XXVII MEETING OF THE BRAZILIAN SOCIETY OF IMMUNOLOGY

V INTERNATIONAL SYMPOSIUM ON ALLERGY AND CLINICAL IMMUNOLOGY

October 20th to 23rd, 2002
Pestana Bahia Hotel

FINAL PROGRAM

ABSTRACTS

INFECTION AND IMMUNITY

Date: October 22nd, 2002

P-285

TITLE: Characterization of the human T cell response against aspartic acid-contained Trypanosoma cruzi antigenic fractions.

AUTHOR(S): Thereza Christina Benévolo de Andrade[1]
CO-AUTHOR(S): Rosa Teixeira Pinho[1], Isabelle do Nascimento de Oliveira[1], Paula De Luca[2], Álvaro Luiz Bertho[2], Alejandro Hasslocher-Moreno[3], Sérgio Salles Xavier[3] and Fátima Conceição-Silva[2]*.
INSTITUTION: Lab. de Imunologia Clinica[1] and Lab. Imunidade Celular e Humoral[2]- Depto de Imunologia- IOC; Instituto de Pesquisas Clinicas Evandro Chagas[3] - IPEC - Fiocruz.

Chagas' disease, a protozoan infection caused by *Trypanosoma cruzi* is endemic in the American Continent and it is the most important and the leading cause of heart failure in Latin America. The pathogenesis of Chagas' disease is still unclear, despite the intense investigations both in humans and in animal models. Several questions remain unanswered such as: What factors may determine whether the disease remains in the indeterminate form or progress to severe ones? What are the mechanisms that may initiate the development of tissue damage? What is responsible: the parasite or the host immune system? The answer for these questions can be addressed in several ways. One of them is the identification of parasite antigens that are object of immune recognition by a wide variety of individuals. Among these antigens, we could include parasite enzymes that play a role in host parasite interaction and are target antigens to the host immune response. The characterization of the cellular immune response to these enzymes could be important to understand the pathogenic mechanisms of the infection. Recently, Pinho (thesis, 2001) identified and purified two antigene fractions with aspartic proteinase properties. In the present study these two enzyme-containing fractions, denominated CZI and CZII were used in *in vitro* assays to detect and characterize the cellular immune response to these antigens in patients with Chagas' disease and in healthy individuals as controls. Our results showed a predominance of a specific cellular Type 1 immune response, with an important IFN-g secretion when compared to IL10. The responding cells were identified as CD4+ T lymphocytes and this response was present only in individuals with Chagas' disease. The cellular response to CZII fraction was considered statistically significant ($p=0,01$) when we compared patients with or without cardiomiopathy form respectively. We also study the immune response to crude *T.cruzi* antigens in the same individuals and no difference was observed between patients with Chagas' disease and healthy individuals. This is the first characterization of the cellular immune response *in vitro* of PBMC from patients with Chagas' disease to aspartic proteinase fractions isolated *from T.cruzi*. Further additional studies will be performed in order to characterize the immune response to peptides representing regions of this fraction to support the findings of this study.

INFECTION AND IMMUNITY

Date: October 22nd, 2002

P-286

TITLE: Characterization of CD4+ T cell hybridomas specific for Trypanosoma cruzi Trans-sialidase.

AUTHOR(S): Hiyane, M. I.
CO-AUTHOR(S): Hiyane, M. I.; Boscardin, S. B.; Rodrigues, M. M.
INSTITUTION: Unifesp

BALB/c mice immunized with a DNA plasmid encoding the Trypanosoma cruzi Trans-sialidase (TS) developed CD4+ Th1, CD8+ Tc1 and protective immune response against infection1. From DNA-vaccinated mice, we obtained CD4+ Th1 which displayed remarkable anti-parasitic activity in vitro2. The aim of the present study was to obtain T cell hybridomas specific for TS protein that would allow us to further characterize the specificity and function the CD4+ Th1 cells. For that purpose, lymph node cells from BALB/c mice immunized with the recombinant TS protein was fused to cells AKR thymoma BW 1100.129.237. Twenty five hybridomas were obtained. Twelve of them were antigen specific, secreting more than 1 ng/ml of IFN-g when stimulated with recombinant TS protein. None of them secreted either IL-4 or IL-10 upon in vitro re-stimulation. By flow cytometry analysis, all hybridomas were positives for CD3 and 8 of them were highly positive for CD4 T cell marker. Four of five hybridomas tested were also positive for the TCRbeta-chain. RNA was obtained from these hybridomas, and we are currently trying to isolate the cDNA encoding the TCR variable region.

1-Costa, F., et al., 1998. Vaccine 16, 768-774.
2-Rodrigues, M.M., et al., 1999. Infect. Immun. 67, 3855-3863.

Supported by grants from FAPESP, PADCT, CNPq, PRONEX and FINEP.

INFECTION AND IMMUNITY

Date: October 22nd, 2002

P-287

TITLE: CD8LOW TCRabLOW CELLS FROM Trypanosoma cruzi CHRONIC INFECTED MICE PRODUCE HIGH AMOUNTS OF INF-g mRNA AND DISPLAY PROTECTIVE ACTIVITY AGAINST THE ACUTE INFECTION UPON IN VIVO TRANSFER

AUTHOR(S): Grisotto, M. G.
CO-AUTHOR(S): Marinho, C. R. F.; D'ImpérioLima, M. R.; Gollob, K. J.; Torres, K. C. L.; Alvarez, J. M.
INSTITUTION: ICB - USP - Depto de Imunologia and Departamento de Morfologia ICB-UFMG, Belo Horizonte, MG, Brasil

CD8+ T lymphocytes expressing low levels of TCRab and CD8 molecules are present in high numbers in the spleen, liver, blood and peritoneal cavity of T. cruzi-infected chronic mice. We have analysed by RT-PCR the IFN-g production capacity of TCRabLOWCD8LOW cells or other CD8+ cell subsets from the spleen of chronic mice. The in vivo protective potential of isolated spleen TCRabLOW CD8LOW was also evaluated by transfer into acutely-infected mice. For RT-PCR experiments, CD8+ cell subsets were sorted in a FACs Vantage and the mRNA extraction carried out directly or after 6 hour in vitro incubation with plate-bound anti-CD3. For transfer experiments, CD8LOWTCRabLOW were negatively-isolated using specific antibodies conjugated with magnetic beads. CD8LOWTCRabLOW cells were injected 1 day after infection with T. cruzi (Y-strain). Control groups were injected with PBS or total chronic spleen cells. Increased amounts of mRNA were present in CD8LOWTCRabLOW cells compared to CD8HIGHCD45RCHIGH and CD8HIGHCD45RCLOW subsets or to CD8+ spleen cells from non-infected mice, both after CD3 stimuli or direct ex vivo measurement. CD8LOWTCRabLOW cells conferred in vivo protection against T. cruzi infection. PBS-injected infected mice showed increased levels of parasitemia that started by day 6 and displayed two peaks, at days 10 and 18 and 100% mortality by day 19. Mice transferred with CD8LOWTCRabLOW cells displayed a smaller parasitemia peak (day 10) that progressively declined and was controlled by day 20. Nevertheless, the animals of this group succumbed by day 30 with negative parasitemias. Mice injected with unseparated chronic spleen cells showed a very small parasitemia peak by day 10 that was totally controlled by day 12, and 100% of the animals survived. Our results indicate, that in spite of receptor/correceptor down-regulation, CD8LOWTCRabLOW from chronic mice are high IFN-g producers and play an important role in in vivo control of T. cruzi parasites. Supported by FAPESP.

INFECTION AND IMMUNITY

Date: October 22nd, 2002

P-288

TITLE: CD8+ T cells infiltrating heart lesions from Chagas' cardiomyopathy patients recognize epitopes from *Trypanosoma cruzi* proteins cruzipain and FL-160

AUTHOR(S): Fonseca, S.G.[1], Teisserenc, H.[3], Clave, E.[3], Toubert, L.[3], Ianni, B.[1], Lopes, V.[1], Mady, C.[1], Sette, A.[4], Sidney, J.[4] Kalil, J.[1,2], Cunha-Neto, E.[1,2]
INSTITUTION: [1] Heart Institute (InCor) and [2] Division of Clinical Immunology and Allergy, University of São Paulo School of Medicine, São Paulo, Brazil; [3] Hôpital Saint-Louis, Paris, France; [4] Epimmune, Inc. San Diego, USA.

Chagas' disease cardiomyopathy (CCC) occurs in 10-30% of the 16 million patients infected with the protozoan *Trypanosoma cruzi* in Latin American. Heart-infiltrating T cells predominantly produce IFN-gamma and TNF-alpha. CD4+ T cells crossreactively recognizing cardiac myosin and a tandemly repetitive *T. cruzi* antigen can be found in heart lesions, but CD8+ T cells are more abundant around areas in heart tissue where *T. cruzi* is found, suggesting that CD8+ T cells infiltrating CCC hearts can be specific to *T. cruzi* antigens. The findings that CD8+ T cells are twice as abundant as CD4+ T cells in CCC heart lesions further suggested that heart infiltrating CD8+ T cells play an important role in pathogenesis. However, there are no direct data showing the antigen specific of heart infiltrating CD8+ T cells. To study CD8+ T cell recognition of *T. cruzi* antigens, we tested the recognition of 26 HLA-A2-binding, 9-and 10-mer peptides derived from the sequences of secreted/membrane proteins cruzipain/FL-160 by PBMC from HLA-A2+ Chagas' disease patients and healthy controls using IFN-gamma ELISPOT. Several peptides were recognized by at least 62.5% of the patients and negligibly by controls. We selected the immunodominant peptides CZ16-24, CZ60-68 and FL457-465 to assemble peptide-HLA-A2 tetramer complexes. We analyzed the binding of tetramer complexes Tet CZ16-24, Tet CZ60-68 and Tet FL457-465 to CD8+ T cells from T cell lines repeatedly stimulated with irradiated PBMC and phytoheamagglutinin (2.5 µg/ml) in presence of IL2+IL7+IL15 (100 IU/ml, 5 ng/ml and 5 ng/ml, respectively), obtained from CCC heart tissue (percutaneous transvenous endomyocardial biopsy of right ventricular septum, EMB) of 3 patients. All intralesional T cell lines had subpopulations binding the 3 tetramers complexes, and up to 3% of CD8+ T cell lines bound to a single peptide-HLA-A2 tetramer complex. Heart-infiltrating T cell line samples displayed higher frequencies of Tet+CD8+ T cells than paired PBMC samples from the same donor, indicating an accumulation of *T. cruzi*-specific CD8+ T cells in heart tissue. Results provide the first direct evidence of the antigen specificity of CD8+ T cells in CCC heart tissue and are consistent with the migration and/or local expansion of *T. cruzi* -specific CD8+ T cells in the heart lesions of CCC patients.

Supported by: FAPESP, COFECUB-INSERM.