Identification of antigenic components of *Toxoplasma gondii* by an immunoblotting technique

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Proteins of *Toxoplasma gondii* were separated by SDS–polyacrylamide gel electrophoresis with subsequent transfer to a nitrocellulose sheet by electrophoretic blotting. Immunologically reactive polypeptides were detected by human sera with previously known toxoplasma antibody levels. Heavy chain-specific, peroxidase-conjugated anti-human immunoglobulins were used as the indicator antibodies for the separate identification of IgG and IgM reactive polypeptides. IgG toxoplasma antibodies reacted with several antigens of M_r ~27000–67000, while toxoplasma-specific IgM seemed to detect only a few polypeptides. The M_r of 35000 for the dominating IgM reactive polypeptide was observed.

Toxoplasma gondii *Antigenic structure* *Immunoblotting technique* *IgG and IgM toxoplasma antibodies* *Specific immunogen*

1. INTRODUCTION

Toxoplasmosis, caused by the protozoan parasite *Toxoplasma gondii*, is a common infection in humans with a world-wide distribution. Most infections are subclinical, or run a mild clinical course but in some cases severe manifestations may be seen; especially in immunocompromised patients and in congenital infections.

A strong humoral-immune response is usually evoked by the toxoplasma infection. The demonstration of specific antibody response constitutes the basis for the diagnosis of toxoplasmosis and several serological methods are available. However, very little is known about the antigenic structure of this parasite.

Polyacrylamide gel electrophoresis is a valuable tool for analysing complex protein mixtures because of its high resolution capacity. The procedure for the transfer of proteins from a polyacrylamide gel to a nitrocellulose sheet as described in [1] has created possibilities to identify, by specific antisera, immunologically reactive polypeptides separated and immobilized on the nitrocellulose replica.

We have applied a modification of this immunoblotting technique for the demonstration of different antigenic components of *T. gondii* as revealed by IgG and IgM toxoplasma antibodies from human sera. Our results suggest that the patterns of antigens eliciting IgG and IgM antibody responses against *Toxoplasma* are strikingly different.

2. MATERIALS AND METHODS

2.1. *Preparation of Toxoplasma lysate*

Toxoplasma lysate for the sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE) was prepared as follows. Toxoplasma trophozoites were obtained from the peritoneal exudates of mice infected 4 days earlier with the RH strain of *Toxoplasma gondii* by intraperitoneal inoculation. Contaminating mouse

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cells were removed by differential centrifugation at 300 rev./min for 10 min followed by 3 washes with 0.9% NaCl solution. The pellet was resuspended in distilled water and sonicated. The lysate was stored in 100 μl aliquots at $-70°C$.

2.2. *Determination of toxoplasma antibodies*

Commercial Toxo-EIA-Kits for the determination of IgG and IgM toxoplasma antibodies were used (Labsystems, Helsinki). The antibody levels of the serum samples are expressed as relative units (EIA units; EIU) derived from known positive references. The World Health Organization anti-toxoplasma reference serum (1000 IU/ml) contains 170 EIU of IgG toxoplasma antibodies.

2.3. *Human sera*

Five serum samples from different persons were used. All these sera had originally been submitted for the routine testing of IgG and IgM toxoplasma antibodies, after which the samples had been stored at $-20°C$. Two of these sera, coded S-1 and S-2, were from patients with clinically acute toxoplasmosis verified by a lymph node biopsy. The 3 other sera, coded S-3, S-4 and S-5, had been collected from laboratory personnel for screening purposes and all of them had been apparently healthy at the time of blood collection. The toxoplasma antibody levels were 150 EIU for IgG and 110 EIU for IgM in S-1; 130 EIU for IgG and 90 EIU for IgM in S-2. The antibody levels for IgG in S-3 and S-4 were 100 EIU and 80 EIU, respectively; both samples were negative for IgM antibodies. S-5 was negative for both IgG and IgM toxoplasma antibodies and was used as a negative control serum.

2.4. *Polyacrylamide gel electrophoresis and the immunoblotting technique*

Toxoplasma lysate was exposed to a SDS–PAGE performed as in [2]. The lysate (1 mg protein/ml) was mixed in a 1:2 ratio with the sample buffer (0.02 M Tris–HCl (pH 6.8), 20% glycerol, 2% sodium dodecyl sulphate, 2% 2-mercaptoethanol, 0.05% bromophenolblue) and boiled for 3 min in a water bath. Disrupted sample preparations (30 μl/track) were separated on a vertical 8% slab gel by electrophoresis at a constant current of 25 mA. Known M_r standards were included (Electrophoresis Calibration Kit, Pharmacia, Uppsala). Transfer of separated polypeptides onto a nitrocellulose paper (Transblot Transfer Medium, Bio-Rad, Richmond CA) was carried out in 25 mM Tris–HCl buffer (pH 8.3) containing 192 mM glycine and 20% (v/v) methanol, with a commercial blotting apparatus (Bio-Rad) as in [1]. For the immunoblotting the nitrocellulose strips were soaked in 0.01 M phosphate-buffered saline, containing 10% (v/v) newborn calf serum and 0.2% (v/v) Triton X-100 (PBS–NCS–TrX100), for 30 min, washed and then exposed to 1:100 diluted human sera in PBS–NCS–TrX100 for 30 min. After washing, the nitrocellulose strips were incubated for 30 min with peroxidase-conjugated rabbit anti-human IgG or IgM, specific for heavy chains (DAKO, Copenhagen) diluted 1:100 in the same buffer as above. After washing, the strips were developed with 3,3-diaminobenzidintetrahydrochloride (DAB; Fluka AG, Buchs), 0.05% DAB made in 50 mM Tris–HCl (pH 7.6) with 0.03% (v/v) H_2O_2, was used. All incubations were carried out at room temperature.

3. RESULTS AND DISCUSSION

The sera S-1, S-2, S-3 and S-4, containing toxoplasma antibodies, revealed patterns of antigenic polypeptides closely resembling each other when tested by peroxidase-conjugated anti-gamma immunoglobulins (fig.1). Complex profiles, each composed of several separate bands, were observed. The range of the estimated M_r-values for these IgG reactive polypeptides was 27000–67000. These results indicate that the IgG antibody response is triggered simultaneously by a wide spectrum of antigenic components. Interestingly, very similar patterns were revealed by IgG toxoplasma antibodies irrespectively whether they were from the acute or chronic stage sera.

Strikingly different patterns presenting only few antigens were observed by using peroxidase-conjugated anti-mu immunoglobulins (fig.1). Only sera with IgM toxoplasma antibody activity (S-1 and S-2) showed visible reactions. Both sera detected a strong band corresponding to an M_r of 35000. The other serum, S-1, revealed an IgM-reactive polypeptide also in the M_r 50000 region. Identical patterns were observed by several other serum samples taken from individuals with recent

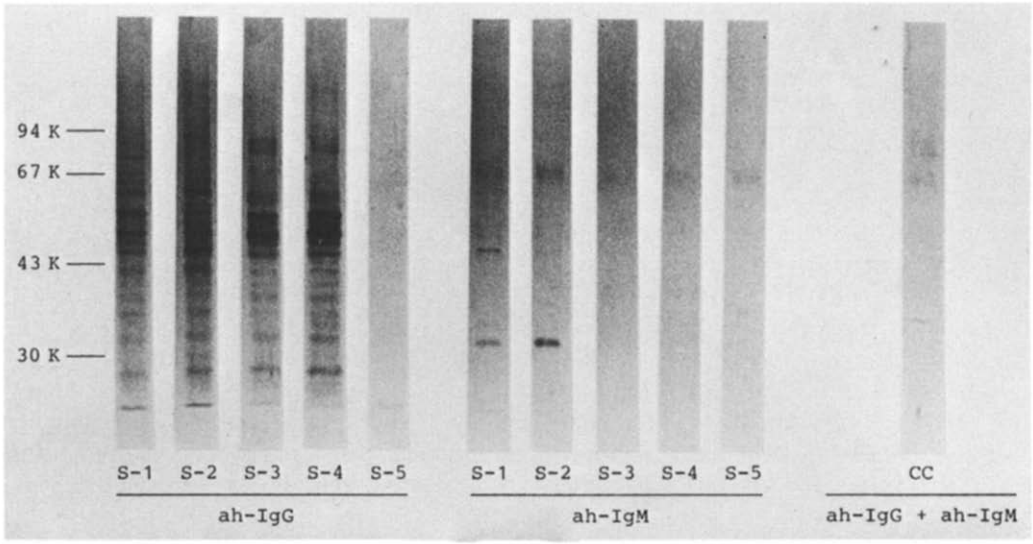

Fig.1. Immunological detection of antigenic polypeptides of *Toxoplasma gondii*. Toxoplasma proteins were separated by SDS–PAGE, followed by electrophoretic blotting onto a nitrocellulose sheet. Antigenic polypeptides were detected by toxoplasma antibodies from human sera and the reactions were separately visualized by peroxidase-conjugated, heavy chain-specific anti-human IgG (ah-IgG) and IgM (ah-IgM). Five human sera were used; S-1 and S-2 contained high levels of IgG and IgM toxoplasma antibodies; S-3 and S-4 had only IgG toxoplasma antibodies; S-5 was negative for toxoplasma antibodies. In the conjugate control (CC) the diluent buffer was used instead of serum. The positions of M_r-standards (kDa) are shown on the left.

toxoplasmosis. This seems to indicate that only few antigenic components were responsible for eliciting the specific IgM antibody response. This has never been reported before. Authors in [3] studied surface-labeled toxoplasma tachyzoites by immunoprecipitation and detected 4 major antigenic polypeptides. One of these had an M_r of 35000 and is probably identical with the dominating IgM-reactive antigen found here.

The specificity of the observed patterns of antigenic components was confirmed by the negative control serum (S-5); only one or two faint nonspecific bands were recognized which could also be seen in the conjugated control lane (fig.1) where buffer had been used instead of serum.

Here, we have demonstrated the suitability of the immunoblotting technique for the investigation of the antigenic structure of *T. gondii*. A special advantage of this method is the possibility to identify separately the immunogens stimulating the production of antibodies in different immunoglobulin classes. The identification and purification of the major antigenic components for an IgM antibody response could be very valuable for diagnostic applications. In earlier studies it has been shown that the demonstration of specific IgM response by an enzyme immunoassay is a most reliable diagnostic marker in acute toxoplasmosis [4]. The use of purified antigen would further improve the specificity and sensitivity of this test. Detailed information about the humoral immune response in toxoplasmosis could be obtained by investigating sequential antigenic profiles as revealed by antibodies from serum samples taken at different stages of infection.

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