higher the v value, the lower the time of extracorporeal circulation. Therefore, the higher value (0.78 cm/min) was selected for the next assays.

Double perfusion did not increase the percentage of adsorbed Igs. Nevertheless, when the column was perfused thrice (Table 3, assay 4), the A% values for total IgE and IgE_{D1} decreased. Probably, the recirculation of plasma worked like a washing solution, removing the weakly bound Igs. Therefore, the single perfusion was preferred for the next assays.

The change of p_v/b_v from 5.7 to 2.85 implies that for the same p_v , the b_v was doubled. As shown in assays 2 and 5 (Table 3), the perfusion of the double b_v increased a little the percentage adsorption for total IgE (from 23.6 to 28.7%) and increased the A% more effectively for IgE_{D1} (from 7.5 to 14.5%). The values for IgA, IgG, and IgM increased slightly.

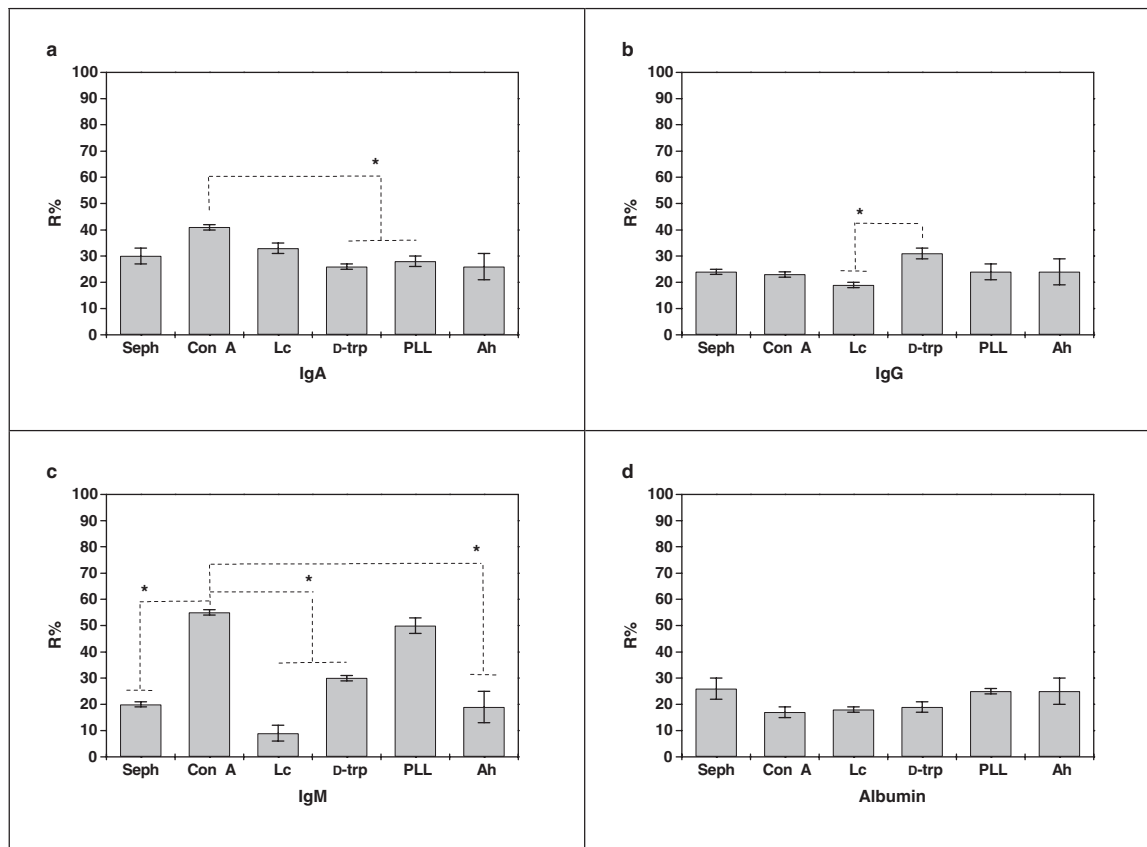


FIG. 2. Removal percentage (R%) for IgA (a), IgG (b), IgM (c), and albumin (d), tested by in vitro batch system, using sera pool. Figures are mean $\pm$ SD of triplicate samples. * $P \leq 0.05$ (Kruskal–Wallis test).

Therefore, the smaller value for pv/bv (2.85) was chosen for the next assay.

The percentage adsorption decreased a little for total IgE and more effectively for IgE_{D1} (Table 3, assays 5 and 6) with increasing T (from 25 to 37°C). The values for IgA, IgG, and IgM decreased slightly.

In all tested experimental conditions, the adsorbed albumin amount was lower than the array protein system detection range. Besides, the A% values for

IgA, G, and M were significantly lower than for total and specific IgE. The higher IgE adsorption was obtained in assay 5; thus, these experimental conditions were chosen for the in vitro simulation of therapeutic immunoadsorption.

In the control assay, the adsorbing material without ligand showed a very low adsorption for total IgE (A%, 0.8%) when compared to that value obtained with the Lc-agarose, at the same experimental

TABLE 3. Percentage adsorption (A%) of total IgE, IgE_{D1}, IgA, IgG, IgM, and albumin as a function of some operating variables

Assay	Operating variables					A%					
	v (cm/min)	np	pv/bv	T (°C)	Time (min) [†]	Total IgE	IgE _{D1}	IgA	IgG	IgM	Albumin
1	0.26	1	5.70	25	90	26.2	9.0	1.9	0.3	0.6	*
2	0.78	1	5.70	25	30	23.6	7.5	1.3	0.2	0.3	*
3	0.78	2	5.70	25	60	24.6	5.6	1.3	0.2	0.6	*
4	0.78	3	5.70	25	90	9.4	1.7	1.1	0.2	0.5	*
5	0.78	1	2.85	25	15	28.7	14.5	2.1	0.3	0.4	*
6	0.78	1	2.85	37	15	22.0	8.4	1.2	0.1	0.3	*

Plasma volume: 4.56 mL for assays 1–4 and 2.28 mL for assays 5 and 6.

*Values lower than the detectable range of array protein system.

[†]Time (min): contact time between plasma sample and bed of Lc-agarose gel.

conditions (Table 3, assay 2). Besides, the amounts of IgE_{D1}, IgA, IgG, IgM, and albumin adsorbed on Sepharose were undetectable by FEIA and nephelometry.

In vitro simulation of therapeutic immunoadsorption

The performance of the Lc-agarose in conditions likely to be encountered in a clinical setting was evaluated by the R% of proteins. The previously chosen experimental conditions were kept constant (v , 0.78 cm/min; np, single perfusion; pv/bv, 2.85; T, 25°C).

The Lc-agarose was able to remove all the Igs studied and albumin. However, the R% values for target molecules total IgE (48.8%), IgE_{D1} (40.9%), and IgE_{D201} (42.8%) were higher than for IgA (25.3%), IgG (26.5%), IgM (11.9%), and albumin (24.4%). Comparing these results with that obtained in batch system assays, the values for total and specific IgE are smaller than those obtained in batch system assays. It may be due to the initial concentrations of IgE in the sample; the sera pool sample contains higher IgE concentrations than plasma sample (see Table 1). This disagreement did not occur for IgA, IgG, IgM, and albumin, whose initial concentrations are similar in both samples employed.

DISCUSSION

The main goal of this work was to investigate (in vitro) the potential use of an alternative adsorbent for IgE selective adsorption therapy. We investigated the adsorption capacity and selectivity for IgE and the complement activation, in order to screen a few adsorbents. Some ligands previously mentioned in the literature, which presented affinity to IgE or potential application for its purification, were chosen (9–11): Con A, Lc, D-trp, PLL, and Ah, all of them commercially available and having attractive costs (Sigma catalog, 2005).

Concerning the adsorption capacity, the highest R% values of total and specific IgE were obtained with Con A-Sepharose and Lc-agarose, which presented a significant difference between the other adsorbents. These results are in agreement with those obtained by Shibasaki et al. (9), who verified that IgE preparations—from allergic subjects—presented high affinity to Con A and Lc. According to them, the human IgE interacts with those lectins through carbohydrate receptors in IgE molecule.

The decrease in the IgE concentration with minimal loss of IgG, IgM, IgA, and albumin is essential in the extracorporeal treatment for IgE removal. Adsorbents used in clinical applications must not

adsorb high amounts of these proteins, in order to avoid replacement procedures after extracorporeal treatment. Unfortunately, the Con A-Sepharose presented a drawback concerning IgA and IgM adsorption. The high IgM adsorption on Con A-Sepharose was already reported by Ray and Raychaudhuri (19), for both myeloma Igs and normal serum Igs. Although the Lc-agarose adsorbed some IgA, it can be considered the most selective concerning IgE removal, as the prevailing protein adsorbed was the targeted Ig.

Regarding the last requirement assessed, it was not the aim of this investigation to analyze all aspects of biocompatibility. Indeed, the complement activation is one of several aspects that can be evaluated (20). Complement activation measurements were conducted indirectly by C3 and C4 consumption studies. The Lc-agarose exhibited the lowest decrease in C3 and C4 concentrations, suggesting that complement activation did not happen. Comparatively, the Con A-Sepharose and PLL-agarose gels exhibited the highest decrease in C3 and C4 concentrations, suggesting complement activation. Therefore, the Con A-Sepharose would be indicated for IgE purification purposes, and the Lc-agarose was considered the most promising for IgE selective adsorption therapy.

Chromatographic experiments were accomplished in order to verify the behavior of Lc-agarose concerning adsorption of IgE and other plasma proteins under continuous flow conditions and to determine favorable experimental conditions.

In clinical apheresis, the np is related with the time of extracorporeal circulation session. In in vitro procedures, it indicates how many times the plasma will go through the column (circulation). The circulation of plasma was not able to increase the adsorption of IgE effectively; there was rather a reduction of bound antibodies after three perfusions. An underlying rinsing effect may be present, like with other affinity systems (18).

The pv/bv indicates how much total pv can be processed on a certain adsorption system; it can be estimated from the maximum protein binding capacity of the adsorbent. As this parameter for the system IgE-Lc was not available, the choice was for the value of 5.7, which is used in apheresis employing pseudo-biospecific ligands (18) and 2.85. The latter value led to the best result for IgE adsorption. Probably, at pv/bv 5.7, the column was saturated.

The influence of T on IgE adsorption was also evaluated, as the extracorporeal circulation clinical procedure is carried out at 37°C. The adsorption of IgE at 37°C was a little lower than at 25°C. In order to improve the performance of the system, a cooling

device could be installed before the column inlet to adjust the T of plasma to 25°C. After IgE adsorption, the outlet plasma stream would be heated by an adequate device to 37°C and returned to the patient.

In the control assay, the Lc-agarose was compared to the matrix without an immobilized ligand, the Seph. This matrix was not able to bind any protein or antibody. However, the results obtained in batch system assays showed significant removal of IgE by Sepharose. Probably, some proteins remained within the pores and interstices of the adsorbent during incubation of sera pool, or had been adsorbed by nonspecific weak interactions.

Regarding the in vitro simulation of therapeutic immunoadsorption, the R% of IgE for Lc-agarose was lower than that for Sepharose-CL-4B-anti-IgE (6). The differences in adsorption behavior between Lc-agarose and Sepharose-CL-4B-anti-IgE are due to the type of interaction between the ligand and adsorbed molecule. Anti-IgE (the ligand used for removal of IgE from the extracorporeal circulation) binds IgE molecules with high affinity through interaction with Fab portion (antibody-antigen binding), whereas Lc probably binds through interaction with D-manose and D-glucose (21), with less specificity. The selectivity of anti-IgE for total IgE was confirmed in vivo by specific adsorptive capacity for IgE from 83 to 98%, after 1200–3400 mL of processed pv (6). Some clinical IgE apheresis sessions were carried out for patients suffering from atopic asthma, containing high initial concentrations of IgE (6). Although it produces good results and has efficiency, the Sepharose-CL-4B-anti-IgE is very expensive. Experiments from plasma samples containing high concentrations of IgE could be accomplished in order to better evaluate the performance of Lc-agarose, as it has more attractive cost than Sepharose-CL-4B-anti-IgE. Data from clinical experiments will be necessary to evaluate the safety and efficacy of Lc-agarose as an immunoadsorbent system.

CONCLUSION

Among the adsorbents studied, the Lc-agarose was the most promising for application in the IgE selective adsorption therapy, as it met all established screening requirements. Although the Con A-Sepharose presented high adsorption of IgE, it may induce complement activation. This adsorbent could be employed for separation purposes.

Regarding the effect of operating variables on IgE adsorption employing Lc-agarose, it was observed that the plasma v (cm/min) did not affect IgE adsorption. In contrast, the np, pv/bv, and T (°C) affected

IgE adsorption. The most suitable experimental conditions for IgE adsorption were v, 0.78 cm/min, np, 1, pv/bv, 2.85, and T, 25°C.

Regarding the in vitro simulation of therapeutic immunoadsorption, the Lc-agarose was able to remove more total and specific IgE than IgA, IgG, IgM, and albumin. However, its performance was not as good as in batch system assays. It may be due to the different initial concentrations of IgE in the samples. The Lc may be an alternative to anti-IgE in the extracorporeal treatment of IgE-related diseases. However, further experiments are necessary in order to evaluate this system.

Acknowledgments: This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (Brazil) and Conselho Nacional de Desenvolvimento Científico e Tecnológico (Brazil). The authors thank Dr. C. Vilella from the Laboratório de Imunologia e Alergia Experimental (UNICAMP, Brazil) for FEIA analysis.

REFERENCES

- Casolaro V, Georas SN, Song Z, Ono SJ. Biology and genetics of atopic disease. *Curr Opin Immunol* 1996;8:796–803.
- Barnes PJ. New directions in allergic diseases: mechanism-based anti-inflammatory therapies. *J Allergy Clin Immunol* 2000;106:5–16.
- Singh MB, Bhalla PL. Hypoallergenic derivatives of major grass pollen allergens for allergy vaccination. *Immunol Cell Biol* 2003;81:86–91.
- Golden DBK. Insect sting allergy and venom immunotherapy. A model and a mystery. *J Allergy Clin Immunol* 2005;115:439–47.
- Schultz A, Stuck BA, Feuring M, Hörmann K, Wehling M. Novel approaches in the treatment of allergic rhinitis. *Curr Opin Allergy Clin Immunol* 2003;3:21–7.
- Lebedin YS, Gorchakov VD, Petrova EN, et al. Ex vivo removal of IgE in atopic asthma by extracorporeal plasmoid-immunoadsorption (EPIA): development of a clinical adsorbent. *Int J Artif Organs* 1991;14:508–14.
- Sato H, Watanabe K, Azuma J, Kidaka T, Hori M. Specific removal of IgE by therapeutic immunoadsorption system. *J Immunol Methods* 1989;118:161–8.
- Gorchakov VD, Sakodinskii KI, Lebedin YS, Chuchalin AG. Immunosorbents for clinical use: ex vivo immunoglobulin E removal in allergy. *J Chromatogr* 1991;563:166–71.
- Shibasaki M, Sumazaki R, Isoyama S, Takita H. Interaction of lectins with human IgE: IgE-binding property and histamine-releasing activity of twelve plant lectins. *Int Arch Allergy Immunol* 1992;98:18–25.
- Zhao Z, Baldo BA, O'Brien RM, Plomley RF. Reaction with, and fine structural recognition of polyamines by human IgE antibodies. *Mol Immunol* 2000;37:233–40.
- Ikononov V, Samtleben W, Schimidt B, Blumenstein M, Gurland HJ. Adsorption profile of commercially available adsorbents: an in vitro evaluation. *Int J Artif Organs* 1992;15:312–9.
- Takeda F, Arakawa T, Toma H, Ishii A, Sato Y. Intranasal sensitization with *Blomia tropicalis* antigens induces allergic responses in mice characterized by elevated antigen-specific and non-specific serum IgE and peripheral blood eosinophil counts. *Rev Inst Med Trop Sao Paulo* 2004;46:1–8.

13. Bradford MM. Rapid and sensitive method for a quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 1976;72:248–54.
14. Hagiwara K, Collet-Cassart D, Kobayashi K, Vaerman JP. Jacalin: isolation, characterization and influence of various factors on its interaction with human IgA1, as assessed by precipitation and latex agglutination. *Mol Immunol* 1988;25: 69–83.
15. Loomes LM, Stewart WW, Mazengera RL, Senior BW, Kerr MA. Purification and characterization of human immunoglobulin IgA1 and IgA2 isotypes from serum. *J Immunol Methods* 1991;141:209–18.
16. Roque-Barreira MC, Praz F, Halbwachs-Mecarelli L, Greene LJ, Campos-Neto A. IgA-purification and characterization of the lectin jacalin. *Braz J Med Biol Res* 1986;19: 149–57.
17. Wu A, Sugii S. Coding and classification of -galactose, N-acetyl-galactosamine, and -Galp-[1→3(4)]—GlcPNAc, specificities of applied lectins. *Carbohydr Res* 1991;213: 127–43.
18. Avenhaus B, Avenhaus W, Schneider M, Domschke W, Gaubitz M. Development of an in vitro miniature model to simulate immunoadsorption in patients with systemic lupus erythematosus. *J Clin Apher* 2002;17:183–9.
19. Ray PK, Raychaudhuri S. Differential binding affinity of immobilized concanavalin A-Sepharose 4B for normal and myelomatous immunoglobulins. *Biomed Pharmacother* 1982; 36:206–10.
20. Cheung AK, Lemke H. Criteria and standardization for biocompatibility. *Nephrol Dial Transplant* 1994;9:72–6.
21. Loris R, Casset F, Bouckaert J, et al. The monosaccharide binding site of lentil lectin: an X-ray and molecular modeling study. *Glycoconjugate J* 1994;11:507–17.