

Immunological screening of asymptomatic *Leishmania* infection in individuals exposed to an endemic area of American Tegumentary Leishmaniasis (ATL)

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Background. Clinical manifestations of American Tegumentary Leishmaniasis (ATL) include cutaneous (CL) and mucous forms (ML); however, it is conceivable that there are individuals who might be infected but not present any clinical manifestations, as reported other types of Leishmaniasis. These individuals, here called asymptomatic, may show a natural resistance to develop ATL diseases. The aim of this study was to characterize the cellular immune response of asymptomatic individuals who were exposed to *Leishmania* by short periods of time during seasonal work in endemic areas (Fig. 1). Their immune response will be compared with CL and ML subjects that acquired the disease under similar conditions.

Methods. Fifty-seven subjects (32 men/25 women) from the highlands of Cusco, Peru (Fig. 2): 28 who have visited an endemic area without any disease sign (SEA), 7 with CL, 12 with ML and 10 healthy subjects from of a non-endemic area (SNEA) were enrolled. The asymptomatic infection was defined in the SEA group by a specific-T cell proliferation using peripheral blood mononuclear cells (PBMC) stimulated for 5 days with a local total soluble leishmanial antigen of *L. braziliensis*. T cell proliferation was evaluated by [³H] thymidine incorporation. Cytokine Th1/Th2/Th17 patterns (IL-17A, IFN- γ , TNF- α , IL-10, IL-6, IL-2) were quantified in the supernatants by a flow cytometry multiplex assay.

Relevance. The search of asymptomatic individuals (Fig. 3) who were exposed to an endemic area of ATL will allow to identify markers of a natural resistance and select a suitable population for vaccine trials.



Figure 1. Seasonal work in the peruvian forest.

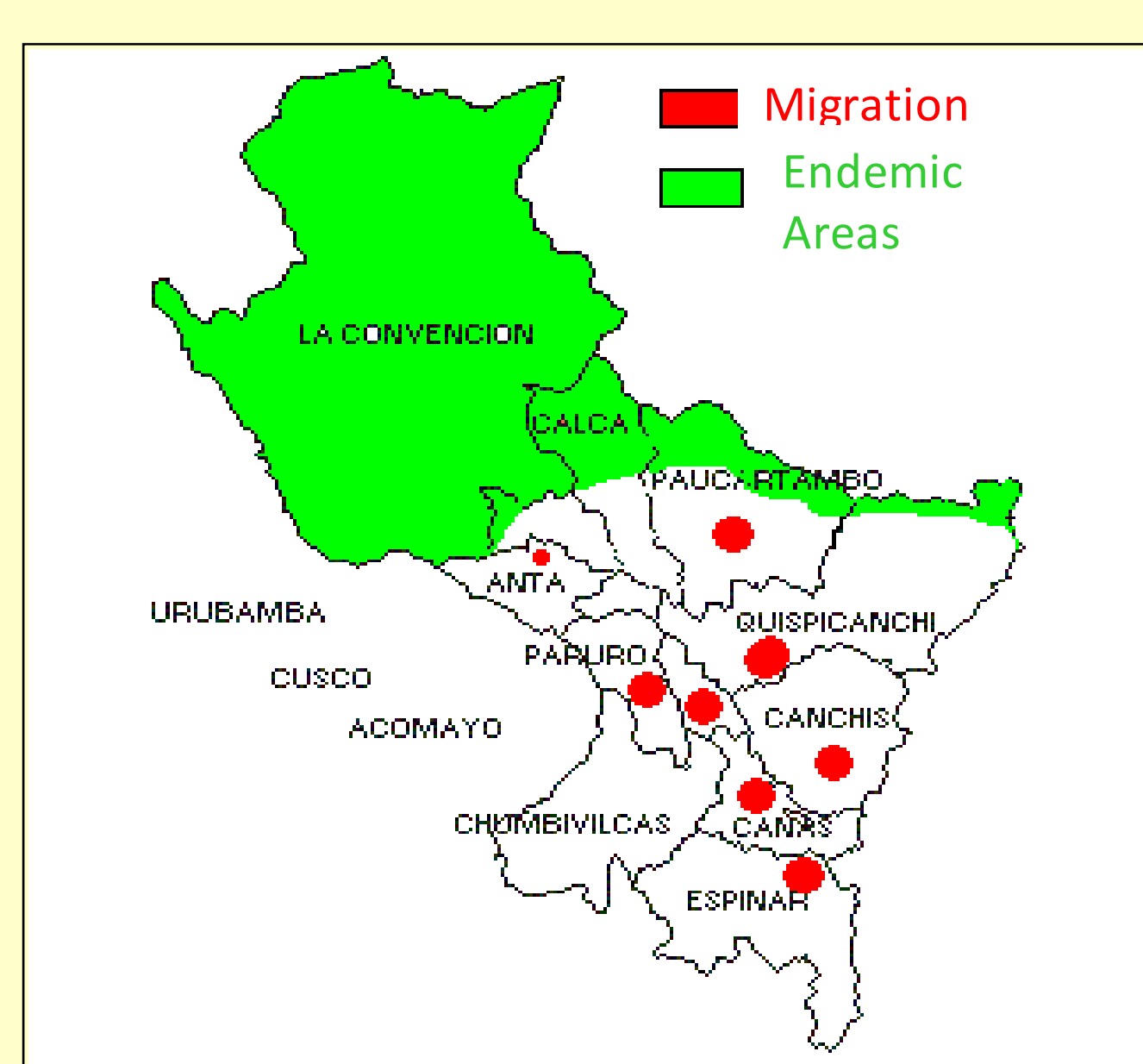


Figure 2 Endemic areas of Leishmaniasis and Migration Areas In Cusco.

Results. T-cell proliferation stimulation index (SI) cut-off above 2.47 was used to define an asymptomatic infection (Fig. 4): 15 out of 28 SEA (53.57%) presented significantly higher SI compared to 13 remaining SEA (4.5 vs. 1.32, $P < 0.001$). CL and ML patients presented SI of 77.6 and 22.2, respectively. An increased specific IFN- γ production was observed in SEA with SI above the cut-off compared to SNEA (18.6 vs. 4.0 pg/ml, $P < 0.05$) (Fig. 5). IFN- γ levels in CL and ML patients were 3259 and 4674 pg/ml, respectively. For the remaining cytokines, there were no significant differences between SEA with SI above the cut-off and SNEA. In CL and ML patients, high levels of TNF- α and IL-10 were observed. No immunological differences were found between SEA with SI below the cut off and SNEA.

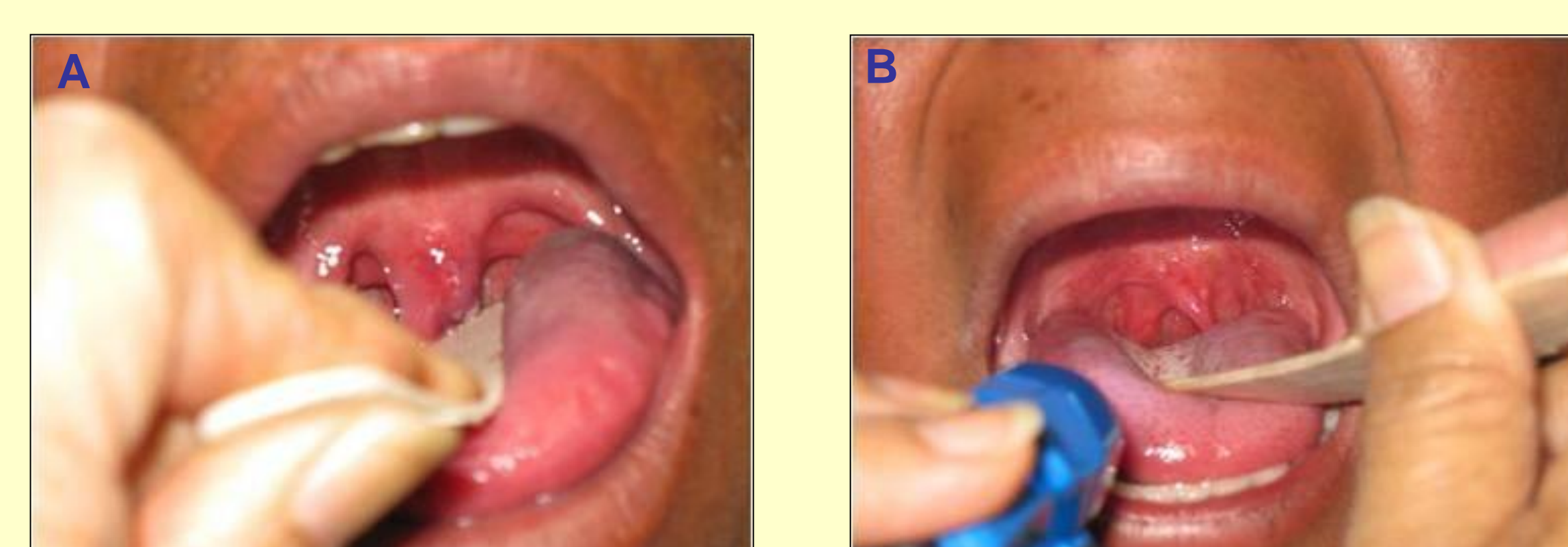


Figure 3. Clinical examination of A. Symptomatic with uvula infiltration and erythema plus edema in palate. B. Asymptomatic candidate. These individuals did not show any observable sign.

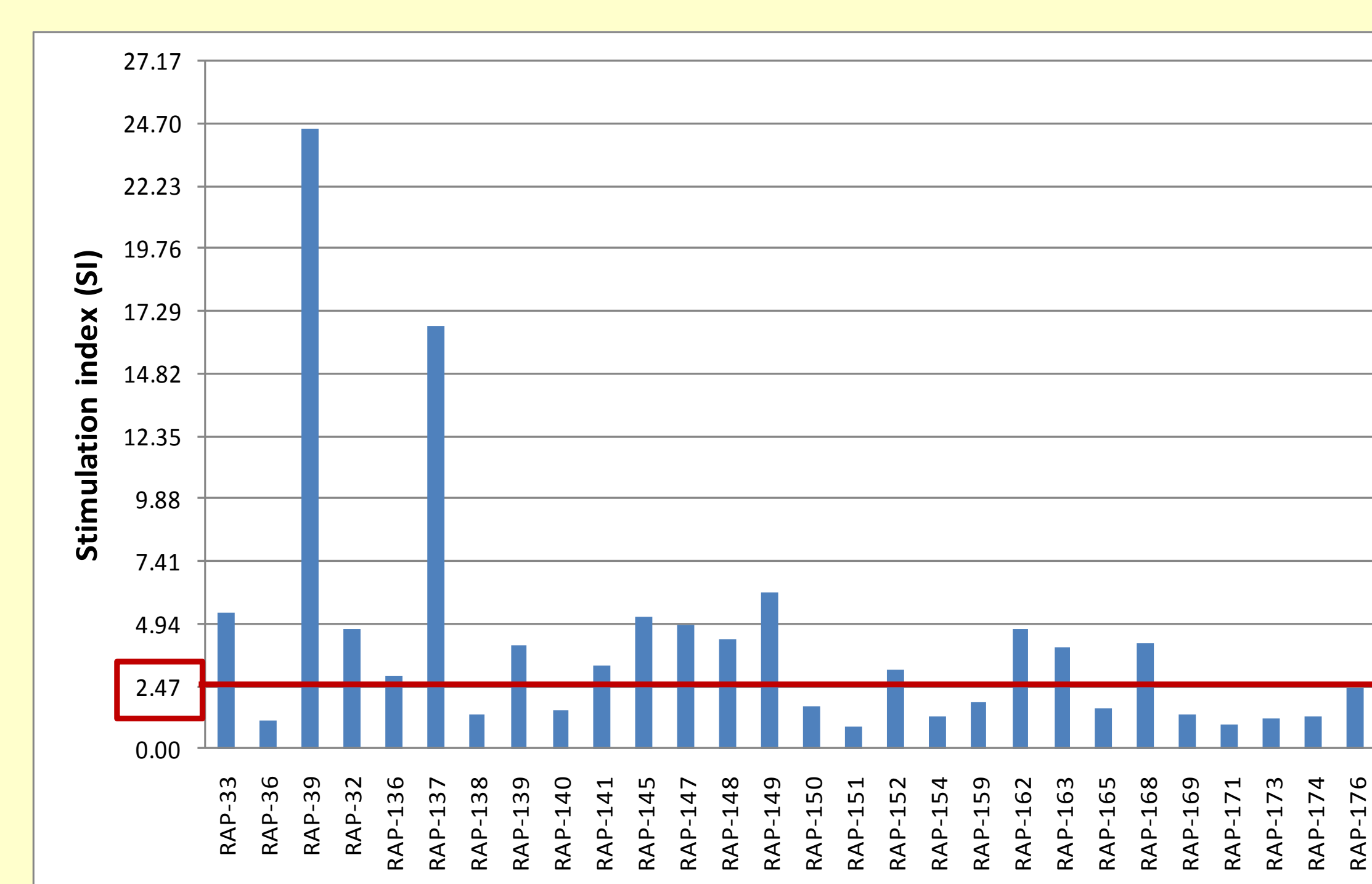


Figure 4. T-cell proliferation stimulation index (SI) in individuals exposed to an endemic area of American Tegumentary Leishmaniasis. Red line limits the cut-off.

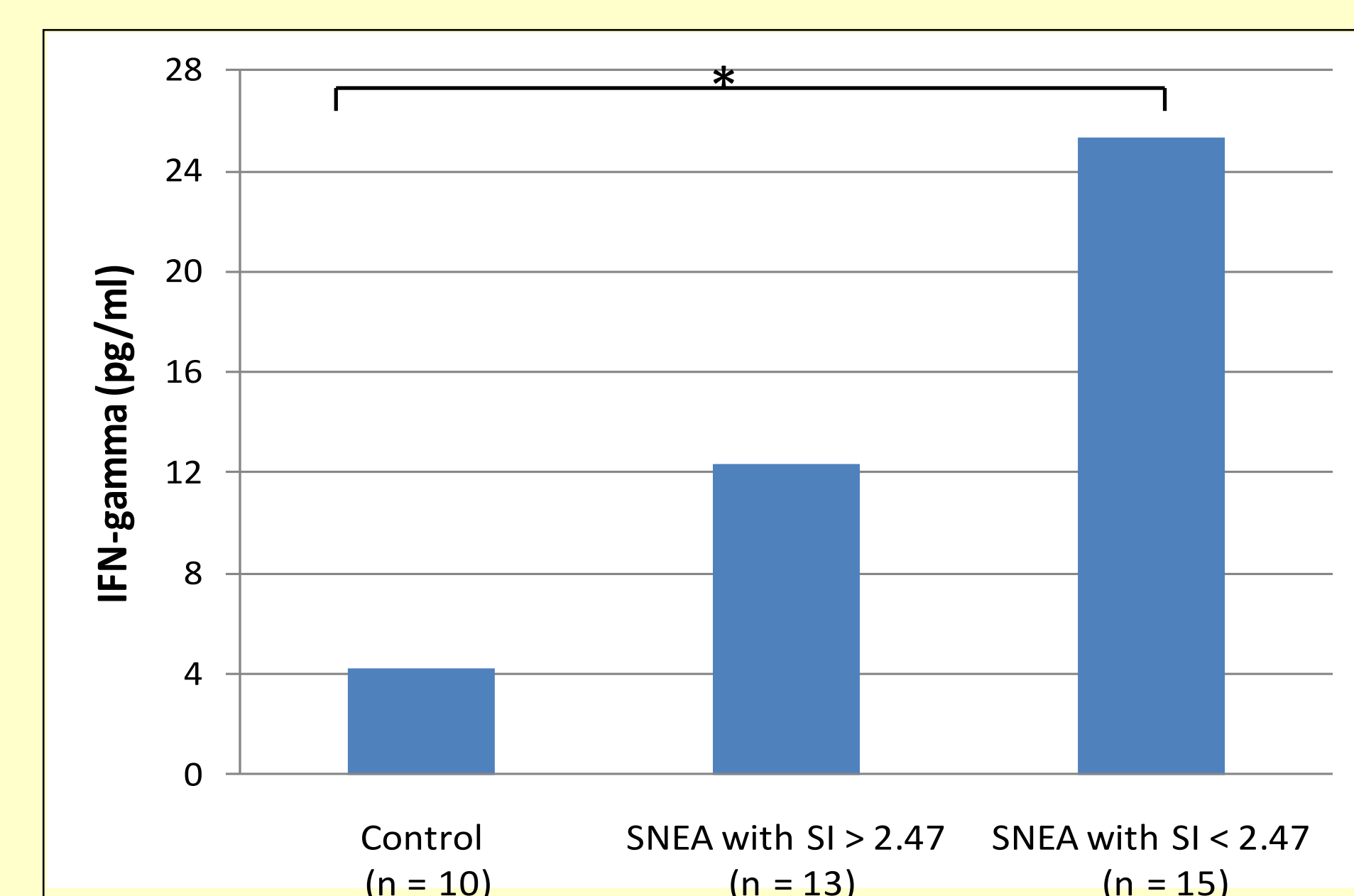


Figure 5. IFN- γ levels in asymptomatic *Leishmania* infection. Only P-values indicating significant differences are shown, * $P < 0.05$.

Conclusions. Our results showed two discrete groups among subjects apparently healthy that might be exposed to *Leishmania* parasites in endemic areas. We propose that T-cell proliferation and IFN- γ discriminated individuals who were *Leishmania* infected but asymptomatic from individuals that we assumed were non infected during seasonal work in the Amazon jungle.

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