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CASE REPORT

Cytokine profiles in *Mycoplasma pneumoniae* infection-associated hemophagocytic lymphohistiocytosis

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A 3-year-old boy with *Mycoplasma pneumoniae* infection associated with hemophagocytic lymphohistiocytosis (MP-HLH) presented with an elevated level of serum interleukin-12 (IL-12) and lower levels of interferon-γ and IL-10 compared to patients with Epstein–Barr virus infection associated with HLH (EBV-HLH). Unlike the patients with EBV-HLH, $CD8^{+}$ $CD5^{low}$ HLA-DR^{++} T cells were not detected in our pediatric patient. Thus, the pathophysiology of MP-HLH may differ from that of EBV-HLH.

Introduction

Hemophagocytic lymphohistiocytosis (HLH) is a hyperinflammatory syndrome characterized by the uncontrolled activation and massive proliferation of macrophages and T cells with hypercytokinemia.[1] Although HLH and *Mycoplasma pneumoniae* (MP) infection can be comorbid, the mechanism of the pathogenesis remains unclear. Here we report on the changes in peripheral blood T-cell subsets and serum cytokine profiles observed in a boy with MP infection associated with HLH (MP-HLH).

Case report

A 3-year-old boy was admitted to a hospital with persisting fever and coughs for 4 days. He had been healthy before

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the onset of fever. A chest radiograph revealed massive infiltration in the right upper lung lobe, suggesting pneumonia. He was initially treated with clarithromycin and ampicillin/sulbactam, which were replaced with meropenem and minocycline, due to the possibility of penicillin-resistant *Streptococcus pneumoniae* and macrolide-resistant *M. pneumoniae* as causative agents. Because of a persistent high-grade fever and elevated serum levels of lactate dehydrogenase (LDH) and ferritin, the boy was referred to our hospital 4 days later. On admission, his laboratory findings were as follows: white blood cell count, 4800/μL (neutrophils, 57.6%); hemoglobin, 11.9 g/dL; platelets, 116,000/μL; serum C-reactive protein, 59.4 mg/L (normal <3.2 mg/L); aspartate

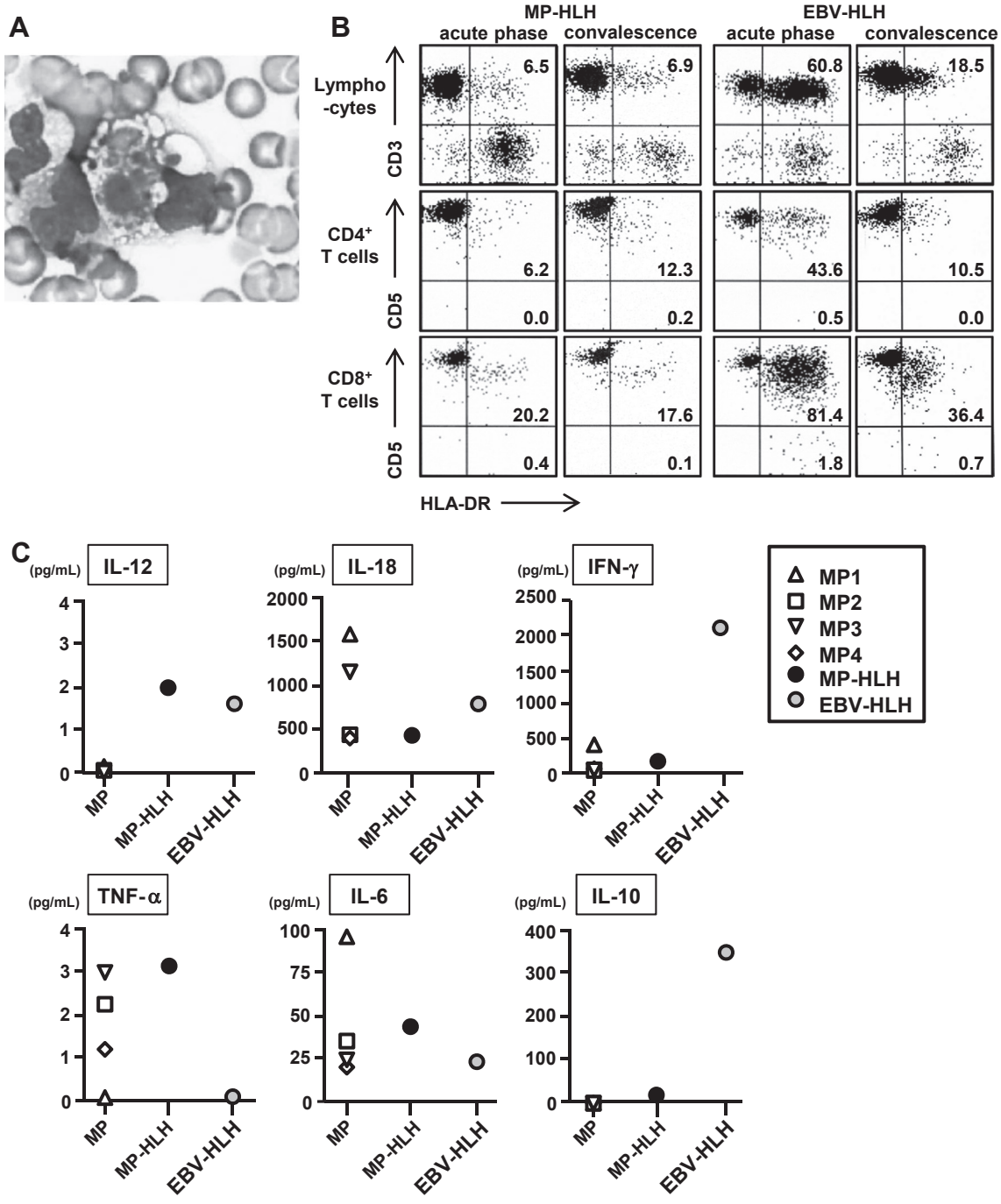

Figure 1. (A) Bone marrow smear revealed hemophagocytosis by macrophage. (B) Flow cytometric analysis of CD5 expression on $CD8^+$ T cells. CD5 downregulation was detected in Epstein—Barr virus-associated hemophagocytic lymphohistiocytosis (EBV-HLH) but not in *Mycoplasma pneumoniae*-associated HLH (MP-HLH). Values represent the percentage of the respective surface molecules expressing T cells. (C) Serum cytokine levels in MP-HLH, EBV-HLH, and severe MP patients before corticosteroid administration. Serum interleukin-2 (IL-2), IL-4, and IL-17 were not detected. IFN-γ = interferon-γ; TNF-α = tumor necrosis factor-α.

aminotransferase, 1788 IU/L; alanine aminotransferase, 332 IU/L; LDH, 3748 IU/L; ferritin, 7718 ng/mL (normal, 18.6—261 ng/mL); soluble interleukin-2 (IL-2) receptor, 2686 U/mL (normal, 144.5—518 U/mL); and fibrinogen, 143 mg/dL (normal, 140—340 mg/dL). The passive agglutination test titers for MP were 1:2560 on Day 10, which increased to 1:20,480 on Day 32. Both cytomegalovirus antigenemia and anti-Epstein—Barr virus (EBV) antibodies were negative. Natural killer (NK) cell cytotoxicity was 18.9% (normal >18%). Bone marrow aspiration exhibited normal cellularity with increased hemophagocytic macrophages (Fig. 1A). The patient was diagnosed with MP-HLH according to the HLH-2004 criteria and was treated with intravenous immunoglobulin (IVIG, 2 g/kg) and prednisolone (2.6 mg/kg/d). His symptoms and laboratory findings improved within a few days. The prednisolone dose was gradually tapered and then stopped when no recurrence was noted 13 days after admission.

To understand the pathophysiology of MP-HLH, we examined the patient's peripheral blood T-cell subsets and serum cytokine profiles. Because an increase in $CD8^{+}$ $CD5^{low}$ HLA-DR^{++} T cells is a characteristic feature of EBV infection associated with HLH (EBV-HLH)[2] and familial HLH type 2 (FHL2),[3] whole blood from the patient was stained with antihuman leukocyte antigen (HLA)-DR (Ancell, Bayport, MN, USA), antihuman CD5, antihuman CD8, or antihuman CD3 (all from Affymetrix eBioscience, San Diego, CA, USA) monoclonal antibodies (mAbs). CD5 expression on $CD8^{+}$ T cells was evaluated with a FACSCalibur flow cytometer using CellQuest (BD Biosciences, San Jose, CA, USA). CD5 expression was considered negative if the level was similar to those of NK cells and CD5-negative B cells. A patient with nonfamilial EBV-HLH diagnosed using the HLH-2004 criteria was used as a positive control (Table 1). As shown in Fig. 1C, lymphocytes from the MP-HLH patient had a lower percentage of HLA-DR^{++} activated T cells than those from the EBV-HLH patient. In contrast to the EBV-HLH case, few, if any, $CD8^{+}$ $CD5^{low}$ HLA-DR^{++} T cells were detected in the peripheral blood of the MP-HLH patient.

We compared the patient's serum cytokine profiles prior to commencement of steroid treatment with those of four patients with severe MP infection who required supplementary steroid therapy due to respiratory distress or fever lasting more than 7 days, and those of a patient with EBV-HLH (Table 1). Informed consent was obtained from the parents in accordance with the Declaration of Helsinki. The serum concentrations of cytokines were determined using a BD cytometric bead array human Th1/Th2/Th17 kit (BD Biosciences) and a sandwich enzyme-linked immunosorbent assay with antihuman IL-18 mAbs (MBL, Nagoya, Japan), according to the manufacturer's instructions. As shown in Fig. 1C, both MP-HLH and EBV-HLH showed higher serum IL-12 levels than severe MP. IL-2, IL-4, and IL-17 were not detectable in any of the sera. There was no significant difference in the serum levels of the other examined cytokines between MP-HLH and severe MP. Of note, the elevated serum interferon-γ (IFN- γ) and IL-10 levels were much lower than those of EBV-HLH.

Discussion

HLH is roughly classified into two forms, namely, familial (genetic) and secondary (acquired). Secondary HLH is associated with a variety of underlying conditions including infections, autoimmune diseases, or malignancy.[1] EBV-HLH is well-known to be prototypic of secondary HLH, whereas MP-HLH is rare. Most cases of MP-HLH have been shown to exhibit relatively mild cytopenia and good therapeutic responsiveness to systemic corticosteroids.[4,5] Consistent with previous reports, the present case was well controlled using a corticosteroid and IVIG.

FHL is caused by mutations that impair the cytotoxicity of NK cells and CD8 T cells, which kill both infected cells and antigen-presenting cells, to remove antigens and to terminate immune responses.[1] Viral infections generally trigger the development of FHL symptoms, resulting in persistent activation and proliferation of lymphocytes, followed by the uncontrolled secretion of proinflammatory cytokines. CD8 T cells producing high levels of IFN-γ play a critical role in the pathogenesis of FHL.[3,6] The down-regulation of CD5 observed in patients with EBV-HLH and FHL2 is considered a consequence of the dysregulated proliferation of $CD8^{+}$ T cells. The increase in $CD8^{+}$ $CD5^{low}$

Table 1 Demographic data of the patients.

Case (age/sex)		MP-HLH 3 y/M	MP1 6 y/M	MP2 11 y/F	MP3 3 y/F	MP4 4 y/M	EBV-HLH 4 y/F
Minimum SpO_2 (%)		90	92	90	96	89	99
Maximum ferritin (ng/mL)		7718	377	n.d.	n.d.	n.d.	1247
Anti-*Mycoplasma pneumoniae* PA	Acute	2560	1280	<40	160	n.d.	EBV-PCR (+)
	Convalescent	>20,480	20,480	1280	2560	5120	
Antibiotics		CAM, ABPC/SBT, MEPM, and MINO	AZM, CLDM, and MINO	CAM, ABPC/SBT, and MINO	CFDN, CAM, and CLDM	CAM, AZM, ABPC/SBT, and CLDM	AZM and MEPM
Immunotherapy		PLS and IVIG	PLS	PLS	PLS	PLS	PLS, CsA, and etoposide

ABPC/SBT = ampicillin/sulbactam; AZM = azithromycin; CAM = clarithromycin; CLDM = clindamycin; CsA = cyclosporine A; EBV-HLH = Epstein—Barr virus-associated HLH; HLH = hemophagocytic lymphohistiocytosis; IVIG = intravenous immunoglobulin; MEPM = meropenem; MINO = minocycline; MP = mycoplasmal pneumonia; MP-HLH = *Mycoplasma pneumoniae* infection-associated hemophagocytic lymphohistiocytosis (HLH); n.d. = not determined; PCR = polymerase chain reaction; PLS = prednisolone; CFDN = cefdinir; PA = passive agglutination.

HLA-DR^{++} T cells is correlated with the severity of FHL2 or EBV-HLH.[2,3] However, the role of these subpopulations in the other form of HLH remains unknown. Animal models of HLH have shown that pathogenic IFN-γ production could be attributed to other cells rather than CD8^{+} T cells, depending on the underlying conditions.[7,8] Considering the lack of CD8^{+} CD5^{low} HLA-DR^{++} T cells, cells other than the CD8^{+} T-cell subpopulation produced low levels of IFN-γ in MP-HLH, resulting in mild HLH.

IL-12 is well-known to enhance IFN-γ production by Th1 cells, CD8^{+} T cells, and NK cells. Although administered antibiotics might modify the cytokine profile, the IFN-γ levels observed in MP-HLH were much lower than that of EBV-HLH despite the similar levels of IL-12, supporting the notion that the extremely high levels of IFN-γ in EBV-HLH could be explained by dysregulation of EBV-infected CD8^{+} T cells.

IL-18 is a proinflammatory cytokine that enhances the activity of NK cells and induces IFN-γ production in concert with IL-12. Elevation of serum IL-18 levels is reported in patients with X-linked inhibitor of apoptosis deficiency-associated HLH.[9] Serum IL-18 levels can also be elevated in patients with MP.[10] Whereas the IL-18 levels of the present case were not as high as those of severe MP, IL-18 levels merely reflect the severity of MP rather than the comorbidity of HLH, as postulated by Tanaka et al.[10]

In the present case, NK cytotoxic activity was close to the lower normal limit. Although the role of NK cell cytotoxicity in protection against mycoplasmal infection is uncertain, poor NK cytotoxic activity might account for vulnerability to mycoplasmal infection and the development of MP-HLH. Overall, the underlying pathophysiology of MP-HLH may differ from that of EBV-HLH.

Conflicts of interest

All authors declare that they have no conflicts of interest related to the material discussed in the article.

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