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## Pathogenesis of systemic juvenile idiopathic arthritis: some answers, more questions

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### Abstract

Systemic juvenile idiopathic arthritis (sJIA) has long been recognized as unique among childhood arthritides, because of its distinctive clinical and epidemiological features, including an association with macrophage activation syndrome. Here, we summarize research into sJIA pathogenesis. The triggers of disease are unknown, although infections are suspects. Once initiated, sJIA seems to be driven by innate proinflammatory cytokines. Endogenous Toll-like receptor ligands, including S100 proteins, probably synergize with cytokines to perpetuate inflammation. These and other findings support the hypothesis that sJIA is an autoinflammatory condition. Indeed, IL-1 is implicated as a pivotal cytokine, but the source of excess IL-1 activity remains obscure and the role of IL-1 in chronic arthritis is less clear. Another hypothesis is that a form of hemophagocytic lymphohistiocytosis underlies sJIA, with varying degrees of its expression across the spectrum of disease. Alternatively, sJIA with MAS might be a genetically distinct subtype. Yet another hypothesis proposes that inadequate downregulation of immune activation is central to sJIA, supporting evidence for which includes 'alternative activation' of monocyte and macrophages and possible deficiencies in IL-10 and T regulatory cells. Some altered immune phenotypes persist during clinically inactive disease, which suggests that this stage might represent compensated inflammation. Despite much progress being made, many questions remain, providing fertile ground for future research.

### Introduction

#### The clinical picture

Systemic juvenile idiopathic arthritis (sJIA) is a unique type of childhood chronic arthritis that is currently classified as a subtype of juvenile idiopathic arthritis (JIA).<sup>1</sup> Unlike the other subtypes of JIA, sJIA shows no sex bias or peak age at onset during childhood. Extra-articular features, including daily spiking fevers, fleeting salmon-colored macular rash,

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lymphadenopathy, hepatosplenomegaly and polyserositis, can overshadow joint inflammation at disease onset. Erythrocyte sedimentation rate, C-reactive protein, and counts of neutrophils and platelets are typically quite elevated, reflecting systemic inflammation. The clinical course at later stages is highly variable. In one study, approximately 40% of patients with sJIA (followed on average for close to 5 years) had a monocyclic course, <10% had a polycyclic course and >50% had a persistent course (Box 1).<sup>2</sup> The proportion of patients with monocyclic disease might be underestimated due to lack of ascertainment. In many patients with polycyclic or persistent sJIA, the systemic features subside during the initial months or years of disease, whereas arthritis is progressive. The joint disease is notable for early destructive changes, ankylosis of the cervical spine, wrists and mid-foot, and reduced responsiveness to treatments that are effective in polyarticular JIA. Responses to other treatments, including among patients. Polyarticular arthritis at 6 months after disease onset, particularly with hip involvement and highly increased platelet counts, is predictive of joint damage by 2 years.<sup>3,4</sup> With persistent disease, significant growth impairment - beyond the extent attributable to steroid therapy - is typical. In North America and Europe, sJIA accounts for roughly 10% of cases of JIA; in India and Japan, where oligoarticular disease is less common, sJIA represents about 30% and 50% of reported JIA cases, respectively.<sup>5,6</sup> Importantly, sJIA accounts for a disproportionate share of JIA-related mortality; this excess mortality is attributable primarily to complications of systemic inflammation (such as macrophage activation syndrome [MAS] and amyloidosis) and of immunosuppressive therapies.

### **Association with macrophage activation syndrome**

The association of sJIA with MAS is a striking feature of the disease. MAS is a potentially fatal condition that is characterized by persistent fever, cytopenias, liver abnormalities, coagulopathy and central nervous system dysfunction. Well-differentiated macrophages with hemophagocytic activity are the pathologic hallmark of MAS.<sup>7</sup> Presumed precipitating factors in children with sJIA include various infections and medications.<sup>7</sup> Approximately 10% of patients with sJIA are diagnosed with MAS, some of whom suffer repetitive episodes.<sup>8,9</sup> However, in as many as 50% of patients with active sJIA, MAS is evident in bone marrow aspirates.<sup>10,11</sup> MAS is an acquired form of hemophagocytic lymphohistiocytosis (HLH), a group of histiocytic disorders that also includes inherited disorders.<sup>7</sup>

### **Pathophysiology of sJIA**

Data from recent genetic and immunologic studies and from clinical trials of biologic therapies have provided substantial new insights into sJIA pathophysiology, but investigations have yet to reveal a primary underlying etiologic pathway for this disease. Indeed, the clinical heterogeneity of sJIA is paralleled by evidence for the involvement of various immune pathways and genetic influences. However, the studies to date have not associated immune variations with specific differences in disease course, response to medication or clinical outcomes, except for immune features associated with MAS. In this article, we review the current literature on sJIA pathogenesis, highlighting alternative mechanistic hypotheses, emerging themes and unanswered questions.

### sJIA as a pathogen-triggered disease

The increased incidence of sJIA compared with that of adult-onset Still disease (the adult equivalent of sJIA) has implications for the etiology of sJIA. One scenario is that infectious agents that are typically encountered in childhood initiate sJIA; no single environmental trigger has been identified, although this lack of an obvious candidate could point to multiple common agents being capable of initiating sJIA. Consistent with the possibility of infectious triggers, onset of sJIA seemed to be seasonal in some studies.<sup>12–14</sup> Alternatively, onset of disease during childhood could be associated with the presence of stronger or increased numbers of disease susceptibility alleles.

### sJIA as an autoinflammatory disease

**Autoimmune versus autoinflammatory diseases**—Advances in elucidating the basic mechanisms of innate immunity have led to a new paradigm for understanding immune-mediated diseases, which is based on the relative contributions of abnormalities in the adaptive and innate immune responses. At one end of the spectrum are classic autoimmune diseases, such as rheumatoid arthritis or type 1 diabetes, in which adaptive immune responses are central. These diseases are associated with autoreactive antigen-specific T lymphocytes and high titers of autoantibodies, and typically show strong associations with MHC class II alleles. By contrast, abnormalities in pathways of innate immunity lead to a distinct group of pathologic conditions known as autoinflammatory diseases, in which monocytes and neutrophils, rather than lymphocytes, are the predominant effector cells.<sup>15</sup> Initially, the autoinflammatory label was applied to a group of diseases with single-gene defects. These diseases have common clinical features including recurrent fevers and multisystem inflammation that usually affects the joints, skin, gastrointestinal tract and eyes, and are complicated by amyloidosis in the long term. A prototypical example, neonatal-onset multisystem inflammatory disease (NOMID), is caused by mutations in the NLRP3 gene, which encodes cryopyrin (also known as NACHT, LRR, and PYD domains-containing protein 3). Recently, the class of autoinflammatory diseases has been widened to encompass multigenic diseases in which innate inflammation is prominent, such as Crohn disease, type II diabetes and sJIA.<sup>15</sup> Many clinical features of sJIA, including fever, arthritis and increased risk of amyloidosis, fit this classification. Notably, however, the occurrence of MAS is very rare in the monogenic autoinflammatory diseases.<sup>16</sup>

**Genetic investigations**—In common with other autoinflammatory conditions, but unlike other subtypes of JIA, strong associations with MHC class II alleles have not been found for sJIA, although in some populations there might be an association with HLA-DR4 in patients with erosive arthritis.<sup>17</sup> The inherited risk factors for sJIA most consistently described are polymorphisms in the promoter elements and genes encoding tumor necrosis factor (TNF),<sup>18</sup> IL-6,<sup>19,20</sup> IL-10,<sup>21,22</sup> macrophage migration inhibitory factor (MIF)<sup>23,24</sup> and the IL-1 family (specifically, IL1A, IL1RN, IL1R2 and possibly IL1F10).<sup>25</sup> In addition, an association has been reported between susceptibility to sJIA with single nucleotide polymorphisms (SNPs) within SLC26A2, mutations in which cause diastrophic dysplasia.<sup>26</sup> Another study found that 2 of 9 patients with sJIA had loss-of-function variants in P2X7, which encodes an ATP receptor that regulates IL-1 processing and secretion.<sup>27</sup> Altogether,

these findings support the model of sJIA as a multigenic disease, with each allele identified thus far providing a small contribution to inherited susceptibility.

Genes responsible for monogenic autoinflammatory diseases have been evaluated in sJIA. An initial study found no notable associations of sJIA with NLRP3, NOD2, MEFV or PSTPIP1, mutations in each of which cause such diseases.<sup>28</sup> In a 2009 report, a subgroup (15%) of Turkish patients with severe sJIA harbored MEFV mutations.<sup>29</sup> MEFV, the causal gene in familial Mediterranean fever, encodes pyrin - a protein that affects the IL-1 $\beta$ -activation pathway but whose precise function is unknown. Many patients with inherited autoinflammatory syndromes do not have defined genetic lesions, so additional overlap between risk factors for these diseases and for sJIA could yet be revealed. A genome-wide association study of sJIA is currently underway.

**Innate immune activity in sJIA**—Consistent with its classification as an autoinflammatory disorder, sJIA is characterized by prominent innate immune activity and a limited role for adaptive immunity. In active disease, circulating innate immune cells - monocytes, neutrophils and immature (CD34<sup>+</sup> CD33<sup>+</sup>) myelomonocytic precursors - are expanded in number.<sup>30–32</sup> Microarray studies of peripheral blood mononuclear cells (PBMCs) from patients with sJIA show increased expression of genes associated with the activation of the monocyte/macrophage lineage.<sup>30,33–36</sup> Expression profiles also demonstrate upregulation of innate immune receptors (for example, CARD12 [NLR family CARD domain-containing protein 4] and Toll-like receptor [TLR] 5) and signaling pathways (such as TLR–IL-1, IL-6 and PPAR- $\gamma$  signaling), as well as downregulation of gene networks involving processes related to natural killer (NK) cells, T cells and MHC.<sup>30,33–37</sup> In one study, gene expression profiles from patients with sJIA overlapped to a greater extent with NOMID-associated transcripts than with those associated with systemic lupus erythematosus, polyarticular JIA or Kawasaki disease.<sup>34</sup>

**Proinflammatory mediators**—Many features of sJIA seem to be explained by the known effects of innate proinflammatory cytokines, IL-1 and IL-6 in particular but also macrophage colony-stimulating factor (M-CSF), TNF and IL-18 (Figure 1). The implicated cytokines are principally, though not exclusively, produced by monocytes, macrophages and neutrophils. Translational studies of patient samples also support the contributions of these mediators to disease.

**IL-1 and S100 proteins**—IL-1, a protein with pleiotropic effects, upregulates its own transcription as well as that of IL-6, among other cytokines. In addition to driving systemic inflammation, IL-1 stimulates the destruction of cartilage and bone; for example, by inducing follistatin-related protein 1, an inflammatory product of joint matrix cells and a possible biomarker of sJIA disease activity.<sup>38</sup> Interestingly, gene expression studies of sJIA PBMCs have not usually shown increased expression of IL1B,<sup>30,33–36</sup> but serum from patients with active sJIA can induce the transcription of various IL-1 related genes, including IL1B, IL1RN, IL1R1 and IL1R2, in PBMCs from healthy individuals.<sup>33</sup> In addition, PBMCs from patients with untreated, new-onset sJIA demonstrated a possible IL-1-driven signature.<sup>30</sup> A central role for IL-1 also emerged in pathway analysis of plasma proteins that are elevated at the time of sJIA flare.<sup>39</sup>

The most compelling evidence for the involvement of IL-1 in sJIA is the successful treatment of sJIA with inhibitors of IL-1 such as anakinra, a soluble IL-1 receptor antagonist (IL-1Ra) that is similar to naturally occurring IL-1Ra. Serum levels of endogenous IL-1Ra are elevated in active sJIA, though not to the degree observed in polyarticular JIA.<sup>40–42</sup> Augmenting IL-1 inhibition above these levels is rapidly therapeutic in a substantial proportion of patients with sJIA.<sup>33,43</sup> Concurrent with a clinical response to anti-IL-1 therapy, many disease-associated changes in gene expression in PBMCs revert to normal.<sup>33,36,37</sup>

In initial studies of sJIA treatment with anakinra, about half of the patients treated were responders, whereas the other half had a transient response, followed by the return of arthritis and elevated levels of acute-phase proteins, although fever and rash did not typically recur.<sup>37,27</sup> The incidence of poor response to anakinra in some patients suggested the possibility of biological subgroups, a hypothesis supported by the finding that this group had higher serum levels of granulocyte colony-stimulating factor and lower serum levels of IL-9 than those who responded to anakinra therapy.<sup>27</sup> However, evidence from a recent retrospective study of 46 patients suggests that, if given as a first-line drug, anakinra leads to the resolution of both systemic symptoms and arthritis and protects against the development of persistent arthritis in approximately 90% of patients, and that the non-responders might have been underdosed.<sup>44</sup> These data, which require confirmation, argue that IL-1 drives the initiating abnormalities in most cases of sJIA. The seemingly reduced success of IL-1 inhibition in patients with refractory arthritis implies that IL-1-independent mechanisms arise later in the disease course, if disease is uncontrolled.

The basis of the observed excess IL-1 activity in sJIA is not known. The initial rapid response and continued dependence on IL-1 inhibition in many patients with sJIA are reminiscent of responses seen in NLRP3-associated autoinflammatory syndromes. In vitro, however, NLRP3-mutant cells show impaired redox homeostasis associated with rapid secretion of IL-1,<sup>45</sup> whereas sJIA monocytes do not.<sup>27</sup> Monocytes from both disease groups are resistant to enhancement by ATP of lipopolysaccharide-stimulated IL-1 $\beta$  secretion in vitro, but probably for different reasons (Figure 2).

The occurrence of a clinical response to IL-1 inhibition, despite the absence of excess IL-1 secretion by sJIA blood monocytes in vitro, suggests that increased IL-1 activity in vivo might involve other cell types, privileged sites, or different triggers or pathways, and some evidence supports these possibilities. IL-1 levels in synovial fluid are higher in active sJIA than in active polyarticular JIA, whereas levels in circulating plasma are lower in sJIA.<sup>46</sup> Pathways other than the caspase-1 - inflammasome pathway that is shown in Figure 2 can contribute to IL-1 $\beta$  secretion in inflammatory diseases (including models of arthritis).<sup>47,48</sup> For example, proteinase-3 in neutrophils,<sup>48</sup> secreted serine proteases of mast cells and neutrophils which, in turn, further increases production of S100 proteins (Figure 3). In vitro, the disruption of this loop reduces the production of IL-1 $\beta$  by PBMCs.<sup>50</sup>

**IL-6**—In addition to IL-1, IL-6 also contributes to sJIA pathogenesis. Serum levels of IL-6 rise and fall in parallel with sJIA fever spikes, and increased IL-6 production is associated with thrombocytosis, microcytic anemia, growth retardation, and osteopenia.<sup>52–54</sup> In some,

but not all, reports, IL-6 is spontaneously released in vitro from circulating immune cells in sJIA,<sup>55,56</sup> although this phenotype is not unique to sJIA.<sup>57</sup> Importantly, neutralizing IL-6 with tocilizumab in sJIA led to clinical improvement in preliminary studies.<sup>58,59</sup> Positive response to this therapy is associated with the upregulation of cartilage oligomeric matrix protein, a marker of growth cartilage, supporting a role for IL-6 in growth impairment in sJIA.<sup>60</sup> Another consequence of elevated IL-6 in active sJIA is modulation of levels of proteases and their regulators (for example, MMP-9 and its inhibitor TIMP-1).<sup>61–64</sup> Presumably as a consequence of these changes, novel fragments of various proteins can be detected in plasma<sup>39</sup> and urine<sup>63</sup> during flare; these peptides might provide a novel source of sJIA biomarkers.

### sJIA as a defect of Immunoregulation

Anti-inflammatory responses are also stimulated in active sJIA, presumably in an attempt to quell inflammation. In untreated early disease, PBMCs show increases in transcripts for negative regulators of innate immune responses, such as IL1RN (encoding IL-1Ra) and SOCS3 (encoding suppressor of cytokine signaling 3), which is induced by IL-1 and IL-6 and inhibits their signaling pathways.<sup>30,34</sup> Additional anti-inflammatory pathways are detected during sJIA, as discussed below. It seems that none are sufficient to resolve the inflammation in sJIA. The exception might be disease resolution in monocyclic sJIA but this possibility has not been investigated.

**IL-10**—Expression of the immunoregulatory cytokine IL-10 is detectable during active disease. IL-10 mediates a broad range of anti-inflammatory action with effects on both innate and adaptive immune responses. The IL10 allele associated with sJIA is a ‘low expressor’,<sup>21,22</sup> and average levels of IL-10 in plasma and synovial fluid during active disease, although elevated compared with inactive disease, are lower than levels in active poly articular JIA.<sup>46,65</sup> These results have suggested that the anti-inflammatory IL-10 response is deficient in sJIA. One downstream effect of IL-10 is induction of the enzyme heme oxygenase 1 (HO-1), which mediates anti-inflammatory effects through its products (discussed below). Serum levels of HO-1 in sJIA correlate with erythrocyte sedimentation rate.<sup>66</sup>

**Alternatively activated monocyte/macrophages**—The monocyte/macrophage lineage is strikingly plastic in response to environmental cues and undergoes different forms of polarized activation. ‘Classically activated’ M1 macrophages are highly proinflammatory, whereas ‘alternatively activated’ M2 macrophages fine-tune or resolve inflammatory responses, perform scavenger functions and promote tissue remodeling and repair.<sup>67</sup> M1 and M2 macrophages also participate in type 1 T helper (TH1) and TH2 responses, respectively. Interferon (IFN)- $\gamma$  is the key cytokine driving the M1 pathway, whereas the addition of IL-4, IL-3, IL-10 or steroids to monocytes in culture promotes their differentiation into M2 macrophages.<sup>67</sup> Subtypes of M2 macrophages have been described, in association with different polarizing conditions (Table 1). sJIA monocytes express transcripts related to the M2 pathway(s), for example, the genes MS4A4A, GPR84, CCL2, ARG1, CD163 and IL10.<sup>30,34</sup> The M2 phenotype in sJIA monocytes is not the result of steroid treatment, because this phenotype is observed in untreated patients.<sup>30</sup> sJIA monocytes are also M2-like

in their resistance to apoptotic stimuli.<sup>68,69</sup> In addition, the absence of IFN-induced gene expression signatures in PBMCs from patients with active sJIA is consistent with a more-M2-like phenotype.<sup>30,34,35,70</sup> Interestingly, anakinra treatment might reverse this aspect of the phenotype; in a recent study, elevated type I IFN-regulated transcripts were observed in PBMCs of the majority of anakinra-treated subjects, regardless of their clinical response.<sup>37</sup> This finding implies that IL-1 might contribute (directly or indirectly) to inhibition of IFN signaling, for example through inducing SOCS-3. As M2 polarization of monocyte/macrophages is a 'deactivating' program, it seems plausible that it is induced to suppress the inflammatory state.

In common with alternatively activated circulating monocytes, hemophagocytic bone-marrow macrophages, observed in occult<sup>10</sup> or overt MAS, express CD163. CD163 can bind hemoglobin-haptoglobin (Hb-HP) complexes, initiating pathways that facilitate adaptation to the oxidative stress that is caused by free heme and iron.<sup>71</sup> Uptake of Hb-HP complexes by CD163<sup>+</sup> macrophages induces intracellular HO-1 activity. HO-1 degrades the heme subunit of Hb into biliverdin, which is subsequently converted to the anti-inflammatory components bilirubin, carbon monoxide and free iron. The free iron is either sequestered with ferritin within the cell or transported to red blood cell precursors in the bone marrow. Thus, the CD163<sup>+</sup> macrophages seem to have a protective, anti-inflammatory role.<sup>10,11,72,73</sup>

**T<sub>REG</sub> cell–T<sub>H</sub>17 cell balance**—Another anti-inflammatory mechanism that might be deficient in sJIA is the action of T regulatory (T<sub>REG</sub>) cells. Reduced frequency of circulating T<sub>REG</sub> cells has been noted in active sJIA.<sup>74</sup> In the setting of autologous stem cell transplantation, clinical improvement correlates with normalized T<sub>REG</sub> cell frequency in blood.<sup>74</sup> Restoration of T<sub>REG</sub> cells by this method implies that T<sub>REG</sub> cell deficiency is not a primary defect in sJIA. In inflamed joints of patients with various subtypes of JIA, including sJIA, T<sub>REG</sub> cells are found in inverse proportion to the highly inflammatory CD4<sup>+</sup> subset of T<sub>H</sub>17 cells,<sup>75,76</sup> which are strongly implicated in destructive arthritis.<sup>77,78</sup> T<sub>H</sub>17 cells develop from naïve T cells or T<sub>REG</sub> cells, and IL-1 $\beta$  is a critical cytokine in T<sub>H</sub>17 differentiation, synergizing with IL-6 and IL-23. The balance of T<sub>REG</sub> cells and T<sub>H</sub>17 cells is implicated as a determinant of joint-related outcomes,<sup>75</sup> and might have a role in the chronic arthritis stage of sJIA.<sup>80,81</sup>

**sJIA as an HLH variant**—The association of sJIA with MAS, which shares features with acquired HLH in other disease settings, has led to the hypothesis that sJIA represents an HLH variant. Evidence for occult MAS in a substantial proportion of patients with sJIA supports this possibility.<sup>10</sup>

**HLH genes and sJIA**—As one test of this hypothesis, genes responsible for familial HLH have been studied in sJIA. Initial SNP analysis limited to four genes, encoding two cytotoxic effectors, perforin (PRF1) and granzyme B (GZMB), and two regulators of secretory cytotoxic granule function (RAB27A and UNC13D, which is also known as Munc13-4), did not reveal any associations with sJIA.<sup>82</sup> However, more extensive SNP typing, albeit in small numbers of patients, has yielded provocative results. For example, Munc13-4 sequences with HLH-associated mutations were found in 2 of 18 patients with sJIA and MAS, and a novel Munc13-4 SNP haplotype was present in more than half of the remaining

sJIA patients with MAS versus ~10% of controls or of those with sJIA but not MAS.<sup>83</sup> In another study, missense mutations in PRF1, many of which were associated with perforin dysfunction, were present in 20% of patients with sJIA and were more frequent in sJIA with MAS than without MAS.<sup>84</sup> Likewise, polymorphisms in IRF5, which encodes a transcription factor in the proinflammatory TLR signaling pathway, were associated with susceptibility to sJIA with MAS but not without MAS.<sup>85</sup> Thus, emerging evidence suggests sJIA with MAS might represent a genetically distinct subtype of sJIA that is an HLH variant.

### HLH-related immune features in sJIA with MAS

**Interferon  $\gamma$  production**—HLH is thought to stem from excessive activation and expansion of (mostly CD8<sup>+</sup>) T cells, which produce IFN- $\gamma$  and stimulate macrophages to produce proinflammatory cytokines. Serum levels of IFN- $\gamma$  and neopterin, a product of IFN-driven macrophages, are higher in sJIA with MAS than without MAS.<sup>86,87</sup> Liver tissue from patients with MAS demonstrates massive infiltration by IFN- $\gamma$ -producing CD8<sup>+</sup> T cells and hemophagocytic macrophages that produce TNF and IL-6.<sup>88</sup> Whether IL-1 has a role in HLH is not clear. MAS can develop in children being treated with anakinra as a first-line drug;<sup>44</sup> conversely, anakinra has been effective in the treatment of MAS.<sup>89</sup>

**Impaired cytotoxic function**—In inherited HLH, the underlying gene defects impair cytotoxic functions,<sup>90</sup> indicating a link between this deficiency and the uncontrolled proliferation of macrophage and activation of T cells. In one possible mechanism for this link, defective killing of infected cells results in persistent antigenic stimulation of T cells, leading them to produce high levels of macrophage-activating cytokines, such as IFN- $\gamma$ . In another mechanism, abnormal cytotoxic cells fail to remove activated macrophages and IFN- $\gamma$ -producing cells during the contraction phase of the immune response, perpetuating inflammation.<sup>91</sup>

Consistent with a mechanistic similarity between the genetic HLH diseases and sJIA, deficient cytolytic activity in circulating NK cells distinguishes sJIA from other subtypes of JIA and is prominent in sJIA with MAS.<sup>92–94</sup> A recent study indicates that NK cell dysfunction in sJIA is associated with defective phosphorylation and signaling by the  $\beta$  chain of the IL-18 receptor.<sup>95</sup> Interestingly, a member of the IL-1 family, IL-1F7b, could contribute to this phenotype, because the complex formed by its attachment to IL-18 binding protein (IL-18BP) binds to the IL-18 receptor  $\beta$  chain and blocks IL-18 signal transduction.<sup>96</sup> Another source of cytolytic dysfunction in sJIA could be polymorphisms in relevant genes, as described above. Along these lines, in a study of new-onset, untreated sJIA, patients in a ‘very high serum ferritin’ subgroup differed from those in a ‘normal ferritin’ group in having higher expression levels of RAB27A and lower expression levels of SH2D1A, genes that are critical for activation of the cytolytic pathway.<sup>30</sup> The ‘high ferritin’ subgroup was also at higher apparent risk for MAS.

**Enhanced red blood cell turnover**—‘High ferritin’ patients are also distinguished by a gene expression signature, originating from CD71<sup>+</sup> erythroid precursors.<sup>32</sup> This erythropoiesis signature and expansion of erythroid precursors are found in HLH, probably



as a result of increased blood cell turnover. In new-onset sJIA, this signature might reflect subclinical hemophagocytosis.<sup>32</sup>

**Excess IL-18 activity**—Uniquely high serum levels of biologically active IL-18 are observed in active sJIA, in comparison with other inflammatory diseases.<sup>65,86</sup> A contribution of this cytokine to MAS is suggested by elevated circulating levels of IL-18 during MAS, and by the role of IL-18 in the induction of IFN- $\gamma$  production by CD8<sup>+</sup> T cells and NK cells.<sup>30,95</sup> IL-18 bioactivity is regulated by its soluble decoy receptor, IL-18BP, and a severe imbalance between levels of IL-18 and IL-18BP has been hypothesized to drive MAS and other forms of acquired HLH.<sup>97</sup> Data also suggest that the IL-18 receptor binds an unknown ligand that delivers an anti-inflammatory signal, and competition from high levels of IL-18 will suppress this response.<sup>98</sup> However, in the absence of IL-12, which is not expressed at very high levels in sJIA, IL-18 can divert TH1 cells to cells that produce IL-13 and IL-4, raising the alternative possibility that IL-18 participates in an immunoregulatory (negative) feedback loop.<sup>99</sup> The cellular source of serum IL-18 in sJIA has not been extensively studied, but is suggested by one report to be bone marrow macrophages.<sup>100</sup> Intriguing, but not well understood, is the strong correlation of IL-18 levels with serum ferritin in sJIA.<sup>101</sup> Ferritin, which is critical in iron homeostasis, is also an acute-phase protein with an antioxidant and cytoprotective role during inflammatory states. IL-18 induces production and secretion of H-ferritin by macrophages,<sup>102</sup> whereas serum ferritin is typically L-ferritin. However, serum ferritin in malignant histiocytosis consists mainly of H-ferritin.<sup>103</sup>

### Insights from a new animal model of MAS

The most recent animal model of MAS, derived in normal mice by chronic TLR9 stimulation, provides several new insights.<sup>104</sup> The mice developed features characteristic of MAS: splenomegaly, pancytopenia, hyperferritinemia, fibrin microthrombi, elevated serum levels of IFN- $\gamma$  and hepatic inflammation. A small population of CD8<sup>+</sup> T cells was activated, but activation of NK cells was more robust. Strikingly, hemophagocytic cells were absent in the bone marrow, arguing against a role for these cells in disease initiation. However, after blockade of the receptor for IL-10, hemophagocytic cells appeared, indicating a critical protective function for IL-10. Interestingly, experiments in this model also suggested a contribution of an as-yet unidentified IFN- $\gamma$ -producing myeloid cell.

### Inactive sJIA as compensated inflammation

Several observations suggest that some immunologic alterations remain during periods of sJIA quiescence. Levels of canonical monocyte subset surface markers, CD14 and CD16, remain elevated during inactive disease, although only a few patients off medication were assayed.<sup>31</sup> Levels of the acute-phase protein serum amyloid A are also elevated in samples obtained from patients with clinically inactive sJIA (off medication).<sup>105</sup> Notably, serum amyloid A is able to induce expansion of T<sub>REG</sub> cells, which may contribute to controlling inflammation.<sup>105</sup> Elevated levels of certain cytokines, notably MIF<sup>65</sup> and IL-18,<sup>65</sup> during inactive disease (with NSAID treatment only or with no medication) suggest some continued immune activity despite clinical quiescence. As these studies did not use a validated definition of inactive sJIA or remission, the results require confirmation in future

studies. Nonetheless, taken together, the results raise the possibility that quiescence (off medication) in sJIA represents a state of compensated inflammation.

## Conclusions

It remains to be determined whether sJIA is primarily a disease of exuberant innate inflammation that outstrips normal anti-inflammatory controls, or whether the primary immune dysfunction is defective downregulation of initially normal inflammatory responses to (probably infectious) triggers. Indeed, although the clinical picture is one of excess inflammation, it is possible that the underlying abnormality causes some degree of immunodeficiency that results in secondary (chronic) inflammation, such as occurs in some HLH disorders and has been proposed for Crohn disease.<sup>106</sup> For example, exuberant IL-1 activity could downregulate the efficacy of type I IFN pathways,<sup>37</sup> attenuating the clearance of viral infections. In any of these scenarios, sJIA might result from an immune abnormality produced by different additive molecular etiologies in different patient subgroups.

A number of additional questions are raised by the available data. What are the initial triggers of sJIA? What are the mechanistic differences between sJIA patients with a monocyclic course and those with a more chronic course? What circulating factors stimulate expression of IL1 family genes in normal PBMCs, and how do these factors relate to the primary sJIA defect? What is the molecular basis of excess IL-1 activity in sJIA? What is the relationship between IL-1 and IL-6 production in sJIA? What is the role of neutrophils? Do the cells that drive inflammation operate from a privileged site? Are there two phases, with an IL-1-dependent process followed by one driven by other cells or mediators? Is there a critical role for T<sub>H</sub>17 cells in persistent sJIA? How is an IL-1-driven disease linked to a complication that is driven by reduced cytotoxic function? Is sJIA with MAS a disease variant that has a distinct genetic profile, or is it a severe (environmentally triggered) subtype of disease along a continuum in which all sJIA includes subclinical MAS? Which antigen-presenting cells have a proinflammatory role in MAS? The next years of sJIA research promise to be very exciting as answers to these questions emerge.

## Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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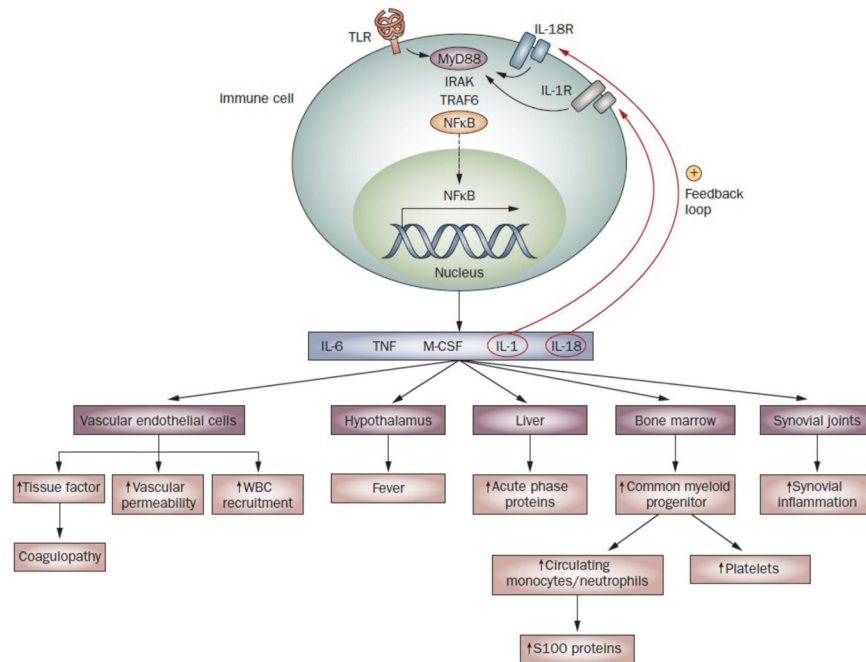
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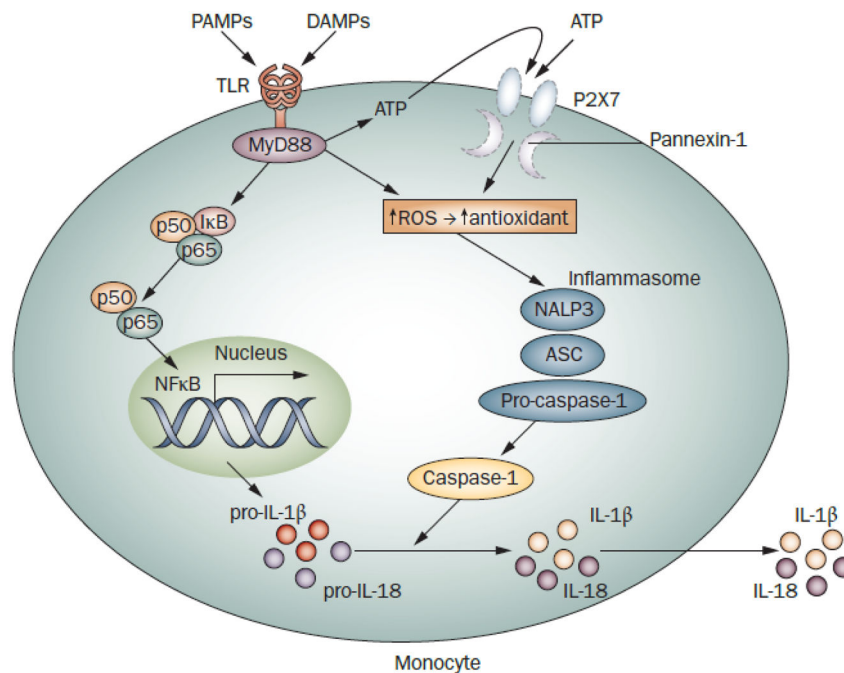
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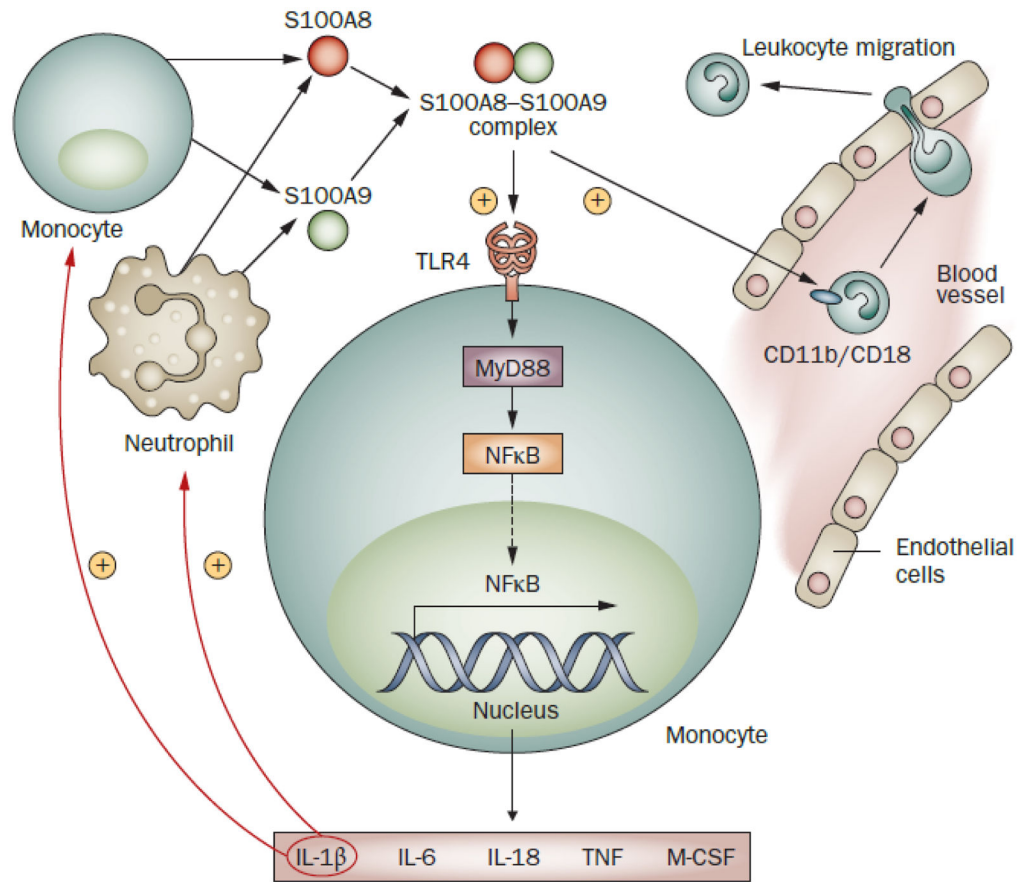
**Figure 1.**

Perpetuation of innate immune responses in sJIA. Innate immune pathways activated in sJIA are normally triggered by the recognition of pathogen-associated molecular patterns by TLRs expressed on innate immune cells, but can also be triggered by endogenous ligands in inflammatory conditions. These intracellular pathways lead to activation of the transcription factor NF $\kappa$ B, which is translocated into the nucleus where it upregulates expression of genes encoding several proinflammatory cytokines. These cytokines initiate the inflammatory cascade through their effects on the hypothalamus, bone marrow, liver and vascular endothelial cells. Activated vascular endothelial cells act as a procoagulant surface and are likely to contribute to the coagulopathy of sJIA.<sup>107</sup> Antibodies against endothelial cells reportedly occur in sJIA,<sup>108</sup> although the relevance of this latter finding is unclear. The cytokines also drive joint inflammation, by stimulating osteoclast-mediated bone resorption, osteoblast apoptosis, inhibition of chondrocyte proteoglycan synthesis and synovocyte secretion of enzymes that degrade matrix and cartilage. Since signaling through IL-1R and IL-18R shares the downstream portion of the TLR4 signaling pathway, IL-1 and IL-18 provide positive feedback loops that further contribute to perpetuation of the inflammatory responses in sJIA. Abbreviations: IL-1R, IL-1 receptor; IL-18R, IL-18 receptor; M-CSF, macrophage colony-stimulating factor; NF $\kappa$ B, nuclear factor  $\kappa$ B; sJIA, systemic juvenile idiopathic arthritis; TLR, Toll-like receptor; TNF, tumor necrosis factor; WBC, white blood cell.



**Figure 2.**

Secretion of IL-1 $\beta$  by monocytes in inflammatory diseases. Ligation of pattern recognition receptors, such as TLRs, induces expression of inactive pro-IL-1 $\beta$ ; its cleavage by caspase-1 leads to mature IL-1 $\beta$  and secretion.<sup>109</sup> Activation of caspase-1 requires assembly of the inflammasome, a multimolecular complex that includes NALP3 (cryopyrin) and the adaptor protein ASC.<sup>110</sup> Exogenous ATP triggers the receptor P2X7 and its associated pore, pannexin-1, inducing a strong redox response (generation of ROS followed by upregulation of antioxidant systems) and facilitating assembly/activation of the inflammasome. TLR signaling also stimulates ROS generation and release of endogenous ATP, resulting in autocrine stimulation. NLRP3 mutant monocytes (from patients with cryopyrin-associated periodic syndromes) have altered basal and TLR-stimulated redox states, causing increased and abnormally fast secretion of LPS-stimulated IL-1 $\beta$ . IL-1 secretion is not increased further by ATP, seemingly due to depletion of this pathway after LPS alone.<sup>27,45,111</sup> sJIA monocytes do not share this overall phenotype,<sup>45</sup> but are resistant to ATP-enhanced IL-1 secretion.<sup>37</sup> Anakinra treatment results in increased P2X7 transcripts in sJIA blood cells,<sup>37</sup> suggesting that excess IL-1 activity leads to suppression of P2X7. IL-18 processing and secretion is similar to that of IL-1; however, pro-IL-18 is constitutively produced by monocytes. Abbreviations: ASC, apoptosis-associated speck-like protein containing a CARD; DAMP, damage-associated molecular pattern molecule; IL, interleukin; LPS, lipopolysaccharide; PAMP, pathogen-associated molecular pattern molecule; P2X7, P2X purinoceptor 7; ROS, reactive oxygen species; sJIA, systemic juvenile idiopathic arthritis; TLR, Toll-like receptor.



**Figure 3.**

A possible positive feedback cycle involving IL-1 $\beta$  and S100 proteins contributes to perpetuation of chronic inflammation in sJIA. Various pro-inflammatory stimuli, including IL-1 $\beta$ , lead to activation of monocytes and neutrophils. The activation of these cells is accompanied by increased secretion of S100 proteins, including S100-A8, S100-A9 and S100-A12. The proteins S100-A8 and S100-A9, which are markedly elevated in sJIA, form a complex called calprotectin that can serve as an endogenous agonist of TLR4 and trigger TLR signaling pathways, leading to activation of the transcription factor NF $\kappa$ B. Translocation of activated NF $\kappa$ B into the nucleus upregulates expression of IL-1 $\beta$ . In turn, increased IL-1 $\beta$  leads to further secretion of S100 proteins. In addition, S100-A8-S100-A9 complexes activate endothelial cells and also bind the integrin receptor CD11b/CD18 on phagocytes and stimulate their transendothelial migration into tissues.<sup>112,113</sup> Abbreviations: IL, interleukin; M-CSF, macrophage colony-stimulating factor; NF $\kappa$ B, nuclear factor  $\kappa$ B; TNF, tumor necrosis factor.

**Table 1**Characteristics of M1 and M2 monocyte/macrophage subsets in humans<sup>67,114–116</sup>

Characteristic	M1	M2a*	M2b*	M2c*
<i>In vitro</i> induction	IFN- $\gamma$ plus lipopolysaccharide	IL-4, IL-13	Immune complex plus lipopolysaccharide or IL-1 $\beta$	IL-10, TGF- $\beta$ , glucocorticoids
Surface expression	MHC class II, CD40, CD80, IL-2R $\alpha$ (CD25), IL-7R $\alpha$ (CD127)	MHC class II, MMR (CD206), CD209 (DC-SIGN), CD20 antigen-like 1 (MS4A4A), CD20 antigen-like 3 (MS4A6A), SRAP, CD302, Dectin 1	No unique markers defined	CD163, IL-21R, TLR1, TLR8
Cytokines	IL-1 $\beta$ , IL-6, IL-12, IL-15, IL-23, TNF	IL-1Ra, IL-10, TGF- $\beta$ , IGF1, Fibronectin 1, $\beta$ -ig-H3, PDGFC, Coagulation factor XIIIa	IL-10	IL-10
CC-chemokine ligands	CCL8, CCL15, CCL19, CCL20	CCL2, CCL13, CCL14, CCL17, CCL18, CCL22, CCL23, CCL24, CCL26	CCL1, CCL20	CCL18
CXC-chemokine ligands	CXCL9, CXCL10, CXCL11, CXCL13		GRO- $\alpha$ (CXCL1), CXCL2, CXCL3	
Transcription factor	IRF5	STAT6, IRF4	IRF4	IRF4

\* Subtypes of alternatively activated M2 cells have been defined,<sup>69,115</sup> Abbreviations:  $\beta$ -ig-H3, TGF $\beta$ -inducible protein ig-H3; CCL CC-chemokine ligand; CXCL, CXC-chemokine ligand; DCL1, dicer-like 1; DC-SIGN, dendritic cell-specific ICAM-3-grabbing non-integrin 1; GRO $\alpha$ , growth-regulated protein  $\alpha$ ; IGF, insulin-like growth factor; IFN, interferon; IL-1Ra, interleukin-1 receptor antagonist; IL-2R $\alpha$ ; interleukin-2 receptor subunit  $\alpha$ ; IL-7R $\alpha$ , interleukin-7 receptor subunit  $\alpha$ ; IL-21R, interleukin-21 receptor; IRF, interferon regulatory factor; MMR, macrophage mannose receptor; PDGFC, platelet-derived growth factor C; SRAR steroid receptor RNA activator protein; STAT6, signal transducer and activator of transcription 6; TGF, transforming growth factor; TLR, Toll-like receptor; TNF, tumor necrosis factor.