[Original]

Binding Property of LDH-linked IgG in Serum to Protein A-Sepharose CL-4B*

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Summary

The binding capacity of LDH-linked IgG to Protein A-Sepharose CL-4B was examined in the sera of 12 patients. Protein A binds to the major part of immunoglobulin G and therefore also to LDH-linked IgG.

Each patient's serum was incubated with Protein A-Sepharose CL-4B immunosorbent, then LDH activity in the sediment was measured. LDH activity on this immunosorbent was demonstrated for every patient, which showed that LDH-linked IgG bound to this immunosorbent.

The method, using Protein A-Sepharose CL-4B, was simple and quantitative for the detection of LDH-linked IgG, because Protein A reacts to IgG at a constant rate and one molecule of LDH linked to one molecule of IgG. Observation of the quantitative dynamics of LDH-linked IgG following the progress of the clinical condition of the disease may be useful to understand the pathophysiological property of LDH-linked IgG.

Key words : LDH-linked IgG, Protein A-Sepharose CL-4B.

Introduction

Protein A binds to immunoglobulin G (IgG) with the exception of IgG₃¹⁾. Thus the application of Protein A-Sepharose CL-4B immunosorbent for the detection of enzyme-linked IgG has been studied. Binding of alkaline phosphatase (ALP)-linked IgG or creatinine phosphokinase(CK)-linked IgG to Protein A-Sepharose CL-4B has been already demonstrated by Kurt Bauer et al.²⁾. However, binding of lactate dehydrogenase (LDH)-linked IgG to this immunosorbent had not been investigated until now. The reason why there was no study for the detection of LDH-linked IgG using Protein A-Sepharose CL-4B is that the number of patients with LDH-linked IgG was few³⁾. A further reason is that the subclass of LDH-linked IgG reported previously is almost all IgG₃. Biewenga found LDH-

linked IgG₃⁴⁾ in 13 out of 14 cases of LDH-linked IgG in 1975. Kano⁵⁾ reported that IgG₃ was the main subclass of LDH-linked IgG.

In the present study, we analyzed LDH-linked IgG in the sera of 12 patients and found binding capacity of LDH-linked IgG to Protein A-Sepharose CL-4B in all of the patients.

Materials and Methods

Sera were obtained from 12 patients with LDH-linked IgG. The clinical data for these patients and results of the serum analysis are noted in Table 1.

Control sera from two patients did not contain LDH-linked Ig. Control serum 1 was obtained from a 37 year-old male patient with acute hepatitis (LDH activity : 1,282 Wróblewski Unit(W.U.), IgG concentration : 791 mg/dl). Control serum 2 was

* LDH 結合性 IgG の Protein A-Sepharose CL-4B への吸着性.

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Table 1. Clinical data and results of serum analysis.

Patient	Sex	Age	Diagnosis	LDH activity (W.U.)	IgG level (mg/dl)	Light chain* type of LDH-linked IgG
Group I						
Nt	m	46	Ulcerative Colitis	353	1,500	L
Hk	m	68	Diabetes Mellitus	890	1,800	K & L
Ms	m	49	Liver Cancer	247	1,038	K & L
Yh	m	87	Pruritus Senilis	706	1,068	L
Yr	f	85	Cerebral Thrombosis	293	1,136	K
Oh	m	20	Ulcerative Colitis	257	1,700	L
Group II						
Iy	m	63	Rheumatism	650	1,655	K & L
Ks	m	71	Arteriosclerotic Obstruction	404	1,245	K & L
Ty	m	54	Liver Cancer	520	2,205	K & L
Sk	f	42	Ulcerative Colitis	429	1,280	K
Group III						
Ny	m	31	Ulcerative Colitis	290	1,901	L
Mt	f	22	Ulcerative Colitis	227	1,750	L

* K; Kappa, L; Lambda

obtained from a 64 year-old female patient with IgG₃ multiple myeloma (LDH activity: 162 W.U., IgG concentration: 1900 mg/dl).

Measurement of LDH activity: LDH activity was measured by the use of a LDH activity assay kit (LDH NEO D, Eiken Kagaku Co., Ltd, Japan) according to the modified Babson-Phillips method⁹⁾. The substrate solution was made up of 250 mM D, L-lithium lactate.

Assay of LDH isoenzyme: The sera were electrophoretically analyzed on cellulose acetate membrane (TITAN III, Helena Laboratories, U.S.A.) with Tris-barbital buffer (pH 8.8, $\mu=0.075$). As Sepharose beads disturbed LDH isoenzymes causing separation on TITAN III membrane, supernate A was subjected to electrophoresis on polyacrylamide disc gel (containing 7% acrylamide, pH 8.9). It was performed for about 1 hour at 3 mA per tube using Tris-glycine buffer (pH 8.9). LDH isoenzymes were stained with LD Vis Reagent (Helena Laboratories, U.S.A.).

Determination of binding to Protein A-Sepharose CL-4B: The procedures followed were those of Kurt Bauer et al.²⁾ as shown in Fig. 1. Each patient's serum separated into 500 μ l was incubated

Fig. 1. Schematic diagram for the detection of LDH-linked IgG which binds to Protein A-Sepharose CL-4B.

with 30, 60 and 90 mg of Protein A-Sepharose CL-4B for 2 hours at room temperature. Control serum was also tested at the same time. After removing the supernate from the above mixture by centrifugation (5 min, 3,000 rpm), the sediment was washed with 1/15 M phosphate buffer (pH 7.4) five times. LDH activity in the first supernate and the last sediment was measured by the method as described above.

Measurement of IgG concentration: IgG concentration in the serum and in the supernate was measured by single radial immunodiffusion method (HYLAND, U.S.A.).

Results

LDH-linked IgG was demonstrated in the sera of 12 patients by the method of immunoprecipitin reaction in free liquid media⁷⁾.

These 12 patients could be divided into 3 groups according to differences in the LDH isoenzyme patterns on TITAN III membrane. In group I, LDH₁, LDH₂, LDH₃ and LDH₄ bands diffused toward the cathode side. As LDH₅ band migrated more anodically, a fraction of LDH₄ overlapped with a fraction of LDH₅ and there seemed to occur an abnormal LDH band between the usual LDH₄ and LDH₅ bands. The LDH isoenzyme pattern in the serum of patient Oh did not show clear abnormality on TITAN III membrane. But on polyacrylamide disc gel, it showed an extra LDH band just to the anode side of the LDH₅ band. In group II, each LDH fraction was very diffuse and migrated more cathodically. In patient Iy, the zymogram of LDH was extremely unusual. It seemed to be composed of one major band between LDH₄ and LDH₅ zones. In group III, the activity of LDH₄ and LDH₅ was characteristically low. The diffuse patterns of three bands, LDH₁, LDH₂ and LDH₃, were the same as those of group I.

Results of binding capacity of LDH-linked IgG to Protein A-Sepharose CL-4B are summarized in Table 2. LDH activity is shown by an optical density at 570 nm. The individual results are shown by graphs in Fig. 2-a and -b.

Results of patients in group I are shown in Fig. 2-a. By addition of 90 mg of Protein A-Sepharose CL-4B, LDH activity in the supernate decreased to about 60% of total serum LDH activity (graph A). LDH activity on 30 mg of Protein A-Sepharose CL-4B was equal to an optical density of 0.035 to 0.090 (graph B). Results of other patients are shown in Fig. 2-b. In group II, LDH activity in the supernate decreased to about 50% of

Table 2. Optical density at 570 nm of the supernate (A) and the sediment (B). LDH activity in the sediment of the control was below 0.002 (optical density).

Patient	LDH activity (O.D. at 570 nm)				
	Serum	After adding Protein A-Sepharose CL-4 B (mg)			
		30	60	90	
Group I					
Nt	0.309	0.243	0.210	0.181	A
		0.051	0.086	0.162	B
Hk	0.639	0.548	0.526	0.424	A
		0.090	0.165	0.202	B
Ms	0.179	0.168	0.155	0.103	A
		0.089	0.199	0.230	B
Yh	0.559	0.519	0.398	0.321	A
		0.088	0.162	0.184	B
Yr	0.208	0.188	0.153	0.114	A
		0.043	0.073	0.122	B
Oh	0.174	0.167	0.139	0.088	A
		0.035	0.079	0.111	B
Group II					
Iy	0.507	0.461	0.326	0.198	A
		0.406	0.536	0.596	B
Ks	0.315	0.271	0.228	0.183	A
		0.234	0.273	0.347	B
Ty	0.362	0.311	0.219	0.167	A
		0.151	0.224	0.291	B
Sk	0.313	0.280	0.178	0.149	A
		0.103	0.260	0.264	B
Group III					
Ny	0.195	0.193	0.191	0.175	A
		0.032	0.041	0.053	B
Mt	0.130	0.121	0.117	0.111	A
		0.015	0.028	0.034	B

serum activity. LDH activity on 30 mg of Protein A-Sepharose CL-4B was very high. It was equal to an optical density of 0.103 to 0.406. That indicated a large amount of LDH-linked IgG. In group III, LDH activity in the sediment was low and the slope of the increasing line (graph B) was very gentle. There seemed to be little LDH-linked IgG.

In every case, IgG in the patient's serum binds to Protein A-Sepharose CL-4B reliably. Addition of 90 mg of Protein A-Sepharose CL-4B caused a

Fig. 2-a. LDH activity in the supernate (graph A) and in the sediment (graph B) of Group I as amount of Protein A-Sepharose CL-4B is increased. Graph A shows percentage of LDH activity and IgG concentration remaining in the supernate.

greater than 60% IgG absorption.

Results of experiments with two kinds of control serum are shown in Fig. 3. IgG concentration in the control serum 1 supernate was decreased by addition of increasing amounts of Protein A-Sepharose CL-4B, while LDH activity varied little in the supernate and was not found in the sediment. Therefore, high level of LDH activity had no effect on the measurement of LDH activity on IgG bound Protein A-Sepharose CL-4B. In the control serum 2 supernate, addition of increasing amounts

Fig. 2-b. LDH activity in the supernate and in the sediment of Group II and Group III as amount of Protein A-Sepharose CL-4B is increased.

of Protein A-Sepharose CL-4B had practically no effect on both IgG concentration and LDH activity. This is because Protein A does not bind to IgG₃. There was no LDH activity in the sediment either.

Each supernate was additionally submitted to polyacrylamide disc electrophoresis. LDH isoenzyme pattern of the control serum supernate did not change by addition of increasing amounts of Protein A-Sepharose CL-4B, whereas, due to the same increase, an abnormal LDH band in every patient's serum was decreased or caused to disappear and become similar to the normal pattern.

Fig. 3. LDH activity in the supernate and in the sediment of control serum. Control serum 1 was from the patient with high LDH activity. Control serum 2 was from the patient with IgG₃ multiple myeloma.

Discussion

Since Ganrot⁸⁾ reported about the patient suffering from lupoid cirrhosis with LDH-linked Ig, the relationship between LDH-linked Ig and autoimmune disease has been suspected. Until now, there had been no close relation found between LDH-linked Ig and any disease. In 1981, G.G. Leroux et al.⁹⁾ found LDH-linked Ig in 2 out of 20 patients with ulcerative colitis. We also found¹⁰⁾ LDH-linked Ig in 4 out of 42 patients with ulcerative colitis and detected the isotype specificity of LDH-linked Ig in these patients, that being IgG-lambda. Consequently, we were further interested in the occurrence of LDH-linked IgG as an autoimmune complex. Nagamine⁹⁾ reported that LDH-linked IgG appeared transiently in hepatic disease. ALP-linked IgG appears or disappears according to changes in the clinical condition of ulcerative colitis. Therefore

the quantitative variations in LDH-linked IgG must be observed in detail following the progression of the disease. Such observation is necessary for the investigation of the nature of LDH-linked IgG.

In this paper, the binding property of LDH-linked IgG to Protein A-Sepharose CL-4 B was demonstrated in all 12 patients, having various diseases. Such a binding property had been also demonstrated in one patient with ALP-linked IgG and one patient with CK-linked IgG by Kurt Bauer et al.²⁾ At present, the most sensitive method of detecting LDH-linked IgG is based on immunoprecipitin reaction in free liquid media. The same sensitivity was obtained by using Protein A-Sepharose CL-4 B. Moreover, LDH-linked IgG could be observed quantitatively from LDH activity on Protein A-Sepharose CL-4 B and also from the reduction of LDH activity in the supernate as IgG concentration was decreased. The measurement of LDH activity in the sediment is particularly reliable for the quantitative detection of LDH-linked IgG. This is because Protein A reacts with IgG at a constant rate (IgG 25 mg/ Protein A-Sepharose CL-4 B gel 1 ml) and one molecule of LDH binds to one molecule of IgG¹²⁾. Therefore LDH activity in the sediment should give approximately a linear increase. A little curve in graph B seemed to be caused by an experimental error. When IgG concentration remaining in the supernate was below 10% of the serum, Protein A-Sepharose CL-4 B bound to all IgG except IgG₃. At this state, LDH activity in the sediment should show the activity of LDH bound to IgG. When the remarkable abnormality of LDH isoenzyme pattern was observed, as in group II, LDH activity in the sediment was very high. On the other hand, when there was a little abnormality, as in group III, LDH activity in the sediment was low and the slope was gentle.

Biewenga et al.⁴⁾ found 13 patients with LDH-linked IgG₃ out of 14 with LDH-linked IgG, while Kano⁵⁾ reported the predominance of the IgG₃ subclass in LDH-linked IgG. The reason, why the binding property of LDH-linked IgG to Protein A-Sepharose CL-4 B was not investigated, seemed to

be these reports. However, LDH-linked IgG in all of our 12 patients had the property of binding to Protein A-Sepharose CL-4 B. If Protein A does not bind to IgG₃, the LDH-linked IgG in our patients might not be IgG₃.

Recently, Ito et al.¹³⁾ reported that IgG₃ carrying different set of Gm allotypes had the property of binding to Protein A-Sepharose CL-4 B. Shimizu et al.¹⁴⁾ also reported the case of IgG₃ myeloma protein binding to Protein A-Sepharose CL-4 B. If the subclass of LDH-linked IgG in all of our patients was IgG₃, it might well be IgG₃ carrying peculiar Gm allotypes, which can react with Protein A.

Regarding the subclass of LDH-linked IgG, further experiments using antisera of the IgG subclass are necessary. We intend to make that our subject for continuing research.

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References

- 1) Kronvall, G., and Williams, R.C. : J. Immunol., **103** : 828, 1969.
- 2) Kurt, B. et al. : Clin. Chem., **26** : 297, 1980.
- 3) Nagamine, M. : Physico-Chem. Biol. (Seibutsu-butsurikagaku), **21** : 193, 1977.
- 4) Biewenga, J. and Feltkamp, T. E. W. : Clin. Chem. Acta, **64** : 101, 1975.
- 5) Kano, S. : Physico-Chem. Biol. (Seibutsu-butsurikagaku), **21** : 201, 1977.

- 6) Babson, A.L. and Phillips, G.E. : Clin. Chem. Acta, **12** : 210, 1965.
- 7) Tozawa, T. et al. : Physico-Chem. Biol. (Seibutsu-butsurikagaku), **26** : 243, 1982.
- 8) Ganrot, P.O. : Experimentia, **23** : 593, 1967.
- 9) Leroux-Roels, G.G. et al. : J. Lab. Clin. Med., **97** : 316, 1981.
- 10) Tozawa, T. and Fujiwara, Y. : Physico-Chem. Biol. (Seibutsu-butsurikagaku), **26** : 79, 1982.
- 11) Miki, K. et al. : Jpn. J. Gastroenterol. (Nisshō-byōkaishi), **73** : 162, 1975.
- 12) Kuwa, K. et al. : Physico-Chem. Biol. (Seibutsu-butsurikagaku), **21** : 209, 1977.
- 13) Ito, S. et al. : Proc. Japan Acad., **56** : 226, 1980.
- 14) Shimizu, A. et al. : Jap. J. Clin. Path. (Rinshō-byōri), **29** : 123, 1981.

要 旨

LDH 結合 IgG の Protein A-Sepharose CL-4 B に対する吸着能を, 12 例の患者血清で調べた。

患者血清を Protein A-Sepharose CL-4 B とインキュベートし, その沈殿物中の LDH 活性を測定した。その結果, 全例に, Protein A-Sepharose CL-4 B 中の LDH 活性が認められ, 12 例すべての LDH 結合 IgG が Protein A-Sepharose CL-4 B への吸着性をもつことが示された。

Protein A-Sepharose CL-4 B を用いた LDH 結合 IgG の検出は簡便で, 検出感度もすぐれている。しかも, IgG は Protein A と一定の割合で結合し, LDH 1 分子が IgG 1 分子と結合していることから, LDH 結合 IgG を定量的に把握しうる。この方法を用い, LDH 結合 IgG の臨床経過に関連した動態を定量的に観察することは, この複合物の病態生理学的な解明に役立つものと期待できる。