

## VI. SUMMARY

The blastogenic response of lymphocytes from twenty-two HIV-negative blood donors, fifteen asymptomatic HIV-infected individuals, fifteen AIDS patients without *P. marneffei* infection, and sixteen AIDS patients with *P. marneffei* infection were studied to determine cell-mediated immune response. Peripheral blood mononuclear cells from each group was separated by Ficoll-Hypaque gradient centrifugation and stimulated with PHA-P, crude sonicated *P. marneffei* protein as antigen, and PPD. Tritiated-thymidine incorporation of suboptimal concentration PHA-P stimulated peripheral blood mononuclear cells from asymptomatic HIV-infected individuals, AIDS patients without and with *P. marneffei* infection were  $7,223 \pm 7,896$  cpm,  $2,742 \pm 5,074$  cpm, and  $596 \pm 946$  cpm, respectively, whereas, HIV-negative donor was  $20,746 \pm 7,594$  cpm. Furthermore, the tritiated-thymidine incorporation of the optimal concentration PHA-P stimulated peripheral blood mononuclear cells from asymptomatic HIV-infected individuals, AIDS patients without and with *P. marneffei* infection were  $17,744 \pm 9,600$  cpm,  $12,923 \pm 9,137$  cpm, and  $7,696 \pm 8,404$  cpm, respectively, whereas, HIV-negative donor was  $29,304 \pm 7,094$  cpm.

In *in vitro* lymphocyte transformation test using crude sonicated *P. marneffei* protein as antigen, the tritiated-thymidine incorporation of the suboptimal concentration of crude sonicated *P. marneffei* antigen stimulated peripheral blood mononuclear cells from HIV negative

donors, asymptomatic HIV-infected individuals, AIDS patients without and with *P. marneffei* infection were  $10,016 \pm 6,922$  cpm,  $2,129 \pm 5,354$  cpm,  $129 \pm 219$  cpm, and  $142 \pm 347$  cpm, respectively. And with the optimal concentration of crude sonicated *P. marneffei* antigen were  $12,280 \pm 7,206$  cpm,  $2,232 \pm 6,247$  cpm,  $193 \pm 469$  cpm, and  $110 \pm 229$  cpm, respectively. This defect was similar to PPD. It was found that the tritiated-thymidine incorporation of the suboptimal concentration PPD stimulated peripheral blood mononuclear cells from HIV-negative donors, asymptomatic HIV-infected individuals, AIDS patients without and with *P. marneffei* infection were  $5,333 \pm 5,849$  cpm,  $1,009 \pm 2,612$  cpm,  $8 \pm 12$  cpm, and  $145 \pm 240$  cpm, respectively, whereas, the optimal concentration PPD were  $8,808 \pm 6,544$  cpm,  $2,089 \pm 5,268$  cpm,  $0.5 \pm 1$  cpm, and  $111 \pm 127$  cpm, respectively. These results showed a significant decreasing of lymphoproliferative response against the suboptimal and optimal concentration of PHA-P, crude sonicated *P. marneffei* antigen, and PPD in the all groups of patients when compared to HIV-negative donors ( $p < 0.001$ ).

In addition the crude sonicated of *P. marneffei* protein separated by using Sephadex G-100 gel filtration column. Two distinct protein peaks were observed. The protein antigen activity of each peak was tested by lymphocyte transformation test. It was shown that the protein antigen activity was in peak 1 fraction.

The presence of inhibitory factor in AIDS patient plasma was also demonstrated. The mean  $\pm$  SD of percent inhibition of lymphocyte

transformation was performed with the suboptimal concentration of PHA-P in the presence of plasma from asymptomatic HIV-infected individuals, AIDS patients without and infected with *P. marneffeii*, compared to the condition of autologous plasma, were  $23 \pm 22\%$ ,  $68 \pm 22\%$ , and  $57 \pm 27\%$ , respectively. Furthermore, stimulation with the optimal concentration of PHA-P was also performed. The percent inhibition in the presence of plasma obtained from asymptomatic HIV-infected individual, AIDS patient without and with *P. marneffeii* infection were  $5 \pm 7\%$ ,  $20 \pm 17\%$ , and  $27 \pm 19\%$ , respectively. It was indicated that, percent inhibition of lymphoproliferative response in suboptimal and optimal concentration of PHA-P in presence of plasma from AIDS patients without and with *P. marneffeii* infection were significant higher than the plasma from asymptomatic HIV-infected individuals ( $p < 0.001$ ). Whereas, it was non significant difference in percent inhibition assay between the plasma from AIDS patients without and with *P. marneffeii* infection in both suboptimal and optimal concentration of PHA-P ( $p > 0.2$ ). Moreover the present study was shown that the percent inhibition was correlated to the concentration of patient plasma. These results showed the suppression of lymphocyte transformation by plasma of AIDS patients without and with *P. marneffeii* infection.