JOURNAL OF CLINICAL MICROBIOLOGY, Dec. 1990, p. 2632–2636
0095-1137/90/122632-05$02.00/0

Vol. 28, No. 12

Toxoplasma Antigens Recognized by Human Immunoglobulin A Antibodies

JAYNE HUSKINSON,[1] PHILIPPE THULLIEZ,[2] AND JACK S. REMINGTON[1,3]*

Department of Immunology and Infectious Diseases, Research Institute, Palo Alto Medical Foundation, 860 Bryant Street, Palo Alto, California 94301[1]; Stanford University School of Medicine, Stanford, California 94305[3]; and Laboratoire de Serologie Neonatale et de Recherche sur la Toxoplasmose, Institut de Puericulture de Paris, 75014 Paris, France[2]

Received 13 April 1990/Accepted 11 September 1990

The immunoglobulin A (IgA) antibody response to antigens of *Toxoplasma gondii* during the acute and chronic stages of *T. gondii* infection was studied by immunoblotting with a nonreduced antigen (NRA) preparation. Serum samples were from individuals with acute or chronic *T. gondii* infection, and sequential samples were from women who seroconverted during gestation or congenitally infected infants. IgA antibodies to a variety of antigens were present in sera from each of the groups, irrespective of the titer in an IgA enzyme-linked immunosorbent assay. The predominant NRA recognized by IgA antibodies in sera from each of the four groups had an approximate molecular weight of 30,000. Other prominent NRAs had approximate molecular weights of 35,000 and 5,000; however, the latter NRA was not present in sera of congenitally infected infants.

The recent description of the immunoglobulin A (IgA) antibody response in patients infected with *Toxoplasma gondii* (1, 2, 6–8, 13, 14) suggests that demonstration of such antibodies may be useful for diagnosis of the acute infection, especially in newborns (1, 13). We considered it of interest to attempt to identify the major antigens of *T. gondii* that are recognized by IgA antibodies in adults with acute and chronic (latent) infections and in neonates with congenital toxoplasma infections.

MATERIALS AND METHODS

***T. gondii* antigen preparation.** Tachyzoites of the RH strain of *T. gondii*, obtained from the peritoneal cavity of 2-day-infected Swiss Webster female mice (15) (Simonsen Laboratories, Gilroy, Calif.), were used to prepare reduced antigen (RA) and nonreduced antigen (NRA) as described previously (4).

Serum samples. Sera were collected from 17 patients; 5 had serologic evidence of recently acquired *T. gondii* infection (group A); 5 had serologic evidence of chronic infection (group B) for 4 (patient B1), 6 (patient B2), 4 (patient B3), 15 (patient B4), and 3 (patient B5) years, respectively; and 4 were pregnant and seroconverted during gestation (group C). The remaining three patients were infants (group D), one of whom was the infant of one of the group C patients (mother C2, infant D2). The criteria for classification of patients were described previously (10). Serial serum specimens were obtained from the women in group C, who had been screened monthly by the Sabin Feldman dye test in a prospective study. Serial serum specimens were also obtained from the three infants in group D whose mothers seroconverted during pregnancy. The patients in group C were treated with spiramycin when the diagnosis of acute toxoplasma infection was made (10). The patients in group D were treated with pyrimethamine and sulfadiazine, beginning at birth in one infant (infant D2) and at 2 months of age in the remaining two (infants D1 and D3).

A pool of serum samples from six clinically normal individuals, each of which was negative (<1:2) when tested by the Sabin-Feldman dye test (11), was used as a control for the immunoblots.

Serologic tests. The serologic tests used in this study were the Sabin Feldman dye test (11), double-sandwich (DS) IgM enzyme-linked immunosorbent assay (ELISA) (DS IgM ELISA) (12), IgM immunosorbent agglutination assay (3), and DS IgA ELISA (13). Results of the serologic tests used in this study are shown in Table 1. The IgM ELISA and IgA ELISA results are expressed on a scale of 1 to 10 as described previously (12). The IgM immunosorbent agglutination assay titers range from 0 to 12, as described previously (3).

Immunoblots. Polyacrylamide gel electrophoresis of RAs or NRAs was performed as described previously (4) on 5 to 15% gradient slab gels by using the discontinuous sodium dodecyl sulfate buffer system described by Laemmli (5).

All sera were diluted 1:100 in 0.01 M phosphate-buffered saline (PBS; pH 7.2)–0.05% Tween 20 containing 5% nonfat dry milk. The conjugates used were peroxidase-labeled goat-anti-human IgA, IgG, and IgM antibodies (Caltag Laboratories, San Francisco, Calif.) that were diluted, according to previously determined optimal dilutions, 1:200, 1:1,000 and 1:3,000, respectively, in PBS–0.05% Tween 20 containing 3% bovine serum albumin. The substrate was 3,3′-diaminobenzidine tetrahydrochloride (Organon Teknika, Durham, N.C.) and was used at a final concentration of 0.1 mg/ml in PBS.

Control immunoblots to test for binding of the three conjugates to the *T. gondii* antigen did not reveal any bands (data not shown). Immunoblots to detect the presence of IgA, IgG, and IgM toxoplasma antibodies against RA with each of the serum samples revealed fewer or fainter bands than were detected with NRA (data not shown). For this reason, only results obtained with the NRA are presented here.

* Corresponding author.

TABLE 1. Serologic data of patients

Patient serum identification	Specimen type[a]	Date of serum collection (mo/day/yr)	Antibody titer by:			
			Dye test[b]	DS IgM ELISA[c]	IgM ISAGA[c,d]	DS IgA ELISA[c]
A1	S		1,600	10.6		5.3
A2	S		3,200	7.2		7.4
A3	S		1,600	6.7		4.7
A4	S		1,600	8.5		9.5
A5	S		200	7.8		2.6
B1	S		50	1.9		4.2
B2	S		10	0.1		−0.1
B3	S		50	0.8		0.2
B4	S		25	0.2		−0.2
B5	S		400	2.5		1.7
C1(a)	S	11/29/88	<2		0	0.7
C1(b)	S	2/17/89	400		12	4.6
C1(c)	S	3/1/89	400		12	4
C1(d)	S	3/21/89	400		12	3.4
C1(e)	S	5/17/89	200		12	7.2
C2(a)	S	3/26/88	<2		5	0.3
C2(b)	S	4/20/88	40		12	5
C2(c)	S	5/11/88	400		12	2.7
C2(d)	S	5/19/88	800		12	3.1
C3(a)	S	10/13/88	<2		0	0.1
C3(b)	S	12/1/88	400		12	2.9
C3(c)	S	12/29/88	800		12	3.5
C3(d)	S	5/17/89	800		12	1.7
C4(a)	S	12/1/88	<2		0	0.1
C4(b)	S	1/5/89	40		12	3.3
C4(c)	S	1/23/89	400		12	4
C4(d)	S	2/15/89	800		12	4.4
C4(e)	S	4/21/89	400		11	2.3
D1(a)	CS	10/1/88	3,200		0	1.8
D1(b)	S	10/5/88	1,600		0	3.5
D1(c)	S	11/17/88	800		0	3.5
D1(d)	S	12/14/88	400		0	1.4
D1(e)	S	1/31/89	200		0	1.1
D2(e)	CS	5/19/88	800		12	4
D2(f)	S	5/25/88	1,600		12	9.3
D2(g)	S	8/31/88	800		0	0.7
D2(h)	S	1/3/89	1,600		0	0.4
D2(i)	S	5/31/89	6,400		0	0.5
D3(a)	FS	2/29/88	80		0	3.4
D3(b)	CS	6/16/88	400		0	1.3
D3(c)	S	8/31/88	40		0	−0.5
D3(d)	S	1/16/89	100		0	−0.1

[a] S, Serum; CS, cord serum; FS, fetal serum.
[b] Titers expressed in international units.
[c] Titers expressed as described in the text.
[d] ISAGA, IgM immunosorbent agglutination assay.

For determination of the approximate molecular weights (MWs) of bands below the 14,400-MW marker, an antibody-positive serum sample was tested against RA and NRA on a 10 to 20% gradient gel, to yield a better separation of this region, by using MW markers ranging from 43,000 to 3,000. The MW markers used were ovalbumin, carbonic anhydrase β-lactoglobulin, lysozyme, bovine trypsin inhibitor, and insulin (α and β chains) (Bethesda Research Laboratories, Gaithersburg, Md.).

RESULTS

Results of the serologic tests in each of the sera are given in Table 1. Dates on which the sera were drawn are given for the women in group C and the infants in group D. Representative immunoblots are shown in Fig. 1 to 4. The predominant antigen (most intensely stained band) detected by IgA antibodies had an approximate MW of 30,000. The antigens detected by IgA antibodies in the sera of each of the

TABLE 2. Number of antigens recognized by IgA antibodies in sera from four patient groups

MW (10^3) of bands	No. in the following groups recognizing antigen[a]:				Recognition of antigen by IgG and/or IgM antibodies[b]
	A	B	C	D	
5	5	3	4	0	IgG and IgM
30	5	4	4	3	IgG and IgM
35	5	3	4	2	IgG and IgM
37	0	0	2	0	IgG
39	2	2	0	0	IgG
42	2	0	1	0	IgG and IgM
45	4	2	4	0	IgG and IgM
54–60	4	3	3	1	IgG and IgM
73	5	3	4	1	IgG
83	5	3	3	1	IgG and IgM
97	3	2	4	2	IgG

[a] Numbers represent the number of patients whose IgA immunoblots revealed bands with the indicated MWs.

[b] Indicates whether the band revealed by IgA antibodies was also revealed by IgG and/or IgM antibodies.

individuals in the four patient groups and their approximate MWs are given in Table 2.

IgA immunoblots of group A and B sera. The patterns of bands developed with IgA antibodies did not reveal remarkable differences between group A and group B patient sera. However, the regularity with which certain bands appeared did differ between the two groups. This was especially true for the prominent antigens which had approximate MWs of 35,000, 30,000, and 5,000. These three bands appeared more frequently in sera from group A patients (Table 2). The band which corresponded to an approximate MW of 30,000 was the most intensely stained in the immunoblots of sera from both groups. The bands which corresponded to approximate MWs of 35,000 and 5,000 varied in intensity. Other noteworthy bands, which were present in sera from both groups A and B but more frequently in sera from group A, revealed antigens with approximate MWs of 97,000, 83,000, and 45,000 (Table 2). Representative examples of the immunoblots of sera from patients in groups A and B are shown in Fig. 1.

IgA immunoblots of group C sera. Serum samples from pregnant women who acquired *T. gondii* infections during pregnancy were available before and after their acute infections. The patterns of bands revealed by IgA antibodies (Fig. 2) were similar in each of the seroconverters. As in the immunoblots of sera from patients in groups A and B, the predominant antigens had approximate MWs of 35,000, 30,000, and 5,000. Of special interest was that of these predominant antigens, the one with an approximate MW of 35,000 either appeared first or was the most intensely stained with the earliest postinfection serum sample with which these bands were first demonstrable. In sera from three of the patients, this band was present before any of the serologic tests were positive. An example of this is seen in Fig. 2, lane a. A band with an approximate MW of 55,000 was also demonstrated in immunoblots of the first serum sample from all four of the group C patients. However, this band was also present in the control (negative pool) serum sample (data not shown).

IgA immunoblots of group D sera. The immunoblots of sera from the three infants in group D revealed relatively few bands (Fig. 3). The only prominent bands corresponded to approximate MWs of 35,000 and 30,000.

Comparison of IgA with IgG and IgM immunoblots. Immunoblots to detect IgG antibodies revealed more bands than the immunoblots to detect IgA antbodies (Fig. 4A). Conversely, the immunoblots to detect IgM antibodies revealed fewer bands than the immunoblots to detect IgA antibodies (Fig. 4B). For group D patients, two serum samples had demonstrable bands with IgM and one did not. Although none of the serum samples from the latter patient in group D (patient D3) had demonstrable IgM antibodies by immunoblotting, IgA antibodies were detected in immunoblots of each of this patient's serum samples. In the immunoblots for IgM antibodies, serum samples g, h, and i from patient D2

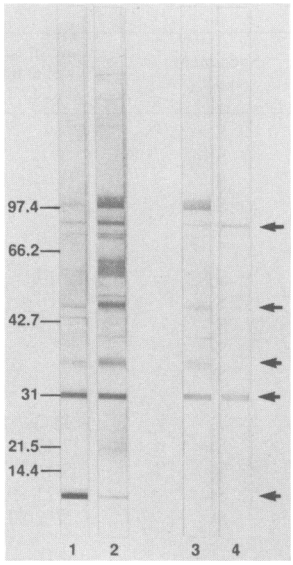

FIG. 1. Immunoblot analysis of *T. gondii* NRAs recognized by IgA antibodies in sera from patients in group A (lanes 1 and 2) and group B (lanes 3 and 4). The lane numbers correlate with the designated patient identification numbers given in Table 1. Arrows show some of the bands mentioned in the text. Numbers on the left represent MW markers (in thousands).

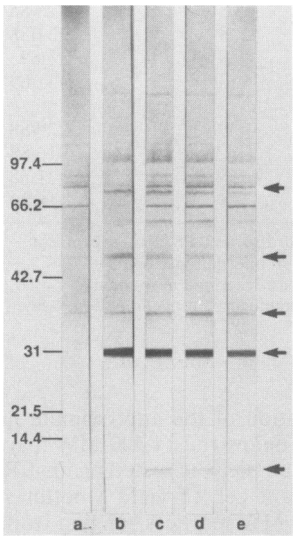

FIG. 2. Immunoblot analysis of *T. gondii* NRAs recognized by IgA antibodies in five serum samples (lanes a through e) from seroconverter C1. Arrows show some of the bands mentioned in the text. Numbers on the left represent MW markers (in thousands).

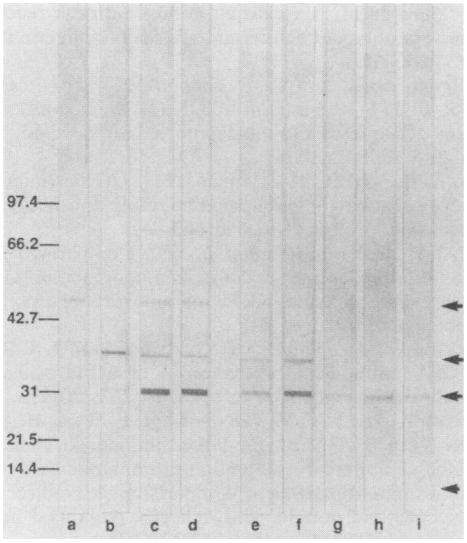

FIG. 3. Immunoblot analysis of *T. gondii* NRAs recognized by IgA antibodies in sera from a mother, C2 (lanes a through d), and her baby, D2 (lanes e through i). Arrows show some of the bands mentioned in the text. Numbers on the left represent MW markers (in thousands).

revealed bands in the area with an approximate MW of 30,000 which was very faint compared with those in the immunoblots for detection of IgA antibodies.

DISCUSSION

The results of the immunoblots described above reveal that an array of antigens of *T. gondii* are recognized by IgA antibodies in sera from both acutely and chronically infected individuals. This was true whether or not they had significant titers in an IgA ELISA (in which the same antigen preparation was used but without pretreatment with sodium dodecyl sulfate). The remarkable intensity of the bands obtained with sera from chronically infected individuals in the area of the immunoblots corresponding to an approximate MW of 30,000 seems surprising in view of the recent report of negative results in sera from such individuals when tested in an IgA ELISA that measures specifically IgA antibody against P30 toxoplasma antigen (1). We have obtained similar negative results in our IgA ELISA (13). Whether this reaction in the immunoblots is due to treatment of the toxoplasma antigens with sodium dodecyl sulfate is not known.

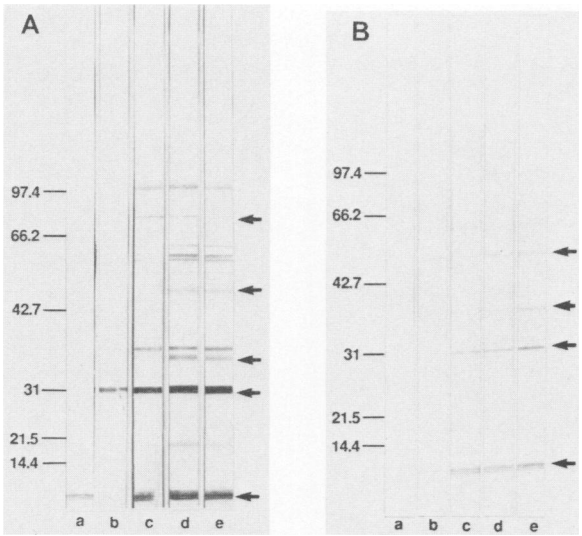

FIG. 4. Immunoblot analysis of *T. gondii* NRAs recognized by IgG (A) and IgM (B) antibodies in five serum samples (lanes a through e) from seroconverter C1. Arrows show some of the bands mentioned in the text. Numbers on the left represent MW markers (in thousands).

Antigens with approximate MWs of 35,000 and 5,000 (measured as an MW of approximately 4,000 by Potasman et al. [9]) were also recognized by IgA antibodies in sera from individuals with acute or chronic infections. Potasman et al. (9) previously reported these antigens to be among the first recognized by IgG and IgM antibodies and the most intensely stained bands in IgG and IgM immunoblots of sera obtained from individuals in the early stage of acute infection. Our results with the earliest sera from acutely infected pregnant women revealed that the antigen with an approximate MW of 35,000 either appeared in the immunoblots before other bands or was the most intensely stained of any of the bands.

Partanen et al. (7) studied, by immunoblotting, the antibody subclass response in sera from a single patient with lymphadenopathic toxoplasmosis. Their results were with reduced toxoplasma antigen only. This likely accounted for the relative paucity of bands observed at 45 weeks after infection compared with those immunoblots developed with sera 4 to 9 weeks after a laboratory accident caused infection of the patient. Of interest was their observation of intensely staining bands in areas of the immunoblots corresponding to approximate MWs of 50,000 and 25,000. These intensely stained bands were not observed in our immunoblots of RAs or NRAs. It is unclear whether these differences were due to the laboratory-acquired infection with the virulent RH strain of toxoplasma.

Differences in the sensitivity between the IgA ELISA and the IgA immunoblots were noted with the infants' serum samples. In infant D1 the IgA ELISA titers would be considered positive (unpublished data), but no bands were demonstrable in the IgA immunoblots of that infant's serum samples a and b. However, bands with an approximate MW of 30,000 were noted with serum samples c and d from this infant. Thus, with serum samples a and b from infant D1, the IgA ELISA appeared to be more sensitive than the immunoblots. However, in sera from infants D2 and D3, bands were demonstrable in the IgA immunoblots, including those treated with serum samples in which the titers in the IgA ELISA were low or negative (Fig. 3). In contrast to the numbers of bands in IgA immunoblots of sera from adults, only antigens with MWs of approximately 35,000 and 30,000 were demonstrable in immunoblots of sera obtained in the early newborn period (Fig. 3).

ACKNOWLEDGMENTS

We thank Dorothy Gibbons and Marie Paule Hajdu for excellent technical assistance.

This work was supported by Public Health Service grant AI04717 from the National Institutes of Health.

LITERATURE CITED

1. **Decoster, A., F. Darcy, A. Caron, and A. Capron.** 1988. IgA antibodies against P30 as markers of congenital and acute toxoplasmosis. Lancet **ii**:1104–1107.

2. **Derouin, F., and J.-J. Sulcebe Ballet.** 1987. Sequential determination of IgG subclasses and IgA specific antibodies in primary and reactivating toxoplasmosis. Biomed. Pharmocother. **41:** 429–433.
3. **Desmonts, G., Y. Naot, and J. S. Remington.** 1981. Immunoglobulin M-immunosorbent agglutination assay for diagnosis of infectious diseases: diagnosis of acute congenital and acquired *Toxoplasma* infections. J. Clin. Microbiol. **14:**486–491.
4. **Huskinson, J., P. N. Stepick-Biek, F. G. Araujo, P. Thulliez, Y. Suzuki, and J. S. Remington.** 1989. *Toxoplasma* antigens recognized by immunoglobulin G subclasses during acute and chronic infection. J. Clin. Microbiol. **27:**2031–2038.
5. **Laemmli, U. K.** 1970. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature (London) **227:**680–685.
6. **Le Fichoux, M. P., and H. Chan.** 1987. Contribution of specific serum IgA assay to the diagnosis of toxoplasmosis. Ann. Pediatr. (Paris) **34:**375–379.
7. **Partanen, P., H. Turunen, R. Passivuo, and P. Leinikki.** 1984. Immunoblot analysis of *Toxoplasma gondii* antigens by human immunoglobulins G, M, and A antibodies at different stages of infection. J. Clin. Microbiol. **20:**133–135.
8. **Pinon, J. M., H. Thoannes, P. H. Pouletty, J. Poirriez, J. Damiens, and P. Pelletier.** 1986. Detection of IgA specific for toxoplasmosis in serum and cerebrospinal fluid using a non-enzymatic IgA-capture assay. Diagn. Immunol. **4:**223–227.
9. **Potasman, I., F. G. Araujo, G. Desmonts, and J. S. Remington.** 1986. Analysis of Toxoplasma gondii antigens recognized by human sera obtained before and after acute infection. J. Infect. Dis. **154:**650–657.
10. **Remington, J. S., and G. Desmonts.** 1990. Toxoplasmosis, p. 89–195. *In* J. S. Remington and J. O. Klein, (ed.), Infectious diseases of the fetus and the newborn infant, 3rd ed. The W. B. Saunders Co., Philadelphia.
11. **Sabin, A. B., and H. A. Feldman.** 1948. Dyes as microchemical indicators of a new phenomenon affecting a protozoan parasite (Toxoplasma). Science **108:**660–663.
12. **Siegel, J. P., and J. S. Remington.** 1983. Comparison of methods for quantitating antigen-specific immunoglobulin M antibody with a reverse enzyme-linked immunosorbent assay. J. Clin. Microbiol. **18:**63–70.
13. **Stepick-Biek, P., P. Thulliez, F. G. Araujo, and J. S. Remington.** 1990. IgA antibodies for diagnosis of acute congenital and acquired toxoplasmosis. J. Infect. Dis. **162:**270–273.
14. **van Loon, A. M., J. T. M. van der Logt, F. W. A. Hessen, and J. van der Veen.** 1983. Enzyme-linked immunosorbent assay that uses labeled antigen for detection of immunoglobulin M and A antibodies in toxoplasmosis: comparison with indirect immunofluorescence and double sandwich enzyme-linked immunosorbent assay. J. Clin. Microbiol. **17:**997–1004.
15. **Wilson, C. B., V. Tsai, and J. S. Remington.** 1980. Failure to trigger the oxidative metabolic burst by normal macrophages: possible mechanisms for survival of intracellular pathogens. J. Exp. Med. **151:**328–346.