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Original Article

Flow Cytometric Analysis of *Leishmania* Reactive $CD4^+$/$CD8^+$ Lymphocyte Proliferation in Cutaneous Leishmaniasis

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Abstract:

Background: Determination of the division history of T cells *in vitro* is helpful in the study of effector mechanisms against infections. Technique described here uses the intracellular fluorescent label carboxyfluorescein diacetate succinimidyl ester (CFSE) to monitor the proliferation.

Methods: In a cross sectional study, blood samples were collected from 7 volunteers with history of cutaneous leishmaniasis (CL) and one healthy control from endemic areas in Isfahan province who referred to the Center for Research and Training in Skin Diseases and Leprosy (CRTSDL), then $CD4^+$/$CD8^+$ lymphocytes and $CD14^+$ monocytes were isolated from peripheral blood mononuclear cells (PBMC) using mAbs and magnetic nanoparticles. CFSE labeled $CD4^+$ or $CD8^+$ lymphocytes cultured with autologous monocytes in the presence of PHA, SLA, live *Leishmania major* or as control without stimulation. Cells were harvested after 7 days and were analyzed using flow cytometry.

Results: Five consecutive divisions were monitored separately. Stimulation of $CD4^+$ or $CD8^+$ lymphocytes from CL subjects with SLA showed a significant difference in proliferation comparing with unstimulated cells ($P< 0.05$). The significant difference in the percentages of $CD4^+$ cells stimulated with SLA was revealed at different divisions for each subject. In $CD8^+$ lymphocyte, significant stronger stimulation of SLA was evident later in the proliferation process. The mean number of divisions in both $CD4^+$/$CD8^+$ lymphocytes stimulated with SLA was significantly greater than when stimulated with live *L. major* (P=0.007 / P=0.012, respectively)

Conclusion: The percentage of divided cells might be calculated separately in each division. The cells remained active following CFSE staining and there is possibility of functional analysis simultaneously.

Keywords:

CFSE ، $CD4^+$/$CD8^+$ T cells ، Proliferation ، Cutaneous leishmaniasis

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