Author's response to reviews

Title: BCG-induced pneumonitis with lymphocytic pleurisy in the absence of elevated KL-6

Authors:

Makoto Tobiume (kawadai698@yahoo.co.jp)
Tsutomu Shinohara (shinoharat@kochi2.hosp.go.jp)
Takahira Kuno (kunot@kochi2.hosp.go.jp)
Shinji Mukai (mukais@kochi2.hosp.go.jp)
Keishi Naruse (narusek@kochi2.hosp.go.jp)
Nobuo Hatakeyama (hatakeyaman@kochi2.hosp.go.jp)
Fumitaka Ogushi (fogushi@kochi.hosp.go.jp)

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Thank you for your useful recommendations for the revision of this manuscript. We have followed the suggestions of the reviewers.

I hope that our responses to the reviewers’ comments will allay their concerns, and place the manuscript in a publishable category.

Sincerely yours,

Tsutomu Shinohara, M.D., Ph.D.
NHO National Kochi Hospital
Department of Clinical Investigation
2-25 Asakuranishimachi, Kochi
780-8077, Japan
Phone 088- 844-3111
Fax 088-843-6385
E-mail: shinoharat@kochi2.hosp.go.jp
ANSWER TO REVIEWER 1

Thank you very much for your comments. Our responses are listed below.

Authors used disease name interchangeably with EAA and HP. What is the difference between EAA and HP?

Response: In principle, there is no difference between EAA and HP. Therefore, we only used the term “HP” as a standard in the revised manuscript in order to avoid confusion.

In your case, BAL fluid analysis showed increased CD4/CD8 ratio, but typical HPs usually reveal decreased CD4/CD8 ratio.

Response: We discussed this issue with a new reference in the revised manuscript as follows. Line 158 page 8 in the revised manuscript

“In contrast to the decreased CD4+/CD8+ ratio reported in typical HP patients, marked T helper cell alveolitis was found by BAL, as reported in previous cases [2]. Since CD4+ alveolar lymphocytosis in the healthy zone of patients with localized pulmonary tuberculosis was reported previously [10], an increased CD4+/CD8+ ratio in BAL may imply intense immunosensitivity toward BCG antigens.”

Did you check echocardiography, and what is the level of adenosine deaminase (ADA) in pleural fluid? Did you perform pleural biopsy?

Response: We did not check echocardiograms because heart failure symptoms did not manifest. ADA in the pleural effusion was 18.8 U/l (5.0-20.0). Pleural needle biopsy was not performed due to the insufficient buildup of effusion (line 129 page 6 in the revised manuscript). We also speculated on the relationship between ADA in the pleural effusion and the antigen-antibody reaction with a new reference in the revised manuscript as follows. Line 210 page 11 in the revised manuscript

“We could not analyze the antigen-antibody reaction in our patient. However, the presence of serum-specific IgG to BCG was reported previously in a patient with BCG-induced pneumonitis [22]. In addition, the level of ADA, which is related to the proliferation of T lymphocytes, in the pleural effusion of our patient was not higher than that in patients with typical tuberculous pleurisy. These findings suggest that the hypersensitivity reaction was induced not only by cell-mediated immunity, but also by humoral immunity in patients treated with BCG.”

TBLB specimen showed not typical finding of HP especially no granuloma?

Response: Unfortunately, as stated clearly in the revised manuscript, a typical epithelioid noncaseating granuloma was not found in the tissue (line 124 page 6 in the revised manuscript). However, a granuloma was not necessarily detected in the lungs of previously reported cases of BCG-induced pneumonitis, and other findings suggested T helper cell alveolitis in this case.
Lymphocyte stimulation test means cell-mediated immune reaction. You did not describe any findings associated antigen-antibody reaction.

Response: As noted above, we discussed the antigen-antibody reaction with a new reference in the revised manuscript.
Line 210 page 11 in the revised manuscript
“We could not analyze the antigen-antibody reaction in our patient. However, the presence of serum-specific IgG to BCG was reported previously in a patient with BCG-induced pneumonitis [22]. In addition, the level of ADA, which is related to the proliferation of T lymphocytes, in the pleural effusion of our patient was not higher than that in patients with typical tuberculous pleurisy. These findings suggest that the hypersensitivity reaction was induced not only by cell-mediated immunity, but also by humoral immunity in patients treated with BCG.”

ANSWER TO REVIEWER 2

Thank you for your helpful comments. The indicated articles were extremely informative. We added all these articles to the References section and the main points of these studies have been incorporated into the revised manuscript. Our responses are listed below.

Although interesting, this phenomenon is not new and has been studied several times before. Of the previously published papers, for example Manfred R et al (Cancer Biother Radiopharm, 2009 24:621-7) describes pleural thickening following BCG therapy in a literature review.

Response: Manfred R et al. reported two cases of pulmonary disease induced by BCG therapy. The first case had extensive fibrosis, diffuse pleural thickening with sparse calcification and some calcified mediastinal lymph nodes, which suggested prior pulmonary tuberculosis, and the second case also had apical pleural thickening, fibrosal hyperdense bands, and multiple calcified lymph nodes as the signs of a previous juvenile tubercular disease. They described that “HRCT of second case pointed out the appearance of a dishomogeneous parenchimal infiltrate at the right side, associated with a modest homolateral pleural reaction”. However, the presence of pleural effusion and details of the “pleural reaction” were not described. We have added these issues to the revised manuscript as follows.
Line 192 page 10 in the revised manuscript
“Although BCG-induced parenchymal infiltration with a modest homolateral pleural reaction was observed in a patient with COPD and a history of previous tuberculosis in one case report, the presence of pleural effusion and details of the “pleural reaction” were not described [17].”

Because the relationship between the pulmonary disease induced by BCG therapy and a history of tuberculosis is informative, we described this issue with a new reference in the revised manuscript as follows.
Line 67 page 3 in the revised manuscript
“…a hypersensitivity reaction rather than a disseminated BCG infection is suspected in the pathogenesis of this disorder [2]. Epithelioid noncaseating granulomas of the lung have been identified in several cases [3]. The frequency of complications associated with BCG immunotherapy was shown to be significantly higher in patients with prior tuberculosis than in patients without a history of tubercular illness, which provides additional support for this hypothesis [4].”


**Response:** We discussed the antigen-antibody reaction and quoted the case reported by Um et al. in the revised manuscript as follows.

Line 210 page 11 in the revised manuscript

“We could not analyze the antigen-antibody reaction in our patient. However, the presence of serum-specific IgG to BCG was reported previously in a patient with BCG-induced pneumonitis [22]. In addition, the level of ADA, which is related to the proliferation of T lymphocytes, in the pleural effusion of our patient was not higher than that in patients with typical tuberculous pleurisy. These findings suggest that the hypersensitivity reaction was induced not only by cell-mediated immunity, but also by humoral immunity in patients treated with BCG.”


**Response:** Although Caramori et al. reviewed 15 cases of the community-acquired pulmonary complications of intravesical BCG immunotherapy, pleural effusion was not detected. Orikasa et al. reported a case of acute eosinophilic pneumonia associated with intravesical BCG therapy. However, eosinophil infiltration was also not clearly observed in the lung tissue of our patient. We described these issues in the revised manuscript as follows.

Line 188 page 10 in the revised manuscript

“Caramori et al. recently reviewed the community-acquired pulmonary complications of intravesical BCG immunotherapy in 15 patients, including their own patient. Chest imaging of these cases revealed a military pattern (n=12), bilateral pulmonary opacities (n=2), and reticulonodular pattern (n=1). However, the pleural effusion was not included [16].”

Line 122 page 6 in the revised manuscript

“Transbronchial biopsies of the right lung (rS3 and rS8) revealed alveolar septal thickening and lymphocyte infiltration with no evidence of mycobacteria (Figure 1c). A typical epithelioid noncaseating granuloma was not found in the tissue. Although a case of acute eosinophilic pneumonia associated with intravesical BCG therapy was reported previously [9], clear eosinophil infiltration was also not observed.”
Finally, Djoba et al. (Cytokine, 2009 47:132-6) has been investigating KL-6 profiles in patients with different forms of tuberculosis and reports differential KL-2 profiles in between pulmonary TB, pleural TB and other form of pleural effusion diseases.

**Response:** Djoba et al. indicated that pulmonary TB patients, pleural TB patients, or patients with other forms of pleural effusions had significantly higher levels of serum KL-6 than those of healthy controls. However, no significant difference was observed in serum KL-6 levels among pulmonary TB patients, pleural TB patients, and patients with other forms of pleural effusions. These results were shown only in a figure and not in the text. Although the actual value could not be resolved by the figure, the mean values of these tubercular illnesses were less than 500 U/ml. We added these findings to the revised manuscript as follows.

Line 173 page 9 in the revised manuscript

“Patients with pulmonary tuberculosis or tuberculous pleurisy have significantly higher levels of serum KL-6 than those of healthy controls. However, the mean values of these tubercular illnesses were reported to be less than 500 U/ml [14]. In contrast, circulating KL-6 levels were shown to be markedly increased in particular types of drug-induced lung injury such as diffuse alveolar damage and chronic interstitial pneumonia patterns [15], and an elevated serum KL-6 level is one characteristic of active HP (1,508 ± 647 U/ml [5]).”

The language needs some editing.

**Response:** The English used in the revised manuscript was rechecked by another native speaker.

**ANSWER TO REVIEWER 3**

Thank you for your comments. Our responses are listed below.

It’s interesting authors not seen any monocytes and macrophages in their BALF but later authors report that pleural effusion of lymphocytic pleurisy contains about 14% macrophages?

**Response:** We have discussed this issue with new references in the revised manuscript as follows.

Line 204 page 10 in the revised manuscript

“The number of macrophages was larger in the pleural effusion than in the BALF in this case. Although the role of macrophages in tuberculous pleurisy remains unclear, stimulated human pleural mesothelial cells were shown to produce monocyte chemotactic protein-1 (MCP-1), which is known to be elevated in tuberculous pleural fluid [20, 21]. Interactions between macrophages and lymphocytes may have been present in the pleural space of our patient.”
Reporting the amount of inflammatory cytokine levels in BALF will definitely strengthen the authors finding.

**Response:** We have added new data on inflammatory cytokine levels in the BALF with a new reference in the revised manuscript as follows.

**Line 118 page 6 in the revised manuscript**

“The concentration of interleukin (IL)-8 in the BALF was 76.7 pg/ml (< 15 pg/ml [8]). IL-1β, IL-10, IL-12, interferon gamma (IFN-γ), tumor necrosis factor alpha (TNF-α), and monocyte chemotactic protein-1 (MCP-1) were under the detection limits (< 10, < 2, < 7.8, < 1.56, < 0.55 and < 62.5 pg/ml, respectively).”

**Line 162 page 8 in the revised manuscript**

“Although IFN-γ, IL-12, and TNF-α, which were shown to be involved in the pathogenesis of HP in animal models and *in vitro* experiments [11, 12], did not reach detectable values in the BALF of our patient, the high level of IL-8, which is a chemoattractant for T lymphocytes and neutrophils, was consistent with that in patients with HP [8].”