Human Immunology 75 (2014) 996–1000

Contents lists available at ScienceDirect

journal homepage: www.elsevier.com/locate/humimm

Rapid Communication

IFNL cytokines do not modulate human or murine NK cell functions ☆

Maria H. Morrison [a,1], Ciara Keane [a,1], Louise M. Quinn [a], Aoife Kelly [b], Cliona O'Farrelly [b], Colm Bergin [c], Clair M. Gardiner [a,*]

[a] *Natural Killer Cell Research Group, School of Biochemistry and Immunology, Trinity College Dublin, Trinity Biomedical Science Institute, 152-160 Pearse Street, Dublin 2, Ireland*
[b] *Comparative Immunology Group, School of Biochemistry and Immunology, Trinity College Dublin, Trinity Biomedical Science Institute, 152-160 Pearse Street, Dublin 2, Ireland*
[c] *Department of GU Medicine & Infectious Diseases, St. James's Hospital, Dublin 8, Ireland*

ARTICLE INFO

Article history:
Received 21 November 2013
Accepted 23 June 2014
Available online 30 June 2014

Keywords:
NK cells
HCV
Interferon lambda

ABSTRACT

The interferon-lambda (IFNL) cytokines have been shown to be important in HCV infection with SNPs in the *IFNL3* gene associated with both natural and treatment induced viral clearance. We have recently shown that rs1299860 (an *IFNL3* associated SNP) and an NK cell gene, *KIR2DS3*, synergised to increase the odds of chronic infection in a homogenous cohort of Irish women infected with HCV. To characterise a biological basis for the genetic synergy, we investigated for any evidence that IFNL cytokines regulate NK cell functions. Using a range of functional responses, we did not find any evidence of NK cell activation by IFNL3, IFNL1 or IFNL2 cytokines. Similar results were found using human and murine NK cells. In addition, and in contrast to our preliminary study, we did not find any evidence that IFNL cytokines inhibited NK cell cytokine production; thus, the biological basis for the genetic synergy remains to be discovered.
© 2014 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

1. Introduction

Hepatitis C virus is a life-threatening infection that affects over 170 million people globally [1,2]. While a minority of individuals clear the virus using both innate and adaptive arms of their natural immune response, most individuals develop chronic HCV infection which leads to associated long term problems such as liver cirrhosis, hepatocellular carcinoma or need for liver transplantation [3]. Understanding how the immune system can eradicate the virus naturally in some people is essential for the rational design of HCV specific vaccines and the appropriate clinical management of patients with chronic infection that are treated using conventional (IFN-α and ribavirin) or newer (direct acting anti-viral) therapeutic agents. While most research has focussed on defining a role for the adaptive arm of the immune response in terms of viral clearance, evidence is mounting that the innate immune system also plays a considerable role in both spontaneous and treatment-induced viral clearance [4,5].

We have previously reported that innate immune genes predicted spontaneous viral clearance in a unique cohort of Irish women infected with HCV from contaminated anti-D immunoglobulin [5]. Analysis of this cohort is extremely informative as the patients are genetically homogenous and lack confounding variables such as gender, age and the presence of other infections [6]. One of these genetic markers identified is the well-characterised interferon-lambda, *IFNL3* (formerly *IL28B*)-associated SNP (rs12979860) [7–9] while the second is a gene, *KIR2DS3*, that is associated with human Natural Killer (NK) cells [5]. Each genetic locus conferred a risk but the presence of both loci synergised to significantly increase the risk of developing chronic HCV infection. We have since confirmed the importance of these genes in HCV viral clearance as they also synergise to predict treatment induced viral clearance in a cohort of Irish patients co-infected with HIV [10]. Previously, we had observed that IFNL2 (IL28A) inhibited IFN-γ production by NK cells in a minority of healthy donors, although findings to the contrary were subsequently reported [11]. We hypothesised that IFNL cytokines could potentially modulate NK cell functions and that this might in part account for the observed genetic synergy [5].

2. Materials and methods

2.1. Patient cohort

The study population consisted of 26 healthy volunteers and 21 patients infected with HCV through receipt of contaminated blood products. Informed written consent was obtained from each

☆ Funding for this project came from Science Foundation Ireland, the Irish Health Research Board and the Irish Haemophilia Society.
* Corresponding author. Fax: +353 16772400.
E-mail address: clair.gardiner@tcd.ie (C.M. Gardiner).
[1] These authors contributed equally to the work.

http://dx.doi.org/10.1016/j.humimm.2014.06.016
0198-8859/© 2014 American Society for Histocompatibility and Immunogenetics. Published by Elsevier Inc. All rights reserved.

volunteer, and the study received ethical approval from the Research and Ethics Committees of St James's Hospital, Dublin 8, Ireland.

2.2. Flow cytometry

Anti-CD56 PE (clone B159), anti-CD3 PerCP (clone SK7), anti-CD69 FITC (clone FN50), anti-IFN-γ FITC (clone 25723.11), anti-CD107a FITC (clone H4A3) against human proteins were purchased from BD Biosciences (Erembodegem, Belgium). Anti-mouse NKp46 PE (clone 29A1.4), anti-mouse CD3 FITC (clone 145-2C11) and anti-mouse IFNγ PECy7 (clone XMG1.2) were purchased from eBioscience and anti-mouse CD69 PECy5 (clone H1.2F3) was purchased from Biolegend. Flow cytometry was carried out on a Dako Cyan flow cytometer and analysed using Flowjo software version 8.8.6 (TreeStar). Lymphocytes were identified on the basis of FSC-SSC; Human NK cells were identified by CD56+CD3− gating, and murine NK cells by NKp46+CD3− gating within the lymphocyte population.

2.3. Cell stimulations and IFN-γ intracellular staining for flow cytometric analysis

Peripheral blood mononuclear cells (PBMCs) were isolated from venous blood of healthy volunteers and HCV infected patients by Ficoll density gradient centrifugation. Cells were stimulated for 18 h at a density of 5×10^6 cells/ml, the last four hours in the presence of Golgi-Plug (BD Pharmingen); 30 ng/ml rhIL12 (Strathmann

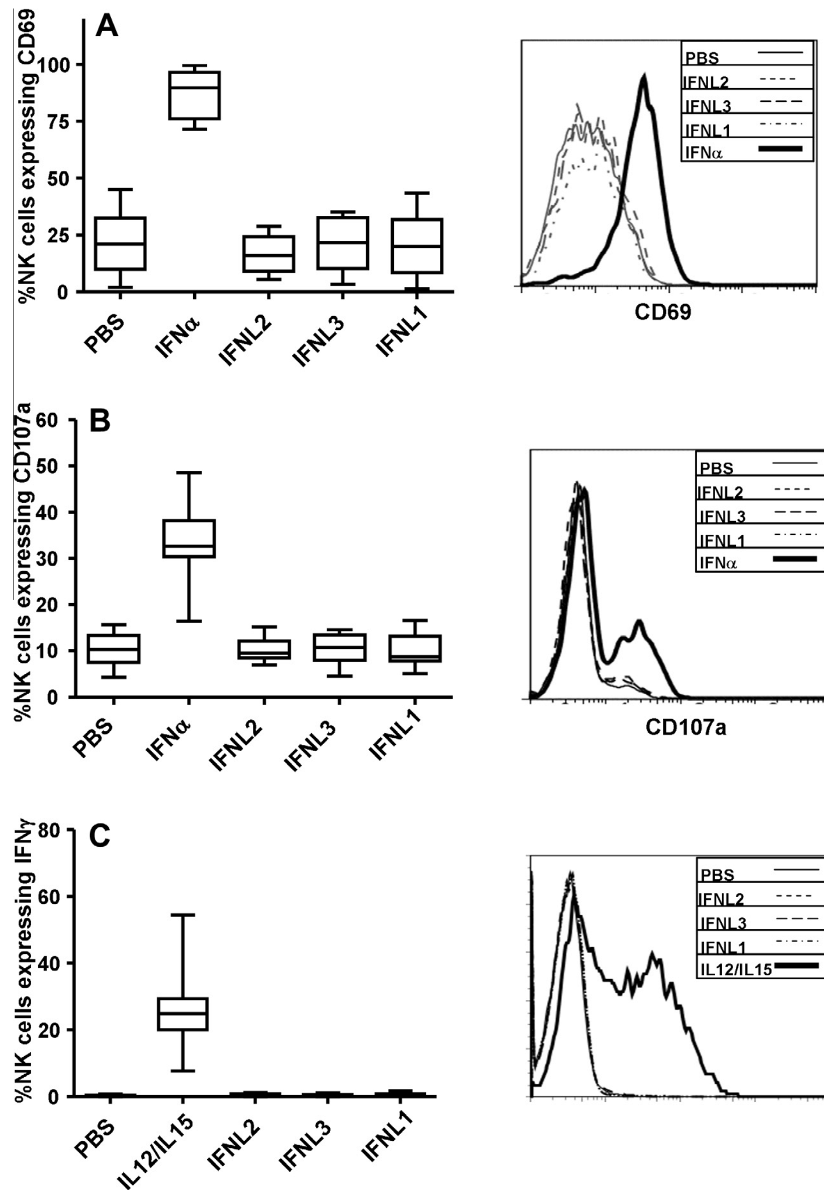

Fig. 1. IFNL does not activate human NK cells. (A) PBMCs were stimulated for 18 h with PBS, IFNL2, IFNL3 and IFNL1 [500 ng/ml] and IFN-α [1000 U/ml], and the frequency of CD56+CD3− NK cells expressing CD69 measured by flow cytometry. A flow cytometry histogram for a representative donor is shown on the right. Horizontal lines in the bar chart indicate median percentages and vertical lines indicate the range of values. A one way ANOVA was used to compare data, $n = 8$. (B) PBMCs were stimulated for 18 h with PBS, IFNL2, IFNL3 and IFNL1 [500 ng/ml] and IFN-α [1000 U/ml]. PBMCs were then co-incubated with K562 target cells. The frequency of CD56+CD3− NK cells expressing CD107a+ was determined by flow cytometry. A flow cytometry histogram for a representative donor is shown on the right and the average data presented in the bar chart. Horizontal lines indicate median percentages and vertical lines indicate the range of values. A one way ANOVA was used to compare data, $n = 5$. (C) PBMCs were stimulated for 18 h with PBS, IFNL2, IFNL3 and IFNL1 [500 ng/ml] and rhIL-12 [30 ng/ml]/rhIL-15 [100 ng/ml]. The percentage of CD56+CD3− NK cells expressing IFN-γ as measured by intracellular staining by flow cytometry is shown. Horizontal lines indicate median percentages, and vertical lines indicate the range of values. A one-way ANOVA was used to compare data, $n = 8$.

Download English Version:

https://daneshyari.com/en/article/3350185

Download Persian Version:

https://daneshyari.com/article/3350185

Daneshyari.com