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Immunoadsorption procedure as a potential method for the specific β2-microglobulin removal from plasma of patients with chronic renal failure

L. Vallar[a], P.M.P. Costa[b], A. Teixeira[b], M. Pfister[a], R. Barrois[a], P.P. Costa[b], C. Rivat[a,*]

[a]*Centre de Recherche INSERM, Plateau de Brabois, C.O. 10, 54511 Vandoeuvre-les-Nancy Cedex, France*
[b]*Centro de Estudos de Paramiloidose, Hospital Sto Antonio, 4000 Porto, Portugal*

Abstract

β_2-Microglobulin (β_2-M), which accumulates in the plasma of patients undergoing long-term dialysis, has been identified as the principal precursor protein of amyloid fibrils in dialysis-related amyloidosis. As no specific treatment for this affection has been yet established, an extracorporeal immunoadsorption procedure appears to be an attractive therapeutic approach to remove β_2-M. Several murine monoclonal antibodies to human β_2-M were developed and compared as affinity ligands. One of them was selected on the basis of its specificity and adsorption capacity. In order to achieve maximum efficiency in protein removal, different parameters of the procedure were studied and optimized: effect of antibody coupling density, determination of maximum adsorption capacity of the immunoadsorbents and influence of antigen concentration and of flow-rate on antigen capture efficiency. The conditions of regeneration of immunoaffinity sorbents were also investigated to allow their multiple use without loss of adsorption capacity. The results show the validity of the proposed technique in removing β-M from plasma of patients with chronic renal failure.

1. Introduction

Long-term dialysis is frequently complicated by various osteoarticular problems, such as carpal tunnel syndrome, trigger finger, bone cysts and destructive spondylarthropathy [1–5]. These new types of osteoarthropathy have been related to the deposition of an amyloid material in which the main protein component is β_2-microglobulin (β_2-M) [6,7].

While this protein is normally catabolized in the renal tubules, it accumulates in the plasma of patients with renal failure in proportion to the degree of renal insufficiency. In dialysis patients, the loss of renal function, combined with the defective removal of β_2-M across conventional dialysis membranes, leads to a marked increase in plasma β_2-M levels which can be elevated up to 60 times the normal range [8]. Although the pathogenesis of dialysis-related amyloidosis is not yet well understood, this long-term and constant elevation of β_2-M in plasma seems to be the key factor in amyloidogenesis. Therefore, it appears that β_2-M removal from blood of

* Corresponding author.

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uraemic patients could certainly be helpful in preventing the disease and halt or slow its progression. Various high-flux synthetic dialysis membranes have been developed that are able to remove β_2-M by filtration and/or adsorption [9–12]. A newly designed adsorbent column, consisting of porous cellulose beads covalently bound to organic compounds, has been proved to adsorb large amounts of β_2-M by hydrophobic interaction [13]. This material, evaluated in a clinical trial in combination with a high-flux dialysis membrane, has given promising preliminary results [12]. Unfortunately, the amounts of β_2-M eliminated, whatever the method of protein removal, are not sufficient to counterbalance its daily generation and so prevent its retention.

In this context, specific immunoadsorption of plasma β_2-M on immobilized anti-β_2-M antibodies, in an extracorporeal circulation, seems to provide an attractive therapeutic alternative. Many studies have already shown the feasibility and effectiveness of such a procedure for the specific removal from blood plasma of pathogenic substances, such as apolipoprotein B [14–16], transthyretin [17,18], antibodies or immune complexes [19–25].

In this paper, we report a comparative in vitro study of several anti-human β_2-M monoclonal antibodies as affinity ligands, by the determination of their specificity and adsorption capacity. The optimum conditions for use of these antibodies in an immunoadsorption procedure have also been determined in order to achieve maximum efficiency in β_2-M removal.

2. Experimental

2.1. Anti-β_2-M monoclonal antobodies

Hybridoma cell lines producing anti-human monoclonal antibodies were developed according to widely used procedures [26]. Spleen cells were obtained from Balb/c mice immunized with β_2-M isolated from human urine (Sigma, St. Louis, MO, USA). They were fused with mouse myeloma cell line SP_2O using polyethylene glycol. Hybrids were tested for production of antibody of the desired specificity using an enzyme-linked immunosorbent assay (ELISA) and cloned by limiting dilution.

For antibody production, selected clones were adapted to and grown in RPMI 1640 medium (Seromed; Biochrom, Berlin, Germany), supplemented with 10% foetal bovine serum.

2.2. Production of monoclonal antibodies

The antibody intended to be used as an affinity ligand for immunoadsorption was produced on a large scale from ascitic fluids and purified by affinity chromatography using an 80×18 mm I.D. protein A–Sepharose CL-4B column (Pharmacia, Uppsala, Sweden) [26]. Purified antibodies were dialysed against 0.1 *M* sodium hydrogencarbonate–0.5 *M* NaCl buffer (pH 8.5) and stored at −20°C.

2.3. Preparation of immunoadsorbents

Purified monoclonal antibodies were immobilized on Sepharose CL-4B (Pharmacia) previously activated with cyanogen bromide (CNBr), according to the modified method of Cuatrecasas et al. [27]. Briefly, a volume of a 10% CNBr solution in distilled water was added to an identical volume of packed Sepharose, and the pH was maintained at 11.0 using 10 *M* NaOH up to stabilization. After extensive washing at 4°C, first using cold distilled water and then cold 0.1 *M* sodium hydrogencarbonate–0.5 *M* NaCl buffer (pH 8.5), the antibody solution in the same buffer was added to the activated Sepharose and the mixture was left overnight at 4°C with gentle stirring. The unbound proteins were eliminated by washing with sodium hydrogencarbonate buffer. The amount of antibodies coupled to the support was deduced from an assay of unbound proteins. The reactive groups in excess were blocked with 1 *M* ethanolamine (pH 8.5) for 2 h. After washing with 0.3 *M* glycine–HCl (pH 2.8)

to disrupt possible protein–protein interactions, the immunoadsorbent was stored at 4°C in 0.15 *M* NaCl–10 m*M* sodium phosphate buffer (pH 7.4) (PBS) containing 0.02% NaN_3.

2.4. Preparation of β_2-M-enriched blood plasma

Immunoadsorption experiments were carried out with citrated human blood plasma supplemented with urinary β_2-M. A concentrated stock solution of β_2-M was prepared from urine of patients with multiple myeloma. Urine samples (24 h) were collected in sterile bottles containing a buffering concentrated medium and NaN_3 as preservative, and were dialysed against distilled water at 4°C. Proteins were precipitated with ammonium sulphate (50 g per 100 ml). Pellets were dissolved in PBS, extensively dialysed against PBS and then concentrated by ultrafiltration. The final product contained high level of β_2-M, and was contaminated with human serum albumin and Bence Jones proteins. Other minor unidentified contaminants of medium molecular masses were also present. Biochemical and immunological identity between urinary and plasmatic β_2-M was confirmed by polyacrylamide gel electrophoresis (PAGE) in the presence of sodium dodecyl sulphate (SDS), isoelectric focusing, immunoelectrophoresis and Ouchterlony immunodiffusion.

2.5. Immunoadsorption procedure

Blood plasma was applied to the immunoadsorbent column previously equilibrated with PBS. Non-specifically bound plasma components were washed out with the same buffer. Bound β_2-M was then desorbed with either 0.3 *M* glycine–HCl (pH 2.8), 3 *M* NaSCN–0.1 *M* CH_3COONa (pH 6.0) or 0.1 *M* triethanolamine–1 *M* NaCl (pH 10.5). The immunoadsorbent was regenerated by extensive washing with PBS containing 0.02% NaN_3 and stored at 4°C between each chromatographic run.

2.6. Analytical methods

β_2-M assay

β_2-M levels in starting blood plasma and in purified fractions were determined by a double-antibody solid-phase ELISA.

Microtitre plates (Costar, Cambridge, MA, USA) were coated overnight with 100 μl of 5 μg/ml of anti-human β_2-M monoclonal antibodies in 0.05 *M* sodium carbonate buffer (pH 9.6) at 4°C. After washing with 130 m*M* NaCl–5 m*M* Na_2HPO_4–1 m*M* KH_2PO_4 buffer (pH 7.2) containing 0.05% Tween 20 (PBS–Tween), 125 μl of 0.5% cold-water fish skin gelatin (Sigma) were added to each well and the plates were incubated at 37°C for 3 h. They were then washed with PBS–Tween. Aliquots (100 μl) of samples diluted in PBS–Tween were added to the wells and incubated for 2 h at 37°C. Titrated normal human plasma was used as a standard. The plates were washed with PBS–Tween, then 100 μl of 1:1500 diluted horseradish peroxidase conjugated sheep anti-human β_2-M antibodies (Binding Site, Birmingham, UK) were added to each well. The plates were incubated at 37°C for 1 h. After washing with PBS–Tween and then with 140 m*M* sodium acetate–citrate buffer (pH 6.0), 100 μl of 0.1 mg/ml 3,3′,5,5′-tetramethylbenzidine and 0.01% hydrogen peroxide in acetate–citrate buffer were dispensed into each well. The enzymatic reaction was stopped by addition of 25 μl of 2 *M* sulphuric acid and the absorbance was measured at 450 mm in a Titertek plus micro ELISA reader (Flow Laboratories, Puteaux, France). The specific characteristics of this assay are presented in Table 1.

Immunoblotting

SDS-PAGE was carried out using a Phastgel gradient from 8 to 25% on a Phastsystem apparatus according to the Pharmacia procedure. Proteins were transferred on a nitrocellulose membrane by diffusion at 70°C for 30 min. The membrane was sliced and the resulting strips were incubated with the different anti-β_2-M monoclonal antibodies. Horseradish peroxidase-conjugated goat anti-mouse IgG antibodies (Cal-

Table 1
Specific β_2-M assay characteristics

Parameter		No. of determinations	Results		
			Mean value (mg/l)	Standard deviation	Coefficient of variation (%)
Precision	Within-series precision (a)	50	0.84	0.059	7.0
	Reproducibility (b)	10	1.26	0.095	7.6
		10	3.42	0.260	7.7
		10	5.90	0.520	8.9
Accuracy	Recovery test (c)	30	$y = 0.952x + 0.176$, $r = 0.989$ (y = observed concentration, x = theoretical concentration)		
	Dilution test (d)	18	$y = 1.048x - 0.064$, $r = 0.994$ (y = observed concentration, x = theoretical concentration)		
Comparison with a radioimmunoassay		33	$y = 0.772x + 0.338$, $r = 0.996$ (y = concentration determined with ELISA, x = concentration determined with RIA kit)		
Sensitivity			0.10 mg/l		
Specificity			No cross-reactivity with human IgG, κ and λ light chains of human immunoglobulins		

The precision of the assay was evaluated by determining within-series precision and reproducibility: (a) was determined by assaying a sample of plasma during the same analytical run and (b) by assaying three different samples of plasma during multiple analytical runs. The accuracy of the assay was measured by performing recovery and dilution tests: (c) the percentage recovery of five different β_2-M concentrations added to six plasma samples was calculated; (d) three elevated plasma samples were diluted in the zero standard, and the percentage recovery was calculated. For comparison purposes, eleven plasma samples were assayed in triplicate for β_2-M with our ELISA and a radioimmunoassay (RIA) kit (Immunotech, Marseille, France). Sensitivity was determined from the dose–response curve.

tag, San Francisco, CA, USA) were used for detection.

3. Results

3.1. Comparative study of the anti-β_2-M monoclonal antibodies

Specificity of the antibodies

Four hybridoma cell lines, producing antibodies to human β_2-M, were selected by immunoblotting, using antibodies purified from culture supernatants. The four developed antibodies, EG_4A_7, EH_1C_5, GB_5C_7 and GG_6A_7, bound specifically to β_2-M transferred on to a nitrocellulose membrane.

To confirm this result, aliquots of each antibody were pre-incubated with various amounts of free β_2-M and then tested for reactivity against isolated β_2-M adsorbed on a microtitre plate in a solid-phase ELISA. Fig. 1 shows that the recognition of immobilized β_2-M by the monoclonal antibodies was fully inhibited by free molecules.

As the sequence and three-dimensional structure of β_2-M present a strong homology with the constant part of the heavy and light chains of immunoglobulins [28], further investigations were performed using an ELISA technique, and proved the lack of immunological cross-reactivity with human IgG and with human κ and λ light chains.

Properties of affinity ligand

Each purified antibody was immobilized on a 4-ml volume of CNBr-activated Sepharose. The coupling yields were found to be similar with GG_6A_7, EG_4A_7 and GB_5C_7 antibodies (80–84%) and slightly less elevated with EH_1C_5 antibody (70%).

To determine the adsorption capacity of these

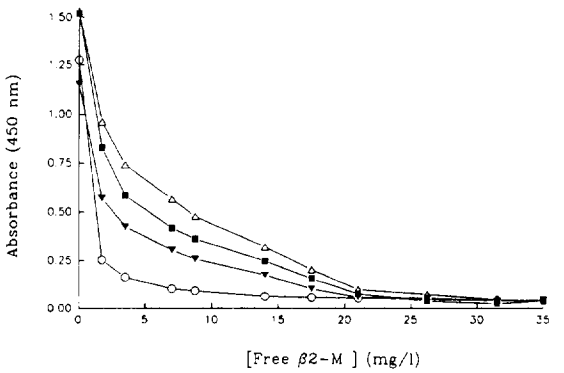

Fig. 1. Inhibition of anti-β_2M monoclonal antibodies by free antigen. Free β_2-M (70.0 μg/ml) was serially diluted and 200 μl of each dilution were pre-incubated with 200 μl of each antibody (10.0 μg/ml) for 2 h at 37°C in glass tubes. The residual activity in 100 μl of pre-incubated mixture was measured by incubation in a β_2-M coated microtitre plate (5 μg/ml) for 2 h at 37°C. ○ = GG_6A_7; ▼ = EH_1C_5; △ = GB_5C_7, ■ = EG_4A_7. Detection was achieved using peroxidase-conjugated goat anti-mouse IgG antibodies.

immunoadsorbents, an excess of plasma, containing high levels of β_2-M, was passed through each column. Desorption of captured β_2-M was achieved using either 0.3 *M* glycine–HCl (pH 2.8), 0.1 *M* triethanolamine–1 *M* NaCl (pH 10.5) or 3 *M* NaSCN–0.1 *M* CH_3COONa (pH 6.0) solutions. The results summarized in Table 2 indicate that two of the monoclonal antibodies (GG_6A_7 and EG_4A_7) lost their ability to fix the protein after immobilization on Sepharose, whereas the other two (EH_1C_5 and GB_5C_7) showed a good adsorption capacity.

Almost complete desorption of β_2-M was obtained for the four tested antibodies in chaotropic conditions, and for only two immunoadsorbents with acidic buffer. The basic solution appeared to be ineffective in removing β_2-M from the GB_5C_7 column.

When analysed by SDS-PAGE, the β_2-M-containing peak, eluted under acidic pH conditions from GB_5C_7 immunoadsorbent, presented very weak, trace amounts of contaminants.

Selected on the basis of its specificity and adsorption capacity, the GB_5C_7 antibody was used in all subsequent experiments.

3.2. Optimization of the immunoadsorption procedure

Different parameters are known to have an influence on the effectiveness of immunoaffinity chromatographic systems [16,18]. In order to study and to optimize them, several immunoadsorbents were prepared with various concentrations of GB_5C_7 antibody coupled to 5-ml volumes of activated Sepharose.

Determination of the maximum antibody-binding activity of immunoadsorbents

Increasing volumes of blood plasma were applied to each immunoadsorbent. Immunoadsorption cycles were all carried out under the same operating conditions. As shown in Fig. 2, the amount of β_2-M desorbed from each im-

Table 2
Comparative study of the anti-β_2-M monoclonal antibodies as affinity ligands

Antibody	Antibody density on the adsorbent (mg/ml of support)	Adsorption capacity (μg of β_2-M/ml of support)	Conditions of desorption: % of β_2-M desorbed with		
			Acidic pH	Basic pH	Chaotropic agent
GG_6A_7	2.18	4.80	50–55	ND[a]	95–100
EH_1C5	2.39	93.00	95–100	ND	95–100
EG_4A_7	1.94	2.00	50–55	ND	95–100
GB_5C_7	2.41	127.50	95–100	10–15	95–100

10 ml of plasma, containing 125 mg of β_2-M/l, were applied to immunoadsorbents (42 × 11 mm I.D.) prepared with the different antibodies. The perfusion flow-rate was 20.0 ml/h. Desorption was achieved with either 0.1 *M* triethanolamine–1 *M* NaCl (pH 10.5), 0.3 *M* glycine–HCl (pH 2.8) or 3 *M* NaSCN–0.1 *M* CH_3COONa (pH 6.0) solutions.
[a] ND = not determined.

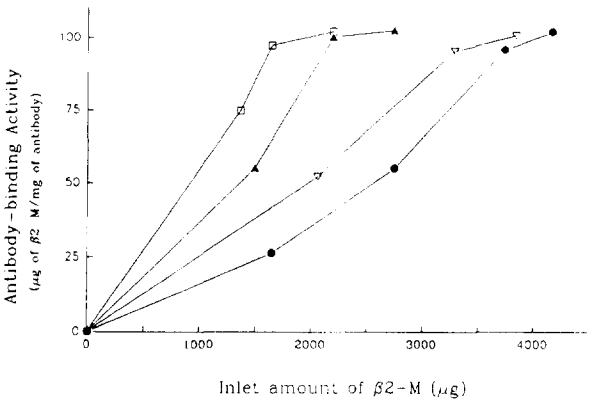

Fig. 2. Determination of the maximum antibody-binding activity of the immunoadsorbents. Increasing volumes of blood plasma, containing 27.2 mg of β_2-M/l, were applied at a flow-rate of 25 ml/h to several immunoadsorbents (53 × 11 mm I.D.) with various immobilized antibody densities. □ = 2.4; ▲ = 4.0; ▽ = 5.8; ● = 7.7 mg of antibody/ml of gel. Elution was achieved with 0.3 *M* glycine–HCl buffer (pH 2.8).

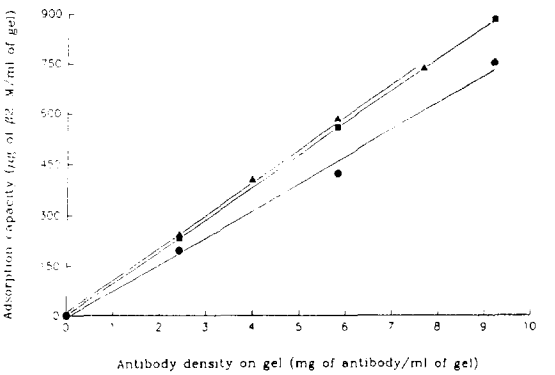

Fig. 3. Effect of antibody density on gel on adsorption capacity. Saturating volumes of plasma were applied at a flow-rate of 25 ml/h to immunoadsorbents (53 × 11 mm I.D.) with various immobilized antibody densities. Each curve corresponds to a given starting plasmatic β_2-M level. ■ = 6.4; ▲ = 27.2; ● = 52.0 mg/l. Elution was performed with 0.3 *M* glycine–HCl buffer (pH 2.8).

munoadsorbent increased with increasing inlet amount of β_2-M until the antibody-binding activity reached a plateau at a level of 100–105 μg of β_2-M/mg of antibody. At this point, the immunoadsorbent was fully saturated by antigen. Whatever the ligand density on the immunoadsorbent, saturation occurred with the same ratio of inlet β_2-M amount to the amount of immobilized antibodies.

Antibody density on the chromatographic support

To study the variations in adsorption capacity with the antibody density on the support, saturating volumes of plasma, determined to ensure a maximum binding of β_2-M, were applied to each immunoadsorbent. All runs were performed under the same experimental conditions, using plasma with different initial β_2-M levels.

The adsorption capacity of the immunoaffinity supports was found to increase linearly with the concentration of ligand on the matrix (Fig. 3). The antigen capture efficiency remained unchanged even for the more densely immobilized antibodies. This result was in good agreement with those mentioned above (Fig. 2). Further, the adsorption capacity of the immunoadsorbents was affected by the initial plasma β_2-M level. The antibody-binding activity decreased from 100 μg of β_2-M/mg of antibody with medium β_2-M levels down to 80 and 53 μg of β_2-M/mg of antibody when tested with plasma containing 52 and 125 mg of β_2-M/l, respectively (Table 2, Fig. 3).

Residence time

The influence of residence time (i.e., the ratio of the volume of adsorbent to flow-rate) on the antibody-binding capacity was investigated by applying saturating volumes of plasma to an immunoadsorbent (4.0 mg of antibody/ml of support) at different flow-rates.

As shown in Fig. 4, the antigen capture efficiency of the ligand remained almost unchanged for a residence time of at least 5 min. Higher flow-rates caused a gradual decrease in capture efficiency, which nevertheless remained effective at all residence times studied.

Multiple use of immunoadsorbents

Two columns were packed with an equivalent 5-ml volume of immunoadsorbent with an antibody coupling density of 4.0 mg of antibody/ml of support. Eight consecutive immunoadsorption cycles were performed over a 2-week period.

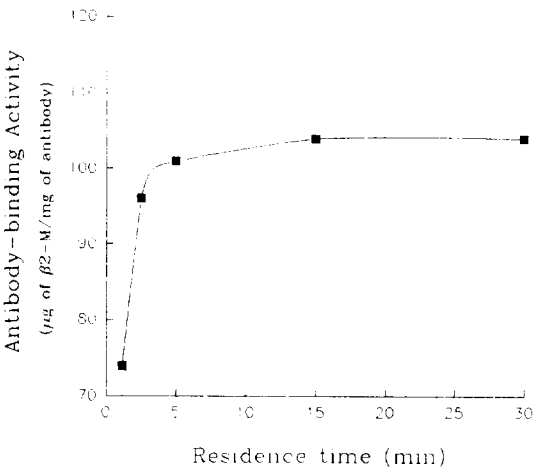

Fig. 4. Influence of the residence time on adsorption capacity. Saturating volumes of plasma, containing 52.0 mg of β_2-M/l, were applied to immunoadsorbent (53 × 11 mm I.D.) at flow-rates varying from 10 to 260 ml/h. The antibody density on the gel was 4.0 mg/ml. Elution was achieved with 0.3 *M* glycine–HCl buffer (pH 2.8).

Each run was achieved by passing through the two columns a saturating volume of the same sample of plasma (52.0 mg of β_2-M/l). The immunoadsorbents were regenerated using always for the same column either 0.3 *M* glycine–HCl (pH 2.8) or 3 *M* NaSCN–0.1 *M* CH_3OONa (pH 6.0) as desorption buffer. Between each run, the immunoadsorbents were stored at 4°C in PBS containing 0.02% NaN_3.

Whereas the adsorption capacity of the immunoadsorbent regenerated with acidic pH remained constant over runs, the desorption under chaotropic conditions led to a substantial decrease in β_2-M removal efficiency during the fourth chromatographic cycle. A slow and progressive decrease of about 2–8% was then observed during subsequent runs. The final adsorption capacity was found to be 55% of the initial performance.

4. Discussion

The development of an immunoadsorption procedure for the removal of β_2-M in patients with chronic renal failure is justified by the lack of specific treatment for dialysis-related amyloidosis. In fact, the strategies that have been developed until now to reduce β_2-M levels in uraemic patients have been based on more or less specific physical processes of protein extraction (filtration and/or adsorption), using highly permeable synthetic dialysis membranes or a hydrophobic porous adsorbent. However, even dialysis sessions three times a week do not lower the β_2-M total body burden to normal values. Under these conditions, immunoadsorption, due to its great efficiency and specificity, seems to be an attractive therapeutic approach.

The choice of antibody is of particular interest as it conditions the performance of the affinity system. Monoclonal antibodies appear to be the most convenient affinity ligands with regard to their homogeneous quality and their well defined characteristics in terms of affinity and specificity.

The four developed monoclonal antibodies are specifically directed against antigenic determinants situated on β_2-M, as indicated by the results of immunoblotting and of antibody inhibition experiments. However, not all of these antibodies can be used in an immunoadsorption procedure. In fact, only two of them conserve their β_2-M binding capacity after being immobilized on Sepharose. The antibody GB_5C_7 appears to be the most suitable affinity ligand for a use in an immunoaffinity system, as it exhibits both a good specificity and the highest adsorption capacity. Under optimum conditions of adsorption, its maximum binding capacity is 100–105 μg of β_2-M/mg of antibody, corresponding to a molar ratio of 1.33 mol of captured β_2-M per mole of antibody. The orientation of the molecules of ligand on the matrix is certainly responsible for the difference established between the theoretical maximum binding activity of IgG (i.e., 2 mol/mol) and the actual functionality of immobilized antibodies. During the coupling period, the antibody molecules are in fact randomly attached to the chromatographic support. The paratopes may become inaccessible to the antigens if the chemical binding between an active group of the support and the antibody occurs in the region of its Fab fragments.

Variations in antibody density on chromatographic supports do not affect the efficiency of adsorption. Even the more densely immobilized antibody molecules conserve their capacity to react with β_2-M molecules. This result shows that there is no steric hindrance phenomenon that could restrain the capture of antigen by the ligand. The optimum amount of antibody to be immobilized on an adsorbent is largely influenced by the molecular size of the antigen, as has been already described for several antibody–antigen systems [16,18]. For the immunoadsorption of LDL cholesterol, which is a very large molecule, the optimum adsorption capacity is observed with 4–6 mg of specific antibody immobilized per millilitre support. With transthyretin (M_r 55 000), this is achieved for antibody concentrations on the support $\geq$9 mg/ml. Taking relative small size of β_2-M molecules (M_r 11 800) into consideration, the result obtained in this study is not surprising. For a future clinical application, the dimensions of the column must be appropriate to the limited extracorporeal blood volume of the patient. The absence of steric hindrance will permit the use of restricted volumes of immunoadsorbent with elevated densities of coupled antibodies. Moreover, the useful volume of immunosorbent, allowing complete removal of β_2-M without being saturated, is easily determined as saturation occurs in proportion to the amount of immobilized antibody.

A common limiting factor for the efficiency of adsorption is the dilution of antigen in circulating plasma. The results of this study show that the immunoadsorbents can be used over a wide range of plasma β_2-M levels (6.4–52 mg/l), corresponding to the concentrations usually found in uraemic patients, without seriously impairing their adsorption capacity. The technique can be applied to all patients independently of the degree of β_2-M retention, and can conserve its efficiency as the β_2-M level is lowered during a plasma recirculation procedure.

β_2-M removal can occur with substantial flowrates with an acceptable decrease in the adsorption capacity of immunoadsorbents. In clinical situations, the duration of a specific plasmatic cleaning session could therefore be considerably reduced.

The possibility of regeneration and of multiple use of the immunoadsorbents with no significant loss of adsorption capacity contributes to minimizing the cost of the method. Two desorption agents appear to be efficient in the complete desorption of captured β_2-M. Of these, only 0.3 *M* glycine–HCl buffer (pH 2.8) allows the remaining of adsorption capacity over chromatographic runs, whereas 3 *M* NaSCN–0.1 *M* CH_3COONa (pH 6.0) solution leads to a progressive decrease in the binding performance of the immunoadsorbents. Further, glycine has the advantage of being less drastic for immobilized antibodies and adsorbed proteins than the chaotropic agent.

Under the optimum conditions, this immunoadsorption system provides a highly specific way to remove β_2-M from plasma of uraemic patients. Nevertheless, some studies are still needed before practical use of this method can be envisaged. An important consideration concerns especially the stability of the immunoaffinity supports over the range of chemical and biochemical conditions to which they are subjected. The fragility of the matrix, and the possible lability of the chemical bonds between the support and the antibody molecules, are responsible, among other factors, for the release of antibodies from carrier material. The corresponding contamination of plasma with released antibodies may lead to side-effects resulting from the development of a humoral antibody response in patients undergoing repeated treatments. The reported observations about LDL cholesterol immunoadsorption, which has been used in clinical practice for many years, indicate that severe unwanted effects requiring interruption of the therapy rarely occur [29]. The development of an immunization is demonstrated in most of the patients, but there is no evidence for a correlation between the presence or absence of immunization and clinical reactions of the patients. With regard to the method described here, and in spite of these encouraging results, the levels of antibody release must be carefully evaluated and

lowered as far as possible, in order to ensure its safety under the conditions applied. Data from the literature show that the leakage of antibody molecules is unavoidable, but the extent of this phenomenon can be significantly minimized by a judicious choice of the matrix, of the pH conditions used during the chromatographic cycle and of the activation procedure employed for the preparation of the immunoadsorbents [30]. In this study, Sepharose CL-4B and CNBr activation were selected because they are the most widely used in clinical trials without reported side-effects. Nevertheless, from the viewpoint of antibody release and adsorption efficiency, several other chromatographic supports and activation methods should be compared. In addition, study of the biocompatibility of the immunoadsorption system should receive particular attention. Undesirable interactions between the immunoaffinity support and plasma components, such as those of the complement cascade or coagulation factors, should be elucidated. This last aspect should not be a hindrance to the development of an extracorporeal immunoadsorption device. In fact, recently reported data about LDL-apheresis show that Sepharose CL-4B coupled to antibodies can be considered to be safe as carrier material under well defined operating conditions [31].

Finally, a parallel study is in progress with the aim of developing immunoadsorbents from hollow-fibre systems, which could be used with complete blood [32]. These matrices would improve the comfort of the patient by allowing the specific removal of β_2-M, directly during dialysis sessions, without needing a previous step of separation of blood cells and plasma.

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