

SUMMARY OF THESIS*

FONSECA, Simone Gonçalves da – **Estudo funcional dos linfócitos T CD8+ na cardiopatia chagásica crônica humana.** São Paulo, 2002. (Tese de Doutorado – Instituto de Ciências Biomédicas da Universidade de São Paulo)

FUNCTIONAL STUDY OF T LYMPHOCYTES CD8+ IN THE HUMAN CHRONIC CHAGASIC CARDIOPATHY

The heart inflammatory infiltrate in human chronic Chagas' cardiomyopathy (CCC) shows a 2:1 predominance of CD8+ to CD4+ T lymphocytes. However, culture of biopsy-derived fragments in the presence of IL-2 and phytohaemagglutinin (PHA) supports the in vitro growth of CD4+ but not CD8+ T lymphocytes. To elucidate this paradox, we searched for cytokines that could allow the growth of CD8+ T cells from intralesional T line obtained from CCC patients. We also studied the recognition of epitopes derived from *T. cruzi* proteins by peripheral and intralesional CD8+ T cells from Chagas' disease patients. We observed that the addition of IL-7, IL-15 or both, to IL-2-containing culture medium increased the growth (up to 10-fold) and maintained the viability of CD8+ T and CD4+ T lymphocytes from a PHA-stimulated PBMC T cell line from a CCC patient (MD). CD8+ T cells grew slowly and only for a short period in response to IL-2; growth was only rescued in the presence of IL-7, IL-15, or both. We observed that the expression of the alpha chain of IL-2 receptor was higher on CD4+ T cells than on CD8+ T cells from this line, suggesting that the partial unresponsiveness of such T cells to IL-2 could be due to a lack of high-affinity IL-2 receptors. Long-term culture with IL-2+IL7+IL15 (several cycles of stimulus) promoted an increase of the CD8+ population in 50% of intralesional lines tested. The mean number of IL-15+ cells / 400x microscopic field in heart tissue biopsies was higher in the heart samples of CCC patients than in the controls samples (7.7 (37.90-0.40) and 0.4 (1.10-0.08), respectively). An IFN- γ ELISPOT analysis of PBMC of HLA-A2+ Chagas' disease patients and normal individuals, tested with cruzipain and FL-160 peptides that bound to HLA-A2, showed a much

higher response in PBMC of Chagas' disease patients than normal controls, and identified several immunodominant epitopes. Analysis of the CD8+ T cells that were specific to the three immunodominant peptides CZ16-24, CZ60-68 and FL457-465 was also performed using the HLA-A2-peptide tetramer approach. CD8+ T cells from PBMC from some Chagas' disease patients that were positive to tetramer HLA-A2/peptide, tetCz16-24, tetCZ60-68 and tetF457-465 were higher than those observed in HLA-A2+ control individuals. CD8+ T cells of an intralesional T cell line from an HLA-A2+ CCC patient (JRS) stained positive for the 3 tetramers tested; tetCZ60-68 stained 0.9% of intralesional line-derived CD8+ T cells, and only 0.2% of PBMC CD8+ T lymphocytes from the same patient. T cell line from CCC patient MD, derived by PHA stimulus from PBMC, was used as control, and showed less than 0.02% of CD8+ tetramer-positive cells. We also observed that T cell lines from two HLA-A2 negative CCC patients contained up to 1.5% tetramer-positive CD8+ T cells. We hypothesize that *T. cruzi*-specific CD8+ T cells migrate to the heart of CCC patients and are kept viable and accumulate in tissue by the action of IL-15 produced by inflammatory cells like macrophages/dendritic cells, as a consequence of continuous production of pro-inflammatory cytokines in the lesion site.

Simone Gonçalves da Fonseca
Laboratório de Imunologia
Instituto do Coração/FMUSP
Universidade de São Paulo

*This thesis is available at the Library of the Instituto de Medicina Tropical de São Paulo