Fc Receptors on the Surface of Toxoplasma gondii Trophozoites: a Confounding Factor in Testing for Anti-Toxoplasma Antibodies by Indirect Immunofluorescence

DELIA B. BUDZKO,* LISA TYLER, AND DONALD ARMSTRONG

Infectious Diseases Service, Memorial Sloan-Kettering Cancer Center, New York, New York 10021

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Negative sera often produce false-positive polar patterns of fluorescence in indirect immunofluorescence tests for serum anti-Toxoplasma gondii antibodies, representing a confounding factor in the diagnosis of toxoplasmosis. In this work, we studied whether T. gondii trophozoites, the antigenic material used in the immunofluorescence tests, expressed surface Fc receptors that could cause the binding of normal immunoglobulins, thus producing false-positive results. We report here that T. gondii trophozoites indeed have Fc receptors on their surface. This was shown by the direct binding of purified human Fc to the parasite, evidenced by the subsequent binding of fluorescein-labeled Fab specific for human Fc. In addition, pretreatment of the parasite with excess purified Fc to saturate the surface Fc-binding sites abrogated the formation of polar fluorescence. The trophozoites appeared to express Fc receptors with different degrees of affinity for the specific ligand, since a diffuse fluorescence pattern was observed following incubation with 1 mg of Fc per ml (10 μg per well), whereas with 0.2 mg (0.2 μg per well), a majority of parasites showed polar fluorescence. This observation suggests that the Fc receptors accumulated at the polar cap are those with higher affinity. The present findings raise intriguing questions regarding the possible biological role(s) of the Fc receptors on T. gondii but, more immediately, indicated that pretreatment of the antigenic material with Fc, a commercially available reagent, constitutes a practical, simple way to avoid false-positive immunofluorescence test results due to the binding of nonspecific immunoglobulin to the parasite.

The interpretation of indirect immunofluorescence tests for circulating antibodies to Toxoplasma gondii is often made difficult by the appearance of the organisms, i.e., the antigenic material used in the test displays polar fluorescence after incubation with negative sera and treatment with fluorescein-labeled antibody specific for human immunoglobulin G (IgG) (3, 4). Because the second antibody does not distinguish between toxoplasma-specific and nonspecific immunoglobulins, we explored whether T. gondii trophozoites could bind immunoglobulin molecules via a Fc receptor-like surface component. This hypothesis seemed plausible because other protozoans, e.g., several Leishmania species and Trypanosoma lewisi (1, 2), have been shown to bind antibody-coated erythrocytes and aggregated rat immunoglobulin, respectively, via a surface component similar to Fc receptors. It is also noteworthy that the diagnosis of herpes simplex virus type 1 infection by indirect immunofluorescence has often been complicated by the fact that the virus induces the expression of Fc receptors on the infected host cells used as the antigenic material (5, 6). We show in this paper that T. gondii trophozoites express receptors for the Fc portion of human immunoglobulin and that the false-positive indirect immunofluorescence tests that result from this fact can be avoided by pretreating the parasite with purified human Fc.

MATERIALS AND METHODS

Parasites. T. gondii was maintained by biweekly passages in female CD1 mice (Charles River Breeding Laboratories, Inc., Wilmington, Mass.), with intraperitoneal injections of 1 × 10⁴ to 2 × 10⁶ organisms per mouse. The animals were sacrificed by cervical dislocation, and the peritoneal fluids were collected by several washings with sterile phosphate-buffered saline (PBS), pH 7.4, containing 5 U of heparin per ml. The peritoneal cell suspension was centrifuged at 220 × g for 20 min to pellet most of the leukocytes, and the supernatant was further centrifuged at 1,400 × g for 10 min to sediment the parasites. The organisms were then washed three times with PBS by centrifugation. After fixation with 1% Formalin for 30 min, parasite concentrations were determined microscopically by using a hemacytometer; concentration adjustments were made with sterile saline solution.

Sera. Human blood samples were obtained by venipuncture. The serum samples were separated by centrifugation and stored at −70°C until used. To promote immunoglobulin aggregation, selected samples were heated at 52°C for 20 min.

Conventional indirect immunofluorescence test. Wells cut on Teflon-coated microscope slides (Cel-line, Newfield, N.J.) were smeared with the suspension of T. gondii described above, adjusted to contain 100 organisms per 5 μl. A 5-μl portion of the suspension was applied to each well and air dried, and the slides were stored at −70°C until used. For the test, parasite smears were incubated at 37°C for 30 min with serial dilutions of the sera to be tested, washed three times with PBS, rinsed with distilled water, and air dried. In the second step, the slides were incubated with 1/60 dilution in PBS of fluorescein-labeled sheep anti-human immunoglobulin (anti-IgG; Wellcome Research Laboratories, Beckenham, England) at 37°C for 30 min. In some instances, this antibody preparation was replaced with fluorescein-labeled Fab from goat anti-human Fab (Organon Teknika, Charlotte, N.C.) or with fluorescein-labeled Fab from goat anti-human Fc (Organon Teknika). After three washes with PBS and a rinse with distilled water, the slides
were air dried, counterstained with Evans blue, rinsed again with water, and mounted with glycerol.

**Modified indirect immunofluorescence test.** Wells on Teflon-coated slides with parasite smears were covered with 10 μl of a solution containing 1 or 0.2 mg of human Fc (Calbiochem-Behring, La Jolla, Calif.) per ml in PBS and were incubated at room temperature for 30 min. After the slides were washed three times with PBS, wells received the various serum dilutions and the test was continued as described above. After being washed, fluorescein-labeled Fab from anti-human Fab was applied to each well and the slides were incubated at 37°C for 30 min. Additional washes and treatment of the slides were as described above.

**Test for Fc receptors on T. gondii.** Parasite smears in the wells of the Teflon-coated slides were incubated with the Fc preparation described above at 37°C for 30 min and then washed three times with PBS. The slides were next incubated with a solution of fluorescein-labeled Fab from goat anti-human Fc (Organon Teknika) (37°C, 30 min) and washed three times with PBS. Further processing and mounting were as described above.

**RESULTS**

**Presence of receptors for human Fc on T. gondii trophozoites.** Incubation of smears of fixed T. gondii with normal human serum (i.e., a serum which produced neither a positive complement fixation test nor diffuse membrane fluorescence in indirect immunofluorescence tests with T. gondii antigens) produced a typical polar pattern of fluorescence (Fig. 1). This polar fluorescence was seen when the trophozoites initially incubated with normal human serum were further treated with fluorescein-labeled anti-human IgG, fluorescein-labeled anti-human Fc, or fluorescein-labeled Fab from anti-human Fab antibodies. In contrast, when purified Fc (10 μg per well) instead of normal human serum was used in step 1 and either fluorescein-labeled anti-human IgG or fluorescein-labeled Fab from anti-human Fc antibody was used in step 2, a well-defined diffuse pattern of membrane fluorescence was seen. No fluorescence was visible when T. gondii were first treated with human Fc and then with fluorescein-labeled anti-Fab. Negative results were also obtained with parasites pretreated with human Fab regardless of which of the three fluorescein-labeled reagents was used in the staining step.

In experiments in which the parasites were pretreated with Fc, the concentration of this reagent appeared to influence the resulting immunofluorescence pattern produced with fluorescein-labeled anti-human Fc. Thus, approximately half of the trophozoites displayed a diffuse fluorescence pattern when 1 mg of Fc per ml was used, whereas 0.2 mg of Fc per ml led to a vast majority of parasites showing polar fluorescence.

**Effects of pretreatment of T. gondii with purified human Fc.** The binding of nonspecific human immunoglobulin by T. gondii trophozoites is consistent with the presence of Fc receptors on the parasite surface and would explain the polar pattern of fluorescence produced by otherwise negative sera. To examine this possibility, we tested negative as well as positive sera which had been shown to either produce or not produce polar fluorescence in the conventional indirect immunofluorescence test. Each sample was tested in replicates by using fluorescein-labeled anti-IgG or fluorescein-labeled anti-Fab in the staining step. Negative sera producing a polar fluorescence pattern (polar negative) behaved as
such when untreated *T. gondii* was used as antigen but did not behave the same way after the parasites were treated with purified Fc (Table 1). In comparing the antibody titers of antibody-positive sera that were obtained by using untreated and Fc-treated *T. gondii* as antigen, we often observed a slight drop. Thus, for example, a serum producing a titer of 1/32 in the conventional indirect immunofluorescence test gave a titer of 1/8 in the modified test with Fc-treated parasites. Interestingly, negative sera in general became polar positive after being heated at 52°C if tested with untreated *T. gondii* trophozoites. However, this artificial result could also be avoided by using Fc-treated organisms in the test. For those clearly positive sera which did not produce artificial polar patterns of fluorescence, the apparent reduction in titer, if any, due to the use of Fc-treated *T. gondii* never exceeded a twofold dilution and was therefore insignificant.

**DISCUSSION**

As far as we know, these results demonstrate for the first time the presence on the surface of *T. gondii* trophozoites of receptors for the Fc portion of human immunoglobulin and show that these receptors are responsible for the nonspecific polar fluorescence that is frequently produced by the otherwise negative sera in conventional indirect immunofluorescence tests.

The presence of Fc receptors on *T. gondii* was evidenced by the diffuse membrane fluorescence produced when the parasites were first incubated with purified Fc and then treated with fluorescein-labeled Fab specific for human Fc. That Fc receptors on the surface of *T. gondii* were the likely cause of the artificial polar fluorescence was indicated by the abrogation of the phenomenon in the modified indirect immunofluorescence test, i.e., when Fc-treated parasites were used as the antigen. The Fc receptors on *T. gondii* appeared to be heterogeneous with respect to their binding affinity, since diffuse membrane fluorescence was seen after the organisms were incubated first with the Fc concentration (1 mg of Fc per ml), but only the polar pattern was produced when the concentration was reduced to 0.2 mg of Fc per ml. This finding suggests that the Fc receptors accumulating at the pole are probably those with the greater affinity. Further studies are necessary to test this possibility and to probe the biological function(s) of these receptors on *T. gondii*. In the meantime, a most practical and useful application emerges from the present findings: the relatively simple manner in which purified Fc can be used to eliminate a confounding factor in the testing of anti-*T. gondii* IgG antibodies by indirect immunofluorescence. This instance is particularly significant with acquired immune deficiency syndrome patients whose sera often have circulating immunocomplexes. Polar-type fluorescence occurs occasionally in the indirect immunofluorescence test for anti-*T. gondii* IgM antibody. Experiments similar to those described in this paper but using Fc purified from IgM should establish whether this phenomenon is due to the presence of a receptor for IgM Fc on the parasite.

**LITERATURE CITED**