Iranian Red Crescent Medical Journal

ORIGINAL ARTICLE

Detection of Mycobacterial antigen and antibody in patients with tuberculosis and their association with therapy

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Abstract

Background: Little is known about changes in total antibodies occurring during the progression of tuberculosis or its treatment. Using passive and reverse passive hemagglutination methods, mycobacterial antibody and antigen were determined in sera of patients with acute smear-positive pulmonary tuberculosis.

Methods: Fifty-nine patients were studied in different groups according to the duration of treatment in various stages of disease. The first group that did not undergo treatment showed a very low level of antibody. The second group with less than 6 months of therapy had a mean antibody titer of about 1:70. The third group underwent therapy for more than 6 months and their mean antibody titer was about 1:970. The fourth group whose disease was under control and their treatments were discontinued, showed the highest level of antibodies (1:2760). **Results:** A significant difference was found between the average antibody titers and the duration of treatment and course of disease. No antigen was detected in any patients except in group 5.

Conclusion: A high antibody response seemed to occur after treatment when the bacteria were disrupted and their antigens released. Absence of antigen may be due to the formation of antigen-antibody complexes.

Keywords: Tuberculosis; PPD; Antituberculous antibody; Reverse passive hemagglutination

Introduction

Mycobacterial antigens and antibodies have been the subject of intensive studies by physicochemical and molecular techniques.[1] The protection against mycobacterial disease is due to cellular immune response although antibodies against various antigenic components are produced during the course of infection and used for the assessment of disease status and recovery.[2] In tuberculosis contrary to other infectious diseases, the humoral immune response is different in various stages of illness. Some investigators used a reverse passive hemagglutination assay (RPHA) and rabbit antimycobacterial antibody to detect

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circulating mycobacterial antigens in CSF. Passive hemagglutination (PHA) and RPHA tests are sensitive and economical immunoassays which can be used by less equipped medical diagnostic laboratories.[3-6] The present study utilized these methods to monitor mycobacterial antigen and antibody in sera of tuberculous patients, investigate their changes in different stages of chemotherapy and determine their relationship to the disease and recovery.

Material and Methods

Antigen preparation:

The culture of Bacillus Calmette Guerin (BCG) grown in Dubos media at 37°C for 7-14 days was centrifuged at 2000g for 10 minutes. The pellet was washed three times with sterile saline (0.85%). BCG suspensions containing $3.5x10^6$/mL and $3.7x10^6$/mL of bacteria were sonicated for 15 minutes (3-4 times) to achieve complete disruption of the cells. They were then used for coating of sheep erythrocytes and immunization of rabbits respectively.

Preparation of anti-mycobacterial serum:

Rabbits received subcutaneous injections of 4 mL of sonicated suspension of BCG in multiple sites. The injections were repeated every three weeks for 7 times. Sera were separated, pooled and absorbed with sheep erythrocytes to remove heterophile antibodies. The total immunoglobulins were extracted by ammonium sulfate precipitation method (40%) and its final concentration was determined by Lowry method and adjusted to 31.67 mg/mL.

Patients Sera:

A total of 59 serum samples were collected from six groups of patients and two control groups as follows:

1. Active pulmonary tuberculosis with positive sputa and less than 6 months of therapy; 2. Active pulmonary tuberculosis with positive sputum and more than 6 months of therapy; 3. Active tuberculosis with positive sputa and refractory to therapy; 4. Pulmonary tuberculosis with negative sputa and less than 6 months therapy; 5. Untreated pulmonary tuberculosis with positive sputa; 6. Pulmonary tuberculosis converted from positive to negative sputum following a complete course of therapy.

Controls:

1) 30 clinically healthy individuals with normal chest x-ray and positive PPD skin test, 2) 50 healthy subjects with negative PPD skin tests.

Adsorption and inactivation of sera:

All sera were inactivated by heating at 56'C for 30 minutes prior to adsorption with sheep erythrocytes (SRBC) to remove heterophyl antibodies and were stored at –20°C until used.[7]

Sheep red blood cells (SRBC):

Sheep blood was collected in Alsever solution (1:2.2 mL v/v) and used after 3 days of incubation at 4 ˚C. The cells were used for up to 2 weeks.[7]

PHA and RPHA tests:

PHA test was done using sensitized RBCs (treated with tannic acid, 1/20000)[8] which were coated with prepared antigen (1/50) and PPD (1/60) using patients, and control sera.[9] RPHA test was then performed on the same sera as follows. RBCs were treated with trypsin (0.25%) for 30 min at 37 ˚C. Fetal bovine serum (40%) or soybean (0.025%) was used for 10 min (25 ˚C) to neutralize residual trypsin. The RBCs thus treated were washed and

Table 1: The mean titer of anti-BCG and PPD in pulmonary tuberculosis patients

Mean Ab titer	Patient groups					
	*<6 month therapy	*>6 month therapy	* Drug Resistant	+ <6 month therapy	*Before therapy	*Complete therapy
Against BCG	1:64	1:1028	1:64	1:1024	1:32	1:4096
Against PPD	1:32	1:512	1:32	1:512	1:8	1:1024

*Sputum Positive Pulmonary Tuberculosis Patients, +Sputum Negative Pulmonary Tuberculosis Patients

coated with prepared antibody by chromic chloride (1/4500), packed and used for tests.[10-12]

Results

Indirect methods of passive and reverse passive hemagglutination were performed on 59 tuberculous patients and 80 negative control groups, to detect mycobacterial antigen and antibodies. As shown in Table 1, group 1 contained moderate level of antimycobacterial antibody with no detectable mycobacterial antigen (Table 2). High titers of mycobacterial antibodies were found in Group 2, without any detectable antigen. Low titers of antimy-

Table 2: Mycobacterial antigen (Ag) and antibody (Ab) titers in patients with pulmonary tuberculosis and positive sputa

No	Less than 6 month therapy (Group 1)			More than 6 month therapy(Group 2)		
	Ab titer against BCG	Ab titer against PPD	Ag titer	Ab titer against BCG	Ab titer against PPD	Ag titer
1	1:512	1:256	-	1:4096	1:1024	-
2	1:16	1:8	-	1:512	1:256	-
3	1:8	1:8	-	1:8192	1:4096	-
4	1:128	1:32	-	1:4096	1:1024	-
5	1:512	1:256	-	1:256	1:256	-
6	1:8	1:4	-	1:2048	1:512	-
7	1:16	1:4	-	1:8192	1:4096	-
8	1:128	1:32	-	1:256	1:128	-
9	1:512	1:512	-	1:32	1:32	-
10	1:128	1:64	-	1:16	1:8	-
11	1:32	1:8	-	1:8192	1:2048	-
12	1:128	1:64	-	1:2048	1:1024	-
13	1:1024	1:512	-	1:1024	1:512	-

Table 3: Mycobacterial antigen (Ag) and antibody (Ab) titers in patients with pulmonary tuberculosis and positive sputa

	Drug resistant Group 3			No therapy (new cases) Group 5			Completed treatment Group 6		
No	Ab titer against BCG	Ab titer against PPD	Ag titer	Ab titer against BCG	Ab titer against PPD	Ag titer	Ab titer against BCG	Ab titer against PPD	Ag titer
1	1:64	1:32	-	1:256	1:64	1:2	1:4096	1:2048	-
2	1:512	1:32	-	1:32	1:16	1:4	1:2048	1:2048	-
3	1:128	1:64	-	1:8	-	1:4	1:8192	1:4096	-
4	1:128	1:32	-	1:16	1:4	1:4	1:16384	1:4096	-
5	1:16	1:16	-	1:4	1:4	1:16	1:512	1:512	-
6	1:64	1:32	-	1:32	1:8	1:4	1:8192	1:2048	-
7	1:32	1:16	-	1:16	1:8	1:8	1:1024	1:512	-
8				1:8	1:4	1:8	1:2048	1:512	-
9				1:512	1:32	-	1:4096	1:1024	-
10				1:32	1:8	1:4			-
11				1:256	1:64	1:2			-

cobacterial antibody, in absence of antigen, were detected in group 3 who were resistant to treatment with 4 antituberculous drugs (Table 3). Group 4 comprised two patients (Table 4), who appeared normal with negative sputa, and were previously presented with pulmonary tuberculosis and received less than 6 months of therapy. They had antimycobacterial antibody with no detectable mycobacterial antigen. The patients in group 5 were new cases who did not receive any antituberculous treatment. Mycobacterial antigen was found in all but one patient in this group with an antibody titer of 1:512. The patients in group 6 received a complete course of therapy and their disease was under control. Except in one patient with moderate antibody titer, others showed high titers of antibody, with no detectable antigen (Table 3).

Table 4: Mycobacterial antigen (Ag) and antibody (Ab) titers in sputum negative pulmonary tuberculosis patients (group 4).

No	Ab titer against BCG	Ab titer against PPD	Ag titer
1	1:2048	1:512	-
2	1:1024	1:512	-
3	1:512	1:128	-
4	1:1024	1:128	-
5	1:2048	1:512	-
6	1:4096	1:1024	-
7	1:1024	1:512	-

Control group1 included 30 healthy individuals, according to their chest rays and clinical manifestations, with positive PPD skin test. An antibody titer of 1:8 was found in 23% of this group, with no detectable antigen. Control group 2 comprised 50 healthy subjects, with negative PPD skin test and undetectable antigen or antibody. According to the

results obtained, the BCG derived preparation was a better antigen and compared to PPD induced a higher antibody titer.

Discussion

This study investigated the presence of mycobacterial antigens and antibodies in tuberculous patients at different stages of treatment and disease progression.

Reports on the presence of mycobacterial antigen in sera are very few and are predominantly determined by ELISA. Using methods introduced during recent years, outstanding progress was made in the detection of mycobacterial antigens and their specific epitopes.[13-19] Moreover, changes in immune response, occurring after treatment were the subject of other investigations.[17,20] The indirect passive and reverse passive hemagglutination methods were comparable in sensitivity to those of radio-immunoassy and ELISA.[9] Furthermore, the detection of mycobacterial antigen by RPHA is the first to be reported herein. On the other hand, the detection of antimycobacterial antibodies in patients and control sera by indirect hemagglutination tests was also described in present investigation. In this regard, it was found that disrupted mycobacterial cell preparation could reveal present detect higher antibody titer than PPD. This was revealed by a comparison between antibody titers shown by SRBCs coated with sonicated suspention of mycobacterium bovis, used at 1:50 concentration, and PPD. On the other hand, the duration of treatment appears to be a crucial factor affecting antibody titers in patient's sera. Patients with active pulmonary tuberculousis and positive sputa and receiving treatment for less than 6 months showed moderate antibody titers. On the whole, maximum antibody titers were found in patient treated for more than 6 months (Table 2). It seems that induction of higher antibody titers in patients of Group 6 could be due to the disruption of mycobacteria and release of cryptic epitopes. In progressive tuberculosis, the antibody response was weak and found in approximately 36% of the patients. Examination of antibody titers in patients of different groups showed that chemotherapy led to the elevation of antibody titer. This was evident that considerable antibody titers were found in patients receiving therapy for more and less than 6 months. Furthermore, significant correlation was found between chemotherapy and elevation of antibody titer ($p<0.05$), which was concurrent with progressive recovery. An explanation for a weak antibody response in patients prior to the initiation of therapy may be due to stimulation of humoral antibody response by mycobacterial lipids surrounding the cell wall glycoproteins. The weak antibody response in a group of new patients, who have not yet received chemotherapy, might be due to interference with natural host defense mechanisms involved in the release of bacterial antigens.

Acknowledgement

We would like to thank the Shiraz Veterinary School for providing us with sheep blood and the Health Center of Fars Province for the supply of patients sera and Dr. D. Mehrabani at Center for Development of Clinical Research of Namazee Hospital for editorial assistance.

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