Cytokines in systemic juvenile idiopathic arthritis and haemophagocytic lymphohistiocytosis: tipping the balance between interleukin-18 and interferon-γ

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Abstract

Objectives. To study the role of IFN-γ in the pathogenesis of systemic JIA (sJIA) and haemophagocytic lymphohistiocytosis (HLH) by searching for an IFN-γ profile, and to assess its relationship with other cytokines.

Methods. Patients with inactive (n = 10) and active sJIA (n = 10), HLH [n = 5; of which 3 had sJIA-associated macrophage activation syndrome (MAS)] and healthy controls (n = 16) were enrolled in the study. Cytokines and IFN-γ-induced genes and proteins were determined in plasma, in patient peripheral blood mononuclear cells (PBMCs) and in lymph node biopsies of one patient during both sJIA and MAS episodes. IFN-γ responses were investigated in healthy donor PBMCs, primary fibroblasts and endothelial cells.

Results. Plasma IFN-γ, IL-6 and IL-18 were elevated in active sJIA and HLH. Levels of IFN-γ and IFN-γ-induced proteins (IP-10/CXCL-10, IL-18BP and indoleamine 2,3-dioxygenase) in HLH were much higher than levels in active sJIA. Free IL-18 and ratios of IL-18/IFN-γ were higher in active sJIA compared with HLH. HLH PBMCs showed hyporesponsiveness to IFN-γ in vitro when compared with control and sJIA PBMCs. Endothelial cells and fibroblasts expressed IFN-γ-induced proteins in situ in lymph node staining of a MAS patient and in vitro upon stimulation with IFN-γ.

Conclusion. Patients with active sJIA and HLH/MAS show distinct cytokine profiles, with highly elevated plasma levels of IFN-γ and IFN-γ-induced proteins typically found in HLH/MAS. In addition to PBMCs, histiocytes, endothelial cells and fibroblasts may contribute to an IFN-γ profile in plasma. Increasing levels of IFN-γ compared with IL-18 may raise suspicion about the development of MAS in sJIA.

Key words: systemic juvenile idiopathic arthritis, macrophage activation syndrome, haemophagocytic lymphohistiocytosis, IFN-γ, IL-18, cytokine, plasma, peripheral blood mononuclear cells.

Rheumatology key messages

- IFN-γ and its downstream proteins distinguish macrophage activation syndrome from systemic JIA.
- High free IL-18 accompanied by low IFN-γ is typically seen in systemic JIA.
- Endothelial cells and fibroblasts can contribute to an IFN-γ profile in haemophagocytic lymphohistiocytosis.
Introduction

Systemic JIA (sJIA) is a rare paediatric immune-inflammatory disorder, characterized by arthritis and systemic features, including fever, rash and lymphadenopathy [1–3]. A striking aspect of sJIA is its strong association with macrophage activation syndrome (MAS), a condition caused by excessive activation of T cells and macrophages, leading to haemophagocytic activity and massive inflammatory responses [4, 5]. MAS is categorized as a form of secondary haemophagocytic lymphohistiocytosis (HLH) [6]. Whereas secondary HLH can be a complication of infections, malignancies and childhood inflammatory disorders, primary HLH has a genetic cause related to defective cytotoxic activity [6].

Several findings support the concept that sJIA is an autoinflammatory disease driven by pro-inflammatory cytokines such as IL-1β, IL-6 and IL-18 [7–16]. Despite the high inflammatory status of both sJIA and HLH, the role of IFN-γ, a key cytokine in inflammation and macrophage activation, is incompletely understood [17]. Gene expression studies performed on peripheral blood mononuclear cells (PBMCs) from sJIA patients revealed an absence of IFN-γ-upregulated genes [18]. Sikora et al. [19] reported an intact transcriptional response of sJIA PBMCs to IFN-γ, suggesting that the absent IFN-γ gene signature might be the result of low in vivo exposure to IFN-γ. Conversely, a major role of IFN-γ in the pathogenesis of HLH is presumed from our observations demonstrating in situ IFN-γ in liver biopsies in a heterogeneous group of HLH patients [20], from elevated IFN-γ plasma levels in HLH [21–23] and from animal models for HLH [24, 25], in which the symptoms were inhibited by anti-IFN-γ antibody treatment. We recently described a new mouse model showing typical clinical and pathological features reminiscent of sJIA [26]. Intriguingly, sJIA-like features, provoked by challenging mice with Freund’s adjuvant, were more evident in IFN-γ-deficient mice than in wild-type counterparts, suggesting a protective role of IFN-γ in this model. Indeed, in addition to its pro-inflammatory activities, IFN-γ also has profound anti-inflammatory effects such as promoting Treg activity, inhibiting development and activity of T helper 17 cells and suppressing IL-1β signalling (i.e. IL-8) [27, 28]. In this study, we investigated the role of IFN-γ in a comprehensive way in patients with sJIA as well as in patients with sJIA-associated MAS and HLH.

Patients and methods

Patients

In total 25 patients and 16 healthy controls were recruited from the University Hospital of Leuven and Hôpital Necker, Enfants Malades, Paris. Informed consent was given according to the Declaration of Helsinki. The study was approved by the Ethics Committee of the University Hospital Leuven. Patient samples were collected at sJIA disease flare or at the occurrence of HLH/MAS before additional therapeutic intervention, and were compared with samples from inactive sJIA patients and age-matched controls. All sJIA patients met the criteria of the ILAR [3] and were grouped according to their disease state into active or inactive sJIA. Inactive sJIA was defined by absence of fever, rash, arthritis and inflammatory parameters [29]. Table 1 summarizes demographic data, diagnosis, treatment, clinical characteristics and laboratory values at the time of sampling. Diagnosis of HLH/MAS was based on the guidelines set by Ravelli et al. [30] and criteria set in the HLH-2004 protocol [31]. Three of the five HLH patients had sJIA-associated MAS. All HLH patients underwent genetic testing for HLH-associated genes, revealing a compound heterozygous mutation in UNC13D (p.Arg83X; p.Ala1018Asp) in one patient presenting with HLH (HLH3). In an EBV-associated HLH patient (HLH4), a combination of two heterozygous unclassified variants were found in both the perforin and Munc13/4 genes.

Plasma isolation and cell cultures

Within 2 h after withdrawal of EDTA-anticoagulated blood, plasma was separated and stored (−80°C). Fresh lympho-prepurified patient/control PBMCs (Axis-Shield PoC AS, Oslo, Norway) were cultured in RPMI-1640 medium containing 10% fetal bovine serum (Lonza BioWhittaker, Walkersville, MD, USA) and stimulated with human recombinant IFN-γ (PeproTech, London, UK). Healthy adult PBMCs, obtained similarly from buffy coats from the Red Cross of Flanders, were frozen in liquid nitrogen and thawed at the time of stimulation with IFN-γ and/or IL-1β (PeproTech). Primary human retinal microvascular endothelial cells (Cell Systems, Kirkland, CA, USA) were cultured in endothelial basal medium-2, supplemented with the endothelial growth medium-2/MV Bullet kit (Lonza BioWhittaker). Primary human diploid skin/muscle-derived fibroblasts (E1SM) were grown in Eagle’s minimal essential medium (Lonza BioWhittaker) containing 10% fetal bovine serum, as previously described [32]. Monolayers were grown to confluence and stimulated with IFN-γ.

RNA extraction, qPCR and ELISA

RNA was purified using the RNeasy Micro kit (Qiagen, Hilden, Germany) for patient material or the PureLink RNA Mini kit (Invitrogen, Carlsbad, CA, USA). cDNA synthesis was performed using SuperScript II Reverse Transcriptase (Invitrogen) according to the manufacturer’s protocols. mRNA levels were analysed in duplicate by qPCR using TaqMan Gene Expression Assays (Applied Biosystems, Foster City, CA, USA; assay IDs in supplementary Table S1, available at Rheumatology Online) and normalized to the housekeeping gene GAPDH. Cytokine levels in plasma were measured by sandwich ELISA according to the manufacturer’s protocols (supplementary Table S2, available at Rheumatology Online) or as described [33, 34]. Levels of free IL-18 were calculated as described [35].

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$sJIA$ patients in an active disease state. $^b$Patients with macrophage activation syndrome complicating sJIA. $^c$Primary HLH patient with a $Munc13/4$ mutation. $^d$EBV-associated HLH.

Cn: canakinumab (anti-IL-1$\beta$); Tc: tocilizumab (anti-IL-6 receptor); AR: active arthritis; HSM: hepatosplenomegaly; LA: lymphadenopathy; WBC: white blood cell count; RBC: red blood cell count; Hb: haemoglobin; PLT: platelet count; AST/ALT: aspartate/alanine transaminase; LDH: lactate dehydrogenase; FTN: ferritin; ND: not determined.
Flow cytometry, western blot, immunohistochemistry and HPLC-MS

PBMCs were stained with anti-CD11c-PE, anti-CD14-PerCp Cy5.5, anti-HLA-DR-APC and anti-CD119-PE (eBioscience). Intracellular indoleamine 2,3-dioxygenase (IDO) staining was performed using the Cytotox/ Cytoperm kit (BD Biosciences) and anti-IDO-alexa633 mouse monoclonal antibody 4.16H1 [36]. Staining of phosphorylated STAT1 (pSTAT1) was performed according to the manufacturer’s protocol using 90% methanol for permeabilization and anti-pSTAT1 (pY701) or isotype control antibodies (BD Biosciences). Detailed methods for western blot analysis (IDO), immunohistochemistry (IDO and IP-10) and tandem LC-MS [tryptophan (Trp) and kynurenine] can be found in the supplementary data, available at Rheumatology Online, section on detailed methods for western blot, immunohistochemistry and HPLC-MS.

Statistical analysis

Statistical analysis was performed using GraphPad Prism 5 software. The Wilcoxon signed rank test was used for induction experiments, the Mann–Whitney U-test for comparison of two groups not showing Gaussian distribution, and for multiple comparisons, Kruskal–Wallis tests were performed with Dunn’s post-test. P-values ≤ 0.05 were considered indicative of statistical significant differences.

Results

Increased cytokine levels in active sJIA and HLH patients

IFN-γ levels in plasma were quantified and compared with other cytokines (Fig. 1A; supplementary Table S3, available at Rheumatology Online). Concentrations of IFN-γ were approximately five times higher in plasma of active sJIA patients than in inactive sJIA patients and healthy controls (Fig. 1A). In HLH patients, IFN-γ levels were >100 times higher than in controls (P < 0.001) and were increased compared with inactive (P < 0.01) and active [not significant (ns)] sJIA patients.

IFN-γ could not be detected in any of the plasma samples, and a barely detectable concentration of IFN-β was detected in only one HLH plasma sample (supplementary Table S3, available at Rheumatology Online).

IL-6 plasma levels were elevated in active sJIA (P < 0.001) and inactive sJIA (ns) patients as compared with healthy controls (Fig. 1A). Median IL-6 levels in HLH patients were even higher compared with patients with active sJIA and were elevated compared with healthy controls (P < 0.001). A 10- to 100-fold difference in IL-6 levels was noted between the three patients with sJIA-associated MAS, and two other patients, one with primary HLH and one with EBV-associated HLH (open squares in Fig. 1A).

IL-1β levels are difficult to detect in the plasma of sJIA patients [9, 13, 15]. Likewise, we could not detect IL-1β in

Fig. 1 Elevated cytokine levels in plasma of active sJIA and HLH patients

Levels of cytokines and IFN-γ-induced proteins were determined by ELISA in the plasma of inactive and active sJIA patients, haemophagocytic lymphohistiocytosis (HLH) patients and healthy controls. (A) Concentrations (pg/ml) of IFN-γ, IL-6 and IL-18 are depicted on a logarithmic scale, IL-1β on a linear scale. (B) IFN-induced proteins IP-10 (pg/ml) and IL-18BP (ng/ml) were analysed in the plasma of patients and both are displayed on a logarithmic scale. (C) Plasma concentrations of tryptophan and kynurenine were determined by HPLC. The ratio of kynurenine to tryptophan is depicted, and reflects a measure of IDO activity in the patients. Dots represent single patients, with open squares representing a patient with primary HLH and one with EBV-associated HLH; horizontal bars represent the median. The horizontal line depicts the lower detection limit of the ELISAs. *P < 0.05; **P < 0.01; ***P < 0.001, Kruskal–Wallis with Dunn’s post-test.
the plasma of active sJIA patients. Yet, IL-1β levels were elevated in all three patients with sJIA-associated MAS (Fig. 1A).

Levels of IL-18, a member of the IL-1β family, were increased in active sJIA patients in comparison with the level in inactive sJIA patients (P < 0.05) and healthy controls (P < 0.001) (Fig. 1A). IL-18 in HLH patients was significantly higher than in controls (P < 0.05).

IL-18 can be a co-inducer of IL-13 in NK and T cells [37]. IL-13 (together with IL-4) can induce an M2 macrophage phenotype, which is typically seen in sJIA [2, 38, 39]. IL-13 was detected in half of the samples, but no significant differences were found between inactive/active sJIA, HLH and controls (supplementary Table S3, available at Rheumatology Online). Together, these data clearly show a cytokine storm in both active sJIA and HLH patients, with raised levels of IFN-γ, IL-6 and IL-18.

**Elevation of IP-10, IL-18BP and IDO activity in plasma of active sJIA and HLH patients**

We next quantified IFN-γ-induced proteins. IFN-inducible protein-10 (IP-10/CXCL10) [40] was detected in four out of seven active sJIA patients and was elevated in HLH patients compared with patients with inactive sJIA (P < 0.001) and compared with healthy controls (P < 0.001) (Fig. 1B).

IL-18 binding protein (IL-18BP), an antagonist of IL-18 that is known to be induced by IFN-γ [41], was higher in active sJIA patients compared with healthy controls (P < 0.05) (Fig. 1B). IL-18BP was elevated 5-fold in patients with HLH compared with those with active sJIA, resulting in significant differences from inactive sJIA patients (P < 0.01) and healthy controls (P < 0.001).

IDO, an intracellular enzyme catalysing the degradation of the essential amino acid Trp to kynurenine, is induced by IFN-γ [42]. Measurement of IDO activity in plasma, by detecting the ratio of kynurenine to Trp, revealed moderately raised IDO activity in active sJIA and a highly elevated ratio of kynurenine:Trp in HLH patients compared with inactive sJIA patients (P < 0.05) and healthy controls (P < 0.01) (Fig. 1C). Based on the above plasma levels, HLH patients have a distinct IFN-γ plasma profile, with high levels of both IFN-γ and its induced proteins, while active sJIA patients show a moderate IFN-γ profile.

**High free IL-18 plasma levels in active sJIA**

IL-18 was originally identified as an IFN-γ-inducing factor [43]. In view of the high IL-18 levels found in active sJIA, the only moderately elevated levels of IFN-γ and related proteins in these patients were surprising. Accordingly, the median ratio of IL-18 to IFN-γ was >200 times higher in active sJIA plasma compared with HLH plasma (Fig. 2A, P < 0.01). Calculated free IL-18 levels were found to be significantly higher in plasma of active sJIA patients (P < 0.01), but not in that of HLH patients, when compared with healthy controls (Fig. 2B). These data show a different balance of IL-18 and IFN-γ in sJIA vs HLH, with an important role for IL-18 in sJIA and an IFN-γ-tipped balance in HLH.

**Defective induction of IDO, IP-10 and MIG upon *in vitro* stimulation of HLH PBMCs with IFN-γ**

In addition to plasma protein levels, we analysed corresponding mRNA levels of IFN-γ-induced proteins in freshly isolated PBMCs of all patients, and confirmed the reported lack of an IFN-γ gene signature in sJIA [18]. In PBMCs of patients with HLH, there was a trend towards higher IFN-γ, IL-10 and MIG mRNA (supplementary Fig. S1, available at Rheumatology Online). The elevated levels of IFN-γ in plasma of active sJIA and especially of HLH patients seem counterintuitive with the absence of a clear-cut IFN-γ signature in their freshly isolated PBMCs. Therefore, we investigated the response of patient PBMCs to IFN-γ stimulation in vitro. Fig. 3A shows a significant increase in IDO and IL-10 mRNA in response to IFN-γ in healthy controls (P < 0.001) as well as in inactive (P < 0.05) and active (P < 0.05) sJIA patients. In HLH patients, IP-10 and MIG mRNA were both induced, however, not significantly and to a lesser extent than in the other groups. The induction of JAK1/2 and STAT1 followed a similar pattern, but with a greater variability for JAK1 (supplementary Fig. S2, available at Rheumatology Online).

To confirm these data at the protein level, the induction of IDO was analysed by intracellular flow cytometry. IFN-γ induced IDO in PBMCs from healthy controls, inactive and active sJIA patients. IDO levels were significantly lower in HLH patients compared with healthy controls (P < 0.05) and inactive sJIA patients (P < 0.05) (Fig. 3B). In addition, lower levels of IFN-γ-induced IDO were found in two active sJIA patients. Western blot analysis confirmed flow cytometric results, with little or no IDO expression in PBMCs of two active sJIA and one HLH patient, after stimulation with a concentration of IFN-γ as low as 1.5 ng/ml (Fig. 3C). In the supernatant of IFN-γ-stimulated PBMC cultures, we found induction of IP-10/CXCL10 and
In healthy controls, inactive and active sJIA patients (Fig. 3D). In contrast, in PBMCs of HLH patients, IFN-γ failed to induce IP-10 and MIG proteins (Fig. 3D).

To look further into the cause of the IFN-γ hyporesponsiveness of HLH PBMCs, the remaining PBMCs of a selected number of patients were thawed for additional exploration. We found no difference in the expression of the IFN-γ receptor 1 (IFN-γ-R1/CD119) on monocytes of one active sJIA patient, one patient with sJIA-associated MAS and three healthy controls (data not shown). In addition, in PBMCs of three active sJIA patients, one patient with sJIA-associated MAS and three controls, we
found that neither active sJIA patients, nor one HLH patient have an intrinsic defect in the phosphorylation of STAT1 in response to IFN-γ (data not shown).

**Endothelial cells and fibroblasts as alternative sources of plasma cytokines**

The IFN-γ profile seen in the plasma of sJIA and HLH patients (Fig. 1) is not in line with gene expression findings of freshly isolated PBMCs of the patients ([18, 44] and supplementary Fig. S1, available at *Rheumatology* Online). As we hypothesized that cells other than PBMCs might be a source of IFN-γ-induced proteins, IFN-γ responses were compared with primary fibroblast and endothelial cells. Induction of IP-10, IL-18BP and IDO mRNA by IFN-γ was 10–100 times higher in fibroblasts and endothelial cells compared with adult donor PBMCs (Fig. 4A). The inductions were confirmed at the protein level for IP-10 and IL-18BP (Fig. 4B). However, PBMCs did not produce IL-18BP in response to IFN-γ. In addition to PBMCs, fibroblasts and endothelial cells respond to IFN-γ and might contribute to an IFN-γ protein profile in plasma.

**Production of IP-10 and IDO by histiocytes, endothelial cells and fibroblasts in a MAS lymph node**

Two years before the development of MAS, during active sJIA, patient HLH2 (Table 1) presented with persistently...
enlarged lymph nodes; a lymph node biopsy was performed to exclude lymphoproliferative disorder. A second lymph node biopsy was taken at the time of overt MAS. Immunohistochemical staining showed a limited number of IP-10- and IDO-positive, mainly monocytic cells, during the stage of active sJIA (Fig. 4C, left panels). During the MAS episode, a prominent increase in both IP-10 and IDO staining was observed, corresponding to the IFN-γ profile in plasma (Fig. 4C, right panels). Next to histiocytes (upper right panel), endothelial cells and fibroblasts in the lymph node sections stained for IP-10 and IDO (arrows, lower right panels), endorsing the importance of alternative sources of IFN-γ-associated proteins.

Anti-inflammatory role of IFN-γ through inhibition of IL-1β signalling in PBMCs

IFN-γ exerts both pro- and anti-inflammatory functions. One of its anti-inflammatory properties is regulation of pro-inflammatory cytokines, including IL-1β signalling [28]. To confirm the IL-1β-inhibiting role of IFN-γ, healthy adult PBMCs were cultured in the presence of recombinant IL-1β and/or IFN-γ, and the induction of IL-1β and IL-8/CXCL8 was checked. As shown in Fig. 5A and B, IFN-γ inhibited IL-1β-induced production of IL-1β as well as the production of IL-8, an important chemokine for attraction and activation of polymorphonuclear neutrophils [45]. Hence, elevated IFN-γ levels observed in the plasma of HLH patients might have an influence on the IL-1β levels in patient PBMCs. Quantification of IL-1β and IL-8 mRNA levels in freshly isolated patient PBMCs demonstrated lower mRNA levels of both IL-1β and IL-8 in HLH PBMCs as compared with other patients and controls, with a significant decrease compared with inactive and active sJIA patients (Fig. 5C). The decreased IL-1β and IL-8 mRNA in HLH PBMCs may be a consequence of the highly elevated IFN-γ plasma levels.

Discussion

As previously described, both active sJIA and HLH patients experience a cytokine storm, with raised levels of pro-inflammatory cytokines, including IL-1β [46]. The findings in this study suggest that IFN-γ may play a role in regulating IL-1β expression, potentially contributing to the anti-inflammatory effects observed in HLH patients. The decreased IL-1β and IL-8 mRNA in HLH PBMCs further supports the notion that IFN-γ may be a key player in the cytokine storm, influencing the production of these cytokines.

**Fig. 5** IFN-γ inhibits IL-1β-induced gene and protein expression in PBMCs

(A and B) The regulation of IL-1β and IL-8 by IL-1β and/or IFN-γ was investigated in healthy adult donor PBMCs. Cells were incubated for 24 h with IFN-γ [1.5 ng/ml (+) or 15 ng/ml (+++) or IL-1β [1 ng/ml (+) or 10 ng/ml (+++) and combination of both cytokines. (A) mRNA levels of IL-1β and IL-8 are depicted normalized to GAPDH and relative to the untreated medium condition. (B) IL-8 protein levels were detected in the culture supernatant by ELISA. Bars represent medians with interquartile range (n = 3). (C) mRNA expression of IL-1β and IL-8 was analysed by qPCR in PBMCs of inactive and active sJIA patients, HLH patients and healthy controls. mRNA expression was normalized to the housekeeping gene GAPDH. Dots represent single patients, with open squares representing a patient with primary HLH and one with EBV-associated HLH; horizontal bars represent the median. *P < 0.05; **P < 0.01, Kruskal-Wallis with Dunns’ post-test. HLH: haemophagocytic lymphohistiocytosis; PBMC: peripheral blood mononuclear cell.
of IL-6 and IL-18 [10–12, 15, 46, 47], which was confirmed in our study. Though IFN-γ is reported to be elevated in primary HLH [21–23] and HLH secondary to infection or malignancy [47], we are the first to report high levels of IFN-γ and its induced proteins in plasma of patients with MAS complicating sJIA. As we confirmed that IFN-γ inhibits IL-1β-signalling in PBMCs [28], decreased IL-1β and IL-8 expression in HLH PBMCs might be linked to high IFN-γ levels in these patients.

Reports concerning IFN-γ plasma levels in sJIA are scarce and not always in agreement. WhileGattorno et al. [13] reported significantly elevated levels of IFN-γ in the serum of active sJIA patients compared with healthy controls, De Jager et al. [15] did not observe raised IFN-γ. We did observe moderately—though not significantly—elevated IFN-γ in the plasma of active sJIA patients. However, we found a marked difference in the balance between IFN-γ and the other cytokines. IFN-γ is more than 20 times higher in HLH patients as compared with active sJIA patients, while IL-6 and IL-18 do not differ significantly between the two patient groups. Conversely, active sJIA patients showed a highly elevated ratio of IL-18 to IFN-γ and high free IL-18 levels compared with HLH patients. Since IL-18 is an important inducer of IFN-γ in NK cells [43], the low IFN-γ levels in active sJIA patients were counterintuitive. In this context, de Jager et al. [48] reported a defective phosphorylation of the IL-18 receptor β-chain in NK cells of sJIA patients, leading to defective IL-18-induced IFN-γ production in vitro. Intriguingly, defective IFN-γ production may be in line with our recently developed mouse model reminiscent of sJIA, in which the clinical, biological and pathological features of sJIA were more prominent in IFN-γ-deficient mice [26]. These data suggest that IFN-γ is not required for the development of sJIA and may exert protective activity in the pathogenesis of sJIA.

The role of IFN-γ in HLH/MAS seems different. Indeed, we observed a substantial increase in the IFN-γ-induced proteins IP-10 and IDO in a lymph node biopsy from a patient during a MAS episode as opposed to the active sJIA phase. IFN-γ plays an essential role in the primary HLH-like syndrome elicited in lymphocytic choriomeningitis virus (LCMV)-infected mice with a genetic defect in cytotoxicity [24, 25]. In contrast, Canna et al. [49] ascribed only a minor role to IFN-γ in a murine model of TLR-9-induced fulminant MAS, in which IFN-γ mediates anaemia but is dispensable for the other manifestations.

Despite the high levels of IFN-γ and its downstream proteins in HLH/MAS, its exact role is yet to be elucidated. Looking at PBMCs ex vivo, we confirmed the gene expression results of Fall et al. [18] and Sumegi et al. [44], who reported the absence of an IFN-γ gene signature in PBMCs of active sJIA and primary HLH patients, respectively. As mRNA expression data in freshly isolated PBMCs did not correspond to the levels of IFN-γ in the plasma of patients, we investigated IFN-γ responses in PBMCs. We report an intact transcriptional response in sJIA patients, as was shown previously by Sikora et al. [19]. In addition, we found that IFN-γ induced IDO, IP-10 and MIG proteins in PBMCs of both controls and sJIA patients. PBMCs of HLH patients showed hyporesponsiveness to IFN-γ, which is unexpected in view of the extremely high levels of IFN-γ, IDO and IP-10 in the plasma. We hypothesize that the defect observed in the in vitro culture might be explained by functional exhaustion, in which PBMCs, which have been producing massive amounts of IDO and IP-10 in plasma, fail to continue this production upon in vitro restimulation. As no decreased IFN-γ-receptor expression nor IFN-γ-induced pSTAT1 was found, we presume the exhaustion might occur at the transcriptional and/or posttranslational level. In systemic diseases like sJIA and MAS, PBMCs are the most suitable cells for research, reflected by their use in many reports. We found a robust IFN-γ responsiveness in primary fibroblasts and endothelial cells, in addition to PBMCs. The production of IFN-γ-induced IP-10 and IDO by these cells was shown in situ on a lymph node of a MAS patient. We propose that in the interpretation of studies in PBMCs, other cell types should also be taken into account.

We recognize that the number of patients (especially HLH patients) tested in this study is limited, which is related to the rarity of the conditions: some population-based studies in Europe showed an annual incidence of sJIA of between 0.3 and 0.8 cases per 100 000 children under 16 years of age [50]; moreover, full-blown MAS only occurs in ~10% of children with sJIA [1]. Although it is well known that primary and secondary HLH have different genetic backgrounds, they both share a final common pathway with a similar clinical and pathological picture [6]. Therefore, for the purpose of our study, all HLH patients were analysed as one group. Furthermore, since our findings in the responses of the five HLH patients were homogeneous despite their different genetic backgrounds, we believe that our findings are of relevance. Nevertheless, we acknowledge that these findings need to be confirmed in larger sample sizes.

In conclusion, sJIA and HLH/MAS patients both show a prominent inflammatory cytokine response, though with a distinct profile. HLH/MAS patients show a clear-cut IFN-γ profile, in contrast to active sJIA patients, in which IL-18 levels greatly outweigh the relatively low IFN-γ levels. IFN-γ responsiveness of PBMCs is equally different between HLH and active sJIA patients, with low responsiveness of HLH/MAS PBMCs. In addition to PBMCs, histiocytes, endothelial cells and fibroblasts may contribute to an IFN-γ profile, and therefore these cells should be taken into account for research into the pathogenesis of sJIA and HLH/MAS. A decreased IL-18/IFN-γ ratio might be an additional signal heightening the suspicion of a MAS episode in sJIA.

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Supplementary data

Supplementary data are available at Rheumatology Online.

References


