[Jpn. J. Parasitol., Vol. 33, No. 5, 369-375, October, 1984]

Serologic Diagnosis of Toxoplasmic Lymphadenitis

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(Received for publication; March 29, 1984)

Key words: toxoplasmosis, lymphadenitis, serologic diagnosis

Introduction

Lymphadenitis is one of some characteristic clinical features of acute or subacute toxoplasmosis, yet for the diagnosis of the disease, performance of certain laboratory examinations is needed. Because of the difficulties and limitations in obtaining tissue for histopathology and detection of the organisms, the diagnosis usually depends on serology.

It was recognized in the earlier studies (Dorfman and Remington, 1973; Karim and Ludlam, 1975; Welch *et al.*, 1980) that the IgM immunofluorescent antibody test and Sabin-Feldman dye test were useful for the diagnosis of toxoplasmic lymphadenitis. In this study an assessment was made on the relative usefulness of four different serologic tests commonly used in Japan.

Materials and Methods

1. Patients

Lymphadenopathic patients subjected to this study were 11 cases who were diagnosed by pathologists as suggestive of or consistent with toxoplasmic lymphadenitis by lymphnode biopsies on the basis of characteristic histopathology (Piringer-Kuchinka *et al.*, 1958; Saxén *et al.*, 1958; Stansfeld, 1961) during the period from 1974 to 1982 in various medical institutions. They were 5 males and 6 females and the average age of these patients was 42 years (range, 13 to 72 years).

To compare and assess the meaning of results of the serologic tests, two other populations of patients were studied. They were 17 cases of malignant lymphoma and 368 general outpatients at Jikei University hospital in Tokyo. The patients with malignant lymphoma comprised 9 males and 8 females and the average age was 44 years (range, 12 to 73 years). The general outpatients, excluding those of toxoplasmic lymphadenopathy and malignant lymphoma, consisted of 251 males and 117 females, and the average age was 46 years (range, 9 to 83 years).

2. Serologic tests

Four different serologic tests applied were the dye test (DT), indirect latex agglutination (ILA) test, indirect hemagglutination (IHA) test and IgM-immunofluorescent antibody (IgM-IFA) test.

The DT employed in this study was a modification of the technique described by Frenkel and Jacobs (1958), in which the plasma was used instead of serum as source of the accessory factor (Kobayashi *et al.*, 1968).

The ILA test was performed as previously (Kobayashi *et al.*, 1977) using the commercial kit product (Eiken Chemical Co. Ltd., Tokyo) prepared by the method of Tsubota and Ozawa (1977). Briefly, 0.025 ml of the buffer solution was applied to each well of a hard U-type microplate with 12×8 wells. 0.025 ml of test sera were added to each of the first wells and mixed to make 1 : 2 dilutions.

This study was partially supported by Grant-in-Aid for Scientific Research, No. 337016, from the Ministry of Education, Science and Culture, Japan.

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This was followed by two-fold dilution technique to make a series of serum dilutions on each serum. The final wells were used for the negative controls. A 0.025 ml of the suspension of the sensitized latex particles was then added to each well. The plate was agitated for several minutes to secure reaction mixture. After standing the plate overnight at room temperature, the agglutination patterns were read. Antibody titers were expressed in terms of the initial serum dilution before addition of the sensitized latex suspension.

The procedure of IHA test was essentially the same as that of the original method (Jacobs and Lunde, 1957) except for the followings; tannic acid concentration was 1 : 80,000 and the optimal concentration of the antigen was defined as the one giving the highest IHA titer to the positive serum (Kobayashi *et al.*, 1971). The IgM-IFA test was conducted as described by Remington *et al.* (1968).

Results

Results of our first serologic tests on each of 11 cases suggestive of toxoplasmic lymphadenitis are shown in Table 1. The serum samples, except for 2 cases, were obtained from the lymphadenopathic patients during one to 4.5 months (average, 2.5 months) after onset of the disease. The two exceptional cases were as follows; patient (Case No. 1) noticed swelling of lymphnodes at various sites of the body since one year ago and marked enlargement since 2 months ago. Another patient (No. 8) had swelling of cervical lymphnodes with repeated remission and exacerbation during past 8 years, and noticed rapid enlargement since about one month ago.

As shown in the table, positive serologic tests were shown in all the cases. DT titers ranged from 1 : 1,024 to 1 : 1,000,000, ILA test titers from 1 : 256 to 1 : 64,000, IHA test titers from 1 : 1,024 to ≧1 : 4,000,000, and IgM-IFA test titers from 1 : 16 to 1 : 128.

Table 2 indicates the frequencies with which patients of three different populations met various serologic criteria. The data are shown on the basis of results obtained by the first trial of these tests at our hands after onset of the disease.

DT : All of 11 cases histologically suggestive

Table 1 Results of various serologic tests on toxoplasmic lymphadenitis patients*

Case No.	Age	Sex	Period†		Reciprocal antibody titer§			
			Biopsy	Sero. test	DT	ILA	IHA	IgM-IFA
1	44	M	1Y (2M)‡	1Y (2M)	1,024	ND	4,000,000	32
2	39	F	1M	1M	4,096	ND	4,096	ND
3	31	F	2M	3.5M	16,000	8,000	4,096	32
4	38	M	2M	2.5M	≧4,096	1,024	≧16,000	16
5	25	M	1M	1M	16,000	1,024	1,024	128
6	60	M	2M	4M	1,000,000	64,000	≧4,000,000	32
7	52	F	1.5M	4M	4,096	512	4,096	32
8	47	F	8Y (1M)	8Y (1.5M)	4,096	≧4,096	≧16,000	16
9	45	F	1M	1M	1,024	256	4,096	64
10	72	M	3M	4.5M	4,096	64,000	ND	16
11	13	F	20D	1M	64,000	256	ND	16

* Patients as diagnosed by histopathology

† Period from onset of illness to the test; D=day, M=month, Y=year

‡ See the text.

§ Antibody titers are those determined by our first tests after onset of the diseases; DT= dye test, ILA=indirect latex agglutination test, IHA=indirect hemagglutination test, IgM-IFA=IgM-immunofluorescent antibody test, ND=not done

Table 2 Frequencies of antibody titers shown by patients in various serologic tests

Serologic test	Reciprocal antibody titer	Toxoplasmic lymphadenitis	Malignant lymphoma	Other diseases (General outpatients)
DT	≧16,000	4/11(36)	0/17	0/368
	≧4,096	9/11(82)	0/17	0/368
	≧1,024	11/11(100)	0/17	7/368(2)
	Posi. (≧16)	11/11(100)	7/17(41)	130/368(35)
ILA	≧4,096	4/9 (44)	0/15	0/368
	≧1,024	6/9 (67)	0/15	31/368(8)
	≧ 256	9/9 (100)	2/15(13)	63/368(17)
	Posi. (≧32)	9/9 (100)	7/15(47)	128/368(35)
IHA	≧16,000	4/9 (44)	ND	9/292(3)
	≧4,096	8/9 (89)	ND	38/292(13)
	≧1,024	9/9 (100)	ND	81/292(28)
	Posi. (≧256)	9/9 (100)	ND	106/292(36)
IgM-IFA	≧64	1/9 (11)	0/15	0/115
	≧32	5/9 (56)	0/15	5/115(4)
	≧16	9/9 (100)	0/15	24/115(21)
	Posi. (≧8)	9/9 (100)	3/15(20)	37/115(32)

Note. Data are given as number (%) of patients who met the criterion for a given test / total number of patients.
Antibody titers represent those determined by our initial tests after onset of the diseases.

of toxoplasmic lymphadenitis had a DT titer of ≧1 : 1,024 and 9 cases (82 %) had a titer of 1 : 4,096 or higher. Although 7 (41 %) of 17 cases of malignant lymphoma were positive, their DT titers were less than 1 : 256.
In the general outpatients, 35 % were seropositive, but only 7 cases (2 %) had a titer of 1 : 1,024 and no cases had 1 : 4,096 or higher.
ILA : All the cases of toxoplasmic lymphadenitis showed a ILA test titer of ≧1 : 256 and 44 % had ≧1 : 4,096. Although less than 20 % of cases of malignant lymphoma or general outpatients had a titer of ≧1 : 256, no case had ≧1 : 4,096.
IHA : All cases of toxolplasmic lymphadenitis had a IHA titer of ≧1 : 1,024, 89 % had ≧1 : 4,096 and 44 % had ≧1 : 16,000. In general outpatients, 28 % of them had a titer of ≧1 : 1,024, 13 % had ≧1 : 4,096 and still 3 % had ≧1 : 16,000. The IHA tests were not performed on malignant lymphoma patients.
IgM-IFA : All the cases of toxoplasmic lymphadenitis showed IgM-IFA test titer of ≧1 : 16 and 56 % had ≧1 : 32. All the malignant lymphoma patients showed a titer of less than 1 : 16. In the general outpatients, 21 % showed a titer of ≧1 : 16, but only 4 % had ≧1 : 32 and none had 1 : 64 or higher.

Four cases of toxoplasmic lymphadenitis could be followed to investigate possible fluctuation of the antibody titers after treatment with Daraprim, Spiramycin, sulfonamide or their combinations. Results are shown in Table 3. Out of 4 cases, no particular changes in antibody titer were shown in two cases (Nos. 5 and 7) through before and after the treatment, while some decreases in IHA test titer (Case No. 1) or in DT titer (Case No. 6). Changes in IgM-IFA test titers in these cases were rather intricate. Diminishing ti-

Table 3 Fluctuation of anti-*Toxoplasma* antibody titers in cases of toxoplasmic lymphadenopathy before and after treatment

Case No.	Age	Sex	Date	Reciprocal antibody titer			
				DT	ILA	IHA	IgM-IFA
1	44	M	'74/12/21	1,024	ND	4,000,000	32
			'75/ 2/27	1,024	ND	4,000,000	64
			Treatment with Spiramycin, Daraprim and Sulfadiazine for one month				
			6/21	1,024	ND	1,000,000	128
			9/11	1,024	ND	16,000	128
5	25	M	'79/ 7/12	16,000	1,024	1,024	128
			7/17	64,000	512	1,024	32
			Treatment with Daraprim and Daimeton for two weeks				
			7/26	250,000	1,024	1,024	128
			Treatment with Daraprim and Daimeton for two weeks				
			8/15	64,000	1,024	1,024	32
6	60	M	'80/ 4/28	1,000,000	64,000	≧4,000,000	32
			Treatment with Daraprim and Daimeton for 20 days				
			6/26	4,096	64,000	ND	<8
7	52	F	'80/ 7/10	4,096	512	4,096	32
			Treatment with Acetylspiramycin and Sinomin for one month				
			7/21	4,096	256	4,096	32
			8/27	4,096	512	4,096	8

ters were observed in cases of Nos. 6 and 7 after the treatment, whereas a tendency to increase was seen in cases of Nos. 1 and 5.

Discussion

Since the initial reports on glandular toxoplasmosis (Siim, 1950; Gard and Magnusson, 1950; Piringer-Kuchinka, 1952) many reports on such cases have been accumulated in Japan (Tsunematsu *et al.*, 1964; Utsumi and Koizumi, 1965; Ojima *et al.*, 1972; Higuchi *et al.*, 1974; Hirabayashi *et al.*, 1974; Suzuki *et al.*, 1979; Kobayashi, 1983). Although the diagnosis of these reported cases of glandular toxoplasmosis was based on serology, histopathology or more rarely demonstration of the organisms in the lymphnode tissue, some cases gave only low titers or even negative serologic test. The present study was aimed to determine titers which should be taken to signify lymphadenopathic toxoplasmosis. In this regard, titers above those found in the normal populations or patients other than toxoplasmic lymphadenopathy would be accepted for the criteria.

Beverley and Beattie (1958) adopted an arbitrary level of a DT titer of 1 : 256 and complement fixation test titer of 1 : 10. They reported that this did not prove toxoplasmosis but made it very highly probable.

In the present study, four different tests were employed for the assessment. These tests were those routinely used at our hands. Among these, ILA was of particular interest to be studied since its commercial kit is available and most commonly used in Japan at various medical institutions.

The sharp contrast in frequency of a DT titer of ≧1 : 1,024 between toxoplasmic lymphadenopathy and the controls indicates that DT is a highly useful diagnostic tool and a titer of ≧1 : 1,024, or more strictly ≧1 : 4,096, should be the criterion for the diagnosis of the disease. These results were very close to

those presented by Dorfman and Remington (1973) in which all of 31 cases of toxoplasmic lymphadenitis as diagnosed by histopathology had positive DT, with titers ranging from 1 : 1,024 to 1 : 32,768 and 81 % of them had a titer of ≧1 : 4,096. In the control populations such as malignant lymphoma or surgical patients, there were no cases with a DT titer of ≧1 : 4,096.

The ILA test, at a titer of ≧1 : 256, appeared to be useful for screening of toxoplasmic lymphadenitis. A titer of ≧1 : 1,024 may be required for the diagnosis of the disease. However, if this level of titer was adopted as the criterion, as many as 33 % of the cases of toxoplasmic lymphadenitis are to be missed. This indicates that ILA test is less useful than DT for the diagnostic purpose.

The IHA test at a titer of 1 : 1,024 may be useful for the screening of toxoplasmic lymphadenitis and a titer of ≧1 : 4,096 for the diagnosis. However, it seemed that this technique is slightly less useful than ILA because of considerably frequent occurrence of high titers in the control populations.

The IgM-IFA test would be useful for the diagnosis of acute toxoplasmosis such as toxoplasmic lymphadenopathy. In the present study, all the cases of toxoplasmic lymphadenitis were positive at a titer of ≧1 : 16. In contrast, non and one fourth of the control populations had positive tests at the same antibody titer level. Dorfman and Remington (1973) reported that IgM-IFA test was positive in 97 % of toxoplasmic lymphadenitis patients at a titer of ≧1 : 10, while in only 0.8% and 1.2 % of control populations. Although IgM-IFA test appeared to be useful, the magnitude of the antibody titers shown in the toxoplasmic lymphadenitis patients in our study was somewhat low as compared with those reported by some other authors. Welch *et al.* (1980) showed that IgM-IFA test titers of ≧1 : 160 were the best indicators of the infection acquired in the past two to four months. Similar results were presented by Karim and Ludlam (1975). On the other hand, Suzuki *et al.* (1979) reported two cases of acute toxoplasmosis with lymphnode swelling in which IgM-IFA test titers were less than 1 : 16. The reason for the discrepancies among these results is not necessarily clear.

Judging from the present study results, it may be concluded that the ILA test is a useful tool for the serodiagnosis of toxoplasmic lymphadenitis. However, for the ultimate diagnosis of the disease, a supplementation by DT or IgM-IFA test would be necessary.

It will be interesting to learn how the administration of anti-*Toxoplasma* agents affect appearance and continuation of the antibody titer. As described in the previous chapter, with a few exceptional occasions, no remarkable changes in titer were observed after treatment. This suggests that the curative effect of an anti-*Toxoplasma* agent can hardly be evaluated by the possible decrease in antibody titer in most cases except for patients who acquired the infection very recently.

Summary

Four different serologic tests were compared one another and assessed for the diagnosis of toxoplasmic lymphadenopathy. Eleven cases of lymphadenopathy were subjected to the study in which the histo-pathology of lymphnodes was consistent with toxoplasmosis. The serum specimens, with two exceptional cases, were obtained from the patients during one to 4.5 months (average, 2.5 months) after onset of the disease. Seventeen cases of malignant lymphoma and 368 general outpatients were served as the controls.

The Sabin-Feldman dye test (DT) titer of ≧1 : 1,024 were reached in all the patients of toxoplasmic lymphadenitis, and 82 % of them had a titer of ≧1 : 4,096. All patients of malignant lymphoma and 98% of patients with general diseases showed titers of ≦1 : 256. In the indirect latex agglutination (ILA) test, all the cases of toxoplasmic lymphadenitis had a titer of ≧1 : 256 and 44 % had ≧1 : 4,096. In the control groups, less than 20 % of the cases had a titer of ≧1 : 256, but no case showed ≧1 : 4,096. The indirect hemagglutination (IHA) tests were positive at a titer of ≧1 : 4,096 in 89 % of cases of toxoplasmic lymphadenitis, while 13 % of the

control populations had ≧1 : 4,096. The IgM-immunofluorescent antibody (IgM-IFA) test titers were positive at ≧1 : 16 in all the cases of toxoplasmic lymphadenitis, and ≧1 : 32 in 56 % of them. All the patients of malignant lymphoma were negative at 1 : 16. Although 21 % of the general outpatients showed ≧1 : 16, those who had ≧1 : 32 were seen in only 4%.

From these results, it was suggested that DT is the most reliable technique for the sero-diagnosis of toxoplasmic lymphadenitis. The IHA may be the least useful among these tests. The significant antibody titers for the possibility of toxoplasmic lymphadenitis are ≧1 : 1,024 for DT, ≧1 : 256 for ILA test, ≧1 : 1,024 for IHA test and ≧1 : 16 for IgM-IFA test. The criteria with much higher titers, e.g., ≧1 : 4,096 for both DT and ILA test and ≧1 : 64 for IgM-IFA test makes the diagnosis more definitive, but on the other hand, it brings increasing possibility of missing the disease.

Acknowledgements

We gratefully acknowledge the help given to us by the many physicians and pathologists who supplied us with materials from, and information about, the patients. Our thanks are due to Mmes. M. Tanizawa and M. Asakura of the Department of Parasitology, Jikei Medical School, for their technical assistance.

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トキソプラズマ性リンパ節炎の血清学的診断について

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トキソプラズマ性リンパ節炎に対する血清学的診断基準を求めることを目的として，リンパ節の病理組織学的所見から本症と診断された11例，対照として悪性リンパ腫17例，上記以外の一般外来患者368例につき，各種血清反応を施行し，得られた結果につき検討した．

リンパ節の腫脹をしめす患者につき，トキソプラズマ性リンパ節炎としての可能性を示唆する有意の抗体価は，色素試験（DT）1,024倍以上，間接ラテックス凝集反応（ILA）256倍以上，間接赤血球凝集反応（IHA）1,024倍以上，IgM 螢光抗体法（IgM-IFA）では16倍以上で，これらのうちDTが最も信頼しうる方法であると考えられた．また，DTおよびILAで4,096倍以上，IgM-IF 64倍以上を診断基準とした場合には，本症確定の精度はきわめて増大するが，その反面見逃しの確率もそれだけ高くなる．