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Inflammatory cytokines in the pathogenesis and treatment of systemic juvenile idiopathic arthritis

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Abstract

Systemic juvenile idiopathic arthritis (s-JIA) accounts for only 10-20% of JIA, but represents the group with the worse long term prognosis. The disease shows clinical and laboratory peculiarities that set this disease apart from the other JIA subsets. In this article we will review the evidence regarding the pathogenesis of s-JIA focusing on the role of dysregulated production of inflammatory cytokines. New therapeutic approaches, aimed at specifically antagonizing anti-inflammatory cytokines, are becoming available. The promising preliminary results of these treatments will be discussed. Albeit preliminary, these results suggest that new tools will be part, hopefully soon, of the therapeutic armamentarium of pediatric rheumatologists.

Introduction

The term juvenile idiopathic arthritis (JIA) encompasses a heterogeneous group of conditions characterized by chronic arthritis of childhood. The various subclassifications of JIA have been developed with the aim of identifying distinct disease groups. Although the present classification scheme [1] is still being debated, some separate clinical subsets have been identified.

Among these, systemic JIA (s-JIA) is defined by the presence of high spiking fever associated with other systemic features, such as an evanescent skin rash, hepatosplenomegaly, lymphadenopathy, and serositis. S-JIA accounts for 10-20% of JIA.

Clinical and laboratory peculiarities of systemic JIA

With respect to other JIA subsets, in addition to the systemic symptoms, patients with s-JIA show other notable clinical features [2]. Systemic osteoporosis and stunted growth are frequent complications of long lasting disease. Secondary amyloidosis is more frequent in s-JIA than in the other JIA subtypes. Routine blood studies also show peculiar features. Erythrocyte sedimentation rate (ESR) and C-reactive protein (CRP) level, as well as other acute phase reactants, are markedly elevated, and leukocytosis, with high neutrophil counts, and thrombocytosis are prominent. Ferritin concentrations are high and correlate with systemic disease activity [3]. A microcytic anemia, characterized by a marked defect in iron supply for erythropoiesis is often present [4].

An additional peculiarity of s-JIA is macrophage activation syndrome (MAS), a life-threatening complication characterized by a systemic inflammatory reaction caused by an excessive activation and proliferation of T cells and macrophages. MAS, which is part of the spectrum of hemophagocytic lymphohistiocytosis, occurs much more frequently in s-JIA than in any other rheumatic disease. The most current pathogenic hypotheses, the clinical features, and treatment approaches have recently been reviewed [5-6].

The disease course of patients with s-JIA is variable. One-half to two-thirds of patients develop a chronic persistent polyarthritis. Approximately half of these patients develop significant disability [7-8]. This group of patients is the subset of JIA with the worst long-term prognosis, and represents a therapeutic challenge. Glucocorticoids are often necessary to control systemic symptoms, but do not affect long-term prognosis and are burdened by severe side effects. Although being routinely used as the first choice for a second-line agent in s-JIA, methotrexate appears to be less efficacious than in the other JIA subsets (9). The experience with other immunosuppressive drugs is often anecdotal. The efficacy of etanercept has been disappointing in comparison with the good results obtained in other JIA subsets [10-11]. In summary, s-JIA is the JIA subset with the potential for the worst long-term prognosis, and the presently available treatments are not as effective as necessary.

Is s-JIA an autoinflammatory disease? The concept of *innate autoimmunity*

The unique clinical characteristics that differentiate s-JIA from other subtypes of JIA suggest differences in the ethiopathogenetic mechanisms. Antinuclear antibodies and rheumatoid factor are generally negative in children with s-JIA. Presence of other autoantibodies, as well as the presence of autoreactive T lymphocytes, has not been consistently demonstrated. Studies aimed at defining genetic association with HLA alleles have consistently failed to find significant associations. There is also an equal predilection for affecting boys and girls. All together this suggests that s-JIA should not necessarily be defined as a classical autoimmune disease.

In the last several years, the work of many laboratories has led to a better understanding of the innate immune response. This was largely driven by the discovery of Toll-like receptors (TLRs). TLRs recognize pathogen associated molecular patterns (PAMPs), largely invariant structure expressed by microorganisms, but not by eukaryotic cells. A total of 10 human TLR genes have been identified so far [12]. TLRs are expressed on several immune cells, but macrophages and natural killer cells express high levels of TLRs, and they appear to play a major role in the innate response. Following engagement of TLRs a fast response is induced, which is mainly characterized by the production of inflammatory cytokines that induce and maintain the inflammatory response.

All immune defence mechanisms must have a mechanism to differentiate self from non-self, which is the basis for tolerance to self. The basis of innate immune tolerance is the failure of TLRs to recognize molecules of the host. It is possible to envision, under pathological circumstances, a failure of this type of tolerance. This has recently led to the concept of “innate autoimmunity”. In this respects, it is worth noting that endogenous ligands of TLRs have been identified, although their role in physiological, as well as pathological, conditions has not yet been established [13].

Following engagement of TLRs, the activation of intracellular signalling pathways leading to effector mechanisms of the innate response (i.e., inflammatory cytokine production) is tightly regulated with checks and balances provided by a variety of inhibitory and control mechanisms [14]. The recent identification of the genetic causes of some of the recurrent fever syndromes has shed light on novel pathways involved in the regulation of cytokine production following inflammatory stimuli. It is beyond the scope of this review to describe in detail the molecular mechanisms involved that have been the subject of recent reviews [15]. These advances provided the proof of principle that abnormalities in the control mechanisms of the production of inflammatory cytokines lead to chronic and/or recurrent inflammation. For this group of diseases, including familiar Mediterranean fever (FMF), TNF receptor associated syndrome (TRAPS), CIAS1 gene-associated disease (e.g., CINCA/NOMID), and PFAPA syndrome, the term recurrent autoinflammatory syndromes has been proposed. [15]

With respect to s-JIA, little if any information is at present available concerning the expression of TLRs or concerning the activation of signalling pathways triggered by engagement of TLRs. Nevertheless, indirect evidence supports the conclusion that s-JIA could be defined as an autoinflammatory disease. S-JIA has been found to be genetically associated with polymorphisms in the promoter of genes coding for the inflammatory cytokines, interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α) and macrophage migration inhibitory factor (MIF) [16-20]. Some of these polymorphisms have been demonstrated to be functionally relevant, being associated with higher expression of the corresponding cytokine gene. This suggests that the predisposition to higher production of inflammatory cytokines is part of the genetic background of s-JIA.

Inflammatory cytokines in s-JIA

The clinical and laboratory features of s-IA suggest that inflammatory cytokines of the innate response may play a role. Since the early 1990's several laboratories have investigated levels of inflammatory cytokines in s-JIA. Studies aimed at identifying correlations with relevant clinical and laboratory features of the disease, together with data from experimental animals, have provided evidence, albeit indirect, of specific involvement. In the following paragraphs, I will discuss the available data concerning the involvement of three main inflammatory cytokines, TNF-α, IL-1, and IL-6, and the recent information gained from the use of cytokine antagonists in the treatment of patients with s-JIA. Table 1 shows the largely overlapping activities of IL-1β, IL-6 and TNF-α and compares the potency and in vivo efficiency of each cytokines in each activity.

Table 1: Biological activities and relative potency of IL-1, IL-6, TNF in the induction of the systemic and local inflammatory response.

	IL-1	IL-6	TNF
Central nervous system			
Fever	++	+++	+
Hypothalamic production corticotrophin releasing factor	+	+++	+
Liver			
Acute phase protein production	++	+++	+
Bone Marrow			
PMN increase	+	+++	++
Thrombocytopoiesis	-	+++	-
Inhibition of erythropoiesis (1)	++	++	++
Endotohelial Cells			
Increase in vascular permeability and vasodilatation	+++	+	++
Induction of chemokines and adhesion molecules	+	++	+++

Induction of procoagulant phenotype (2)	**+**	**+**	**+++**
Fibroblast			
Release of metalloproteinases and prostaglandins	**+++**	**-**	**++**
Proliferation	**+++**	**++**	**-**

(1) Inhibition of erythropoiesis by Il-1 and TNF is mediated by inhibition of erythropoietin production and of bone marrow erythroid precursors. Inhibition of erythropoiesis by IL-6 is mediated through induction of hepcidin production that causes blockade of iron in reticuloendothelium and inhibition of intestinal iron absorption
(2) TNF induces a procoagulant phenotype by increasing endothelial expression of tissue factor and by decreasing endothelial expression of thrombomodulin, which in turns leads to decreased levels of activated protein C.

<u>TNF-α in s-JIA</u>

TNF-α is the first inflammatory cytokine to be targeted as a therapeutic attempt in chronic inflammatory diseases. A vast body of evidence pointed to a role for TNF-α in chronic joint inflammation, but some key findings provided the rationale for the use of TNF-α inhibitors: i) TNF-α and TNF receptors are highly expressed in rheumatoid synovium, ii) inhibition of TNF-α in cultures of synovial fibroblasts leads to suppression of other proinflammatory cytokines and chemokines, implying that TNF-α is upstream in the cytokine cascade, and iii) TNF-α transgenic mice overexpressing TNF-α in monocytes developed spontaneously inflammatory arthritis [21].

With respect to s-JIA, high circulating levels of TNF-α have been reported by several authors. However, no correlations with the peculiar clinical or laboratory features of the disease have been reported with one notable exception. Activation of the TNF system, evaluated by elevated soluble TNF receptor levels, was found to be associated with sub-clinical coagulation abnormalities and markedly increased levels of soluble TNF receptors were found in patients with MAS [22]. Since TNF-α is well known to induce activation of coagulation [23], it was hypothesized that TNF-α could play a role in the sub-clinical coagulopathy present in many patients with active s-JIA, and possibly in MAS. However, this hypothesis has been recently challenged by clinical observations showing the development of MAS during etanercept treatment, with etanercept treatment even being suggested as a possible MAS triggering factor [24].

In the joints of s-JIA patients, TNF-α appears to be activated in a manner similar to the other forms of JIA or adult RA as suggested by comparable synovial tissue expression and synovial fluid levels [25,26]. However, as previously mentioned, patients with s-JIA do not show a favorable response to TNF-α inhibition by etanercept and their response rate, in terms of improvement of arthritis, as well as of systemic features, is markedly lower that that observed in the other JIA onset types [10,11]. This shows that TNF-α may not be as relevant in s-JIA, as it is in polyarticular JIA or in adult RA. An obvious implication of these clinical observations is that in the apparent chaos of the cytokine network a hierarchy exists, and this may be different in different diseases, even in the presence of similar expression patterns in blood and synovium for a given cytokine. Data in mice support this concept. As shown by the use of IL-6-/- mice, deletion of the IL-6 gene resulted in total protection from collagen-induced arthritis, while did not affecting the incidence and severity of the spontaneous arthritis of TNF-α transgenics mice (27). Similarly, again using cytokine knock-out mice, a differential involvement of IL-1, IL-6 and TNF-α was demonstrated in a more recent model of spontaneous chronic arthritis (28).

IL-6 in systemic JIA

In contrast to TNF-α, evidence obtained from many laboratories points to IL-6 as a potential pivotal cytokine in s-JIA. Serum and synovial levels of IL-6 in patients with s-JIA are markedly increased and significantly higher than those present in polyarticular and oligoarticular JIA and in adult patients with rheumatoid arthritis (26,29). In addition, peripheral blood and synovial mononuclear cells from patients with s-JIA spontaneously express high levels of IL-6 [30-31]. The association of s-JIA with a functionally relevant single nucleotide polymorphism of the IL-6 promoter [16-17] further suggests that the polymorphism in the IL-6 gene itself may contribute to the over-production of IL-6.

IL-6 appears to be involved in synovial inflammation and in the damage to peri-articular cartilage and bone. In s-JIA, circulating levels of IL-6 correlate with the extent and severity of joint involvement. [29] IL-6 appears to play an important role in the induction and maintenance of chronic synovial inflammation. In several animal models of arthritis, IL-6 knock-out animals are protected [27-28,32-34], with this protection being associated with absence or significant reduction of the inflammatory infiltrate. In animals, IL-6 has been demonstrated to induce the switch from an acute neutrophilic infiltrate to a chronic type infiltrate composed of lymphocytes and macrophages. [35] IL-6 also affects endothelium expression of adhesion molecules and particularly induces endothelial cell production of the chemokine monocyte chemoattractant protein-1, responsible for monocyte recruitment. Additionally, IL-6 is involved in the recruitment of mesenchymal vascular cells and neoangiogenesis in vivo [36], and induces synovial fibroblasts proliferation. [37] Furthermore, IL-6 decreases aggrecan protein and collagen-II production by chondrocytes [38] and induces activation of osteoclasts [39] suggesting a role for synovial IL-6 in articular cartilage degradation and bone resorption. Thus, IL-6 has a wide range of pleiotropic effects on the synovium.

A number of studies have addressed the association of IL-6 over-production with the extra-articular manifestations of s-JIA. An increase in IL-6 levels occurs during the fever peak [29,40-41] and IL-6 is pyrogenic both in animals and humans. [42-43] Acute phase proteins are markedly elevated in s-JIA, and CRP levels significantly correlate with circulating levels of IL-6. IL-6 is also a major inducer of liver production of acute phase proteins. Lastly, IL-6 promotes thrombocytopoiesis in vivo [43] and in s-JIA IL-6 levels are significantly correlated with platelet counts. [29]

In contrast to the anemia of adult rheumatoid arthritis, anemia in s-JIA is characterized by a marked defect in iron supply for erythropoiesis while growth of erythroid colonies is normal and erythropoietin production appropriate. [4] Moreover, some patients with s-JIA and long lasting severe anemia show iron malabsorption. [44] Several observation suggest that these features may be caused by overproduction of IL-6: i) IL-6 increases ferritin expression and hepatic uptake of serum iron [45-46], resulting in reticuloendothelial iron block, ii) IL-6, but not IL-1 or TNF-α, induces liver production of hepcidin, a liver peptide that inhibits both the release of iron from macrophages and intestinal iron absorption [47-48], iii) microcytic anemia is present in IL-6 transgenic mice [49-50], and iv) administration of IL-6 causes a marked decrease in serum iron and an increase in ferritin levels in humans. [51]

Growth impairment in s-JIA is well known since the first description of the disease. [52] It occurs during active disease independently from glucocorticoid administration. Over-expression of IL-6 alone in mice is sufficient to cause growth defects. IL-6-transgenic mice with high circulating levels of IL-6 since birth show decreased growth rates leading to adult mice 50-60% the size of their wild-type littermates [53]. These IL-6 transgenic mice have normal growth hormone production, but low levels of insulin-like growth factor-I (GF-I) and of IGF binding protein-3 (IGFBP-3), similar to what is found in patients with s-JIA. [54] IL-6 over-expression in mice also causes a marked delay in the appearance and maturation of epiphyseal ossification centres (De Benedetti et al, submitted), reflecting the delayed skeletal age frequently observed in patients.

IL-6 has also profound effects on bone. It induces osteoclast formation and activation, and IL-6 knock-out mice are protected from ovariectomy-induced osteoporosis. [39,55] Patients with s-JIA are particularly prone to the development of systemic osteoporosis. [56] In IL-6 transgenic mice, we have recently observed: i) decreased trabecular bone volume and decreased trabecular numbers, ii) reduced cortical thickness, iii) ossification defects, and iv) increased osteoclast numbers and surfaces, with biochemical evidence of increased bone resorbing activity (De Benedetti et al., submitted). This shows that overexpression of IL-6 alone can lead to secondary osteoporosis.

In summary, a possible pivotal role of IL-6 is supported by a vast, albeit indirect, body of evidence. Yokota and coworkers have recently reported results obtained in a small number of patients with steroid-dependent s-JIA treated with escalating doses (2 to 8 mg/kg every two weeks) of tocilizumab, a humanized monoclonal antibody to the IL-6 receptor (57). Treatment of s-JIA patients with tocilizumab leads to a rapid response with marked improvement in the core set criteria. The three patients treated with the highest dosage reached an ACR70 response. In addition to a marked amelioration in joint symptoms, fever was rapidly controlled. Erythrocyte sedimentation rate values and C-reactive protein levels improved quickly in all patients reaching normal values in the majority after the first administration of the drug. By the end of the treatment period, in addition to normalization of acute phase reactants, including thrombocytosis, a marked improvement in anemia was observed. These results with tocilizumab provide proof of principle that indeed over-production of IL-6 plays a central role in the clinical and laboratory features of s-JIA and open promising therapeutic perspectives in the treatment of severe s-JIA.

IL-1 in s-JIA

The laboratory evidence pointing to a role for IL-1 in s-JIA is disappointingly scarce and contradictory. Circulating levels of IL-1β have been reported to be increased, but when compared with levels in polyarticular JIA they were found to be lower by some groups or higher by other groups. [40, 58-61] No apparent correlation of circulating IL-1β levels with clinical and laboratory features have been consistently reported. In particular, in a study aimed at defining the relation of cytokine levels with the fever peak, serum levels of IL-1β and IL-6, as well as of TNF-α and IL-8, did not show changes in temporal association with changes in body temperature, supporting the conclusion that IL-1 was not involved in the induction of the fever in s-JIA patients (41). In addition, in patients with s-JIA synovial fluid (SF) levels of IL-1β were similar to those present in pauciarticular JIA or adult RA. [26]. A very recent reports showed, in peripheral blood mononuclear cells, increased expression of the IL-1β gene, as well as of other genes of innate immunity, in patients with active s-JIA. Expression of IL-1β was higher in patients with active systemic features at sampling [62].

Recent clinical data suggest that IL-1 may be important, possibly pivotal, in the pathogenesis of s-JIA. Indeed, it has been reported that therapeutic use of recombinant IL-1 receptor antagonist led to very satisfactory results in some patients. The results in a small number of patients have been recently reported [62,63]. Similar to what has been observed with the treatment directed to antagonizing IL-6, the response was quick with normalization of ESR and CRP in a short time. A marked improvement in clinical manifestations was also reported. These reports suggest that the IL-1 receptor antagonist is an efficacious treatment for severe s-JIA.

These clinical results open new ways of investigation in the pathogenesis of s-JIA. The previously mentioned discovery of the molecular abnormalities causing genetic autoinflammatory diseases has revealed a new mechanism of regulation of IL-1 release, based on the inflammasome [64]. The inflammasome is a multiple adaptor protein complex that regulates activation of caspase-1. Caspase-1 is the protease that cleaves pro-IL-1β, that cannot be secreted, to mature IL-1β that is exported to the extracellular medium. Several proteins, some of which are involved in the recurrent autoinflammatory syndromes, have been shown to interact with components of the inflammasome and to affect caspase-1 activation. However, to the best of our knowledge, there are no available data on the inflammasome in patients with s-JIA. It is worth noting that the inflammasome regulates the release not only of IL-1β, but also of IL-18. IL-18, besides being a pivotal Th1 inducing factor, has direct proinflammatory effects inducing monocyte production of inflammatory cytokines and chemokines [65]. Interestingly, high levels of IL-18 have been found in patients with s-JIA. [66 and De Benedetti, unpublished]. In addition, the relationship, between IL-6 and IL-1 production and the inflammasome is still to be investigated and may possibly exist in patients with s-JIA.

Conclusion and future perspectives

The knowledge of the role of inflammatory cytokines in the pathogenesis of s-JIA has significantly increased in last few years. However, several questions remain unanswered. What are the mechanisms leading to the increased production of inflammatory cytokines? Despite the genetic associations with functionally relevant polymorphisms of the promoters of proinflammatory cytokines, it is highly conceivable that the genetic predisposition to higher cytokine production may not be the only mechanism responsible for the occurrence of s-JIA. What is the potential role for environmental factors? Moreover, at present we do not have any information on the possible role of the activation of TLRs and of the downstream signalling pathways in s-JIA, as well as of the inflammasome.

Despite this lack of information, there is sufficient preliminary data to suggest that two new promising treatments are now available for patients with severe s-JIA. In one case (i.e., IL-6 blockade) the rational for the new treatment was provided by a vast body of laboratory evidence. In the other (i.e., IL-1 blockade) new information was made available from clinical observations. Controlled clinical trials are now necessary to demonstrate clinically efficacy and to define the safety profile of the two new drugs in the treatment of s-JIA. Based on the promising preliminary results, it is indeed reasonable to expect that the availability of these new treatments will lead to a significant improvement of the management of severe s-JIA with a better long-term prognosis of a disease that at present is still burdened by a severe functional impairment.

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