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Trypanosoma cruzi-infected individuals demonstrate varied antibody responses to a panel of *trans*-sialidase proteins encoded by SA85-1 genes

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Abstract

Chronic infection with *Trypanosoma cruzi* causes significant morbidity and mortality. The parasite expresses on its surface and sheds into the extracellular milieu a large superfamily of *trans*-sialidase proteins. Previous studies have demonstrated that during *T. cruzi* infection, the *trans*-sialidase superfamily stimulates an antibody response, but how individuals respond to different proteins of the *trans*-sialidase superfamily remain poorly defined. In this report, we present an analysis of the antibody response of chronically infected individuals and inbred strains of mice to a panel of 11 different *trans*-sialidase proteins encoded by surface antigen 85 kD (SA85-1) genes. These data indicate that: (1) 90% of the individuals tested generated antibodies to one or more *trans*-sialidase proteins; (2) the individuals develop different patterns of antibody responsiveness to the panel of *trans*-sialidase proteins; (3) three inbred strains of mice develop *trans*-sialidase antibody responses, but each strain develops a different pattern of antibody response to the panel of *trans*-sialidase proteins; (4) the differences in the pattern of antibody response by the mouse strains are independent of MHC differences; and (5) *trans*-sialidase proteins that do not stimulate an antibody response during *T. cruzi* infection can stimulate a response following immunization. Together these data indicate that during *T. cruzi* infection individuals develop a diverse *trans*-sialidase antibody response that appears to be affected by genetic and environmental factors.

Keywords: *Trypanosoma cruzi*; Chagas disease; Antibody; Human; Mice

Abbreviations: SA85, surface antigen 85 kD; GST, glutathione S-transferase protein

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1. Introduction

Trypanosoma cruzi chronically infects approximately 18 million people in Latin America, and causes Chagas disease, a chronic inflammatory disease, in approximately one-third of the infected individuals (WHO, 2002; WorldBank, 1993). During the acute infection, *T. cruzi* replicates and disseminates in the mammalian host and parasites are easily detected in the blood. In most cases during the acute infection, the parasite is controlled and parasites become difficult to detect, but individuals remain infected for their lifetime. What mechanisms the parasite utilizes to enable its persistence remains unclear. Furthermore, the persistent parasite appears to trigger the chronic inflammatory disease, but the mechanisms causing the inflammation, which may include bystander damage, molecular mimicry, or epitope spreading, are also unclear (Brener and Gazzinelli, 1997; Buckner and Van Voorhis, 2000; Tarleton and Zhang, 1999).

In recent years, efforts to decrease vector transmission of the parasite in some endemic areas have demonstrated success (Moncayo, 2003; WHO, 2002). Insect vector transmission continues unabated in other areas, however (Monroy et al., 2003). In addition, infections can occur independently of insect vectors, e.g., following blood product transfusion, organ transplantation or to the fetus during pregnancy (Blanco et al., 2000; Hermann et al., 2004; Schmunis, 1999; Schmunis et al., 1998, 2001). To continue to decrease the transmission of *T. cruzi*, accurate data on the incidence and prevalence of *T. cruzi* infection, and *T. cruzi* contamination of blood products and organs, are required.

Diagnosis of *T. cruzi* infection based on clinical observations is difficult as the manifestations, before the onset of Chagas disease, are non-specific. Thus, diagnosis of *T. cruzi* infection has relied predominantly on serological tests (da Silveira et al., 2001). Initial diagnostic tests included ELISA, indirect immunofluorescence (IFA), and indirect hemagglutination assay (IHA), all of which utilized crude preparations of epimastigote antigens; epimastigotes are the form of the parasite that replicates in insects (Camargo et al., 1986). These tests are difficult to standardize because of the complex nature of the antigens. Furthermore, these tests lack sufficient sensitivity and specificity (McCarthy, 2003). Currently, DNA technology is being used to select and express specific recombinant *T. cruzi* proteins to improve serological diagnosis (Meira et al., 2002; Pereira-Chioccola et al., 2003; Umezawa et al., 2004). To date, however, adequate serological diagnostic tests have not been developed.

The *trans*-sialidase superfamily includes several genes that encode functional *trans*-sialidases, and many genes that lack *trans*-sialidase function (Affranchino et al., 1989; Kahn et al., 1990, 1991, 1993; Parodi et al., 1992; Todeschini et al., 2004; Uemura et al., 1992). Among those that encode non-functional *trans*-sialidases are the surface antigen 85 kD (SA85-1) genes (Kahn et al., 1993). Our previous studies indicate that the *T. cruzi* genome encodes greater than 100 SA85-1 genes, and that many SA85-1 genes are simultaneously expressed by each parasite (Kahn et al., 1990; Kahn et al., 1999). The first SA85-1 gene, designated *SA85-1.1*, was selected from a gene expression library with chronic-infected mouse serum (Kahn et al.,1990). The antigenic region selected was not homologous to the catalytic region of the *trans*-sialidase proteins. To better understand the genetic diversity and antigenicity of the SA85-1 family, the non-catalytic region of two other SA85-1 genes (*SA85-1.2* and *SA85-1.3*) were cloned and studied (Kahn et al., 1990). Sequence analyses indicated that these genes and proteins are 80% homologous to each other (Kahn et al., 1990). These studies and others suggest that during infections many SA85-1 proteins and *trans*-sialidase proteins are expressed simultaneously by each parasite, and that many of the proteins are antigenic (Affranchino et al., 1989; Cetron et al., 1992; Frasch, 1994; Kahn et al., 1990). Furthermore, the DNA sequences of the different SA85-1 proteins suggest that their sequence variation could generate altered T cell epitopes that might contribute to immune evasion. To further study T cell epitope variation, the homologous region of eight additional SA85-1 genes were cloned and expressed (Kahn and Wleklinski, 1997). These studies demonstrated that the *trans*-sialidase SA85-1 antigenic variation does generate a variety of T cell epitopes (Kahn and Wleklinski, 1997). The sequence analyses of these genes are limited to the region of the T cell epitope and do not provide information concerning their overall homology (Kahn and Wleklinski, 1997). It remains unclear how the antigen and epitope variation of the *trans*-sialidase SA85-1 proteins affects individuals' antibody responses to the proteins. Here, we present data on the antibody response of 20 *T. cruzi*-