This issue of The Journal carries an important contribution by Cairns Smith and colleagues, describing a large and unique epidemiological study carried out in three leprosy endemic villages on Maharashtra State in India. The study addresses the role of the upper respiratory tract in the transmission of *Mycobacterium leprae*, using two assays: PCR for *M. leprae* antigen detection on nasal swabs and salivary IgA for mucosal immune response to a crude whole *M. leprae* antigen. The results are interpreted as consistent with an hypothesis that *M. leprae* persists in endemic communities as transient, benign, commensal infections (carriage) in the upper respiratory tract of clinically healthy individuals. If true, this would entail a radical revision of the general view of the natural history of leprosy. It would also provide an explanation for the observation that case finding and treatment programs have little demonstrable impact upon the future incidence of disease, and hence have major implications for our expectations concerning leprosy control. Given the importance and difficulty of these issues, everyone with an interest in leprosy should be keen to see this latest study.

The results are not straightforward. The independent variables against which the PCR and IgA results are presented are age, sex, season, contact status (defined as having lived in the same household with a leprosy patient at some time in the previous 10 years), and BCG scar status. Leprosy status was assessed, but the paper says nothing about this beyond the comment that there were 42 cases in all, and that three were picked up in the baseline survey (we are not told in which villages). We are not told if the assays were carried out on the patients, let alone any results. We are not given an age distribution of the population, or of any of the other variables, but are told in the text that BCG scars were far more frequent among younger than older individuals (this is not surprising, as BCG is likely to have been introduced into the population in relatively recent years).

Considering just the polymerase chain reaction (PCR) results, according to Table 1, the prevalence of PCR positivity was low overall (1.6%), and its trend by village was, if anything, exactly the opposite of the implied leprosy prevalence (the paper does not tell you this, but it is evident from the distribution of contacts that cumulative leprosy prevalence was highest in village 2 and lowest in village 3; whereas PCR prevalence was highest in village 3, lowest in village 2). From Table 2, PCR positivity seems to be, if anything, higher in non-contacts than in contacts (one might suppose that contacts would be older on average than non-contacts, but there is no discussion of this in the paper). From Table 3 we see no association between PCR status and BCG scar. Table 4 shows no evidence of association between PCR and IgA ($\chi^2 = 0.36; p > 0.5$). Table 5 shows that PCR positivity appeared to be higher from August to November than at other times of year. Tables 6 and 7 add little, except to show that only 1 out of 47 follow-up samples taken from those initially PCR positive was positive a second time (as the data are shown grouped, we cannot see the patterns of consistency in individuals).

These results, in particular the negative association with prevalence or contact status, provide no evidence that the PCR results have anything to do with leprosy or with *M. leprae*. The paper does not mention contacts of current MB patients, which is a group in which one might a priori expect to find positivity. One notes that the prev-

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1 This is a Commentary on an article by Cairns Smith, *et al.* published in this issue of the Journal entitled “An Approach to Understanding the Transmission of *M. leprae* Using Molecular and Immunological Methods: Results from the MILEP2 Study,” which can be found on page 269.

Mailing address: Paul E. M. Fine, Professor of Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, Keppel Street, London WC1 7HT, U.K.
lence in follow ups of positives is the same as that in the initial survey, which could reflect either that PCR positivity is transient, or that the assay is at the limit of specificity, and is just picking up some other cross reacting nucleic acid in the population or in the laboratory. Other studies with this assay have experienced problems with its specificity (2), but this crucial issue is not dealt with explicitly here. The authors tell us that they discarded 16% of results because they failed quality control—but this statistic is not explained, and there is no discussion of its implications for the extent of false positives in the series reported (i.e., if x% of negative controls were assessed as positive in the laboratory, leading to discard of whole batches, and these manifest false positives were randomly distributed throughout the series, this proportion should have been subtracted from the proportion positive in their test series).

Concerning the IgA assay, two-thirds of all samples were considered to be positive “for mucosal responses to *M. leprae*.” We are shown no data by age, but are told in the text that “no difference was found between age groups.” This might be surprising, insofar as one might expect cumulative exposure to increase with age, but may be consistent with other evidence that IgA responses can be short-lived. The village distributions in Table 1 show little evidence of an association with leprosy contact status (villages 2 and 3 have highest and lowest proportions of contacts, respectively, but similar prevalences of IgA positivity). An association of IgA positivity with contact status is indicated in Table 2, but we are not told if the prevalence was higher in contacts of MB patients than in contacts of PB cases, or whether it was higher in contacts of recent compared to contacts of previous patients. Table 3 suggests higher IgA levels in individuals without BCG scar, compared to those with a scar. This may not make sense, unless we note that those without BCG scar are considerably older than those with a scar—and thus one might think the prevalence difference reflects age, and a higher IgA in older individuals. But we were told in the text there was no relation by age (though shown no data), so this relationship with absence of BCG poses a puzzle. There is something peculiar going on here, as the claim of no relationship of IgA with age implies a transient response (otherwise prevalence reflects cumulative incidence and must increase by age), whereas the absence of a BCG scar is unlikely to be a transient characteristic. I have no explanation for this! The most striking finding is shown in Table 5: an extraordinary, almost 10-fold, increase in prevalence of (strong) IgA positivity in the month of November. We are shown no evidence to indicate whether this was a once-off or a consistent phenomenon. Furthermore, this huge excess suggests that the month of collection is an important risk factor, and means that none of the other analyses can be interpreted without appropriate adjustment for the month of collection (i.e., the peculiar association with lack of BCG scar could be attributable to differential selection by age or BCG scar status during the November period). No such adjustments have been made.

What do these IgA data mean? Is it likely that two-thirds of all individuals were experiencing a short lived “mucosal response to *M. leprae*” at the time they were sampled? The IgA assay was performed with a crude whole *M. leprae* assay, and thus one would not expect it to be specific for *M. leprae* infection, but we are given no data on sensitivity or specificity (e.g., in currently infected MB patients, or in current contacts of MB patient). Cross reactivity is well known for T cell assays of different mycobacteria. The association with contact status may reflect some level of association of the assay with true *M. leprae* exposure, but further discussion of sensitivity and specificity, and detailed multivariate analysis is necessary to show convincingly whether any of the associations here reflects more than confounding.

Many readers will, like me, find this a tantalizing paper. They will struggle, and will wonder at the end of their effort if these data really do reflect *M. leprae*. I, for one, am not convinced that they do. The only internally consistent observation is the tendency for higher prevalence of both PCR and IgA positivity in the rainy season, though we have the irony of no association between the assays as shown in Table 4. Do the positive results reflect glimpses of *M. leprae*—or some other influence or artifact, perhaps some environmental mycobacterium? There is no way to tell from the data
presented here. Given the immense amount of work which went into the collection of these data, their complexity, and their susceptibility to confound, a more detailed presentation of the data and quality controls, including results on the cases and contact groups, detailed descriptions by age and contact status, and appropriate multivariate analysis, is called for.

If the hypothesis that *M. leprae* is a common, benign, transient resident of nasal cavities in endemic communities were correct, it would have major implications for our understanding of leprosy, and for expectations of control based upon case finding and treatment. But it remains an hypothesis, and criticisms similar to those above can be levelled at the other papers which have appeared on this subject (1). Given the importance of the issue at stake, it is important to test the hypothesis more rigorously than it has been to date. In addition to the analytical suggestions above, here are two proposals: first, in order to make results of such PCR-based studies convincing, a large number (appreciably greater than the reciprocal of the prevalence rate of carriage) of nasal swabs from a non-endemic control population should be blind-coded and randomly mixed with the test series before they are sent to the testing lab. The results on these should be reported explicitly, and the percentage positives in these samples should be subtracted from the percentages declared positive in the test series. Second, individual nasal swabs from healthy subjects in the same endemic community could be pooled and inoculated into susceptible animals (mouse footpads or armadillos). These suggestions are not trivial, and would be expensive. However, given the importance of the issue at stake, I can think of few better ways to further our understanding of leprosy at this point, and hope that some research group will take up the challenge.

—Paul E. M. Fine, VMD, Ph.D.

Professor of Communicable Disease Epidemiology, London School of Hygiene and Tropical Medicine, London, U.K.

REFERENCES
