Russian Journal of Infection and Immunity = Infektsiya i immunitet
2023, vol. 13, no. 4, pp. 663–674

Инфекция и иммунитет
2023, Т. 13, № 4, с. 663–674

FEATURES OF NK CELL PHENOTYPE VIRUS GENOTYPE-DRIVEN CHRONIC VIRAL HEPATITIS C

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Abstract. Elimination of the hepatitis C virus (HCV) due to direct antiviral drug (DAD) action affects alteration in virus phenotype and, accordingly, NK cell functional activity. However, the published data are very contradictory. The aim of the study was to investigae alterations in NK cell subset phenotype after DAD treatment of HCV genotype-dependent chronic viral hepatitis C (CVHC) patients. *Materials and methods.* 111 CVHC patients and 21 healthy volunteers were examined. The diagnosis was established on epidemiological, clinical and laboratory data. All 111 subjects with CVHC received direct antiviral drugs Sofosbuvir and Velpatasvir for 12 weeks. The study of the NK cell phenotypes wwas analyzed by multicolor flow cytometry. *Results.* A decreased count of cytokine-producing along with increased frequency of cytotoxic NK cells were found in CVHC patients blood samples with various HCV genotypes prior to DAD treatment. The imbalance of cytotoxic cells with a high level of functional activity was also found in CVHC patients regardless of HCV genotype. The patients with HCV genotypes 1 and 3 showed significantly increased level of immunoregulatory NK cells. In addition, increased count of glycohydrolase (CD38) and ecto-5'-nucleotidase (CD73)-expressing NK cells were found in patients with HCV genotypes 1 and 3. Hence, such alterations in NK cell phenotype in CVHC patients were presented as sustained high viral load which peaking at carriers of HCV genotype 1 that was minimal in patients with HCV genotype 2. The most prominent change in NK cells after DAD treatment was found in CVHC patients with HCV genotype 2 (normalization of CD8-expressing NK cell subset composition and count). Only patients with HCV genotype 2 after treatment had increased frequencies of peripheral blood double-negative $CD38^{-}CD73^{-}$ NK cells. Patients with HCV genotypes 1 and 3 also showed minimally improved in NK cell subset composition after DAD treatment. *Conclusion*. Evaluation of specific changes in NK cell phenotype during DAD treatment of CVHC patients driven by HCV genotype undoubtedly is of importance and high relevance. The results obtained are novel and complement the insights into CVHC immunopathogenesis. Analysis of NK cell phenotypes and functional activity in patients with CVHC may promote development of new methods for treating HCV infection.

Key words: *chronic viral hepatitis C, hepatitis C virus, genotype, NK cells, phenotype, subsets, treatment.*

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Для цитирования:
Савченко А.А., Тихонова Е.П., Анисимова А.А., Кудрявцев И.В., Беленюк В.Д., Борисов А.Г. Особенности фенотипа NK-клеток у больных хроническим вирусным гепатитом С в зависимости от генотипа вируса // Инфекция и иммунитет. 2023. Т. 13, № 4. С. 663–674. doi: 10.15789/2220-7619-HCV-8047

Citation:
Savchenko A.A., Tikhonova E.P., Anisimova A.A., Kudryavcev I.V., Belenjuk V.D., Borisov A.G. Features of NK cell phenotype virus genotype-driven chronic viral hepatitis C // Russian Journal of Infection and Immunity = Infektsiya i immunitet, 2023, vol. 13, no. 4, pp. 663–674. doi: 10.15789/2220-7619-HCV-8047

DOI: http://dx.doi.org/10.15789/2220-7619-HCV-8047

ОСОБЕННОСТИ ФЕНОТИПА NK-КЛЕТОК У БОЛЬНЫХ ХРОНИЧЕСКИМ ВИРУСНЫМ ГЕПАТИТОМ С В ЗАВИСИМОСТИ ОТ ГЕНОТИПА ВИРУСА

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Резюме. Элиминация вируса гепатита С (ВГС), вызванная действием препаратов прямого противовирусного действия (ППППВД), влияет на изменение фенотипа и, соответственно, функциональную активность NK-клеток. Однако имеющиеся в литературе данные весьма противоречивы. Целью настоящего исследования было изучение особенностей изменения фенотипа NK-клеток (с учетом субпопуляционного состава) после лечения больных хроническим вирусным гепатитом С (ХВГС) ППППВД в зависимости от генотипа ВГС. *Материалы и методы.* Обследовано 111 больных ХВГС и 21 здоровый человек в качестве контрольной группы. Диагноз ХВГС устанавливали на основании эпидемиологических и клинико-лабораторных данных при выявлении специфических серологических маркеров хронического гепатита С и РНК ВГС, включая генотипирование ВГС. Все 111 человек с ХВГС получали противовирусные препараты прямого действия (Софосбувир и Велпатасвир) в течение 12 недель. Изучение фенотипа NK-клеток крови проводили методом проточной цитометрии с использованием прямой иммунофлуоресценции цельной периферической крови с моноклональными антителами. *Результаты.* В крови больных ХВГС с различными генотипами ВГС до начала лечения ППППВД выявлено снижение количества цитокин-продуцирующих и увеличение количества цитотоксических NK-клеток. Дисбаланс цитотоксических клеток с высоким уровнем функциональной активности также был выявлен у больных ХВГС вне зависимости от генотипа ВГС. У пациентов с генотипами 1 и 3 ВГС наблюдалось достоверное увеличение NK-клеток с иммунорегуляторной активностью. Кроме того, у больных ВГС генотипов 1 и 3 выявлено увеличение количества NK-клеток, экспрессирующих гликогидролазу (CD38) и экто-5'-нуклеотидазу (CD73). Результатом выявленных нарушений в фенотипе NK-клеток у больных ХВГС было сохранение выраженной вирусной нагрузки, которая была максимальной при инфицированности генотипом 1 ВГС и минимальной у больных с генотипом 2 ВГС. Наиболее выраженные изменения фенотипического состава NK-клеток после лечения ДАД выявлены у больных ХВГС с генотипом 2 ВГС (нормализация субпопуляционного состава и количества NK-клеток, экспрессирующих CD8). Кроме того, только у больных с генотипом 2 ВГС после лечения наблюдалось повышение содержания дубль-негативных (по CD38 и CD73) NK-клеток в крови. У пациентов с генотипами 1 и 3 ВГС также наблюдалось улучшение состава NK-клеток с различными фенотипами после лечения ДАД, но эти изменения были минимальными. *Выводы.* Оценка особенностей изменения фенотипа NK-клеток при лечении в зависимости от генотипа имеет несомненную значимость и высокую актуальность. Полученные результаты обладают новизной и дополняют информацию об иммунопатогенезе ХВГС. Анализ фенотипов NK-клеток и их функциональной активности у пациентов с ХВГС может помочь в разработке новых методов лечения HCV-инфекции.

Ключевые слова: *хронический вирусный гепатит С, вирус гепатита С, генотип, NK-клетки, фенотип, субпопуляции, лечение.*

Introduction

Hepatitis C virus (Hepatitis C virus, HCV) is one of the main causes of chronic liver infections in the world. The consequences of chronic viral hepatitis C (CVHC) are highly variable, ranging from minimal histological changes to extensive fibrosis and cirrhosis with or without hepatocellular carcinoma [5, 13]. According to available estimates, the number of patients with CVHC in the world is about 180 million people and most patients are unaware of the presence of this infection in them [37]. Treatment of HCV-associated liver injury has improved significantly over the past 20 years [4, 24]. It could be closely linked with our recent data on disease pathophysiology as well as the improvement of methods for diagnostics, treatment and disease prevention.

Currently, there are two main mechanisms of viral infection development. The first one is determined by the pathogenic action of the virus itself against the background of immune disorders associated with a lack of immunity components and/or with a lack of activation for a specific pathogen (tolerance) [1, 2]. The second mechanism is realized by the activation of immune system on virus-infected cells. Consequently, the prognosis of development, the nature of the course and the outcome of an infectious disease vary significantly in depending on the state of the immune system and the characteristics of its response to an infectious pathogen [6, 9].

NK cells (Natural Killer) are defined as a separate population of lymphocytes that perform the functions of innate immunity. Cytolytic activity without prior stimulation of virus-infected and some tumor cells is the initially defined and main function of NK cells [19, 33]. The killer activity of NK cells is regulated by the expression of MHC I molecules. Infected or malignant cells can downregulate MHC I (also known as "missing-self hypothesis"), to become invisible for $CD8^+$ T cells but the loss of MHC I antigens for inhibitory receptors on NK cells sensitizes these cells for NK-mediated killing [12, 29]. However, at present, the regulation of some processes of innate and adaptive immunity is also determined by the function of NK cells which is realized by these cells during functional activation due to the secretion of a wide range of cytokines [7]. Thus, Pallmer K. and Oxenius A. (2016) showed that NK cells stimulated the maturation and activation of dendritic cells, secrete cytokines that promoted the differentiation of 'naïve' Th lymphocytes into Th1 cells [29]. In a study by Anuforo O.U.U. et al. (2018) showed that NK cells maintained apoptosis and regulated the functional activity of neutrophils against the background of antigen-induced inflammation [8]. In this regard, there is a need to study the phenotype of NK cells and the characteristics of their functional activity in patients with CVHC.

A number of studies have shown that the treatment of the disease with direct antiviral drugs (DADs) is effective and allows achieving complete elimination of HCV from the body [4, 30]. However, disturbances in the immune system may persist while levels of inflammation and fibrosis in the liver have been reduced. It was found that the treatment of CVHC patients with DADs led only to a temporary restoration of the functional activity of cells and then the reactivity of these cells decreased significantly below the norm [39]. It is assumed that this phenomenon was determined by the differentiated response of various NK cell subsets to treatment as well as the influence of the changing functional and metabolic state of the liver on the immune system as a whole. In addition, we assume that the change in the phenotype and functional activity of NK cells during DAD treatment may vary depending on the virus genotype.

Thus, the aim of the study was to investigate the features of changes in the NK cell phenotype (including subset composition) after DAD treatment of CVHC patients in depending on the genotype of HCV.

Materials and methods

Study participants. 111 CVHC patients (62 men and 47 women) aged 45.3±15.2 years were examined on the basis of the clinical gastroenterological department of Scientific Research Institute of Medical Problems of the North (Krasnoyarsk). The diagnosis was established on the basis of epidemiological and clinical and laboratory data upon detection of specific serological markers of chronic hepatitis C and HCV RNA according to the recommendations of the European Association for the Study of the Liver (EASL) [14, 15]. Liver fibrosis was studied by shear wave transient elastometry using ultrasound systems Aixplorer (France) or Siemens Acuson S2000 (Germany). Fibrosis was assessed using the METAVIR scale. There were 4 degrees of fibrosis in depending on the indicators of liver elasticity detected: F0 — no fibrosis (≤ 5.8 kPa); F1 — portal and periportal fibrosis without septa (5.9–7.2 kPa); F2 — portal and periportal fibrosis with single septa (7.3–9.5 kPa); F3 — portal and periportal fibrosis with multiple bridging porto-portal and porto-central septa (9.6–12.5 kPa); F4 — cirrhosis (≥ 12.6 kPa).

The HCV RNA content was determined by quantitative real-time PCR on a Biorad CFX96 Real Time System instrument (BioRad Laboratories, USA) using an Abbott RealTime HCV test® test system (Abbott, USA). The HCV genotype was determined using the VERSANT® HCV Amplification 2.0 (LiPA) kit (Siemens, Germany). The degree of liver fibrosis in patients with chronic hepatitis C was assessed using ultrasonic elastography using a Fibroscan 502 device (Echosens, France).

Treatment of patients with CVHC was carried out on the basis of the 2016 EASL recommendations [15]. All 111 examined patients were "naive" (not previously treated with antiviral drugs) and did not have liver cirrhosis (stage F0–F3 by METAVIR). 53 patients with CVHC had HCV genotype 1 (35 patients had liver fibrosis F0–F1 by METAVIR, 9 patients had fibrosis F2 by METAVIR, 9 patients had fibrosis F3 by METAVIR), 9 patients were diagnosed with HCV genotype 2 (3 patients had F0–F1 liver fibrosis according to METAVIR, 3 patients had F2 fibrosis by METAVIR and 3 patients had F3 fibrosis by METAVIR), genotype 3 (subtypes were not determined) HCV was detected in 49 people (25 patients had liver fibrosis F0–F1 by METAVIR, 9 people had F2 fibrosis according to METAVIR and 15 patients had F3 fibrosis by METAVIR). All 111 people with CVHC received direct antiviral drugs Sofosbuvir (400 mg) and Velpatasvir (100 mg) once a day for 12 weeks. Clinical and laboratory control with the determination of the amount of HCV RNA by polymerase chain reaction (PCR) was carried out before the start of treatment, after 4 weeks of therapy, at the end of treatment and 24 weeks after the end of therapy. Drug adherence was assessed using the Morisky–Green Test [10].

The control group included 21 practically healthy individuals who were excluded during the preventive examination of chronic diseases of various organs and systems, including infectious diseases, there were no complaints about the state of health, there were normal indicators of clinical and biochemical blood tests, there were no markers for viral hepatitis B and C in the anamnesis of life, the absence of bad habits (alcohol abuse) was indicated.

All studies were performed with the informed consent of the subjects and in accordance with the Declaration of Helsinki of the World Association "Ethical principles for conducting scientific medical research involving humans" as amended in 2000 and "Rules of Clinical Practice in the Russian Federation", approved by order of the Ministry of Health of the Russian Federation No. 266 dated June 19 2003.

Flow cytometry. The study of the NK cell phenotype was carried out by direct immunofluorescence of whole peripheral blood using monoclonal antibodies (Beckman Coulter, USA) labeled with FITC (fluorescein isothiocyanate), ECD (phycoerythrin-Texas Red-X), APC (allophycocyanin), AA700 (ale-xa fluor 700) and AA750 (alexa fluor 750) in the following panel: CD38-FITC/CD94-PE/CD73-ECD/CD56-PC5.5/CD16-PC7/CD8-APC/CD3-AA700/CD45-AA750. The distribution of antibodies along the fluorescence channels was carried out in accordance with the principles of panel formation for multicolor flow cytometry studies [3]. Sample preparation was performed according to the standard procedure [31]. Stained cells were analyzed on a Navios flow cytometer (Beckman Coulter, Inc., USA) of the Krasnoyarsk Regional Center of Research Equipment of Federal Research Center "Krasnoyarsk Science Center SB RAS". At least 5000 lymphocytes were analyzed for each blood sample. The obtained data were analyzed using the Kaluza software package (Beckman Coulter, Inc., USA).

Statistical analysis. The results were presented using the median (Me) and interquartile range as 25th (Q1) and 75th (Q3) percentiles. The significance of differences between the indicators of independent samples (when comparing the indicators of patients with control values) was assessed using the nonparametric Mann–Whitney U test. The significance of differences in indicators in groups of CVHC patients before and after treatment (dependent samples) was determined by the Wilcoxon matched pairs test. Spearman's rank correlation coefficient was calculated to assess the strength of the relationships of the studied indicators. Statistical analysis was carried out using the Statistica 8.0 software package (StatSoft Inc., USA, 2007).

Results

The level of viral load in CVHC patients is presented in Table 1. It was found that the maximum level of viral load was detected in CVHC patients with HCV genotype 1 but no statistically significant differences in this indicator between patients with different genotypes of HCV were found. Treatment with DADs for 12 weeks in "naïve" patients with CVHC without liver cirrhosis led to a sustained virological response (SVR, determined 6 months after the end of treatment). We didn't find differences in response to drug treatment in CVHC patients with different HCV genotypes. However, 2 patients with HCV genotypes 1 and 2 were identified after treatment with DADs with high levels of viral load (respectively 1.16×10^5 MU/ml and 0.06×10^5 MU/ml). The both patients were declared non-compliant after the Morisky-Green test. In this regard, the analysis of the NK cell phenotype characteristics after treatment for these two patients was not performed.

Assessing NK cell level in patients with CVHC, it was found that before the onset of DAD treatment, the percentage of NK cell total fraction ($CD3^-CD56^+CD45^+$) in the blood was reduced in patients with HCV genotypes 1 and 3 compared with control values (Table 2). CVHC patients with HCV genotype 2 during this period of the survey had an increased absolute number of NK cells in the blood relative to the control range. The percentage of $CD56^{bright}CD16^-$ and $CD56^{bright}CD16^+$ NK cells in patients with all three HCV genotypes was reduced while the level of $CD56^{dim}CD16^-$ NK cells was increased compared to control values. At the same time, the most pronounced increase in the percentage of $CD56^{dim}CD16^-$ NK cells was found in patients with HCV genotype 2. A high level of $CD56^{dim}CD16^+$ NK cells relative to control values was found in all patients with CVHC and didn't depend on the HCV genotype. Only patients with HCV genotype 1 before DAD treatment had a reduced number of $CD56^{dim}CD94^+$ NK cells relative to the control level. At the same time, an increase in the number of $CD56^{dim}CD94^+$ NK cells in patients with HCV genotype 2 was found relative to the level detected in patients with HCV genotype 1.

It is known that about 30% of peripheral blood NK cells express the CD8 receptor, the functional activity of such cells is considered high and they demonstrate increased survival in the process of target cell lysis [18, 24]. We found that the maximum (among CVHC patients) percentage of NK cells expressing CD8 ($CD3^-CD56^+CD8^+CD45^+$) before DAD treatment was detected in patients with HCV genotype 2 (Table 3). However, there were practically no differences in the percentage of different subsets of NK cells expressing and not expressing the CD8 marker in CVHC

Table 1. The level of HCV genotype-driven viral load (MU/ml) in patients with chronic hepatitis C before treatment [Me (Q_1–Q_3)]

Genotypes	Viral load	p
Genotype 1	2.92×10^5 (0.44×10^5 — 7.62×10^5)	
Genotype 2	0.72×10^5 (0.35×10^5 — 1.56×10^5)	$p_1 = 0.127$
Genotype 1	1.4×10^5 (0.48×10^5 — 5.20×10^5)	$p_1 = 0.499$ $p_2 = 0.226$

Note. p_1 — significant differences versus patients with HCV genotype 1; p_2 — significant differences versus patients with HCV genotype 2.

patients in depending on the HCV genotype. The content of $CD56^{bright}CD16^{-}CD8^{-}$, $CD56^{bright}CD16^{-}CD8^{+}$, $CD56^{bright}CD16^{+}CD8^{-}$ and $CD56^{bright}CD16^{+}CD8^{+}$ NK cells in all patients with CVHC was reduced relative to the control values while the number of $CD56^{dim}CD16^{-}CD8^{-}$, $CD56^{dim}CD16^{-}CD8^{+}$, $CD56^{dim}CD16^{+}CD8^{-}$ and $CD56^{dim}CD16^{+}CD8^{+}$ NK cells was increased. At the same time, the maximum levels of $CD56^{dim}CD16^{-}CD8^{-}$ and $CD56^{dim}CD16^{-}CD8^{+}$ NK cells were detected in the blood of patients with genotype 2.

Table 2. HCV genotype-driven NK cell subset composition in patients with chronic hepatitis C before treatment [Me (Q_1–Q_3)]

Parameters	Control n = 21	Patients with CVHC		
		Genotype 1 n = 53	Genotype 2 n = 9	Genotype 3 n = 49
$CD3^{-}CD56^{+}CD45^{+}$, %	11.09 (8.07–17.80)	9.34 (5.85–12.47) $p_1 = 0.042$	10.97 (9.38–20.06)	8.08 (6.65–12.36) $p_1 = 0.026$
$CD3^{-}CD56^{+}CD45^{+}$, 10^9/L	0.17 (0.12–0.27)	0.21 (0.12–0.28)	0.28 (0.14–0.72) $p_1 = 0.047$	0.19 (0.13–0.25)
$CD56^{bright}CD16^{-}$, %	1.26 (0.63–1.96)	0.27 (0.14–0.49) $p_1 < 0.001$	0.20 (0.09–1.73) $p_1 = 0.046$	0.23 (0.12–0.38) $p_1 < 0.001$
$CD56^{bright}CD16^{+}$, %	6.67 (4.77–14.45)	0.35 (0.09–3.23) $p_1 < 0.001$	0.43 (0.08–2.23) $p_1 < 0.001$	0.51 (0.16–2.07) $p_1 < 0.001$
$CD56^{dim}CD16^{-}$, %	0.43 (0.25–1.38)	1.67 (1.15–3.45) $p_1 < 0.001$	4.66 (2.87–8.37) $p_1 < 0.001$ $p_2 = 0.007$	1.60 (1.16–2.70) $p_1.3 < 0.001$
$CD56^{dim}CD16^{+}$, %	1.86 (0.80–2.11)	3.05 (1.39–6.00) $p_1 = 0.006$	4.80 (1.49–13.43) $p_1 = 0.036$	4.68 (1.60–8.09) $p_1 = 0.002$
$CD56^{bright}CD94^{-}$, %	0.06 (0.02–1.76)	0.05 (0.01–0.18)	0.04 (0.01–0.05)	0.04 (0.02–0.10)
$CD56^{bright}CD94^{+}$, %	0.24 (0.13–0.74)	0.36 (0.20–0.54)	0.41 (0.21–0.55)	0.35 (0.23–0.52)
$CD56^{dim}CD94^{-}$, %	4.26 (2.52–5.99)	4.39 (2.76–6.69)	5.28 (4.46–5.68)	4.15 (2.93–5.48)
$CD56^{dim}CD94^{+}$, %	6.11 (2.81–8.74)	3.20 (2.28–5.30) $p_1 = 0.034$	5.45 (3.78–7.22) $p_2 = 0.013$	4.32 (2.45–6.39)

Note. p_1 — significant differences versus control; p_2 — significant differences versus patients with HCV genotype 1; p_3 — significant differences versus patients with HCV genotype 2.

Table 3. HCV genotype-driven peripheral blood CD8-expressing NK cell level (in %) in patients with chronic hepatitis C before treatment [Me (Q_1–Q_3)]

Parameters	Control n = 21	Patients with CVHC		
		Genotype 1 n = 53	Genotype 2 n = 9	Genotype 3 n = 49
$CD3^{-}CD56^{+}CD8^{-}CD45^{+}$	5.44 (4.73–9.27)	5.24 (3.21–6.96)	5.95 (3.45–6.67)	4.95 (3.20–6.78)
$CD3^{-}CD56^{+}CD8^{+}CD45^{+}$	4.23 (2.40–6.52)	3.45 (1.81–5.19)	6.24 (3.46–13.06) $p_2 = 0.030$	3.17 (1.94–5.70) $p_3 = 0.029$
$CD56^{bright}CD16^{-}CD8^{-}$	0.53 (0.31–0.86)	0.14 (0.06–0.23) $p_1 < 0.001$	0.09 (0.03–0.18) $p_1 = 0.017$	0.12 (0.07–0.20) $p_1 < 0.001$
$CD56^{bright}CD16^{-}CD8^{+}$	0.48 (0.21–0.89)	0.11 (0.04–0.24) $p_1 < 0.001$	0.10 (0.06–0.45) $p_1 = 0.047$	0.09 (0.04–0.19) $p_1 < 0.001$
$CD56^{bright}CD16^{+}CD8^{-}$	3.68 (2.13–4.41)	0.17 (0.03–1.57) $p_1 < 0.001$	0.16 (0.09–0.34) $p_1 < 0.001$	0.18 (0.05–0.78) $p_1 < 0.001$
$CD56^{bright}CD16^{+}CD8^{+}$	2.14 (1.38–5.42)	0.22 (0.04–1.73) $p_1 < 0.001$	0.21 (0.02–1.40) $p_1 = 0.003$	0.31 (0.11–1.19) $p_1 < 0.001$
$CD56^{dim}CD16^{-}CD8^{-}$	0.21 (0.07–0.84)	1.28 (0.81–2.19) $p_1 < 0.001$	2.20 (1.57–2.89) $p_1 < 0.001$	1.25 (0.71–1.78) $p_1 < 0.001$ $p_3 = 0.020$
$CD56^{dim}CD16^{-}CD8^{+}$	0.18 (0.07–0.25)	0.40 (0.19–0.96) $p_1 = 0.006$	3.00 (0.91–5.42) $p_{1,2} < 0.001$	0.38 (0.20–0.74) $p_1 = 0.005$ $p_3 < 0.001$
$CD56^{dim}CD16^{+}CD8^{-}$	1.14 (0.44–1.51)	1.85 (0.74–3.47) $p_1 = 0.012$	2.76 (0.91–4.14) $p_1 = 0.048$	2.51 (0.54–4.60) $p_1 = 0.031$
$CD56^{dim}CD16^{+}CD8^{+}$	0.38 (0.22–0.47)	1.19 (0.45–2.22) $p_1 < 0.001$	1.73 (0.44–9.16) $p_1 = 0.012$	1.66 (0.49–3.43) $p_1 < 0.001$

Note. See footnote to Table 2.

In recent years, studies have appeared that characterize the features of the reactivity of NK cells in the expression of CD38 and CD73 receptors [17, 28]. It was found that the number of $CD56^+CD38^-CD73^-$ and $CD56^+CD38^+CD73^-$ NK cells was reduced in CVHC patients with HCV genotypes 1 and 3 before DAD treatment while the content of $CD56^+CD38^+CD73^+$ NK cells in patients with the same HCV genotypes was increased relative to control values (Table 4). No changes in the number of NK cells expressing CD38 and CD73 markers were found in patients with HCV genotype 2.

Relationships between the number of NK cells with different phenotypes and the level of viral load in patients with CVHC were studied using correlation analysis. It was found that the level of viral load was negatively correlated with the number of $CD56^{dim}CD16^-$ NK cells ($r = -0.32$, $p = 0.016$) and was positively correlated with the content of $CD56^{dim}CD16^-CD8^+$ NK cells ($r = 0.26$, $p = 0.048$) in patients with HCV genotype 1. The level of viral load was only negatively correlated with the number of $CD56^{bright}CD16^-$ ($r = -0.94$, $p = 0.005$) and $CD56^{bright}CD16^+CD8^+$ NK cells ($r = -0.96$, $p = 0.004$) in patients with HCV genotype 2. At the same time, the level of viral load only positively correlated with the number of $CD56^+CD38^+CD73^-$ ($r = 0.30$, $p = 0.035$) and $CD56^+CD38^-CD73^+$ NK cells ($r = 0.38$, $p = 0.007$) in patients with HCV genotype 3.

Some changes in the subset composition of NK cells in CVHC patients persisted even after DAD treatment. Thus, an increased number (relative to control values) of $CD56^{dim}CD16^-$ NK cells was detected in the blood of patients after treatment regardless of HCV genotype (Table 5). It was also found that a low number of $CD56^{bright}CD16^-$ and $CD56^{bright}CD16^+$ NK cells, as well as an increased content of $CD56^{dim}CD16^+$ NK cells, persisted in patients after treatment with HCV genotypes 1 and 3. At the same time, the number of $CD56^{bright}CD16^-$,

Table 4. HCV genotype-driven peripheral blood CD38- and CD73-expressing NK cell level (in %) in patients with chronic hepatitis C before treatment [Me (Q_1–Q_3)]

Parameters	Control n = 21	Patients with CVHC		
		Genotype 1 n = 53	Genotype 9 n = 9	Genotype 3 n = 49
$CD56^+CD38^-CD73^-$	2.34 (1.76–5.25)	1.12 (0.62–2.51) $p_1 < 0.001$	1.35 (1.01–6.47)	1.07 (0.56–1.75) $p_1 < 0.001$
$CD56^+CD38^-CD73^+$	0.013 (0.011–0.082)	0.047 (0.020–0.096)	0.055 (0.016–0.086)	0.034 (0.017–0.072)
$CD56^+CD38^+CD73^-$	7.93 (6.10–15.01)	5.87 (4.21–8.29) $p_1 = 0.009$	7.69 (6.77–13.57)	5.56 (4.17–8.58) $p_1 = 0.009$
$CD56^+CD38^+CD73^+$	0.039 (0.020–0.088)	0.156 (0.074–0.341) $p_1 = 0.010$	0.179 (0.031–0.335)	0.114 (0.055–0.251) $p_1 = 0.018$

Note. p_1 — significant differences versus control.

Table 5. HCV genotype-driven peripheral blood NK cell subset composition in patients with chronic hepatitis C after treatment [Me (Q_1–Q_3)]

Parameters	Control n = 21	Patients with CVHC		
		Genotype 1 n = 52	Genotype 2 n = 8	Genotype 3 n = 49
$CD3^-CD56^+CD45^+$, %	11.09 (8.07–17.80)	13.10 (7.63–15.91)	18.63 (17.81–22.48)	9.19 (8.27–12.41)
$CD3^-CD56^+CD45^+$, 10^9/L	0.17 (0.12–0.27)	0.30 (0.16–0.46)	0.40 (0.26–0.66)	0.21 (0.15–0.30)
$CD56^{bright}CD16^-$, %	1.26 (0.63–1.96)	0.20 (0.09–0.54) $p_1 < 0.001$	0.86 (0.20–3.76)	0.17 (0.11–0.37) $p_1 < 0.001$
$CD56^{bright}CD16^+$, %	6.67 (4.77–14.45)	0.90 (0.18–4.48) $p_1 < 0.001$	8.95 (0.63–15.61)	1.00 (0.29–6.22) $p_1 < 0.001$
$CD56^{dim}CD16^-$, %	0.43 (0.25–1.38)	1.85 (0.81–3.97) $p_1 < 0.001$	1.70 (1.33–6.20) $p_1 = 0.046$	2.13 (1.24–2.73) $p_1 < 0.001$
$CD56^{dim}CD16^+$, %	1.86 (0.80–2.11)	3.39 (0.86–6.68) $p_1 = 0.037$	4.61 (0.30–14.79)	3.07 (1.26–6.30) $p_1 = 0.037$
$CD56^{bright}CD94^-$, %	0.06 (0.02–1.76)	0.04 (0.02–0.20)	0.06 (0.03–1.63)	0.08 (0.02–0.28)
$CD56^{bright}CD94^+$, %	0.24 (0.13–0.74)	0.29 (0.18–0.51)	3.74 (0.90–11.61) $p_1 < 0.001$ $p_2 = 0.002$	0.42 (0.19–0.75) $p_3 = 0.004$
$CD56^{dim}CD94^-$, %	4.26 (2.52–5.99)	5.93 (3.33–8.78)	6.02 (2.72–6.76)	5.46 (4.24–6.75)
$CD56^{dim}CD94^+$, %	6.11 (2.81–8.74)	4.50 (2.52–6.49)	6.43 (4.25–14.79)	3.43 (1.87–4.78) $p_1 = 0.015$

Note. See footnote to Table 2.

$CD56^{bright}CD16^{+}$ and $CD56^{bright}CD94^{+}$ NK cells in the blood of CVHC patients with the HCV genotype 2 after DAD treatment increased significantly compared to the initial (before treatment) values (by Wilcoxon matched pairs test: $p < 0.001$, $p = 0.038$ and $p < 0.001$ accordingly) which led to the normalization of the content of $CD56^{bright}CD16^{-}$ and $CD56^{bright}CD16^{+}$ NK cells and a significant increase in the level of $CD56^{bright}CD94^{+}$ cells relative to control values (see Tables 2 and 5). Only patients with HCV genotype 3 after treatment had a decrease in the level of $CD56^{dim}CD94^{+}$ NK cells relative to control values.

The most pronounced changes in the blood levels of expressing and non-expressing CD8 NK cells after DAD treatment were found in CVHC patients with HCV genotype 2 (Table 6). A statistically significant increase in the number of $CD56^{bright}CD16^{-}CD8^{-}$, $CD56^{bright}CD16^{-}CD8^{+}$, $CD56^{bright}CD16^{+}CD8^{-}$ and $CD56^{bright}CD16^{+}CD8^{+}$ NK cells was found in patients of this group after treatment relative to baseline values: by Wilcoxon matched pairs test: $p = 0.044$, $p = 0.010$, $p = 0.037$ and $p = 0.004$ accordingly (see Tables 3 and 6). This resulted in an increase in the content of cells with these phenotypes to the level of control values. In addition, the number of $CD3^{-}CD56^{+}CD8^{+}CD45^{+}$ cells in the blood of patients with HCV genotype 2 after treatment was increased by almost 3.5 times relative to control values. Almost complete agreement with the initial values in terms of the number of expressing and non-expressing CD8 NK cells was found in patients with HCV genotype 1 after DAD treatment. The only exception was the normalization of the number of $CD56^{dim}CD16^{+}CD8^{-}$ NK cells. Normalization of the content of $CD56^{dim}CD16^{-}CD8^{+}$ and $CD56^{dim}CD16^{+}CD8^{-}$ NK cells was also found in patients with genotype 3 after treatment. In addition, an increase in the number of $CD56^{bright}CD16^{+}CD8^{-}$ cells in patients of this group after treatment was detected using the Wilcoxon matched pairs test ($p = 0.045$), but their level still remained below the control. Other features in the number of expressing and non-expressing CD8 NK cells identified before treatment in patients with genotype 3 remained unchanged after DAD treatment.

The number of $CD56^{+}CD38^{+}CD73^{-}$ and $CD56^{+}CD38^{+}CD73^{+}$ NK cells in the blood of CVHC patients with HCV genotype 1 was normalized after DAD treatment while the content of $CD56^{+}CD38^{-}CD73^{-}$ cells remained low relative to the control range (Table 7). Patients with genotype 2 after treatment had the number of expressing and non-expressing CD38 and CD73 NK cells in the norm as well as before treatment. However, the median content of $CD56^{+}CD38^{-}CD73^{-}$ cells was increased in patients of this group after treatment by 5 times compared with the initial level. All changes in the number of expressing and non-expressing CD38 and CD73 NK cells detected in CVHC patients with HCV genotype 3 before treatment were also found by us after DAD treatment.

Table 6. HCV genotype-driven peripheral blood CD8-expressing NK cell level (in %) in patients with chronic hepatitis C after treatment [Me (Q_1–Q_3)]

Parameters	Control n = 21	Patients with CVHC		
		Genotype 1 n = 52	Genotype 2 n = 8	Genotype 3 n = 49
$CD3^{-}CD56^{+}CD8^{-}CD45^{+}$	5.44 (4.73–9.27)	6.72 (3.88–9.78)	7.38 (3.23–7.43)	5.50 (4.16–7.26)
$CD3^{-}CD56^{+}CD8^{+}CD45^{+}$	4.23 (2.40–6.52)	4.29 (2.47–6.34)	14.68 (9.77–15.01) $p_1 = 0.012$ $p_2 = 0.001$	3.29 (2.82–4.87) $p_3 = 0.004$
$CD56^{bright}CD16^{-}CD8^{-}$	0.53 (0.31–0.86)	0.12 (0.04–0.28) $p_1 < 0.001$	0.13 (0.09–0.34)	0.10 (0.06–0.18) $p_1 < 0.001$
$CD56^{bright}CD16^{-}CD8^{+}$	0.48 (0.21–0.89)	0.08 (0.05–0.33) $p_1 < 0.001$	0.50 (0.11–3.56) $p_2 = 0.046$	0.07 (0.04–0.19) $p_1 < 0.001$ $p_3 = 0.041$
$CD56^{bright}CD16^{+}CD8^{-}$	3.68 (2.13–4.41)	0.34 (0.12–2.34) $p_1 < 0.001$	0.25 (0.19–5.60)	0.56 (0.13–2.68) $p_1 < 0.001$
$CD56^{bright}CD16^{+}CD8^{+}$	2.14 (1.38–5.42)	0.54 (0.08–2.00) $p_1 < 0.001$	8.58 (0.43–9.46)	0.55 (0.11–2.58) $p_1 = 0.005$
$CD56^{dim}CD16^{-}CD8^{-}$	0.21 (0.07–0.84)	1.30 (0.51–3.05) $p_1 < 0.001$	1.52 (0.45–2.26)	1.53 (0.79–2.17) $p_1 < 0.001$
$CD56^{dim}CD16^{-}CD8^{+}$	0.18 (0.07–0.25)	0.39 (0.19–1.14) $p_1 = 0.004$	0.81 (0.11–3.92)	0.19 (0.10–0.54)
$CD56^{dim}CD16^{+}CD8^{-}$	1.14 (0.44–1.51)	1.78 (0.46–4.38)	2.41 (0.22–4.59)	1.67 (0.73–4.10)
$CD56^{dim}CD16^{+}CD8^{+}$	0.38 (0.22–0.47)	0.95 (0.60–2.75) $p_1 = 0.002$	1.74 (0.08–10.16)	0.92 (0.29–2.54) $p_1 = 0.016$

Note. See footnote to Table 2.

Table 7. HCV genotype-driven peripheral blood CD38- and CD73-expressing NK cell level (in %) in patients with chronic hepatitis C after treatment

Parameters	Control n = 21	Patients with CVHC		
		Genotype 1 n = 52	Genotype 2 n = 8	Genotype 3 n = 49
$CD56^+CD38^-CD73^-$	2.34 (1.76–5.25)	1.76 (0.80–3.40) $p_1 = 0.035$	6.75 (0.90–16.48)	0.98 (0.57–1.78) $p_1 < 0.001$
$CD56^+CD38^-CD73^+$	0.013 (0.011–0.082)	0.036 (0.021–0.076)	0.038 (0.018–0.072)	0.027 (0.017–0.059)
$CD56^+CD38^+CD73^-$	7.93 (6.10–15.01)	7.60 (4.37–11.12)	12.65 (5.01–16.84)	6.58 (5.19–7.86) $p_1 = 0.021$
$CD56^+CD38^+CD73^+$	0.039 (0.020–0.088)	0.101 (0.052–0.218)	0.412 (0.145–0.678)	0.132 (0.071–0.243) $p_1 < 0.001$

Note. p_1 – significant differences versus control.

Discussion

NK cells are a heterogeneous population, have natural cytolytic activity and are capable of producing a wide range of cytokines [12, 19, 33]. We carried out a study of the content of the main fractions of blood NK cells in CVHC patients according to CD16 and CD56 markers. CD16 is a low-affinity G III type immunoglobulin receptor (FcγRIII), which mediates the mechanism of cellular antibody-dependent cytotoxicity [11, 19]. The CD56 marker (NCAM, Leu-19, NKH-1) is a glycoprotein belonging to the immunoglobulin superfamily and is involved in the implementation of intercellular contacts [11]. Two main subpopulations of NK cells are distinguished based on the level of CD56 expression. $CD56^{bright}$ NK cells are actively proliferating cells, show minimal cytotoxic activity but intensively synthesize and secrete cytokines such as IFNγ, TNF and GM-CSF [26]. Accordingly, this subset of NK cells is defined as cytokine-synthesizing. There is also evidence that $CD56^{bright}$ NK cells can be defined as regulatory (iNK cells) due to the pleiotropic function of cytokines in various immune and non-immune processes [23, 37]. Cells with the $CD56^{bright}CD16^+$ phenotype are mature NK cells, cells with the $CD56^{bright}CD16^-$ phenotype are defined as less mature and predominantly localized in secondary lymphoid organs (through the expression of CCR7) [22, 23, 35]. The degree of proliferation of $CD56^{dim}$ NK cells in response to activation stimuli is much lower, the cells produce an insignificant amount of cytokines (including IFNγ), but have a high level of cytotoxicity [23]. Accordingly, this subset is defined as cytotoxic. At the same time, the fraction of $CD56^{dim}CD16^-$ cells is characterized as maturing NK cells, cells with the $CD56^{dim}CD16^+$ phenotype predominantly circulate in the blood and, expressing CXCR1, CX3CR1, and ChemR23, migrate to the zones of immune-inflammatory processes [22, 23, 35].

We found that the restructuring of the subset composition of NK cells in CVHC patients before the start of DAD treatment occurred with all three HCV genotypes while patients with genotypes 1 and 3 also had a decrease in the percentage of the total fraction of NK cells and patients with HCV genotype 2 had an increase in the absolute number of NK cells. The restructuring of the subset composition of NK cells was determined by a decrease in the amount of cytokine-producing NK cells ($CD56^{bright}$) in the blood of patients and an increase in the level of cytotoxic cells ($CD56^{dim}$). The revealed changes characterized the reactive reaction of NK cells to HCV infection. The number of mature cytokine-producing cells ($CD56^{dim}CD16^-$) in patients with HCV genotype 2 was minimal in the blood. At the same time, the maximum content of $CD56^{dim}CD16^-$ NK cells was found in patients with HCV genotype 2 (more than 10 times higher than control values). Moreover, it was with this subset of NK cells that the cells with the most pronounced cytotoxic activity was associated [4]. Therefore, a feature is revealed in the reaction of NK cells in CVHC in depending on the genotype of the HCV. It should also be noted that a high level of $CD56^{dim}CD16^-$ NK cells in patients with HCV genotype 1 was associated with an increase in viral load while a low level of $CD56^{bright}CD16^-$ NK cells in patients with HCV genotype 2 correlated with a decrease in viral load (according to the results of correlation analysis). This result determines the low functional activity of NK cells with the $CD56^{dim}$ phenotype (the number of which has been increased) in CVHC patients and the increased significance in the mechanisms of antiviral immunity of cells with the $CD56^{bright}$ phenotype (the number of which has been reduced).

The percentage and absolute number of NK cells in the blood of CVHC patients returned to normal after DAD treatment for all three types of HCV genotype. Against this background, the changes described above in the subset composition of NK cells in patients with HCV genotypes 1 and 3 were completely preserved after treatment. At the same time, patients with HCV genotype 2 after DAD treatment had a complete normalization of cytokine-producing cells of NK cells while the number of cells with the $CD56^{dim}CD16^+$ phenotype also increased to the level of control values. The level of $CD56^{dim}CD16^-$ cells in CVHC patients of this group after treatment remained elevated relative to control values (but already 3.95 times) but was already the lowest compared to the detected number in other HCV genotypes. Accordingly, it can be con-

cluded that a more pronounced improvement in the subset composition of NK cells after DAD treatment was in patients with HCV genotype 2.

We also examined the number of cytokine-producing and cytotoxic NK cells expressing the CD94 marker in the blood of CVHC patients before and after DAD treatment. CD94 is a 30 kDa type II transmembrane glycoprotein belonging to the family of Ca^{2+}-dependent lectins (type C) [27]. The CD94 receptor binds to a member of the NKG2 family to form a disulfide-coupled NK cell receptor for class I MHC molecules which have higher specificity than inhibitory/activating killer cell receptors (KIR/KAR) belonging to the Ig superfamily [38]. It has been proven that the level of synthesis of functional molecules (perforins and granzymes) is carried out depending on the expression of the CD94 receptor: $CD94^+CD56^{dim}$ NK cells had lower expression levels of granzyme B and perforin and, accordingly, were characterized by a lower level of cellular cytotoxicity than $CD94^-CD56^{dim}$ NK cells [38]. When examining patients with CVHC before starting DAD treatment, we found minor changes in the content of blood NK cells expressing the CD94 receptor. Only patients with HCV genotype 1 had low levels (compared with control values and those found in patients with HCV genotype 2) of $CD56^{dim}CD94^+$ NK cells. At the same time, after DAD treatment, the content of this fraction of NK cells was already reduced in patients with HCV genotype 3. However, patients with HCV genotype 2 after DAD treatment had a significant increase (more than 15 times compared to control values) in the number of $CD56^{bright}CD94^+$ NK cells in the blood. In the Hughes T. et al. (2014) study, NK cells with the $CD56^{bright}CD94^+$ phenotype were characterized as IFNγ producing cells with cytolytic activity [20]. Therefore, we can conclude that the treatment of CVHC patients with HCV genotype 2 led to an increase in the functional activity of NK cells.

In recent years, attention has been drawn to the fraction of NK cells expressing the CD8 receptor [16, 25]. It has been proven that $CD8^+$ NK cells are highly sensitive to activation stimuli, have an increased level of cytotoxicity, and co-express molecules such as IFNγ, CD107a, TNFα, and MIP-1β [16, 25]. Our examination of CVHC patients showed that the maximum number (but corresponding to control values) of NK cells expressing the CD8 receptor was detected before the start of DAD treatment in patients with HCV genotype 2. In general, changes in the number of NK cells expressing the CD8 receptor relative to control values in CVHC patients with different HCV genotypes before DAD treatment corresponded to the above changes in the content of $CD56^{bright}$ and $CD56^{dim}$ NK cells: the level of $CD56^{bright}$ cells expressing and non-expressing CD8 was reduced relative to control values, while the number of $CD56^{dim}$ cells expressing and not expressing CD8 was increased. At the same time, patients with genotype 2 had during this period of examination had the maximum number of $CD56^{dim}CD16^-CD8^-$ and $CD56^{dim}CD16^-CD8^+$ NK cells (10.5 and 16.7 times higher than the control level, respectively). Correlation analysis showed no relationship between the content of $CD8^+$ NK cells and the level of viral load. A positive correlation was found with the number of $CD56^{dim}CD16^-CD8^+$ cells in HCV genotype 1 and a negative correlation with the level of $CD56^{bright}CD16^+CD8^+$ cells in HCV genotype 2 with the level of viral load which made it possible to once again note the functional insufficiency of NK cells in CVHC patients before the start of DAD treatment.

The number of $CD8^+$NK cells in the blood after DAD treatment in CVHC patients with HCV genotypes 1 and 3 remained at the level of control values. At the same time, the content of $CD8^+$NK cells in patients with HCV genotype 2 increased after treatment and exceeded the control values by almost 3.5 times. The patients with this genotype after treatment also had changes in the number of $CD56^{bright}$ and $CD56^{dim}$ NK cells expressing CD8 receptor which brought the content of these cells in the blood in line with the control values. Only the number of $CD56^{dim}CD16^+CD8^-$ NK cells returned to normal in patients with HCV genotype 1 after treatment. At the same time, the number of $CD56^{dim}CD16^-CD8^+$ and $CD56^{dim}CD16^+CD8^-$ NK cells in the blood of patients with HCV genotype 3 also returned to normal after treatment. At the same time, the number of $CD56^{dim}CD16^-CD8^+$ and $CD56^{dim}CD16^+CD8^-$ NK cells in the blood of patients with HCV genotype 3 also returned to normal after treatment. Consequently, the result of the treatment of CVHC patients was the normalization of the number of individual fractions of NK cells with cytotoxic activity for individuals with genotypes 1 and 3 as well as the restoration to control values of NK cells with cytokine-producing and cytotoxic activity for persons with genotype 2.

Also, the number of NK cells expressing and non-expressing CD38 and CD73 in CVHC patients before and after DAD treatment was studied. CD38 is a about 45 kDa glycoprotein that is expressed on the surface of many cells of the immune system and is defined as a glycohydrolase (EC 3.2.2.6) that catalyzes the degradation of NAD^+ or $NADP^+$ to form cyclic ADP-ribose and nicotinamide [32]. The products of this reaction are necessary for the regulation of the intracellular pool of Ca^{2+}. CD38 is involved in the regulation of cellular metabolism through the regulation of the NAD pool and in the pathogenesis of many conditions, including aging, obesity, diabetes, heart disease, asthma, and inflammation. It was shown that the expression of the CD38 receptor on the membrane of NK cells led to a decrease in their functional activity [34]. CD73 (NT5E) is an ecto-5'-nucleotidase (EC 3.1.3.5) – an enzyme that cleaves adenosine monophosphate (AMP) to adenosine [36]. It has been proven that NK cells expressing CD73 have reduced cytotoxic activity and can implement the functions of regulatory cells [28].

We found a decrease in the number of double-negative NK cells as well as positive for CD38 and negative for CD73 in CVHC patients with HCV genotypes 1 and 3 before the start of DAD treatment. At the same time, the revealed changes in patients HCV genotypes 1 and 3 were manifested in the blood of patients at this stage of the examination against the background of a pronounced increase in the number of double-positive NK cells, that is, cells with the most pronounced regulatory activity. It should also be noted that only patients with HCV genotype 3 had positive correlations between viral load and the number of CD38 and CD73 positive NK cells. Patients with HCV genotype 2 before DAD treatment had no changes in the number of NK cells expressing CD38 and CD73 relative to control values. The patients with HCV genotype 1 after treatment had a normalization of the number of regulatory NK cells and that were positive for CD38 which undoubtedly determined the increase in the functional activity of NK cells. At the same time, there were no changes in the number of NK cells in the blood with the expression of CD38 and CD73 in patients with HCV genotype 3 after the treatment which made it possible to conclude that the immunoregulatory activity of NK cells was preserved in patients of this group. The level of double-negative for CD38 and CD73 NK cells in patients with HCV genotype 2 after treatment increased by 5 times relative to the initial values (but within control range). Consequently, the number of positive for CD38 and CD73 NK cells in CVHC patients before and after DAD treatment differed significantly depending on HCV genotype, and the most pronounced immunoregulatory activity of NK cells was manifested in HCV genotype 3.

It should be noted that data on changes in the phenotype and functional activity of NK cells after DAD treatment of CVHC patients are very contradictory. For example, in the work of Zhang X. et al. (2022) was shown that the functional activity of NK cells was restored during treatment but then decreased to a level below the control values. At the same time, the authors noted (as in our study) the number of $CD56^{dim}$NK cells in patients constantly increased in the course of DAD treatment [39]. At the same time, the study of Jiang H.J. et al. (2019) was showed that the functional activity of NK cells in CVHC patients was restored during DAD treatment due to an increase in the number of $CD56^{bright}$NK cells and a decrease in the content of NK cells expressing the NKG2A receptor [21].

Conclusion

Thus, a decrease in the number of cytokine-producing and an increase in the number of cytotoxic NK cells was found in the blood of CVHC patients with various HCV genotypes before the start of DAD treatment. Such changes in the subset composition of NK cells can be regarded as a functional response to the persistence of the virus in the body. However, imbalance imbalance of cytotoxic cells with a high level of functional activity was found in CVHC patients regardless of HCV genotype. On the one hand, before DAD treatment, patients had an increase in the number of NK cells expressing the CD8 receptor, on the other hand, there was no increase in the content of terminally differentiated cytotoxic NK cells with a maximum intracellular level of perforins and granzymes ($CD56^{dim}CD94^{-}$). The patients with HCV genotypes 1 and 3 had a significant increase in NK cells with immunoregulatory activity. In addition, an increase in the number of NK cells expressing glycohydrolase (CD38) and ecto-5'-nucleotidase (CD73) (cells with immunoregulatory function) was found in patients with HCV genotypes 1 and 3. The result of the identified disorders in the phenotype of NK cells in CVHC patients was the preservation of a pronounced viral load which was maximum in HCV genotype 1 and minimal in patients with HCV genotype 2. The most pronounced changes in the phenotypic composition of NK cells after DAD treatment were found in CVHC patients with HCV genotype 2 (normalization of the subset composition and the number of NK cells expressing CD8). In addition, only patients with HCV genotype 2 after treatment had an increase in the content of double-negative (for CD38 and CD73) NK cells in the blood. The patients with HCV genotypes 1 and 3 also had an improvement in the composition of NK cells with different phenotypes after DAD treatment but these changes were minimal. Accordingly, evaluation of the features of changes in the phenotype of NK cells during DAD treatment the CVHC patients in depending on the genotype have of undoubted significance and high relevance. The results obtained are novel and complement the information on the immunopathogenesis of chronic viral hepatitis C. Analysis of the phenotypes of NK cells and their functional activity in patients with CVHC can help in the development of new methods of treating HCV infection.

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Поступила в редакцию 17.03.2023
Принята к печати 21.06.2023

Received 17.03.2023
Accepted 21.06.2023