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Comparison of Different Serotests for Specific Toxoplasma IgM-Antibodies (ISAGA, SPIHA, IFAT) and Detection of Circulating Antigen in Two Cases of Laboratory Acquired Toxoplasma Infection

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Summary

Two symptomatic *Toxoplasma* infections of laboratory personnel have been serologically followed up for 5.5 and 10 months, respectively. Results obtained by commonly used test systems (indirect fluorescent antibody tests for IgG and IgM antibodies, complement fixation test) were compared with those of two recently developed and improved tests for IgM detection (immunosorbent agglutination assay [ISAGA] and solid-phase indirect haemadsorption assay [SPIHA] as well as with those of a test designed for the detection of circulating antigen (cag-ELISA).

Zusammenfassung

Zwei klinisch apparente Laborinfektionen mit *Toxoplasma gondii* wurden 5,5 bzw. 10 Monate lang serologisch verfolgt. Die in den herkömmlichen Testsystemen (Indirekter Immunfluoreszenztest für IgG-, IgM-Antikörper, Komplementbindungsreaktion) erbrachten Ergebnisse wurden mit jenen Ergebnissen verglichen, die in zwei kürzlich entwickelten und verbesserten Tests zur Bestimmung von IgM-Antikörpern (immunosorbent agglutination assay [ISAGA], solid-phase indirect haemadsorption assay [SPIHA]) erzielt wurden. Außerdem wurden die Ergebnisse eines Enzymimmuntests zum Nachweis von zirkulierendem Antigen (cag-ELISA) in die vergleichenden Untersuchungen einbezogen.

Introduction

Current concepts in the serodiagnosis of toxoplasmosis are directed towards a more rapid and more accurate detection of acute Toxoplasma infections. Improved test systems have been recently described for the determination of specific IgM-antibodies (6, 8, 9, 12, 19, 21, 26) and new serotests have been developed to detect circulating

Toxoplasma antigens in serum (17, 20, 25). A reliable and rapid diagnosis of acute toxoplasmosis is of eminent importance in cases where clinical symptoms are indicating an acute infection (e.g. in cases with lymphadenitis, in immunocompromised patients with encephalitis), in the toxoplasmosis-surveillance during pregnancy, and in the detection of transplacental *Toxoplasma* infections of newborns.

Inquiring the quality of new or improved serotests in the diagnosis of acute toxoplasmosis, conclusions are difficult to draw from the use of common patient's sera, because the precise point of infection is not known. On the other hand, results from animal experiments cannot be unreservedly transferred to the situation in man.

We have taken advantage of two laboratory acquired *Toxoplasma* infections to compare test results obtained by commonly used serological test systems (indirect fluorescent antibody test, complement fixation test) with such of a test for the detection of circulating antigen and such of tests for specific 104 that do not bring about the problems of antibody competition or interference of autoantibodies (immunosorbent agglutination assa^{y}, solid phase indirect haemadsorption assay, indirect fluorescent antibody test with preceding separation of IgM from IgG).

Material and Methods

Patients

Case 1: A 23 years old medical assistent (I.G.), seronegative for *Toxoplasma gondii,* accidentally injected herself parasites (strain BK) into the thumb of the left hand. 3 days after infection pain of the left hand occurred and a lymph node became palpable in the elbow area. 24 days p.i. an axillary lvmphadenitis together with a lymphadenitis along the musculus sternocleidomastoideus developed. The body temperature was subfebrile. On day 30 p.i. a therapy with pyrimethamine (Daraprim®) was initiated, during which the complaints disappeared.

Case 2: Manipulating a syringe with a defect piston, some drops of a mixture of saline and *Toxoplasma* tachyzoites (strain BK) spurted into the left eye of a 19 years old laboratory assistent (M.B.), seronegative for *Toxoplasma gondii.* Despite immediate rinsing of the eye, the area around the left ear turned sensitive to pressure 4 days p.i., and on day 9 p.i. a unilateral edema of the left eye and left side of the face occurred. On da^{y} 11 p.i. the retromandibular lymph nodes became palpable, on day 12 p.i. a nuchal lymphadenitis evolved. From day 21 on the patient was without clinical symptoms. A therapy was not initiated.

Serological tests

Indirect fluorescent antibody test (IFAT)

a) for the detection of IgM-antibodies (IgM-IFAT): carried out according to the recommendations of the FRG-Bundesgesundheitsamt (4), but with an extended serum incubation time: 120 min. Conjugate: goat anti-human IgM/FITC, Atlantic antibodies, Scarborough, USA. To prevent false reactions the test has been performed after separation of IgM from IgG antibodies by immunoabsorption with anti-human IgG (27).

b) for the detection of IgG-antibodies (IgG-IFAT): carried out according to the recommendations of the FRG-Bundesgesundheitsamt (4). Conjugate: goat anti-human IgG/FITC, Behringwerke AG, Marburg, FRG.

Complement fixation test (CFT): carried out according to the recommendations of the FRG-Bundesgesundheitsamt (4).

*Immunosorbent agglutination assay (ISAG*A): Bio Mérieux, France: carried out as instructed by the manufacturer.

Solid-phase indirect haemadsorption assay (SPINA): performed as described (19), except that a monoclona] anti-human IgM antibody was used to coat the microwell plates.
Enzyme-linked immunosorbent assay for circulating antigen (cag-ELISA): performed as formerly described (15).

Results

The serological follow-up of the two symptomatic *Toxoplasma* infections of laboratory personnel was carried out for 5.5 and 10 months, respectively. The time course of antibody titers as well as the detection of cag are shown in Figs. 1 and 2. (Antibodies detected by ISAGA are recorded in ISAGA-values according to the manufacturer's prescription).

In both cases maximal ISAGA values were yielded soon after infection. ISAGA-values remained positive for the whole recording period, while antibody titers measured by SPINA decreased about 55-80 days p.i. Antibody titers measured by IgM-and IgG-IFAT and CFT showed the usual pattern. Circulating antigen was detectable in the serum of patient I.G. at day 30 and 33 p.i., in patient M.B. from day 18 through day 37 p.i.

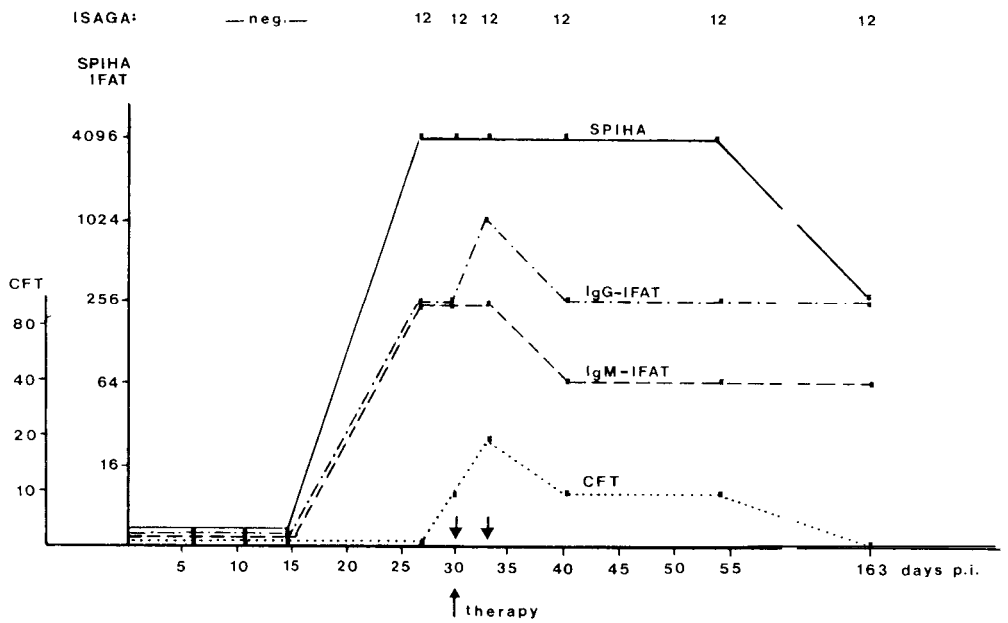

Fig. 1. Time-course of antibody immune response after laboratory-acquired *Toxoplasma* infection (patient I.G.)
CFT: Complement Fixation Test, IFAT: Indirect Fluorescent Antibody Test, ISAGA: Immunosorbent Agglutination Assay (numbers are ISAGA values according to the manufacturer's prescription), SPIHA: Solid-Phase Indirect Haemadsorption Assay, ↓ : detection of circulating antigen.

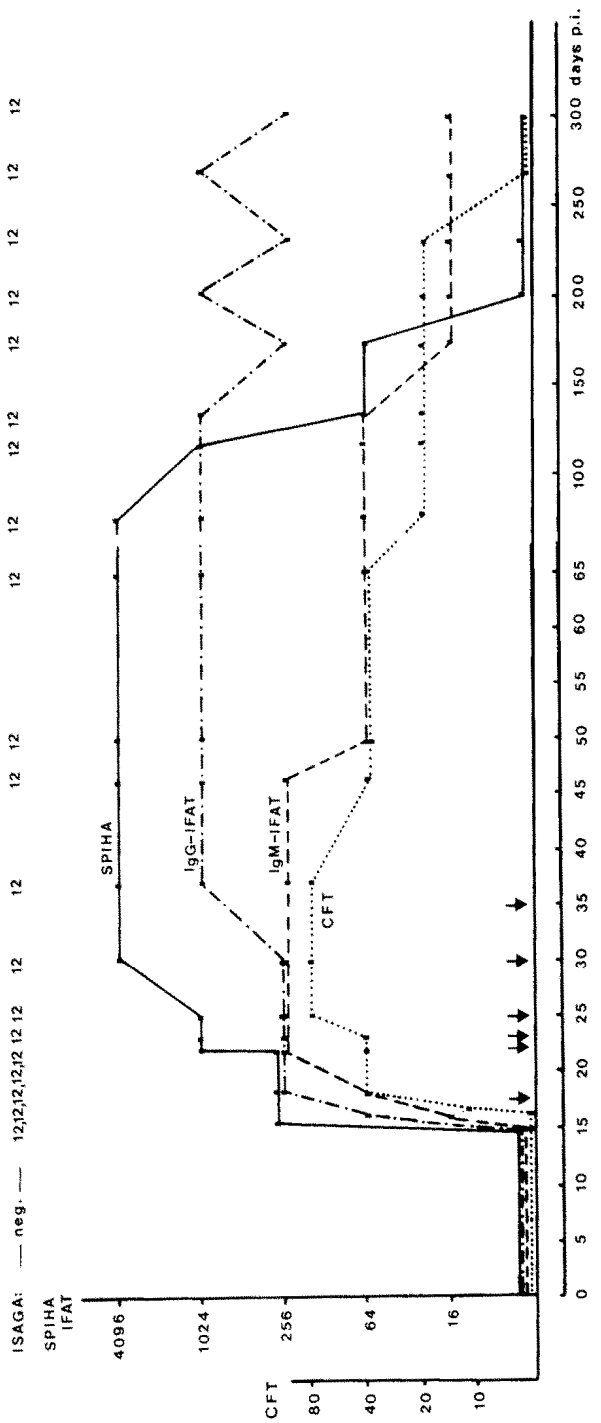

Fig. 2. Time-course of antibody immune response after laboratory-acquired *Toxoplasma* infection (patient M.B.). The abbreviations are the same as in Fig. 1.

Discussion

The most common serological tests for the detection of human IgM antibodies against *Toxoplasma gondii* use anti-human IgM conjugates in "indirect assays". False-positive reactions may be obtained in such tests due to the presence of rheumatoid factor or antinuclear antibodies. False-negative results or weakened positive reactions are likely to occur with sera presenting high concentrations of specific anti-Toxo*plasma* IgG antibodies which compete with specific IgM antibodies for antigenic binding sites (2, 5, 7, 18).

To exclude these sources of error improved test systems use anti-human IgM catching antibodies coated onto the solid-phase (e.g. ISAGA, SPIHA, Double Sandwich ELISA), or common tests are carried out after having separated the IgM from the IgG antibodies.

Comparative studies of test results have been variously performed with IgM-IFAT + IgM-ELISA (21), ISAGA + IgM-IFAT (23), IgM-indirect haemagglutination test + IgM-IFAT (10), SPIHA + IgM-IFAT (1, I9), and SPINA + ISAGA (22) on the basis of sera from various groups of patients. However, no exact data are available concerning the time course of IgM antibodies and the time course of cag-detectability during acute toxoplasmosis from the very beginning of the infection.

By means of a serological follow-up of two laboratory acquired *Toxoplasma* infections we intended to supply additional information on the significance of the recently described tests for IgM- and cag-detection. Surprisingly, both patients remained seronegative until day 14t^{h} p.i. and 15`^{h} p.i., respectively. Usually, the first test becoming positive after infection is the ISAGA (22), in our study this test initially reacted similar to the SPIHA (Fig. 1, 2). Maximal ISAGA values were yielded from the first positive reaction till the end of the recording period (Fig. 1, 2). The SPIHA, performed with the identical monoclonal anti-human IgM catching antibody, showed a rise not as steep as depicted by ISAGA but more distinct than depicted by IgM-IFAT. Antibody-titers in SPIHA decreased from about 55-80 days p.i. on and became negative 200 days p.i. Since the ISAGA values did not decline for a long period after infection, an observation which has repeatedly been confirmed during our toxoplasmosis surveillance, we consider the SPIHA more suitable to distinguish an acute *Toxoplasma* infection from a chronic one. We, therefore, prefer the use of the SPIHA for the diagnosis of fresh, acute *Toxoplasma* infections. On the other hand, the high sensitivity of the ISAGA makes the test very useful in those cases, where no course of the antibody levels can be determined and a very sensitive IgM-test is required, *i.e.* in the detection of connatal *Toxoplasma* infection in newborns. A real advantage of the ISAGA is its availability as a commercial test kit. The SPIHA is not commercially available; accurate block titrations have to be carried out to define the adequate concentration of catching antibody and erythrocytes. The quality of the anti-human IgM antibody (at hest a monoclonal antibody) and the exact concentration of erythrocytes are crucial for obtaining good results (19).

In a comparative study of ISAGA and SPIHA using the identical monoclonal antihuman IgM antibody on 100 human sera (22), in 83% corresponding test results were obtained. 8% were positive by ISAGA but negative by SPIHA, 9% were positive by SPIHA but negative by ISAGA. The authors concluded that the discrepancies between the two methods are not due to differences in sensitivity or reproducibility of IgM binding, but arise solely from the different antigens utilized (surface and/or cytoplasmic antigens), reflecting the heterogeneity of the IgM antibody immune response.

Our results confirm these findings: despite identical catching antibodies used, results

obtained by ISAGA and SPIHA differ during the course of *Toxoplasma* infection. Presumably the SPIHA preferentially detects those IgM antibodies which arise against antigen released during parasite destruction. This would also explain why the SPINA turns to negative some months after infection in contrast to the ISAGA, where positive reactions are obviously obtained for a much longer period.

Compared to the IgM-IFAT, both SPIHA and ISAGA showed a more intense reaction. Since in IgM-IFAT separation of IgM from IgG is necessary to prevent false reactions, additional processing of the serum samples is required. Therefore, the IgM-IFAT rendered somewhat inconvenient. Easy-to-perform separation procedures are rare. Chromatographic equipment usually is not available in routine laboratories and the preadsorption of serum with protein A of *Staphylococcus aureus* has its shortcomings (11, 14). We effected separation by immunoabsorption of serum with anti-human IgG antibodies and consider this procedure a valuable tool for a routine laboratory. An alternative to the detection of IgM antibodies for the diagnosis of an acute *Toxoplasma* infection presumably is the demonstration of cag (3, 15, 16, I7, 24, 25). But, although the appearance of cag in serum has been described to coincide with an early stage of an acute *Toxoplasma* infection (25), it is doubtful whether cag may be detected prior to the appearance of specific IgM antibodies (3, I5, I6).

In both of our patients with laboratory acquired *Toxoplasma* infections cag was detectable between week 3 and 6 p.i. (i.e. detectability concurred with the rising of IgG antibodies). Results of the same kind were obtained when we tested sera of pregnant women (16) and when we investigated the appearance of cag in laboratory animals (15). Thus, it should be emphazised that the appearance of cag in serum is an indication of acute toxoplasmosis, although it does not reflect the very beginning of the infection. We suggest that the demonstration of cag may be attributed to a release of antigens due to the destruction of parasites by the host's immune response. However, it might well be that other types of cag will be selected in similar test systems when catching antibodies of different specificity are used.

The detection of cag in serum gains great importance in cases where antibody titers are not conclusive (16), and in cases where the patient's immune system is suppressed or deficient (e.g. AIDS patients (17)).

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