Rapid Communication

Immunological Studies on LATS-immunoglobulin by the Reaction with Staphylococcal Protein A

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Synopsis

The reaction of LATS activity with Staphylococcal Protein A, a specific binding protein with the Fc part of human IgG(1), IgG(2) and IgG(4), was examined. When IgG(1), IgG(2) and IgG(4) subclasses were removed from LATS positive sera or LATS-IgG fractions by affinity chromatography on Protein A-Sepharose, LATS activity decreased. Almost all LATS activity was found in the fraction that reacted with Protein A. It is suggested that LATS has an expression of a very distinct immunoglobulin G structure, and that LATS activity is distributed mainly in the fraction containing IgG(1), IgG(2) and IgG(4) in LATS positive serum.

Since LATS (long acting thyroid stimulator) was discovered in the serum of patients with hyperthyroidism, this substance has been considered to be an important factor in Graves' disease. LATS activity is located in the fraction of immunoglobulin G(IgG) in serum from hyperthyroid patients. It is believed that LATS may be an antibody (McKenzie, 1967; Ochi and DeGroot, 1968 a, b; Burke, 1971). It is, however, still unclear to which IgG subclass the LATS-immunoglobulin belongs.

Among the 4 subclasses of human IgG, Staphylococcal Protein A is known for its peculiar property of reacting non-immunologically with the Fc-region of subclass IgG(1), IgG(2) and IgG(4), whereas IgG(3) molecules are nonreactive (Kronvall and Williams, 1969; Kronvall and Frommel, 1970). In addition, IgA, IgM have been reported to be nonreactive (Kronvall *et al.*, 1970). No other human serum proteins combine with Protein A (Kronvall *et al.*, 1970).

The present work was examined the distribution of LATS activity when IgG molecules of subclass 1, 2 and 4 were isolated from LATS-positive sera or the LATS-IgG fraction by affinity chromatography on Protein A-Sepharose.

Materials and Methods

LATS assay was performed according to McKenzie's method (Ochi and DeGroot, 1968a). Immunoglobulins (IgG, IgA, IgM) were determined by single radial immunodiffusion using an immunoplate (Eikenkagaku Lab.). Rabbit antisera specific for each 4 subclasses of human IgG were obtained from the Nordic Immunological Lab. (Tilburg, The Netherlands).

Protein A was partially purified (Arai *et al.* 1974) by DEAE-Cellulose column from *Staphylococcus aureus* Cowan 1 strain according to the method of Jensen. Protein A-Sepharose CL-4B was obtained from Pharmacia Fine Chemicals (Uppsala, Sweden).

Experiments using 2 different staphylococcal Protein A preparations were performed.

Exp. (1) Absorption experiment of IgG molecules from LATS-positive sera by Protein A.

Various amounts (5-40 mg) of partially purified

Protein A preparation were mixed with LATS positive sera (1.0 ml) and allowed to stand at 5°C overnight. The visible precipitate was removed after centrifugation at 6,000 rpm for 20 min., and the supernatant fraction was used for bioassay and for immunoglobulin determination.

Exp. (2) Affinity chromatography on Protein A-Sepharose CL-4B.

According to the Pharmacia's direction, 3 g of freeze-dried powder of Protein A-Sepharose having a binding capacity for 250 mg of human IgG was used. Three ml of LATS-positive serum was applied to Protein A-Sepharose columns (2.0×5.0 cm) that had been equilibrated with 0.05 M phosphate buffer solution (PBS) pH 7.8. Fraction rate was 75 ml/hr and each eluted fraction was measured its absorbance of OD 280 nm by Automatic UV recorder (Toyo-Uvicon). The first eluted fraction (unabsorbed fraction; Fr(1)) was collected, and the second eluted Fr(2) was obtained with 0.1 M Glycine-HCl, pH 2.8. Fr(2) was adjusted to pH 7.8 by a few drops of 1 N NaOH solution. Similar affinity chromatography methods were carried out LATS-IgG fraction instead of LATS-positive serum. The components of each Fr(1) and Fr(2) were examined using an antibody specified for each human IgG subclass and an antibody specified for IgA or IgM.

Fig. 1. LATS activity and immunoglobulin in supernatant fraction. Protein A was added to LATS positive serum. After centrifugation LATS activity and immunoglobulin level was expressed by supernatant fraction (S) divided total (T).

Results

Exp. (1) Typical experimental results when LATS-positive serum was treated with Protein A are shown in Fig. (1). When Protein A for 1 ml of test serum was increased from 5 to 20 mg, the amount of IgG gradually disappeared from the supernatant fraction. On the other hand, the amount of the supernatant fraction of both IgM and IgA did not decrease. LATS activity in these supernatant fractions disappeared with the disappearance of IgG. Even if more than 20 mg of Protein A was used, about 10% of IgG still remained in the supernatant fraction. Slight LATS activity also remained in the supernatant fraction.

Exp. (2) When LATS-positive serum was fractionated by affinity chromatography on Protein A-Sepharose, Fr(1) and Fr(2) were obtained. Fr(1) showed a visible precipitation line against anti-IgG(3) by the Ouchterlony method, but no reaction was observed with anti-IgG(1), IgG(2) and IgG(4). The component observed in Fr(1) appeared to be serum protein except IgG(1), IgG(2)

Fig. 2. Separation of LATS by Affinity Chromatography on Protein A-Sepharose. PBS; Phosphate buffer solution.

and IgG(4). Fr(2) appears to be IgG(1), IgG(2) and IgG(4). Almost all LATS activity was found in Fr(2) from the sera of 5 patients (Fig. 2-A).

A similar affinity chromatography experiment was performed using the LATS-IgG fraction (Fig. 2-B). Fr(1) consisted of IgG(3). Fr(2) was confirmed as IgG(1), IgG(2) and IgG(4). LATS activity in Fr(1) was negative in 2 cases and slightly detectable in 2 other cases. A high LATS activity was found in Fr(2) in all test cases (Table (1)).

Discussion

It is well known that Protein A precipitates by reacting with the Fc part of subclass IgG(1), IgG(2) and IgG(4) (Kronvall and Williams, 1969). Therefore, the immunological properties of LATS was examined by the interaction with LATS-positive sera and Protein A. Almost all LATS activity reacted with Protein A.

It is possible to isolate immunoglobulin of type IgG(3), when the IgG fraction of serum has previously been isolated by affinity chromatography on Protein A-Sepharose. Therefore, LATS-positive serum or LATS-IgG fraction was applied to this column. In both experiments the effluent (unabsorbing fraction) from column con-

tained IgG(3), while the other IgG subclasses were retained in the column and could subsequently be eluted with 0.1M glycine buffer, pH 2.8. Although the distribution of LATS activity in the IgG subclass could not be definitively determined in this experiment, it is suggested that LATS activity is distributed mainly in any subclass of IgG(1), IgG(2) and IgG(4), but that its distribution in IgG(3) is barely observable amounts.

It is generally accepted that the distribution percentage of each subclass of human IgG is 64–70% of IgG(1); 23–28% of IgG(2); 4–7% of IgG(3) and 3–4% of IgG(4) (Schur, 1972). Similar distribution percentages in antibody against thyroglobulin were also reported (Hay and Torrigiani, 1974). In the present experiment, a high distribution of LATS activity was found in the fraction containing these 3 subclasses of IgG(1), IgG(2) and IgG(4) except for IgG(3).

References

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Table 1. Distribution of LATS activity by affinity chromatography on protein A-sepharose

LATS-positive patient	Test sample	Fr(1)		Fr(2)	
		mg	Assay response (%)	mg	Assay response (%)
A.S.	Serum	20	108±13	3	470±55*
	LATS-IgG	3	182±17*	3	550±62*
I.T.	Serum	20	107±12	3	400±52*
	LATS-IgG	3	108±13	3	422±58*
T.T.	Serum	20	100±7	3	528±82*
	LATS-IgG	3	110±12	3	608±77*
H.S.	Serum	20	98±14	3	620±77*
	LATS-IgG	3	180±22*	3	708±88*
K.M.	Serum	20	108±15	3	402±38*

All values are shown as mean±standard deviation.

*; $p < 0.01$, from control (100%).

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Book Review

Pioneers in Neuroendocrinology (Perspectives in Neuroendocrine Research)

Edited by J. Meites (Michigan), B. T. Donovan (London) and S. M. McCann (Dallas)
Plenum Press, New York and London 1975, \$ 27.00

Published by Plenum Publishing Corporation, 277 West 17th Street, New York
N. Y. 10011

The book is an unique collection of essays by the pioneering investigators in neuroendocrinology who provide personal accounts of the events that led them to study relationships between the brain and endocrine system. The contents are as follow: Neuroendocrine feedbacks (C. Aron); The Story of Some Neuroendocrinological Studies in Cats (P. Bard); A Marvelous Region (W. Bargmann); My Research in Neuroendocrinology Study of the Photo-Sexual Reflex in the Domestic Duck (J. Benoit); Development of the Convergent Themes of Neuroendocrinology (C. McC. Brooks); Wanderings in Neuroendocrinology (E. W. Dempsey); Contribution to the Substructure of Neuroendocrinology (J. W. Everett); Ned Opes Ned Vires Sed Artis Aceptra Permanent (H. Hellers); Recollections About the Neurological Science with Emphasis on Neuroendocrinology (J. C. Hinsey); Reflection (H. Hoagland); The Regulatory Centers of Endocrine Glands in the Hypothalamus (W. Hohlweg); Personal Neuroscientific Development with Remarks on the Other Events and People (W. R. Ingram); My Way from Hypophysectomy to Hypophyseal Portal Vessels (D. Jacobsohn); Stimuli that Release Hormones of the Pars Nervosa (M.

Pickford); Feedback Control of Gonadal and Hypophyseal Hormones, Evolution of the Concept (D. Price); Twenty-Five Years in Neuroendocrinology of Reproduction (1945-1970) (C. H. Sawyer); Neurosecretion and its Role in Neuroendocrine Regulation (B. Scharrer); Comments (M. C. Schelenyak); How I Did Discover Neurosecretion (F. S. Stutinsky); Under the Spell of Hypothalamic Feedback (J. Szentagothai); Nervous Influences in Endocrine Activity (M. Vogt).

This books deals with some of the early work on neurosecretion, neurotransmitter, releasing factors, locus and control of ADH and oxytocin secretion, neural control of the anterior pituitary, the role of the portal vessels, brain neuroanatomy and neurophysiology, effects of lesions and electrical stimulation of discrete brain areas, electrophysiological recordings and correlates, feedback mechanism, reproductive neuroendocrinology, effects of hormones on behavior and other aspects. There is hardly an area of modern neuroendocrinology that is not represented here by earlier investigations. We believe that the readers of the collection of attractive essays will be given some encouragement, advice or hint from the pioneers in neuroendocrinology.