The Role of Natural Killer Cells in Inflammation in Active Adult-onset Still Disease

Adult-onset Still disease (AOSD) is a systemic inflammatory disease characterized by spiking fever, arthralgia, rash, leukocytosis, and hepatosplenomegaly, similar to systemic-onset juvenile idiopathic arthritis (sJIA)^{1,2}. The etiology of AOSD is unclear but the disease is thought to result from a combination of genetic and environmental factors. The disease pathology is characterized by widespread immune dysregulation, involving cytokines and their receptors, immune cells, and tissues. Activation of the innate immune system plays a major role in the systemic inflammation that characterizes AOSD and sJIA³. Activated macrophages and neutrophils induce increased levels of proinflammatory cytokines, such as interleukin (IL)-1 β , IL-18, IL-6, and interferon (IFN)- γ . These proinflammatory cytokines provoke activation of macrophages and neutrophils, and differentiation of T cells. Decreased numbers and functional impairment of natural killer (NK) cells have also been implicated in the pathogenesis of AOSD and sJIA^{4,5}.

NK cells are an important lymphocytic component of the innate immune system, providing a cytotoxicity defense against malignant, infected, and autoreactive cells^{6,7}. NK cells exert their effects by secreting cytolytic granules such as perforin, granzyme, and serine proteases, and induce caspase-dependent apoptosis through death receptors including Fas/FasL. Impaired cytotoxic function of NK cells leads to excessive activation of macrophages and T cells, as seen in macrophage activation syndrome (MAS), and is a major, life-threatening complication of AOSD and sJIA^{8,9}. Primary hemophagocytic lymphohistiocytosis (HLH), a hereditary disorder of MAS, occurs as a result of genetic defects in the perforin- or granzyme-dependent pathways of NK cells or cytotoxic T cells¹⁰. Additional genetic mutations associated with inhibitory or stimulatory NK cell receptors have also been observed in secondary HLH. The resulting failure of NK cells to lyse activated antigen-presenting cells results in sustained stimulation of lymphocytes and macrophages, leading to a cascade of inflammatory cytokines. Taken together, these data suggest that the dysfunction of NK

cells or NK T cells may be important in the underlying systemic inflammation and macrophage activation seen in chronic inflammatory disorders, such as sJIA and AOSD. Alteration of NK cells has been well documented in AOSD and sJIA¹¹, with active AOSD patients exhibiting decreased circulating NK T cells along with NK cells with poor proliferative function and impaired cytotoxicity^{4,5}. Such defective NK cytotoxicity was confirmed by the impaired IL-18-induced IFN- γ production seen in patients with sJIA¹². Further, downregulated NK T or NK cell populations recovered during remission of sJIA or AOSD^{4,13}.

In this issue of *The Journal*, Shimojima, *et al* investigated the characteristics of circulating NK cells, including IFN- γ production, in patients with active AOSD¹⁴. Based on flow cytometry data, Shimojima, *et al* showed that the number of IFN- γ expressing NK cells was increased in acute AOSD compared to healthy controls (HC), although the proportion of circulating NK cells as a percentage of total lymphocytes was decreased. Further, expression of IL-12 and IL-15 receptors, but not of IL-18 receptors, was significantly increased on NK cells in acute AOSD. The cytotoxicity of NK cells depends on IFN- γ , with IL-18, IL-12, and IL-15 modulating the secretion of IFN- γ by interacting with their receptors on NK cells¹⁵. A defect in the phosphorylation of IL-18 receptor- β on NK cells has been reported in sJIA¹⁶, and may contribute to impaired NK cell expansion in AOSD despite the high levels of IL-18 seen in these patients. Similarly, that study also found that increased IL-18 levels were not correlated with the number of IFN- γ -expressing NK cells. The accompanying increases in IL-12 and IL-15 receptor expression on NK cells could be regarded as a compensatory reaction against high levels of intracellular IFN- γ . These data show that the persistent upregulation of IL-18 in acute AOSD does not affect NK cell expression levels, despite the higher numbers of IFN- γ -expressing NK cells in these patients.

NK cells are divided into 5 phenotypes based on differences in surface receptor expression and cytotoxic

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functions¹⁷. CD56^{dim} cells exhibit more potent cytolytic activity and contain more cytoplasmic granules than CD56^{bright} cells, which have higher proliferative potential and show enhanced cytokine production¹⁸. Shimojima, *et al* divided NK cells into 2 distinct subsets according to CD56 expression, and enhanced their IFN-γ-producing ability¹⁴. IFN-γ expression in CD56^{bright} cells was significantly decreased in acute AOSD compared to HC, while IFN-γ expression in CD56^{dim} was significantly increased. This result suggested 2 basic hypotheses: that CD56^{dim} NK cells develop their ability to produce IFN-γ during AOSD development in response to other immunopathogenic cells, and that the absolute number of NK cells expressing IFN-γ can be altered during the transition from CD56^{bright} to CD56^{dim} in acute AOSD. While the lower proportion of CD56^{bright} cells could be regarded as a paradoxical phenomenon due to their higher IFN-γ expression, this effect may be due to a lack of immunomodulating cytokines such as IL-10 and IL-13, which are induced by CD56^{bright} cells¹⁹. Further, the lack of regulatory CD56^{bright} NK cells might exacerbate the inflammatory response in AOSD. To confirm this hypothesis, *in vitro* experiments should be conducted under various conditions.

The data from Shimojima, *et al* revealed some limitations regarding the extent of the role of NK cells in the pathogenesis of AOSD. First, they evaluated the total number of NK cells and IFN-γ-expressing NK cells, along with IL-12, IL-15, and IL-18 receptor expression levels in each NK cell population; however, functional studies of each NK cell population were not performed. NK cells are a critical cytotoxic regulator of the immune response, with NK cell dysfunction thought to be an important factor in AOSD. A better understanding of differences in cytotoxic function based on IFN-γ production in NK cells, and of NK cell frequency, may be important for evaluating the role of these cells in AOSD pathogenesis. Second, the study evaluated only the association between NK cell characteristics and acute inflammation in AOSD. Recently, AOSD was shown to exhibit 2 distinct patterns of disease progression: systemic inflammatory and chronic articular patterns². The systemic inflammatory pattern is characterized by recurrent systemic flares, such as fever, rash, and myalgia, while the chronic articular pattern is characterized by persistent symptoms of erosive arthritis, similar to rheumatoid arthritis³. Many researchers have suggested that NK cell dysfunction could be related to systemic inflammation, rather than the chronic articular pattern of AOSD pathogenesis². However, in the cited study¹⁴, patients experiencing AOSD flares during followup were not included, and the patterns of AOSD were not described. Third, Shimojima, *et al* showed that circulating NK cell counts were negatively correlated with AOSD activity when comparing active and remission status; however, the authors did not consider the influence of treatment, particularly with glucocorticoids. Glucocorticoids

were shown to suppress initial release of IFN-γ from NK cells, and to enhance cell proliferation and survival in the presence of IL-2 and IL-12²⁰. Considering that glucocorticoids are the treatment of choice in AOSD, changes in the population or function of NK cells might be affected by administration of these agents. Intriguingly, the levels of IFN-γ-producing NK cells were decreased after remission in almost all patients with AOSD, with 4 of 7 patients in remission currently receiving glucocorticoids as a maintenance therapy. Therefore, to rule out an effect of glucocorticoids, greater numbers of followup patients in remission not treated with corticosteroids should be recruited, to confirm the number of IFN-γ-producing NK cells in AOSD remission¹⁴. Although Shimojima, *et al* suggested a possible connection between changes in the levels of cytokines (such as IL-18) and NK cell characteristics, they failed to demonstrate a clear linkage mechanism because of the absence of *in vitro* functional studies. Finally, the number of subjects in their study was small, and peripheral blood cells in patients with remission status could be affected by a variety of factors, such as the types of drugs administered.

Several analyses revealed impaired NK cell cytotoxicity and decreased NK cell number in acute AOSD (Table 1); however, the cause of impaired NK cell cytotoxicity and decreased NK cell numbers remains unclear. Evidence regarding the underlying cause of such changes in NK cells, both in patients with active AOSD and those in remission, and of the associations between cytokine levels and NK cell cytotoxicity in AOSD and sJIA, is sorely lacking. Possible causes of these phenomena include pathologic alterations,

Table 1. Current findings regarding the role of natural killer cells in adult-onset Still disease (AOSD) and systemic juvenile idiopathic arthritis (sJIA).

Disease	Findings	Ref.
AOSD	Lower NK T cell counts	4
	Defect in GalCer-mediated NK cytotoxicity	
AOSD	Lower NK cell counts	5
	Lower NK cytolytic function	
sJIA	Lower NK cytolytic activity	9
	Lower circulating CD56 ^{bright} NK cell levels	
sJIA	Impaired upregulation of cell-mediated perforin and IFN-γ in NK cells	16
	Defect in IL-18 receptor β phosphorylation	
sJIA	Lower cell counts, cytotoxicity, perforin, and granzyme B expression in NK cells	13
	Lower frequency of KIR2DS4	
sJIA	Increased expression of innate genes and decreased expression of immune-regulating genes of NK cells	12
	Alterations in inhibitory and excitatory receptors of NK cells	
	Decreased granzyme K expression in CD56 ^{bright} NK cells and defective IL-18-induced IFN-γ production	
sJIA	Impaired NK cell activation by IL-18	21

NK: natural killer; IL: interleukin; KIR: killer cell immunoglobulin-like receptor; IFN: interferon; KIR2DS4: killer cell immunoglobulin-like excitatory receptors.

including the release of lower levels of IFN- γ from NK cells, as well as effects of genetic susceptibilities on NK cell function; however, data on the effects of genetic factors on NK cell cytotoxicity in sJIA and AOSD remain scarce.

The NK cell machinery includes a variety of receptors with both inhibitory and excitatory actions, which together control NK cell activation. A study examining gene expression in patients with AOSD identified a specific gene polymorphism in NK cell receptors¹³. Similarly, an *in vitro* assay examining killer cell immunoglobulin-like receptors (KIR), which are composed of inhibitory and excitatory receptors, revealed lower levels of the excitatory receptor KIR2DS4 in sJIA compared to polyarticular and pauciarticular JIA. While such genetic differences may lead to uncontrolled innate immune activation, more conclusive evidence regarding the genetic susceptibility of NK cells in AOSD is required.

A better understanding of the role of NK cells in the pathogenesis of AOSD is necessary for proper diagnosis and treatment of patients with AOSD. Despite certain limitations in the data, this study has improved our understanding of NK cell characteristics and IFN- γ production patterns in AOSD¹⁴. The data presented in this article help to clarify the details regarding IFN- γ production according to the expression of various receptors and NK cell subsets in AOSD. However, it remains unclear whether the observed changes in NK cells are associated with disease etiology or arise as a result of the chronic immune dysregulation seen in this disease. Ongoing genetic and molecular investigations into these various aspects of AOSD pathology may help to explain the changes in NK cells seen therein, and their roles in disease pathogenesis.

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