HUMORAL IMMUNE RESPONSE AGAINST MYCOBACTERIAL ANTIGENS IN BRONCHOALVEOLAR FLUID FROM TUBERCULOSIS PATIENTS

Resistance to tuberculosis (TB) is cell-mediated but a humoral response is common and may be correlated with the lack of effective local cellular defense mechanisms. The goal of the study was to evaluate IgG, IgA, and IgM-mediated humoral immune response against 38-kDa+16-kDa and 38-kDa+lipoarabinomannan (LAM) mycobacterial antigens in bronchoalveolar fluid (BALF) from patients with pulmonary TB. Non-tuberculosis (NTB) patients were used as control. 179 BALF samples (56 TB and 123 NTB) were examined. Commercially available ELISA-based assays against proteins 38-kDa and 16-kDa or 38-kDa plus LAM were used. Three different dilutions of BALF: 1:1; 1:10, and 1:50 (100) were tested. Only the results obtained with the 1:10 dilution allowed distinguishing TB and NTB groups. The mean IgG level for 38-kDa+LAM was significantly higher in the TB than that in the NTB group (P<0.0001). The mean IgA level for 38-kDa+LAM also was higher in the TB group (P<0.05). No difference was observed between TB and NTB groups in the titer of IgM antibodies. These findings indicate that TB is associated with the presence of detectable levels of antibodies in BALF. The antibody response is highly heterogeneous. This phenomenon results from the balance between pathogen and host immune system. The tests examined for detection of IgG in BALF can be used in combination with other diagnostic methods to increase diagnostic accuracy of pulmonary TB.

Key words: bronchoalveolar lavage, humoral immune response, tuberculosis
INTRODUCTION

Tuberculosis (TB) is a major health problem throughout the world causing three million of deaths annually. TB is spread from person to person by aerosolized Mycobacterium tuberculosis. The majority of infected individuals readily control primary infection that does not progress to clinical tuberculosis (1). Cell-mediated immunity in the lung, mediated by T cells and macrophages, controls infection. Granulomas are the pathological hallmark for this protective immune response (2-4). Recruitment and activation of T cells is critical for the protective immunity to M. tuberculosis (2-4). Other components of the immune response, such as natural resistance-associated macrophage protein (Nramp), neutrophils, natural killer cells, and antibodies may also contribute to the immune resistance to TB (2-5). Although the immunopathology of TB is largely regulated by the cell-mediated immune response, the knowledge of the humoral immune response at various stages of infection and in different compartments of the body may help us to elucidate the complex interaction between pathogen and host (6, 7). A better understanding of immune mechanisms in the lung microenvironment facilitates the understanding of the basic biology of host defenses to M. tuberculosis and also vaccine development, immunotherapy, and efforts to identify surrogate markers for protective immunity (2-4, 6). The aim of the present study was to evaluate the antimycobacterial antibody level in BALF from patients with pulmonary TB and compare it with those in other lung pathologies.

MATERIAL AND METHODS

Subjects

The study was performed on Caucasian subjects who were treated at the National Institute of Tuberculosis and Lung Diseases in Warsaw. All subjects gave informed consent and the project was approved by a local Ethics Committee. All subjects were HIV negative and previously BCG vaccinated. 179 BALF samples were examined from 56 pulmonary TB, 74 non-TB (NTB) infections of respiratory tract, 19 lung cancer, 14 sarcoidosis, 14 MOTT patients, and 2 healthy volunteers. All TB cases were confirmed by microbiological methods. The examination of acid-fast bacilli was made using the Ziehl-Neelsen stain and culture on Löwenstein-Jensen solid egg-based medium or Bactec system. The diagnosis of sarcoidosis was confirmed by histological testing (evidence of non-caseating granulomas). None of patients was receiving antituberculosis or steroid treatment at the time of BALF collecting. Lung cancer was diagnosed on the basis of histopathological examination. NTB infections were diagnosed on the basis of clinical and radiological signs followed by culture results.

All patients underwent BAL under local anesthesia with the use of a flexible fiberoptic bronchoscope with approximately 200 ml of saline according to the procedure described previously (8). The BAL fluid was passed through a filtration gauze to remove mucus, was then centrifuged, and the supernatant stored at -40°C until the day of use.
**ELISA test**

Immmunoenzymatic tests to measure IgG antibodies against 38-kDa and 16-kDa recombinant mycobacterial antigen expressed in and purified from *E. coli* (Pathozyme Tb complex plus, Omega Diagnostics, Scotland) or IgG, IgA, and IgM antibodies against 38-kDa and LAM (MycoG, Myco A and Myco M Omega Diagnostics, Scotland) were used. The 38-kDa and 16-kDa proteins are recombinant proteins (9-11). 38-kDa is an immunodominant lipoprotein antigen, isolated as a component of antigen 5 by affinity chromatography, specific only for *M. tuberculosis* complex (9-11). 16-kDa antigen is immunodominant antigen related to family of low molecular weight heat shock proteins. This antigen contains epitopes of *M. tuberculosis* complex specific for B-cells (9). The tests are based on a solid double antibody sandwich ELISA. BALF samples, after dilution with saline 1:1, 1:10, 1:50 for Pathozyme plus and 1:1, 1:10, 1:100 for Myco tests, were added to microwells precoated with antigens. Dilutions 1:50 or 1:100 are recommended to detect antibodies in serum. As BALF contains less protein than the serum does, we decided to use the dilution 1:10 and also an undiluted sample. All samples were assayed in duplicates. In the positive case, antigen-antibody complex reacted with the peroxidase-labeled antihuman IgG (IgA or IgM) conjugate. Using H$_2$O$_2$/TMB as substrate, the enzymatic activity of peroxidase was measured at 450 nm with the use of automated reading system. IgG and IgA results were referred to the standard curve. IgM results were expressed as the optical density index and compared with the calculated cut-off level.

**Statistical analysis**

Statistical analysis was performed with the use of S-PLUS 2000 (10). Results are expressed as means ±SD. Results of antibody titers were compared with Wilcoxon's rank sum test. Statistical significance was accepted at a level of P<0.05.

**RESULTS**

IgG against 38-kDa+16-kDa and IgG, IgA, and IgM anti 38-kDa+LAM were detected in BALF. Comparisons of the antibody levels at the dilutions studied are shown in *Fig. 1*. The mean IgG level anti 38-kDa+16-kDa in dilution 1:1 was 773 ±154 U/ml in TB (n=49) and 506 ±70 U/ml in the control group (n=98). For dilution 1:10, the mean antibody level was 212 ±1 U/ml in TB and 21.5 ±1.6 U/ml in controls (P<0.05). For dilution 1:50, the calculated antibody levels were 94 ±14 U/ml and 67 ±5 U/ml, respectively.

For IgG against 38-kDa+LAM, the mean antibody levels for TB and control were the following: dilution 1:1, 234 ±63 U/ml (n=54) and 125 ±11 U/ml (n=112); dilution 1:10, 47.6 ±7 U/ml and 3.2 ±0.2 U/ml, respectively. The differences between TB and control were highly significant (P<0.0001). For the greatest dilutions, the calculated titers were 25 ±3.0 U/ml and 21±0.5 U/ml.

For the IgA class, the following results were obtained: dilution 1:1, 1091 ±140 U/ml (n=41) and 874 ±87 U/ml (n=89); dilution 1:10, 144 ±88 U/ml and 19 ±2.6 U/ml (P<0.05); and dilution 1:100, 62 ±15 U/ml and 47.5 ±4 U/ml for TB and control, respectively.

For the IgM class, the following optical density indices were obtained: dilution 1:1, 0.5 ±0.09 (n=41) and 0.28 ±0.02 (n=89); dilution 1:10, 0.08 ±0.008
DISCUSSION

Pathological changes in TB usually represent a local inflammatory reaction limited to one or few organs (12). Disseminated TB constitutes a low percentage of all cases. A local inflammatory reaction may not lead to systemic changes. A well known phenomenon in TB is compartmentalization of the immune response with local activation and systemic depression of immune reactions (13). This phenomenon is highlighted in sarcoidosis but it is also observed in a number of granulomatous diseases including TB (13, 14). The distribution of locally activated cells from periphery to the inflammation site may explain negative tuberculin reaction in some patients with TB (13). In some TB patients, the antimycobacterial antibody level may also be very low.

In the present study, a local immune response in the lungs of pulmonary TB patients was evaluated. In those cases in which the antibody response in serum is weak, local antibody productions in the lungs may reflect the disease activity. As the tests applied were standardized to measure the antibody level in sera, three different dilutions of BALF were performed. The analysis of the results indicates that the optimal BALF sample dilution was 1:10. This dilution is 5 or 10 times lower than that required for serum. The difference may stem from a lower protein level in BALF compared with serum. Only was the 1:10 dilution effective in

and 0.08 ±0.007; dilution 1:100, 0.06 ±0.001 and 0.006 ±0.0006 for TB and control, respectively. All differences here between the TB and control groups were insignificant (Fig.1).
showing up differences between the TB and control groups. An undiluted sample was unworkable to see the differences, being too concentrated. A very high optical density of undiluted control samples likely resulted from some background interference related to non-specific antigen-antibody reactions. On the other hand, dilution recommended for serum was too high in relation to the antibody level in the BALF sample. In this case, in both TB and control groups, the optical density values were very low, below the detection limit, and no difference between the groups could be noted. With the use of the optimal dilution, significant differences in the antibody level between TB and controls came to light in all examined tests, with the exception of IgM-based assay. An increased antimycobacterial antibody level in BALF from TB patients has also been observed by other authors (15, 16). Grubek-Jaworska et al (15) have examined IgG anti A60 in BALF. The authors found no difference between TB and sarcoidosis, lung cancer, or healthy volunteers. However, the examined groups were relatively small, the TB group consisted of 13 persons and all other groups were of 10 or fewer. These authors used undiluted BALF, which could be the cause of non-specifically increased optical density in the control groups. In the same patients, a significant difference in the antibody titer in sera diluted 1:100 was observed between the TB and control groups (15). Raja et al (16) have observed increased IgG and IgA antibody levels against antigen 5 in BALF. The main component of antigen 5 is antigen 38-kDa. BALF was undiluted. Those results are in line with our observations. Our experience suggests that the way of BALF immunoassay might be useful, but further studies are needed. Although an antibody-mediated immune response is thought to have a small influence on immunopathogenesis of TB, the antibody may play a local role in the bronchial tree (17). As the transbronchial spread of the disease is a relatively uncommon event, it is possible that the local humoral reaction may play some protective role in this process.

In conclusion, the findings of this study indicate that TB is associated with the presence of detectable levels of antibodies in BALF. The antibody response is highly heterogeneous resulting from the balance between pathogen and host immune system. The examined tests detecting IgG and IgA in BALF diluted 1:10 can be used in combination with other diagnostic methods to increase diagnostic accuracy of pulmonary TB.

REFERENCES


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