Comparison of A60 and three glycolipid antigens in an ELISA test for tuberculosis

Clin Microbiol Infect 1997; 3: 595–596

Lagrange and his coworkers [1] have compared the serologic reactions to three mycobacterial glycolipid antigens prepared by the Pasteur Institute with the reaction to a commercially available protein antigen (A60). This study presents several interesting observations but the conduct of the investigation and the sweeping conclusion drawn deserve comment.

First, the mode of use of the A60 test clearly exposes the interspecific character of the protein antigen, the detection of inapparent mycobacterial infections in healthy exposed groups and the need to evaluate the serologic results in conjunction with other laboratory and clinical observations. Emphasis is placed on the sero-analysis of immunodepressed and immunosuppressed subjects, who produce small amounts of IgA antibodies against A60; the search for IgA antibodies is warranted and would increase the diagnostic power in extrapulmonary cases, in HIV-seropositive cases and in the AIDS syndrome. The reproach cannot be made that the limitations of the commercial ELISA test have been concealed by the manufacturer. It is not possible that Lagrange et al were ignorant of these established limitations, and one would have expected that a study that confirmed them would have included these previous observations in the introduction.

Second, the corrective measures to be applied in the serologic A60 analysis of immunodepressed cases are clearly set out in the instructions for use of the antigen. A comparison purporting to demonstrate the absolute superiority of the three non-peptide antigens, which are not generally available, over this commercial protein antigen should have followed these recommendations, when indicated. However, no search for IgA antibodies was made in HIV-positive cases, as recommended, while the cut-offs for IgM and IgG antibodies were not decreased but considerably increased, to 100% specificity.

Third, I note that the ordinates of the figures have no scale and thus suggest that the serologic responses were identical in intensity for all the antigens used—when in fact they varied widely within the same patient groups. For example, the mean absorbance for HIV-negative, sputum smear-positive extrapulmonary cases was 0.041 with the PGLTb antigen and 0.527 with A60. The presentation of these data as 41 and 527 and their statistical manipulation is misleading: an ELISA absorbance value of 0.041 is, for all practical purposes, a blank. The *P* value included under this entry in Table 1 should in fact be 0.05 rather than 0.001. All these values were obtained at a wavelength of 414 nm, which is inappropriate for the A60 test, the optimum for which is 450 nm. At 414 nm the intensity of the absorbance is reduced two- to three-fold, and uncertain results are obtained in the low-density range, precisely where the cut-off is estimated.

Another discrepancy is the claim that all the patients infected by *Mycobacterium* spp. other than tuberculosis (MOTT) included in this study were HIV positive with disseminated disease (page 215) set against the observation that the specificity of A60 was about 80% (in truth 83.3%) in HIV-negative patients with localized MOTT infection (page 219).

Fourth, the statistical analysis relies on the premise that all TB-culture-positive cases are 'tuberculosis (TB) cases' while all TB-culture-negative cases are 'controls'. The data given in the tables show a few differences among patient groups for some antigens, but most groups gave similar mean absorbances, at least if their SEM is taken into account. Lagrange et al understand that TB patients may differ in their response and subdivide them according to bacteriologic and clinical criteria but they do not apply this concept to the various extrapulmonary locations analyzed by A60 and LOS antigens. The SEM of the A60 absorbance is very large (0.344 for a mean of 0.527) and is even larger for the LOS antigen: 0.0749, almost equal to the mean, which could bring the mean 0.0910 down to 0.016, where it would rejoin blank values. These two large SEM values indicate that, for these two antigens, all the extrapulmonary cases cannot be drawn into a single group. Similarly, Lagrange et al do not apply the concept either to control groups; they pool all 'negative' cases and define a cut-off for serologic data at 100% specificity. This runs counter to the spirit of a statistical analysis which deliberately takes into account the unavoidable variations occurring in biological material and among individual samples. In doing so, they considerably diminish the sensitivity and usefulness of a recognized interspecific antigen. They disregard the possibility of abacillary TB, ignore the possibility of positive serology due to MOTT, do not take into account the possibility of abortive infections in control groups at risk of infection but prone to be blood donors (airline stewards, policemen, social workers, supermarket employees, clinicians, nurses), neglect the impact of immunodepression on HIV-positive controls and claim species specificity for the three in-house lipid antigens, although DAT antigen reacts with leprosy sera (reference 19) and anti-PGL antiserum reacts with lipidic extracts of *M. bovis*, BCG, *M. xenopi*, *M. flavescens* and *M. fallax*.

Fifth, the dynamic aspects of antibody production are not considered. The poor sensitivity of the protein antigen reported by Lagrange et al. in contrast to the

results reported in the literature, is not due solely to statistical misuse but is real and is interesting yet is not discussed by Lagrange et al. It is most probably due to the timing of the serologic tests. A60 antigen is dominant during human disease and serologic testing with A60 is applied to patients who consult because they have symptoms; the serologic results obtained must be evaluated together with clinical clues, as recommended. The criteria for selection of patients and controls applied by Lagrange et al are not stated. Presumably the search was diverted towards asymptomatic TB cases in conjunction with suspicion of HIV infection. One may safely predict that the frequency of anti-A60 seropositives would increase with the appearance of symptoms and also in HIV-positive cases with chronic TB, while the frequency of positive cases detected with the non-peptide antigens would be higher in healthy people with no other sign of infection, with an extension of the search to TB-risk groups.

Sixth, a most disturbing aspect of this comparative analysis is the absence of publication of the DTH data that were collected on TB patients. Lagrange et al. omitted this search in control groups, thereby ignoring the possibility of BCG vaccinations and inapparent or past infections. Equally worrying is the absence of statistical analysis on the usefulness of combing protein antigens with non-peptide antigen in ELISA. Such information could have been provided and would have contributed substantially to our understanding of the immunologic processes and also, if an additive effect were observed, improved the system of detection.

Research into human tuberculosis is difficult and frustrating. The investigation of Lagrange et al represents a welcome departure from traditional research concerns, even if it presents substantial deficiencies. It contributes to a better understanding of the immunopathology of the disease. In the face of discrepant findings, what must be avoided now is a grand sweep of induction that extracts general laws from limited observations with preparations the properties of which have not yet been completely defined.

R. Maes
Anda Biologicals,
37 rue de la Course,
F-6700 Strasbourg,
France

Reference

1. Simmonney N, Molina JM, Molimard M, Oksenhendler E, Lagrange P. Comparison of A60 and three glycolipid antigens in an ELISA test for tuberculosis. Clin Microbiol Infect 1996; 2: 214–22.

Response to Dr Maes

Clin Microbiol Infect 1997; 3: 596–597

The object of this study was to compare the relative efficiency of different peptide and non-peptide antigens in an ELISA test in hospitalized patients presenting with mycobacterial disease and with a bacteriologically proven diagnosis. The controls were healthy blood donors without diagnosed tuberculosis and not treated during the preceding 5 years. The controls with pulmonary afflictions were patients whose samples (expectoration, bronchoscopy, bronchoalveolar lavage), including the repeats, turned out to be negative. None of them had received antituberculosis antibiotic therapy. The notion of HIV seropositivity was included in this study only retrospectively, on the basis of patient records, in order to verify whether these ELISA tests were applicable to all cases in which active tuberculosis had been diagnosed, and independently of the knowledge of the immune status with respect to HIV. Demonstration of anti-A60 IgA was not attempted in this study since the only document published at the time of our study was that by Gupta et al [1] which showed equivalence between IgG and IgA. This has been subsequently confirmed in an Italian study [2]. Furthermore, the recommendation to test for anti-A60 IgA in HIV-positive subjects does not seem to be documented, and in any case, does not appear in the two above-mentioned publications.

As far as the absence of graduation of the ordinate in Figures 1 and 2 is concerned, this is a typographical error. At any rate, this is of little importance and the cut-off levels are indicated. These have not been chosen randomly, but have been calculated according to established procedures using the mean of the results of the ODs of the blood donors plus two standard deviations. These cut-off levels, and in particular those for anti-A60 IgGs, are identical to those found by other authors [2]. They have been given in Tables 2 to 5. The conditions for reading the ELISA A60 plates were those recommended by the manufacturer and readings were done at 450 nm, and not at 414 nm, the wavelength used exclusively for the reading of ELISAs using the glycolipid antigens. The use of these cut-off levels has allowed the comparison of the sensitivities of the different tests, using one comparable specificity. Furthermore, the comparison of the means of the antibody titers, in various clinical–biological situations (pulmonary and extrapulmonary tuberculosis, seropositivity and -negativity with respect to HIV, positive or negative direct examination) has been carried out in order to attempt the evaluation of the influence of these situations on the antibody response measured by the OD with respect to each antigen tested. As has