

Evidence of Structural Proteins of *Toxoplasma gondii* in Sera of Experimentally Infected Mice*

ANDREAS HASSL, HORST ASPÖCK, and HEINZ FLAMM

Abteilung für Medizinische Parasitologie (Leiter: Univ.-Prof. Dr. *Horst Aspöck*) des Hygiene-Instituts der Universität Wien (Vorstand: Univ.-Ptof. Dr. *Heinz Flamm*), A-1095 Wien

Abstract

The present study was performed to clarify whether structural proteins are constituents of the antigens of *Toxoplasma gondii* which circulate in the sera of experimentally infected mice. Rabbits were immunized with mice sera containing circulating antigen, the rabbit sera were then tested for antibodies against *Toxoplasma gondii*. Low titers of specific antibodies, directed against cell wall proteins, could be detected. Thus, the circulating antigen must at least partially consist of structural proteins.

Zusammenfassung

Es sollte geklärt werden, ob die Antigene von *Toxoplasma gondii*, die im Serum experimentell infizierter Mäuse zirkulieren, Strukturproteine beinhalten. Zu diesem Zweck wurden Kaninchen mit antigenhaltigem Mäuseserum immunisiert, die Kaninchenserum wurden anschließend auf spezifische Antikörper gegen *Toxoplasma gondii* untersucht. Es konnten niedrige Titer von Antikörpern, die gegen Zellwandproteine gerichtet waren, gefunden werden. Zirkulierendes Antigen muß daher, zumindest teilweise, aus Strukturproteinen bestehen.

Circulating antigen of *Toxoplasma gondii* (cag) has repeatedly been demonstrated in sera of experimentally infected animals (3, 6, 9, 10). However, the mechanism of cag release is still unclear. There is strong evidence that the destruction of parasite cells is the major contribution to cag formation (6, 7). Thus, cag probably consists of structural proteins which become soluble after the burst of *Toxoplasma* cells.

Mice (Him: OF1 (Swiss) SPF) were intraperitoneally infected with 4×10^7 trophozoites of three different strains of *Toxoplasma gondii* (BK, KB, and 928) each. Three days p.i. blood was collected by heart puncture and serum was prepared by centrifugation (1710 g, 10 min). The sera were tested for their contents of cag ($0.1 \mu\text{g} \times \text{ml}^{-1}$) according to (3). 0.5 ml of serum was mixed with 0.5 ml complete Freund's adjuvant and sc. and im. injected into 3 rabbits (Iva: CHB, SPF). One more rabbit was

* Dedicated to Professor Dr. Georg *Henneberg* on occasion of his 80th birthday.

immunized in the same way with serum of non-infected mice, and one rabbit was not immunized at all. The rabbits obtained three booster injections on days 10, 21, and 28. At day 34 p.i. blood was collected from all rabbits by heart puncture. The Ig fraction was purified by precipitation with 4 M ammonium sulfate at 20 °C and dialysis against PBS. Then, the antibodies directed against mouse proteins were removed by adsorption with normal mouse serum fixed to nitrocellulose stripes.

The Ig fractions of the five rabbits were tested for their contents of specific antibodies against *Toxoplasma gondii* in an indirect hemagglutination assay (IHA; Cellognost Toxoplasmosis; Behringwerke, Marburg, FR Germany), in an indirect fluorescent antibody test using trophozoites as antigen produced in the usual way from mouse ascites (IFAT; done according to [2]), and in an IFAT using *Toxoplasma* trophozoites as antigen which were prepared from a serum-free cell culture (4) (IFAT-sf; the test, except antigen production, was performed according to [2]).

antibody-titers in: immunized with	IHA	IFAT	IFAT-sf rabbit
cag from strain: BK	1 : 8000	1 : 128	1 : 256
KB	neg	neg	1 : 16
928	neg	1 : 32	1 : 256
mouse blood	neg	neg	neg
rabbit not immunized	neg	neg	neg

Although cag of *Toxoplasma gondii* could regularly be demonstrated in sera of experimentally infected mice (1, 9, 10), there is a strange inconsistency in literature on the nature of this cag (8). Different types of antigen release have been taken into consideration: immunolysis followed either by a wash out of inner antigens (6) or by a disintegration of the membrane (3), secretion of proteins by the parasite cells (7), or enzyme release (1). As the cag is most likely a mixture of different parasite proteins, the detectability of particular components influences strongly the specificity and reliability of tests for cag detection.

By immunizing rabbits with mouse serum containing cag, antibodies were produced which were directed against cell wall proteins of *Toxoplasma gondii*. The adsorption of the Ig fractions before testing and the use of IFAT-antigen produced in a medium not contaminated with any mouse proteins guarantee specific results. Serotests performed with antigens raised in serum-free environments seem to be more specific and more sensitive due to more exposed antigens on the parasite cell wall (5). The IHA is commonly considered to detect antibodies against inner antigens preferably (e. g. 6). The lack of detectable IHA-antibodies in two strains seem to point out that wall antigens must be more prominent ingredients of cag than inner antigens. The high IHA-titers found in the rabbit immunized with cag of strain BK may be explained by particular antigenic affinities with the strain used as reagent in the test.

The detection of cell wall proteins as constituents of the cag in mice is an evidence for cell lysis as a major cause for cag formation. Moreover, it offers the possibility of a production of more specific test reagents by isolating these cell wall proteins on one hand and of studies on cag by genetic engineering on the other.

We thank Dr. *K. Hermentin*, Mrs. *I. Gerstl*, and Ms. *E. Berger* for their pleasing cooperation and Dr. *J. P. Overdulve/Utrecht* for supplying with strain KB and 928.

References

1. *Asai, T., T. Kim, M. Kobayashi, and S. Kojima*: Detection of nucleoside triphosphate hydrolase as a circulating antigen in sera of mice infected with *Toxoplasma gondii*. *Infect. Immun.* 55 (1987) 1332-1335
2. Bundesgesetzblatt: Empfehlungen für die Durchführung der Toxoplasma-Seroreaktionen mittels Mikromethode. *Bundesgesundhbl.* 20 (1976) 108-112
3. *Hassl, A., H. Auer, K. Hermentin, O. Picher, and H. Aspöck*: Experimental studies on circulating antigen of *Toxoplasma gondii* in intermediate hosts: Criteria for detection and structural properties. *Zbl. Bakt. Hyg. A* 263 (1987) 625-534
4. *Hermentin, K., H. Auer, and H. Aspöck*: In vitro cultivation of *Toxoplasma gondii* under defined, serum-free conditions. *J. Parasit.* 73 (1987) 1276-1277
5. *Hermentin, K., H. Auer, and H. Aspöck*: Serodiagnosis of toxoplasmosis: Influence of host deriving proteins attached to trophozoites. *Trop. Med. Parasit.* 38 (1987) 255
6. *Hughes, H. P. A.*: Characterization of the circulating antigen of *Toxoplasma gondii*. *Immunol. Lett.* 3 (1981) 99-102
7. *Hughes, H. P. A., and F. van Knapen*: Characterisation of a secretory antigen from *Toxoplasma gondii* and its role in circulating antigen production. *Int. J. Parasit.* 12 (1982) 433-437
8. *Johnson, A. M.*: The antigenic structure of *Toxoplasma gondii*: A review: *Pathology* 17 (1985) 9-19
9. *Knapen, F. van and S. O. Panggabean*: The demonstration of circulating antigens and complexes in experimental toxoplasmosis in mice. *Trop. Geogr. Med.* 32 (1980) 84
10. *Raizman, R. E. and F. A. Neva*: Detection of circulating antigen in acute experimental infections with *Toxoplasma gondii*. *J. Infect. Dis.* 132 (1975) 44-48

Dr. A. Hassl, Univ.-Prof. Dr. H. Aspöck, Univ.-Prof. Dr. H. Flamm, Institute of Hygiene, Kinderspitalgasse 15, A-1095 Vienna, Austria