Available online at www.sciencedirect.com
SCIENCE DIRECT®
Experimental Parasitology 111 (2005) 87–96
Experimental Parasitology
www.elsevier.com/locate/yexpr

Trypanosoma cruzi: Influence of predominant bacteria from indigenous digestive microbiota on experimental infection in mice

R. Duarte [a], A.M. Silva [a], L.Q. Vieira [b], L.C.C. Afonso [c], J.R. Nicoli [a,*]

[a] *Departamento de Microbiologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[b] *Departamento de Bioquímica-Imunologia, ICB, Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil*
[c] *Departamento de Ciências Biológicas, ICEB/NUPEB, Universidade Federal de Ouro Preto, Ouro Preto, MG, Brazil*

Received 4 March 2005; received in revised form 2 June 2005; accepted 7 June 2005
Available online 20 July 2005

Abstract

To verify the influence of some predominant components from indigenous microbiota on systemic immunological responses during experimental Chagas disease, germ-free NIH Swiss mice were mono-associated with *Escherichia coli*, *Enterococcus faecalis*, *Bacteroides vulgatus* or *Peptostreptococcus* sp. and then infected with the Y strain of *Trypanosoma cruzi*. All the mono-associations predominantly induced a Th1 type of specific immune response to the infection by *T. cruzi*. A direct correlation was observed between a higher survival rate and increased IFN-γ and TNF-α production ($P < 0.05$) in *E. faecalis*-, *B. vulgatus*-, and *Peptostreptococcus*-associated mice. Moreover, higher levels of anti-*T. cruzi* IgG1 and anti-*T. cruzi* IgG2a were also found in mono-associated animals after infection. On the other hand, with the exception of *E. faecalis*-associated mice, mono-association induced a lower IL-10 production after infection ($P < 0.05$) when compared with germ-free animals. Interestingly, spleen cell cultures from non-infected germ-free and mono-associated mice spontaneously produced higher levels ($P < 0.05$) of IL-10 than cultures from infected mono-associated mice, except again for *E. faecalis*-associated animals. In conclusion, the presence of the components of the indigenous microbiota skews the immune response towards production of inflammatory cytokines during experimental infection with *T. cruzi* in gnotobiotic mice. However, the degree of increase in production of cytokines depends on each bacterial component.

Keywords: Microbiota; *Trypanosoma cruzi*; Cytokines; Immunoglobulins; Gnotobiotic mice

1. Introduction

The human gastrointestinal tract harbors one of the most complex ecosystems known in microbial ecology with bacterial populations reaching 10^{10}–10^{11} viable cells/g of contents in its lower portions. These organisms may belong to about 400 different bacterial species, although it is believed that only 20–40 of them reach predominant levels, consisting of 99% of the total community (Berg, 1996). Concerning the human predominant fecal bacteria, two population levels can be distinguished: (i) the dominant microbiota (10^9–10^{11} cells/g of contents) constituted only by obligate anaerobes (*Bacteroides*, *Bifidobacterium*, *Eubacterium*, *Peptostreptococcus*, and *Fusobacterium*) and (ii) the sub-dominant microbiota (10^7–10^8 cells/g of contents) containing predominantly facultative anaerobic and microaerophilic bacteria (*Escherichia coli*, *Enterococcus*, and *Lactobacillus*). In healthy hosts, the presence of this microbiota has a very large impact on various aspects of function and metabolism such as metabolic rate, gastrointestinal function, specific and quantitative aspects of immune function, and the many aspects of biochemical homeostasis. Only the predominant species have population levels high enough to be considered

* Corresponding author. Fax: +55 31 3499 2730.
E-mail address: jnicoli@icb.ufmg.br (J.R. Nicoli).

0014-4894/$ - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.exppara.2005.06.002

as responsible for the three main functions of the intestinal microbiota which have considerable importance for the host health: (i) the colonization resistance, (ii) the immunomodulation, and (iii) the nutritional contribution for the host (MacFarland, 2000). Presently, available data also indicate that this indigenous microbiota almost always has a profound influence on the host–parasite relationship. As an example, it is well known that the presence of intestinal microbiota is essential for the pathogenicity of some protozoa and helminthes such as *Entamoeba histolytica* (Phillips and Wolfe, 1959), *Nippostrongylus brasiliensis* (Wescott and Todd, 1964), *Nematospiroides dubius* (Wescott, 1968), *Trichinella spiralis* (Przyjalkowski and Wescott, 1969), *Eimeria tenella* (Visco and Barnes, 1972), *Ascaridia galli* (Johnson and Reid, 1973), *Trichuris suis* (Rutter and Beer, 1975), *Eimeria falciformis* (Owen, 1975), *Eimeria ovinoidalis* (Gouet et al., 1984), and *Giardia duodenalis* (Torres et al., 2000). In contrast, this microbiota can reduce the pathological consequences of other infectious diseases as described for experimental infections with *Trypanosoma cruzi* (Silva et al., 1987), *Cryptococcus neoformans* (Salkowski et al., 1987), *Strongyloides venezuelensis* (Martins et al., 2000), and almost all enteropathogenic bacteria (*Clostridium difficile*, *Clostridium perfringens*, *E. coli*, *Pseudomonas aeruginosa*, *Salmonella typhimurium*, *Shigella flexneri*, and *Vibrio cholerae*) (Wilson, 1995). Experimental infections with *Raillietina cesticillus* (Reid and Botero, 1967) and *Isopora suis* (Harleman and Meyer, 1984) are two of the very few cases, where the normal microbiota has no influence on the course of a disease.

Trypanosoma cruzi is the causative agent of Chagas disease in man and determines a systemic infection that is controlled, although not completely eliminated, by T cell-dependent immune responses. Control of parasitemia in the acute phase of infection is critically dependent on intracellular killing by cytokine-activated macrophages. In this way, different studies indicate the crucial role of IFN-γ, IL-12 (Michailowsky et al., 2001), and TNF-α (Silva et al., 1995) as well as NO (Vespa et al., 1994) in host resistance to infection with *T. cruzi*.

As cited above, infection with the intracellular parasite *T. cruzi* is more severe in germ-free animals, as shown by a higher mortality when compared with conventional controls (Silva et al., 1987). Germ-free mice also displayed earlier and higher parasitemia than conventional controls. Moreover, tissues from germ-free mice were more intensively parasitized and presented a more aggressive inflammatory response. Germ-free mice infected with *T. cruzi* presented a stronger local reaction to subcutaneous injection of formalin-killed parasites as determined by footpad swelling than conventional animals (Furarah et al., 1991). Recent data showed higher IFN-γ, TNF-α, and NO production by spleen cell cultures, and higher blood levels of IgG1 in conventional mice infected with Y strain of *T. cruzi* when compared to their germ-free counterparts (Duarte et al., 2004). However, these data concern the whole microbiota and there is no information about the role of individual components of the indigenous bacterial ecosystem on these differences.

To investigate the role of individual components of the indigenous microbiota on the immune response to a systemic parasitic infection, germ-free mice were monoassociated with Gram positive (*Peptostreptococcus* sp.) and Gram negative (*Bacteroides vulgatus*) obligate anaerobic bacteria or Gram positive (*Enterococcus faecalis*) and Gram negative (*E. coli*) facultative bacteria, pertaining to the predominant human fecal microbiota, and subsequently infected with *T. cruzi*. Survival rate, cytokine, and NO productions by spleen cell cultures, and IgG1 and IgG2a serum concentrations were determined.

2. Materials and methods

2.1. Mice

Germ-free 21-day-old NIH mice of both sexes were used in this study. The matrices were obtained from Taconic Farms (Germantown, NY, USA) and maintained in the germ-free facility of the Instituto de Ciências Biológicas (Universidade Federal de Minas Gerais, Belo Horizonte, MG, Brazil) for several generations. Germ-free animals were housed in flexible plastic isolators (Standard Safety, McHenry, IL, USA) and handled according to established procedures. Experiments with gnotobiotic mice were carried out in micro-isolators (UNO Roestvastaal B.V., Zevenar, The Netherlands). Water and commercial autoclavable diet (Nuvital, Curitiba, PR, Brazil) were steam sterilized and administered ad libitum. Microbiological status of germ-free and gnotobiotic mice was performed by routine culture of recently collected feces in brain heart infusion (BHI) broth medium (Oxoid, Hampshire, England) and fluid thioglycollate medium (Difco, Detroit, MI, USA). Fecal cultures were incubated at 37 and 25 °C during 72 h. Controlled lighting (12 h light, 12 h dark) was used for all the animals. All the animals received humane care as outlined in the "Guide for the Care and Use of Laboratory Animals" of the National Research Council (1996).

2.2. Bacteria

Escherichia coli, *Enterococcus faecalis*, *Bacteroides vulgatus*, and *Peptostreptococcus* sp. were all isolated from feces of healthy human volunteers. The bacteria were maintained at −86 °C in BHI broth medium (Oxoid) supplemented with glycerol 10%. Identity of