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**Production of Monoclonal Antibodies
for *Toxoplasma gondii* Lactate
Dehydrogenase**

by

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in Partial Fulfillment of the Requirements for the Degree of

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- ABSTRACT-

Production of Monoclonal Antibodies for *Toxoplasma gondii* Lactate Dehydrogenase

Background/Purpose: *Toxoplasma gondii* is one of the most common parasitic infections of human and other warm blooded animals. Because of the geographical distribution of toxoplasmosis, there is a need to develop a sensitive, rapid, simple and an inexpensive test for the early screening of patients. In this study, to create the dipstick kit that has high sensitivity against LDH (lactate dehydrogenase) of *T. gondii*, at the first of all, hybridoma cell lines producing monoclonal antibodies with high affinity and specificity against *T. gondii* LDH (TgLDH) were established.

Methods: After 6-8 week old BALB/c mice were immunized with synthetic peptides of TgLDH, splenocytes were harvested. To get the monoclonal antibodies (McAbs), splenocytes were fused with myeloma cells using PEG by a cell fusion technique. Hybridoma cells were selected by ELISA and subcloned through a limiting dilution method. McAbs were characterized by isotyping.

Results: Eight McAb-producing hybridoma cells were selected. After three times of limiting dilution, eight clones, 1A10, 1B12, 3B2, 3B6, 1C12, 3C8, 3G6 and 3H1, were selected. OD values of eight monoclonal antibodies were ranged from 0.938 to 1.269 by ELISA. All eight McAbs isotypes were IgM class.

Conclusion: These results indicated that eight hybridoma clones had strong reactivity to TgLDH and may be useful for diagnosis to *T. gondii* infection.

Key Words: *Toxoplasma gondii*, lactate dehydrogenase, cell fusion technique, monoclonal antibodies.

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I. INTRODUCTION

Toxoplasma gondii is one of the most common pathogen in the world. A huge portion of the world's population (over 30 million in France, and over 80 million people in the USA) is infected with *T. gondii*. The parasites were first recovered in the North African rodent called *Ctenodactylus gondi*, hence the species was named *gondii*. It can infect virtually all mammals but the definitive host is the house cat and other members of the Felidae (Garcia and Bruckner, 1997a).

This organism is an obligate intracellular parasite and is found in two forms in humans. The actively proliferating trophozoites or tachyzoites (endozoites) are usually seen in the early, more acute phases of the infection. The resting forms or bradyzoites (cystozoites) are primarily found in muscle and the brain (Garcia and Bruckner, 1997b).

Although *T. gondii* infection is usually asymptomatic in individuals with normal immune systems, it can cause severe syndromes including congenital malformations such blindness, mental retardation, and hydrocephaly in fetuses exposed in uterus. Recently, more attention has been given to *T. gondii* because toxoplasmosis is the most common opportunistic infection in immunocompromised patients with AIDS or in transplant patients (Luft and Remington, 1992).

A. Morphology

Various strains of *T. gondii* differ in virulence and certain biological and genetic characteristics. The life cycle of *T. gondii* includes various stages:

Tachyzoites (endozoites) are proliferative forms that reproduce rapidly within a parasitophorous vacuole in nucleate host cells by means of endodyogeny (endodyogeny: formation of two daughter cells from a mother cell by endogenous budding) (Fig. 1A, 1B). The tachyzoites are sickle-shaped (toxon: bow) cells about 4~7 μm long and 2~4 μm wide. An apical complex is located at the anterior pole, consisting of several components, including the conoid (a conical structure of spirally arranged microtubulin), a pole ring complex, the rhoptries, and micronemes (Fig. 1B). The apical complex contributes to parasite penetration into host cells. The nucleus is located in the posterior half of the cell. More recent investigations have revealed that *T. gondii* and several other apicomplexan protozoa (e.g., *Plasmodium*, *Sarcocystis*) contain, in addition to the chromosomal and mitochondrial genomes, a further genome consisting of circular DNA (35 kb) localized in a special organelle called the apicoplast. This organelle, usually located anterior to the nucleus and close to the Golgi apparatus, is surrounded by several membranes and possesses outer and inner membrane complexes. There is evidence that the apicoplast has evolved from the plastids of endosymbiotic green or red algae. The function of the apicoplast remains uncertain but is indispensable for the host cell and may be a new and specific target for chemotherapy. Tachyzoites also multiply in experimental animals and cell cultures. This stage is highly labile outside of a host and usually does not survive the stomach passage following ingestion.

Bradyzoites (cystozoites) are stages produced by slow reproduction within the cysts (4~8 x 2~4 μm). The cysts develop intracellularly in various tissues, have a relatively resistant wall, grow as large as 150 μm , and can contain up to several thousand bradyzoites

(Fig. 1C). They have a long lifespan in the host. Humans and animals can be infected by peroral ingestion of meat containing cysts (Fig. 2).

Oocysts are rounded and encysted stages of the organism, surrounded by a resistant cyst wall, and are approximately 9 x 14 μm in size. They are the final product of a sexual reproductive cycle in the intestinal epithelia of *Felidae*. They contain a zygote and are shed in feces (Fig. 2). Sporulation takes place within two to four days, producing two sporocysts with four sporozoites each. Sporulated oocysts are infective for human and animals.

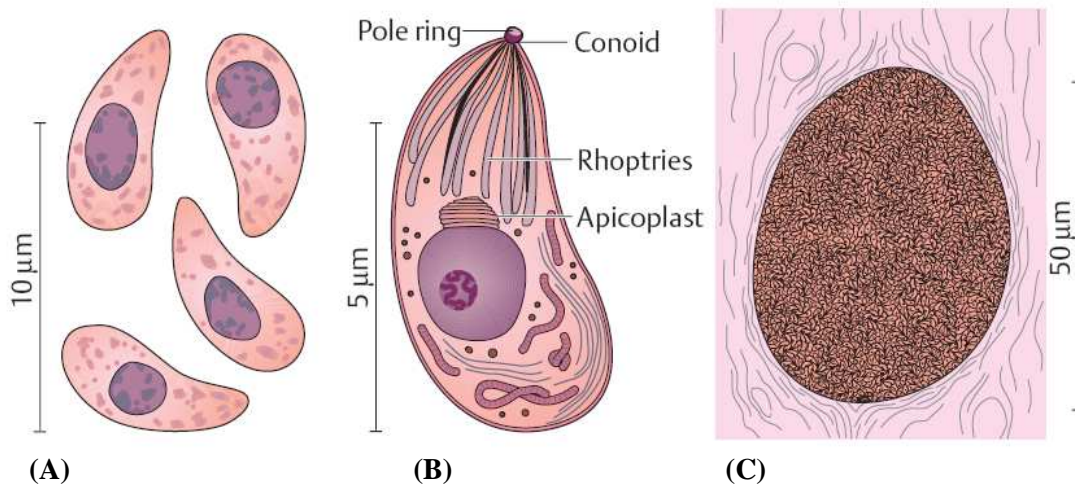


Fig. 1. Morphology of *T. gondii*. (A) Tachyzoites; (B) Ultrastructure of a tachyzoite; (C) Cyst with bradyzoites (Kayser, 2005).

B. Life cycle

The developmental cycle of *T. gondii* involves three phases: intestinal, external, and extraintestinal (Fig. 2).

The intestinal phase: Production of sexual forms (gamogony) takes only place in enterocytes of definitive hosts. Only domestic cats, and several other felid species of little epidemiological significance, can function as definitive hosts for *T. gondii*. Only extraintestinal development is seen in intermediate hosts (pigs, sheep, and many other animal species) as well as in dead-end hosts (humans). Following primary infection of a cat with cysts in raw meat, asexual reproductive forms at first develop in the small intestine epithelium, with sexually differentiated stages and oocysts following later. The oocysts are shed in feces after a prepatent period of three to nine days. When cats are infected with sporulated oocysts, the prepatent period is extended to 20–35 days because in these cases the intestinal development of *T. gondii* is preceded by an extraintestinal asexual reproduction. Oocyst shedding lasts for only a few days to a maximum of three weeks, but can be highly intensive (up to 600 million oocysts per eat during the patent period).

External phase: Oocysts excreted in cat feces sporulate at room temperature within two to four days, rendering them infective. Kept moist, they remain infective for up to five years and are not killed by standard disinfectant agents. They die within a few minutes at temperatures exceeding 55°C or by cooling to -13°C.

Extraintestinal phase: This phase follows a peroral infection with oocysts or cysts and is observed in intermediate hosts (humans, dogs, sheep, pigs, other vertebrates and birds), as well as in the definitive hosts (cats). Starting from the intestine, *T. gondii* travel in blood

or lymph to various organs and multiply in nucleate host cells, especially in the reticulohistiocytic system, in musculature, and in the CNS. Repeated endodyogenic cycles produce as many as 32 individual daughter cells in the expanding host cell before it bursts. The tachyzoites thus released attack neighboring cells. These processes result in focal necroses and inflammatory reactions in affected tissues. Generalization of the infection can lead to colonization of the placenta and, about three to four weeks later, infection of the fetus. Bradyzoites that elicit no inflammatory reactions in the near vicinity are produced early in the course of the infection. Such bradyzoites (cystozoites) are found above all in the CNS, in the skeletal and heart muscles as well as in the retina, the uterine wall, and other organs. They can remain viable for years without causing noticeable damage to the host.

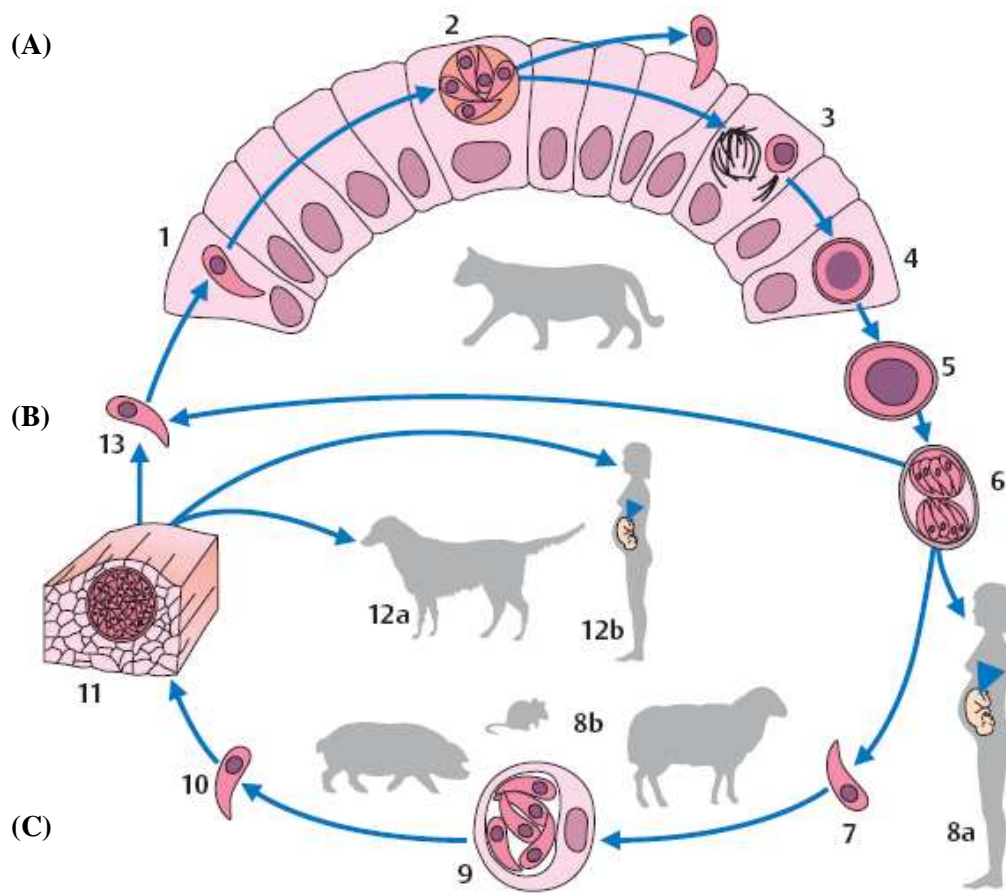


Fig. 2. Life cycle of *T. gondii*. (A) Development in the definitive host (cat): 1 *Toxoplasma* that has penetrated into a small intestine epithelial cell; 2 asexual reproductive stage with merozoites. 3 production of sexual forms (gamogony) and formation of the zygote; 4 oocyst. (B) External phase with sporogony: 5 unsporulated oocyst, shed in cat feces; 6 sporulated oocyst with two sporocysts each containing four sporozoites. (C) Development in an intermediate host (mammals, birds, humans): 7 sporozoite released in organism following oral ingestion of oocysts; 8a human infection; 8b infection of various animal species; 9 asexual reproductive stages (tachyzoites) in a somatic cell; 10 free tachyzoite; 11 cyst with bradyzoites in musculature; 12a infection of a dog with *Toxoplasma* cysts in animal meat; 12b infection of a human with *Toxoplasma* cysts; 13 infection of cat with infective stages of cysts in meat or with oocysts (Kayser, 2005).

C. Diagnosis

Diagnosis of toxoplasmosis can be aided by biologic, serologic, histologic or molecular methods or by combination of the above. Clinical signs of toxoplasmosis are nonspecific and cannot be depended on for a definite diagnosis; toxoplasmosis clinically mimics several other infectious diseases.

Many serologic tests have been used to detect antibodies to *T. gondii*; these include the Sabin-Feldman dye test, the indirect hemagglutination assay, the indirect fluorescent antibody assay (IFA), the direct agglutination test, the latex agglutination test (LAT), the enzyme-linked immunoabsorbent assay (ELISA) and the immunoabsorbent agglutination assay test (IAAT). The Sabin-Feldman dye test, which is still considered as the “gold standard” for detection *T. gondii* in human, is labour-intensive and has the disadvantage that it requires a continuous supply of live parasites. Therefore, most epidemiological studies on *T. gondii* infection now use different tests for antibody detection (Dubey, 1988; Tenter, 2000). However, these tests are all time consuming and require a laboratory equipped with proper instruments and trained personnel. Therefore there is a need to develop a sensitive, rapid, simple and an inexpensive test for the early screening of patients.

D. Lactate dehydrogenase of parasites

It is well known that parasites, within hosts, derive much of their energy from anaerobic glycolysis. Glycolytic enzymes are essential for the survival of the parasites and appear to be potential targets for chemotherapeutic attacks. LDH is a terminal glycolytic

enzyme, which catalyzes the interconversion of pyruvate and lactate in the presence of the nicotinamide adenine dinucleotide coenzyme. Compounds that inhibit LDH have also been shown to inhibit growth of *Plasmodium falciparum* in cultured erythrocytes (Royer et al., 1986) and *T. gondii* in cultured fibroblasts (Dando et al., 2001). It has been suggested that lactate dehydrogenase (LDH), therefore represents a judicious target for drug therapy against these organisms (Kavanagh et al., 2004).

E. Purpose of this study

To create the rapid diagnostic dipstick kit that has high sensitivity against lactate dehydrogenase (LDH) of *Toxoplasma gondii*, first of all, hybridoma cell lines producing monoclonal antibodies with high affinity and specificity against *T. gondii* LDH (TgLDH) were established.

II. MATERIALS AND METHODS

A. Culture of myeloma cells

F0 cells (a mouse myeloma cell line) were cultured in Roswell Park Memorial Institute (RPMI)-1640 medium (Welgene, Korea) containing 10% fetal bovine serum (FBS) (GIBCO, New York, USA) at 37°C in 5% CO₂ incubator.

B. Antigen

Five synthetic peptide of *T. gondii* lactate dehydrogenase (TgLDH) were produced, but only fraction 5 was only used for immunization in this study.

Table 1. Amino acids sequence of synthetic peptide specific for TgLDH-5

Fraction	Amino acids sequence
#5	Q-F-Q-K-S-V-D-D-V-M-A-L-N-K-A-V-A-A-L-Q-A-4MAP

C. Immunization of mice with TgLDH

To produce the immune splenocytes, TgLDH (50 µg/100 µl) was mixed with an equal volume of Freund's complete adjuvant (Sigma laboratories, UAS), and mixture was injected intraperitoneally (i.p.) into 6~8 week old BALB/c mice. At second and third time, the mice were boosted biweekly for another 4 weeks with TgLDH (25 µg/100 µl) mixed with an equal volume of Freund's incomplete adjuvant. After the third immunization, each

immunized mouse serum was assayed by indirect enzyme-linked immunosorbent assay (ELISA). For the final boost, the TgLDH (5 µg/100 µl) was injected intravenously without the adjuvant.

D. Cell fusion

To produce cell lines that secrete McAb, we practiced a cell-fusion technique by the method of previous paper (Kohler and Milstein, 1975; Lee et al., 2007). After blood was harvested, the spleen was extracted from an immunized mouse, and splenocytes were harvested. Then, myeloma cells were harvested from culture flask. After washing 3 times with incomplete RPMI-1640 by centrifuge at 1,500 rpm for 3 min, myeloma cells and spleen cells were suspended together in incomplete RPMI-1640. The cell suspension was centrifuged at 1,500 rpm for 3 min and the pellet was mixed with 50% polyethylene glycol (PEG) (Sigma laboratories, USA). Essentially, for effective cell fusion, the mixture was mixed with 1 ml of 50% PEG solution for 1 min at 37°C in water bath by gently shaking, and then diluted with 19 ml incomplete RPMI-1640. The cell suspension was centrifuged at 1,000 rpm for 5 min, and the supernatant was removed. After cell fusion, the cells were distributed with 100 µl of RPMI-1640 medium containing 20% FBS in individual wells of 96 well culture plate and maintained at 37°C in 5% CO₂ incubator. At one day after cell fusion, 100 µl of Hypoxanthine-Aminopterin-Thymidine (HAT) medium were added to each well (Littlefield, 1964). HAT medium was changed at second and third days after fusion. Then culture medium was replaced with 100 µl of Hypoxanthine-Thymidine (HT) medium. The hybridoma cells were checked with an inverted microscope after cell fusion.

E. ELISA screening

Antigen (synthetic TgLDH peptide and *T. gondii* lysate) was diluted (0.1 µg/100 µl) with 0.05 M carbonate-bicarbonate buffer (pH 9.6), then added to each well of an ELISA plate (Nunc, Denmark) and incubated for over night at 4°C. After washing three times with PBS containing 5% Tween 20 (PBST), it was blocked by 3% bovine serum albumin (BSA) for 2 h at 4°C. After washing three times with PBST, immune serum diluted at 1:500 ratio or hybridoma cell culture supernatant were added on each well (100 µl/well) and incubated for 2 h at 37°C. After 3 times washing with PBST, secondary antibody (100 µl/well) conjugated to alkaline phosphatase (Sigma laboratories) diluted at 1:5,000 ratio was added and incubated for 1 h at 37°C. After washing three times with PBST, substrate buffer (100 µl/well) containing p-NPP tablet (p-nitrophenyl phosphate 20 mg) was added. After incubating for 10~20 min at room temperature, absorbance was read at 405 nm.

F. Selection of hybridoma cells

Hybridoma cells producing monoclonal antibodies were selected by the limiting dilution method. Hybridoma cells were seeded on a 96 well culture plate with 0.2 cells per well. After incubation about 10 days, the hybridoma cells were checked with an inverted microscope and then screened by ELISA. Colonies showing high antibody titer were selected, and then transferred to 24 well culture plates. Selected hybridoma clones were moved into 75-cm² culture flask.

G. Large scale production of McAbs

6~8 week old BALB/c mice were injected intraperitoneally with 200 μ l of Freund's incomplete adjuvant. After four days, mice were injected intraperitoneally with hybridoma cells. For 1~2 weeks, ascites were collected from mice peritoneum by using syringe. After collection, ascites were stored for 30 min at 4 $^{\circ}$ C, and centrifuged at 12,000 rpm for 5 min at 4 $^{\circ}$ C. Collecting supernatant was filtered. The affinity of antibodies was checked by ELISA.

H. Monoclonal antibody isotyping

The McAb isotypes were determined by using the IsoStrip mouse monoclonal antibody isotyping kit (Roche, Indiana, USA) according to the manufacturer's instructions. All isotypes of IgA, IgM, IgG2a and IgG2b, IgG3 were checked.

III. RESULTS

A. Antibody formation of immunized mice

Sera obtained from immunized mice were assayed for antibody formation by indirect ELISA with synthetic peptide of TgLDH-5 (fraction 5) as the antigen (Table 1). Sera from unimmunized mice were used as the negative control. Fig. 3 showed the polyclonal antibody formation for TgLDH-5 antigen. OD values of sera from immunized mice were 0.903 after third immunization. After intravenous injection, the OD values of sera were increased to 1.204, otherwise OD values of normal mouse serum and PBS were 0.16 and 0.13, respectively (Fig. 3).

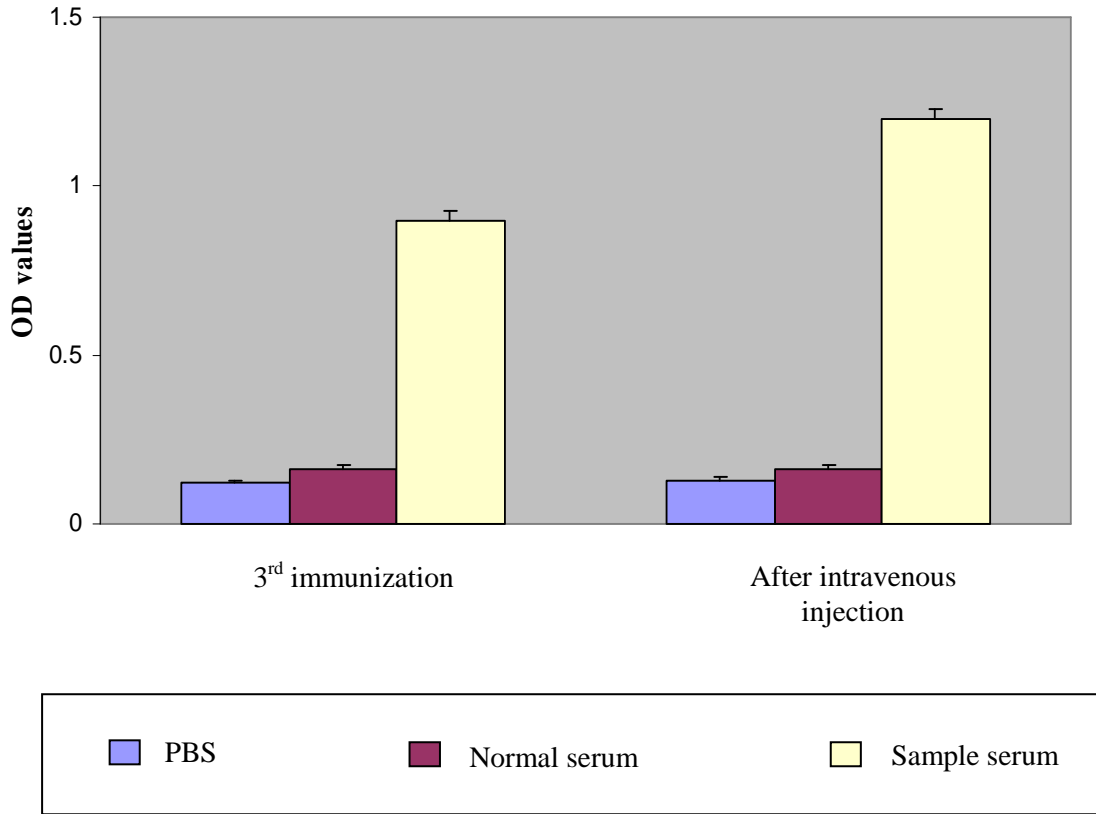


Fig. 3. Polyclonal antibody titers from the TgLDH-5 immunized mice. OD₄₀₅ values of each serum were screened by indirect ELISA (n=3). Sample and normal serum were diluted in PBS at 1:500 ratio, respectively. PBS; control.

B. Cell fusion

Microscopic examination of the 96 wells revealed the growth of hybridoma cells (Data is not shown). Approximately 60% fusion rate was accomplished in a plate. After final cell fusions, twelve hybridoma cell clones were produced, which the antibody formation for TgLDH-5 were detected by indirect ELISA. After three times of limiting dilution, eight hybridoma clones were selected. The OD values of them ranged from 0.435 to 1.035 and control was 0.192. Eight hybridoma cell clones were named as 1A10, 3B2, 3B6, 1B12, 1C12, 3C8, 3G6, and 3H1.

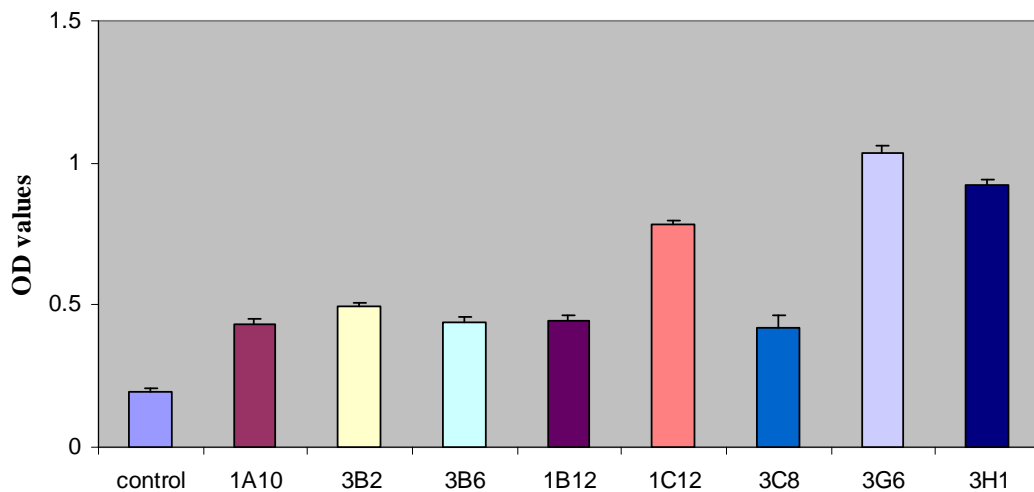


Fig. 4. Antibody titers of the hybridoma cell culture supernatant after cell fusion. After hybridoma cells were checked by microscopy, OD_{405} values of each culture supernatant were screened by indirect ELISA (n=3). Control; normal F0 cell culture supernatant.

C. Isolation of antibody-producing hybridoma cells

All of eight clones were selected by three times of limiting dilution. Using hybridoma cell culture supernatant after the third limiting dilution, the OD values of them ranged from 0.938 to 1.269 and control was 0.156 (Fig. 5). Among eight clones, 1C12 indicated the highest value.

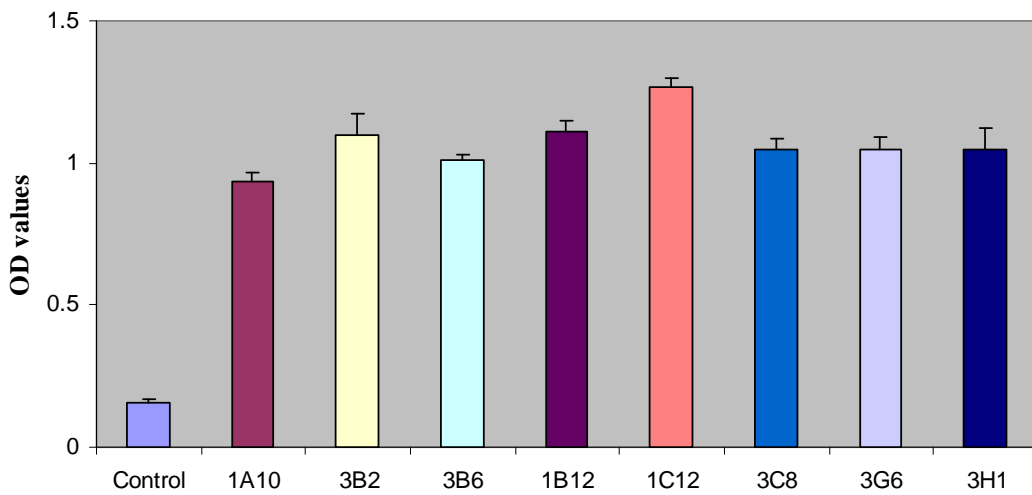


Fig. 5. Antibody titers of the hybridoma cell culture supernatant after third limiting dilution. OD₄₀₅ values of each culture supernatant were screened by indirect ELISA (n=3). TgLDH-5 peptide was used as antigen. Control; normal F0 cell culture supernatant.

D. Producing large scale of antibodies

To produce large scale of antibodies, hybridoma cells were intraperitoneally injected to BLAB/c mice and ascitic fluids containing McAbs were harvested. The ascites were diluted at 1:500 ratio with PBS and screened by indirect ELISA. OD values ranged from 1.089 to 1.314, otherwise OD values of normal and PBS were 0.127 and 0.116, respectively (Fig. 6).

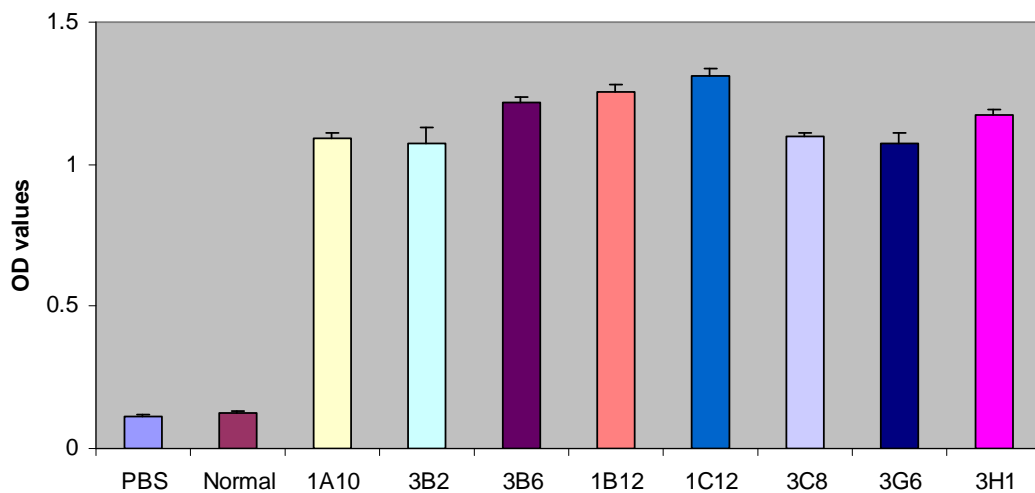


Fig. 6. Antibody titers of McAb-containing ascitic fluids. OD₄₀₅ values of ascetic fluids were screened by indirect ELISA (n=3). All ascites and normal serum were diluted in PBS at 1:500 ratio. TgLDH-5 peptide was used as antigen. Normal; serum from normal mouse. PBS; control.

E. The specificity of McAbs with *T. gondii* lysate

To investigate whether the McAbs were responsive to lysate of *T. gondii*, indirect ELISA was performed with *T. gondii* lysate used as antigen. The OD values of cell culture supernatants from eight hybridoma clones ranged from 0.986 to 1.239 and control was 0.203 (Fig. 7). In cases of eight ascites, the antibody titers ranged from 1.025 to 1.445, otherwise OD values of PBS and normal were 0.162 and 0.212, respectively (Fig. 8).

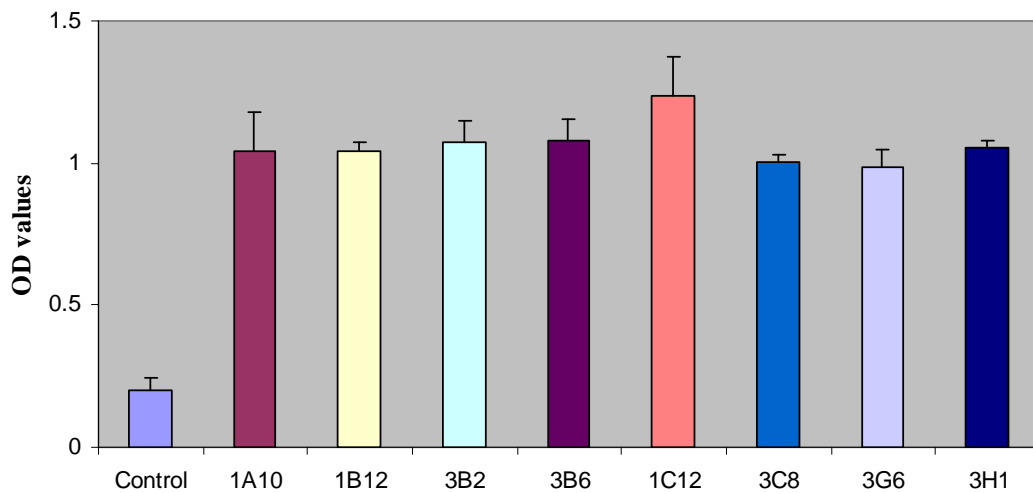


Fig. 7. Antibody titers of the hybridoma cell culture supernatant using *T. gondii* lysate as antigen. OD₄₀₅ values of each culture supernatant were screened by indirect ELISA (n=3). Control; normal F0 cell culture supernatant.

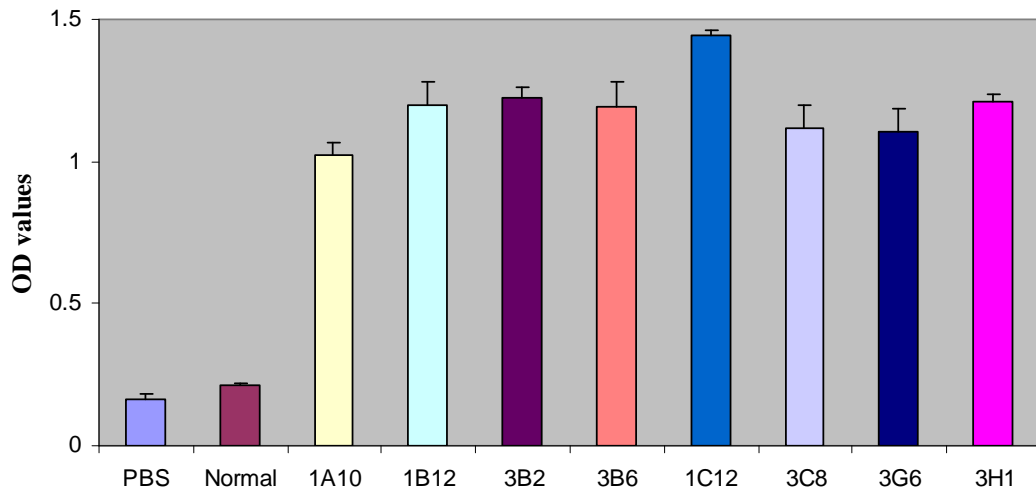


Fig. 8. Antibody titers of McAb-containing ascitic fluids using *T. gondii* lysate as antigen. OD₄₀₅ values of ascetic fluids were screened by indirect ELISA (n=3). All ascites and normal serum were diluted in PBS at 1:500 ratio. Normal; serum from healthy mouse. PBS; control.

F. Isotyping of monoclonal antibodies

The McAb isotypes were determined with a mouse monoclonal antibody isotyping kit (Roche, Indiana, USA). All of eight monoclonal antibodies were IgM class.

Table 2. Isotype profile of eight hybridoma clones producing monoclonal antibody

Clones	Isotype
1A10	IgM
1B12	IgM
3B2	IgM
3B6	IgM
1C12	IgM
3C8	IgM
3G6	IgM
3H1	IgM

IV. DISCUSSION

T. gondii, an intracellular protozoan parasite, is the etiological agent of the worldwide human and animal toxoplasmosis. Until now, there is no vaccine to prevent toxoplasmosis in humans. Clinical signs of toxoplasmosis are non-specific and are not sufficiently characteristic for a definite diagnosis. In fact, toxoplasmosis mimics several other infectious diseases. Therefore, diagnosis of the infection is important not only for treatment but from the point of view epidemiology and prevention. But diagnosis of any infectious disease depends on isolation of the organism and demonstration of an antibody response to the infection. In the case of toxoplasmosis the organism is difficult to isolate because it is very difficult to culture. The number of organism seen in histological sections or isolated from enlarged lymph nodes is very small. Isolation of organisms from other tissues is also a difficult and time consuming procedure. Diagnosis therefore depends on serological tests (Fleck, 1989).

The most popular test has proved to be the toxoplasma dye test. The disadvantages of this test are that it requires the use of live organisms, is complicated, and needs fresh frozen human plasma. These difficulties have led to a search for specific and sensitive substitutes (Fleck, 1989). Many different antibody tests have been investigated such as the indirect hemagglutination assay, indirect fluorescent antibody assay, the direct agglutination test, the latex agglutination test, ELISA and the immunosorbent agglutination assay test. These tests are quite sensitive and specific and have been proved very useful in confirming the diagnosis and in seroepidemiological studies (Remington, 2000). However, these tests are all time consuming and require a laboratory equipped with proper instruments and

trained personnel. These distinct drawbacks make them unsuitable for direct use in the field and in laboratories where no refrigeration, incubator or expensive spectrophotometric equipment are available. Because of the geographical distribution of toxoplasmosis, there is a need to develop a sensitive, rapid, simple and an inexpensive test for the early screening of patients. With the development of monoclonal antibody in 1980s, assays for detecting *T. gondii* antigens were developed (Hafid, 1995).

Recently, immunochromatographic tests, rapid diagnostic tests, have been developed. Lactate dehydrogenase (LDH) are the most common antigens used in Immunochromatographic tests (Makler and Hinrichs, 1993).

In the present study, McAbs against the TgLDH were produced and characterized. Eight hybridoma cells with high ELISA titer were selected. After three times of limiting dilution, all eight clones had strong reactivity to TgLDH by indirect ELISA.

All eight McAbs generated in this study were IgM class. Serologic test for detection of IgM antibodies are commonly performed for the diagnosis of acute acquired toxoplasma infection in the immunocompetent patients (Remington, 1995). Because IgM antibodies may persist for many months or even years following the acute infection (Bobic, 1992; Bono, 1989; Handman, 1980).

In this study, the eight hybridoma cell lines producing TgLDH specific McAbs were established. Although the further studies to investigate the sensitivity against patient sera are needed, these McAbs may be used to create the rapid diagnostic dipstick kit.

V. CONCLUSION

To create the rapid diagnostic dipstick kit that has high sensitivity against LDH (lactate dehydrogenase) of *T. gondii*, first of all, hybridoma cells producing monoclonal antibodies with high affinity and specificity must be generated. This study established hybridoma cells producing McAbs against *T. gondii* LDH (TgLDH).

After cell fusion, hybridoma clones secreting antibodies against TgLDH were screened by ELISA. After three times of limiting dilution, eight clones, 1A10, 1B12, 3B2, 3B6, 1C12, 3C8, 3G6 and 3H1, were selected. The OD values of cell culture supernatant and ascites from eight hybridoma clones ranged from 0.936 to 1.239 and 1.089 to 1.314, respectively. All eight McAbs generated in this study were IgM class.

These results indicated that eight hybridoma clones had strong reactivity to TgLDH and may be useful for diagnosis to *T. gondii* infection.

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특소포자충의 lactate dehydrogenase를 이용한 단클론 항체의 생산

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목적: 특소포자충의 lactate dehydrogenase에 대하여 높은 민감성과 정확도를 가지는 진단 키트를 만들기 위해서 특소포자충의 lactate dehydrogenase에 맞는 펩타이드를 합성한 후 세포 융합 기술을 이용하여 단클론 항체를 만들었다.

방법 : 6-8주령의 BALB/c 마우스에 TgLDH5를 면역시킨 후 비장을 적출하여 PEG를 사용하여 세포 융합을 시켰다. 그리고, 세포 융합 된 세포들의 isotyping을 확인하였다.

결과: 8개의 융합된 세포들을 얻을 수 있었는데 그 이름을 1A10, 1B12, 3B2, 3B6, 1C12, 3C8, 3G6 그리고 3H1이라 명명하였다. 또한, 8개의 단클론 항체들의 O.D값은 0.938에서 1.269를 보였고 isotyping을 확인한 결과 모두 IgM이었다.

결론: 8개의 단클론 항체들 모두 TgLDH와 강하게 반응하였다.

핵심어: 독소포자충, lactate dehydrogenase, 세포 융합 기술, 단클론항체