Short Communication

Expression of co-stimulatory molecules CD80 and CD86 is altered in CD14\(^+\)HLA-DR\(^+\) monocytes from patients with Chagas disease following induction by Trypanosoma cruzi recombinant antigens

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Abstract

Introduction: The relationships between monocytes and lymphocytes through MHC class II molecules and costimulatory, are of utmost importance for the production of an efficient immune response. In this work, we assessed the expression of surface molecules CD80 and CD86 on CD14\(^+\)HLA-DR\(^+\) monocytes from patients with Chagas disease. Methods: The study population consisted of 31 patients with chronic clinical forms of Chagas disease. Patient blood samples were cultured in the presence of recombinant cytoplasmic repetitive antigen (CRA) and flagellar repetitive antigen (FRA). Results: We found considerable differences in the expression profile of surface molecules involved in antigen presentation. Conclusions: CRA and FRA may contribute to host immune response evasion by Trypanosoma cruzi.

Keywords: Chagas disease. Immune response. Recombinant antigens.

Chagas disease (CD) remains one of the most important infectious and parasitic diseases in Latin America that is still related to poverty\([1]\). The chronic phase of this disease manifests in three main clinical forms: indeterminate (IND), cardiac (CARD), and digestive\([4]\). CARD is considered to be responsible for the high morbidity and mortality in infected people, occurring in most symptomatic patients\([1]\). It is believed that the pathological manifestations of the disease are a consequence of multifactorial mechanisms involving both parasite and the patient\([2]\). Several studies have reported the importance of the host immune response in combating the parasite and its role in the clinical evolution of the disease\([3]\). Accordingly, activation of the immune system is responsible for controlling parasitemia in the acute phase and the inflammatory reaction in specific organs\([5]\). Not surprisingly, some organisms have evolved mechanisms to escape the host immune system, such as decreasing the modulatory response of antigen-presenting cells (APCs) and expressing co-stimulatory molecules\([4]\).

The human leukocyte antigen-antigen D related (HLA-DR) receptor found on the surface of APCs stimulates the adaptive immune response by presenting peptides to cluster of differentiation 4\(^+\) (CD4\(^+\)) T lymphocytes\([6]\). Co-stimulatory molecules CD80 and CD86 play an important role in promoting activation and differentiation of T lymphocytes\([6]\). These molecules are recognized by the CD28 receptor, which is expressed on almost all T cells\([7]\). The signaling cascade elicited by the binding of co-stimulatory molecules to CD28 acts in conjunction with the one triggered by the interaction of the T-cell receptor and co-receptor with the peptide-major histocompatibility complex (MHC) on the surface of APCs\([8]\).

Most studies on the immune response of patients with CD have used soluble parasite antigen preparations. This may prevent the detection of a specific immune response as these preparations include a complex mixture of antigens. Therefore, the use of defined parasite reagents such as recombinant antigens becomes an important requirement for the identification of molecules critical for parasite-host interactions and in studies of the immunopathology of CD\([9],[10]\). Cytoplasmic repetitive antigen (CRA) and flagellar repetitive antigen (FRA)\([9],[10]\) have been used by our group to study the development of immunological markers for the prognosis of chronic clinical forms of CD.
We believe that in chronic human infection, the development/maintenance of immunological memory is determined primarily by CRA rather than by FRA, which is present in the flagellum of trypomastigotes. Here, we evaluated the antigen-presenting ability of CD14+ HLA-DR+ monocytes obtained from peripheral blood of patients chronically infected with Trypanosoma cruzi. To do so, we assessed the expression of CD80 and CD86 before and after in vitro stimulation with T. cruzi CRA and FRA.

The study population consisted of 31 patients with chronic clinical forms of CD recruited at the Emergency room Cardiology of Pernambuco (PROCAPE), University of Pernambuco (UPE), Recife, Pernambuco. The population represented a convenience non-probabilistic sample and was recruited between January 2011 and January 2012. Inclusion criteria were: positive serology for Chagas infection in two tests with different methodological principles; clinical examination for proper characterization of the clinical form; lack of previous etiological treatment; no digestive complaints, such as dysphagia and constipation; no reported changes in leukocyte count; and no blood transfusion or organ transplantation. Additionally, based on their medical records and survey forms, patients did not report any other diseases. The patients included in the CARD2 group (n = 14, 24 to 71 years old, three men and seven women) presented Echocardiography. Patients included in the CARD1 group (n = 10, 41 to 70 years old, three men and four women) not infected by T. cruzi and selected according to the following criteria: they had not alterations. The control group (NI) consisted of individuals (n = 7, 36 to 60 years old, four men and ten women) had dilatation of the cardiac area, with an ejection fraction < 40%, and/or augmented left ventricular area. Characterization of the cardiac dilatation was performed through posteroanterior chest radiography and the ejection fraction was estimated by the Teichholz method using an echo-Doppler cardiogram, following guidelines by the American Society of Echocardiography. Patients included in the CARD1 group (n = 10, 41 to 70 years old, three men and seven women) presented electrocardiogram alterations and no dilatation of the cardiac area, with an ejection fraction > 55% in the echo-Doppler cardiogram. Patients included in the IND group (n = 7, 38 to 63 years old, four men and three women) did not show any cardiac and digestive alterations. The control group (NI) consisted of individuals (n = 7, 36 to 60 years old, three men and four women) not infected by T. cruzi and selected according to the following criteria: they had not lived at any time in an area endemic for CD; they had never received changes in leukocyte count; and no blood transfusion or organ transplantation. Additionally, based on their medical records and survey forms, patients did not report any other diseases. The patients included in the CARD2 group (n = 14, 24 to 71 years old, three men and seven women) presented Echocardiography. Patients included in the CARD1 group (n = 10, 41 to 70 years old, three men and four women) not infected by T. cruzi and selected according to the following criteria: they had not alterations. The control group (NI) consisted of individuals (n = 7, 36 to 60 years old, three men and four women) not infected by T. cruzi and selected according to the following criteria: they had not lived at any time in an area endemic for CD; they had never received a blood transfusion; the serological test for CD was negative; and they did not present alterations in leukocyte blood count.

CRA and FRA were obtained as described by Krigger et al. and were prepared at the Department of Diagnostic Reagents of Bio-Manguinhos before being sent to the Immunoparasitology Laboratory at Centro de Pesquisa Aggeu Magalhães/Fundação Oswaldo Cruz (CPqAM/FIOCRUZ). They were further evaluated for any contamination with bacterial proteins and carbohydrates, as described by Pereira et al.

Heparinized peripheral blood was cultured for about 24h and diluted 1:1 in 14mL polypropylene tubes (BD Biosciences, San.Jose, CA, USA) containing RPMI 1640 medium supplemented with L-glutamine, 1% antibiotics (stock solution of 10,000U penicillin and 10,000U streptomycin; Sigma-Aldrich, St. Louis, MO, USA), and 10% fetal bovine serum (Sigma-Aldrich). Blood was stimulated as described by Lorena et al.

After in vitro incubation, cells were treated with 20mM ethylenediaminetetraacetic acid (EDTA) (Sigma-Aldrich), washed with phosphate-buffered saline (PBS)-Wash (containing 0.5% bovine serum albumin and 1% sodium azide; Sigma-Aldrich), and centrifuged at 400 × g for 10 min at 22-25°C. The supernatant was discarded leaving a final volume of 1mL in which the pellet was resuspended. Aliquots (100µL) of the cell suspension were placed in polystyrene tubes (5mL; BD Biosciences) containing fluorescein isothiocyanate-conjugated anti-CD14 (Cat #: MA 1-82074; 5µL), PerCP-conjugated anti-HLA-DR (Cat #: MHLDR31; 2.5µL), phycoerythrin-conjugated anti-CD80 (Cat #: MA1-10288; 2.5µL), or allophycocyanin-conjugated anti-CD86 (Cat #: MA1-10294 or MHCD8605; 2.5µL; Invitrogen Corporation, Carlsbad, CA, USA) monoclonal antibodies and incubated for 30 min at room temperature. After incubation, erythrocytes were lysed by the addition of 2mL lysing solution (2.8% sodium citrate, 30% diethylene glycol, 54% formaldehyde 37%, 0.04% heparin - United States Pharmacopeia - USP 100,000). Next, cells were washed with PBS-Wash and fixed with 200µL BD Cytofix™ (BD Biosciences). Samples were stored at 4°C until further analysis by flow cytometry.

Sample acquisition and analysis were performed on a FACScalibur flow cytometer (BD Biosciences Immunocytometry Systems). The population of CD14+ cells was selected by side scatter (SSC) versus FL1 dot plot; a total of 1,000 events were acquired within the R1 window. After selecting the window of interest (R1), CD14+ monocytes presenting the surface HLA-DR receptor were analyzed by plotting two-dimensional graphs of point fluorescence distributions, from which the percentage of labeled cells was derived. To analyze the expression of surface molecules on CD14+ HLA-DR+ monocytes, a window of interest (R2) was selected in the FL1 versus FL3/FL4 two-dimensional graphs, allowing triple-labeled cells to be detected.

Statistical analysis of cell profile data was performed using PRISM 5.0 (GraphPad Software, San Diego, CA, USA). To confirm the assumption of homogeneity, the Bartlett test was used. To compare values of surface molecule expression ex vivo and in vitro, the Wilcoxon test for paired samples was used. All conclusions were based on a 5% significance level.

First, we assessed the expression of CD80 on CD14+ HLA-DR+ monocytes before and after antigen stimulation. We observed that after stimulation with CRA and FRA, expression of CD80 in vitro increased significantly in all groups compared to ex vivo samples (Figure 1A-H). Only in IND patients treated with FRA, statistical difference could not be found (Figure 1E). Analysis of CD86 expression on CD14+ HLA-DR+ monocytes (Figure 2) revealed a significant decrease in individuals with the CARD form of CD, i.e., CARD1 and CARD2, following stimulation with CRA (Figure 2B – Figure 2C) compared to ex vivo samples (Figure 2A – Figure 2D). Again, no statistical difference was observed in the IND group (Figure 2A). When stimulated with FRA (Figure 2E – Figure 2H), we found that only the CARD1 group showed a statistically significant decrease of CD86 compared to ex vivo samples (Figure 2F).

The induction of an effective immune response depends directly on activation of APCs, since stimulation of T cells requires interaction between their receptors and the
FIGURE 1. Expression of CD80 on the surface of CD14+HLA-DR+ peripheral blood cells from patients with CD after stimulation with CRA or FRA. Patients were grouped according to the clinic form of CD: (A and E) IND (n = 7); (B and F) CARD1 (n = 10); (C and G) CARD2 (n = 14); and (D and H) NI individuals, (n = 7). Statistically different values with p < 0.05 are indicated. CD: cluster of differentiation; HLA-DR: human leukocyte antigen-antigen D; CD: Chagas disease; CRA: cytoplasmic repetitive antigen; FRA: flagellar repetitive antigen; IND: indeterminate; CARD: cardiac; NI: non-infected individuals.

Next, we carried out functional studies of CD14+HLA-DR+ monocytes by monitoring the expression of co-stimulatory molecules involved in immunopathological processes of CD.

During antigen processing and presentation, APCs need stimuli to express co-stimulatory molecules, such as CD80 and CD86, which are important to promote the activation and differentiation of T lymphocytes(4) (5). CD86 is constitutively expressed and, therefore, it is more abundant on the surface of monocytes than CD80, which is expressed only following stimulation(10)(11).

When we evaluated the expression of these molecules on the surface of CD14+HLA-DR+ monocytes stimulated with CRA or FRA, we observed that expression of CD80 was higher in all groups (except for the IND group after stimulation with FRA), compared to ex vivo readings. Our results are in agreement with...
FIGURE 2. Expression of CD86 on the surface of CD14+HLA-DR+ peripheral blood cells from patients with CD after stimulation with CRA and FRA. (A and E) IND (n = 7), (B and F) CARD1 (n = 10), (C and G) CARD 2 (n = 14), and (D and H) NI (n = 7). CD: cluster of differentiation; HLA-DR: human leukocyte antigen-antigen D; CD: Chagas disease; CRA: cytoplasmic repetitive antigen; FRA: flagellar repetitive antigen; IND: indeterminate; CARD: cardiac; NI: non-infected individuals. Statistically different values with p < 0.05 are indicated.
with those of Souza et al.\textsuperscript{(10)}, who also reported an increased expression of CD80 on the surface of monocytes following \textit{T. cruzi} infection of adherent cells. Our results showed a significant increase in the percentage of CD80-expressing CD14+HLA-DR\textsuperscript{+} monocytes after stimulation with both antigens in patients with CARD. This finding suggests that increased expression of CD80 favors T cell stimulation, leading to a possible biased Th1 profile\textsuperscript{(12)}.

When evaluating the expression of the co-stimulatory molecule CD86 on the surface of CD14+HLA-DR\textsuperscript{+} monocytes, we found it was lower in patients with cardiac clinical forms (CARD1 and CARD2) stimulated with either CRA or FRA. These findings are in agreement with the results reported by Souza et al.\textsuperscript{(3)}, in which a decrease in the expression of CD86 was observed in monocytes of patients with CARD forms. Our data, together with findings reported by other authors, suggest that alterations in the expression of co-stimulatory molecules are an important step in the development of a cellular immune response against \textit{T. cruzi}. The latter may release soluble immunosuppressive factors to disrupt the immune system and provide an escape mechanism to evade the host immune response\textsuperscript{(12)(13)(14)}. Further studies evaluating the effect of co-stimulatory and inhibitory molecules on T cell activation will be required to fully elucidate the host evasion mechanisms developed by \textit{T. cruzi}.

In summary, here we show that during the course of chronic CD, there are important differences in the expression profile of surface molecules involved in antigen presentation. Thus, prospective studies linking the expression of stimulatory (CD28) and inhibitory (CTLA-4) T cell proteins, and of co-stimulatory molecules CD80 and CD86, may be important to assess the evolution of CD immunopathology.

**Ethical considerations**

The recruitment of individuals and the experimental protocols were approved by the Research Ethics Committee of CPqAM/Fiocruz (CAEE: 0155.0.095.000-08). All patients included in this study signed an informed consent form.

**Conflict of interest**

The authors declare that there is no conflict of interest.

**Financial Support**

The study received financial support from the Conselho Nacional de Desenvolvimento Científico e Tecnológico-CNPq (Universal Edital nº 478572/2009-3 and CNPq/Papes VI/Universidade Oswaldo Cruz Edital nº407763/2012-0). Y.M. Gomes is a research fellow of CNPq (No. 304896/2009-8). During the study, AKA Soares received a Fundação de Amparo à Ciência e Tecnologia do Estado de Pernambuco-FACEPE MSc scholarship.

**REFERENCES**


