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Detection of Tuberculous Lesion by Immunoscintigraphy Using Radiolabeled Specific Polyclonal Antibody Against M. bovis BCG in Rabbit: A Preliminary Result

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가토에서 방사면역 신티그래피를 이용한 결핵병변의 진단 : 예비보고

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결핵성 병변의 단순 x-ray 촬영이나 CT, MRI 소견은 매우 다양하며, 결핵과 전이암 혹은 원발성 암과 감별이 어려운 경우가 있어 결핵으로 확진하기 위하여서 조직 생검이나 수술 등 침습적인 진단 방법을 이용하여야 하였다. 그러므로 이러한 결핵 병변을 비 침습적인 방법으로 정확히 감별할 수 있는 방법을 연구하던 바, 결핵균에 대한 항체를 동위원소에 부착시켜 신티그래피로 진단할 수 있는지의 가능성을 동물실험을 통하여 알아보고자 하였다.

15마리의 가토에서 M.tuberculosis H37Rv를 슬관절에 주입시켜 결핵병변을 유발시키고, 대조군으로 2마리의 가토의 고환에 T.pallidum을 주입하여 매독병변을 유발시킨 후 M.bovis BCG에 대한 특이항체(specific polyclonal antibody)와 정상 가토의 immunoglobulin을 I-131에 부착시켜 각각의 가토에 주입하여 preset time 10분간 감마카메라로 주사한 결과 다음과같은 결과를 얻었다.

① 8마리의 결핵에 감염된 가토에 M.bovis BCG에 대한 $F(ab')_2$를 1 mCi의 I-131 labeling 시킨 후 주사한 결과 모두에서 주사후 2시간 부터 72시간까지 병소가 hot uptake으로 보였으며 주사후 24시간에 가장 높은 target/background ratio를 보였다.

② 2마리의 매독에 감염된 가토에서 anti-BCG $F(ab')_2$를 주사한 결과 2시간에서는 병소에 hot activity를 보였으나 24시간부터 급격히 activity가 감소하였다.

③ $F(ab')_2$ 대신에 intact antibody를 결핵에 감염된 가토에 투여한 결과 specific polyclonal antibody나 정상가토의 immunoglobulin 모두 결핵병소에 96시간까지 hot uptake를 보였다.

그러므로 결핵균에 대한 specific antibody fragment를 이용하면 방사면역 신티그램으로 진단이 가능하리라 사료되었고, intact antibody를 사용할 경우 sensitivity는 높으나 specificity는 적을 것으로 사료되었다.

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INTRODUCTION

Despite efforts in the past to control tuberculosis, it remains as an important medical problem worldwide. It has emerged as an area of the clinical and research interest due to increasing its incidence related to the epidemic of human immunodeficiency virus (HIV) infections.

The definite diagnosis of tuberculosis depends upon the isolation or odentificantion of the etiologic agent, M.tuberculosis, which is often protracted. In addition, a greater proportion of tuberculosis associated with HIV infection is extrapulmonary so that bacteriologic or pathologic confirmation is diffecult[1]. The standard imaging method such as roentgenograms, computerized tomograms, and magnetic resonance images are all useful for the diagnosis, although not pathognomonic. thus, a specific, noninvasive clinical test that can be performed to assess the presence and extent of tuberculous lesion is greatly needed.

There have been attempts to diagnose tuberculosis by immunoassay[2~6], however it was found to have a limited ability to localize and quantify the extent of inflammation.

This study was tested to assess its potential for specific targeting of the tuberculous lesion by immunoscintigraphy using radiolabeled rabbit polyclonal antibody specific for M.bovis BCG in preclinical animal model.

MATERLALS AND METHODS

1. Antibody Preparation and Labeling Procedures

Rabbit polyclonal antibody against M.bovis BCG cross-reacting with M.tuberculosis[7], and normal rabbit intact immunoglobulin (Dakopatts, Glostrup, Denmark) were purchased. The F (ab')$_2$ of the anti-BCG antibody was prepared by pepsin digestion in Ph 4~4.5 of acetate bufferat 37°C overnight[8]. The pFc was completely removed by gel filtration on Sephacryl S-200 (Pharmacia, Uppsala, Sweden). the purity of the preparation was tested by polyacrylamide gel electrophoresis[9].

The F (ab')$_2$ of the anti-BCG antibody, intact anti-BCG and normal rabbit antibodies were radioiodinated by chloramine-T method. 200 microliter of 0.2 M phosphate buffer (PH 7.4), 500 microliter of antibody solution (1~1.5 mg/ml), 3~5 mCi (111~185 MBq) of NaI-131 (Korea Atomic Energy Research Institute, Seoul, Korea), 200 microliter of chloramine-T solution (0.7 mg/ml) were, respectively, added in sequence in a small polystylene tube (12×75 mm), then vortex mixed. After 30 minutes, 200 microliter of sodium metabisulfite solution (0.7 mg/ml) was added to the mixture to terminate the reaction. Unreacted iodide was eliminated by washing the protein twice with 2.0 ml of saline using a microconcentrator, Centricon-30 (Grace Co., Beverly, USA). ITLC-SG developed with 75% methanol was utilized to investigate the labeling yield and radiochemical purity of the antibodies, which were proven to be 60~70% and radiochemical purity of the antibodies, which were proven to be 60~70% and above 97%, respectively. The specific activity of the final product was 1.2~3.5 mCi/mg. The immunoreactivity of the radioiodinated intact and F (ab')$_2$ of the anti-BCG antibodies was determined by ELISA method, which were greater than 90% and 60% respectively.

2. Animal Studies

Tuberculous lesion was developed in 15 rabbits by inoculation of 1 mg of heat-killed, sonicated M. tuberculosis H37Rv (3×10^9 bacilli) in the knee joint[10]. The tuberculous infection was allowed to develop for 6 weeks. Three-phase bone scan using Tc-^{99}m-MDP was performed in all cases, which demonstrated early increased uptake at the knee joint without delayed bone uptake (data not shown).

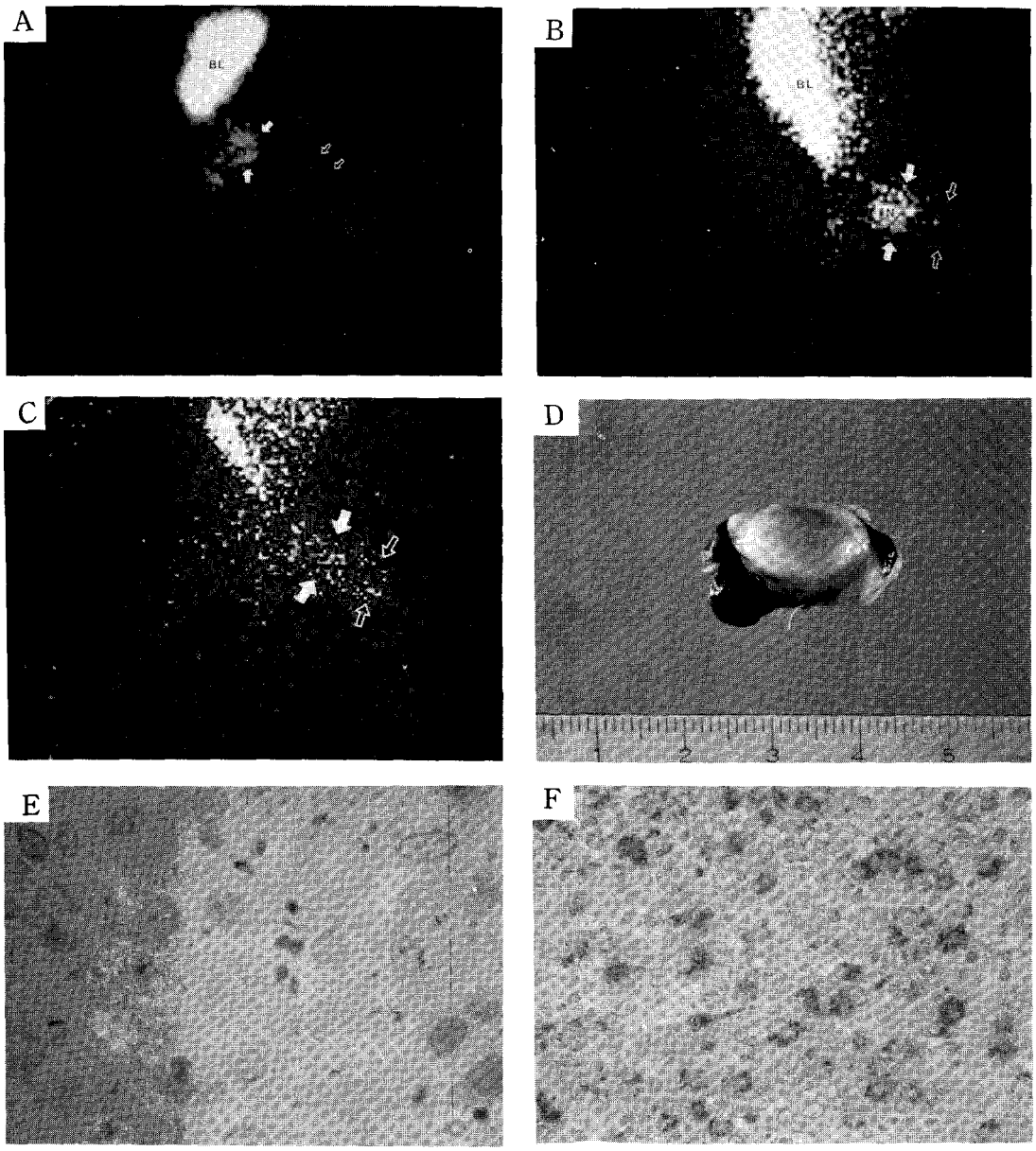

Fig. 1. Immunoscintigraphy using I-131-labeled anti-BCG F $(ab')_2$ in a rabbit with tuberculous lesion: Pathologic correlation.

(a) Scintigraphy 2 hr after injection shows increased uptake over the left thigh (open arrows) and at left inguinal area (solid arrow) with high background activity.

(b,c) Prolonged retention of radioactivity at the infection site can be seen until 48 hrs (c), howevcr, contrast between lesion and background is more apparent on 24 hr image (b).

BL: bladder N: lymph node

(d) An enlarged lymph node was removed from the left inguinal area corresponding to the site of the nodular increased uptake on scintigraphy.

(e) Many fragmented AFB are seen in the cytoplasm of histiocytes (Ziel-Neelson stain, ×1000).

(f) Immunohistochemical staining depicts positive reactions at the cytoplasm of histiocytes as brown colors (×400).

Nontuberculous lesion was developed in two rabbits by inoculation of T. pallidum (2×10^7 bacilli) in each testes for the control study.

1 mCi of I -131-labeled F $(ab')_2$ against BCG was injected intravenously to each of the eight rabbits having tuberculous lesions and the two rabbits having syphilitic orchitis.

Same amount of the radiolabeled intact anti-BCG (n=4) or normal rabbit immunoglobulin (n=3) was given to each of the rabbits having tuberculous lesion.

All of the animals were subjected to the pathologic examinations including immunohistochemical and Ziel-Neelson's stain.

3. Scintigraphy

Images were obtained for a preset time of 10 minutes at 2, 24, 48, and 72 hours after injection of the labeled F $(ab')_2$ and, in addition, 96 hour delayed images from the rabbits injected with the labeled intact antibodies.

The animals were placed supine position under the standard field-of-view scintilation camera, Siemens Orbiter 7500, (Siemens GammaSonics, Illinois, USA) with a high energy, parallel hole collimator at 364 KeV with a 20% window. Matrix size used for the acquisition was 64×64. Target/background (T/B) ratio was obtained by counting the activities of the regions of interest over the infection sites, and over the comparable zones of the contralateral thigh.

RESULTS

All of the tuberculous lesions (n=8) were clearly demonstrated as early as 2 hrs after injection of F $(ab')_2$ with prolonged retention of radioactivity until 48 hrs. Maximum intensity of the lesions was observed at 24hr. the T/B ratio were 1.78±0.25 at 2 hr, 2.51±0.63 at 24 hr, and 1.87±0.16 at 48 hr respectively. The images were faintly positive at 72 hrs. In addition to the primary infection sites, an enlarged draining lymph node possessing multiple acid-fast bacilli was clearly demonstrated in a rabbit. Positive reactions can be seen at the cytoplasm of histiocytes on the immunohistochemical staining using horseradish peroxidase-conjugated anti-BCG antibody (Fig. 1). In contrast to the tuberculous lesions, the syphilitic foci (n=2) demonstrated initial hot uptake but rapid washout of their radioactivity from the lesions (T/B ratio; 3.51±0.43 at 2 hr, 1.44±0.37 at 24 hr and 1.39±0.04 at 48 hr) (Fig. 2).

When the intact antibodies were used, not only the

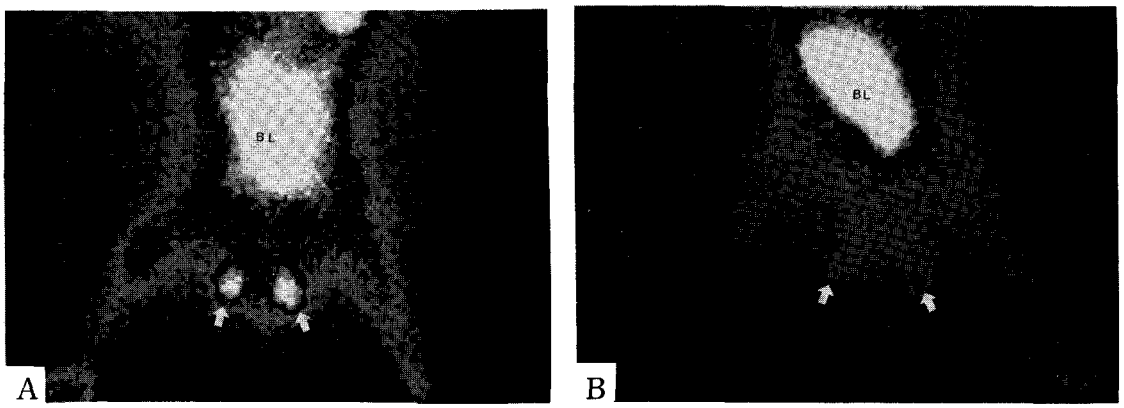

Fig. 2. Immunoscintigraphy using anti-BCG F $(ab')_2$ in a rabbit with syphilitic orchitis.
(A, B) Hot uptake can be seen over the bilateral testes (solid arrows) at 2 hr, which diminished makedly ag 24 hr (b) with minimal retention.

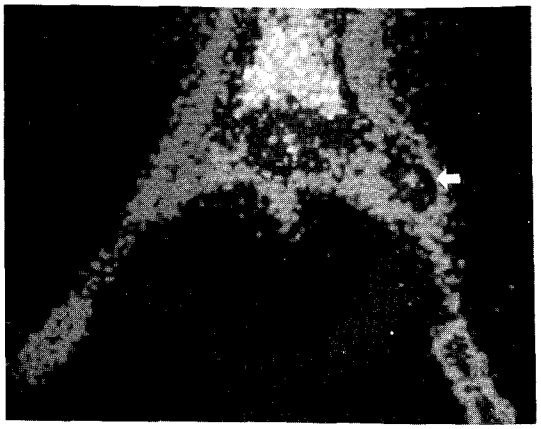

Fig. 3. Immunoscintigraphy 96 hr after injection of the radiolabeled intact normal rabbit immunoglobulin.
A focal infection site (solid arrow) is apparently seen despite the high background activity.

specific (n=4) but irrelevant immunoglobulins (n=3) sustained retention at the tuberculous lesions until 96 hrs (Fig. 3).

DISCUSSION

Rabiolabeled polyclonal or monoclonal antibodies and their fragments provided a powerful approach to diagnosis of various tumors such as colorectal, ovarian and breast carcinomas, melanoma and lymphoma[11]. Immunoscintigraphy is also known to be a good imaging modality in the localization of venous thrombi, myocardial infarction and focal infection sites[12~15]. However it has not been widely used in the specific diagnosis of an infection. Although demonstration of the anatomic site of an infection may be more important than identifying the causative agents, it is essential for the targeted therapy especially in tuberculosis. And the need for the specific in vivo localization is increasing since the radiologic findings of tuberculosis are not pathognomonic and sometimes cancer patients may have tuberculosis foci mimicking primary or metastaytic lesions.

An experimental study concerning the specific immune imaging in infectious disease has been carried out by Rubbin et al. using intact monoclonal antibody spcific for a microbial agent[160]. They described that specific immune imaging was possible because the antibody sustained retention longer than 72 hrs. The mechanism of localization is still uncertain but it was thought to be due to the fact that immunoglobulins accumulate at the infection site by initial exudation of plasma protein from the leaking capillary and by binding of immunoglobulins to the microbial antigens or the Fc receptors on leukocytes. But the irrelevant antibody bound to the leukocytes will egress more rapidly because the specific antibodies have higher affinity than that of the nonspecific Fc receptor binding or the rate of turnover of leuckocytes.

In our study, both the specific and the normal rabbit intact immunoglobulins sustained retension equally at the tuberculous lesions untill 96 hrs which is comparable to the results of Rubin et al. One of the possible explanation for the result was assumed to be due to the differences in the cellular responses between tuberculosis and the acute inflammations components in tuberculosis, also have the Fc receptors as neutophils. Histiocytes become incorporated into granuloma in infected foci, having longer life span than neutrophils[17~18]. The immunoglobulins bound to the macrophages may not egress as rapidly as those bound to the neutrophils in acute inflammation. By the results, we thought specific immune imaging of tuberculosis using intact antibody is difficult and may require longer time, which is limited in its clinical use because the results of the diagnostic procedures are required much sooner.

Howerver one might speculate that fragmented antibody such as F $(ab')_2$, Fab or Fab' would be more useful for the specific localization of inflammation by eliminating the chance of interactions between nonspecific Fc receptors on leukocytes and immunoglobulins. Indeed, earlier reports have described that the antigen binding fragment (Fab) of

the irrelevant antibody is not necessary for the localization of inflammation[19~20]. Our results demonstrating initial transient accumulation of the anti-BCG F $(ab')_2$ in the syphilitic foci, but prlonged its retention at the tubercluous lesions, support the results of the reports. The positive reaction on the immunohistochemical staining may provide the evidence that the retention of the radiolabeled F $(ab')_2$ in the tuberculous lesions is largely due to the antigen-antibody reaction.

Based on this experiment, we have concluded that specific immune imaging of an infection, including chronic inflammations, is possible with specific antibody fragments. It may be more useful than the schintigraphy using radiolabeled leuckocytes, which is known to have high false negative rate in the detection of chronic soft tissue inflammation[21]. We have the plans to proceed further investigation in the localization of tuberculosis using specific monoclonal antibodies against various epitopes in M. tuberculosis.

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