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Usefulness of *Toxoplasma gondii*-Specific Recombinant Antigens in Serodiagnosis of Human Toxoplasmosis

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***Toxoplasma gondii* SAG1, GRA1, and GRA7 recombinant antigens may be regarded as tools for the detection of *T. gondii* immunoglobulin G antibodies in persons with chronic and acute toxoplasmosis. GRA7 is more correlated with acute toxoplasmosis. A combination of these antigens will increase the sensitivity of enzyme-linked immunosorbent assays.**

Laboratory diagnosis of *Toxoplasma* infection is usually based on the detection of specific antibodies. The enzyme-linked immunosorbent assay (ELISA) is one of the easiest tests to perform, and many serological tests for the detection of *Toxoplasma gondii*-specific immunoglobulin are commercially available. Most of these commercial kits use a native parasite antigen prepared from tachyzoites. The use of recombinant antigens would allow better standardization of the tests and reduce the costs of production. In spite of the potential advantages of using recombinant antigens in serology tests, only a limited number of studies have combined more than one antigen in an ELISA (1, 5, 6).

In this study we have evaluated the diagnostic usefulness of the SAG1, GRA1, and GRA7 *T. gondii* recombinant antigens. We chose SAG1 because it is one of the most immunogenic *T. gondii* antigens, GRA1 because it is a major secretory antigen recognized in chronically *Toxoplasma*-infected humans, and GRA7 because it gives a very strong antibody response in the acute phase of infection. These antigens were obtained by the method described previously (3). Production of the *T. gondii* native antigen was based on the standard procedure, by freezing and thawing tachyzoites obtained from a parasite (RH strain) maintained in a mouse. Each antigen was used at a concentration of 10 μg per ml. We used two in-house immunoglobulin G (IgG) ELISAs: (i) an IgG ELISA (rec-ELISA) performed with *T. gondii* recombinant antigens and (ii) an IgG ELISA (nat-ELISA) performed with the *T. gondii* native antigen. The ELISA was carried out as described previously (3). Each serum was examined twice. Results were determined for each serum by calculating the mean value of the optical density (OD) reading for duplicate wells. A positive result was estimated as any value higher than the average OD reading plus three standard deviations (cutoff) obtained with 10 sera from the negative control serum group. The cutoff values were as follows: 0.976 for SAG1, 0.865 for GRA7, 0.669 for GRA1, 0.905 for a combination of three recombinant antigens, and 1.095 for the native antigen. The results of rec-ELISAs were compared with those obtained from a group of sera examined by (i) an IgG in-house indirect fluorescent-antibody assay (IFA), which was performed according to the standard method of Goldman (2) by using tachyzoites as a *T. gondii* antigen (bioMérieux, Marcy l'Etoile, France), (ii) a commercial IgG ELISA (com-ELISA) (Organon-Teknika, Boxtel, The Netherlands), and (iii) the TOXO-ISAGA PLUS IgA/IgM and VIDAS TOXO IgG AVIDITY commercial tests (bioMérieux). All commercial tests were performed according to the manufacturer's procedures. Serum samples were obtained from two diagnostic laboratories in Poland and one laboratory in Denmark during routine diagnostic procedures. Written informed consent was obtained from each study participant.

In the preliminary examination, pooled IFA-negative or -positive sera with different titers were used. For further detailed examination, the groups of sera from toxoplasmosis were as follows: (i) group I, 206 sera from patients with indicative infections acquired in the distant past (chronic toxoplasmosis), examined for the first time with IgG IFA ($n = 82$) or with IgG com-ELISA ($n = 124$), all IgM negative, and (ii) group II, 117 sera from patients suspected of having acute toxoplasmosis, IgM and/or IgA positive, with low or high avidity. All sera were reexamined with nat-ELISA, and only sera that were positive in this test were used in the rec-ELISAs.

The results for pooled sera with different titers in the IFA showed that OD readings were proportional to the tested serum titers. With native SAG1 alone and a combination of recombinant antigens, the OD readings obtained were similar and much higher than those obtained with the GRA1 and GRA7 antigens. An ELISA performed with the control *Escherichia coli* antigen, produced and purified in the same way as the recombinant antigens (3), showed an OD consistently below 0.3, which demonstrates that possible contamination of the

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TABLE 1. The results of the IgG rec-ELISAs obtained with *T. gondii* recombinant antigens compared with those of IgG IFA or IgG com-ELISA (serum group I)

Test and titer	No. of sera examined	No. (%) of positive sera			
		GRA7	GRA1	SAG1	GRA7 + GRA1 + SAG1
IgG IFA					
Negative (<1:32)	20	0 (0)	0 (0)	0 (0)	0 (0)
1:128–1:256 (25–50 IU)	20	16 (80.0)	18 (90.0)	14 (70.0)	18 (90.0)
1:512–1:1,024 (100–200 IU)	26	18 (69.2)	20 (76.9)	24 (92.3)	26 (100)
1:2,048–1:8,192 (400–1,600 IU)	36	30 (83.3)	28 (77.8)	36 (100)	36 (100)
Total (1:128–1:8,192)	82	64 (78.0)	66 (80.5)	74 (90.2)	80 (97.6)
IgG ELISA					
Negative (<1:100)	41				0 (0)
1:100	16				11 (68.7)
1:200	24				21 (87.5)
1:400	31				29 (93.5)
1:800	25				24 (96.0)
1:1,600	28				28 (100)
Total (1:100–1:1,600)	124				113 (91.1)

recombinant antigens with *E. coli* antigens does not influence the rec-ELISA results. Only a few sera, which were low positive by IFA, gave ODs that were higher with the *E. coli* antigen than with GRA7 or GRA1, and these sera were considered to be negative (data not shown).

Sera with no reactivity by the IFA or com-ELISA were also negative in the rec-ELISA with each of the recombinant antigens used. For sera from cases of chronic toxoplasmosis, the sensitivity of antibody detection obtained with rec-ELISA was higher when ranked by IFA or com-ELISA titers, reaching 78.0% for all sera with GRA7, 97.6% with the combination of recombinant antigens in comparison to IFA, and from 68.7 (sera at titers of 1:100) to 100% (all sera at titers of ≥1:1,600) in relation to the serum titers obtained by using com-ELISA (Table 1).

In the group of sera from persons suspected of having acute toxoplasmosis, sensitivity was high in subgroup A (the low or borderline avidity), from 83.3% with GRA1 antigen to 98.6% with SAG1 and 100% with the combination of recombinant antigens. With the sera of subgroup B (high avidity), the results obtained were identical to those for the combination of the recombinant antigens and similar to those for SAG1 and GRA1. In contrast, when GRA7 was used, the sensitivity was significantly lower (only 68.9%, $\chi^2 = 16.1705$, $P = <0.001$). All sera from the control group, IgG and IgM negative, were also negative with rec-ELISAs (Table 2).

The availability of large quantities of *T. gondii* recombinant antigens will facilitate their use in diagnostic tests. We found that a combination of three *T. gondii* antigens increased the sensitivity of rec-ELISAs, especially with low-positive sera (Table 1). Also, it has been our experience that the purity of the recombinant proteins obtained from our *E. coli* expression system and purification procedure is very important for the accuracy of the rec-ELISA. Only a few sera that were low positive by IFA gave ODs that were higher with control *E. coli* antigen than with GRA1 and GRA7 antigens.

The best results reaching 100% were obtained with a combination of the three recombinant antigens. These results are consistent with the results of other investigators (1, 4) who have found that combining complementary recombinant *T. gondii* antigens together in the same immunoassay improves the relative assay sensitivity.

Sera from patients with acute toxoplasmosis with low avidity (group IIA) reacted with the GRA7 antigen with high sensitivity (95.7%), whereas a much lower sensitivity (68.9%, $P < 0.001$) was obtained with sera from patients with previous infection and sera with high avidity (group IIB). The sensitivity obtained with GRA7 antigen was also lowest (78%) for group

TABLE 2. The results of the IgG rec-ELISAs obtained with *T. gondii* recombinant antigens with sera positive in IgM ISAGA and/or IgA ISAGA (serum group II)[a]

Serum group	No. of sera examined	No. (%) of positive sera			
		GRA7	GRA1	SAG1	SAG1 + GRA7 + GRA1
Subgroup A (IgM and/or IgA ISAGA positive, low or borderline avidity)	72	69 (95.9)[b]	60 (83.3)	71 (98.6)	72 (100)
Subgroup B (IgM and/or IgA ISAGA positive, high avidity)	45	31 (68.9)[b]	35 (77.8)	45 (100)	45 (100)
Total (IgM and/or IgA ISAGA positive, low or high avidity)	117	100 (85.5)	95 (81.2)	116 (99.1)	117 (100)
Negative control (IgG and IgM negative)	19	0 (0)	0 (0)	0 (0)	0 (0)

[a] ISAGA, immunosorbent-agglutination assay.
[b] $\chi^2 = 16.1705$, $P < 0.001$.

I sera (chronic toxoplasmosis). The conclusion reached herein is consistent with the results of Jacobs et al. (4) and Aubert et al. (1), in which a similar sensitivity was obtained with GRA7 antigen in IgG rec-ELISA by using sera from acute and chronic toxoplasmosis.

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