INT J TUBERC LUNG DIS 4(6):562–569

Antibody response to culture filtrate antigens of *Mycobacterium tuberculosis* during and after treatment of tuberculosis patients

M. S. Imaz, E. Zerbini

Instituto Nacional de Enfermedades Respiratorias 'Emilio Coni', ANLIS 'Carlos Malbran', Santa Fe, Argentina

SUMMARY

SETTING: Many authors have shown rising titres of anti-mycobacterial antibodies after a few months of anti-tuberculosis treatment. This humoral response might persist for years, making the discrimination between current and old disease difficult.

OBJECTIVE: Characterisation of the humoral response to culture filtrates of *Mycobacterium tuberculosis* before and after treatment of tuberculous patients in order to identify those antigens that could provide information about disease activity.

METHODS: Anti-mycobacterial IgG response was determined during and after treatment of tuberculous patients by ELISA and immunoblot. Serum was taken from 71 active tuberculous patients (59 newly acquired and 12 relapse), 15 old tuberculous patients and 45 non-tuberculous control subjects.

RESULTS: By ELISA, antibody response increased after 2 months of treatment. After chemotherapy was completed, the estimated number of antibodies remained at the same level. The level of specific antibodies in patients seems to reach the same level as that of control subjects 3 years after initiation of treatment. In Western blot, although each patient serum had its own characteristic banding pattern, differences between tuberculous patients and control subjects were found in the area below 20 kDa. Serum from tuberculous patients showed high levels of antibodies at the 14 kDa region. After the beginning of treatment, the intensity of the 14 kDa region band and the percentage of positive recognition tended to decrease. Therefore, one year after initiation of treatment, only seven of 13 cases who demonstrated anti-mycobacterial antibodies in ELISA revealed a mild but still positive reaction at the 14 kDa region; this reactivity disappeared 2 years after initiation of chemotherapy.

CONCLUSIONS: The 14 kDa region antigen seems to induce a humoral response that evolves in relation with the disease activity.

KEY WORDS: tuberculosis; ELISA; Western blot; post-treatment serology

IN DEVELOPING COUNTRIES, many physicians encounter the problem of correctly diagnosing an early relapse or correctly treated and healed tuberculosis (TB) in a patient whose clinical alteration is secondary to another, unrelated disease. Recently, enzyme-linked immunosorbent assay (ELISA) techniques employing various mycobacterial antigens have been used in an attempt to perform serological diagnosis of TB, with variable success.[1] To be of use, a serologic test should give information about disease activity. Although some authors have shown rising titres of anti-mycobacterial antibodies after a few months of well-conducted treatment,[2–4] there is limited information on the persistence of antibodies after successful treatment.

In Argentina, Barrera et al.[5] found that an enzyme immunoassay using mycobacterial culture filtrate as antigen might have a potential use as a presumptive diagnostic test in patients with suspected TB long before culture results are available. The development of immunoblotting techniques stimulated new interest in the study of humoral responses for the diagnosis of tuberculous infection, and might help to identify antigens that allow better discrimination between active and healed TB.

In this study, we looked for antibody response to culture filtrate antigens of *Mycobacterium tuberculosis* during and after successful treatment of tuberculous patients by ELISA and immunoblot.

MATERIALS AND METHODS

Patients

Blood samples were obtained from 131 adult subjects (>15 years of age): 71 patients with active pulmonary tuberculosis (59 newly acquired and 12 relapses), 15

Correspondence to: María Susana Imaz, Instituto Nacional de Enfermedades Respiratorias 'Emilio Coni', Belgrano 6396, Santa Fe 3000, Argentina. Tel: (+54) 342-4892-830/ 4602-503. Fax: (+54) 342-4892-827. e-mail: rfernand@fcjs.unl.edu.ar

Article submitted 22 June 1999. Final version accepted 31 January 2000.

old tuberculous patients and 45 non-tuberculous control subjects. Tuberculosis patients had been diagnosed based on clinical, radiological and bacteriological findings (culture was positive in all cases). Five groups were distinguished.

Group 1

Active, untreated pulmonary TB: 59 patients with current pulmonary TB whose bacteriologic examinations were culture positive and/or smear positive after acid-fast staining. These subjects had no prior documented or treated TB. Serum specimens were obtained from these patients on their first visit to the hospital; the patients were treated with combination antituberculosis chemotherapy with either streptomycin or ethambutol in addition to isoniazid, rifampicin and pyrazinamide during the first 2 months, followed by isoniazid and rifampicin for 4 months.

To study the evolution of the antibody levels during treatment, serum samples were obtained from 42 of the patients at about 2 months after the beginning of treatment, and 26 samples were obtained at the end of treatment. The remaining 16 patients did not return within the designated time period for blood collection and were therefore not submitted to serum sample testing at the end of treatment.

Group 2

Inactive, treated pulmonary TB: 21 of the 59 patients with newly acquired TB and no current disease who submitted to multiple serum testing at years 1, 2 and 3 following combination antituberculosis chemotherapy. Of the 38 patients with newly acquired TB who did not submit to multiple serum testing, two refused blood sample collection, 29 did not return within the designated time period for blood collection, and seven moved from Santa Fe city.

Of these 21 patients with inactive, treated pulmonary TB, 18 could also be evaluated at the end of treatment. At the time blood samples were collected, their bacteriological examinations were smear and culture negative. Three subgroups were distinguished: Subgroup 1, 21 serum samples obtained from patients started on antituberculosis treatment 1 year ± 1 month prior to collection of the blood sample for serological analysis. Subgroup 2, 12 serum samples obtained from patients started on antituberculosis treatment 2 years ± 2 months prior to collection of the blood sample for serological analysis. Subgroup 3, 21 serum samples obtained from patients started on antituberculosis treatment 3 years ± 2 months prior to collection of the blood sample for serological analysis.

Group 3

The relapse TB group consisted of 12 patients who after having followed a correct course of treatment had been considered cured, but had again developed bacteriologically active TB.

Group 4

The old TB group consisted of 15 patients with no current disease who had a definite history of pulmonary TB more than 5 years previously. Their bacteriological examinations were smear and culture negative.

Group 5

The non-TB control group consisted of 45 patients, of whom 20 had respiratory diseases other than TB and 25 had non-respiratory diseases (diabetes and cardiovascular diseases).

Clearance by the local ethics committees, and, where appropriate, informed consent were obtained. The serum specimens were assayed without knowledge of patients' clinical characteristics.

Antigen preparation

M. tuberculosis antigen was prepared at the Instituto Panamericano de Protección de Alimentos y Zoonosis (INPPAZ), as described elsewhere.[5,6] Briefly, *M. tuberculosis* H37Rv was grown for 8 weeks in Dorset Henley's medium. Bacteria were killed by treating the suspension with 1% phenol at 37°C for 3 days. Culture was filtered through cellulose asbestos membranes. The filtrate was then precipitated with trichloroacetic acid (4%) and dissolved in a phosphate buffer pH8 at a concentration of 2 mg/ml. The preparation was aliquoted, lyophilised and stored at 4°C. Before use it was resuspended in carbonate buffer, pH 9.6, at an optimal concentration of 25 μg/ml.

Methods for ELISA

The levels of immunoglobulin G (IgG) antibodies to *M. tuberculosis* culture filtrate antigens were determined by ELISA as described previously.[5] Briefly, polystyrene microtiter plates (Immulon II, Dynatech Laboratories, Alexandria, VA) were coated with 1.87 μg/well of antigen. Serum samples were examined at 1:200 dilution. Protein A coupled with horseradish peroxidase (Sigma Chemical Co., St. Louis, MO), 2,2′-azino-bis (3-ethylbenzthiazoline-6-sulfonic acid) and hydrogen peroxide were used in the process of revealing the immune reaction. The optical density (OD), at 410 ηm, was read in an automatic micro ELISA spectrophotometer (Dynatech MRX, Chantilly, VA, USA). The threshold of positivity (0.25) was set by adding two standard deviations to the mean OD value of the non-TB control group.

SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis)

SDS-PAGE was performed as described by Laemmli.[7] on 13.5% (w/v) gel. The antigen was diluted in a sample loading buffer (0.064M Tris-HCl pH 6.8, 2% (w/v) SDS, 10%(v/v) glycerol, 0.002% bromophenol blue, 5%(v/v) mercaptoethanol) and was heated to 100°C for 5 min. Gel was either stained with Coomassie Brilliant Blue or used for immunoblotting. Molecular

weight markers (Pharmacia, Uppsala, Sweden) were also subjected to electrophoresis along with the antigen.

Western blot analysis

Antigens separated by SDS-PAGE were transferred to nitrocellulose[8] and used for immunoblotting. In brief, nitrocellulose paper containing culture filtrate antigen was cut into fine strips of around 5 mm diameter (15 μg antigen/strip) and made to react with different sample sera (1:50), followed by anti-human IgG peroxidase (1:750, Sigma) and 4-chloronaphthol (Sigma) as chromogen.

Data analysis

Wilcoxon test for paired values was performed to compare the antibody levels measured at each time of sampling, and the McNemar test was used to determine the significance of differences of positivity frequencies found at each time of sampling. To evaluate the significance of differences between mean antibody levels of independent samples, the Wilcoxon test was used, and the Fisher's exact or χ^2 tests were employed to evaluate the significance of differences of positivity frequencies between independent groups.

RESULTS

Human antibody response in ELISA

A cut-off point of 0.25 OD was determined using serum from the 45 non-TB controls. Before starting treatment, 36 (61.0%) of the 59 patients with active, untreated TB had antibody levels of more than 0.25. The frequency of positive values was significantly higher in the relapse TB group than in the active, untreated TB group (91.7% vs 61.0% respectively, Fisher's exact test, $P = 0.048$). Moreover, mean antibody levels in relapse TB patients were significantly higher than in patients with newly acquired TB (Wilcoxon test, $P = 0.019$) (Figure 1).

In 42 of the active, untreated TB group, the antibodies were titrated before and after 2 months of chemotherapy; for 26 of these, antibodies were titrated after 6 months of treatment. As shown in Figure 2, the levels rose after 2 months of treatment (Wilcoxon pair test, $P = 0.0001$), and the percentage of positive values increased from 61.9% (26/42) to 85.7% (36/42) (McNemar test, $P = 0.02$). These levels remained unchanged until the end of treatment, with no statistical difference between the values measured after 2

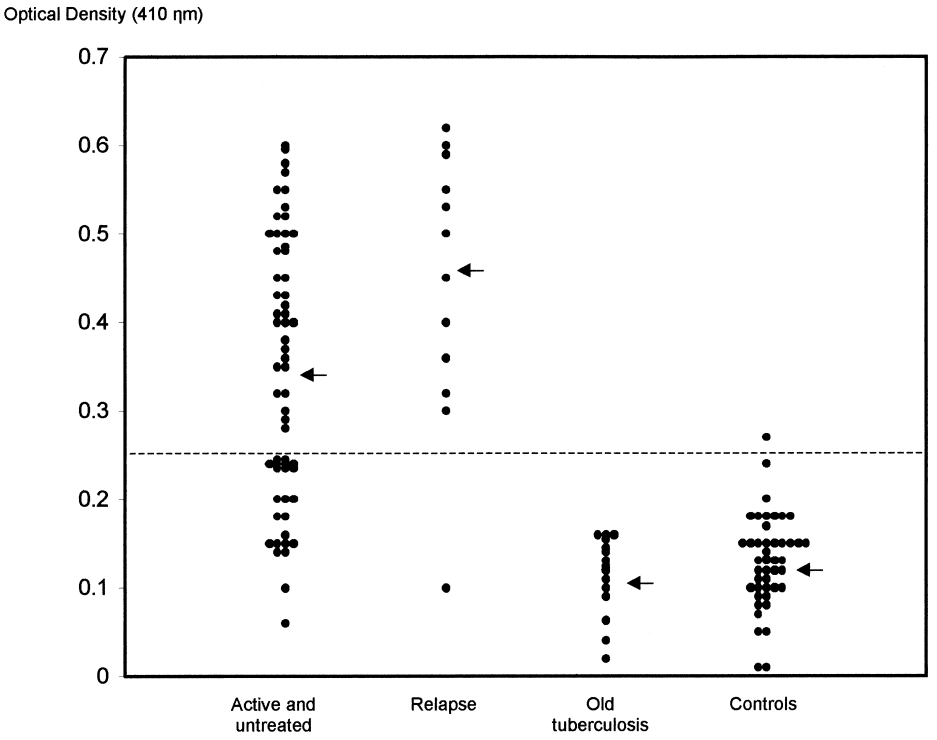

Figure 1 Levels of antibody to culture filtrate antigens in patients with active pulmonary tuberculosis (TB), patients with old TB and non-tuberculous control subjects. The normal upper limit, set by adding two standard deviations to the mean OD value of the control group (0.25), is presented as a dashed line. Mean levels are shown by arrows. Active and untreated = patients with active pulmonary TB before antituberculosis chemotherapy. These subjects had never had prior documented or treated TB. Relapse = patients who, after having finished suitable treatment, were considered cured but again developed bacteriologically active TB. Old tuberculosis = patients with no current disease who had had a definitive past history of pulmonary TB more than 5 years previously. Controls = patients with respiratory diseases other than TB or with non-respiratory diseases.

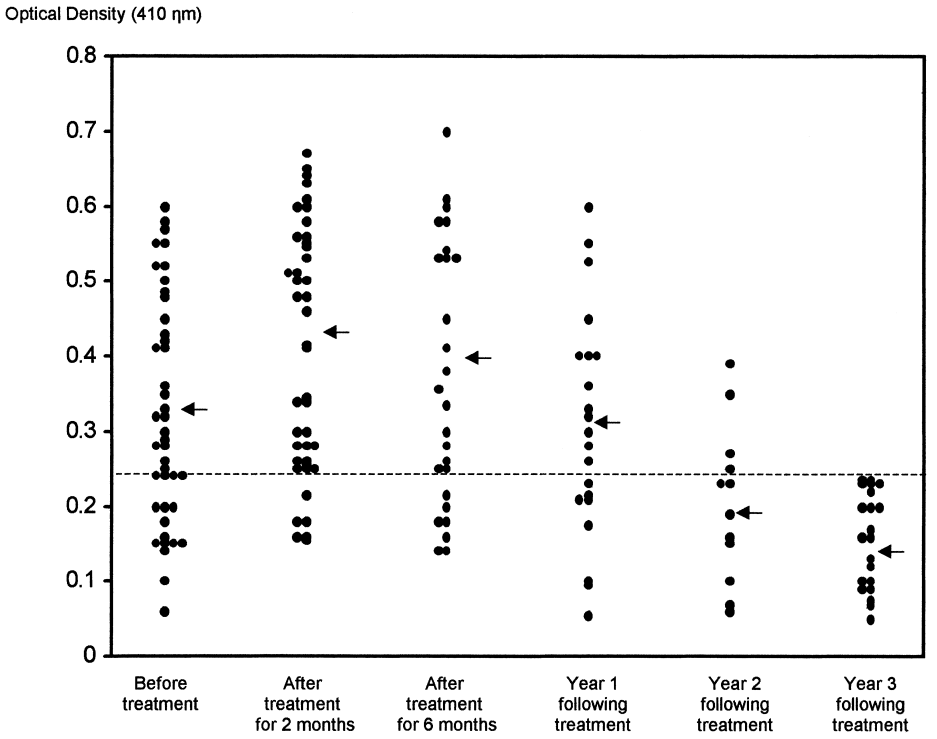

Figure 2 Levels of antibody to culture filtrate antigens before, during and after antituberculosis chemotherapy. Antibody levels were measured before and during the 6-month treatment period and at various times after the end of treatment. For other explanations see Figure 1.

months and those measured after 6 months, so that the values of the levels of antibody to culture filtrate antigens were significantly higher at the end of treatment than at the time of diagnosis (0.31 OD vs 0.40 OD before and after 6 months of treatment, respectively; Wilcoxon pair test, $P = 0.0014$).

In 21 of the 59 patients with newly acquired TB, the time-course changes in the levels of antibody to culture filtrate antigens were examined at years 1, 2 and 3 following treatment. The antibody levels measured at the time of diagnosis in these 21 patients submitted to multiple serum testing did not differ from those measured in the remaining patients with newly acquired TB whose samples could not be obtained at years 1, 2 of 3 following treatment (mean levels: 0.35 OD vs 0.34 OD for patients followed up and those not followed up, respectively, Wilcoxon test, $P = 0.71$; frequencies of positivity: 61.9% vs 60.5% for patients followed up and those not followed up, respectively, χ^2 test, $P = 0.86$). The mean antibody levels were 0.31 for the 1 year ± 1 month period and 0.20 for the 2 year ± 2 months period. After 3 years, the levels reached almost the same value obtained in the non-TB control subjects (Wilcoxon test, $P = 0.128$). The percentages of positive values at years 1, 2 and 3 following treatment were 61.9% (13/21), 33.3% (4/12) and 0% (0/21), respectively (Figure 2). None of the patients from the old TB group showed positive values (Figure 1).

Human antibody response on Western blot

The extreme chemical complexity of the culture filtrate antigen is evident on analysis on Coomassie-Blue stained SDS-PAGE (Figure 3A). A large number of bands of different sizes can be observed, with molecular weights ranging from 10 to 80 kDa.

All the serum samples were tested on Western blot, with *M. tuberculosis* culture filtrate as antigen; a representative example is given in Figure 3B. Although each patient's serum sample had its own characteristic banding pattern, serum from both active TB patients (newly acquired or relapse) and controls reacted with bands in the area above 20 kDa in culture filtrate. Many of the bands recognised were faint.

Some differences between patients with active TB and controls were found in the area below 20 kDa. Thirty-five (59.3%) of the 59 serum samples from active, untreated TB patients revealed immune reactivity in the region of 14 kDa. When treatment was started and the patients began to recover, the percentage of positive recognition and the intensity of the 14 kDa region band tended to decrease. Positive reaction in the region of 14 kDa was obtained in 13 (50.0%), seven (33.3%) and 0 (0%) patients after 6 months of treatment and 1 and 2 years after treatment, respectively. Seven out of 13 cases who demonstrated positive values on ELISA one year after treatment revealed a mild but still positive reaction at the 14 kDa region; this reactivity had totally disappeared 2 years after chemotherapy. Serum from inactive TB patients 3

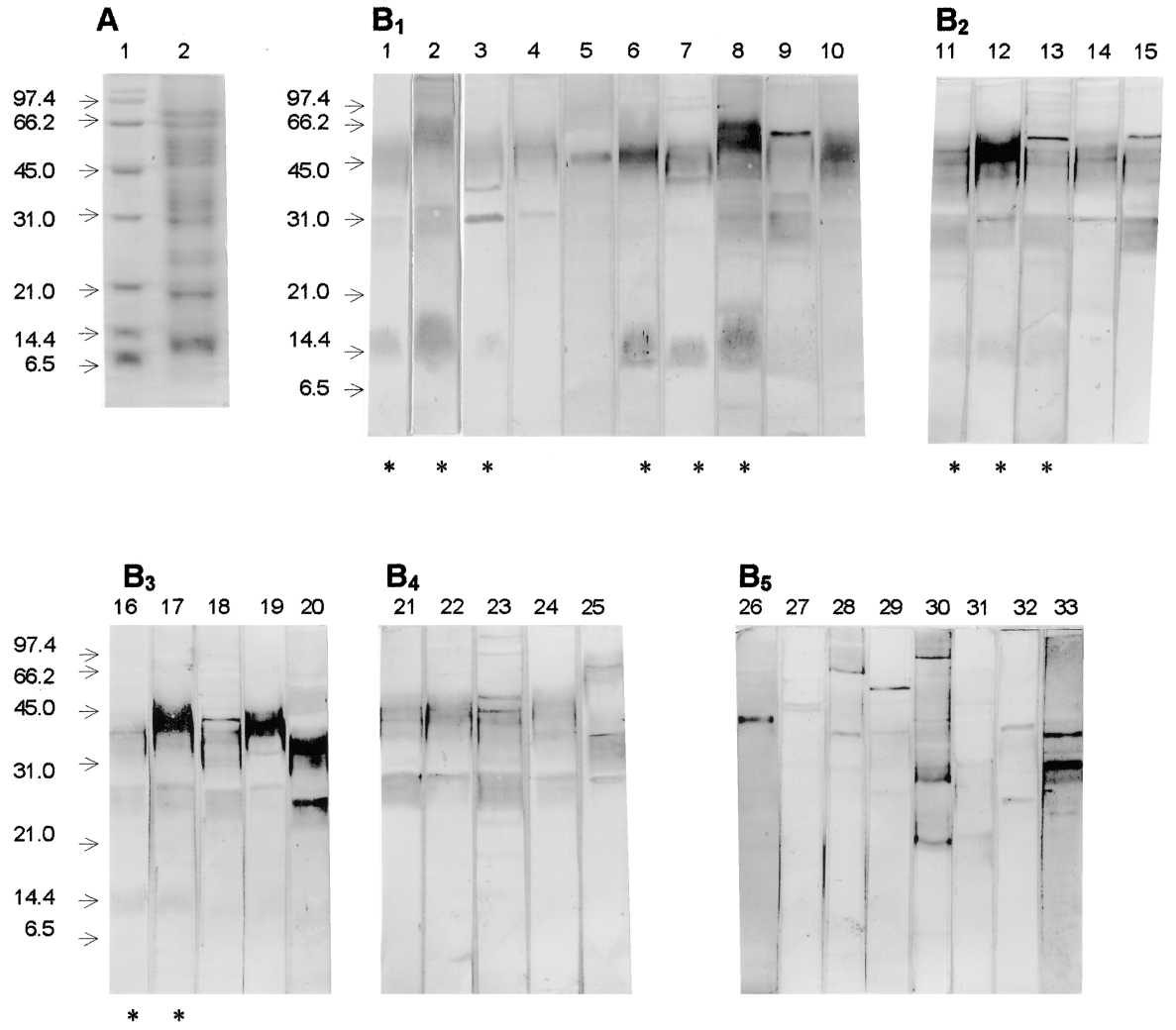

Figure 3 SDS-PAGE and Western blot with *M. tuberculosis* culture filtrate. A. Coomassie staining. Lane 1: molecular mass markers; Lane 2: mycobacterial culture filtrate. B. Antibodies to *M. tuberculosis* culture filtrate in serum from tuberculous patients at different stages of development of the disease and from control subjects as determined by Western blot. B1: Lanes 1–5, serum from newly acquired TB; Lanes 6–10, serum from relapse patients. B2: Lanes 11–15, serum from TB patients after 6 months of treatment. B3: Lanes 16–20, serum from inactive, treated patients 1 year after chemotherapy. B4: Lanes 21–25, serum from inactive, treated patients 2 years after chemotherapy. B5: Lanes 26–33, serum from control subjects. Lanes 1, 11, 16, 21 and 2, 12, 17, 22, show the reaction with serum from the same patient obtained at various times after the beginning of treatment. Molecular weights are indicated by arrows. * recognition of the 14 kDa region band.

years after chemotherapy and that from old TB subjects gave no reaction in the 14 kDa region (data not shown). When serum from the 12 relapse TB patients was analysed, it was found that eight (66.7%) samples showed reactivity at the 14 kDa region.

DISCUSSION

Antigens may occur in culture fluids due to active secretion from mycobacterial cells or because of release of cytoplasmic proteins into culture medium after bacterial lysis. Secreted antigens are likely to provide the first stimulus in vivo for humoral and cellular response to mycobacteria, and thus may be more valuable in a serological test than *M. tuberculosis* antigens derived from dead bacilli.

It has already been shown that serum from patients with relapse TB had higher concentrations of antibodies (native culture filtrate of *M. tuberculosis* H37Rv and lipoarabinomannan) than serum from patients with newly acquired TB.[9,10] Our data confirm the results of these previous studies as far as culture filtrate antigens are concerned; the antibody levels from relapse TB patients, whether considered as means or as frequencies of positivity, were higher than those found in newly acquired TB.

Levels of antibody to culture filtrate antigens were elevated at the beginning of treatment in 26 (61.9%) of 42 untreated TB patients. As has been observed by others,[2,3] these levels rose after a few months of well-conducted treatment, allowing, in many cases, retrospective confirmation of the diagnosis. Several mech-

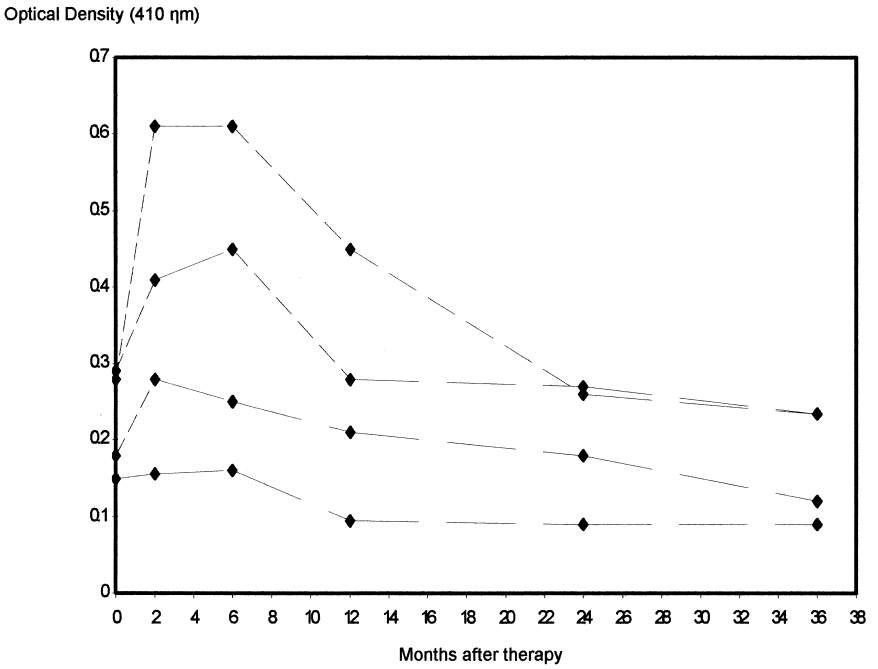

Figure 4 Changes in levels of antibody to culture filtrate antigens during and after anti-tuberculosis chemotherapy of patients with active pulmonary TB.

anisms can explain this phenomenon, such as intense stimulation of the humoral response by antigens released from killed bacteria combined with disinhibition of the immune system, and/or the disappearance of circulating mycobacterial antigens so that specific antibodies are no longer trapped in immune complexes. In contrast with these results are the findings from six patients (one of whom is represented in Figure 4) in whom the initial low antibody level showed no significant changes during the 6-month treatment period in spite of successful treatment. Several interpretations have been proposed to explain these findings: generalised immune suppression, immune complex formation, and specific inhibition of lymphocyte subsets.[11]

It has been reported that both anti-A60[4] and anti-cord factor[2] antibody levels persist for some years after treatment. In our experience, the levels of culture filtrate antibodies diminished progressively after the end of chemotherapy, reaching the same level as that of the non-tuberculous controls after 3 years, thus inhibiting discrimination between current (early relapse) and old disease during this period.

In an attempt to identify those antigens that give information about disease activity, we studied the immune response against culture filtrate antigens by Western blot technique, using serum from patients at different stages of the disease, and found relatively high levels of antibodies to tubercle bacilli in control subjects, as reported by others.[12,13] In agreement with Verbon et al.,[14] differences between patients with active TB and controls were found in the area below 20 kDa. It can be assumed that epitopes recognised on mycobacterial antigens in Western blot by serum from both control subjects and patients with tuberculosis are ubiquitous epitopes, also present on normal commensal bacteria.

When analysing the reactivity of serum according to the evolutionary patterns of the disease, we found that serum from active TB patients (newly acquired or relapse) showed a high level of antibodies directed against the 14 kDa area. These antibodies progressively decreased in recovering and cured patients, suggesting that this antigen might induce a humoral response that evolves in relation with disease activity. Bothamley et al.[15] found that patients who developed a positive sputum culture during treatment showed a significant increase in antibody titres to epitope TB68 of the 14 kDa antigen when compared to titres obtained when the subject's sputum had been negative on sputum culture.

Contrasting with our results, an earlier study using a similar approach, but with *M. tuberculosis* sonicate as antigen,[16] reported that serum from recovering and cured patients showed a high level of antibodies against a 13 kDa molecule; these authors systematically showed the disappearance of these antibodies during relapse or at acute stages of tuberculosis, suggesting the involvement of this molecule in the control process of the disease. A difference in the composition of molecules in the antigen used is likely to be a significant variable contributing to these disparate observations. Verbon et al.[14] reported that some antigens present in sonicate were not detectable in culture supernatant. Although this factor would aid in explaining the lack of reactivity observed with mole-

cules below 20 kDa in cured patients in this study, the determinants associated with the absence of reaction in the area below 20 kDa in active TB patients, reported by Patarroyo et al.,[16] remain to be elucidated.

The results of this study are promising. Measurement of antibodies to culture filtrate antigens of molecular weight <20 kDa might be useful in providing information about disease activity. It may be useful to purify the 14 kDa region band in order to quantitatively follow up the disappearance of antibodies to the 14 kDa region antigen. This could allow a better discrimination between active and healed tuberculosis.

References

1 Bothamley G H. Serological diagnosis of tuberculosis. Eur Respir J 1995; 8: 676–688.

2 Maekura R, Makagawa M, Nakamura Y, et al. Clinical evaluation of rapid serodiagnosis of pulmonary tuberculosis by ELISA with cord factor (Trehalose-6, 6'-dimycolate) as antigen purified from *Mycobacterium tuberculosis*. Am Rev Respir Dis 1993; 148: 997–1001.

3 Drowart A, Huygen K, De Bruyn J, Yernault J C, Farber C M, Van Vooren J P. Antibody levels to whole culture filtrate antigens and to purified P32 during treatment of smear-positive tuberculosis. Chest 1991; 100: 685–687.

4 Fadda G, Grillo R, Ginesu F, Santoru L, Zanetti S, Dettori G. Serodiagnosis and follow up of patients with pulmonary tuberculosis by enzyme-linked immunosorbent assay. Eur J Epidemiol 1992; 8: 81–87.

5 Barrera L, Ritacco V, Eisele C, et al. Evaluación del enzimoinmunoensayo para el diagnóstico rápido de tuberculosis paucibacilar del adulto. Medicina (Buenos Aires) 1989; 49: 561–566.

6 Centro Panamericano de Zoonosis (OPS/OMS). Preparación y estandarización de tuberculinas PPD, Nota Técnica N° 17 (Rev. 1). Buenos Aires: Ramos Mejía, 1980.

7 Laemmli U K. Cleavage of structural proteins during the assembly of the head of bacteriophage T4. Nature 1970; 227: 680–685.

8 Towbin H, Staehelin T, Gordon G. Electrophoresis transfer of proteins from polyacrylamide gel to nitrocellulose sheets: procedure and some applications. Proc Natl Acad Med Sci USA 1979; 76: 4350–4354.

9 Kaplan M H, Chase M W. Antibodies to mycobacteria in human tuberculosis. I. Development of antibodies before and after antimicrobial therapy. J Infect Dis 1980; 142: 825–834.

10 Julian E, Matas L, Ausina V, Luquin M. Detection of lipoarabinomannan antibodies in patients with newly acquired tuberculosis and patients with relapse tuberculosis. J Clin Microbiol 1997; 35: 2663–2664.

11 Grange J M. The humoral immune response in tuberculosis: its nature, biological role and diagnostic usefulness. Adv Tuberc Res 1984; 21: 1–78.

12 Bardana E J, McClatchy J K, Farr R S, Minden P. Universal occurrence of antibodies to tubercle bacilli in sera from non tuberculous and tuberculous individuals. Clin Exp Immunol 1973; 13: 65–77.

13 Turneer M, Van Vooren J P, Nyabenda J, et al. The humoral immune response after BCG vaccination in humans: consequences for the serodiagnosis of tuberculosis. Eur Respir J 1988; 1: 589–593.

14 Verbon A, Kuijper S, Jansen H M, Speelman P, Kolk A. Antigens in culture supernatant of *Mycobacterium tuberculosis*: epitopes defined by monoclonal and human antibodies. J Gen Microbiol 1990; 136: 955–964.

15 Bothamley G H, Rudd R, Festenstein F, Ivanyi J. Clinical value of the measurement of *Mycobacterium tuberculosis* specific antibody in pulmonary tuberculosis. Thorax 1992; 47: 270–275.

16 Patarroyo M E, Parra C A, Pinilla C, et al. Immunogenic synthetic peptides against mycobacteria of potential immunodiagnostic and immunoprophylactic value. Lep Rev 1986; 57: 163–168.

RÉSUMÉ

CADRE : Beaucoup d'auteurs ont démontré des titres croissants d'anticorps antimycobactériens après quelques mois de traitement antituberculeux. Cette réponse humorale pourrait persister pendant des années, ce qui rend difficile la discrimination entre une maladie en cours ou ancienne.

OBJECTIF : Caractériser la réponse humorale à des filtrats de culture de *Mycobacterium tuberculosis* avant et après traitement chez les patients tuberculeux et tenter d'identifier les antigènes qui pourraient fournir des informations au sujet de l'activité de la maladie.

MÉTHODES : La réponse antimycobactérienne en IgG a été déterminée pendant et après traitement chez les patients tuberculeux au moyen de l'ELISA et de l'immunoblot. On a prélevé le sérum chez 71 patients atteints de tuberculose active (59 nouveaux cas et 12 rechutes), chez 15 anciens tuberculeux et chez 45 sujets-contrôle non tuberculeux.

RÉSULTATS : Par la méthode ELISA, la réponse en anticorps a augmenté après une période de traitement de 2 mois. Une fois que cette antibiothérapie a été terminée, la quantité estimée d'anticorps est restée au même niveau. Le niveau d'anticorps spécifiques observé chez les patients 3 ans après le début du traitement rejoint celui des sujets-contrôle. Dans la technique du Western blot, quoique le sérum de chaque patient ait ses propres caractéristiques de type de bande, les différences entre les patients tuberculeux et les sujets-contrôle ont été observées dans la zone en dessous de 20 kDa. Les sérums de patients tuberculeux montrent des taux élevés d'anticorps dans la région de 14 kDa. Après le début du traitement, l'intensité de la bande de la région de 14kDa et le pourcentage de positivité tendent à diminuer. Dès lors, après une période de traitement d'un an, sept seulement des 13 cas chez qui l'ELISA avait démontré des anticorps antimycobactériens montraient encore une réaction légère mais toujours positive dans la région de 14kDa et cette réactivité disparaissait 2 ans après le début de la chimiothérapie.

CONCLUSIONS : L'antigène de la région 14kDa semble induire une réponse humorale qui évolue en relation avec l'activité de la maladie.

RESUMEN

MARCO DE REFERENCIA: Después de algunos meses de tratamiento antituberculoso, varios autores han evidenciado que el título de anticuerpos anti-micobacterianos se incrementa. Esta respuesta humoral podría persistir durante años, dificultando así la discriminación entre enfermedad actual y pasada.

OBJETIVO: Caracterizar la respuesta humoral frente a un filtrado de cultivo de *Mycobacterium tuberculosis* antes y después del tratamiento de pacientes con tuberculosis, con el fin de identificar antígenos que proporcionen información acerca de la actividad de la enfermedad.

MÉTODOS: Utilizando las técnicas de ELISA e inmunoblot, se estudió la respuesta sérica IgG anti-micobacteriana durante y después del tratamiento de pacientes con tuberculosis. Las muestras de suero fueron recolectadas a partir de 71 pacientes con tuberculosis activa (59 con tuberculosis recientemente adquirida y 12 reactivaciones), 15 pacientes con tuberculosis antigua y 45 sujetos controles con enfermedades diferentes a tuberculosis.

RESULTADOS: Utilizando la técnica ELISA, se evidenció que luego de dos meses de tratamiento la respuesta de anticuerpos se incrementaba. Después de finalizada la quimioterapia, la cantidad estimada de anticuerpos se mantuvo al mismo nivel. Recién luego de 3 años de iniciado el tratamiento, las muestras de los pacientes con tuberculosis alcanzaron un nivel de anticuerpos específicos similar al obtenido en las muestras de los pacientes controles. Mediante la técnica de Western blot, aun cuando cada paciente evidenció un patrón de bandas característico, se observó que entre los pacientes con tuberculosis activa y los controles existían diferencias en el patrón de reconocimiento en el área por debajo de 20 kDa. Los sueros de los pacientes con tuberculosis activa evidenciaron altos niveles de anticuerpos hacia la región de 14 kDa. Después del inicio de la terapia, la intensidad de la banda de 14 kDa y el porcentaje de reconocimiento de esta región tendieron a disminuir. Así, luego de un año de iniciado el tratamiento antituberculoso, sólo siete de los 13 casos que habían resultado positivos por ELISA revelaron una reacción débil pero aún positiva en la región de 14 kDa y esta reactividad desapareció después de 2 años de iniciada la quimioterapia.

CONCLUSIONES: El antígeno de la región de 14 kDa parece inducir una respuesta humoral que se desenvuelve en relación con la actividad de la enfermedad.