Rare Transcripts of Interferon-γ Detected in Lepromatous Leprosy Cases

TO THE EDITOR:

The presence of alternatively spliced or extra "illegitimately spliced transcripts" of interleukins and colony stimulating factors that may arise in diseased situations ([3]) in low copy numbers has been reported earlier. In leprosy lesions, a striking difference in the cytokine mRNA profile has been shown by Yamamura, *et al.* ([4]). The mRNA for gamma interferon (IFN-γ) was found to be abundant in tuberculoid lesions as compared to the anergic lepromatous lesions.

In our report, we have analyzed nine representative cases of leprosy (five tuberculoid and four lepromatous) for the presence of low copy number transcripts of IFN-γ by reverse transcription polymerase chain reaction (RT-PCR). We employed nondenaturing polyacrylamide gel electrophoresis (PAGE) followed by silver staining as a simple, economical and sensitive alternative to radiolabeling amplicons. Peripheral blood lymphocytes isolated from the patients were challenged with *Mycobacterium leprae* whole cell-free extract antigens (CD225; kindly provided by Dr. R. J. W. Rees, WHO/IMMLEP) *in vitro* for 48 hr and used for the isolation of RNA ([1]) and cDNA amplification as described ([4]) at an annealing temperature of 55°C. A single band of the expected size of 355 bp was detected in both 1.5% agarose (Fig. 1) and 8% nondenaturing polyacrylamide (Fig. 2a) gels in all of the leprosy cases. The same

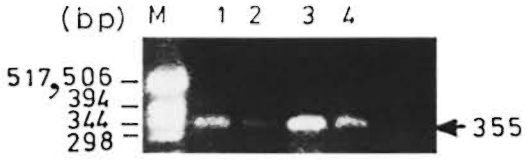

FIG. 1. A 1.5% agarose gel depicting a single RT-PCR product of 355-bp size in all the lanes.

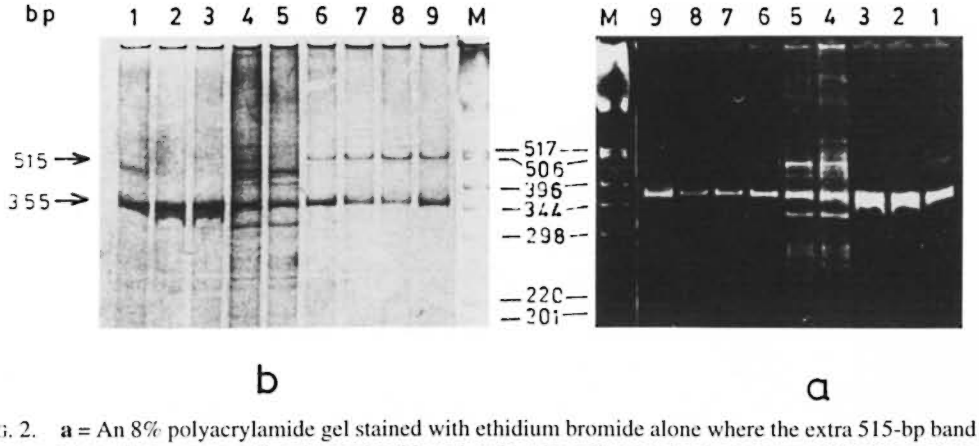

FIG. 2. **a** = An 8% polyacrylamide gel stained with ethidium bromide alone where the extra 515-bp band remained undetected; **b** = same 8% polyacrylamide gel as in **a** but silver stained, showing RT-PCR products of IFN-γ obtained in tuberculoid (lanes 1–5) and lepromatous (lanes 6–9) leprosy patients. Besides a major band of 355-bp size an extra 515-bp product (lanes 1, 6, 7, 8, 9) of RT-PCR was also detected. A ladder of misprimed products amplified at less stringent annealing temperature (50°C) was observed in lanes 4 and 5. Lane M depicts 1-kb ladder molecular size marker.

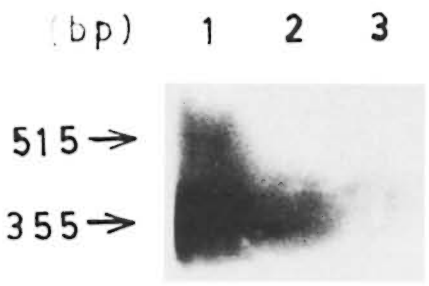

FIG. 3. Southern hybridization of IFN-γ RT-PCR products with nonradioactively labeled IFN cDNA probe. Lane 1 shows both 355-bp and 515-bp hybridized bands (corresponding to lane 6 of Figure 2). Lanes 2 and 3 were loaded with the ladder of misprimed RT-PCR products (lanes 5 and 4 of Figure 2) but only the 355-bp product hybridized.

polyacrylamide gel, when processed for modified silver staining ([2]), detected an extra 515 bp band (lanes 1,6,7,8,9; Fig. 2b). Interestingly, this extra band of 515 bp was detected in all four of the lepromatous leprosy cases. When subjected to hybridization, the IFN-γ cDNA probe hybridized strongly to both the original 355-bp as well as the extra 515-bp amplicons (Fig. 3) but to none of the numerous amplicons generated by design (lanes 4 and 5; Fig. 2) under low stringent conditions (annealing at 50°C) because of mispriming.

Our observation of an extra transcript of IFN-γ corresponding to 515-bp size detected as a rare, low copy number RNA molecule in lepromatous cases raises the possibility of the presence of an alternate population of IFN-γ. However, further extensive studies are required to be performed on a large number of patients to establish this distinct population of IFN-γ mRNA that may contribute toward the *M. leprae*-specific cellular anergy in the multibacillary leprosy patients.

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