

22184

Zhikang Peng
Allan B. Becker
F. Estelle R. Simons

Section of Allergy and Clinical Immunology,
Department of Paediatrics,
University of Manitoba, Winnipeg, Canada

Binding Properties of Protein A and Protein G for Human IgE

Key Words

Protein A
Protein G
IgE, human

Abstract

We studied the binding of protein A and protein G to human monoclonal and polyclonal IgE. An ELISA was used to measure human IgE. Protein A-Sepharose did not bind to monoclonal IgE, but bound to 12–14% of serum polyclonal IgE and to ~3% of the polyclonal IgE purified from the serum. No difference in binding to protein A was found between IgE from an allergic patient and that from a non-allergic subject. Protein G bound to neither polyclonal IgE nor monoclonal IgE. Thus, protein G is better than protein A with respect to separation of IgE from IgG.

Protein A, a cell wall component purified from *Staphylococcus aureus*, is able to bind with immunoglobulins, especially with IgG, in at least 65 different mammalian species known to date [1]. This has made protein A a valuable reagent which has been utilized successfully for a wide variety of laboratory techniques [2]. In human immunoglobulins, protein A binds to IgG subclasses 1, 2 and 4 [3], IgA2 [4], a group of IgM [5], and a small part of polyclonal IgE [6, 7]. Protein A does not bind to myeloma IgD [8], but perhaps to the IgD on the lymphocyte surface [9].

During the past 10 years it has been recognized that a cell wall protein from certain species of streptococci, especially Lancefield group C and group G, exhibited a broader range of binding to IgG than protein A [10, 11]. For human immunoglobulins protein G has been shown to bind all four subclasses, including IgG3 (to which protein A does not bind), but does not bind to IgM, IgA and IgD [10]. The binding of protein G to human IgE has not yet been studied.

In the present study we describe the binding of protein A and protein G to human IgE. We compare the binding differ-

ences between monoclonal IgE and polyclonal IgE, between IgE from an allergic patient and from a non-allergic subject, and between purified and unpurified serum IgE.

Serum IgE was purified mainly according to previously described methods [12]. Briefly, serum (2 ml) from an allergic patient with a high IgE level was precipitated between 30 and 55% saturation of $(\text{NH}_4)_2\text{SO}_4$, dissolved in 0.02 M phosphate-buffered saline (PBS) pH 7.2 and applied to a Sephadex G-25 column equilibrated with PBS. This IgE-containing fraction was then applied to a Con A-Sepharose column (bed volume 12 ml). After washing with PBS, IgE was eluted with 0.2 M methylmannoside in PBS from the column and applied to a protein G-Sepharose column to remove IgG. The purified IgE in the effluent from the protein G column was pooled and concentrated for the use of protein A-binding analysis.

Total IgE was measured by a sandwich ELISA. Polystyrene microtiter plates were coated with 0.1 ml/well of a 1:2,000 goat anti-human IgE (a gift from Dr. N.F. Adkinson, Jr. which was affinity purified with PS myeloma-Se-

pharose 4B) in 0.05 M carbonate buffer (pH 9.6) overnight at 4°C, and then washed three times with washing buffer (0.05% Tween 20 in PBS). A test sample or IgE reference serum (No. 6015, also a gift from Dr. N.F. Adkinson, Jr.), diluted in ELISA buffer (0.2% bovine serum albumin in washing buffer), was added (0.1 ml/well) and incubated for 2 h at 37°C. After washing, 0.1 ml/well of diluted rabbit anti-human IgE (Dakopatts, Denmark) was added and incubated for 2 h at 37°C. After washing, 0.1 ml/well of alkaline phosphatase-conjugated goat anti-rabbit IgG (H+L; Jackson Immunoresearch Laboratories, Inc.) was added and incubated for 2 h at 37°C. After a final washing, 0.1 ml/well of substrate, *p*-nitrophenylphosphate 1 mg/ml in diethanolamine buffer (pH 9.8), was added and incubated overnight at 4°C. The reaction was stopped by addition of 0.1 ml of 1 N NaOH. Absorbance was read at 410 nm on an automated MR700 microplate reader. The level of total IgE was calculated by interpolation from the dilution curve of reference serum. This assay was shown to be specific to human IgE; its sensitivity was 1 ng/ml. Human IgG, IgA, IgM and IgD were not detected by this assay (table 1).

The following IgE-containing samples were analyzed for binding to protein A and protein G: (1) 25 µg of PS myeloma IgE (a gift from Dr. T. Ishizaka) diluted in 1 ml of 0.05 M phosphate buffer (PB) pH 7.2; (2) 0.1 ml of allergic patient serum diluted in 0.9 ml of PB; (3) 0.4 ml of non-allergic subject serum diluted in 1.6 ml of PB, and (4) two IgE fractions. Each was purified from 2 ml of allergic patient serum as described above. These samples were separately applied to a protein A-Sepharose CL-4B (Pharmacia) column (bed volume 6 ml) and a protein G-Sepharose 4 Fast Flow (Pharmacia) column (bed volume 6 ml), which were equilibrated with PB and then allowed to stand in the column for 15 min. After washing with PB, the fractions were pooled. Proteins binding to the column were eluted with 0.1 M glycine buffer (pH 2.7) and neutralized with 0.5 M Tris buffer (pH 8.0). The fractions eluted from the protein A or the protein G column were pooled and dialyzed against PB overnight. Total IgE was assayed by ELISA for these starting samples, effluents and eluents. The percentages of IgE in the effluent and the eluent were calculated (table 2). The results showed that protein A-Sepharose essentially did not bind to monoclonal IgE (0.12%), but did bind to 12–14% of serum polyclonal IgE and 1–3% of purified serum polyclonal IgE. Protein A bound 12% serum IgE from an allergic patient and 14% IgE from a non-allergic subject. In contrast to protein A, protein G bound to neither serum polyclonal IgE nor monoclonal IgE.

Studies have demonstrated that 6–7% of polyclonal human IgE bound to protein A [6, 7]. We have shown that a

Table 1. Cross-reactivity of total IgE assay to immunoglobulin G, A, M, and D measured by ELISA (OD 410 nm)

Concentration ng/ml	Myeloma protein				
	IgG	IgA	IgM	IgD	IgE
0.01	0	0	0	0	>2.0
0.1	0	0	0	0	>2.0
1	0	0	0	0	>2.0
10	0	0	0	0	>2.0

In this ELISA, microplates coated with mAb anti-human IgE were sequentially incubated with different dilutions of myeloma IgG or IgA, IgM, IgD, and IgE, then rabbit anti-human IgE, and finally enzyme-conjugated goat anti-rabbit IgG.

subpopulation of polyclonal dog IgE bound to protein A, but not to protein G [13]. Our present studies show that protein G is better than protein A with respect to separation of IgE from IgG, as protein A binds to a small portion of polyclonal IgE. Also, protein A does not bind to IgG subclass 3 [3].

In our studies, the purified IgE bound to protein G (1–3%) less than the unpurified IgE (12–14%). During the purification procedure, the IgE fraction was passed through a protein G column to remove IgG. If there was any IgG-anti-IgE in the fraction, these immune complexes would have been removed by binding to protein G. This may explain why the purified IgE fraction bound to protein A less than the unpurified IgE. Even after purification, however, still some IgE (1–3%) bound to protein A. This excludes the possibility of the presence of IgG-anti-IgE immune complexes which may lead to the binding of the small portion of polyclonal IgE to protein A.

The mechanisms of the different binding properties of polyclonal human IgE between protein A and protein G are unknown. Previous studies of IgG have shown that the Fc and Fc' fragments were more strongly bound to protein A than Fab fragments and that the heavy chains, but not the light chains, were capable of binding to protein A [14]. The mechanisms for the binding of IgE to protein A are not very clear, but studies with human IgE have reported that the protein A-binding sites on the IgE molecule were not heat labile [6] and were located on the region of Fab'_{2c} rather than Fc_e [15].

Binding assays involving purified Fc fragments from human IgG suggest that protein G has specificity for a Fab region determinant(s) not recognized by protein A, whereas the Fc determinants for protein A and protein G are identical

Table 2. Chromatography of human IgE on protein A- and protein G-Sepharose

	Protein A				Protein G			
	effluent		eluent		effluent		eluent	
	IgE ¹	%	IgE ¹	%	IgE ¹	%	IgE ¹	%
Purified myeloma IgE (PS)	131,487	100	154	0 (0.12)	201,519	100	116	0 (0.06)
Serum								
Allergic patient	187	88	26	12	266	100	0	0
Non-allergic patient	1,126	86	177	14	1,366	100	0	0
Purified serum IgE ²								
Allergic patient No. 1	1,143	97	34	3	—	—	—	—
Allergic patient No. 2	2,213	99	27	1	—	—	—	—

¹ The amount of IgE (ng) was obtained by multiplying the concentration by the volume.

² The purified IgE fraction was passed through a protein G column to remove IgG during its purification procedure. Therefore, the analysis of binding to protein G was not required for the IgE fraction.

or overlapping [11]. The strongest binding to protein G for IgG from various species occurred at pH 4 and 5, but the highest binding to protein A occurred at pH 8. This supports the concept of different molecular mechanisms for immunoglobulin binding by these two bacterial proteins [16].

Acknowledgments

This work was supported in part by funds from the Manitoba Health Research Council and the Children's Hospital Research Foundation.

References

- Kronvall G, Seal US, Svensson S, Williams RC Jr: Phylogenetic aspects of staphylococcal protein A-reactive serum globulins in birds and mammals. *Acta Pathol Microbiol Scand* 1974; 82:12-18.
- Lindmark R, Thorén-Tolling K, Sjöquist J: Binding of immunoglobulins to protein A and immunoglobulins levels in mammalian sera. *J Immunol Methods* 1983;62:1-13.
- Kronvall G, Williams RC Jr: Differences in anti-protein A activity among IgG subgroups. *J Immunol* 1969;103:828-833.
- Saltvedt E, Harboe M: Binding of IgA to protein A containing staphylococci: Relationship to subclass. *Scand J Immunol* 1976;5:1103-1108.
- Harboe M, Fölling I: Recognition of two distinct groups of human IgM and IgA based on different binding to staphylococci. *Scand J Immunol* 1974;3:471-482.
- Johansson SGD, Inganäs A: Interaction of polyclonal human IgE with protein A from *Staphylococcus aureus*. *Immunol Rev* 1978;41:248-260.
- Zikán J, Zavázal V, Krauz V: Heterogeneity of human polyclonal IgE reacting with staphylococcal protein A. *Folia Microbiol* 1984;29:264-268.
- Forsgren A, Grubb AO: Many bacterial species bind human IgD. *J Immunol* 1979;122:1468-1472.
- Romagnani S, Giudizi MG, Almerigogna F, Ricci M: Interaction of staphylococcal protein A with membrane components of IgM- and/or IgD-bearing lymphocytes from human tonsils. *J Immunol* 1980;124:1620-1626.
- Björck L, Kronvall G: Purification and some properties of streptococcal protein G, a novel IgG-binding reagent. *J Immunol* 1984;133: 969-974.
- Eliasson M, Andersson R, Olsson A, Wigzell H, Uhlén M: Differential IgG-binding characteristics of staphylococcal protein A, streptococcal protein G, and a chimeric protein AG. *J Immunol* 1989;142:575-581.
- Peng Z, Arthur G, Kierek-Jaszczuk D, Thomas E, Sehon AH, Rector ES, Simons FER, Becker AB: Purification and identification of polyclonal IgE antibodies from ragweed-sensitized dog sera. *Int Arch Allergy Immunol* 1993;102:176-184.
- Peng Z, Simons FER, Becker AB: Differential binding properties of protein A and protein G for dog immunoglobulins. *J Immunol Methods* 1991;145:255-258.
- Forsgren A, Sjöquist J: 'Protein A' from *S. aureus*. I. Pseudo-immune reaction with human γ -globulin. *J Immunol* 1966;97:822-827.
- Inganäs M, Johansson SGO, Bennich HH: Interaction of human polyclonal IgE and IgG from different species with protein A from *Staphylococcus aureus*: Demonstration of protein A-reactive sites located in the Fab'2 fragment of human IgG. *Scand J Immunol* 1980;12:23-31.
- Åkerström B, Björck L: A physicochemical study of protein G, a molecule with unique immunoglobulin G-binding properties. *J Biol Chem* 1986;261:10240-10244.