Gene expression profiling of peripheral blood mononuclear cells from children with active hemophagocytic lymphohistiocytosis

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**Familial hemophagocytic lymphohistiocytosis (FHL)** is a rare, genetically heterogeneous autosomal recessive immune disorder that results when the critical regulatory pathways that mediate immune defense mechanisms and the natural termination of immune/inflammatory responses are disrupted or overwhelmed. To advance the understanding of FHL, we performed gene expression profiling of peripheral blood mononuclear cells from 11 children with untreated FHL. Total RNA was isolated and gene expression levels were determined using microarray analysis. Comparisons between patients with FHL and normal pediatric controls (n = 30) identified 915 down-regulated and 550 up-regulated genes with more than or equal to 2.5-fold difference in expression (P ≤ .05). The expression of genes associated with natural killer cell functions, innate and adaptive immune responses, proapoptotic proteins, and B- and T-cell differentiation were down-regulated in patients with FHL. Genes associated with the canonical pathways of interleukin-6 (IL-6), IL-10 IL-1, IL-8, TREM1, LXR/RXR activation, and PPAR signaling and genes encoding of antiapoptotic proteins were overexpressed in patients with FHL. This first study of genome-wide expression profiling in children with FHL demonstrates the complexity of gene expression patterns, which underlie the immunobiology of FHL. (Blood. 2011;117(15): e151-e160)

### Methods

**Subjects, sample collection, and genetic analysis**

Parents of patients provided written informed consent for enrollment in this study approved by the Institutional Review Boards of Cincinnati Children’s Hospital Medical Center, in accordance with the Declaration of Helsinki. Whole blood was collected from 11 patients with active FHL before therapy was started. The control group included 30 healthy children (11 males and 19 females) with a median age of 4.5 years. The technical details of the mutational analysis of *PRF1, UNC13D, Rab27A, Sh2D1A*, and *STX1* using polymerase chain reaction (PCR) and direct sequencing have been described previously.12-14

**Total RNA isolation, Affymetrix GeneChip hybridization, image acquisition, and data analysis**

PBMCs were separated by Ficoll gradient centrifugation, placed in Trizol (Invitrogen), and stored at −80°C. Total RNA was isolated and purified.
Table 1. Genetic and clinical characteristics of patients with FHL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Age</th>
<th>Mutations</th>
<th>UNC13D</th>
<th>STX11</th>
<th>NK function in lytic units*</th>
<th>Perforin MCF NK cells†</th>
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<tr>
<td>P33</td>
<td>Female</td>
<td>2 months</td>
<td>50delT + Q481P</td>
<td>ND</td>
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<td>0.0</td>
<td>0.0</td>
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<td>7 years</td>
<td>hV50M</td>
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<td>ND</td>
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<tr>
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<td>h50delT</td>
<td>ND</td>
<td>ND</td>
<td>0.0</td>
<td>0.0</td>
</tr>
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<td>wt</td>
<td>wt</td>
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<td>501</td>
</tr>
<tr>
<td>P101</td>
<td>Male</td>
<td>6 years</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>15.3</td>
<td>440</td>
</tr>
<tr>
<td>P66</td>
<td>Female</td>
<td>15 years</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
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<td>100</td>
</tr>
<tr>
<td>P76</td>
<td>Male</td>
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<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>13.3</td>
<td>634</td>
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<tr>
<td>P92</td>
<td>Female</td>
<td>1 months</td>
<td>1993(−2) a &gt; c + wt</td>
<td>wt</td>
<td>wt</td>
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<td>wt</td>
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<tr>
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<tr>
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<td>16 months</td>
<td>wt</td>
<td>wt</td>
<td>wt</td>
<td>7.4</td>
<td>404</td>
</tr>
</tbody>
</table>

*Normal controls: 5-35 lytic units (mean, 11.3 lytic units).
†Normal controls: 244-502 MCF.

Results

Patient characteristics

Patients in this study fulfilled the diagnostic criteria for FHL according to the Histiocyte Society’s diagnostic guidelines (supplemental Table 1, available on the Blood Web site; see the Supplemental Materials link at the top of the online article). Three patients (P35, P59, and P101) were born to consanguineous parents. Nucleotide sequence analysis identified biallelic disease-causing mutations in PRF1 in patients P33, P59, and P35 (Table 1). Patients P66, P76, P94, P96, P98, P101, and P1002 carried wild-type PRF1, UNC13D, and STX11 and were considered to carry disease-causing mutations of as-yet-to-be identified gene(s). Patient P92 had a 1993(−2) a > c splice site mutation in intron 21 of one allele of UNC13D. UNC18-2 was not tested for the presence of mutations, as this study was carried out before the discovery of that genetic defect in FHL patients.

PCR assays failed to detect the presence of Epstein-Barr virus, cytomegalovirus, or other herpes viruses in the serum of all patients in this study.

Subpopulations of PBMC, NK-cell cytolytic activity, and perforin expression

When the overall number of isolated PBMCs was sufficient, the cellular composition of each sample was determined. As shown in Figure 1, the relative cellular composition of PBMC samples was similar in patients with FHL and controls, with the exception of the T-cell composition in patients P66 and P76 and NK-cell composition in patients P66 and P76. The percentages and absolute number of B cells in patients P76 and P96 were lower than those in controls. As shown in Table 1, an absence or decrease of NK-cell cytolytic activity was noted in 6 of the 11 patients.

Differences in genome-wide expression between patients with FHL and controls

To test the hypothesis that FHL is characterized by distinctive alterations in gene expression, gene expression profiling was performed. Two-group analysis of variance comparisons were carried out (5% Benjamini-Hochberg false discovery rate) on both controls and FHL patients, followed by the use of an expression filter that selected only genes with at least 2.5-fold expression difference. These statistical and expression filters identified 2054 (1284 down- and 870 up-regulated) probe-sets representing 1465 unique and predicted genes that are differentially expressed in patients with FHL compared with that in controls. These 2054 probe-sets were then subjected to two-dimensional cluster analysis, as shown in Figure 2. All patients with FHL are clustered together on the left half of the map in a homogeneous manner, demonstrating the relative unity of gene regulation in PBMCs. Inspection of the data for these 1465 genes showed that 915 had decreased expression and 550 were up-regulated in patients with FHL.
FHL compared with controls (a list of up-regulated and down-regulated genes is shown in supplemental Tables 2 and 3). The number of down-regulated genes was 2 times more than the number of up-regulated genes. Differences in fold changes for differentially expressed genes on the microarray ranged from a decrease of 26.3-fold in the expression of \textit{CX3CR1} (fractalkine receptor gene) to an increase of 192.3-fold in the expression of \textit{CXCL3} (encoding a chemokine ligand with c-x-c motif; supplemental Tables 2 and 3).

**Signaling pathway and functional ontology analyses of genes differentially expressed in patients with FHL**

To determine the biologic meaning of the gene lists, the down- and up-regulated genes, which had at least a 2-fold expression difference, were uploaded to the IPA application, and the enrichment of canonical pathways and molecular networks was inspected. A pathway was considered over-represented if the $P$ value was $\leq .05$, as determined by Fisher exact test. The $P$ value was proportional to the number of genes in a list that corresponds to a given canonical pathway. It should be noted that interpreting the pathways and functions of a set of differentially expressed genes could assess only genes of known functions.

IPA-dependent analyses yielded 39 biologically relevant, over-represented, canonical pathways of the down-regulated gene list related to FHL. Fifteen of these (based on $P$ values) are shown in Figure 3A. These pathways are primarily related to the adaptive immune system, apoptosis, immunodeficiency signaling, NFkB, lipid antigen presentation by CD1, B-cell development, CTLA4 signaling, as well as calcium-mediated apoptosis and other processes related to amino acid metabolism. Three canonical pathways associated with amino acid metabolism were also over-represented in the down-regulated gene list (Figure 3A).
Identical analyses were also conducted on up-regulated genes, and 48 over-represented pathways were identified. Fifteen of these pathways are shown in Figure 3B and include IL-10, IL-6, IL-8, IL-17, TREM, and PPAR pathway signaling; acute phase response signaling; and LXR/RXR activation pathway. The 15 canonical pathways shown in Figure 3 are represented as 92 down-regulated and 85 up-regulated genes (supplemental Tables 4-5).

**Comparative analyses of networks assembled by down-regulated genes in FHL and control patients**

IPA analysis of the down-regulated genes identified several gene networks with IPA scores more than 7, indicating a less than $10^{-7}$ chance that the genes in the network were associated together solely because of random events. The top 5 networks with down-regulated genes are shown in supplemental Table 6. Network 1 received a score of 34 and was assembled from 30 genes that correlate with cell-to-cell signaling and hematologic system development and function, as well as immune and lymphatic system development and functionality. This network had NFkB in the center and was overlaid with canonical pathways of primary immunodeficiency signaling, B-cell development, and communication between innate and adaptive immune cells. Network 2 was associated with metabolic diseases, protein trafficking, and cell cycles and was overlaid with canonical pathways, including propanoate metabolism; valine, leucine, and isoleucine degradation; and aminophosphonate metabolism. Network 3 had TP53 in the center and was found to be correlated with genes associated with DNA replication, recombination, and repair; nucleic acid metabolism; and small molecule biochemistry. Network 4, with 27 focus molecules and a computed IPA score of 29, correlated with functions, such as cellular assembly and organization, molecular transport, and protein trafficking. Network 5, with an IPA score of 28 and assembled from 27 genes, correlated with programmed cell death and muscular and genetic disorders.

**Comparative analyses of networks assembled by up-regulated genes in FHL and control patients**

Network 1 of the up-regulated gene list had an IPA score of 43 and was assembled from 31 genes (supplemental Table 7). This network correlated with cellular functions, such as cellular growth and proliferation, gene expression, RNA damage, cell signaling, genetic disorders, and metabolic diseases. In network 2, TNF and TNF-induced genes (TNFAIP3 and TNFAIP6), TNFAIP1 interacting protein 1 (TNIP1), and tumor necrosis factor superfamily member 9 (TNFSF9) were found in the center connecting to other up-regulated genes in this network. Network 3 had NFkB in the center, which plays an important role in intracellular regulation of immune response, inflammation, and cell cycle regulation. Network 4 correlated with cancer and dermatologic diseases and conditions and was assembled from ERK in the center and various up-regulated genes coding for transcriptional regulators (ZNF165, HEY1, RUNX1, KLF5, and BRG2) and growth factors (JAG1, EREG, HBEGF, and INHBA). Network 5 correlated with cell death, cell cycle, and cellular development and was assembled from CDKN1A and E2F1 in the center and various components regulating cell cycle and cell proliferation.

In a complex disease, such as FHL, multiple interactions of diverse components in the immune system and the complexity of
the various intracellular pathways must be considered in the interpretation of genome-wide gene expression experiments. Although it is presently difficult to understand the biologic outcomes of these interaction patterns in the FHL pathomechanism, some of the genes identified by microarray analysis that are of potential interest in relation to this disease will be highlighted here. To ensure that the gene expression profiles accurately reflected the down-regulation of immune function-related genes, representative genes that were differentially expressed in patients with FHL were assessed using real-time RT-PCR analysis. These included some genes key to the canonical pathways and gene networks. Real-time PCR was performed on samples from patients P33, P35, and P1002, in addition to 2 patients (EH-05 and MW) who were not included in the microarray analysis (Figures 4, 5). The real-time RT-PCR results were in close agreement with microarray data.

**Genes associated with a wide range of innate immune defense mechanisms are down-regulated in FHL**

Microarray analysis identified several immune function-related genes not previously known to be affected in patients with FHL. The expression of Toll-like receptor genes,\(^{21}\) including TLR4, TLR5, TLR7, TLR8, and TLR10, was reduced by more than 3-fold regardless of the genetic cause of the disease (Figure 4A; supplemental Table 2). Genes, such as TIRAP, SARM1, and TRAM, which are activated in signaling cascades by individual TLRs, were also down-regulated. The expression of CD1 and its isoforms\(^{22}\) (CD1A, CD1C, and CD1D) was also reduced in all 11 patients with FHL. The expression of CD1A and CD1D was also analyzed by quantitative RT-PCR in 6 patients and compared well with the microarray observed gene expression changes (Figure 4A; supplemental Table 2). CD1 and its isoforms mediate the presentation of nonpeptide, lipid, and glycolipid antigens to T cells, an important role in the detection and effective clearance of various pathogens.\(^{22}\)

The down-regulation of CD1D has attracted particular attention because the encoded protein restricts NK T cells and has potent immunomodulatory properties, including tumor surveillance, maintenance of self-tolerance, and anti-infectious defenses.\(^{22}\)

**Genes that enhance NK and CTL function and responsiveness are down-regulated in patients with FHL**

Four of the 15 canonical pathways associated with down-regulated genes were related to NK and CTL functions and cytotoxicity. KLRF1 showed decreased expression (supplemental Table 2). KLRF1 encodes a lectin-like receptor, which is involved in NK-mediated cytolysis and is expressed on essentially all human NK cells and a subset of effector memory CD8\(^+\) T cells with an inflammatory NK-like phenotype.\(^{23}\) All 11 patients demonstrated down-regulated expression of KLRG1 (supplemental Table 2).
**KLRG1** encodes a member of the superfamily of inhibitory receptors, which binds to major histocompatibility complex class I ligands on target cells and inhibits NK cells from attacking cells expressing class I antigens at normal levels, such as healthy tissues as opposed to virally infected or transformed cells.\(^\text{24}\)

**CX3CR1**, one of the most down-regulated genes, codes for 7 transmembrane-spanning G protein-coupled receptors that exhibit reduced expression in patients with FHL (Figure 4A; supplemental Table 2). It is expressed in NK cells, monocytes, activated macrophages, and some lymphocyte subpopulations.\(^\text{25}\) The protein encoded by this gene is a receptor for fractalkine (CX3CL1), a transmembrane protein and chemokine involved in the adhesion and migration of leukocytes.\(^\text{26}\) CX3CR1/CX3CL1, a crucial component of optimal host defense against microbial infection, activates the killing functions of phagocytes and augments iNOS-mediated NO generation and proinflammatory cytokine production through the NFκB signaling pathway.\(^\text{27}\) CX3CR1/CX3CL1 signaling is also required for DC-mediated NK-cell activation.

The relative expression of **2B4** (CD244), **PLCγ1**, and **LAT**, which encode for proteins known to be involved in the NK-cell signaling pathway and NK cell-mediated cytotoxicity,\(^\text{28}\) was also suppressed (supplemental Table 2).

**B-cell functions are also impaired in patients with all forms of FHL**

A number of genes with well-characterized roles in B-cell differentiation and function (**BTK, BLNK, CD19, CD79A, CCR2, FCGR2B, CD22, CR2/CD21, and CD72**) were found to be suppressed in the 11 patients with FHL (Figure 4B; supplemental Table 2). Proteins encoded by **FCGR2B**, **CD22**, and **CD72** contain immunoreceptor tyrosine-based motifs in their cytoplasmic tails; these are known as B-cell immune response regulators.\(^\text{29}\) BLNK (adaptor protein B-cell linker) is required for B-cell proliferation.\(^\text{30}\) B-lymphocytes lacking BLNK do not proliferate in response to B-cell antigen receptor engagement.\(^\text{30}\) A recent review of B-cell numbers and functions in newly diagnosed patients with FHL indicated that a significant proportion of patients demonstrate low B-cell counts and hypogammaglobulinemia (A.H.F., unpublished data, July 1, 2010).

**Overexpression of genes coding for pro-inflammatory cytokines**

Defects in cytolytic function in FHL patients lead to the expansion of CD8\(^+\) T cells, which secrete large quantities of cytokines and shed off their receptors. Among the signaling pathways studied,
data revealed a unique landscape where the induction of certain pathways can limit the inflammatory response (eg, the induction of IL-10 signaling and the apparent inhibition of the inflammatory response was coupled with the activation of certain pathways, which promote the inflammatory response [eg, IL-6 and acute phase response signaling]). Genes coding for CSF2, IL-10, IL-1A, IL-1B, IL-1RN, and IL-6 showed more than a 10-fold increase in expression levels (Figure 4C; supplemental Table 3). Genes coding for chemokines (CCL2, CCL20, CCL3, CXCL1, and IL-8) and chemokine receptors (CXCR1, IL-1R1, and IL-1RN) showed high levels of expression in FHL patients. Inflammatory response genes, such as NFKB1 and PTGS2, showed more than a 4-fold increase in expression levels (Figure 4C-D; supplemental Table 3). These increased expression levels of chemokines, cytokines, and their receptors contribute to the proinflammatory responses observed in activated macrophages.6

Three members of the IL-1 family31 (IL-1A, IL-1B, and IL-18) and 2 genes coding for receptors of IL-1 (IL-1R1 and IL-1R2) were overexpressed by more than 10-fold in patients with FHL. Genes known to be induced by IL-1B (pentraxin) or potentially involved in IL-1B secretion (KCNIJ5) were also up-regulated. In FHL patients, IL-1B expression may elicit the rapid activation of a cellular network of genes particularly implicated in inflammatory responses that may create a cellular environment favorable for the inhibition of apoptosis.

The pentraxin-related gene (PTX3) and TNAIP3/A20, which code for TNF-α-induced protein 3/A20 and TNAIP6, were also among the overexpressed genes (Figure 5C; supplemental Table 3). The protein encoded by TNAIP3/A20 is a zinc finger protein and has been shown to inhibit FxNβ activation as well as TNF-mediated apoptosis.32 A previous knockout study on Tnfaip3, a similar gene in mice, suggested that its genetic product is critical for controlling inflammation by terminating TNF-induced FxNβ responses.32 TREM1 (a triggering receptor expressed on myeloid cells-1) and genes in the TREM1 signaling pathway33 (CD83, ITGB1, ICAM1, and TNF) were found to be up-regulated (Figure 4C; supplemental Table 3). TREM1 amplifies acute inflammatory responses by enhancing degranulation and secretion of proinflammatory mediators. Genes associated with TREM1 signaling (CCL2, CCL7, FCGR2B, and NKb2) were also up-regulated and FCGR2B was down-regulated (supplemental Tables 2, 3). Genes in 2 signaling pathways related to lipid metabolism34 (LXR/RXR activation and PPARα/RXRα signaling), which paralleled stimulation of ABCA1, the ATP-binding cassette transporter, were up-regulated in FHL patients (supplemental Table 3). The genes of the PPAR canonical signaling pathway,35 including PPARD, PPARG, and other genes associated with the peroxisome proliferator-activated receptor-δ pathway, were among the up-regulated genes (supplemental Table 3). Liver dysfunction and damage to liver cells were indicated by the overexpression of EDN1, ICAM1, and MMP9 involved in the hepatic fibrosis/hepatic stellate canonical pathway.36

**IFN-γ and IFN-γ-responsive genes were not among the genes differentially expressed in PBMCs of patients with FHL and controls**

Comparison of transcript levels of patients with FHL and controls revealed that IFNs and IFN-inducible genes were not among the differentially expressed genes. Based on the microarray analysis, to further investigate the significance of this observation, we examined the expression of IFNs and IFN-inducible genes. IFN-responsive genes with IFN-responsive elements, such as ADAR, EIF2AK2, IIF16, and TNSF10, were down-regulated (Figure 5B; supplemental Table 3). Others, such as MX1, MyD88, NMI, STAT1, and TRAF3, were not among the differentially expressed genes. IFN-γ and IFN-γ-responsive genes may have been actively suppressed. IFN-γ receptor 2 was also found to be down-regulated (Figure 5A; supplemental Table 3). CCL5, which is induced by IFN-γ and TNF-α, was also down-regulated (Figure 5A; supplemental Table 5). Moreover, the function of one of the up-regulated genes, TNFAIP3/A20 (Figure 5B) is to down-regulate the activity of IRF-3, a key transcription factor for the induction of IFN-γ.37 Another highly up-regulated gene in FHL, SOCS3 (Figure 5B), which operates through the JAK/STAT signaling pathways, lessens the responsiveness of monocyte/macrophages to IFN-γ.38

**Decreased expression of proapoptotic genes and increased expression of antiapoptotic genes in FHL patients**

Apoptosis is a highly regulated process, which is essential for the maintenance of immune homeostasis by rapid disposal of cells that are abnormal, misplaced, nonfunctional, or potentially dangerous to the organism. FHL is characterized by the nonmalignant accumulation of immune cells. Apoptosis-related pathways were found to be one of the most significant biologic processes involved in FHL. The expression of genes (TRAF5, TRAF3IP2, and CASP6) related to the positive regulation of apoptosis and the expression of perforin and granzymes A, K, and H found in CD8+ T cells and NK cells were markedly down-regulated (Figure 5C; supplemental Table 2). The perforin/granzyme system is impaired in these patients either by the inactivating of mutations in PRF1 (observed in patients P33, P59, and P35) or by down-regulation of the perforin/granzyme system, secondary to an unknown genetic cause of the disease.

The genes TNFSF12 (TWEAK) and TNFSF13, which belong to the TNF ligand family and are expressed in hematopoietic tissues, were found to be down-regulated in FHL patients (Figure 5C; supplemental Table 2). These cytokines can induce apoptosis via multiple pathways of cell death in a cell type-specific manner and promote the transition to Th1-based adaptive immunity.39

One of the overexpressed antiapoptotic genes was SERPINB2 (Figure 5C; supplemental Table 3), which encodes plasminogen activator inhibitor type 2, a cytotoxic protective retinoblastoma-binding protein that protects retinoblastoma from calpain cleavage, consequently increasing retinoblastoma levels and enhancing cell survival.40 Another overexpressed gene was BRE (Figure 5C; supplemental Table 3). The BRE protein binds to the cytoplasmic domains of TNFR-1 and Fas and can attenuate death receptor-initiated apoptosis.41 Transgenic BRE mice are significantly more resistant to Fas-mediated lethal apoptosis than wild-type mice.41

**Discussion**

To the best of our knowledge, the present study is the first report of gene expression signatures in patients with active FHL and offers new insights into the molecular pathogenesis of this disease. In FHL, autosomal recessive genetic defects disrupt the mechanisms responsible for target cell and activated T-cell apoptosis, and undermine the elimination of antigen-presenting cells and contraction of immune response. As a consequence, CD8+ T cells, NK cells, and macrophages remain activated and mutually stimulate each other. Therefore, the use of PBMCs is a reasonable starting point for the analysis of the global gene expression of this disease. Comparisons of patients with FHL and controls revealed that
approximately 8% of the gene probes of the entire array was differentially expressed in PBMCs of patients with FHL (both up- and down-regulated) compared with that in PBMCs of controls, reflecting broad alterations in the gene expression of these cells. Some of the genes (IL-6, IL-8, IL-10, TNF, and M-CSF) identified in this study as overexpressed in patients with FHL confirm previous findings, accentuate basic principles on FHL, and support the analytic methods used and the new findings presented here.

It was hypothesized that the PBMC, the most accessible tissue for analysis of gene expression, would reflect pathologic events occurring in FHL patients. However, gene expression patterns might be influenced by quantitative variations within PBMC subsets. No significant changes were observed in the subcell counts of the patients analyzed, demonstrating that even without separation of peripheral blood cells into subsets, subtle and distinguishable differences in the gene expression profiles of patients with FHL and healthy subjects can be readily identified.

The expression levels of more genes were down-regulated than up-regulated in FHL patients. The down-regulated genes code for proteins involved in signaling and regulation of transcription, as well as immune responses and defense mechanism pathways, suggesting that extensive genetic suppression of immunity in FHL patients is central to the disease process and presents at an early stage of FHL. Consequently, susceptibility to secondary infections in FHL patients may be intrinsic to the disease and not directly related to the immunosuppressive drugs used to target hyperactivated T cells and histiocytes, as has been assumed for several years.

The “cytokine storm” has been considered as a hallmark of the immunologic phenotype of FHL. Gene expression data suggest that the underlying mechanism of FHL includes an imbalance of cytokine homeostasis, massive up-regulation (> 50-fold) of genes encoding proinflammatory proteins (IL-8, IL-6, CCL3, and CCL4), and a moderate (0.58- to 10-fold) increase in the expression of genes coding for anti-inflammatory proteins (TGF-β, IL-1RA, TNFAIP3, and IL-10). This pattern has also been observed in graft-versus-host disease, acute respiratory distress syndrome, and SIRS. There were no significant increases in the up-regulation of genes that include IFN (α, β, or γ). The presence of increased levels of IFN-γ in the plasma of patients with FHL has been indicated in several independent case studies of FHL. Abnormal and excessive production of IFN-γ has also been observed in a murine model of FHL type 2, although the genetic background of this model is uniquely prone to viral induction of FHL. Osugi et al reported no increase in IFN-γ levels in patients with FHL, regardless of the level of IL-10 in serum. In another study by Nagasawa et al, 2 of 4 patients with FHL showed no elevated levels of plasma IFN-γ. Serum level of IFN-γ was not elevated in 2 of 5 patients with FHL. In a recent review of 33 patients with FHL, at the Cincinnati Children’s Hospital Medical Center, eligible for hematopoietic cell transplantation, 18 showed low serum level (< 5 U/mL) of IFN-γ in the active stage of the disease before the prehematopoietic cell transplantation therapy started (A.H.F., unpublished data, July 1, 2010). In summary, these observations suggest that PBMCs from patients with FHL display different gene expression program for IFN genes, which may depend on the diversity of triggering agents of the disease.

Patients with severe acute respiratory syndrome showed a pattern similar to FHL, with several up-regulated genes in the innate immune system, but not IFN-γ or IFN-γ-induced genes. After measles virus infection, IFN-γ and IFN-γ-regulated genes are not up-regulated. Similarly, gene expression profiling of PBMCs in patients with systemic juvenile idiopathic arthritis showed a low level of IFN gene expression and a relative paucity of genes whose expression is induced by IFN-γ.

Recent gene expression studies have shown that SjIA is associated with the up-regulation of innate immune pathways, including IL-6, TLR/IL-1R, and PPAR signaling pathways, and the down-regulation of gene networks involving NK-cell, T-cell, and major histocompatibility complex-related biologic processes, including antigen presentation. An unexpected finding in SjIA patients was the highly up-regulated erythropoiesis signature of 67 genes, which normally expressed only in immature red blood cells. The presence of this signature is thought to be a reflection of increased turnover of red blood cells, perhaps secondary to hemophagocytosis. Of these 67 genes, 12 (ANXA3, CA1, CD34, ELL2, HBB, HBG1, HBM, PINK1, PLEK2, SESN3, a member of the solute carrier family [SLC], and SNCA) were also highly up-regulated in FHL patients. These 12 genes include erythropoiesis-related genes that code for fetal and embryonic hemoglobin. Members of the SLC family of transporter genes are also among the genes up-regulated in both FHL and SjIA patients. According to the literature, to date, the overexpression of genes unique to erythropoiesis have only been observed in patients with FHL or SjIA and do not overlap with the list of erythropoiesis genes up-regulated in patients with sickle cell anemia or with severe hemolysis and consequent prolonged erythropoiesis.

Several up-regulated signaling pathways (TREM1, LXL/RXR, PPAR, and acute phase response signaling) in FHL are also over-represented in SIRS and are associated with poor outcomes after pediatric septic shock. Of the 26 down-regulated genes, 7 (ABHD, DNNJC3, DPEP, HRB2, RGS2, SLC39A, and SRPK2) expressed in nonsurvivors of pediatric septic shock were among the down-regulated genes observed in FHL patients, whereas 18 (CCL2, CEACAM1, CLEC, DIT4, EMP1, ENPP2, G0S2, GPR171, IL3, MAFF, PDE4D, RGS1, ROCK, SLC39A8, SOCS1, THBS1, TNFAIP3, and TRIB1) of the 34 up-regulated genes in nonsurvivors of pediatric septic shock were among the up-regulated genes observed in FHL patients, underscoring the similarities between these 2 conditions.

The data of this study reveal some gene expression patterns common between FHL, SIRS, and pediatric septic shock. Signaling pathways represented by down- and up-regulated genes in patients with FHL related to innate immunity, immune response, defense response, and inflammation were also represented by differentially expressed genes in patients with SIRS and pediatric septic shock. In addition, 24 genes differentially expressed in FHL, compared with controls, were also a part of the 60 differentially expressed genes found in both survivors as well as nonsurvivors after pediatric sepsis. CX3CR1, which was found to be down-regulated in both nonsurvivors and survivors, was one of the most suppressed genes in patients with FHL. Cells presenting a proinflammatory, Th1, and/or cytotoxic phenotype preferentially express CX3CR1. Therefore, the down-regulation of CX3CR1 expression in patients with FHL or septic shock might be related to decreased Th1 immune responses that are present in both groups. These data corroborate, at the genomic level, the longstanding concept that FHL, SIRS, sepsis, and septic shock share common features. Gene expression in FHL reflects the delicate balance between extremely complex proinflammatory and anti-inflammatory events reminiscent of SIRS and septic shock.
FHL is a challenge for the immune system. The autosomal recessive form of the disease results from a defect in the granule-dependent cytotoxic pathway, followed by an intrinsic imbalance in negative and regulatory networks involved in pathogen detection and innate and adaptive immunity. In summary, the gene expression pattern observed in patients with active FHL suggests that the primary disease-causing mutation generates a self-fueling process of dysregulated immune responses with extensive down-regulation of genes related to innate and adaptive immune responses, as well as proapoptotic signals, along with the up-regulation of genes coding for proinflammatory cytokines and antiapoptotic factors.

Acknowledgments

The authors thank Drs Hector Wong and Michael Jordan for helpful discussions regarding this study. This work was supported by the National Institute of Allergy and Infectious Diseases (grants R21AI079759-02 and R21AI076746-01) and the Histiocytosis Association of America.

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