ASSOCIATION BETWEEN CIRCULATING FULL-LENGTH OSTEOPONTIN AND IFN-\(\gamma\) WITH DISEASE STATUS OF TUBERCULOSIS AND RESPONSE TO SUCCESSFUL TREATMENT

Chutharut Ridruechai\(^1,2\), Shinsaku Sakurada\(^2\), Hideki Yanai\(^3\), Norio Yamada\(^3\), Pacharee Kantipong\(^4\), Surachai Piyawarawong\(^5\), Panadda Dhepakson\(^6\), Srisin Khusmith\(^1\) and Naoto Keicho\(^2\)

\(^1\)Department of Microbiology and Immunology, Faculty of Tropical Medicine, Mahidol University, Bangkok, Thailand; \(^2\)Department of Respiratory Diseases, Research Institute, National Center for Global Health and Medicine, Shinjuku-ku, Tokyo, Japan; \(^3\)TB/HIV Research Project, Research Institute of Tuberculosis, Japan Anti-Tuberculosis Association, Chiang Rai; \(^4\)Department of Medicine, Chiang Rai Regional Hospital, Ministry of Public Health, Chiang Rai; \(^5\)Mae Chan District Hospital, Ministry of Public Health, Chiang Rai; \(^6\)Medical Biotechnology Center, National Institute of Health, Department of Medical Science, Ministry of Public Health, Nonthaburi, Thailand

Abstract. The T helper type 1 (Th1) immune response plays an important role in protective immunity, pathophysiology and development of tuberculosis (TB). To investigate whether osteopontin (OPN) and other Th1 response-related molecules are associated with TB disease status, including co-infection with HIV, and response to anti-TB treatment, circulating levels of full-length OPN (F-OPN), thrombin-cleaved N-terminal fragment of OPN (N-half OPN), IFN-\(\gamma\), IP-10, IL-18, IL-12/IL-23 (p40), IL-10, IL-15 and C-reactive protein (CRP) were measured before and after anti-TB treatment. Patients with newly active pulmonary TB had significantly higher plasma levels of F-OPN, IFN-\(\gamma\) and CRP than healthy controls (HC). F-OPN, N-half OPN, IFN-\(\gamma\), IP-10, IL-18 and IL-10 levels were higher in patients with extensive TB/HIV co-infection than in patients with a single disease of TB or HIV. Plasma levels of F-OPN correlated well with those of IP-10, IL-18 and N-half OPN among patients with active TB. The F-OPN, IFN-\(\gamma\), IP-10 and CRP levels decreased significantly after effective anti-TB treatment. These data suggest that circulating OPN and Th1 response-related molecules, including IFN-\(\gamma\), may be regulated in response to expansion of active TB and could serve as markers of disease activity before and during treatment.

Keywords: osteopontin, IFN-\(\gamma\), CRP, tuberculosis, HIV/TB

INTRODUCTION

Tuberculosis (TB) is one of the most important infectious causes of death worldwide (WHO, 2009). Despite its long historical interaction with humans, our understanding of host response to the TB
pathogen remains incomplete. Investigation of the molecular differences in host immune status between patients with active TB, co-infected with HIV and control subjects may provide a clue to understand the disease process.

In response to *M. tuberculosis*, activated macrophages and CD4+ T lymphocytes produce Th1 cytokines, including IFN-γ, IL-12 and IL-18 (Schluger and Rom, 1998; van Crevel *et al*, 2002). IFN-γ triggers initiation of the major effector mechanism for the Th1 immune response (Flynn *et al*, 1993). IL-12 induction is observed following uptake of *M. tuberculosis* by dendritic cells and macrophages, which drives the production of IFN-γ in NK and T cells (van Crevel *et al*, 2002). Similarly, IL-18 exhibits strong IFN-γ inducing activity synergistically with IL-12 (Dinarello and Fantuzzi, 2003). The expression of IL-10 mRNA has been demonstrated in lymph nodes of TB patients, particularly in those with HIV/TB co-infection (Lin *et al*, 1996). Although IL-10 may down-regulate the immune response to mycobacterial infection (van Crevel *et al*, 2002), the exact role of IL-10 in TB remains controversial. IP-10, an IFN-γ inducible chemokine, is also predominant in active TB lymph nodes and the lung (Ferrero *et al*, 2003). Elevated circulating IP-10 levels have been reported in patients with active TB and their levels are generally high initially and decrease after anti-TB treatment (Koguchi *et al*, 2003; Inomata *et al*, 2005). Although these OPN levels have been reported to be correlated with Th1 cytokines, IFN-γ and IL-18 (Yamada *et al*, 2000; Inomata *et al*, 2005), the results of measuring circulating Th1 cytokine levels in human TB patients have often been inconsistent or unclear (Yamada *et al*, 2000; Morosini *et al*, 2003; Deveci *et al*, 2005; Inomata *et al*, 2005; Aktas *et al*, 2009). Immune reconstitution syndrome occurs after commencement of highly active antiretroviral therapy (HAART), at a stage when the *M. tuberculosis*-specific Th1 response is partially restored (Lawn *et al*, 2005). In HIV infected individuals, elevated OPN levels are found in cerebrospinal fluid and plasma and correlate with neurocognitive abnormalities (Burdo *et al*, 2008). OPN is the only pro-inflammatory cytokine found to increase after 1 month of HAART in lymph nodes (Li *et al*, 2004) and persists for 6 months of HAART (Chagan-Yasutan *et al*, 2009). OPN is susceptible to proteolytic fragmentation and a thrombin-cleaved N-terminal fragment of OPN (N-half OPN) is known to
affect its biological activity (O’Regan and Berman, 2000).

In this study, we attempted to address three questions unsolved by previous studies: 1) is OPN associated with TB even with HIV co-infection (CD4+ T cell-depletion) in which granulomatous formation is generally poor? 2) is the N-half form, presumably cleaved by thrombin at the site of disease, more accurately connected with parameters of disease activity? 3) Do a variety of Th1-related molecules all coordinate with OPN levels? We investigated the concentrations of both full-length and N-half OPN, cytokines and a chemokine, including IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), IL-10 and IL-15, in the plasma of patients with newly active pulmonary TB, HIV/TB co-infection, HIV single infection and healthy controls and their levels within and between groups were compared. OPN and Th1 response-related molecules in patients with newly active pulmonary TB were also evaluated before and after anti-TB treatment. C-reactive protein (CRP) was simultaneously measured as a marker to monitor response to anti-TB treatment and an indicator of inflammation (Sahiratmadja et al, 2007; Peresi et al, 2008).

MATERIALS AND METHODS

Subjects

Twenty-three patients with pulmonary TB and 6 HIV/TB co-infected patients without highly active antiretroviral therapy (HAART) (HIV+TB+HAART) were recruited from the outpatient and inpatient clinics of Mae Chan and Chiang Rai hospitals, Chiang Rai Province, northern Thailand. HAART was defined as the regular use of two nucleoside reverse transcriptase inhibitors, NRTI [Stavudine (d4T) and Lamivudine (3TC)] plus a non-nucleoside reverse transcriptase inhibitor, NNRTI [Nevirapine (NVP) or Efavirenz (EFV)]. The patients with TB and HIV+TB+HAART-(HAART-) were all newly diagnosed pulmonary TB patients with sputum smears positive for acid-fast bacilli and confirmed by positive cultures for M. tuberculosis and abnormal chest radiographic findings. The patients had never received anti-TB treatment or had taken anti-TB drugs for less than 7 days at the time of enrollment. They had never received any immune-suppressive drugs or other immunomodulators. None of them had diabetes mellitus or other acute infections. On enrollment, the HIV/TB co-infected patients had not previously received antiretroviral therapy but were positive for HIV antibodies detected by particle agglutination assay (Serodia-HIV-1/2, Fujirebio, Tokyo, Japan) and/or immunochromatographic rapid test (Determine HIV-1/2, Abbott Laboratories, Abbott Park, Ill) followed by a confirmation test using enzyme-linked immunosorbent assay (ELISA) (Enzygnost Anti-HIV 1/2 plus ELISA, Dade Behring, Marburg, Germany).

Ten HIV patients not taking HAART (HIV+HAART-) and 17 HIV patients receiving HAART (HIV+HAART+) were recruited from the HIV Care and Treatment Project (Daycare clinic), Mae Chan Hospital. These patients had no previous history of TB. One patient who was HIV+HAART+ was taking isoniazid preventive therapy (IPT) for active TB on enrollment. Their sputum smears were negative for acid-fast bacilli and the cultures were negative for M. tuberculosis. They were negative (induration<5 mm) for tuberculin skin test and had no concomitant active AIDS-related opportunistic infections during the 30 days prior to enrollment. None had diabetes mellitus or
was receiving immune-suppressive drugs or other immunomodulators during the 90 days prior to enrollment.

Twenty-five Thai healthy controls (HC) were recruited through the blood bank at Mae Chan Hospital and served as controls. They had no previous history of TB or risk factors for TB. Their chest radiographs were normal. None of them had diabetes mellitus. All were negative for hepatitis B surface antigen, hepatitis C antigen and HIV antibodies using particle agglutination assay (Serodia-HIV-1/2, Fujirebio, Tokyo, Japan) and/or ELISA (Enzygnost Anti-HIV 1/2 plus ELISA, Dade Behring, Marburg, Germany).

The baseline characteristics of this patients and healthy controls are summarized in Table 1. Patients with TB had significantly higher white blood cell (WBC) counts \((p<0.05)\) than HC; patients with HIV+TB+HAART- tended to have higher WBC counts. Patients with HIV+HAART-had significantly lower WBC counts than HC \((p<0.01)\). The CD4+ cell counts in TB patients were significantly higher than in HIV+HAART- patients \((p<0.01)\), but were not significantly different from those with

---

### Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>HC</th>
<th>TB</th>
<th>HIV+ HAART-</th>
<th>HIV+HAART+</th>
<th>HIV+TB+HAART-</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age, median (range), years</strong></td>
<td>35.0 (21-52)</td>
<td>46.0 (18-64)</td>
<td>37.5 (31-53)</td>
<td>39.0 (27-52)</td>
<td>43.0 (30-47)</td>
</tr>
<tr>
<td><strong>Sex, number of males/females</strong></td>
<td>15/10</td>
<td>15/8</td>
<td>6/4</td>
<td>8/9</td>
<td>5/1</td>
</tr>
<tr>
<td><strong>WBC x 10^3, median (range), cells/µl</strong></td>
<td>6.80 (3.64-11.20)</td>
<td>9.60 (3.10-15.80)</td>
<td>5.21 (3.31-6.06)</td>
<td>5.48 (2.82-9.11)</td>
<td>8.62 (5.70-12.80)</td>
</tr>
<tr>
<td><strong>CD4+ T cell count, median (range), cells/µl</strong></td>
<td>1,050 (451-1,580)</td>
<td>564 (226-1,081)</td>
<td>274 (30-789)</td>
<td>437 (104-843)</td>
<td>146 (19-344)</td>
</tr>
<tr>
<td><strong>≤ 200, No. (%)</strong></td>
<td>3 (30.0)</td>
<td>4 (23.5)</td>
<td>4 (66.7)</td>
<td>7 (41.2)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td><strong>201-500, No. (%)</strong></td>
<td>1 (4.0)</td>
<td>10 (43.5)</td>
<td>5 (50.0)</td>
<td>7 (41.2)</td>
<td>2 (33.3)</td>
</tr>
<tr>
<td><strong>&gt;500, No. (%)</strong></td>
<td>24 (96.0)</td>
<td>13 (56.5)</td>
<td>2 (20.0)</td>
<td>6 (35.3)</td>
<td>146 (19-344)</td>
</tr>
<tr>
<td><strong>CXR findings, No. (%)</strong></td>
<td>23 (92.0)</td>
<td>9 (90.0)</td>
<td>17 (100.0)</td>
<td>5 (83.3)</td>
<td>1 (16.7)</td>
</tr>
<tr>
<td><strong>Normal</strong></td>
<td>20 (87.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td><strong>Infiltrate /non-cavitary</strong></td>
<td>3 (13.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td><strong>Cavitary</strong></td>
<td>2 (8.0)</td>
<td>3 (13.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
<td>1 (10.0)</td>
</tr>
<tr>
<td><strong>No definite infiltration</strong></td>
<td>22</td>
<td>3</td>
<td>3</td>
<td>3</td>
<td>3</td>
</tr>
<tr>
<td><strong>Site of TB infection by member</strong></td>
<td>Pulmonary</td>
<td>Extra-pulmonary</td>
<td>Both</td>
<td>Both</td>
<td>Both</td>
</tr>
</tbody>
</table>

HAART, highly active antiretroviral therapy; HC, healthy control; TB, patients with tuberculosis; HIV+HAART-, HIV patients without HAART; HIV+HAART+, HIV patients with HAART; HIV+TB+HAART-, HIV/TB co-infected patients without HAART.
HIV+HAART+ ($p=0.07$). Among patients with HIV+HAART+, the median time interval between initiation of HAART and enrollment was 35 months (range 14-56 months). The baseline and follow-up characteristics of the 6 patients with HIV+TB+HAART- are shown in Table 2. Of these 6 patients, 3 had pulmonary TB and 3 had both pulmonary and extrapulmonary TB, 2 of them died during anti-TB treatment with a principal diagnosis of disseminated TB. Among the remain patients, 3 were considered to be cured and 1 patient was still undergoing TB treatment after 6-9 months based on National Tuberculosis Program (NTP) guidelines. Of the 3 patients that could be followed-up, 1 patient with a baseline CD4+ cell count<200 cells/µl had started HAART 2 months after anti-TB treatment. Twelve patients with TB and 3 patients with HIV+TB+HAART- were able to be followed-up after 6-9 months of anti-TB treatment and were considered as cured according to the standard criteria.

This study was approved by the Ethical Review Committee for Research on Human Subjects, Ministry of Public Health, Thailand (Reference number 15/2550) and the National Center for...
Global Health and Medicine, Japan (Reference number 415). Written informed consent was obtained from all subjects prior to enrollment.

**Blood samples**

Blood samples were collected in ethylene diaminetetraacetic acid (EDTA) vacutainer tubes from patients and healthy controls at the time of enrollment and after 6-9 months of anti-TB treatment when they were considered as cured. After centrifugation at 1,000g for 10 minutes at room temperature, the plasmas were collected and kept at -80°C until used.

**Determination of full-length and N-half OPN by ELISA**

The levels for full-length (F-OPN) and N-terminal fragment OPN (N-half OPN) were determined with a sandwich ELISA kit according to the manufacturer’s instructions (IBL, Gunma, Japan). The tests were done in duplicate and the concentrations of F-OPN/N-half OPN were calculated from a linear equation for each standard curve developed with recombinant human F-OPN/N-half OPN. The subtracted absorbance below zero was considered as zero. The lower detection limits of the F-OPN and N-half OPN assay kits were 3.3 ng/ml and 92.7 pg/ml, respectively.

**Determination of cytokines, a chemokine and CRP**

IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), IL-10 and IL-15 levels in plasma were determined using sandwich ELISA kits according to the manufacturer’s instructions. The tests were done in duplicate and the concentrations of cytokines/chemokines were calculated from a linear equation for each standard curve. The subtracted absorbance below zero was considered as zero. The lower detection limits of the assays were 4.7 pg/ml for IFN-γ (BD Biosciences Pharmingen, San Diego, CA), 7.8 pg/ml for IP-10 (BD Biosciences Pharmingen), 12.5 pg/ml for IL-18 (MBL, Nagoya, Japan), 62.5 pg/ml for IL-12/IL-23 (p40) (BioLegend, San Diego, CA), 3.9 pg/ml for IL-10 (BioLegend) and 4.0 pg/ml for IL-15 (BioLegend).

Highly sensitive C-reactive protein (CRP) levels in plasma were measured by means of particle enhanced immunonephelometry using the BN system (CardioPhase® hsCRP, Dade Behring, Newark, DE). The lower detection limit was 148 ng/ml. Values below this level were considered equal to 148 ng/ml. A level of 3,000 ng/ml in the serum was considered as the upper limit of normal.

**Statistical analysis**

Statistical analysis was performed using SPSS software version 17.0. The data were expressed as medians and ranges. Since not all the parameters exhibited normal distribution, comparison between two independent groups was performed using the nonparametric Mann-Whitney U test, and comparison between the two dependent groups was performed using the nonparametric Wilcoxon signed-ranks test. The correlations among the F-OPN, N-half OPN and T cell response-associated molecules were analyzed using a Spearman’s rank correlation test. A p-value <0.05 was considered significant.

**RESULTS**

**Circulating F-OPN levels in TB**

The plasma F-OPN levels from patients with TB (251.9-959.9 ng/ml) and HIV+TB+HAART-(853.2-4,005.4 ng/ml) were significantly higher than in patients with HIV+HAART- (209.5-450.8 ng/ml) (p<0.01, p<0.01, respectively), HIV+HAART+ (141.2-655.1 ng/ml) (p<0.01, p<0.001, respectively) and HC
Changes in circulating IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), CRP and IL-10 in TB

Before anti-TB treatment, the plasma levels of IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40) and CRP in patients with TB tended to be higher than in patients with HIV+HAART- and HIV+HAART+ and HC. Half of patients with HIV+TB+HAART- had even higher N-half OPN levels than patients with TB (p<0.01).

Correlations among circulating F-OPN, N-half OPN, IFN-γ, IP-10, IL-18, CRP and clinical parameters in tuberculosis cases

Correlations among plasma F-OPN, N-half OPN, IFN-γ, IP-10, IL-18, IL-12/IL-23 (p40), IL-10, IL-15 and CRP levels before anti-TB treatment were analyzed in patients with TB. Plasma F-OPN correlated significantly with N-half OPN (r=0.508, p<0.05), IP-10 (r=0.500, p<0.05) and IL-12 (r=0.568, p<0.01); whereas plasma F-OPN did not correlate with IFN-γ, IL-12/IL-23 (p40), IL-10, IL-15 or CRP. Positive correlations were also found between plasma levels of IP-10 and IFN-γ (r=0.525, p<0.05), IP-10 and IL-12 (r=0.527, p<0.05) and IL-12 and CRP (r=0.519, p<0.05). In patients with HIV+TB+HAART-, plasma F-OPN levels correlated significantly with IP-10 and IL-18 levels (r=0.943, p<0.01 and r=0.829, p<0.05, respectively).

The correlations between T cell response-associated molecules and the number of WBCs, lymphocytes, monocytes, CD4+ T cells, CD8+ T cells and CD4+/CD8+ ratio were analyzed in patients with TB. There were significant positive correlations between plasma F-OPN levels and WBC counts (r=0.508, p<0.05), CRP and WBC counts (r=0.651, p<0.01) and negative correlations between IFN-γ and CD4+/CD8+ ratios (r=-0.474, p<0.05), IP-10 and CD4+/CD8+ ratios (r=-0.69, p<0.001).

Circulating OPN, IFN-γ, IP-10 and CRP levels after anti-TB treatment

Plasma F-OPN, IFN-γ, IP-10, IL-18,
Fig 1—Circulating full-length OPN (a), N-half OPN (b), IFN-γ (c), IP-10 (d), IL-18 (e), IL-12/IL-23 (p40) (f), IL-10 (g) and CRP (h) levels in patients with tuberculosis (TB) and HIV/TB co-infection without HAART (HIV+TB+HAART-), HIV patients without HAART (HIV+HAART-) and with HAART (HIV+HAART+) were tested in comparison. Healthy individuals (HC) were used as controls. Bars represent the median values. The horizontal lines represent the lower limits of each measurement.
Fig 2–Circulating full-length OPN, IFN-γ, IP-10, IL-18, IL-10 and CRP levels among patients with active pulmonary TB before and after anti-TB treatment.
IL-12/IL-23 (p40), IL-10, IL-15 and CRP levels before and after 6-9 months of anti-TB treatment in the 12 patients with TB and in the 3 patients with HIV+TB+HAART were evaluated. Significant decreases in plasma F-OPN, IFN-γ, IP-10 and CRP levels were seen in patients with TB after treatment (p<0.01, p<0.05, p<0.01 and p<0.01, respectively) (Fig 2). Although plasma IL-18 levels decreased in some TB patients after treatment, the change was not significant.

Plasma F-OPN, IFN-γ and CRP levels in patients with HIV+TB+HAART tended to decrease after anti-TB treatment. After treatment, clinical improvement, negative sputum microscopy examinations and normal chest radiographs were observed.

**DISCUSSION**

To address the role of OPN in patients with TB, circulating F-OPN, N-half OPN and other cytokines and chemokine levels were evaluated along with clinical parameters in Thai patients with active pulmonary TB and HIV/TB co-infection. Circulating F-OPN, IFN-γ and CRP levels were significantly elevated in patients with active pulmonary TB and the levels decreased after effective anti-TB treatment. High concentrations of F-OPN, N-half OPN, IFN-γ, IP-10, IL-18 and IL-10 found in the plasma of patients with HIV/TB co-infection were unexpected, although this was a small-scale study. Levels of N-half OPN were much lower than those of F-OPN in all groups. Plasma levels of F-OPN correlated well with IP-10, IL-18 and N-half OPN levels among patients with active TB.

The high F-OPN levels in TB patients suggested a role for circulating F-OPN in disease activity among TB patients. Elevated circulating F-OPN levels in pulmonary TB patients is consistent with previous studies (Koguchi et al, 2003; Inomata et al, 2005). This may be partly due to leakage from granuloma sites evidenced by accumulation of OPN proteins in lung tissue sections from TB patients (Nau et al, 1997) and by abundant OPN expression in lymph nodes with well-formed granulomas (Nau et al, 2000). However, elevated circulating F-OPN and N-half OPN in patients with HIV/TB co-infection was not expected. HIV/TB co-infection is known to be associated with failure of granuloma formation and failure to control *M. tuberculosis* infection, thereby leading to mycobacterial dissemination (Corbett et al, 2003). The contribution of HIV infection to elevated circulating F-OPN is known and these levels correlate with HIV-induced CNS dysfunction, particularly in HIV-associated dementia, a severe neurocognitive abnormality that commonly occurs during the late stages of HIV infection (Burdo et al, 2008). Without receiving HAART, HIV infection chronically activates the host immune system to maintain a defense that only partially controls infection (Fauci, 1996), but chronic activation and replication, as well as storage of virus, leads to pathological consequences that may stimulate the production of various mediators of immune activation, including OPN. Collectively, prominent levels of circulating F-OPN in HIV/TB co-infection may not indicate disease status of effective granuloma formation but rather reflect spread of active TB lesions, large numbers of pathogens in the body or synergistic immune activation due to HIV/TB co-infection. F-OPN levels may not be equivalent to TB-associated inflammation simply measured by CRP because F-OPN levels did not correlate with CRP levels in the TB group.

The introduction of HAART among
HIV-infected patients usually results in the gradual reconstitution of the immune system (Weiss et al., 1999). HAART induced changes in the expression of many pro-inflammatory cytokines, including OPN in lymph nodes of HIV infected individuals 1 month after initiation (Li et al., 2004) but persistently elevated levels of circulating F-OPN during 6 months of HAART were observed (Chagan-Yasutan et al., 2009). In line with the latter findings, in this study, no differences in circulating F-OPN levels between HIV patients with or without HAART were found, despite a possible alteration in immune status with HAART. Different results are possibly due to differences in disease stage, regimen and duration of HAART.

Levels of circulating N-half OPN were much lower than those of F-OPN among all groups, and may not be helpful for monitoring disease activity. N-half OPN is generally more potent in causing cell migration and adhesions at the site of disease than in the uncleaved full-length form (Senger et al., 1994). In the synovial fluid of patients with rheumatoid arthritis (RA), N-half OPN has been detected at lower levels than F-OPN (Hasegawa et al., 2009). This indicates that N-half OPN exists at lower levels than its full form even at the site of inflammation. N-half OPN was detected in urine but not plasma from patients with RA at much lower levels than F-OPN (Shio et al., 2010). N-half OPN may not be stable in body fluids, including plasma, or is barely produced in tissues through strict regulation of thrombin/anti-thrombin balance. Thus, investigation regarding the functional form of OPN in TB and HIV/TB co-infection is further necessary when a more sensitive assay system is developed.

Elevation of circulating F-OPN, IFN-γ, IP-10 and IL-18 levels was documented in patients with active pulmonary TB. The results of circulating F-OPN, IFN-γ and IL-18 levels in patients with TB are consistent with other studies (Verbon et al., 1999; Morosini et al., 2003; Inomata et al., 2005). The finding of lower circulating IL-10 levels among TB patients than healthy controls is in contrast to some other studies (Verbon et al., 1999; Morosini et al., 2003; Deveci et al., 2005). This variability may result from a different status of healthy controls, in that all were negative on the interferon-gamma release assay (IGRA) in our study, whereas other studies consisted of controls with both positive and negative tuberculin skin tests (TST) (Morosini et al., 2003; Inomata et al., 2005). IL-10 levels in healthy controls in this study may have been affected by simultaneous infection with helminthes or tropical diseases, as is often seen in developing countries (Borkow and Bentwich, 2004). TB patients have different clinical characteristics, but only pulmonary TB patients with sputum smears positive for acid-fast bacilli (AFB) were recruited into this study, whereas another study included patients with both pulmonary and extra-pulmonary TB (Verbon et al., 1999).

The present results showed elevated IFN-γ and IP-10 levels were found in TB patients similar to previous studies (Juffermans et al., 1999; Azzurri et al., 2005; Djoba Siawaya et al., 2009). The present study demonstrated, for the first time, positive correlations between levels of F-OPN and IP-10, between IP-10 and IL-18 and between IP-10 and IFN-γ in patient with TB. Our findings of no correlations between circulating F-OPN and IFN-γ, between F-OPN and IL-12 and between IFN-γ and IL-12 are in contrast with some previous studies (Inomata et al., 2005; Pokkali and Das, 2009). Further studies are needed. OPN was found to be elevated...
along other Th1-related molecules in patients with active TB.

In patients with TB, a significant decrease in circulating F-OPN, IFN-\(\gamma\), IP-10, CRP levels and a trend toward a decrease in IL-18 levels were observed 6 to 9 months after anti-TB treatment. Furthermore, a decrease in circulating F-OPN, IFN-\(\gamma\) and CRP in 3 HIV/TB co-infected patients after completing treatment suggests these molecules may be useful for evaluating TB disease activity and monitoring response to treatment, as has been shown in previous studies (Koguchi et al., 2003; Inomata et al., 2005). However, discrepancies may occur (Verbon et al., 1999; Inomata et al., 2005; Djoba Siawaya et al., 2009) and caution is needed to interpret the results.

In conclusion, the present study confirmed the possible contribution of OPN for evaluating pulmonary TB disease activity, particularly in HIV/TB co-infected patients in association with Th1 response-related molecules. Clinically, the elevated OPN, IFN-\(\gamma\) and CRP levels and their decline after successful anti-TB treatment suggests circulating levels of F-OPN and Th1 response-related molecules, including IFN-\(\gamma\), may be useful to determine expansion of active TB lesions and/or pathogens and may serve as markers of disease activity before and during treatment.

ACKNOWLEDGMENTS

We thank all the subjects for their kind participation in the study and the staff of the TB/HIV Research Project, Thailand, a collaborative research project between the Research Institute of Tuberculosis (RIT) and the Japan Anti-tuberculosis Association, and the Thai Ministry of Public Health for collecting the blood samples and obtaining the clinical information.

This study was supported by Health and Labor Science Research Grants for Research on Emerging and Re-emerging Infectious Diseases (H17-shinko-021 and H20-shinko-014), Ministry of Health, Labor and Welfare, Japan, Grants for Research on Global Health and Medicine (17C-1 and 20C-3), Ministry of Health, Labor and Welfare, Japan and Faculty of Tropical Medicine, Mahidol University, Thailand.

REFERENCES


Deveci F, Akbulut HH, Turgut T, Muz MH. Changes in serum cytokine levels in ac-


Sahiratmadja E, Alisjahbana B, de Boer T,


