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Changes of Serum IgG Antibody Reactivity to Protein Antigens of *Treponema pallidum* in Syphilis Patients after Treatment

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The changes of serum IgG antibody reactivity to protein antigens of Treponema pallidum after treatment of syphilis were observed using sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and Western blot. Until 9 to 12 months after treatment, it was seen that there was a loss of several antibodies and some diminution in their reactivity in primary, secondary and early latent syphilis, but no changes occurred in late latent and reinfected syphilis. In primary syphilis, there was a significant loss of two IgG antibodies to the treponemal antigens of molecular weights 68,500 and 47,000 at 11 months after treatment. According to our previous study, the treponemal antigen of molecular weight 68,500 was T. pallidum specific and appeared only in primary syphilis, and that of molecular weight 47,000 was one of the major antigens of T. pallidum.

The reaction between serum IgG antibodies of 14 patients who had been treated for secondary, early latent and late latent syphilis 2 to 14 years ago and major antigens of T. pallidum was observed and any loss or decrease in reactivity was not discovered.

From the results obtained, it was concluded that the observation of serum IgG antibody reactivity to protein antigens of T. pallidum is not helpful in evaluating the efficacy of treatment in secondary, early latent, late latent and reinfected syphilis. However, serum IgG antibodies to treponemal antigens of molecular weights 68,500 and 47,000 could possibly be useful in the assessment of the efficacy of treatment in primary syphilis.

Key Words: *Serum IgG antibody, protein antigens, T. pallidum, treated syphilis*

INTRODUCTION

Nowadays, many researchers have been attempting to characterize the protein antigens of syphilis reacting with antibodies in the sera of patients with various stages of untreated syphilis (Hanff et al., 1982; Hanff et al., 1983; Moskophidis and Müller, 1984; Baker-Zander et al., 1985; Baker-Zander et al., 1986). We also examined the IgG antibodies reacting with the protein antigens of *Treponema pallidum* in the sera of patients with various stages of syphilis before treatment, and also the antigens common to both *T. pallidum* and nonpathogenic *T. phagedenis* employing sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting (Kim et al., 1988).

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However, the changes of serum antibody reactivity to treponemal antigens after treatment have not yet been thoroughly investigated. Moskophidis and Müller (1984) demonstrated that serum IgG antibodies against a wide spectrum of protein antigens of *T. pallidum* persisted in patients with syphilis treated 2-3 years prior to their study, but showed only weaker reactivity compared to untreated patients. Baker-Zander et al. (1986) also showed that IgG antibody reactivity declined slightly after treatment but continued to show reactivity against all molecules detected initially.

For the above reasons, this study was conducted to examine the changes of serum IgG antibody reactivity to treponemal antigens at 3 month intervals for 6-12 months, and at 2-14 years after therapy in patients with various stages of syphilis employing SDS-PAGE and immunoblotting.

MATERIALS AND METHODS

1. Sources of treponemes

T. pallidum , Nichols strain, was provided by Centers for Disease Control (CDC), U.S.A. This was passed intratesticularly in rabbits every 10 days as previously described (Lee et al., 1987). *T. pallidum* was inoculated into testes of white rabbits with negative VDRL and TPHA in a concentration of $2\text{-}3\times10^7$ *T. pallidum* per testis. The infected rabbits were sacrificed on day 10 and the testes were removed. The testes were sliced, and the organisms were extracted in a solution containing 10 ml of phosphate buffered saline for 10 minutes with agitation. The solution without testicular tissue debris was centrifuged at 1,000×g for 5 minutes and the organisms in the supernatant were purified by Percoll density gradient centrifugation (Hanff et al., 1984). After centrifugation, the treponemal band was removed with a pipette, and Percoll was separated by centrifugation at 100,000×g for 1 hour at 4°C.

2. Sera

The sera were obtained from patients with various stages of syphilis, (primary, secondary, early latent, late latent and reinfected syphilis), diagnosed on the grounds of the patient's history, clinical manifestations, and serologic tests for syphilis which included VDRL, FTA-ABS, 19S (IgM)-FTA and IgM-TP-ELISA. The VDRL and FTA-ABS tests were performed as described in the Manual of Tests for Syphilis (1969). The TPHA test was done according to the instructions of the company (Fujirebio pharmaceutical Co., Ltd., Tokyo, Japan), and the 19S (IgM)-FTA tests were performed following the method of Müller (1982). The IgM-TP-ELISA test was performed using the modified method (Lee et al., 1987).

The sera were obtained from the syphilis patients in groups of 3 at each clinical stage before and after treatment at 3 month intervals for 6-12 months, when each group of 3 was pooled. Another group of sera examined was from 14 patients who had been treated for secondary (1), early latent (3) and late latent (10) syphilis 2-14 years ago. The sera were stored at −70°C before using.

3. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis

All prepared samples were solubilized in 20μl of sample buffer containing 0.5ml sample dissolving buffer, 0.5ml distilled water, 50μl ß-mercaptoethanol, 150mg sucrose, and a trace of bromophenol blue, and were boiled for 5-10 minutes. After centrifugation for 5 minutes, the supernatants were loaded into each well for electrophoresis. The sample dissolving buffer was made of 0.15M Tris (pH 6.8) and 4.6% sodium dodecyl sulfate. Protein standards (Bio-Rad, Pichmond, CA) were included on each gel (phosphorylase b, 92.5 kdaltons; bovine serum albumin, 66.2 kdaltons; ovalbumin, 45 kdaltons; carbonic anhydrase, 31 kdaltons; soybean trypsin inhibitor, 21.5 kdaltons; and lysozyme, 14.4 kdaltons). Approximate molecular weights were determined by the method of Weber and Osborn (1969). The prepared proteins were electrophoresed on 10% polyacrylamide gels in the discontinuous Tris-glycine system as described by Laemmli (1970). After electrophoresis, the gels were fixed and stained with 0.25% Coomassie Brilliant Blue dye for 18 hours and destained with a solution containing 10% acetic acid and 30% methanol for 36 hours.

4. Western blot and autoradiography

Electrophoretic transfer of proteins to nitrocellulose paper was performed by the method of Towbin et al. (1979) using an electrophoretic transfer apparatus (Hoeffer Scientific Instruments, San Francisco, CA). With the nitrocellulose paper facing the anode, Western blot was performed at 0.8mA for 3 hours in a buffer solution (pH 9.18) containing 20% methanol and 25mM Tris. Blotted papers were washed 3 times with 0.05% Tween 20-PBS (mixing 0.5ml of Tween 20 with 1000ml of PBS) and incubated with 3% bovine serum albumin at room temperature for 18 hours for blockage of nonspecfic protein binding sites. Before incubation with antisera, the blots were cut into 8 mm

strips.

The blots were incubated with 1:25-1:50 diluted antiserum for 2 hours with constant rocking, and washed for 2 hours with 0.05% Tween 20-PBS. After washing, the blots were incubated with ^{125}I-protein A (^{125}IPA, Amersham Corp., Arlington Heights, IL) with rocking. ^{125}I-protein A was used at a concentration of $1.2\text{-}1.8\times10^6$ cpm/ml. The blots were washed as before for 2 hours, dried and exposed to Kodak T-MAT G X-ray film (Eastman Kodak Co., Rochester, NY) at −70°C for 24-48hrs with an enhancing screen cassette.

RESULTS

1. Changes of serum IgG antibody reactivity to protein antigens of *T. pallidum* at regular intervals after therapy in patients with various stages of syphilis

On observing IgG antibody reactivity against protein antigens of *T. pallidum* in the sera of patients with various stages of syphilis, the following results were obtained. In primary syphilis there was no loss or change of IgG antibody reactivity to treponemal antigens until 9 months after therapy, and serum IgG antibodies to antigens of molecular weights 68,500, 47,000, 43,000 and 27,000 disappeared at 11 months after therapy (Table 1, Fig. 1).

In secondary syphilis, serum IgG antibody reactivity to several treponemal antigens began to decrease from the third month after treatment, and the antigens of molecular weights 80,000, 71,500, 64,000, 61,000, 43,000, 34,000, 32,000, 23,500 and 21,500 reacting with serum IgG antibodies were not detected 9 months after treatment (Table 2, Fig. 2). In early latent syphilis, antigens of higher molecular weights 71,500 and 64,000 and lower molecular weight 43,000 reacting with serum IgG antibody disappeared from 6 months and 9 months after treatment respectively (Table 3, Fig. 3). In late latent syphilis, serum IgG antibody reactivity to several treponemal antigens was partially decreased after therapy, but no definite loss of antigens was observed until 9 months after therapy (data not shown). In reinfected syphilis there was also no loss of antigens reacting with serum IgG antibodies until 6 months after therapy.

2. Changes of serum IgG antibody reactivity to major protein antigens of *T. pallidum* in patients with treated syphilis.

Serum IgG antibody reactivity to major protein antigens of *T. pallidum* (MW 47,000, 36,500, 15,500, 14,000) was observed in 14 patients who had been treated 2-14 years ago for secondary (1), early latent (3) and late latent (10) syphilis, but no evident decrease in reactivity was detected (Table 4).

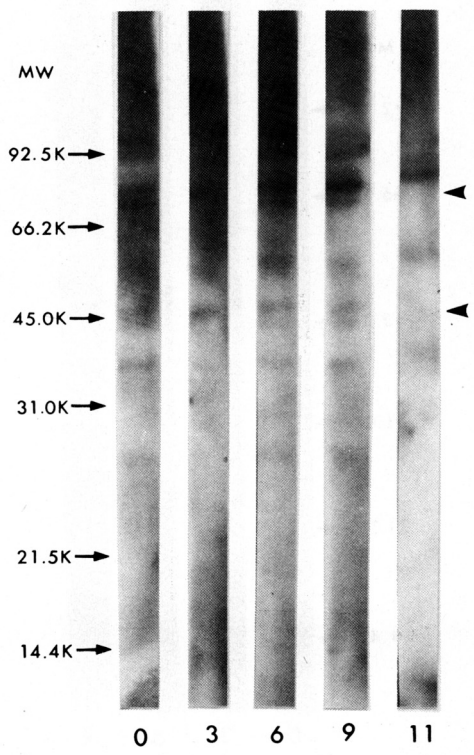

Fig. 1. IgG antibody reactivity to protein antigens of *T. pallidum* in pooled sera from patients with primary syphilis before and after treatment. The antigens transferred to nitrocellulose paper were incubated with sera followed by ^{125}I-protein A and identified by autoradiography. Positions of molecular weight standards are shown on the left. Numbers below indicate months after treatment. The arrows (▲) indicate the lost antibodies of molecular weights 68,500 and 47,000.

DISCUSSION

Since the technique to transfer polypeptides separated by SDS-PAGE to solid-phase matrix (Western blot) for antigenic analysis was developed (Towbin et al., 1979), many studies have been performed on the humoral immune response in human syphilis to individual proteins of *T. pallidum* (Hanff et al., 1982; Hanff et al., 1983; Moskophidis and Müller, 1984; Baker-Zander et al., 1985; Baker-Zander et al., 1986; Kim et al., 1988).

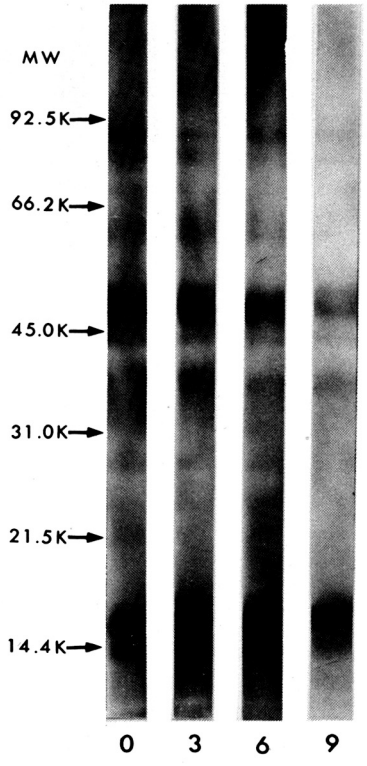

Fig. 2. IgG antibody reactivity to protein antigens of *T. pallidum* in pooled sera from patients with secondary syphilis before and after treatment. The antigens transferred to nitrocellulose paper were incubated with sera followed by ^{125}I-protein A and identified by autoradiography. Positions of molecular weight standards are shown on the left. Numbers below indicate months after treatment.

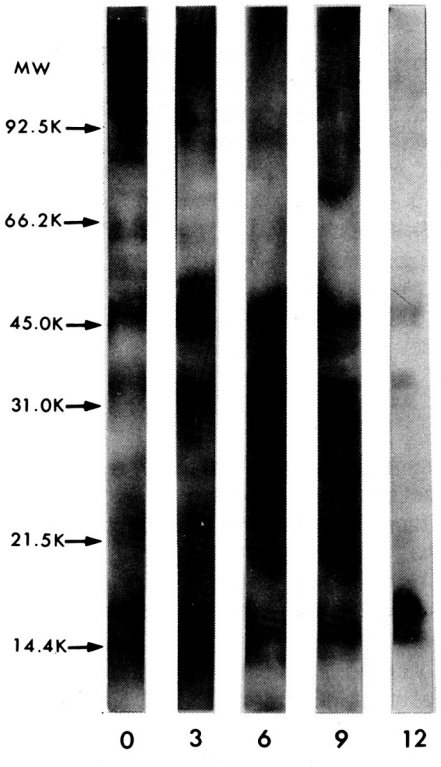

Fig. 3. IgG antibody reactivity to protein antigens of *T. pallidum* in pooled sera from patients with early latent syphilis before and after treatment. The antigens transferred to nitrocellulose paper were incubated with sera followed by ^{125}I-protein A and identified by autoradiography. Positions of molecular weight standards are shown on the left. Numbers below indicate months after treatment.

Table 1. Changes of IgG antibody reactivity to protein antigens of *T. pallidum* in pooled sera from patients with primary syphilis after treatment

MW of antigens (kDa)	Sera*				
	Before treatment	After treatment (months)			
		3	6	9	11
80.0	+ +	+ +	+ +	+ +	+
71.5	+ + +	+ +	+ +	+ +	+ +
68.5	+ +	+	+ +	+ +	−
53.5	+	+	+	+	+
47.0	+ +	+ +	+ +	+ +	−
43.0	+	+	+	+	−
39.0	+ +	+	+ +	+ +	+
32.0	+	+	+	+	+
27.0	+	±	+	+	−

*Reactivity of IgG with individual *T. pallidum* antigens: + + +, strong; + +, intermediate; +, weak; ±, equivocal

Table 2. Changes of IgG antibody reactivity to protein antigens of *T. pallidum* in pooled sera from patients with secondary syphilis after treatment

MW of antigens (kDa)	Sera*			
	Before treatment	After treatment (months)		
		3	6	9
86.5	+	+	+	±
80.0	+	+	±	−
71.5	+	+	−	−
64.0	+	+	−	−
61.0	+	+	−	−
47.0	+ + +	+ + +	+ + +	+ + +
43.0	+ +	+	+	−
36.5	+ +	+ +	+ +	+ +
34.0	+	+	+	−
32.0	+	+	+	−
27.0	+	+	+	+
23.5	+	+	+	−
21.5	+	−	−	−
15.5	+ + +	+ + +	+ + +	+ + +
14.0	+ + +	+ + +	+ + +	+ +

*Reactivity of IgG with individual *T. pallidum* antigens: + + +, strong; + +, intermediate; +, weak; ±, equivocal

Table 3. Changes of IgG antibody reactivity to protein antigens of *T. pallidum* in pooled sera from patients with early latent syphilis after treatment

MW of antigens (kDa)	Sera*				
	Before treatment	After treatment (months)			
		3	6	9	12
86.5	+	+	+	+	+
80.0	+	+	+	+	+
71.5	+	+	−	−	−
64.0	+	+	−	−	−
61.0	+	+	+	+	+
53.5	+	+	+	+	+
47.0	+ + +	+ + +	+ + +	+ + +	+ + +
43.0	+	+	+	+	−
39.0	+	+	+	±	±
36.5	+ +	+ +	+ +	+ +	+ +
34.0	+	+	+	+	+
32.0	+	+	+	+	+
27.0	+	+	+	+	+
23.5	+	+	+	+	+
21.5	+	+	+	+	+
15.5	+ + +	+ + +	+ + +	+ + +	+ + +
14.0	+ + +	+ + +	+ + +	+ +	+ + +

*Reactivity of IgG with individual *T. pallidum* antigens: + + +, strong; + +, intermediate; +, weak; ±, equivocal

Table 4. IgG antibody reactivity to major protein antigens of *T. pallidum* in sera of patients with treated syphilis*

MW of antigens (kDa)	Before treatment	After treatment
47.0	+ + +	+ + +
36.5	+ +	+ +
15.5	+ + +	+ + +
14.0	+ + +	+ + +

*Reactivity of IgG with major protein antigens of *T. pallidum*: + + +, strong: + +, intermediate: +, weak

However, most previous studies were devoted mainly to characterizing the antigens detected during the different clinical stages of syphilis before treatment, and only limited examinations have been performed on the changes of serum antibody reactivity to protein antigens of *T. pallidum* after treatment (Van Eijk and Van Embden, 1983; Moskophidis and Müller, 1984; Hensel et al., 1985; Baker-Zander et al., 1986). Moskophidis and Müller (1984) investigated serum antibodies to treponemal antigens in patients with treated secondary syphilis who had been treated 2 to 3 years prior to their study. They detected IgG antibodies against a wide spectrum of treponemal proteins with molecular weights between 15,500 and 115,000, and which showed only weaker reactivity compared to those of patients with untreated secondary syphilis. Baker-Zander et al. (1986) observed the changes of IgG reactivity to antigens of *T. pallidum* after treatment at different clinical stages of syphilis. In primary syphilis, IgG reactivity persisted despite some diminution in staining intensity. In secondary and early latent syphilis, IgG antibody also declined slightly in staining intensity but continued to show reactivity against all molecules detected initially. Van Eijk and Van Embden (1983) and Hensel et al. (1985) emphasized the importance of IgG antibody against treponemal antigens of molecular weights 16,000 and 17,000 respectively, which persisted for an unreported period of time after sufficient treatment.

In our study, only a loss of IgG antibody reactivity to several antigens and a decrease in staining intensity was seen in primary, secondary and early latent syphilis. In primary syphilis, there was a significant loss of two IgG antibodies to the treponemal antigens of molecular weights 68,500 and 47,000 at 11 months after treatment. According to our previous study (Kim et al., 1988), the treponemal antigen of molecular weight 68,500 was *T. pallidum* specific and appeared only in primary syphilis, and that of molecular weight 47,000 was one of the major antigens of *T. pallidum*. Hence, the observation of serum IgG antibody reactivity to treponemal antigens of molecular weights 68,500 and 47,000 could be useful in evaluating the efficacy of treatment in primary syphilis. The remainder of the IgG antibodies which disappeared in primary, secondary and early latent syphilis were reacting with common treponemal antigens of *T. pallidum* and nonpathogenic *T. phagedenis*, and were consequently thought to be insignificant. However, no changes occurred in late latent and reinfected syphilis.

In secondary, early latent, and late latent syphilis the most strongly reacting antigens of *T. pallidum* precipitated by IgG antibodies before treatment in the sera of patients were the polypeptides of molecular weights 47,000, 36,500, 15,500 and 14,000. It was consequently observed that these were the major antigens of *T. pallidum* (Kim et al., 1988). In this study, we also observed the reaction of serum IgG antibodies of patients who had been treated 2 to 14 years previously for secondary, early latent and late latent syphilis with major antigens of *T. pallidum* to see the longterm changes of reactivity, but no loss or decrease in reactivity was detected.

It was therefore concluded that even the characterization of IgG antibody reactivity to individual protein antigens of *T. pallidum* could not contribute to the assessment of treatment of syphilis in secondary, early latent, late latent and reinfected syphilis. On the other hand, IgG antibodies to treponemal antigens of molecular weights 68,500 and 47,000 could possibly be useful in the assessment of the efficacy of treatment in primary syphilis. But we cannot concretely emphasize the importance of the aforementioned significant antibody to treponemal antigens, because the results were obtained using polyvalent patients' sera. If we were to take it one step further, the production of monoclonal antibodies would enable us to characterize the above mentioned treponemal antigens of molecular weights 68,500 and 47,000, so that they could be utilized to confirm the efficacy of treatment in primary syphilis.

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