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CASE REPORT

Cytokines and neutrophils responses in influenza pneumonia

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Abstract This case report shows a striking correlation of remarkable brief high levels of pro- and anti-inflammatory cytokines coupled with increased neutrophil activation, followed by a sharp decrease in cytokine levels and increased neutrophil apoptosis associated with the favorable clinical outcomes of a patient with severe influenza infection. The host response examined in our case is not complete, given it did not assess the full spectrum of host response. The brief neutrophil and cytokine response seen in our case in the absence of antiviral therapy and in the presence of methotrexate immunosuppressive therapy rise the question as to whether the latter optimally modulated the macrophage function, resulting in a favorable outcome of severe influenza viral infection.

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Background

Community-acquired pneumonia (CAP) is a serious complication of influenza viral infection [1]. The poor clinical outcomes seen in some patients with influenza CAP have been related to a cytokine storm and an uncontrolled neutrophil activation induced by the influenza virus [2, 3]. Unique examples of the severity of influenza pneumonia have been the histopathological studies of H5N1 and 1918 H1N1 influenza viral infections, revealing severe lung consolidation and destruction of lung anatomy [4, 5]. From the pathogenesis perspective, the lungs of mice infected with H5N1 and 1918 H1N1 influenza virus showed an early and excessive infiltration of macrophages and neutrophils in the presence of high levels of pro-inflammatory cytokines [6]. These findings indicate that an excessive pro-inflammatory response is likely to result in severe lung damage and suggest that the successful regulation and balance of the pro-inflammatory cytokine response is, thus, essential for a favorable outcome in patients with influenza pneumonia.

We examined the inflammatory responses in a patient with pneumonia and the degree of neutrophil activation, neutrophil life span, and the levels of pro-inflammatory and anti-inflammatory cytokines at hospitalization days 1 and 4. In this case report, we present the correlation of different markers of inflammatory responses with the clinical outcomes in one of the patients enrolled in our Community-Acquired Pneumonia Inflammatory Study Group (CAPISG).

Case report

A 54-year-old African-American female with a history of chronic obstructive pulmonary disease (COPD) and rheumatoid arthritis was admitted to the hospital with shortness of breath and worsening cough for 1 day. She had been treated until the hospitalization day with oral methotrexate 2.5 mg weekly, fluticasone/salmeterol combination one puff twice daily, and montelukast 10 mg orally daily. The patient refused influenza immunization for the current season. On examination, the lung exam showed bibasilar crackles. Pulse oximetry showed an oxygen saturation of 80 % in room air. The white blood cell (WBC) count was 18,300 cells/mm^3. A chest radiograph showed bibasilar infiltrates. The nucleic acid amplification tests for respiratory viruses was positive for influenza virus A, H3N2. Cultures for typical bacteria, urinary *Legionella* antigen, and DNA amplification assays for atypical bacteria were negative. The patient had received piperacillin–tazobactam, levofloxacin, and vancomycin since admission day for 3 days and then switched to oral levofloxacin and oral trimethoprim–sulfamethoxazole for 2 days. She improved and reached clinical stability by day 3 of hospitalization and was discharged home by day 5.

The patient's inflammatory response was assessed on day 1 and day 4 of her hospitalization. Evaluation of the cytokine response was performed for pro- and anti-inflammatory cytokines in plasma and sputum samples. Blood and sputum cytokines were measured by a multiplex assay (MILLIPLEX, Millipore, Billerica, MA). Blood neutrophil function was evaluated by measuring the increased plasma membrane expression of secretory vesicles (CD35) and specific granules (CD66b), which were stimulated by basal and formyl-methionyl-leucyl-phenylalanine (fMLF). Hydrogen peroxide production, which measures respiratory burst, and the capability of neutrophils to phagocytose bacteria were analyzed by flow cytometry. Sputum neutrophil apoptosis and markers of neutrophil survival were measured by caspase-3 activation and phosphorylated extracellular signal-regulated kinases (pERK 1/2), respectively. Evaluation of neutrophil function was performed by the determination of phagocytosis, oxidative burst, secretory vesicles, specific granules, and apoptosis in both plasma and sputum samples.

Neutrophil function and apoptosis/survival activity

On day 1, markers of neutrophil function indicated significant neutrophil activation, with minimal apoptosis of neutrophils (Figs. 1, 2, on-line supplementary). Markers of neutrophil activation decreased, and neutrophil apoptosis increased by day 4 when the patient reached clinical stability (Figs. 1, 2, on-line supplementary).

Cytokines

On day 1, extremely high levels of interferon gamma-induced protein 10 (IP-10) and interleukin 6 (IL-6) (1,000 pg/ml) were documented in plasma, which also had elevated levels of other proinflammatory cytokines, such as TNF-α and IFN-γ. Interestingly, there were also high levels of IL-10 and IL-1 receptor antagonist (IL-1ra), which are both cytokines with anti-inflammatory activity. Although in the sputum there were also high levels of pro-inflammatory cytokines, including IL-8 (80,000 pg/ml), IL-1β, TNF-α, and IFN-γ (4–8,000 pg/ml), the relative cytokine composition of sputum did not mirror that of plasma, suggesting compartmentalization of cytokine responses. All cytokine levels, both in plasma and sputum, had decreased significantly by day 4 when the patient reached clinical stability (Figure 3, on-line supplementary).

Comments

Our patient had a confirmed influenza virus A H3N2 pneumonia with advanced hypoxemia, indicating a severe illness. Despite the lack of antiviral therapy, this patient had an impressive clinical recovery within 3 to 5 days of hospitalization, suggesting an effective host response to the H3N2 influenza viral infection. There are many factors to take into consideration of our patient's favorable outcome. The presence of rheumatoid arthritis and COPD are expected to weaken the host response to influenza, which may increase the likelihood of poor outcome. The effect of her immunosuppressive and anti-inflammatory therapy on her host inflammatory response remains unknown. Her favorable clinical outcome suggests that her immunosuppressive and anti-inflammatory therapy may have modulated the cytokine storm and the neutrophil hyperactivation induced by the influenza virus [7].

Macrophages are target cells of influenza viral infection and a source of virus-induced cytokine cascades [8]. Influenza A virus-infected macrophages and virus-induced pro-inflammatory gene expression were related to the severity of influenza A illness [3]. On the other hand, methotrexate has been reported to modulate the cytokine production by macrophages [9]. Specifically, methotrexate has been shown to reduce the production of TNF-α. The treatment of rheumatoid arthritis with methotrexate leads to a decline of the percentage of TNF-α-producing T-cells and an increase of IL-10-producing T-cells [10]. In fact, our patient had increased levels of IL-10 (and IL-1ra) at the same time, rather than following, pro-inflammatory cytokines such as TNF-α and IFN-γ, suggesting a balanced host inflammatory response. Methotrexate may downregulate

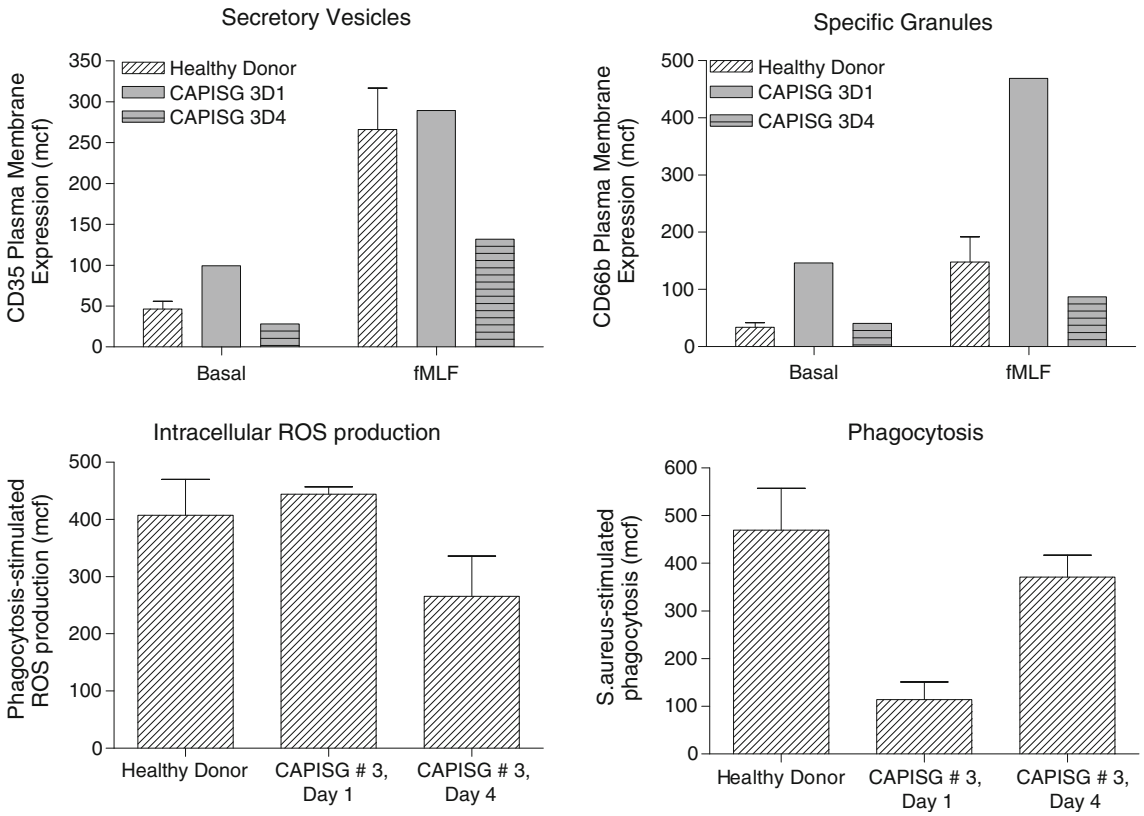

Fig. 1 Whole blood neutrophil activation of the CAPISG patient on days 1–4. *CAPISG* Community-Acquired Pneumonia Inflammation Study Group, study patient is called CAPISG # 3, *ROS* reactive oxygen species, *fMLF* formyl-methionyl-leucyl-phenylalanine

macrophage activation and priming, leading to a brief inflammatory response, likely an optimal response to influenza viral infection. The kinetics of cytokine secretion followed a similar course for both the plasma and sputum, decreasing sharply on day 4. For example, our patient showed a respiratory TNF-α of 8,000 pg/ml on day 1 of hospitalization and <500 pg/ml on day 4. Our patient also showed a high level of IFN-γ in sputum, with a similar decreasing trend over time. These substantially declined levels of respiratory cytokines showed a remarkable correlation with increased neutrophil apoptosis and clinical recovery of our patient with severe influenza illness. TNF-α is an important factor modulating neutrophil activity, and a high level of TNF-α has been linked to the hyperresponsiveness of neutrophils and acute respiratory distress syndrome lung injury [11]. The brief high levels of TNF-α (at the same time as an elevated plasma IL-10) may have contributed to an optimal neutrophil activity, leading to resolution of the severe influenza illness. Thus, a brief high level of IFN-γ may have modulated macrophage and other T-cell functions.

In summary, here, we show a striking correlation of remarkable brief high levels of some pro-inflammatory cytokines with decreased neutrophil survival and the favorable clinical outcomes of a patient with severe influenza infection. The host response examined in our case is not complete, given it did not assess the full spectrum of host response. Our patient's cytokines and neutrophils responses in the absence of antiviral therapy and in the presence of methotrexate immunosuppressive therapy rise the question as to whether the latter optimally downregulated the macrophage function, resulting in a favorable outcome of severe influenza viral infection.

Conflict of interest This work does not have any funding sources and all authors declare that they do not have any conflicts of interest.

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