



# Immunoglobulin M and G Antibodies in Mice in Response to *Toxoplasma gondii*(S-273)Infection and Their Antigen Recognition Patterns in Western Blotting on Various Post-Infection Days

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## 論文内容の要旨

## INTRODUCTION

Detection of *Toxoplasma* specific IgM, IgA and IgE has been suggested to be more helpful in the diagnosis of acute, reactivated and congenital *T. gondii* infection. The lymphocytes both from acquired immunodeficiency syndrome (AIDS) patients and primarily infected immunocompetent patients have been known to synthesize *Toxoplasma* specific antibodies *in-vitro*, and has been thought to be useful biological markers for the diagnosis of toxoplasmic encephalitis. Investigators previously have shown various different types of antigen recognition patterns on Western blotting (WB) during acute and chronic infections, and in congenitally infected newborns and their mothers. We, therefore, became interested to study the IgM and IgG antibody responses in mice experimentally infected with *T. gondii* (S-273) through oral route and to determine their antigen recognition patterns on WB on various post-infection days (PIDs) in order to see whether the antigen recognition patterns by IgM and IgG differ even during an acute infection.

## MATERIALS AND METHODS

A total of five ICR albino mice were infected orally with weakly pathogenic strain of *T. gondii* (S-273) tissue cyst. Mouse brain tissue containing approximately 20 cysts/4 mg of tissue was gently homogenized in 5ml of minimum essential medium (MEM) and 0.1 ml of brain tissue homogenate was fed to each mouse used in this study. From each mouse about 70ul of blood sample was collected from tail vein cut using capillary tubes on 2th, 4th, 6th, 8th, 12th, 16th, 20th, 24th, 29th and 36th, PIDs and were pooled immediately into microtube. Serum samples were stored at -20C till the tests were performed.

*Toxoplasma* antibodies of IgM and IgG classes were measured by ELISA method. Results were read at 492nm in ELISA reader. Serum samples were pre-treated as described by manufacturer prior to performing the IgM-ELISA test. The specificity of ELISA was checked by inhibition ELISA using *T. gondii* (RH) whole cell and sonicated antigens absorbed sera.

Sonicated *Toxoplasma* antigen prepared from *T. gondii* (RH) tachyzoites harvested from ICR albino mice which had been intraperitoneally infected three days earlier was subjected to sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE). The molecular weight (mol. wts.) markers used for SDS-PAGE were phosphorylase b (94kDa), albumin (76kDa), ovalbumin (43kDa), trypsin inhibitor (20kDa) and alphasalalbumin (14kDa). Proteins separated by SDS-PAGE were transferred to polyvinylidene difluoride (PVDF) membrane using an electroblotter at 100 mA for 50 minutes. The PVDF membrane (blots) were saturated with 5.0% skim milk in Tris-tween-20 buffered saline (blocking buffer) followed by drying with the use of blotting paper.

The blots were cut into strips and incubated overnight with the test sera of various PIDs diluted 1:100 in blocking buffer. After washing in washing buffer (Tris-NaCl-Tween-20), the blot strips were incubated at room temperature with horseradish peroxidase labeled rabbit anti-mouse IgM and goat anti-mouse IgG antibodies separately at a dilution of 1:1000. After another wash, the blot strips were soaked in 0.05% 4-chloro-m-naphthol and 0.1% hydrogen peroxide in Tris-buffered saline (TBS) for 10 minutes. Colour reaction was stopped by washing with the washing buffer. Then a diagrammatic representation of the bands observed on the blot strips was made on tracing paper.

## RESULTS

1. *Toxoplasma* antibodies were detectable by ELISA on 12th PID and onwards.
2. Antibody appeared on 12th PID was of IgM type which reached its peak level on 16th PID and began to decline thereafter. However, it persisted at significantly high level until 36th PID.
3. IgG antibody appeared on 16th PID and showed a steady increase even until 36th PID. However, IgG positivity on WB was observed only on 29th and 36th PIDs.
4. On WB, following antigen recognition patterns of mouse IgM and IgG were observed:
  - a) IgM on 12th PID recognised antigens of mol. wts. 53kDa, 50kDa and 21kDa, of which 53kDa, and 21kDa bands were bigger and intenser. Same was true for 16th PID but with the appearance of additional bands of 30kDa, 20kDa, 19kDa and 14kDa.
  - b) The 20kDa band was seen only on 16th PID. The 50kDa, 30kDa and 14kDa bands disappeared on 24th PID and thereafter, while, the 53kDa, 21kDa and 14kDa bands persisted until 29th PID.
  - c) It was interesting that the high intensity bands of 53kDa and 21kDa showed a thinning tendency on 20th PID and thereafter along with the increase of IgG.

- d) IgM recognised a total of seven antigens having mol. wts. of 14kDa to 53kDa.  
e) IgG on 29th PID recognised antigens of 30kDa, 28kDa, 23kDa, 21.5kDa and 19kDa.  
On 36th PID, three additional bands of 53kDa, 50kDa and 17kDa appeared. Of these, 19kDa band was major one.
5. Antigen recognition patterns of IgM and IgG differed remarkably on various PIDs.

## DISCUSSION

In this study, we demonstrated the IgM and IgG response in mice infected with *T. gondii* (S-273) through oral route. Additionally, we also demonstrated the different types of antigen recognition patterns of IgM and IgG on various PIDs for the first time in the literature. The very slow decline of IgM after attaining its peak level on 16th PID was in support of the persistence of IgM antibody for a long period after an acute infection making IgM detection alone not much helpful in the diagnosis of acute *Toxoplasma* infections. However, our WB failed to show the bands on 36th PID. This could be due to the competition between large amounts of IgG and relatively low amount of IgM in the serum samples. However, our finding showed domination of IgM over IgG even when the IgM level was either equal or less than the IgG level (on 24th and 29th PIDs). This appears to be due to the more antigen binding sites of IgM compared with those of IgG.

Our WB finding clearly showed that the antigen recognition patterns by *Toxoplasma* IgM and IgG produced during a primary infection remarkably differs on various PIDs. Furthermore, the size and intensity of bands also differs to a great extent indicating that some of the molecules are more immunogenic than others. One of the major antigen (21kDa) recognised by IgM corresponds to P30 antigen, while the antigen of 53kDa was one of the unique finding of this study. The major antigen, on the otherhand, recognised by IgG was of 19kDa. Use of these major antigens appears to be one of the useful tool in the differential serodiagnosis of acute and chronic toxoplasmosis.

## 論文審査の結果の要旨

### 研究の背景

トキソプラズマ原虫（以下Tpと略）は、ヒトを含む哺乳類・鳥類に感染し、世界中いたるところに分布している。そして、猫科動物が終宿主である病原性原虫である。近年AIDS、臓器移植のための免疫抑制剤療法、抗癌剤療法などの免疫不全の際のTp症や妊婦感染による先天性Tp症の病原体として、その急性期および慢性期のTp症診断法が重要課題の一つになっている。

本申請者は、この重要課題の研究グループの中心となり、実験動物としてマウスを用いて、Tpを感染させ、感染マウスのIgM・IgG抗体のウェスタン・ブロット法（以下WB法と略）による対応抗原パターンによって、特異性の高い急性期Tp症診断法を検討した。

### 成績

本申請者は、実験的にTp（S-273株）シストを経口感染させたICRアルビノマウスのIgMおよび

IgG抗体の変動と種々の感染経過日（2～36日）におけるTp（RH株）タキゾイト抗原に対する態度をELISA法とWB法により検討した。ELISA法による抗Tp・IgM抗体は、感染12日後から出現し、16日後に最高となった後徐々に減少し続け、36日後にいたってなお検出された。しかし、抗Tp・IgM抗体の出現は、感染36日以前でWB法により確認された。ELISA法による抗Tp・IgG抗体は、感染16日後に出現し感染36日後まで着実に増加した。しかし、WB法における抗Tp・IgG抗体は、感染29日後とそれ以降にのみ確認された。

WB法における抗Tp・IgM抗体とIgG抗体は、種々の感染経過日において興味ある抗原認識パターンを示した。すなわち、抗Tp・IgM抗体は少なくとも14kDaから53kDaの間に7本の抗原バンドを示すが、IgG抗体では17kDaから53kDaの間に8本の抗原バンドを示した。主要な抗原バンドはIgM抗体では21kDaと53kDaであったのに対して、IgG抗体では19kDaであった。このように抗Tp・IgM抗体とIgG抗体によって検出された全ての抗原は同一ではなく、感染経過日数によって変化を示した。

以上の実験成績から、Tp・IgM抗体によって認識される21kDaと53kDaの主要抗原およびTp・IgG抗体によって認識される19kDaの主要抗原は、マウスTp症に特異的な抗原であり、これらの抗原の消長を検討することは、急性期および慢性期Tp症の鑑別診断のために有意な方法の一つであると考えられ、価値ある集積と認める。よって本申請者は、博士（医学）の学位を得る資格があると認める。