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Selective Adsorption of Immunoglobulin G and Immunoglobulin M from Plasma without Adsorption of Fibrinogen by Using Thienyl Amino Acids as Ligands

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Abstract

In vitro adsorption of plasma proteins such as immunoglobulin (Ig)-G and IgM without the removal of albumin and fibrinogen forms the basis of the field of therapeutic immunoadsorption. In the present study, we investigated the activities of adsorbents with thienyl amino acids, tryptophan, or phenylalanine as ligands. IgG adsorption by an adsorbent with a thienyl amino acid ligand was lower than that by an adsorbent with a tryptophan ligand but higher than that by an adsorbent with a phenylalanine ligand. On the other hand, IgM adsorption by an adsorbent with a thienyl amino acid ligand was higher than that by adsorbents with tryptophan and phenylalanine ligands. The adsorption of fibrinogen onto the adsorbent with a thienyl amino acid ligand was far lesser than its adsorption onto the adsorbents with tryptophan and phenylalanine ligands. Adsorbents with thienyl amino acid ligands may therefore be useful for removing antibodies produced in autoimmune diseases. Further *in vitro* biocompatibility studies, *in vivo* animal studies, and clinical trials are required to confirm these results.

Keywords: Immunoadsorption; Immunoglobulin G; Immunoglobulin M; Fibrinogen; Adsorbent; Thienyl amino acid

Introduction

In the recent decades, many technologies for separating and purifying antibody proteins have been reported.

In the field of antibody and protein purification, columns that have a ligand with affinity for antibodies and proteins, where the ligand may be an antibody [1], protein A [2-5], protein G [6], or a peptide [7-8], have been developed. However, these ligands are unstable and costly. Purification columns with low-molecular-weight compounds as ligands have also been developed [9-12]. These low-molecular-weight compounds contain the sulfur atom and have been reported to adsorb IgG antibodies through "thiophilic interactions."

Recently, therapeutic immunoadsorption, an extracorporeal treatment in which plasma antibodies are selectively adsorbed, has been used to successfully treat various patients with autoimmune diseases by using adsorbents with ligands such as protein A [13,14], tryptophan [15,16], phenylalanine [17,18], synthetic peptides [19,20], or polyclonal sheep antibodies against human IgG [21]. These adsorbents have been used as a treatment option for several autoimmune disorders such as rheumatoid arthritis, idiopathic thrombocytopenic purpura, hemophilia with inhibitors, systemic lupus erythematosus, myasthenia gravis, and Guillain-Barré syndrome. These adsorbents contain ligands such as protein A, synthetic peptides, and polyclonal antibodies, which are expensive and enzymatically unstable because the adsorbent ligands are biological molecules.

In contrast, the adsorbents Immusorba TR (IM-TR) and PH (IM-PH) (Asahi Kasei Medical, Tokyo, Japan) have tryptophan and phenylalanine as simple ligands, respectively. These ligands are small molecules that are stable and inexpensive. Therefore, they have been widely used in clinical settings for the medical treatment of autoimmune diseases.

IM-TR and IM-PH adsorb and remove autoantibodies (mainly IgG and IgM) from the blood plasma through hydrophobic interactions of their aromatic ring with tryptophan or phenylalanine, respectively. However, the risk of bleeding increases with the use of IM-TR and IM-PH as the number of treatments increases; this is because the amount of fibrinogen in the blood also decreases through adsorption. This necessitated the development of a stable and inexpensive ligand that adsorbs and removes antibodies such as IgG and IgM selectively without adsorbing fibrinogen.

We have searched for a small-molecule ligand that fulfills the abovementioned requirements. We have previously reported that thiophen, which is a simple compound containing a sulfur atom, has high affinity for autoantibodies in pemphigus [22]. However, the adsorption of fibrinogen was not investigated in our previous study.

Therefore, the purpose of this study was to investigate whether an adsorbent with a low-molecular-weight ligand containing the above mentioned thienyl group could produce a hydrophobic and thiophilic interaction to fully adsorb antibodies without adsorbing fibrinogen.

For this purpose, we prepared porous beads of IM-TR and IM-PH that were covalently complexed with 4 thiol compounds (L-α-3-thienylglycine, β-2-thienyl-DL-serine, β-2-thienyl-D-alanine, and thiophene-2-ethylamine) or 5 types of amino acids (glycine, L-alanine, L-serine, L-phenylalanine, and L-tryptophan). The beads were covalently bound with a low-molecular-weight compound, and then plasma protein adsorption was examined *in vitro*. An adsorbent containing thienyl amino acid as the ligand was found to adsorb IgG and IgM without fibrinogen adsorption. These results suggest that an

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absorbent with a thienyl amino acid ligand is suitable for eliminating antibodies produced in autoimmune diseases or removing IgG and IgM in ABO-incompatible organ implantation. Further *in vitro* biocompatibility studies, *in vivo* animal studies, and clinical trials are necessary.

Materials and Methods

Blood plasma collection

Plasma obtained from 3 healthy volunteers was subjected to adsorption experiments for plasma proteins (albumin, IgG, IgM, and fibrinogen). Written informed consent was obtained from all subjects.

Preparation of adsorbents

A gel containing 100 g of porous beads made from polyvinyl acetate (Asahi Glass Co., Ltd. Japan; average particle diameter, 100 μ; elimination limit molecule quantity, more than 1 million) was diluted in 1 L of dimethyl sulfoxide (DMSO; Wako Pure Chemical Inc., Japan).

Subsequently, 750 mg of sodium borohydride, 780 mL of epichrolohydrin, and 120 g of sodium hydroxide, all from Wako Pure Chemical Inc., were poured into the bead-DMSO mixture and reacted at 30°C for 5 H, and epoxy groups were introduced to the porous beads.

After the reaction, the porous beads were washed with methanol (Wako Pure Chemical Inc.) and then washed with distilled water.

More than 110 μeq of epoxy groups was introduced per milliliter of the porous bead gel by a titration method, and 2 drops of 1% phenolphthalein in ethanol (Wako Pure Chem, Inc., Japan) were dropped into 4 mL of 1.3 mmol/L sodium thiosulfate solution (Wako Pure Chemical Inc.) containing 2 mL of the activated porous bead gel. Next, 0.1N chloride was added until red coloration was no longer observed at 70°C. The quantity of epoxy groups introduced was calculated by the following formula: amount of activation (quantity of epoxy group)=chloride titer/amount of resin×100.

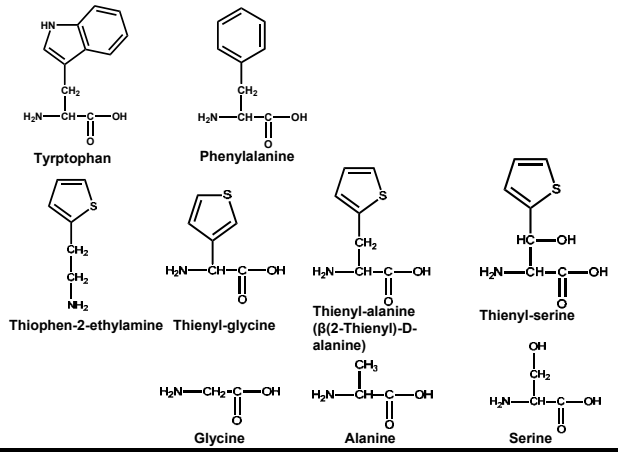
Ligand immobilization

A carbonic acid buffer solution, pH 9.3 (sodium carbonate/sodium bicarbonate; Wako Pure Chemical Inc.), was used as the solvent, and the ligand was dissolved so that 50 μeq/mL gel would be obtained as the amount of immobilization.

The ligand solution and the activated porous beads gel were mixed to permit reaction at 50°C for 16 h. Covalent bonds between amino groups of the ligand and the epoxy groups of the porous bead gel were formed, and the adsorbent was obtained.

Thienyl-glycine (L-α-3-thienylglycine),thienyl-serine (β-(2-thienyl)-DL-serine), thienyl-alanine (β-(2-thienyl)-D-alanine), thiophene-2-ethylamine, glycine, L-alanine, L-serine, L-phenylalanine, and L-tryptophan were used as ligands. All compounds were purchased from Sigma-Aldrich, St. Louis, USA.

The amount of immobilized ligands per milliliter of gel (eq/mL gel) was calculated using the wavelength between 200 and 280 nm with the highest absorption in the solution before and after the immobilization reaction. Almost 50 μeq of each compound was covalently immobilized per milliliter of gel.



Ligand	adsorption rate (%) Alb	IgG	Fbg	IgM	IgG, IgM adsorption ratio against Fbg IgG/Fbg	IgM/Fbg
Tryptophan	19.0	57.7	85.9	41.6	0.7	0.5
Phenylalanine	19.3	32.3	35.7	37.3	0.9	1.0
Thiophene-2-ethylamine	25.7	53.5	93.1	30.0	0.6	0.3
Glycin	16.9	22.6	17.6	21.6	1.3	1.2
Thienyl-glycine	17.7	39.6	6.2	44.9	6.3	7.2
Alanine	16.3	20.7	31.1	19.9	0.7	0.6
Thienyl-alanine	14.1	42.4	9.6	42.7	4.4	4.5
Serine	14.7	22.2	27.7	21.2	0.8	0.8
Thienyl-serine	15.4	37.3	19.3	41.0	1.9	2.1

Figure 1: Chemical formula of ligands and adsorption rates for albumin, IgG, IgM, and fibrinogen.
The chemical structure of ligands is shown. There were 4 thiol compounds (L-α-3-thienylglycine, β-2-thienyl-DL-serine, β-2-thienyl-D-alanine, and 2-thiophene ethylamine) and 5 types of amino acids (glycine, L-alanine, L-serine, L-phenylalanine, and L-tryptophan). The adsorbent (1.0 mL) was placed into a 2.5-mL column, and 3.0 mL of healthy human plasma was run through the column at a flow velocity of 0.3 mL/min for 10 min, and all of the plasma that was eluted from the column was collected. Protein adsorption rate (%) was calculated as (Cin−Cout)/Cin×100, where Cin is the concentration of the solute before adsorption and Cout is the concentration of the solute after adsorption. Mean adsorption rates for samples from 3 healthy volunteers are shown. IgG and IgM adsorption relative to fibrinogen adsorption was evaluated using the mean adsorption rates for IgG, IgM, and fibrinogen.

Adsorption experiment by column flow

The adsorbent (1.0 mL) was placed into a 2.5-mL laboratory column (Molecular Biotechnology, USA), and 3.0 mL of plasma obtained by centrifuging from healthy human blood supplemented with CPD at a ratio of 1:8 (capacity ratio) was run through the column at a flow velocity of 0.3 mL/min for 10 min by using a syringe pump, and all of the plasma that was eluted from the column was collected.

Analysis

The plasma was sampled before and after being run through the column. The concentration of IgG and IgM was measured by immunonephelometry, the concentration of albumin was determined by nephelometry, and the concentration of fibrinogen was measured by the thrombin clotting time method.

The adsorption rate (%) was calculated as (Cin−Cout)/Cin×100, where Cin is the concentration of the solute before adsorption and Cout is the concentration of the solute after adsorption.

Statistical analysis

Data are presented as the mean ± standard deviation (SD) and analyzed using the Student's *t*-test. P<0.05 was considered statistically significant.

Results and Discussion

The purpose of this study was to determine whether an adsorbent with a low-molecular-weight compound with a thienyl group as a ligand could fully adsorb an antibody without adsorbing fibrinogen.

To evaluate ligands with different chemical structures, porous IM-TR and IM-PH beads were used. The chemical structure of the ligands are shown in Figure 1. The ligands used were tryptophan and phenylalanine, which are normally used in IM-TR and IM-PH; thiophene-2-ethylamine, which adsorbs antibodies against pemphigus; thienylalanine, which is thiophene-2-ethylamine with a carboxyl group attached; thienylserine, which is thienylalanine with a hydroxyl group attached to the side chain; thienylglycine, which is thienylalanine without the methylene chain; and the 3 amino acids (glycine, alanine, and serine) corresponding to these thienyl amino acids.

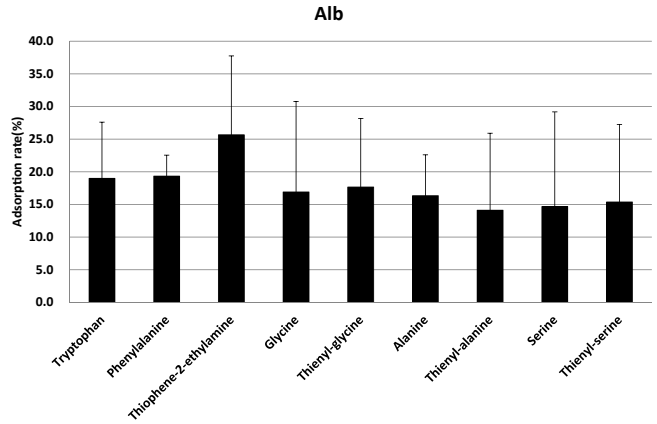


Figure 2: Column flow adsorption for albumin.
The adsorbent (1.0 mL) was placed into a 2.5-mL column, and 3.0 mL of healthy human plasma was run through the column at a flow velocity of 0.3 mL/min for 10 min, and all of the plasma that was eluted from the column was collected. Protein adsorption rate (%) was calculated as (Cin−Cout)/Cin×100, where Cin is the concentration of the solute before adsorption and Cout is the concentration of the solute after adsorption. The mean ± SD values for the adsorption rates in samples from 3 healthy volunteers are presented.

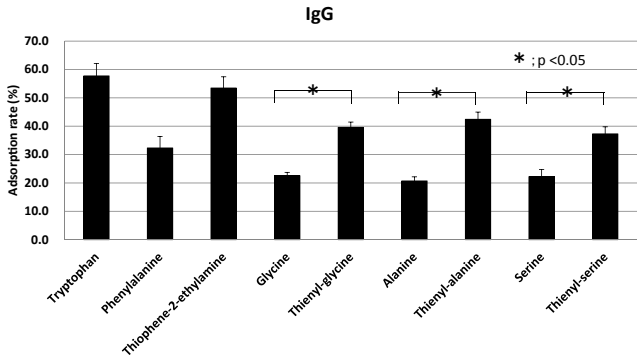


Figure 3: Column flow adsorption for IgG.
The adsorbent (1.0 mL) was placed into a 2.5-mL column, and 3.0 mL of healthy human plasma was run through the column at a flow velocity of 0.3 mL/min for 10 min, and all of the plasma that was eluted from the column was collected. Protein adsorption rate (%) was calculated as (Cin−Cout)/Cin×100, where Cin is the concentration of the solute before adsorption and Cout is the concentration of the solute after adsorption. The mean ± SD values for the adsorption rates in samples from 3 healthy volunteers are given.

Additionally, the average adsorption of albumin, IgG, fibrinogen, and IgM by each ligand, the ratio of the adsorption rate of IgG against the adsorption rate of fibrinogen, and the ratio of the adsorption rate of IgM against the adsorption rate of fibrinogen are shown in Figure 1.

Albumin adsorption

The adsorption rate for albumin is shown in Figure 2. Although thiophene-2-ethylamine showed slightly higher absorption of albumin, but there was no difference among the used adsorbents. Thus, the thienyl amino acid ligands showed the same level of adsorption of albumin as IM-TR and IM-PH, which are clinically used.

IgG adsorption

The adsorption rate for IgG is shown in Figure 3. The order of the adsorption rate for IgG was tryptophan, thiophene-2-ethylamine, thienyl-alanine, thienyl-glycine, thienyl-serine, and phenylalanine; the other ligands showed little adsorption of IgG. It has been reported that thiophene-2-ethylamine shows higher absorption of the antibody against pemphigus than tryptophan [22]. However, with regard to the adsorption of all IgGs, thiophene-2-ethylamine was inferior to tryptophan. It therefore appears that thiophene-2-ethylamine has specific affinity for the IgG antibody against pemphigus. The 3 thienyl amino acids showed slightly higher adsorption of IgG than phenylalanine. However, 3 amino acids (glycine, L-alanine, L-serine) that did not have a thienyl group did not adsorb IgG. Thus, the thienyl group interacts strongly with IgG. It was reported "thiophilic interaction" that a ligand that contains a sulfone group in proximity to a thioether group highly interacts immunoglobulinsby Porath et al. [9,10]. However, that mechanism of interaction between a ligands and proteins The mechanism of "thiophilic interaction", which is not possible fully understood, but Porath et al. speculated that it may involve an electro-donor/acceptor mechanism or proton transfer between protein surface-accessible aromatic amino acids and the sulfone-thioether sulfur atoms in the ligand [23].

Recently, Ren et al. demonstrated that a 4-mercaptoethylpyridine-based adsorbent could remove antibodies against double-stranded DNA and rheumatoid factor from patient serum samples *in vitro* [24]. 4-Mercaptoethylpyridine is a heterocyclic aromatic compound containing nitrogen with a thiol group. In our study, adsorbents whose ligand is an aromatic compound tended to have a strong affinity to

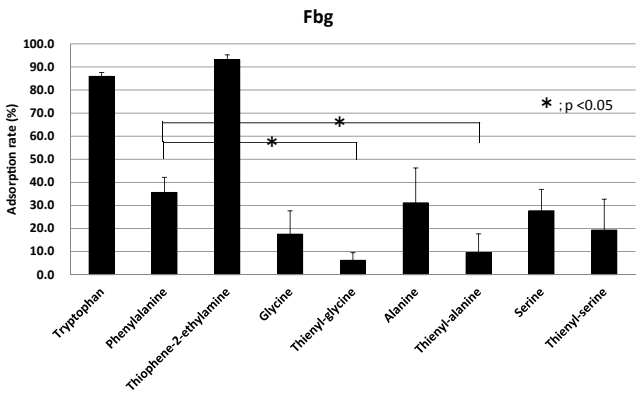


Figure 4: Column flow adsorption for IgM.
The adsorbent (1.0 mL) was placed into a 2.5-mL column, and 3.0 mL of healthy human plasma was run through the column at a flow velocity of 0.3 mL/min for 10 min, and all of the plasma that was eluted from the column was collected. Protein adsorption rate (%) was calculated as (Cin−Cout)/Cin×100, where Cin is the concentration of the solute before adsorption and Cout is the concentration of the solute after adsorption. The mean ± SD values for the adsorption rates in samples from 3 healthy volunteers are given.

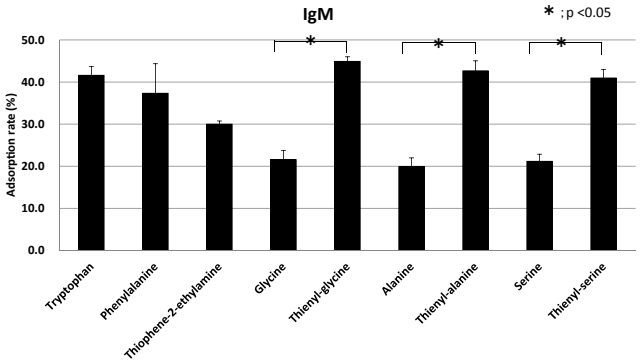


Figure 5: Column flow adsorption for fibrinogen.
The adsorbent (1.0 mL) was placed into a 2.5-mL column, and 3.0 mL of healthy human plasma was run through the column at a flow velocity of 0.3 mL/min for 10 min, and all of the plasma that was eluted from the column was collected. Protein adsorption rate (%) was calculated as (Cin−Cout)/Cin×100, where Cin is the concentration of the solute before adsorption and Cout is the concentration of the solute after adsorption. The mean ± SD values for the adsorption rates in samples from 3 healthy volunteers are given.

autoantibodies. In addition to the aromatic ring, sulfur may also be involved in the affinity between the thienyl group and autoantibodies.

Fibrinogen adsorption

The adsorption rate for fibrinogen is shown in Figure 4. The fibrinogen adsorption rates of the 3 thienyl amino acids (thienyl-glycine, thienyl-alanine, and thienyl-serine) were lower than those of the 3 corresponding amino acids (glycine, alanine, and serine). Surprisingly, thienyl-glycine and thienyl-alanine hardly adsorbed fibrinogen in this experiment. In contrast, the adsorption rate of fibrinogen was high for thiophene-2-ethylamine, tryptophan, and phenylalanine, in that order.

Although the fibrinogen adsorption rate of thiophene-2-ethylamine was high, low fibrinogen adsorption by thienyl-alanine, which is thiophene-2-ethylamine with a carboxyl group, was observed.

Thus, the adsorption of fibrinogen seems to be controlled when carboxylic acid is on the surface of the adsorbent, probably because the pKa of fibrinogen in the plasma is more acidic side than that of the other proteins (i.e., Alb, IgG, and IgM).

In contrast, the higher preference of tryptophan for fibrinogen is thought to arise from tryptophan's bulky indole ring because the hydrophobic interaction and electrostatic interaction between the indole ring and fibrinogen are stronger than the interaction between carboxylic acid and fibrinogen.

IgM adsorption

The adsorption rate for IgM is shown in Figure 5. The 3 thienyl amino acids (thienyl-glycine, thienyl-alanine, and thienyl-serine) adsorbed IgMmore than equivalent as same as tryptophan. IgM was adsorbed by phenylalanine and thiophene-2-ethylamine, in that order; the other ligands hardly adsorbed IgM. This finding shows that the thienyl group strongly interacted with IgM. In contrast, unexpectedly, IgM adsorption of thiophene-2-ethylamine was low. As described above, interactions with carboxyl groups likely contributed to adsorption of IgM. Thus, the lack of a carboxyl group in thiophene-2-ethylamine may result in a weaker interaction with IgM.

Although the hydrophobic interaction mediated by the aromatic ring of tryptophan and phenylalanine and the "thiophen" interaction mediated by thienyl groups likely contributed the most to the interaction with IgM, contribution by the electrostatic interaction mediated by carboxyl groups is also possible.

IgG/Fbg and IgM/Fbg

The ratio of the adsorption rate for IgG to the adsorption rate for fibrinogen (IgG/Fbg) and the ratio of the adsorption rate for IgM to the adsorption rate for fibrinogen (IgM/Fbg) are shown in Figure 6.

The 3 thienyl amino acids (thienyl-glycine, thienyl-alanine, and thienyl-serine) had high IgG/Fbg and IgM/Fbg ratios relative to the other ligands. As described above, these amino acids did not adsorb fibrinogen, and they selectively adsorbed IgG and IgM.

Conclusion

Thienyl amino acids (thienyl-glycine, thienyl-alanine, and thienyl-serine) hardly adsorb fibrinogen, but they selectively adsorb IgG and, especially, IgM. This finding is relevant for efficient plasma purification

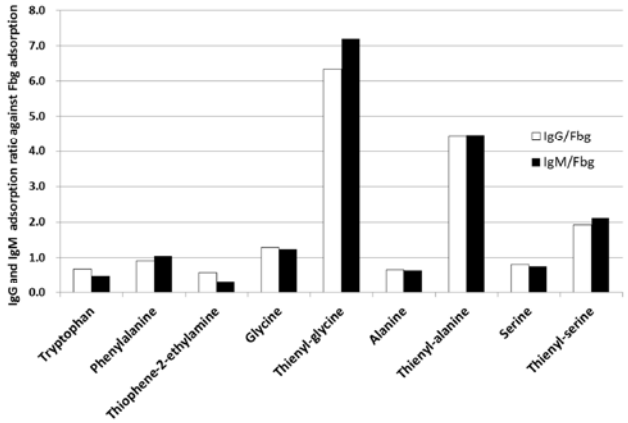


Figure 6: Ratio of IgG and IgM adsorption to fibrinogen absorption measured by column flow adsorption.
The ratio of IgG and IgM adsorption to fibrinogen adsorption was evaluated from the mean adsorption rates for IgG, IgM, and fibrinogen obtained using a column flow method with adsorbents containing the 4 thiol compounds and 5 types of amino acids.

in clinical settings. Because, temporarily, although the IgG adsorption ratio of thienylglycin is about 2/3 of IM-TR, if volume of adsorbent is increased 1.5 times, for example, IgG adsorption ratio estimate almost the same as IM-TR. In that case, although the fibrinogen adsorption ratio of thienylglycine is 1.5 times (fibrinogen adsorption rate is 9.3%) and it is low enough as compared with the fibrinogen adsorption rate of IM-TR. Therefore, adsorbents with thienyl amino acids may be useful for removing antibodies produced in autoimmune diseases or removing IgG and IgM in ABO-incompatible organ implantation. Further *in vitro* biocompatibility studies, *in vivo* animal studies, and clinical trials are needed.

References

1. Mellman IS, Unkeless JC (1980) Purificaton of a functional mouse Fc receptor through the use of a monoclonal antibody. J Exp Med 152: 1048-1069.
2. Nakayama H, Withy RM, Raftery MA (1982) Use of a monoclonal antibody to purify the tetrodotoxin binding component from the electroplax of Electrophorus electricus. Proc Natl Acad Sci 79: 7575-7579.
3. Ansari AA, Chang TS (1983) Immunochemical studies to purify rabbit and chicken immunoglobulin G antibody by protein A-Sepharose chromatography. Am J Vet Res 44: 901-906.
4. Schmerr MJ, Goodwin KR (1991) Separation of ovine IgG1 and IgG2 on protein A-sepharose. Comp Immunol Microbiol Infect Dis 14: 289-295.
5. Zusman R, Beckman DA, Zusman I, Brent RL (1992) Purification of sheep immunoglobin G using protein A trapped in sol-gel glass. Anal Biochem 201: 103-106.
6. Faulmann EL, Boyle MD (1991) A simple preparative procedure to extract and purify protein G from group G streptococci. Prep Biochem 21: 75-86.
7. Palombo G, Rossi M, Cassani G, Fassina G (1998) Affinity purification of mouse monoclonal IgE using a protein A mimetic ligand (TG19318) immobilized on solid supports. J Mol Recognit 11: 247-249.
8. Yang H, Gurgel PV, Carbonell RG (2009) Purification of human immunoglobulin G via Fc-specific small peptide ligand affinity chromatography. J Chromatogr A 1216: 910-918.
9. Porath J, Maisano F, Belew M (1985) Thiophilic adsorption--a new method for protein fractionation. FEBS Lett 185: 306-310.
10. Hutchens TW, Porath J (1986) Thiophilic adsorption of immunoglobulins--analysis of conditions optimal for selective immobilization and purification. Anal Biochem 159: 217-226.
11. Belew M, Juntti N, Larsson A, Porath J (1987) A one-step purification method for monoclonal antibodies based on salt-promoted adsorption chromatography on a 'thiophilic' adsorbent. J Immunol Methods 102: 173-182.
12. Qian H, Li C, Zhang Y, Lin Z (2009) Efficient isolation of immunoglobulin G by paramagnetic polymer beads modified with 2-mercapto-4-mythyl-pyrimidine. J Immunol Methods 343: 119-129.
13. Belak M, Borberg H, Jimenez C, Oette K (1994) Technical and clinical experience with protein A immunoadsorption columns. Transfus Sci 15: 419-422.
14. Samuelsson G (2001) Extracorporeal immunoadsorption with protein A: technical aspects and clinical results. J Clin Apher 16: 49-52.
15. Yoshida M, Tamura Y, Yamada Y, Yamawaki N, Yamashita Y (2000) Immusorba TR and Immusorba PH: basics of design and features of functions. 1998. Ther Apher 4: 127-134.
16. Sato Y, Kimata N, Miyahara S, Nihei H, Agishi T, et al. (2000) Extracorporeal adsorption as a new approach to treatment of botulism. ASAIO J 46: 783-785.
17. Hirata N, Kuriyama T, Yamawaki N (2003) Immusorba TR and PH. TherApher Dial 7: 85-90.
18. Sugimoto K, Yamaji K, Yang KS, Kanai Y, Tsuda H, et al. (2006) Immunoadsorption plasmapheresis using a phenylalanine column as an effective treatment for lupus nephritis. Ther Apher Dial 10: 187-192.
19. Ranspeck W, Brinckmann R, Egner R, Gebauer F, Winkler D, et al. (2003) Peptide based adsorbers for therapeutic immunoadsorption. Ther Apher Dial 7: 91-97.
20. Rech J, Hueber AJ, Kallert S, Leipe J, Kalden JR, et al. (2008) Remission of demyelinating polyneuropathy with immunoadsorption, low dose corticosteroids and anti-CD20 monoclonal antibody. Ther Apher Dial 12: 205-208.
21. Kiseleva EA, Afanasieva OI, Kosheleva NA, Pokrovsky SN (1996) Immunosorbent for IgG apheresis: an *in vitro* study. Transfus Sci 17: 519-525.
22. Yamada H, Itoh A, Hatanaka Y, Tsukiji M, Takamori K (2010) Screening and analysis of adsorbents for pemphigus autoantibodies. Ther Apher Dial 14: 292-297.
23. Hutchens TW, Porath JO (1987) Protein recognition of immobilized ligands: promotion of selective adsorption. Clin Chem 33: 1502-1508.
24. Ren J, Jia L, Xu L, Lin X, Pi Z, et al. (2009) Removal of autoantibodies by 4-mercaptoethylpyridine-based adsorbent. J Chromatogr B Analyt Technol Biomed Life Sci 877: 1200-1204.