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Absence of Interferon- γ -Inducible Gene IGTP Does Not Significantly Alter the Development of Chagasic Cardiomyopathy in Mice Infected with *Trypanosoma cruzi* (Brazil Strain)

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ABSTRACT: Interferon- γ (IFN- γ) contributes to host resistance during acute infection with *Trypanosoma cruzi*, the causative agent of Chagas' disease. Inducibly expressed guanosine triphosphatase (IGTP), a 48-kDa guanosine triphosphatase (GTPase), is a member of a family of GTPase proteins inducibly expressed by IFN- γ . The expression pattern of IGTP suggests that it may mediate IFN- γ -induced responses in a variety of cell types. IGTP has been demonstrated to be important for control of *Toxoplasma gondii* infection but not for resistance against *Listeria monocytogenes*. We evaluated the role of IGTP in development of chronic chagasic cardiomyopathy in IGTP null mice and C57BL/6 (wild type [WT]) mice infected with the Brazil strain for 6 mo. There was no significant difference in parasitemia or cardiac histopathology between null and WT mice. Right ventricular remodeling was observed in infected IGTP null mice, suggesting that IGTP does not significantly alter the course of *T. cruzi* infection.

Chagas' disease, caused by the hemoflagellate parasite *Trypanosoma cruzi*, affects approximately 18 million people, principally among poor areas of Mexico and Central and South America, and is a major cause of heart disease (WHO, 1997). During chronic chagasic cardiomyopathy, microvascular alterations and ventricular remodeling with extensive fibrosis may lead to enlargement of the heart and sudden death (Higuchi, 1997).

Interferon- γ (IFN- γ) is one among the cytokines that contribute to resistance in mammalian hosts during early acute infection with *T. cruzi* (Torrico et al., 1991; Cardillo et al., 1996; Hunter et al., 1996; Romanha et al., 2002). During cell-mediated immunity, induced by intracellular pathogens, IFN- γ is produced by Th1 cells (Boehm et al., 1997). In addition, IFN- γ also stimulates innate cell-mediated immunity through natural killer cells (Cardillo et al., 1996). Furthermore, interleukin (IL)-12 stimulates production of IFN- γ (Cardillo et al., 1996; Hunter et al., 1996). In humans with Chagas' disease, a potent IFN- γ response is generated in mononuclear cells by *T. cruzi* trans-sialidase (Ribeirão et al., 2000) and by *T. cruzi* cruzipain on T-cell lines (Arnholdt et al., 1993). Inflammatory mediators such as tumor necrosis factor- α , IFN- γ , IL-1 β , and nitric oxide are synthesized in the myocardium of infected experimental animals, as well as individuals with chagasic chronic cardiomyopathy (Huang et al., 1999; Abel et al., 2001; Dos Santos et al., 2001; Chandra et al., 2002).

Inducibly expressed guanosine triphosphatase (IGTP), a 48-kDa guanosine triphosphate-binding protein, is one member of a group of at least 6 proteins induced by IFN- γ (Taylor et al., 1996). This family also includes LRG-47 (Sorace et al., 1995) and GTPI (Boehm et al., 1998). Members of this family have been implicated in an IFN- γ -induced mechanism that confers resistance against intracellular pathogens. IGTP is highly expressed in the thymus and is expressed at lower intensity in the spleen, lung, and small intestine (Taylor et al., 1996). After stimulation by IFN- γ , macrophages and fibroblasts exhibit enhanced IGTP expression (Taylor et al., 1996). The physiological function of IGTP is not known, but it may be associated with processing or trafficking of proteins because of its localization in endoplasmic reticulum (Taylor et al., 1997). IGTP null mice exhibit normal immune cell development and normal clearance of *Listeria monocytogenes* and cytomegalovirus infections; however, the mice exhibit loss of host resistance to acute experimental *Toxoplasma gondii* infection (Taylor et al., 2000). IGTP

has also been reported to be important in resistance against, and in IFN- γ -mediated inhibition of, *T. gondii* in astrocytes (Halonen et al., 2001). Recently, it has been shown that host resistance mediated by IGTP is a Stat1-induced function that acts predominantly to restrict acute as opposed to chronic *T. gondii* infection (Collazo et al., 2002). Based on these findings, we investigated the effect of IGTP deficiency on parasitological parameters and cardiac pathology in mice chronically infected with *T. cruzi*.

Male IGTP null (bred on a C57BL/6 $\times$ 129sv genetic background) and C57BL/6 $\times$ 129sv wild type (WT) mice (6–8 wk old) were infected intraperitoneally with 1×10^3 trypomastigotes of the Brazil strain of *T. cruzi*. The IGTP null mice were bred in our own facility from a founding pair obtained from G.A.T. Parasitemia, mortality, and histopathology were evaluated. There were 4 groups of mice: uninfected IGTP null mice ($n = 6$), infected IGTP null mice ($n = 8$), uninfected WT mice ($n = 7$), and infected WT mice ($n = 7$).

Parasitemia was assessed by counting parasites in a hemocytometer chamber and was evaluated beginning on day 25 postinfection and every 15 days thereafter. The mean parasitemia was 8×10^4 trypomastigotes/ml and was not different in IGTP null mice compared with WT mice. Parasitemia waned and was undetectable by 40 days postinfection. All mice survived acute infection and were monitored by *in vivo* cardiac magnetic resonance imaging (MRI) at 6 mo postinfection. To perform these studies, mice were anesthetized with ketamine-xylazine, and electrocardiographic leads were attached to their limbs. Mice were placed in animal holders designed to position the heart at the center of the MRI coil. Cardiac gating was accomplished as previously described (Jelicks et al., 1999), by using the rising phase of the QRS complex of the electrocardiogram to generate a standard 5-V square-wave gating signal that triggered data acquisition. Images were acquired using a 9.4-T wide-bore GE Omega spectrometer operating at a proton frequency of 400 MHz and equipped with 50-mm shielded gradients and a 35-mm ¹H home-built imaging coil. After locating the heart in scout images, multislice spin echo imaging was performed to obtain 4 short-axis images within approximately 5 min. Data were transferred to a PC, where images were analyzed using home-designed software using MATLAB technical computing language (The Mathworks, Natick, Massachusetts). Left ventricle (LV) and right ventricle (RV) dimensions (in mm) were determined. The left ventricular inner dimension (LVID) corresponds to the average of the septal-lateral and anterior-posterior LVID. The LV wall is the average of the anterior, posterior, lateral, and septal walls. The right ventricular inner dimension (RVID) is the widest point of the right ventricular chamber. Fig. 1 shows examples of the MRI images of uninfected and infected IGTP null mice and WT mice. Statistical significance ($P < 0.05$) was evaluated using a parametric Student's *t*-test for the MRI data.

At the end of the MRI study, mice were killed, and hearts were fixed in 10% phosphate-buffered formaldehyde, paraffin-embedded, and stained with hematoxylin and eosin. The hearts of infected and uninfected mice were examined in a blinded fashion and assessed semi-quantitatively for the degree of inflammation and fibrosis as previously described (Morris et al., 1989). All infected mice exhibited chronic inflammation, fibrosis, focal necrosis, and vasculitis. There was no significant difference between the degree of pathology observed in infected IGTP null mice and infected WT mice.

FIGURE 1. Cardiac-gated MRI images of IGTP null and WT mice. **A.** Uninfected WT mouse. **B.** Infected WT mouse. **C.** Uninfected IGTP null mouse. **D.** Infected IGTP null mouse. Axial images show the heart at midlevel. The white arrows indicate the RV wall.

The cardiac dimensions of IGTP null and WT mice measured with MRI are reported in Table I. The LVID of uninfected IGTP null mice was significantly larger than that of uninfected WT mice, although LV wall thickness was not significantly different from that of uninfected WT mice. The RVID of uninfected IGTP null mice was also larger than that of uninfected WT mice. It is not known at this time whether the observed dilatation of both ventricular chambers in the uninfected IGTP null mice is related to the IGTP gene. To determine whether the increased ventricular chamber dimensions observed in uninfected IGTP null mice were a specific effect of the deficiency of the IGTP protein, we measured the cardiac dimensions of a group of 4 uninfected LRG-47 null mice. LRG-47 is another IFN- γ -induced protein. The LVID (3.77 ± 0.1 mm) and the RVID (2.44 ± 0.1) of LRG-47 null mice were not significantly different from the values measured for the uninfected IGTP null mice reported in Table I. However, LV wall thickness (0.96 ± 0.06 mm) in uninfected LRG-47 null mice was significantly smaller than in either uninfected IGTP null or uninfected WT mice. The LVID and the RVID of both IGTP null and LRG-47 null mice were significantly larger than those of WT mice. Taken together, these results suggest that left and right ventricular chamber dilatation is a feature of deficiency of either IGTP or LRG-47, whereas LV wall thickness appears to be affected only by the LRG-47 gene and not by IGTP.

IGTP null mice were infected with the Brazil strain to determine

whether this gene might contribute to the pathogenesis of murine chagasic cardiomyopathy. Chronic *T. cruzi* infection is associated with a significant increase in the RVID of both WT mice and IGTP null mice; however, infection did not appear to have a significant effect on LV dimensions (see Table I). Our data indicate that infected IGTP null mice exhibit chronic cardiomyopathy as determined by histopathology and cardiac MRI. These results are similar to those of our previous studies on mice infected with Brazil strain of *T. cruzi*. Infected mice of various mouse strains typically exhibit dilated RV. For example, we have previously shown that infected WT mice, CD1 or C57BL/6 $\times$ 129sv, exhibit almost doubling of the RVID (1.95 ± 0.13 vs. 3.12 ± 0.35 for CD1 and 1.59 ± 0.04 vs. 3.00 ± 0.38 for C57BL/6 $\times$ 129sv) (Jelicks et al., 1999). This effect is reduced in infected iNOS null mice (which exhibit approximately 40% increase in RVID). Although the RVID of infected IGTP null mice was significantly increased, the 1.25-fold increase in RV dilatation in IGTP null mice was significantly less than the 2.0-fold increase observed in WT mice. Because the uninfected IGTP null mice already exhibit an enlarged RV relative to uninfected WT mice, it is possible that a further increase in RVID beyond ~ 3.0 mm is not attainable.

IGTP null mice are bred on a C57BL/6 $\times$ 129sv genetic background, which we have demonstrated is ordinarily resistant to acute infection with the Brazil strain. Infection of C57BL/6 $\times$ 129sv mice with the Brazil strain does not usually result in acute mortality, but rather the mice gradually develop chronic cardiomyopathy (Trischmann et al., 1978; Huang et al., 1999; Jelicks et al., 1999, 2002). Therefore, the strain of the parasite, as well as the genetic background of the mouse, determines the mortality and progression to chronic disease.

Cardiac-gated MRI is an important tool for the serial evaluation of cardiac structural abnormalities in normal, null, and transgenic mice. We have used both echocardiography and cardiac MRI to evaluate the effects of *T. cruzi* infection in a variety of mouse strains and during therapeutic interventions (Huang et al., 1999; Jelicks et al., 1999; Chandra et al., 2002). In our previous studies, we observed increased RVID in mice during the chronic phase. In this study, absence of the IFN- γ -inducible gene IGTP appears to attenuate the degree of right ventricular remodeling but does not ameliorate the severity of the cardiac pathology associated with chronic *T. cruzi* infection.

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TABLE I. Cardiac dimensions in mice at the chronic phase of *Trypanosoma cruzi* infection.^{†,‡}

	WT uninfected	WT infected	IGTP null uninfected	IGTP null infected
RVID (mm)	1.50 ± 0.1	$2.91 \pm 0.2^*$	$2.30 \pm 0.1^*$	$2.94 \pm 0.2^{**}$
LVID (mm)	3.34 ± 0.1	3.34 ± 0.3	$3.74 \pm 0.2^*$	3.61 ± 0.1
LV wall thickness (mm)	1.13 ± 0.04	1.2 ± 0.1	1.12 ± 0.1	1.04 ± 0.03
n	7	7	6	8

[†] Data are mean $\pm$ SEM.

[‡] Abbreviations: RVID, right ventricular inner dimension; LVID, left ventricular inner dimension; LV, left ventricle; WT, wild type.

* $P < 0.05$ as compared with WT control value.

** $P < 0.05$ as compared with uninfected IGTP and uninfected WT control values.

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Seroepidemiology of *Toxoplasma gondii* Infection Among Chinese Aboriginal and Han People Residing in Mountainous Areas of Northern Thailand

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ABSTRACT: A seroepidemiological survey of *Toxoplasma gondii* infection among Chinese refugees, including Akka and Yau aborigines and Han people living in mountainous areas at elevations of 1,100–1,400

m in Chiang-Rai Province of northern Thailand, was conducted during January 2003 using the latex agglutination test. The overall seroprevalence of *T. gondii* infection was 9.1% for Akka aborigines, 37.9% for