Significant Role of Interleukin-8 in Pathogenesis of Pulmonary Disease Due to Mycoplasma pneumoniae Infection

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We found elevated levels of interleukin-8 in pleural fluid samples from patients with pleural effusion and with a sustained fibrotic change of the lung due to Mycoplasma pneumoniae infection. This result suggests a critical role of interleukin-8 in the pathogenesis of a certain type of pulmonary disease caused by M. pneumoniae.

An increasing number of reports on clinical observations have disclosed the beneficial effects of corticosteroids on the treatment of severe lower respiratory tract diseases due to Mycoplasma pneumoniae infection (1, 2, 9). This strengthens the concept of immunological mechanisms underlying these disease manifestations associated with M. pneumoniae infection. In this context, while many cytokines have been implicated in the pathomechanisms, it has not been clear which cytokine in the inflammatory cascade is predominantly responsible for the lung histopathology (7).

Researchers have reported that the cell-mediated immune response of the host, specifically through the function of Th1-type cytokines, has a critical role in the development of clinical manifestations and pathologic features of M. pneumoniae infection (11, 12). More recently, it was reported that interleukin-18 (IL-18), a regulatory cytokine for Th1-type cytokines, plays an important role in pulmonary disease manifestation due to M. pneumoniae (5). In that study, significantly elevated levels of IL-18 were found in pleural fluid (PF) samples from patients with massive pleural effusion and with a sustained fibrotic change of the lung on chest roentgenograms, presumably as a result of combination of mainly intraluminal organization (10) and, to a lesser extent, pulmonary fibrosis. Moreover, these PF samples were positive for M. pneumoniae by PCR (4). On the other hand, the gamma interferon levels in the PF samples had no correlation with those of IL-18; in addition to that, the tumor necrosis factor alpha (TNF-α) levels in the PF samples were found to be elevated only sporadically (5). Taken together, while IL-18 was found to be a pivotal factor in the development of pulmonary disease manifestation characterized by massive pleural effusion and with a sustained fibrotic change of the lung associated with M. pneumoniae infection, active mediators directly responsible for the pathologic change remained unclear.

In this study we focused on two cytokines, IL-8, in terms of a neutrophil chemoattractant possibly responsible for the formation of intraluminal organization, and transforming growth factor β1 (TGF-β1), in terms of a promoter of fibroblasts possibly responsible for the formation of fibrosis.

Thirteen PF and two serum samples were obtained from 11 patients (ages, 11 months to 15 years), who were already presented and characterized in previous reports (cases 2 to 10 in reference 4 and cases 11 and 12 in reference 5). Mycoplasmal infection was diagnosed serologically (Serodia Myco II; Fujirebio, Tokyo, Japan), and no bacterial pathogens were grown from the PF samples. In cases 4, 7 (the right lung of bilateral involvement), 9, and 11, a sustained fibrotic change on the chest roentgenogram remained for more than 4 weeks. The PF samples from these four cases were positive for the M. pneumoniae DNA by PCR (4) and contained elevated levels of IL-18 (5). The other PF samples were from the patients with transient roentgenographic abnormalities and were negative for the M. pneumoniae DNA, except patient 8, who was an infant with Down syndrome, and contained lower levels of IL-18. In addition, sequential serum samples (n = 11) were obtained from five patients with pneumonia without pleural effusion at intervals of 4 to 7 days.

IL-8 and TGF-β1 were measured by commercially available enzyme-linked immunosorbent assay kits (Amersham International, Amersham, United Kingdom), and all assays were performed according to the manufacturer’s recommendations. The minimal significant level of detection in serum is set by the manufacturer at 5 pg of IL-8/ml. And the normal upper limit in serum was set at 74 ng/ml (mean + 3 × standard error of the mean) for TGF-β1, due to the fact that apparently normal sera (n = 20) gave means ± standard errors of the means of 49.4 ± 8.3. Values greater than these were taken as significantly elevated for serum and were tentatively taken as such for PF samples, due to the fact that “normal” PF cannot be obtained.

First we sought levels in serum IL-8 and TGF-β1 by using sequential samples from patients with M. pneumoniae pneumonia. As shown in Table 1, although samples from case Pn-1 with encephalitis showed detectable levels of IL-8, this was not a consistent finding for serum samples from other patients with encephalitis due to M. pneumoniae (data not shown). This suggests that IL-8 in the systemic circulation might not play a significant role in the pathogenesis of M. pneumoniae pneumo-
TABLE 1. Time course of IL-8 and TGF-β1 in sera from patients with *M. pneumoniae* pneumonia

| Case no. | Cytokine or antibody measured | Amt of cytokine or MpPAa measured in: | Remarks |
|----------|-----------------------------|---------------------------------|---------|
|          |                             | sample 1 | sample 2 | sample 3 | |
| Pn-1     | IL-8                        | 246      | 105     | 10      | Transient |
|          | TGF-β1                      | 126      | 179     | 148     | |
|          | MpPA                        | 80       | 640     | 1,280   | |
| Pn-2     | IL-8                        | 13       | <5      |         | Transient |
|          | TGF-β1                      | 108      | 110     |         | |
|          | MpPA                        | 80       | 2,560   |         | |
| Pn-3     | IL-8                        | <5       | <5      |         | Transient |
|          | TGF-β1                      | 131      | 195     |         | |
|          | MpPA                        | 80       | 640     | 5,120   | |
| Pn-4     | IL-8                        | <5       | <5      |         | Transient |
|          | TGF-β1                      | 79       | 142     |         | |
|          | MpPA                        | 320      | 20,480  |         | |
| Pn-5     | IL-8                        | <5       | <5      |         | Transient |
|          | TGF-β1                      | 94       | 140     |         | |
|          | MpPA                        | 320      | 20,480  | 40,960  | |

a Intervals between sample collections were 4 to 7 days. Case Pn-1 was complicated by encephalitis, while the other case were uncomplicated. IL-8 and TGF-β1 levels are shown in picograms per milliliter and nanograms per milliliter, respectively. Values greater than 5 pg/ml for IL-8 and 74 ng/ml for TGF-β1 can be considered significantly elevated.

b MpPA, an antibody titer measured by a microparticle agglutination test for *M. pneumoniae*.

TABLE 2. Values of IL-8 and TGF-β1 in PF and serum samples from patients with pleural effusions due to *M. pneumoniae* infection

| Case no. | Amt of IL-8 (pg/ml) | Amt of TGF-β1 (ng/ml) | PCR result | Remarksa |
|----------|---------------------|-----------------------|------------|----------|
| 2        | 159                 | 39                    | –          | Transient |
| 3        | <5                  | 104                   | –          | Transient |
| 4 (sample 1) | 5,600               | 47                    | +          | Fibrotic change |
| 4 (sample 2) | 60                 | 120                   | +          | |
| 5        | 81                  | 30                    | –          | Transient |
| 5 (serum) | <5                 | 116                   | –          | |
| 6        | <5                  | 56                    | –          | Transient |
| 7 (right) | 4,360               | 59                    | +          | Fibrotic change |
| 7 (left)  | 105                 | 69                    | =          | Transient |
| 7 (serum) | 23                 | 48                    | –          | |
| 8        | 807                 | 48                    | +          | Transient |
| 9        | 1,422               | 82                    | +          | Fibrotic change |
| 10       | 45                  | 46                    | =          | Transient |
| 11       | 569                 | 70                    | +          | Fibrotic change |
| 12       | 376                 | 43                    | –          | |

a Case number corresponds to that of previous reports (4, 5). For case 4, sample 2 was obtained 4 days after sample 1. Values greater than 5 pg/ml for IL-8 and 74 ng/ml for TGF-β1 can be considered significantly elevated.

b Transient, the retention of PF was transient and a chest roentgenogram became normal within 4 weeks; fibrotic change, a sustained fibrotic change on chest roentgenogram was observed for more than 4 weeks.

right lung of patient 7), four of which also had a fibrotic change of the lung, showed distinctly greater levels of IL-8 than did the other cases. In case 4, the highly elevated initial level of IL-8 (5,600 pg/ml) rapidly fell to 60 pg/ml in sample 2, which was obtained 4 days after sample 1. In case 7, the levels of IL-8 in the left lung (105 pg/ml) and serum (23 pg/ml) were significantly lower than in the right lung (4,360 pg/ml). These results of IL-8 were quite similar to those of IL-18 (5).

Although the levels of TGF-β1 were below the tentative normal upper limit in most of the PF samples, in case 4, where two sequential PF samples could be obtained, the second sample showed greater and significantly elevated levels of TGF-β1.

The results for TGF-β1 obtained in this study suggest that this cytokine plays some role later in the process of *M. pneumoniae* pneumonia. This activity is seemingly associated neither with the presence or absence of pleural effusion nor with a sustained fibrotic change of the lung. In addition, the levels of TGF-β1 were rather lower in the PF samples than in the systemic circulation. Taken together, although this cytokine might work in the repair mechanism of *M. pneumoniae* pneumonia, its contribution must not be large, since the magnitude of elevation was not so large for both the PF and the serum samples when it was compared with the normal value.

On the other hand, the results for IL-8 obtained in this study strongly suggest that this cytokine, specifically produced in the lung, plays a significant role in the pathomechanism of a sustained fibrotic change of the lung which we suspect to be a consequence of intraluminal organization (5). It has been widely recognized that the pathologic feature of *M. pneumoniae* pneumonia is characterized by the mononuclear cell or lymphoplasmacytic cell infiltration at the site of inflammation (1, 2, 7, 9). In this respect, Rollins et al. (10) reported that while the bronchiolar infiltrates were mainly plasma cells and lymphocytes, it was polymorphonuclear leucocytes that were predominantly found in bronchiolar luminal contents and intra-alveolar contents. From this point of view, it must be reasonable to assume that IL-8 is involved in the pathomechanism of lower respiratory tract diseases caused by *M. pneumoniae*.

In a previous study, IL-18, which was formerly called gamma interferon-inducing factor, was also found to be strongly associated with the fibrotic change of the lung (5). In this respect, it is quite interesting that IL-8 could be induced by IL-18 through the intermediate production of TNF-α (8). Although a concomitant elevation of TNF-α in the PF samples could not be found (5), this can be explained by a time lag between the production of TNF-α and IL-18 and the extremely short half-life of TNF-α. In addition, there is a possibility that *M. pneumoniae* has the ability to directly promote IL-8 production through the function of Toll-like receptors (3), on the basis of *Mycoplasma fermentans*’s ability to induce cell activation via Toll-like receptor 2 (6).

In conclusion, our results suggest that IL-8 is involved in the pathomechanism of pulmonary disease manifestations by *M. pneumoniae* at least in a particular population of patients. Because of the well-known pathologic feature of mononuclear cell predominance, the contribution of IL-8 might have been underestimated.

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