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Altered natural killer cell cytokine profile in type 2 autoimmune hepatitis

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ABSTRACT

Type 2 autoimmune hepatitis (AIH-2) is a rare disease presenting in early childhood. The immunopathogenetic mechanisms are poorly characterized, although a defect of regulatory T cells (Treg) has been shown. There is virtually no information on innate immune responses and natural killer (NK) cells in particular. We have performed an extended immunophenotypic and functional analysis of NK cells in children with AIH-2. We show that NK cell frequency is reduced in this setting and that the balance between NK activating and inhibitory receptors is skewed toward activation. More importantly, NK cells display an altered cytokine pattern characterized by increased IFNγ and reduced IL2 production which could contribute to impaired Treg function. Exposure of mononuclear cells to IL2 resulted in normalization of NK IFNγ production. Thus, our findings support treatment of AIH-2 with low-dose IL2, which would result in normalization of NK cell function and expansion of the Treg cell subset.

1. Introduction

Autoimmune hepatitis (AIH) is a rare disease affecting predominantly women, characterized by elevated aminotransferases, polyclonal hypergammaglobulinemia, positive autoantibodies (autoAb), and interface hepatitis featuring abundant plasma cells in portal tracts (reviewed in [1]). The disease shows a favorable response to steroids but, if untreated, can lead to cirrhosis and liver failure. The incidence of AIH varies between 0.2 and 1.9/100,000/Y [2] with a trend toward an increase in recent years particularly in Northern European countries [3]. Most studies reported a bimodal age pattern at presentation, being diagnosed in childhood or adolescence and in middle-age/elderly people. Autoantibodies are the hallmark of AIH and allow subclassification into two types of AIH. AIH-1 is the most frequent, accounting for approximately 90% of the cases, and is associated with positive antinuclear antibody (ANA), smooth muscle antibody (SMA) or anti-soluble liver antigen/liver pancreas antibody (SLA/LP). AIH-2 accounts for up to 10% of the cases and is characterized by the presence of liver-kidney microsomal antibody type 1 (LKM-1) and anti-liver cytosol type 1 antigen (LC1) [4]. Although much has been achieved in clarifying the complex pathogenetic mechanisms involved in liver damage, most available information pertains to adaptive immune responses. Thus, CD4+ T cells and plasma cells prevail in the hepatic inflammatory cell infiltrate and, among the former, Th1 and Th17 seem to play an important role, as in most autoimmune diseases [5–7], whereas classical CD4+/CD25+ regulatory T cell function is impaired [8,9]. However, there is a distinct lack of knowledge on innate immunity, particularly natural killer (NK) cells in AIH.

Children are not spared by AIH, the global incidence in pediatric age being 0.23 per 100,000 [10]. LKM-1 antibody positive AIH-2 is approximately 5 times less frequent than AIH-1 and typically presents in early childhood as severe acute or advanced liver disease (50% of the cases) that may respond poorly to immunosuppressive treatment [11]. There is virtually no information available on NK cell phenotype and function in this disease subgroup, particularly since only a handful of children

Abbreviations: AIH-2, autoimmune hepatitis; Treg, regulatory T cells; NK, natural killer; TRAIL, tumor necrosis factor–related apoptosis inducing ligand; GITR, glucocorticoid-induced tumor necrosis factor-related receptor; IFNγ, interferon gamma; IL2, interleukin 2; TNFα, tumor necrosis factor-alpha; ANA, positive antinuclear antibody; SMA, smooth muscle antibody; SLA/LP, anti-soluble liver antigen/liver pancreas antibody; LKM-1, liver-kidney microsomal antibody type 1; LC1, anti-liver cytosol type 1 antigen; ADCC, antibody-dependent cell-mediated cytotoxicity; SLE, systemic lupus erythematosus.

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Table 1
Chacteristics of the patients and control subjects studied.

	Age (years)	Sex	AST[a] (mU/ml)	ALT[b] (mU/ml)	T. Bilirubin[c] (mg/dl)	IgG[d] (mg/dl)
AIH-1 patients						
1	2	F	858	604	0.14	950
2	12	F	1675	597	13.3	8860
AIH-2 patients						
1	2	F	52	40	0.61	1080
2	4	F	52	48	0.24	1290
3	4	M	785	492	5.79	2140
4	1	F	1160	840	6.3	
5	3	F	35	24	0.44	756
6	6	F	64	120	0.39	
Control						
1	1	F				
2	2	F				
3	5	M				
4	4	F				
5	2	M				
6	1	M				
7	1	M				

ALT: Alanine Aminotransferase; AST: Aspartate Aminotransferase; T. Bilirubin: Total Bilirubin; IgG: Immunoglobulin G.
[a] Normal values 39 mU/ml.
[b] Normal values 34 mU/ml.
[c] Normal values 1.1 mg/dl.
[d] Normal values 1430 mg/dl.

first-diagnosed with AIH-2 per year are seen in tertiary referral centers. We report here an extended NK phenotypic and functional study in a small group of children with AIH-2 prior to enter immunosuppressive therapy.

2. Materials and methods

2.1. Patients and controls

Peripheral blood mononuclear cells (PBMC) were obtained from 6 untreated children with autoimmune hepatitis type-2 (AIH-2), 2 untreated children with AIH-1 diagnosed according to internationally agreed criteria [12], 7 healthy controls of similar age (Table 1) and stored in liquid nitrogen until use. All AIH-2 patients had detectable LKM-1 autoantibody. Written informed consent was obtained from the parents of each child. The study protocol conformed to the ethical guidelines of the 1975 Declaration of Helsinki and was approved by our institutional ethics committee.

2.2. Cells

Cryopreserved peripheral blood mononuclear cells (PBMC) were used for immunophenotypic and functional studies. Immunophenotyping was performed by means of the following antibodies: CD3 Brilliant Violet-421, CD4 FITC, CD16 (clone 3G8) PE-Cy7, CD25 PE-Cy7, CD27 FITC, CD94 FITC, Fas-L PE, CD57 APC, CD127 PE, DNAM FITC, NKG2D APC, NKp46 Brilliant Violet-421, NKp30 Alexa Fluor-647, KIR3DL1 (clone DX9) FITC (BD Biosciences, San Josè, CA, USA), CD56 Pc5.5, NKp44 PE, NKG2A APC (Beckman Coulter, Brea, CA),CD69 APC (Immunotools, Friesoythe, Germany), Siglec-7 PE, TRAIL PE, KIR2DL2/DL3 PE, glucocorticoid-induced tumor necrosis factor-related receptor (GITR) Alexa 488 (BioLegend, San Diego, CA). LIVE/DEAD® Fixable Near-IR Dead Cell Stain Kit (Life Technologies, ThermoFisher Scientific, Waltham, MA, USA) was used to determine the viability of the cells. Treg were identified as $CD4^+CD25^+CD127^{low/neg}$.

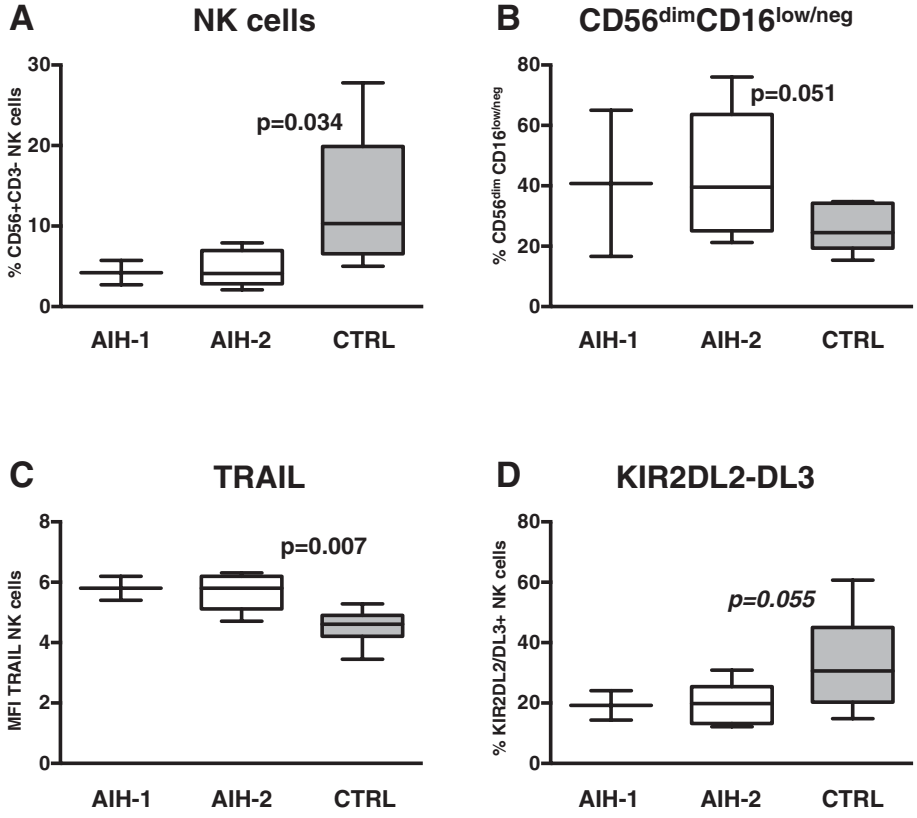

Fig. 1. Frequencies and phenotype of circulating NK cells in AIH-1, AIH-2 and control children. The proportion of $CD56^+/CD3^-$ NK cells (A) was reduced in children with AIH-2 and AIH-1 compared with control subjects. Increased proportions of $CD56^{dim}$ $CD16^{low/neg}$ (B) and TRAIL expressing (C) NK cells were instead observed in patients. There was a trend toward decreased frequencies of KIR2DL2/DL3 (D) NK cells. Box and whiskers show medians, 25th and 75th percentiles and ranges.