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Immunoblot Analysis of *Toxoplasma gondii* Antigens by Human Immunoglobulins G, M, and A Antibodies at Different Stages of Infection

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The *Toxoplasma gondii* antigenic components eliciting the immunoglobulin G (IgG), IgM, and IgA antibody responses were studied by using follow-up sera from a laboratory worker who developed an acute glandular toxoplasmosis after an accidental infection with the protozoa. IgG toxoplasma antibodies reacted with multiple components over a wide molecular weight range from 6,000 to 150,000. In contrast, IgM toxoplasma antibodies reacted predominantly with polypeptides of 6, 25, and 35 kilodaltons, which might be useful in new diagnostic procedures. The general pattern of antigenic components in the IgA toxoplasma antibody response closely resembled that in the IgM response, even though some characteristic features were constantly observed. The possibility that the restricted IgM and IgA antibody responses relate to the pathogenetic events in human toxoplasmosis is considered.

New demands for accurate diagnostic methods of human toxoplasmosis have been imposed. Serological diagnosis of toxoplasmosis has proved to be the most reliable method thus far (10), and the introduction of new sensitive and specific methods, like enzyme immunoassay (EIA), has offered additional valuable support for the serological diagnosis of toxoplasmosis (11). We have reported the suitability of immunoblotting for the study of the antigenic structure of *Toxoplasma gondii* (5). Sharma et al. (6) observed comparable results by using the same method.

The aim of the present study was to obtain a more detailed picture of the time-course relations of the antigenic patterns in different immunoglobulin classes by using sequential serum samples from a patient with acute glandular toxoplasmosis. In addition to immunoglobulin G (IgG) and IgM reactive antigenic components, special attention was directed toward the antigenic components of *Toxoplasma gondii* eliciting the specific IgA antibody response.

A previously described procedure for purification of *Toxoplasma* antigen preparation was used (5). In brief, the trophozoites were purified from peritoneal exudates of mice infected with the RH strain of *Toxoplasma gondii* by intraperitoneal inoculation. IgG and IgM antibodies were measured by using the Labsystems Toxoplasma EIA kit (Labsystems Oy, Helsinki, Finland). A four-layer modification of EIA was used for the determination of IgA toxoplasma antibodies as described earlier (9). The antibody levels in the serum samples are expressed as relative units (EIA units) derived from known positive and negative reference sera. The World Health Organization anti-toxoplasma reference serum (1,000 IU/ml) contains 170 EIA units of IgG toxoplasma antibodies.

Four sequential serum samples from a patient with an acute glandular toxoplasmosis were used. The patient was a laboratory worker who accidentally had spilled ascites fluid from toxoplasma-infected mice onto small scratches on his left hand. He developed a febrile illness with left axillary lymphadenopathy 10 days after the exposure. The symptoms subsided spontaneously within 2 weeks. The first serum sample, drawn 4 weeks after the exposure, was strongly positive for toxoplasma antibodies. The follow-up sera were obtained 6, 9, and 45 weeks after the exposure. The antibody responses of the follow-up sera are shown in the Table 1. A serum sample which had been taken from the patient before the exposure was negative for toxoplasma antibodies and was used as a negative control throughout the study. All serum samples were stored at −20°C.

For immunoblotting, the toxoplasma antigen preparation was run in 10 or 15% sodium dodecyl sulfate-polyacrylamide slab gel electrophoresis (SDS-PAGE) in reducing conditions, and then separated toxoplasma polypeptides were transferred to a nitrocellulose sheet (Fig. 1). The detailed method for immunoblotting has been described earlier (5, 7). The patient sera were diluted 1:100 for IgG and IgM assays and 1:50 for IgA assays. Heavy chain-specific, peroxidase-conjugated rabbit anti-human immunoglobulins were used (DAKO, Copenhagen, Denmark).

The IgG toxoplasma antibodies from human sera revealed complex patterns of antigenic components (Fig. 2). IgG toxoplasma antibodies reacted with several polypeptides over a wide molecular weight range of from 14,000 to 150,000. In separate experiments with 15% slab gels, an additional low-molecular-weight component of approximately 6,000 could be demonstrated (Fig. 3), which is consistent with the results of Sharma et al. (6). The dominating bands were from 30 to 67 kilodaltons (kD) as seen with different sera. The preimmune serum, negative by EIA (Table 1), was also negative by immunoblotting (Fig. 2).

In contrast to the complex antigenic pattern by IgG toxoplasma antibodies, a very different pattern with only few antigenic components was obtained by IgM toxoplasma antibodies from sequential serum samples from the patient with acute toxoplasmosis (Fig. 2). Major reactivity was with polypeptides of 35 and 25 kD. In addition, polypeptides of 14, 30, 40, and 50 kD were observed. These seemed to be most clearly recognized by sera from the early stages of infection. In experiments with 15% slab gels, the diffuse but intensively staining band of 6 kD could be detected (Fig. 3) as also shown by Sharma et al. (6). In contrast to the permanent nature of IgG antibody reactive antigens, the

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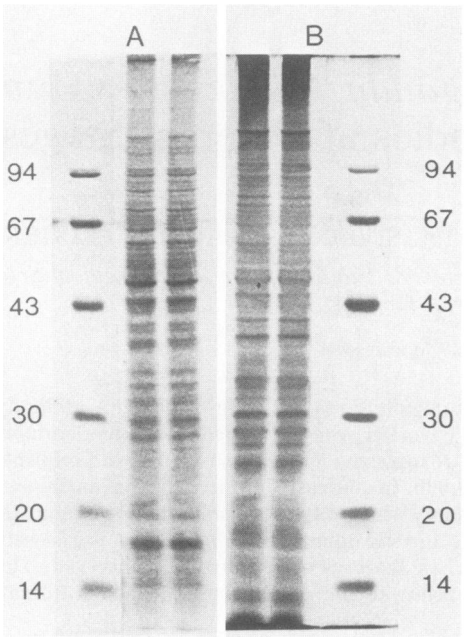

FIG. 1. SDS-PAGE of *Toxoplasma gondii* proteins separated on 10% acrylamide slab gels. (A) Coomassie blue stain. (B) Silver stain. Molecular weight standards are shown ($\times 10^3$) in the margins.

antigenic components recognized by IgM toxoplasma antibodies gradually disappeared along with the declining antibody levels in the follow-up sera. The last follow-up serum, taken at week 45, did not react any of the antigens, further confirming the specificity of the observed reactions.

IgA antibodies gave a pattern closely resembling that given by IgM antibodies. Only a few antigenic components (25, 35, and 50 kD) as well as polypeptides of 30, 32, and 40 kD were recognized by IgA toxoplasma antibodies from early-stage sera. The most obvious difference between the specific IgA and IgG responses was the restricted number of IgA antibody reactive polypeptides, even though all the

TABLE 1. Toxoplasma antibody levels as measured by EIA in sera from a patient with acute toxoplasmosis

Sample	Time after exposure (wk)	Toxoplasma antibody level (EIU)[a]:		
		IgG	IgM	IgA
S0	0	0	0	0
SI	4	75	110	130
SII	6	90	90	120
SIII	9	120	40	100
SIV	45	60	0	20

[a] EIU, Enzyme immunoassay units (see text).

separate antigenic components could be detected by IgG antibodies. The low-molecular-weight component which had reacted strongly with IgG and IgM class toxoplasma antibodies was weaker with IgA when compared with the other IgA reactive components (Fig. 3). Also, here the antigenic components were transient, disappearing during the course of infection (Fig. 2).

The exact number and function of antigenic components in toxoplasma trophozoites are not known. Earlier studies by surface labeling (1, 2) suggested that the number of antigenic membrane components is limited. It is also known that intracellular structures are important in eliciting of hemagglutinating antibodies (3). Probably most of the IgG reactive antigens are derived from the inner structures of disrupted trophozoites.

The smallest antigenic component, 6 kD, suggested by Sharma et al. (6) to be located on the surface of *Toxoplasma gondii*, seemed to migrate as a broad band in SDS-PAGE, similar to bacterial lipopolysaccharides (8).

The restricted number of antigenic components reacting with IgM and IgA toxoplasma antibodies is of great interest. Although the immunoblotting technique is only poorly suited for quantitative studies, the relative intensities of the various IgM and IgA reactive bands compared with the IgG reactive bands prove that there is a true qualitative difference in IgM and IgA antibody responses compared with IgG. These findings raise the possibility that some of the IgG responses

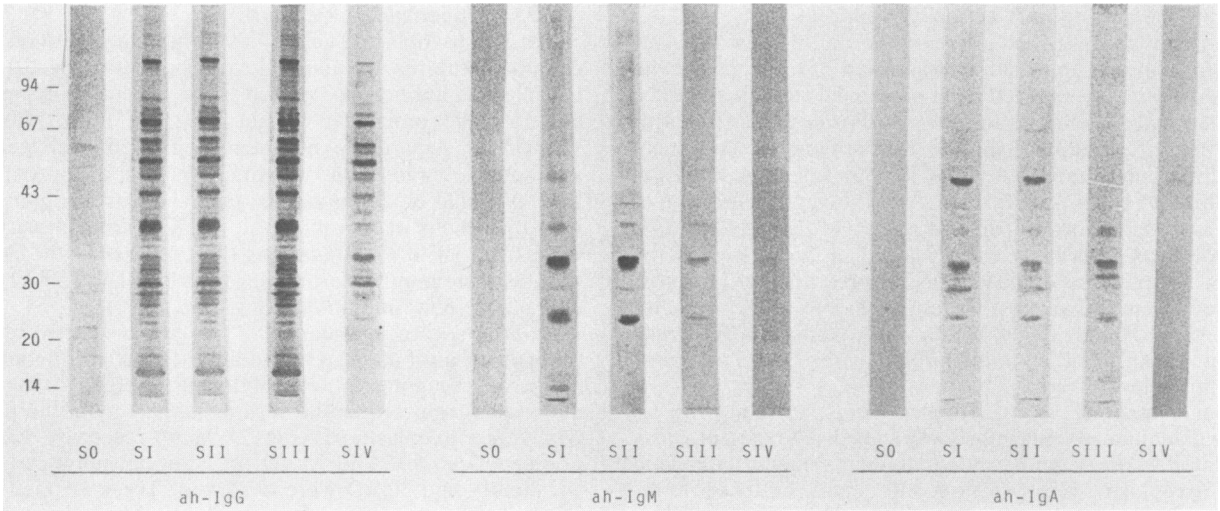

FIG. 2. Immunodetection of *Toxoplasma* antigens by human IgG, IgM, and IgA antibodies during the course of acute toxoplasmosis. Acrylamide slab gels (10%) were used in SDS-PAGE. Sequential sera (S0 through SIV, taken before and at 4, 6, 9, and 45 weeks, respectively, after infection) from a patient with acute toxoplasmosis were used. Peroxidase-conjugated, heavy chain-specific anti-human IgG (ah-IgG), anti-human IgM (ah-IgM), and anti-human IgA (ah-IgA) were used for selective detection of reactions in different immunoglobulin classes. S0 was used as a negative control. Molecular weight standards are shown on the left ($\times 10^3$).

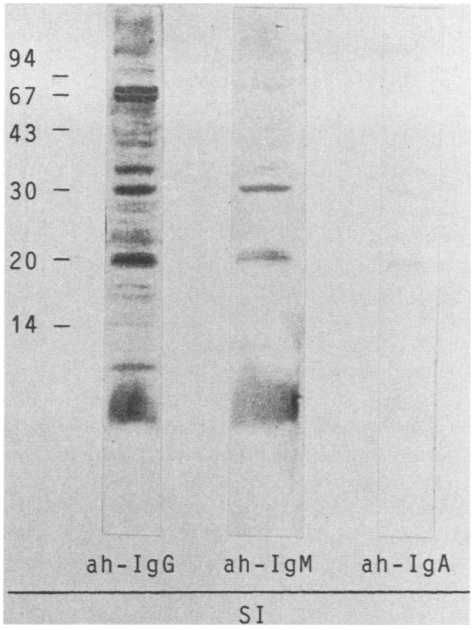

FIG. 3. Immunodetection of *Toxoplasma* antigens by human IgG, IgM, and IgA antibodies with 15% acrylamide slab gels. Molecular weight standards are shown on the left ($\times 10^3$). Abbreviations are the same as in the legend to Fig. 2.

were not preceded by IgM responses, a possibility that needs to be verified by using *Toxoplasma gondii* polypeptides separated without the denaturing conditions, SDS and 2-mercaptoethanol, inherent in the present immunoblotting procedure.

The IgA response has usually been connected with the route of infection, i.e., contact with mucous membranes, rather than with the antigen structure. In the present case the route was transcutaneous, suggesting that the specificity of the bands depended on the antigen structure only.

Also the IgM and IgA responses support the idea that in the present case the course of infection was uncomplicated. No additional bands appeared during the follow-up and the rapid disappearance of IgM and IgA toxoplasma antibodies suggests that the corresponding antigenic components existed for a short time only. It will be of interest to study whether the same components of *Toxoplasma gondii* elicit specific IgM and IgA antibody responses in relapsing infections or in reinfections.

Based on these observations, the following pathways seem possible. In the early stages of infection, intact trophozoites are available for the immunological repertoire. Certain antigenic components located in the membranes of the intact trophozoites elicit an immune response of IgM and IgA antibodies. Most of the IgG reactive antigens are derived from disrupted trophozoites, which are available for a longer time. Accordingly, the IgM and perhaps also the IgA reactive antigens would be the most important structures in the immunological protection against infection.

Purification of one or all of the IgM reactive components may provide antigen for immunoassay of toxoplasma IgM antibodies. Theoretically, it may be possible to use another selection of antigenic polypeptides for preferentially measuring IgA antibodies. Such tests would be of great value for improved laboratory diagnosis of serious toxoplasma infections like congenital toxoplasmosis. The purification of pathogenetically important antigenic components would be of importance in efforts to develop vaccines against *Toxoplasma* infection.

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