

Selective Adsorbent for the Removal of Immunoglobulin E in Bronchial Asthma

Yaoting Yu, Hongmei Zhang, Weichao Wang, and Shenqi Wang

The Key Laboratory of Bioactive Materials, Ministry of Education, Nankai University, Tianjin, 300071, P.R.China

Abstract: Agar-gel resin with tryptophan as ligand was synthesized as an adsorbent for the removal of Immunoglobulin E (IgE) in bronchial asthma. Factors affecting the adsorption properties of the adsorbent, such as the different kind of amino acid linked to the carrier, NaOH concentration and amount of epichlorohydrin used in activation, were studied in detail. The effect of spacer on the adsorption capacity was also investigated. Results showed that the agar-tryptophan adsorbent with a spacer could enhance the adsorption capacity to 70.73% (1309.403IU/g agar-gel), which was higher than that without spacers (53%). The selectivity and hemocompatibility of the adsorbent were also examined, which showed good selectivity for IgE and satisfactory blood compatibility.

Keywords: bronchial asthma, immunoabsorbent, IgE, Agar gel beads, blood compatibility

INTRODUCTION

Bronchial asthma is a kind of tracheal over-reacted disease stimulated by antigen or non-antigen. The pathology and mechanism is still unknown. However, it is believed that asthma is an I-type allergic disease that is conducted by the antibody of immunoglobulin E (IgE); therefore, the total level of IgE in patients is remarkably higher than the normal level (430 ng/ml) [1]. It is a worldwide and high incidence disease, and in China there are about 10–20 million patients [2].

At present, the treatment of bronchial asthma is mainly by drugs; immunotherapy and gene therapy are under investigation [3–7].

Since the 1980s, Sato[8–11] studied blood perfusion for asthma using immunoabsorbents based on PEO and controlled porous glass beads containing

The support of the “Construction Foundation of Tianjin and Nankai University” is acknowledged.

Address correspondence to Shenqi Wang, Division of Bioengineering, School of Chemical and Biomedical Engineering (SCBE-2), Nanyang Technological University, 70 Nanyang Drive, Singapore 637457. E-mail: sanyiwang131@hotmail.com

anti-IgE antibody ligand. Behm[12] synthesized IgE specific PVA beads containing tryptophan ligands, and they were able to remove pathogenic IgE from plasma effectively, but the preparative procedure was difficult, especially on a large scale, and the treatment was expensive.

Recently, our group developed an immunoadsorbent for removal of IgA in IgA-Nephrology [13, 14]. In order to remove IgE in bronchial asthma, the present paper studied an adsorbent based on agar gel beads containing succinic anhydride as spacer and tryptophan as the ligand. The adsorbent has a higher adsorption capacity for anti-IgE antibody and selectivity than the PVA-tryptophan adsorbents and the preparation procedure is simple and cost effective.

MATERIALS AND METHODS

Materials

Agar powder was purchased from Beijing Yili Fine Chemicals (Beijing, China). L-tryptophan was purchased from China Medicine Co. (Beijing, China). Test-kit for IgE was purchased from Sigma. Test-kits for IgG, IgA and IgM, Immunodiffusion plates for C₃ and C₄ were purchased from Institute for Blood Disease, Chinese Academy of Medical Sciences (Tianjin, China). Serum of high level IgE was supplied by Tianjin Changzheng Hospital (Tianjin, China). All other reagents were analytical grade made in China.

Preparation of Adsorbent with L-tryptophan Ligand

Synthesis of agar gel beads [13, 14]

Fifty milliliters of chloroform and a few drops of tween-80 were added to 150 ml of toluene, stirred and kept at 60°C, then 6.0 g of agar powder and 94 ml of distilled water were autoclaved to dissolution and poured into the mixture of chloroform and toluene, which was stirred for 3–5 minutes, then cooled to room temperature; the beads were filtered (20–40 meshes) and washed thoroughly to remove all the solvents.

Activation of beads

1.0 ml of agar gel beads was added to 0.4 ml of epichlorohydrin and 3.0 ml of sodium hydroxide (0.6mol/L) solution, stirred at 40°C for 2 h, then washed excessively with distilled water.

Immobilization of L-tryptophan Ligand

3.0 ml of saturated tryptophan solution (boric acid-borax buffer (pH 7.5)) and 1.0 ml of activated agar gel beads were mixed and stirred for 20 h at room temperature, then washed excessively with distilled water.

Preparation of Beads with Succinic Anhydride Spacer

The wet agar gel beads were washed with ethanol and dried. 1.0 g of the beads was washed several times with pyridine, then added to the mixture of 4.0 g of succinic anhydride and 20 ml of pyridine, stirred for 6 h at 60°C; after filtration the beads were washed with distilled water, and placed in methanol overnight.

Preparation of Beads with Phthalic Anhydride Spacer

The above preparation method was followed, except phthalic anhydride was substituted for succinic anhydride.

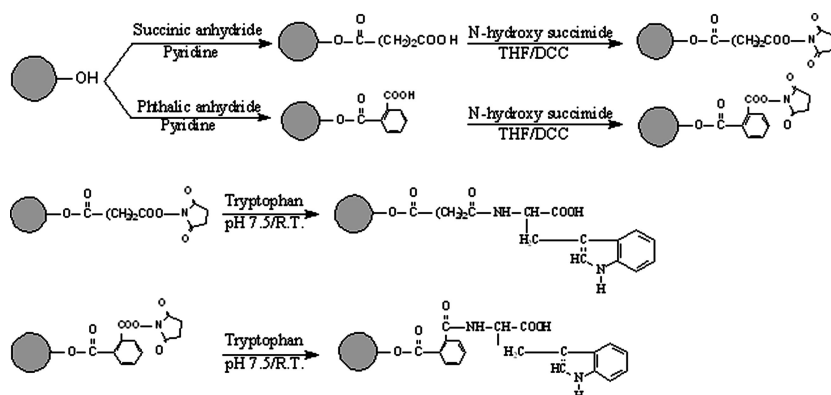
Activation of Carriers

1.0 g of beads containing the spacer was washed three times with absolute tetrahydrofuran (THF), and added to 50 ml of dioxane containing N-hydroxy succinimide and dicyclohexylcarbodiimide (DCC) of iso-molar ratio to the carrier. After stirring for 20 h at room temperature, the carrier was filtrated and washed with distilled water.

Immobilization of L-tryptophan Ligand

L-tryptophan was saturated in boric acid-borax buffer (pH 7.5), then 1.0 ml of activated carrier was added to 3.0 ml of L-tryptophan buffer solution, and stirred for 20 h at room temperature, filtrated and washed with distilled water, and finally stored at 4°C for further use.

The preparation scheme for adsorbents containing spacers is shown in Scheme 1.



Scheme 1. Preparation of adsorbents containing spacers and tryptophan ligand.

Determination of Adsorption Capacity

The adsorption capacity determination was carried out in static state: 1.0 ml of adsorbent was washed with normal saline, incubated with 3.0 ml of high lever of IgE plasma. The mixture was shaken for 2 h at 37°C; the concentration of IgE before and after adsorption was examined by corresponding test-kid. Adsorption percentage was calculated according to the following equation:

$$AP = \frac{[C]_B - [C]_A}{[C]_B} \times 100\% \quad (1)$$

Where AP stands for adsorption percentage, $[C]_B$ and $[C]_A$ stand for the concentrations of IgE before and after adsorption.

Determination of Blood Compatibility

The blood compatibility of IgG, IgM and IgA were examined by corresponding test-kits, C_3 , C_4 were examined by immunodiffusion plates, and the amount of leucocyte, erythrocyte and platelet were countered by an auto hematocyte counter (COULTER T-540).

RESULTS AND DISCUSSION

Effect of Activation Conditions on Activation

The effect of activation was determined by measuring the amount of the epoxy group. As we can see (Figure 1), the concentration of NaOH plays an important role in the activation effect. At high concentrations of NaOH the ring opening of the epoxy group is preferential but the hydrolysis of the chlorine groups is also enhanced; therefore, in order to achieve a high activation efficiency, we needed to optimize the concentration of NaOH. The optimal concentration was 0.5 mol/L NaOH solution and the epoxy group content was 37.09 $\mu\text{mol/ml}$ beads.

Figure 2 shows the effect of epichlorohydrin amount on activation efficiency; the epoxy group in the beads increased as the amount of epichlorohydrin increased and reached an optimal condition at 0.4 ml.

Effect of Amino Acids Ligands on Adsorption Capability

The following amino acids—DL-glycine, L-lysine, DL-phenylalanine, L-alanine, DL-tryptophan and L-tyrosine—were immobilized on agar-gel beads

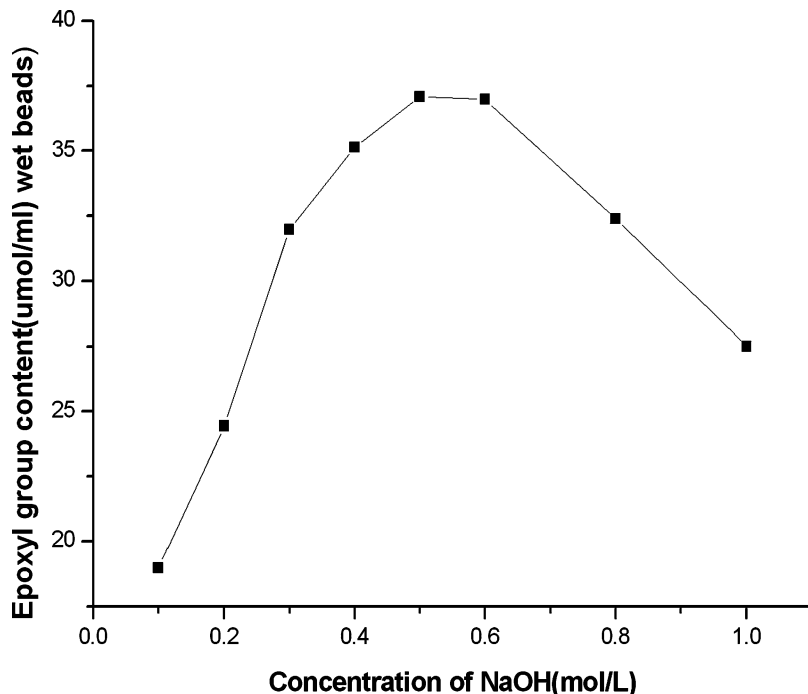


Figure 1. Effect of NaOH concentration on activation.

separately, and their adsorption capacities were evaluated (see Figure 3). It is shown in Figure 3 that highest adsorption capability was obtained when the tryptophan ligand was immobilized on agar-gel beads. Tryptophan is a non-polar amino acid having an indole group in the molecule, which is hydrophobic in character and IgE molecule is also a hydrophobic molecule in character; therefore, we assume that the binding force between tryptophan ligand and IgE would be a hydrophobic interaction.

Effect of Buffer Solutions and pH During the Preparation of the Adsorbent on its Adsorption Capability for IgE

In the preparation process of the adsorbent, tryptophan was immobilized on activated agar-gel beads under various pH values and buffer solutions (Figure 4). Results showed that the highest adsorption capability of the adsorbent for IgE was achieved in boric acid-borax buffer solution at pH 7-8 and the lowest in phosphate buffer at pH 5-7. The above results may be related to the solubility of tryptophan in buffer solution, i.e. low solubility in acidic solution and high in basic solution, therefore the amount of tryptophan immobilized on

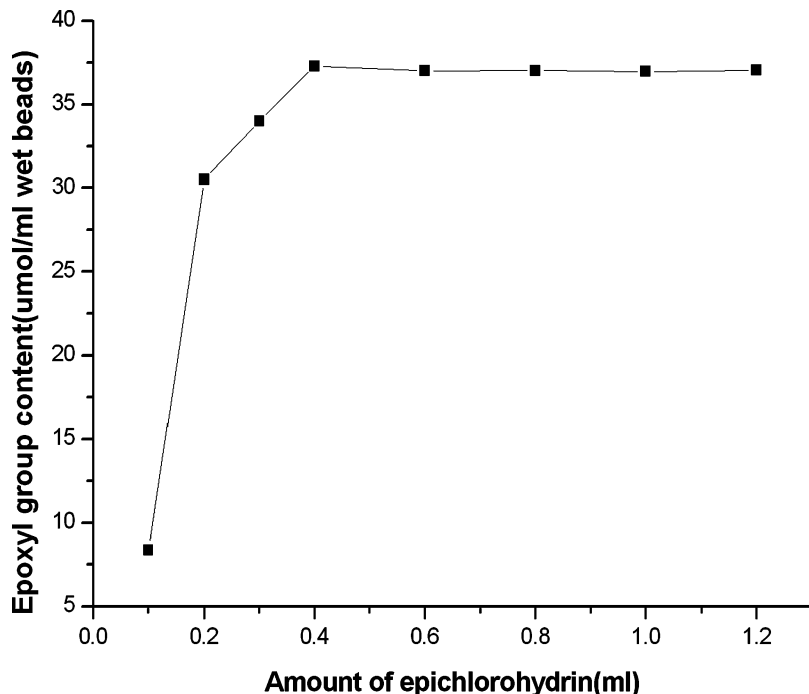


Figure 2. Effect of epichlorohydrin amount on activation.

the carrier was higher in the basic solution than in the acidic medium; consequently the adsorbent with a high adsorption capacity for IgE was prepared in basic buffer solution.

Effect of Spacers on Adsorption Capability

The adsorption capability of adsorbent containing succinic anhydride spacer, phthalic anhydride spacer and without a spacer was evaluated and the results are shown in Figure 5. The purpose of introducing a spacer to the adsorbent is to improve the accessibility of the ligand to the targeted substance (IgE), which could decrease the steric hindrance. Two representative spacers were introduced: succinic anhydride and phthalic anhydride; one is aliphatic hydrocarbon substituent and another is aromatic hydrocarbon substituent. Both spacers showed good accessibility of the ligand to the targeted substance and could enhance adsorption capability to IgE compared to adsorbent without spacers.

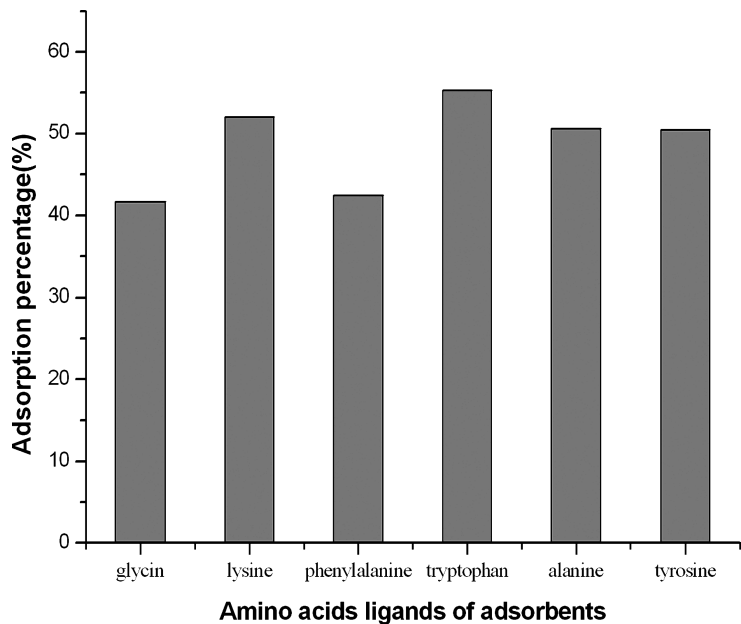


Figure 3. Adsorption percentages of adsorbents with different amino acids ligands.

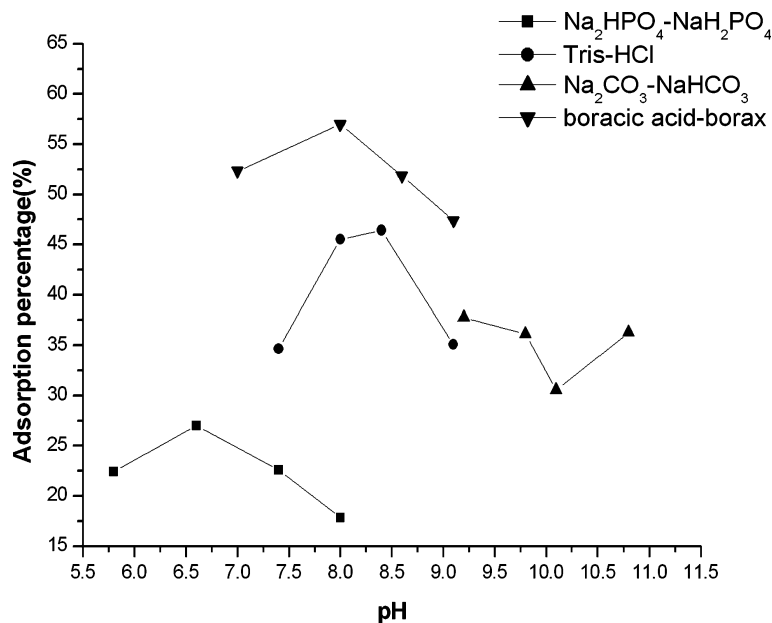


Figure 4. The adsorption percentage of adsorbents for IgE versus different buffer solutions and pH in the preparation process of the adsorbent.

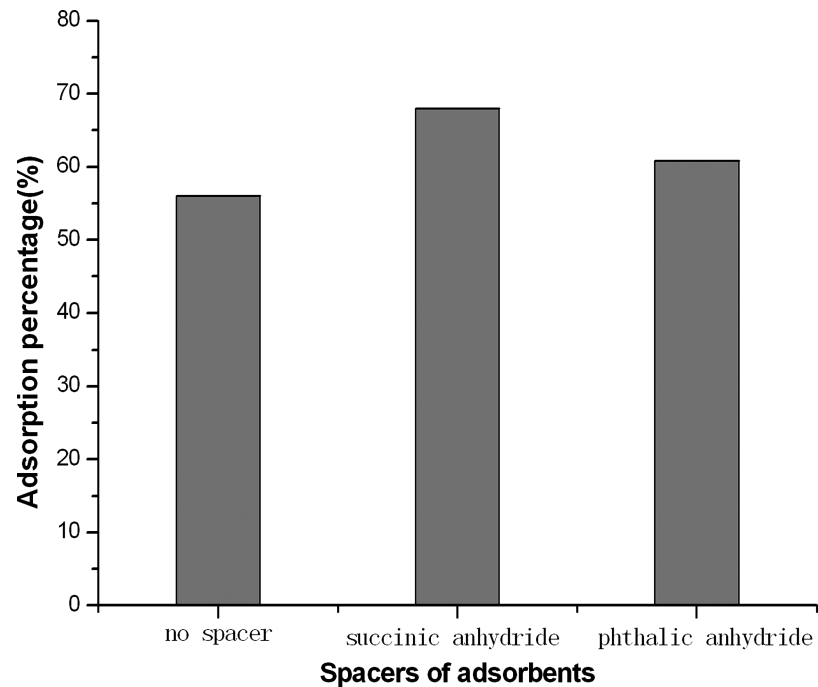


Figure 5. Effect of spacers on adsorption capability.

Adsorption Dynamics of Adsorbent Containing Succinic Anhydride Spacer and Tryptophan Ligand

According to the optimal condition above, the adsorbent was prepared based on the agar-gel beads containing succinic anhydride spacers and tryptophan as ligands in boric acid-borax buffer (pH 7.5); the adsorption capability was evaluated in vitro static condition, and results showed the adsorption percentage of IgE was 70.73% (see Figure 6) after two hours interaction.

Blood Compatibility of the Adsorbent

The changes of erythrocyte, leucocyte, platelet amount (Figure 7), IgG, IgA, IgM and C₃, C₄ content in blood were evaluated before and after adsorption (Table 1).

The maximal adsorption percentage for IgA was 26.17% (Table 1) and is much lower than IgE 70.73%, which signifies the adsorbent has a good selectivity for IgE.

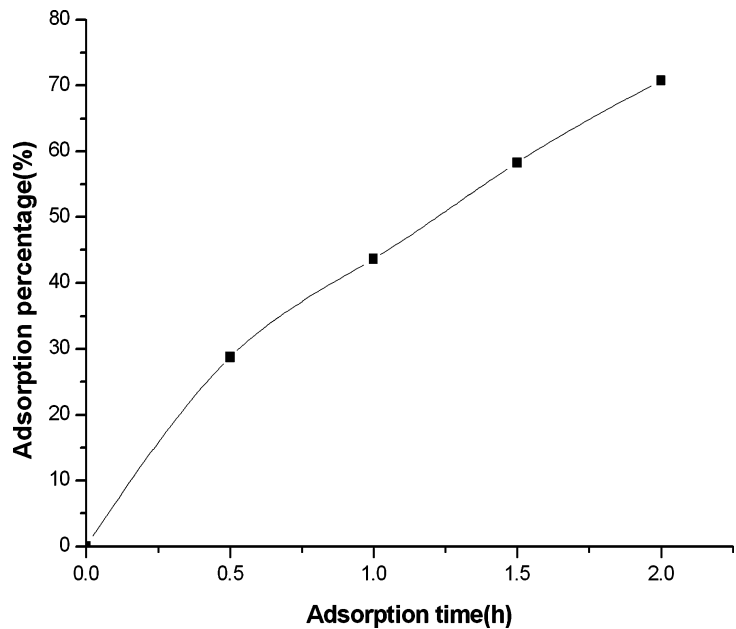


Figure 6. Dynamics of IgE adsorption.

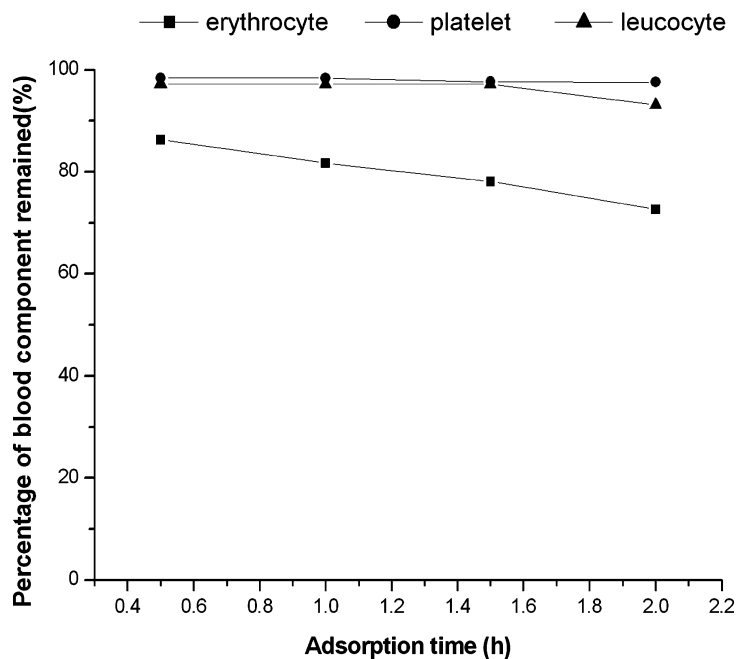


Figure 7. Changes of leucocyte, erythrocyte and platelet during adsorption.

Table 1. Changes of IgG, IgA, IgM, C₃, C₄ before and after adsorption (mg/ml)

Adsorption time (h)	0	0.5	1.0	1.5	2.0	Adsorption percentage(%)
IgG	12.30	11.26	11.26	11.24	10.18	17.24
IgA	2.14	1.95	1.77	1.58	1.58	26.17
IgM	0.51	0.51	0.49	0.48	0.48	5.88
C ₃	1.12	1.12	1.11	0.99	0.97	13.39
C ₄	0.44	0.40	0.40	0.36	0.36	18.18

The adsorption percentage of leucocyte, erythrocyte and platelet was 27.3%, 2.4% and 6.9%, respectively (Figure 7), which signifies that the blood compatibility of the adsorbent is satisfactory, and has a great potential to be used in blood purification.

CONCLUSIONS

Immunoabsorbent for IgE was prepared based on agar-gel beads with a diameter of 20–40 meshes, activated by epichlorohydrin, and L-tryptophan ligand was immobilized on the carrier. The adsorption percentage of IgE in whole blood was 70.73%. Blood compatibility of the adsorbent was satisfactory and has a great potential to be used for whole blood purification in removal of excessive IgE in bronchial asthma patients.

REFERENCES

1. Xuan-Zi, L.I., Ying-Zi, D.I.A.O., Wei, L.I. et al. (1996). Radioimmunoassay of serum IgE, IgG, SIgA in patients of bronchial asthma. *Journal of Isotopes*. **9**(4): 246–248.
2. Jun, X.U. (2001). Progress in bronchial asthma research. *Guangdong Medical Journal*. **22**(2): 93–95.
3. Cantani, A, Micera, M. (2005). Significant decrease of IgE antibodies after a three-year controlled study of specific immunotherapy to pollen allergens in children with allergic asthma. *Eur Rev Med Pharmacol Sci*. **9**(2):103–11.
4. Barnes, P.J. (2004). New drugs for asthma. *Nat Rev Drug Discov*. **3**(10): 831–44.
5. Factor, P. (2003). Gene therapy for asthma. *Mol Ther*. **7**(2): 148–52.
6. Xu, Qing, Chen, Yong. (2002). Research progress in anti-IgE for the therapy of bronchial asthma. *Chinese Journal of Clinical Pharmacy* **11**(2): 120–122.
7. Chen, Xue-Jun, Wu, Bin, Liang, Biao. (2002). Asthma responsive genes affecting IgE level. *Sect Respir Sys Foreign Med Sci*. **22**(5): 249–251.
8. Sato, H., Kidaka, T., Hori, M. (1985). Sterilization of therapeutic immunoabsorbents with aqueous propylene oxide solution. *The International Journal of Artificial Organs* **8**: 109–114.

9. Sato, H., Koichi, W. Azuma, J., Kidaka, T., Hori, M. (1989). Specific removal of IgE by therapeutic immunoadsorption system. *Journal of Immunological Methods* **118**: 161–168.
10. Sato, H., Kidaka, T., Hori, M. (1986). Effect of pore size of porous bead carriers immobilizing antibody on IgE adsorption. *Journal of Biomedical Materials Research* **20**: 853–858.
11. Sato, H., Kidaka, T., Hori, M. (1986). Sterization of therapeutic immunoadsorbents by ionizing radiation. *The International Journal of Artificial Organs* **9**: 131–136.
12. Behm, E., Kuroda, T., Yamawaki, N. et al. (1987). In vitro investigations with selective adsorbents for IgE and IgM. *Biomater. Art. Cells, Art. Org.* **15**: 101–111.
13. Chu, Jia-Qiang, Yu, Yao-Ting, Zhang, Wei-Hua et al. (2003). In vitro studies on the immunoadsorbent for removal of IgA in IgA-Nephropathy(II). *Chem. J. Chinese Universities* **24**(4): 636–638.
14. Chu, Jia-Qiang, Zhang, Wei-Hua, Yu, Yao-Ting et al. (2004). In vitro studies of the immunoadsorbent for removal of IgA in IgA-nephropathy (III). *Chem. J. Chinese Universities* **25**(8): 1454–1457.

Copyright of Artificial Cells, Blood Substitutes, & Biotechnology is the property of Taylor & Francis Ltd and its content may not be copied or emailed to multiple sites or posted to a listserv without the copyright holder's express written permission. However, users may print, download, or email articles for individual use.