Journal of Chromatography, 563 (1991) 166–171
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam

CHROMBIO. 5602

Note

Immunoadsorbents for clinical use: *ex vivo* immunoglobulin E removal in allergy[a]

V. D. GORCHAKOV* and K. I. SAKODYNSKII

Research Production Association "IREA", Bogorodski val. 3, 107258 Moscow (U.S.S.R.)

and

Yu. S. LEBEDIN and A. G. CHUCHALIN

Institute of Pulmonology, Academy of Medical Sciences, Moscow (U.S.S.R.)

(First received April 26th, 1990; revised manuscript received August 8th, 1990)

Immunoadsorbents have a high specificity for the *ex vivo* removal of certain components from plasma. In some instances the effective removal of a pathological component from plasma may be achieved only by means of affinity chromatography, including extracorporeal immunoadsorption. This is particularly true for high-molecular-mass substances of low plasma concentration and broad cross-reactivities of physico-chemical properties, such as immunoglobulin E (IgE). This type of Ig (relative molecular mass *ca.* 190 kilodalton), which plays a major pathogenetic role in atopic diseases, has an extremely low plasma level (several μg/ml); other Igs with very similar relative molecular masses (IgG, monomeric IgA) are present in plasma at much higher concentrations (up to 25 mg/ml).

Hence a therapeutic immunoadsorbent for *ex vivo* IgE removal must meet very strict requirements, such as extremely high specificity, plasma compatibility, good flow performance and sterilizability. As atopic patients are obviously prone to non-specific allergic reactions, there must also be specific requirements for minimum ligand leakage.

Attempts to elaborate effective IgE *ex vivo* removal systems were made earlier [1–3]. In this paper we describe the use of anti-IgE antibody immunoadsorbents on agarose (Sepharose CL-4B) and synthetic (Toyopearl AF Tresyl) supports.

---

[a] Presented at the *11th International Symposium on Biomedical Applications of Chromatography and Electrophoresis, Tallinn, April 24–28th, 1990.*

EXPERIMENTAL

Agarose carrier (Sepharose CL-4B; Pharmacia, Uppsala, Sweden) was activated by cyanogen bromide using standard method. Free reactive groups were blocked by ethanolamine (Serva, Heidelberg, F.R.G.). A 1-ml volume of gel was reacted with 5 mg of rabbit polyclonal anti-human IgE (γ-globulin fraction) from Dakopatts (Denmark).

Synthetic carrier (Toyopearl AF Tresyl 650M; Toyo Soda, Japan) was incubated in 0.1 *M* sodium hydrogencarbonate + 0.6 *M* saline solution for 10 min; 10 mg of anti-IgE preparation were added to 1.0 g of carrier and the mixture was incubated for 18 h at room temperature. The immunoadsorbent was washed on a sintered-glass filter with an excess of 0.5 *M* saline and 0.1 *M* Tris–HCl buffer (pH 8.0). Final washing was performed with 0.14 *M* saline until the eluate pH was $\geqslant 7.3$.

The equilibrium-specific adsorptive capacity (ESAC) was determined by incubation of a small volume of immunoadsorbent (*ca.* 0.2 ml) with 2.0 ml of standard plasma with a high IgE concentration and 50–100 μl of ^{125}I-labelled myeloma IgE ND (Pharmacia). Incubation was maintained for 2 h at room temperature with continuous mixing, then the immunoadsorbents were washed three times with 0.1 *M* phosphate-buffered saline plus 0.1% Tween-20 and counted in a gamma-counter. ESAC was expressed as μg of IgE per gram of immunoadsorbent dry weight.

The flow-through specific adsorption capacity was determined as follows: 1.0 ml of immunoadsorbent was placed in a plastic chromatographic column (Econo-Column; Bio-Rad Labs., Richmond, CA, U.S.A.) and perfused with standard plasma at a constant flow-rate (0.83 ml/min). The outlet IgE concentration in the fractions was determined with a commercial radioimmunoassay kit (IgE RIA; Pharmacia).

Clinical anti-IgE immunoadsorption was performed as described previously [4].

RESULTS AND DISCUSSION

The immobilization of antibodies, including anti-IgE antibodies, on cyanogen bromide-activated Sepharose has been well characterized [1]. However, such data for the newly introduced matrix Toyopearl AF Tresyl were not available.

Fig. 1 shows the kinetics of antibody immobilization on Tresyl carrier. The major part of the protein becomes immobilized during the first 12–15 h of incubation.

The dependence of the ESAC on the antibody protein load is shown in Fig. 2. The ESAC value increases proportionally with the antibody load. In order to determine the optimum antibody load, we calculated the relative ESAC (adsorbed IgE/immobilized antibody protein, w/w). The U-shaped curve shown in

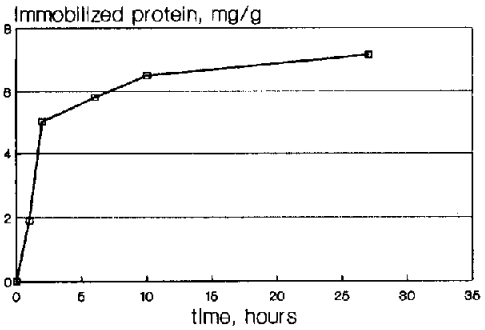

Fig. 1. Immobilization of polyclonal anti-IgE antibodies on activated Toyopearl AF Tresyl matrix.

Fig. 3 was obtained. Presumably, at low antibody load the optimum ESAC is determined by the good steric availability of immobilized protein, whereas at high load poor steric properties may be compensated for by less denaturing (single-point) fixation of the antibody molecule.

The adsorption isotherm of IgE on Toyopearl at 20°C (Fig. 4) shows a clear pseudo-linear pattern with a calculated distribution coefficient $D = 96 \pm 3$, indicating that the Toyopearl immunoadsorbent is highly effective. However, under non-saturating (flow-through) conditions, the Toyopearl immunoadsorbent showed a wave-shaped adsorption curve (Fig. 5). The ESAC of the tested immunoadsorbent was calculated from the adsorption curve (916 μg/g) and the total volume perfused (898 μg/g).

Therefore, under flow-through conditions, the Toyopearl immunoadsorbent,

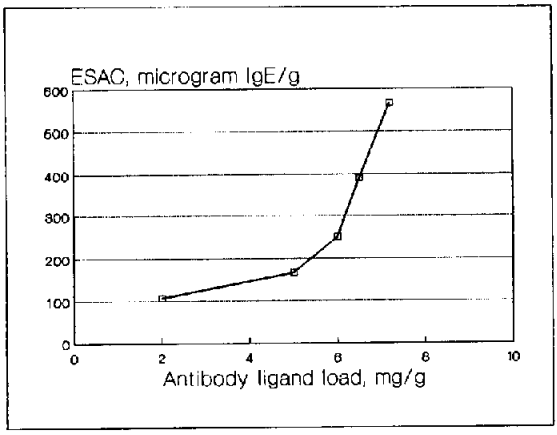

Fig. 2. Equilibrium-specific adsorptive capacity (ESAC) of Toyopearl anti-IgE immunoadsorbents with different antibody load.

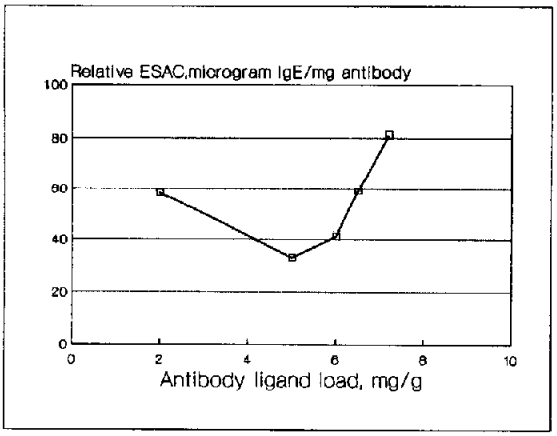

Fig. 3. Relative ESAC of Toyopearl anti-IgE immunoadsorbents with different antibody load.

is less effective than the agarose immunoadsorbent and is comparable to the Bionit polymer matrix described in a previous paper [5]; the wave-shaped outlet concentration curve is possibly related to the steric hindrances during immune complex formation owing to an insufficient pore diameter.

The clinical immunoadsorption procedures were performed using an agarose carrier (Sepharose CL-4B), prepared under aseptic conditions, in an atopic asthma patient with an elevated plasma IgE level.

The efficiency and kinetics of *ex vivo* IgE removal were determined using a previously developed mathematical model [6].

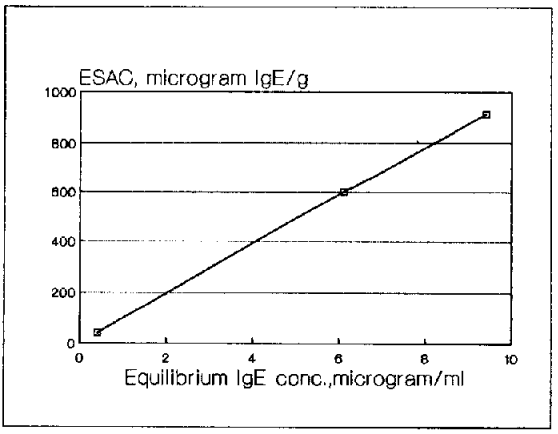

Fig. 4. Adsorption isotherm of IgE on polyclonal anti-IgE Toyopearl immunoadsorbents at 20°C.

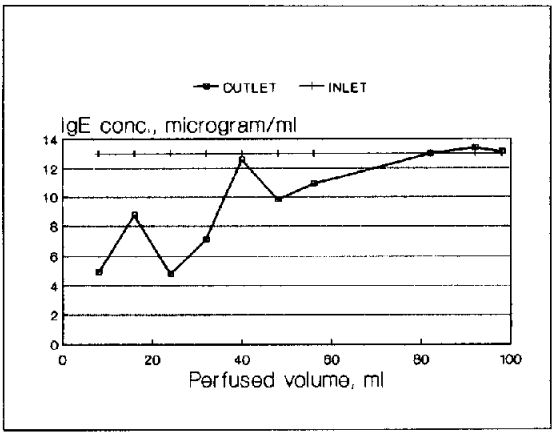

Fig. 5. IgE adsorption curve of polyclonal anti-IgE Toyopearl immunoadsorbent column (immunoadsorbent volume 0.5 ml, plasma flow-rate 0.83 ml/min). The initial IgE concentration was 13.1 μg/ml (+); □, outlet concentration.

The absolute amount of IgE removed from plasma may be calculated in two ways: from the difference in serum concentrations before and after treatment (M_d) [1] and from the in-procedure adsorption curve (M_c) (see Fig. 6) [2]. Considering a single immunoadsorption treatment and assuming the circulating plasma volume of the patient to be 2.2 l, the calculated M_d value was 781 μg. The calculation of M_c using the equation

$$M_c = \int_0^V (C_{in} - C_{out})\, dV$$

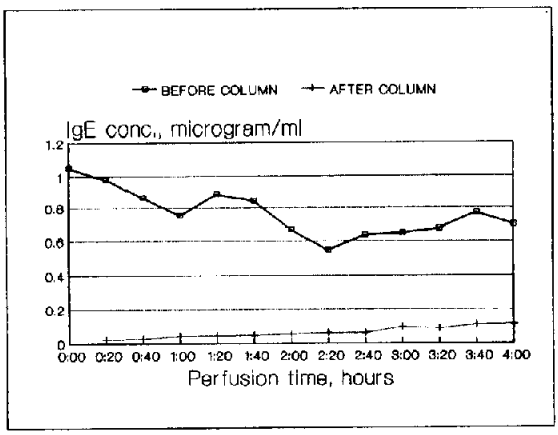

Fig. 6. *Ex vivo* IgE adsorption curve by anti-IgE Sepharose CL-4B. Perfusion rate, 12–15 ml/min. □, Before column; +, after column.

where C_{in} and C_{out} are the concentrations of IgE at the column inlet and outlet and V is the perfused volume, gave a value of 2352 μg.

This simple calculation indicates the possible involvement of tissue depot in *ex vivo* IgE removal by immunoadsorption.

CONCLUSIONS

The relative adsorptive capacity of anti-IgE antibodies, immobilized on the polymer carrier Toyopearl AF Tresyl, shows two optima, at low and high ligand load. The equilibrium-specific adsorptive capacity of Toyopearl anti-IgE immunoadsorbent is comparable to that of agarose-based immunoadsorbent; however, Toyopearl immunoadsorbent is much less effective under flow-through (non-equilibrium) conditions. A Sepharose CL-4B matrix is preferential for *ex vivo* IgE removal by immunoadsorption. A clinical trial with anti-IgE immunoadsorbent showed its high efficiency in terms of *ex vivo* IgE removal.

ACKNOWLEDGEMENT

This work was supported in part by the Council for Molecular Biology and Medical Biotechnology, Academy of Medical Sciences, U.S.S.R., Grant No. 072/1988–89.

REFERENCES

1 H. Sato, T. Kidaka and M. Hori, *Artif. Organs*, 7A (1983) 703.
2 R. Behm, T. Kuroda, N. Yamawaki *et al.*, *Biomater. Artif. Cells Artif. Organs*, 15 (1987) 101.
3 H. Sato, K. Watanabe, J. Azuma, T. Kidaka and M. Hori, *J. Immunol. Methods*, 118 (1989) 161.
4 Yu. S. Lebedin, T. Kormos, Yu. I. Zimin and A. G. Chuchalin, *Immunologiya*, No. 2 (1988) 69.
5 A. G. Chuchalin, V. D. Gorchakov, Yu. S. Lebedin, T. K. Avdyushkina and K. I. Sakodynskii, *Bioorg. Khim.*, 14 (1988) 1524.
6 V. D. Gorchakov, V. I. Sergienko and V. G. Vladimirov, *Selective Haemosorbents*, Meditsina, Moscow, 1989, pp. 94–98.