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Short review

Early detection of *Toxoplasma gondii* infection by using a interferon gamma release assay: A review

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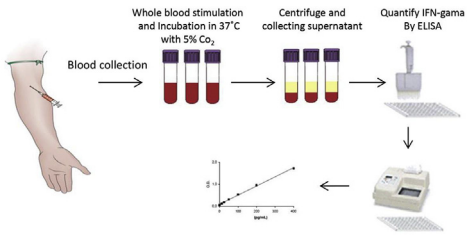
HIGHLIGHTS

- Interferon-gamma release assay (IGRA) was introduced as an in vitro test detecting *T. gondii* infection.
- Few studies have investigated the potential role of cell immunity in diagnosis of toxoplasmosis.
- IGRA accurately distinguished infected from uninfected individuals after in vitro stimulatoion with *T. gondii* antigens, even during the first days of life.
- IGRA is an easy-operation and low-cost method to measure cell mediated immunity against *T. gondii*.
- These results underline the importance of evaluating cellular immunity for early diagnosis of toxoplasmosis.
- ELISA-based IGRA holds the potential to become a useful diagnostic tool for early detection of *T. gondii*.

GRAPHICAL ABSTRACT



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ABSTRACT

Antibody-based serological tests are currently the most common diagnostic methods for detection of *Toxoplasma gondii*; however, these tests bear several limitations. Recently, Interferon-gamma release assay (IGRA), a T-cell-based test, was introduced as an in vitro test for detection of *T. gondii* infection. Few studies have investigated the potential role of cell immunity in diagnosis of toxoplasmosis. IGRA accurately distinguished infected from uninfected individuals, showing strong lymphocyte activation after in vitro stimulation with *T. gondii* antigens, even during the first days of life. IGRA is an easy-operation and low-cost method to measure cell mediated immunity against *T. gondii*. The results of this review underline the importance of evaluating cellular immunity to establish an early diagnosis particularly for

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congenital toxoplasmosis. Therefore, ELISA-based IGRA holds the potential to become a useful diagnostic tool for early detection of *T. gondii* infection.

Contents

1. Introduction

Toxoplasma gondii, a ubiquitous protozoan parasite, was discovered 100 years ago Weiss and Dubey, 2009. Although toxoplasmosis is a worldwide issue, policy on diagnostic strategies substantially differ among countries (Murat et al., 2013). Diagnosis of toxoplasmosis had been established by several methods including the isolation of *T. gondii* from blood or body fluids, demonstration of the parasite in tissues, detection of specific nucleic acids with DNA probes, and serological tests for detection of *T. gondii*-specific immunoglobulins synthesized by the host in response to infection (Holec-Gąsior, 2013).

Different serological examinations such as ELISA, indirect fluorescent-antibody (IFA), latex-agglutination, hemagglutination tests, IgG avidity immunoblotting and hemagglutination tests have been introduced (Mohammadi et al., 2015; pour Azami et al., 2011; Saraei et al., 2010; Ali-Heydari et al., 2013). Antibody-based serological tests are currently the most common diagnostic tools for detection of *T. gondii* infection (Remington et al., 2004). Serological methods are most often designed for IgG and IgM antibodies (Murat et al., 2013). IgA shows similar kinetics to IgM and is less used in routine testing (Gilbert et al., 2007; Pinon et al., 2001). However the serological tests are commonly used, they bear limitations in estimating the time of *T. gondii* infection. Low titers of IgM might persist long after the acute phase of disease in most cases. On the other hand, *T. gondii*-specific IgM and IgG are usually detectable after two weeks post infection (Chardes et al., 1990), that means a seropositive test commonly discloses tissue cyst formation.

It has been reported that *T. gondii* infection elicits a strong Th1 response (Denkers, 1999; Suzuki et al., 1988). The immune response is characterized by parasite-induced IL-12 and T lymphocyte IFN- γ production (Denkers, 1999; Gazzinelli et al., 1994).

Recently, Interferon-gamma release assay (IGRA), a T-cell-based test, was introduced as an in vitro test for detection of *T. gondii* infection. In this study we reviewed the studies which had used IGRA for diagnosis of toxoplasmosis.

2. Material and methods

2.1. Strategy for literature search

The available literature on the IGRA for diagnosis of *T. gondii* was obtained through the PubMed with the search themes of "Toxoplasma gondii or toxoplasmosis or T. gondii", combined with "IFN-gamma -gamma release assay" or IFN- gamma AND diagnosis. The literature was restricted to English language, and searched from their inception until January 2016.

2.2. Blood samples stimulation and quantifying of IFN-γ

According to previous reports, samples of 1 ml of peripheral blood were drawn into tubes contained lithium heparin anticoagulant. Aliquots of diluted blood were cultured in sterile propylene tubes in the presence of *T. gondii* antigens. Positive and negative controls consisted of PHA at a final concentration of 20 μg/ml and PBS, respectively. All cultures were incubated for 24 h at 37 °C in 5% CO2 in a humidified atmosphere. Culture supernatants were collected from each tube after centrifugation at room temperature and stored at −40 °C until the IFN-γ assay was carried out. IFN-γ was assayed using a commercial ELISA kit. The mean OD of the PBS controls was subtracted from the mean OD of antigen-stimulated samples. The amount of IFN-γ released, expressed in pg/ml, was obtained by converting the OD using the standard curve from the kit (Chapey et al., 2010; Di Cristina et al., 2004).

3. Results

Several studies had used whole blood-based IGRA for diagnosis of toxoplasmosis (Chapey et al., 2010; Di Cristina et al., 2004; Ciardelli et al., 2008; Guglietta et al., 2007; Fatoohi et al., 2002; Yamamoto et al., 2000; Meira et al., 2014) (Table 1). In the study by Ciardelli et al., IFN- γ production were significantly higher in infected infants than uninfected cases ($P < 0.001$) and the sensitivity and specificity of IGRA in these patients was 90.3% and 85.7%, respectively (Ciardelli et al., 2008).

Guglietta et al. reported the increased level of IFN- γ (20- to 40-fold), in the presence of GST-GRA1, in subjects with acquired or congenital infection (Guglietta et al., 2007). There was no statistical difference in T-cell activation between individuals with acquired and congenital infection. However, considerable differences in subgroups of children with congenital toxoplasmosis (cases less than 4 years old and cases more than 4 years old) were found. In addition, the GRA1 stimulation index (SI) displayed significant differences when healthy adults with acquired infection and children with congenital infection less than 4 years old were compared. The other recombinant protein (GST-cl16.2) was the most reactive protein inducing PBMC proliferation in 54% of samples from subjects with acquired infection and in 70% of samples from patients with congenital toxoplasmosis (Guglietta et al., 2007).