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The Response of Hibridomas Containing Antibodies Anti Adhesin Mycobacterium tuberculosis

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ABSTRACT

Tuberculosis is a major infectious disease in the world until recently, but efforts to develop diagnostic tests based on antigen-antibody on antigen-antibody reaction to the present results are not encouraging. The purpose of this study was to develop antibodies adhesin Pili isolated from sputum of tuberculosis patients through hybridoma fusion. In this study, Mycobacterium tuberculosis (Mtb) Pili as an antigen and IgG antibody as secondary antibody for the diagnosis of Tb. Research method is Pili isolated with ultrahigh centrifugation at 120,000 rpm in a stratified at 4°C and cuts with pili cuter (omnimixer). Molecular weight determined by electrophoresis, SDS Page (Sodium Dodecyl Sulfate polyacrylamide gel electrophoresis), Electroelution and produce antibodies by fusion between lymphocyte b cell and hybridoma cells that have been induced by adhesion protein pili of Mtb. The study show that pili adhesion proteins of Mtb as a hemagglutinin molecule 38 kDa is used for process production of monoclonal antibodies with form hybrid cells. Results of antibody testing produced from hybridoma cells showed that hybrid cell that contain protein of Mtb 38 kDa cross-reacted with the test sample of 96 sputum samples of TB patients using dot blot, ELISA and western blott. The conclusion is hybrid cell on the culture can results medium culture that reaction with protein adhesion pili of 38 kDa as epitope that binding with antibody that produced by hybridoma cells.

Key words: adhesin, antibody, pili, Hibridoma response, M.tuberculosis

Introduction

Tuberculosis (TB) is a major public health problem. The number of tuberculosis patients in Indonesia is the 3rd largest in the world after India and China with the number of patients approximately 10% of the total number of TB patients worldwide. It is estimated that in 2004, every year there are 539,000 new cases and 101,000 deaths of people. The incidences of smear positive TB cases were around 110 per 100,000 populations (Aditama T Y. 2006.). The numbers of TB cases were found increased significantly in recent years. In the province of DKI Jakarta in 2003 the TB cure rate is still below the national target (<85%) (Firdaus,Umar. 2007).

Tuberculosis is caused by the bacterial pathogen Mycobacterium tuberculosis complex (TBC) consisting of M. tuberculosis (Mtb), M. africanum, and M. bovis. Including M. microti and M. canetti are rarely found. Differentiation of Mycobacterium tuberculosis complex is very important to do because it deals with the success of patient therapy and for epidemiological purposes, especially in areas where tuberculosis has become an epidemic and is a major health problem for humans (Paul W. 2003).

Germs that cause tuberculosis are rod-shaped slender straight or slightly bent with both ends rounded. Colony dry with cauliflower-shaped surface and yellow grow slowly even in optimal conditions. It is known that the optimal pH for growth is 6.8 to 8.0. To maintain virulence growing conditions must be maintained at a pH of 6.8, while stimulating the growth needed for carbon dioxide content of 5% - 10%. Generally the new colony appeared after culture was 14 - 28 days but usually have to wait until the age of 8 weeks (Sandjaja, B.).

Mtb is inhaled into the lungs and be ingested by alveolar macrophages. Macrophages then perform three important functions, namely: 1) produce proteolytic enzymes and other metabolites that have the effect microbacterisidal; 2) produce soluble mediators (cytokines) in response to Mtb in the form of IL-1, IL-6, TNF (tumor necrosis factor alpha), TGF (Transforming Growth Factor beta) and 3) process and present mycobacterial antigens to T lymphocytes Cytokines produced by macrophages have the potential to suppress the effects immunoregulator and cause clinical manifestations of tuberculosis. IL-1 is an endogenous pyrogen causing fever as a characteristic of tuberculosis. IL-6 would increase the production of immunoglobulin by B cells are activated, causing hyperglobulinemia which are often found in patients with tuberculosis. TGF to function together with IFN to increase the production of nitric oxide metabolites and kill bacteria and is required for the formation of granuloma to resolve mycobacterial infections. In addition, TNF can cause the pathogenesis of effects such as fever, weight loss and tissue necrosis that are characteristic of tuberculosis. In tuberculosis
patients, TNF also acts to enhance T cell susceptibility to apoptosis either spontaneously or by stimulation Mtb in vitro. IL-10 inhibits cytokine production by monocytes and lymphocytes, while TGF suppress T cell proliferation and inhibit effector functions of macrophages (Raja A. 2004).

Early diagnosis of Mtb infection is often based on clinical data, but to establish a definitive diagnosis required the isolation and identification of Mtb laboratory. There are limitations to this culture requires a long time and not all patients can get positive results, we need a diagnostic tool to detect active TB, especially in developing countries (Nugraha Yusak, 2007).

Many of Mycobacterial antigens have been identified either semi purified or purified and used as a commercial immune dominants kits such as antigen protein 16kDa, 24 kDa, 38 kDa, ESAT-6, but less sensitivity. 38 kDa proteins is a lipoprotein antigen immune dominant which is part 5 of the antigen with affinity chromatograhi specific only to Mtb complex (Senoi G., 2007).

On several studies, the using of Mtb antigen provides information for other diagnostic techniques. Detection using secretory IgA (mainly slgA2) is immunoglobulin on airway mucosa where IgA provides protection against harmful antigens, but IgA could also cause a systemic response through the role of cell surface receptors on macrophages and leukocytes. IgA mainly play a role in mucosal defense.

Therefore, this study we want to show the response protein adhesin pil Mtb 38 kDa in hibridomas cell for the development of monoclonal antibodies as diagnostic material.

Material and Methods

This research is experimental laboratory, including the local isolation of Mycobacterium tuberculosis antigens with the following activities: 1. Method to get the bacteria Mtb; 2. Staining Procedure Sputum smear Ziehl-Neelsen method; 3.Isolasi Pili Mtb; 4. By SDS Page electrophoresis method for the propagation of protein; 4. Hemagglutination test methods 5. Myeloma cell culture was fusion with spleen cells balb / c mice for production of hybridomas and monoclonal antibodies; 6. Measurement of hibridomas responses by immunochemistry technique.

Isolation M.tuberculosis Pili (MTP):

Initially, Mtb grown in 7H9-OADC-Tw at 37 °C with vibration for 48-72 hours. At the beginning of the culture used for inoculation of 100 7H11 agar plate, as the media, modified by the expenditure of OADC and incubated for 2 weeks as described above. Bacteria are harvested by using sterile glass spreader and diluted into 200 ml PBS. Dilution is divided into 25 ml aliquot, and pili are cut mechanically from the bacterial surface by vigorous vortexing for 5 minutes in 50 ml conical tubes containing 1 cc of sterile 3 mm glass beads. After cutting, the suspension of bacteria was centrifuged at 3000 rpm for 1 hour, and the resulting supernatant was collected. Bacterial pellets were washed with PBS to obtain more pili, and the supernatant collected. Residual bacterial cells and debris cleared by two centrifugation at 3000 rpm, followed by centrifugation at 18,000 rpm for 10 minutes. Contamination of fat has been discarded after mixing with the volume ratio of chloroform / methanol (2:1) for one hour. After centrifugation at 18,000 rpm for 30 minutes to the partition 35 ml centrifuge tube and liquid interphase fraction was collected carefully and extracted two more times, and soluble material (lipids) in an organic solvent removed. Finally, the liquid fraction, interphase ultracentrifuged by 120 000 rpm for 3 h at 4 ° C in the Ti-56 fixed angle rotor. The results of MTP-pellets have been resuspension in PBS and analyzed by 16% SDS-PAGE and Tricine-Page. The concentration of protein was determined by protein assay (Christoper, 2007).

**Sodium Dodecyl Sulfate Gel Polyacrylamid Electrophoresis (SDS-PAGE) and Electroelution:**

Monitoring was done using molecular weight SDS-PAGE method of Laemml (1970). Protein C for 5 minutes in a buffer solution containing 5% sample is heated 100 mM Tris HCL pH 6.8, 5% 2-mercapto ethanol, 2.5% w/v sodium dodecyl sulfate, 10% v/v glyserol with tracer color bromophenol blue. Selected 12.5% mini slab gel with the tracking gel 4%. 125 mV voltage used. As the material is coomassie brilliant blue color and low range sigma standard molecular marker.

Electrophoresis methods used to obtain protein band determined molecular weight. Protein band was measured molecular weight (kDa) and then cut one by one, pieces of protein bands in the gel and then inserted in a cellophane bag that had been preheated with 0.5 M EDTA and sterile PBS. Samples of proteins in gel pieces that are ready to put in a cellophane bag, entered by way of clamp on one end of the cellophane and then clamp the other end, and after wedged Electrophoresis instrument that is inserted in a horizontal mini protein in running buffer solution, then after completion included in the solution containing the dialysate solution sterile and dialisasa aquades overnight at 4 degrees celsius, and the next day the sample in the bag was replaced and dialysis in sterile PBS solvent and incubation was repeated in 4 degree temperatures during the night. Further
samples shall be appointed and do by using acetone precipitation overnight, and samples were collected were centrifuged at 10,000 rpm, the supernatant removed and added to Tris HCL plet as protein solvent, and concentration of protein samples prepared was measured by spectrophotometry.

**Hemagglutination test:**

Hemagglutination test as prescribed by Hanne and Finkelstein (1982). Dilution of the sample made at microplant V ½ concentration of each well 50 ul volume. Each well is added the red blood of mice at concentrations of 0.5% volume. Then, it was shook using a rotator plate for 1 minute. Subsequently, it placed at room temperature for 1 hour. The amount of titer was determined by observation of red blood agglutination at the lowest dilution. The samples tested were intact bacteria, pili proteins and erythrocyte cell. Type of red blood used is the blood of mice.

**Westernblotting examination method was against specific adhesion proteins M tb:**

Westernblotting was using the method of Towbin (1979). Protein bands on the electrophoresis gel transferred on paper using a semi-dry nitrocellulosa bioter (biorad) after the protein samples was carried out SDS-Page. The electric current of 300 mA used within 30 minutes, then, the stained by dye poncho TCA containing 2% to 3% to determine whether the protein sample has been moved to the paper nitrocellulose and marked to determine the molecular weight. Nitrocellulose paper cut according to the column sinks. To remove poncho color, it rinsed with dH2O. Next on, it blocked with TBE containing albumin 3% in TBE pH 7.4 plus 1% BSA, shook for 2 hours and washing again as much as 2 times within 5 min using TBE pH 7.4, containing 0.05% Tween 20. Further, it coupled secondary IgG antibody concentration 1 / 1000 in TBE pH 7.4 and BSA 1%, and protected against the influence of light. Shaked for 2 hours, then washing 2 times for 5 minutes using TBE pH 7.4 0.05% Tween 20. H2O dissolved in 10 ml. As a color material, it used Cip tablets. This solution was poured on the paper nitrocellulose and observed the occurrence of red color. If the reaction was occurred, it is washed with H2O and then dried with filter paper.

**Production of Antibodies from the hybridomas cell:**

Step in the isolation of monoclonal antibodies which will be prepared in this study, include the following activities: immunization of mice, preparation of lymphocytes, macrophages preparation, preparation of myeloma cells, fusion, Elisa, cloning, and antibody production.

**Immunization of mice:**

Immunization of mice refers to the method of Kosraviani. Receptor protein that has been purified by Electrophoresis as much as 0.2 ml (50 ug) emulsified with 0.2 ml of Freund's complete adjuvant, then injected in mice Balb/c aged range 8-10 weeks of female in a intra-peritoneal manner. 2nd Immunization was performed 14 days later with the same volume and manner the results of protein emulsified with Freund's incomplete adjuvant. Three days before fusion it was done injection without ajuvant in an intravenous manner, for 3 consecutive days. Prior to fusion mice were taken to see the production of serum antibodies using Elisa. Negative control mice used Balb / c which not immunized with same both age and sex.

**Preparation of lymphocytes:**

Mice that had been immunized were taken to measure serum antibody production. Positive mice produce antibodies that contain anti-protein adhesion or anti-receptor proteins as shown by the existence of barriers to the process of cell adhesion in macrophages, reduced the population by taking blood erythrocyte nya through orbital plexus approximately 30 minutes before eutination. Mice disinfected using 70% alcohol before placed in a sterile cabinet. Lien taken cleaned from surrounding tissues, if it looks clean, it done spray a few times in the spleen tissue using a solution of RPMI medium until spleen tissues appear transparent. The liquid spray results are expected to contain many lymphocytes. The liquid is then performed by centrifugation at 1000 rpm for 10 minutes at room temperature. Supernatant discarded RPMI media plus to taste, to be computed set of lymphocytes in the room counters.

**Preparation of macrophages:**

Mice Balb / c which not immunized, was intraperitoneally with RPMI medium as much as 5 ml. Stomach of mice was shaked gently to obtain macrophages in peritonelium. The fluid in the peritoneal cavity is taken
which is then performed by centrifugation at 1000 rpm for 10 minutes at room temperature, then performed macrophage cell count in the counter room.

**Myeloma cell:**

Prior to fusion, the myeloma cells were prepared by growing cells using growth media (RPMI + 10% FBS). Penicillin, streptomycin and fungizone added to the media to avoid bacterial and fungal contamination. For myeloma cells prepared for cell fusion is growing at logarithmic phase. Tues myeloma obtained centrifuged at 1000 rpm for 10 minutes at room temperature. Deposition results centrifugation grower media added to taste which then was counted in computing rooms.

Lymphocyte cells and myeloma cells are mixed with a ratio 1: 2. The result of the mixture centrifuged at 1000 rpm, for 10 minutes at room temperature using the RPMI media without serum. Tube of centrifugation results was taken, then tap on the tube wall a few times so that the cells become loose sediment. From the edge of the tube were added 1 ml of PEG (poly ethylen glycol) 6000 with a concentration of 45% at warmer temperatures 45 °C dripped slowly through the tube wall which require an interval not exceeding 3 minutes. Subsequently the tube was shaked in a water bath for 1 minute at 37 °C. Then through the wall of added into the tube with PBS at 37 °C in slowly. The tube with a volume as follows in this order: 1 ml within 60 seconds, 3 ml in 60 seconds and 16 ml in 60 seconds. Subsequently the tube was centrifuged at 1000 rpm for 10 minutes at room temperature. Supernatant was sucked slowly and discarded, while the sediment was washed with RPMI medium by centrifugation at 1000 rpm for 10 minutes at room temperature. Supernatant discarded, the sediment plus HAT media (hyposantine aminopterine thymidine) at sufficiently, then calculated using counters and made room in which the suspension every 100ul cells contain 10,000 to 20,000 cell. In each well plate filled with 100 μl titermikro cell suspension and in each well were added in 1500 macrophage cells. Cell incubated at 37 ° C using a pressure of 5% C02. After hybridoma cells grow, the medium was replaced with fancier medium (RPMI + 20% FBS). With the change in the culture medium color become yellow, then the supernatant was taken for testing the power constraints on the ability of the bacterium Mtbb in macrophage cells that had been prepared.

**Cloning hybridomas:**

Positive hybridomas that produce antibodies, which were grown in titermikro plate contains 24 wells, using a cultivation medium. Hybridomas that have been grown in the microtiter plate antibody production were tested against the ability to inhibit bacterial adhesion in epithelial cells of grouper that had been prepared. Test method was same as in the method of experimentation stage. Good Hybridomas which produce the desired antibody was cloned. Hybrid cell that grows in each microtiter plate using a room computing diluted. Dilution was made 3 times, with an estimated per 100 ul containing 10 cells, 5 cells, and 1 cell. Cloning performed on 96 micro titer wells plate. Selection is done to see the growth of hybridomas that actually originated from one cell to see the production of antibodies. Recloning conducted to obtain a homogeneous colony.

**Production of Antibodies from the cell hybridoma:**

Hybridoma clones producing monoclonal antibodies were grown in 75 cm2 grower tube, using a medium-raiser. Antibodies with high titers obtained by making a ascites in mice Balb/c. Mice were injected intraperitoneally 0.5 ml pristan. Giving pristane repeated on day 6 after the first injection. Hybridoma cell is injected on the day of the 6th day after second administration pristan with a dose of 5 x 107 cells. Further purification of antibodies was performed.

**Antibody Characterization:**

*The determination of the cell hybridoma specificity and sensitivity of Tb Patients by dot blotting:*

Nitrocellulose membrane (NC) cut 7.5 x 11 cm and first soaked in sterile H2O for 30 minutes. Then it mounted on the dotblot tool. Through hole device, the membrane that has been moistened with TBS, drops by 50 mL antigen (in tris buffered saline pH 7.4), incubated overnight at 4°C until completely absorbed antigen into NC membrane. Then performed blocking with blocking buffer TBS (containing 50 mM Tris base, 0.2 M NaCl, 0.5% skim milk pH 7.4), incubated overnight at 4°C, blocking solution was discarded. The next stage in the membrane drops sputum was tested by 50 mL, incubated for two hours at room temperature and placed on a shaker. Solution was discarded, and then washed three times with TBS-0.05% Tween-20. In-add anti-mouse secondary Ab with 1:2500 dilution in tris saline solution, incubated at room temperature for one hour, on a shaker. Washed again three times with TBS-0.05% Tween-20, then incubated at room temperature for 30”. The reaction was stopped by adding H2O.
**Immunoglobulin Isolation:**

Immunoglobulin (antibody) was isolated from the sputum of patients suspected of being infected Mtb. Sputum of patients added to PBS containing 4 mM PMSF and 0.02% NaN3 with a ratio of 1:1, vortex until homogeneous and then centrifuged 3000 rpm, 15 min, 4°C. Pellets that were removed form. Supernatant was done pre-sipitasi with cold absolute ethanol, 4°C overnight, then centrifuged 12 000 rpm, 15 min, 4°C. Formed supernatant discarded. Pellets stored in Buffer 0.5 M Tris-Cl, pH 6.8,-40°C.

**Labeling antibodies from hybrid cells with secondary anti-mouse antibody conjugate biotin using SEM:**

Labeling of antibodies from hybridoma cells generated do antigen detection test for Mtb Immunocytochemistry on head squash (smear of cells / tissue). Head squash preparations (smears of cells / tissue) were fixed with cold methanol, 3-5 minutes, then poured peroxidase blocking solution (H2O2) 10 minutes. Given prediluted blocking serum (protein blocker) 15 minutes, then dried, and incubated with primary antibody (monoclonal antibody positive hybridoma cells) overnight at 40°C. Further washing with PBS (phosphate buffered saline) 3x fresh each 2 minutes then dry with paper towels immediately pour into the glass objects on paper towels. Incubated again with Biotinylated universal secondary antibody (anti-mouse secondary antibody) 20 minutes at room temperature, then wash with fresh PBS 3x each 2 minutes then drain, drops by Trekavidin-HRP 10 min. Wash with fresh PBS 3x each 2 minutes then drain for 3 minutes. Prepare betazoid DAB chromogen solution by mixing 1 mL of DAB chromogen with 100 mL substrate buffer and then dropped into the preparations for 5 minutes washing under tap water, then dry for 3 minutes. Used as drops of Mayer counterstain hematoxilen 1 minute as the blue core. Washing under tap water, then dehydrated with 100% ethanol once, clearing the Xylen or Xylol once. Used as drops of entelan as mounting media, cover with cover slip, check under the microscope.

**Data Analysis:**

Data analysis was performed with a description of quantitative and qualitative data.

**Results:**

**M. Identification tuberculosis:**

Identification of bacteria Mtb as acid-resistant bacteria performed with acid-resistant staining Ziehl - Neelsen in sputum after homogenization and decontamination processes. Staining results seen rod-shaped bacteria (bacilli), red with a blue background. Preview colony Mtb such as Figure 1.
Fig. 1: (A) MTB culture, (b) The results of smear-positive staining is indicated by a black dash

Bacterial culture was further propagated to produce pili and adhesion proteins Mtb by electrophoresis SDS-page.

Electrophoresis SDS-page:

Profile protein of antigen Mtb emerging was calculated molecular weight by regression calculation of correlation compared with the markers. Protein band was determined reproduced by Electroelution for obtaining protein.

Fig. 2: Pili electropherogram stained with CBR-22

Results of haemaglutinin test:

Aims hemagglutination test is whether pili with receptors on red blood cells found on the surface of red blood cells can bind. This test also can identify optimal levels of pili that can react with the antibody on the surface of red blood cells that can agglutinate red blood cells. At this time haemaglutinin test study used blood cells of mice and adhesion proteins Mtb namely 38 kDa.

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<th>Titer</th>
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Based haemaglutinin test gives results that pili adhesin protein of Mtb 38 kDa to agglutinate blood cells (erythrocytes) mice to concentration dilution 1/512 as Table 1.

Examination western blotting specificity of adhesion proteins Mtb 38 kDa:

Examination western blotting performed to test whether pili adhesin protein 38 kDa M.tb sensitive and specific to antigens of Mtb contained in sputum of patients. This examination is carried out on M.tb pili adhesin protein 38 kDa with antibody anti-adhesion pili 38 kDa. Produced by serum of mice, the results of such Figure 3
Fig. 3: Response pili adhesin protein of Mtb 38 kDa with anti-mouse secondary antibody using technique of western blotting Preparation of Hybridomas cell

Production of monoclonal antibodies produced through a process of fusion between myeloma and lymphocytes b cells from spleen cells of mice that have been immunized with pili adhesion proteins M.tb. 38 kDa to form hybridoma cells. Hybridoma cells will be measured antibody titers produced by dot blot technique to determine the hybrid cell clones that have a high antibody titer. Furthermore, hybrid cell clones will be multiplied to produce ascites in mice. Ascites will be taken and tested its response as a monoclonal antibody produced. Preview myeloma cell culture and cell fusion to generate hybridoma cells for production of monoclonal antibodies as Figures 4.

Fig. 4: The fusion of myeloma cells and lymphocytes b cells from spleen balb/c

Measurement of antibody response produced by hybridoma cells by dot blot technique:

Dot blot test results carried out to test whether antibodies Pili Mtb generated is sensitive and specific to antigens of Mtb who reacted. The result of the specificity of antibodies to M. tb. 38 kDa from the hybrid cells with antigens of Mtb like Figure 5.

Fig. 5: Specificity of anti-adhesion antibody examination pili M.tb. 38 kDa with Mtb through blott dot technique.

Based on the examination with a dot blott showed that the antibodies produced by hybrid cells via cloning hybridoma clones of each of 14 clones gave a positive cross-reaction with compared to controls.

These results also indicate that hibridomas carrying antibody anti-adhesin pili M. tb. 38 kDa labeled with anti-mouse secondary antibody biotin conjugat in erythrocyte of mice induced by Mtb show cross-reaction between the antibodies produced and the secondary antibody.
Discussion:

Early diagnosis of infection of Mtb patients are often based on clinical data, but to establish a definitive diagnosis required the isolation and identification of Mtb in the laboratory. One of the diagnosis has been developed using a monoclonal antibody that is using the material pili or fimbriae adhesin protein of Mtb.

Further explained that the effort to develop diagnostic tests Mtb by antigen antibody reaction was started several decades ago, but the results are not encouraging, this is caused by these bacteria express a variety of different antigens at various stages of different diseases. So serodiagnosis of TB should focus on identifying antigens expressed in vivo when the disease becomes manifest and at the same antigen is recognized by the immune system.

An ideal serological test to diagnose various types of TB patients, for example with smear positive TB or TB with negative AFB. For this purpose a diagnostic test to use specific antigens and specific epitopes of the antigen that cross react with Mtb.

The increase in TB cases and deaths by an impact study in the development of diagnostics (Loddenkemper, D. Sagebiel and A. Brendel. 2002). For this purpose it is necessary to develop diagnostic materials based on specific local ingredients with zone transmission of TB. One of these is there any diagnostic material development of the adhesin molecule. Adhesion molecule is a component of bacteria that facilitate bacterial adhesion between cells and target cells in the host, referred to also as a factor of bacterial colonization that started a process of infection, the adhesion is located on the bacterial fimbriae or pili.

The discovery of diagnostic materials from many of them namely Mycobacterial antigens have been identified as protein 16kDa, 24 kDa, ESAT-6, but less sensitivity. In addition, 38 kDa protein is an anti-gene immune dominant lipoprotein which is part 5 of the antigen with affinity chromatography specific only to Mtb-complex.

25-50% of patients infected with MTB, raised and where bacterial infection lam mycobacteria replicate inside macrophages and intracellular immune response. Lymphocytes capable of antigen presenting cells that play a role for CD4 + immune response and DTH (delayed type hypersensitivity within 2-4 weeks at the beginning of infection. Next Interleukin (IL-12) will directly instruct T helper lymphocyte cell 1 (Th1) to secrete interferon (IFN)-γ, which has the potential to activate macrophage cells, which in turn will release chemokines such as interleukin 8 (IL-8), and almost all of which are proinflammatory cytokine tumor necrosis factor (TNF)-α, each of which facilitates the activation mononuclear cells (Lawn, S.D., et al., 2002).

The results showed that the protein adhesin pili isolated from Mtb 38 kDa protein is an immunogenic protein and haemaglutinin. Protein with 38 kDa has made its monoclonal antibody and the results give a cross reaction between antibodies produced by antigen Mtb are familiar. Similarly, the test results on blood cells (erythrocytes) of mice that have been induced by Mtb, showed cross-reaction between the monoclonal antibody produced by the antigen epitopes Mtb that infects the blood cells of mice with SEM examination.

Qualitatively, the results of this hibridomas response has also been demonstrated through an examination of the results of dot blott hybrid cell clones propagated through the ascites of mice that carry bacterial epitopes Mtb. Further development of these antibodies should be tested with various samples of tuberculosis from various different areas where there are M.tb so that could be developed as raw materials locally-based diagnostics for tuberculosis are generally based on shared epitope it recognizes.

In the future, this hibridomas response to pili adhesin protein of M.tb 38 kDa can be developed to produce monoclonal antibodies anti adhesin 38 kDa of bacterial pili.

Acknowledgments


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