Phenotypic Characteristics and Function of NK Cell Subsets in cART-Treated HIV-1-Infected Individuals

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Abstract

Natural killer (NK) cell plays an important role in an innate immune response against viral infection. The kinetics regulation and functional consequences of NK cells in the pathogenesis of diseases are uncertain. We analyzed NK cell distribution and function of successfully combination antiretroviral therapy (cART)-treated HIV-1 infected individuals in Khon Kaen Regional Hospital, Thailand. The results demonstrated that increased percentage and the total number of NK cell in cART-treated HIV-1 infected patients with preferential high levels of CD56dimCD16+ and CD56−CD16+ subsets when compared with a control group even in undetectable viral load (<40 copies per milliliter). Concomitantly, decreased cytotoxic activity measured by CD107a surface expression with maintained IFN-γ production implied the impairment of cytolytic activity was not recovered after cART treatment. Thus, altered NK cell frequency and function by HIV-1 infection are not completely recovered with cART, which may contribute to impaired cellular immune response and persistence of HIV-1.

Keywords

HIV-1, Combination Antiretroviral Therapy (cART), Natural Killer Cells, IFN-γ, CD107a, Cytotoxic Activity, Cytokine Production

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1. Introduction

Natural killer (NK) cells are large lymphocytes functioning in the innate immune response against viral infection and tumors, and modulation of the subsequent adaptive immune response through their cytokine production and cytotoxicity [1]. The NK population consists of approximately 5% - 15% of peripheral blood mononuclear cells. Human NK subsets are defined by the absence of CD3 expression and the expressions of CD56 and/or CD16. NK cells are classified into 4 subsets based on marker expressions and functions [2] [3]: The major NK cell subset is CD56dimCD16+ with cytolytic activity; the second subset, CD56bright, secretes cytokines; the third subset, CD56dimCD16−, is rarely detected in healthy adults but extensively observed in cord blood; and the last subset, CD56dimCD16+, is functionally unclear but seems to respond to tumor cell-stimulated degranulation [4]. NK cell subset alteration in virus-infected individuals has been previously demonstrated, suggesting the functional significance of the NK subset in disease progression. The current study focused on human immunodeficiency virus-1 (HIV-1) infection. Even though the role of NK cells in the course of HIV-1 infection remains to be elucidated, it was recently well documented that NK cells isolated from HIV-1-infected individuals are impaired in their ability to kill HIV-1-infected cells as well as tumor cells [5] [6]. Numerous studies have characterized the effects of HIV viremia on NK cell phenotype and function. Expansion of the CD56 CD16− subset as well as depletion of CD56dimCD16+ has been demonstrated in viremic individuals [7] [8]. The CD56 CD16− subset becomes more prominent in active virally replicated subsets at the expense of CD56bright and CD56 dimCD16+ NK cells [9] [10]. It is likely that the redistribution of NK subsets may contribute to the observed loss of NK cell function over the course of HIV-1 infection.

Human immunodeficiency virus type 1 (HIV-1) causes a persistent infection in human immune competent cells that is ultimately associated with the development of acquired immune deficiency syndrome (AIDS). Around 35.3 million people are infected with this virus worldwide, with approximately 1.6 million deaths occurring per year due to AIDS. Since the discovery, AIDS has caused an estimated 36 million deaths worldwide [11]. Thus, AIDS is the life-threatening pandemic disease that has had a great impact on society as an illness, as a source of discrimination, and as economics [12]. Treatment with combination antiretroviral therapy (cART) has obviously reduced the risk of AIDS development and death. However, despite efficient treatment with cART in the suppression of viral replication, HIV-1-infected individuals still had higher risks of morbidity and mortality than the uninfected population [13]. Moreover, because patients live longer, non-AIDS-related disorders are becoming significant, not only among those with impaired immune responses, but also among those with higher CD4+ cell counts [14]. Thus, the incomplete immune recovery of innate immunity including NK cells is speculated for disease progression [15].

The present study analyzed the phenotypic and functional characteristics of NK cells from cART-treated individuals. It was found that CD56dimCD16+ as well as CD56dimCD16− subsets were significantly increased in patients; however, cytotoxic activity was not recovered even after cART. These data suggested that the impairment of NK function may contribute to decreased total immune function in cART-treated individuals.

2. Methodology

2.1. Study and Subjects

Peripheral blood samples were obtained from sixteen HIV-1-infected individuals attending the ARV clinic, Department of Internal Medicine, Khon Kean Hospital. Patients were treated with cART for at least 1 year and had an undetectable viral load (<40 copies per milliliter). Twelve age-matched HIV-1 seronegative subjects who visited the Department of Internal Medicine, Khon Kean Hospital were selected as controls (Table 1). The study was approved by the Ethics Committee of Khon Kaen Regional Hospital, Khon Kaen, Thailand. All samples were collected with written informed consent.

2.2. Cell Separation and Culture Stimulation with K562 Cell Line

Peripheral blood mononuclear cells (PBMC) were isolated from heparinized blood by Ficoll-Hypaque gradient centrifugation. The MHC class I-devoid erythroleukemic cell line, K562 (obtained from RIKEN Cell Bank, Tsukuba, Japan), was cultured in RPMI1640 medium (Wako Chemical, Osaka, Japan) supplemented with 10% fetal bovine serum (FBS) (HyClone, UT, USA), 100 U/mL penicillin (Meiji Seika Pharma, Tokyo, Japan) and 100 µg/mL streptomycin (Wako Chemical).
Figure 1. Frequency and absolute number of NK cells in cART-treated HIV-infected individuals. (A) Total NK cells were selected by CD3-negative (CD3−) lymphocyte gating followed by determining CD56 and CD16 expression. CD56 CD16− population was excluded and 4 NK cell subsets, 1) CD56 bright, 2) CD56dimCD16+, 3) CD56dimCD16−, and 4) CD56− CD16+, were divided; (B) Total NK cell percentage in lymphocytes; (C) Absolute number of NK cells in PBMC; (D) Absolute NK subpopulation numbers in PBMC. CD56dimCD16+ subset is plotted against the left Y-axis and the other subsets are plotted against the right Y-axis. Plots show the results in healthy controls (n = 12, closed circles) and cART-treated HIV-1-infected individuals (n = 16, open circles). Bars in A-C represent the means.
2.3. Phenotypic Analysis of PBMC by Flow Cytometry

The following antibodies were used in the current study: CD3-PECy7 (UCHT1) from eBioscience (San Diego, CA); CD56-APC (NKH-1) from Beckman Coulter (Paris, France); CD16-Pacific Blue (3G8) from Biolegend (San Diego, CA); CD107a-PE (H4A3), and anti-IFN-γ FITC (4S.B3) from BD Pharmingen (San Diego, CA). For surface staining, cells were stained and incubated for 30 min on ice protected from light. For intracellular staining, cells were then washed in FACS washing medium (Phosphate buffered saline (PBS) containing 3% FBS and 0.1% NaN₃), fixed with 4% paraformaldehyde, washed and permeabilized using 0.1% Saponin (Sigma), and then stained for IFN-γ production. Stained cells were fixed with 4% paraformaldehyde, and analyzed by LSRII flow cytometry (BD Bioscience, San Jose, CA) and FlowJo software (Tree Star, San Carlos, CA).

2.4. Degradation and Intracellular Cytokine Production Analysis

PMBCs and K562 cells were co-cultured at effect or to target ratio of 10:1. Cells were cultured for 1 hour in a 5% CO₂ incubator, after which breedfeldin A (10 μg/ml; Sigma, St. Louis, MO) was added to block cytoplasmic protein transportation, incubated for an additional 5 hours, then cells were stained for surface markers and intracellular IFN-γ [16] [17]. Stained cells were fixed with 4% paraformaldehyde and permeabilized with 0.1% saponin, and then analyzed by flow cytometry.

2.5. Statistical Analysis

GraphPad Prism software (GraphPad Software, Inc., La Jolla, CA, USA) was used with the non-parametric Mann-Whitney U test to analyze the difference between 2 groups. *P* < 0.05 was considered significant.

3. Results

3.1. Clinical Characteristics

Sixteen cART-treated HIV-1-infected patients with an undetectable viral load (<40 copies per milliliter) and 12 controls were recruited for this study. Fifty percent of patients and 42% of controls were male. The average ages of patient and control groups were 33.1 ± 6.5 and 26.5 ± 11.7 years, respectively. The clinical characteristics of cART-treated HIV-1-infected and control subjects are shown in Table 1. In cART-treated HIV-1-infected patients, the average white blood cell counts (WBC), % lymphocytes, total CD4 and % CD4 were 6.919 ± 2.461 × 10⁹/L, 32% ± 9.3%, 39% ± 4% and 0.765 ± 0.197 × 10⁹/L while there were 6.509 ± 2.014 × 10⁹/L, 32.0% ± 6.2%, 17% ± 8.1% and 0.351 ± 0.199 × 10⁹/L in controls. There was no difference in WBC count and % lymphocytes between the 2 groups but total CD4 count and % CD4 were significant lower in cART-treated HIV-1-infected individuals.

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<th>Description of cART-treated HIV-1-infected patients and control subjects enrolled in this study.</th>
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<tr>
<td><strong>Control</strong> (n = 12)</td>
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<td>WBC count (×10⁹/L)</td>
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When NK subset distribution was determined, CD56<sup>dim</sup>CD16<sup>+</sup>, CD56<sup>bright</sup>, CD56<sup>dim</sup>CD16<sup>+</sup>, CD56 CD16<sup>+</sup> of cART-treated HIV-1-infected individuals were 0.392 ± 0.259, 0.008 ± 0.006, 0.022 ± 0.029 and 0.029 ± 0.053 × 10<sup>9</sup>/L, respectively, and there were 0.215 ± 0.120, 0.008 ± 0.004, 0.012 ± 0.010 and 0.005 ± 0.006 × 10<sup>9</sup>/L in controls. Thus, significantly increased CD56<sup>dim</sup>CD16<sup>+</sup> and CD56 CD16<sup>+</sup> subsets were observed in cART-treated HIV-1-infected individuals (P = 0.0274 and P = 0.0148) (Figure 1(D)).

3.3. Impaired Cytotoxic Function of NK Cells during HIV-1 Infection

NK functions in each subset were determined by the detection of CD107a expression and IFN-γ production after stimulation with MHC-class I devoid K562. CD107a is a degranulation marker which represents cytotoxic function [17]. CD107a was detected in 11.91% ± 4.93% of cART-treated HIV-1-infected patients and 27.22% ± 20.04% of controls after K562 stimulation. It was significantly decreased in cART-treated HIV-1-infected individuals (P = 0.0009) (Figure 2(A) and Figure 2(C)) whereas IFN-γ production was maintained (Figure 2(B) and Figure 2(D)) in cART-treated HIV-1 infected individuals when compared to controls (6.71% ± 4.97% vs. 8.45% ± 7.82%, P = 0.3579).

4. Discussion

In this study, we focused on the phenotypic characteristics of NK subsets and their functions in cART-treated HIV-1-infected individuals. cART-treated individuals with an undetectable viral load (<40 copies per milliliter) were recruited and cytotoxic and cytokine release functions were determined with surface CD107a expression and IFN-γ production. The results showed that in cART-treated individuals, total NK cells, CD56<sup>dim</sup>CD16<sup>+</sup> and CD56 CD16<sup>+</sup> NK subsets were increased. Although the primary cytotoxic NK subset, CD56<sup>dim</sup>CD16<sup>+</sup>, was expanded, the cytotoxic function demonstrated by CD107a expression was suppressed in NK cells from cART-treated individuals.

With the introduction of cART, the incidence of opportunistic infection and the occurrence of AIDS-defining malignancies have markedly decreased due to improved immunological function; however, the incidence of non-AIDS-defining malignancies (e.g. lung cancer and Hodgkin lymphoma) is increasing and becoming a major cause of death in cART-treated HIV-1-infected individuals [18]. This could be partially explained by the incomplete recovery of NK cell function. In the current study, phenotypic analysis of NK cells subsets demonstrated significant increases of NK cells in both percentage and absolute number in cART-treated individuals (Figure 1). Among the NK cell subsets, CD56<sup>dim</sup>CD16<sup>+</sup> and CD56 CD16<sup>+</sup> anergic NK subsets were increased whereas no significant differences were detected in the others. These data suggested that NK cells were expanded with preferential increases of CD56<sup>dim</sup>CD16<sup>+</sup> and CD56 CD16<sup>+</sup> subsets during HIV-1 infection and remained increased even after cART therapy, in agreement with previous studies [19] [20]. In contrast, CD107a expression but not IFN-γ production was suppressed in cART-treated individuals (Figure 2). This functional suppression might be due to the redistribution of NK subsets in cART-treated individuals. Impaired NK functions during HIV-1 infection have been documented. HIV-1 viremia induces several phenotypic and functional abnormalities in NK cells [21]-[23]. Expansion of a highly dysfunctional CD56 CD16<sup>+</sup> subset in HIV viremic individuals is known to impair the function of the total NK population [8]. Our study suggested that the dysfunctional CD56 CD16<sup>+</sup> subset remains even after cART treatment until viremia reaches an undetectable level. Hence, HIV-1 affects the NK subset distribution as well as functioning independently of viral load.

It has been shown that administration of IL-2 together with cART augments the number and function of NK cells as well as CD4<sup>+</sup> T cells [24]. The augmented function of NK cells was also demonstrated with IL-7 and IL-15 in an animal model [15] and IL-21 <i>in vitro</i> [25]. This cytokine-supporting therapy is expected to enable complete recovery of the immune system including NK cell function in cART-treated HIV-1-infected individuals, which may contribute to the suppression of remaining HIV-1 and the onset of malignancies.

5. Conclusion

In conclusion, our findings showed that total NK cell number and a dysfunctional CD56<sup>+</sup>CD16<sup>+</sup> subset were not recovered even after cART treatment. Cytotoxic function but not IFN-γ was also kept impaired after cART treatment. Cytokine supplementation therapy inducing the modulation of NK function may change the clinical
fate and prognosis of HIV-1-infected individuals [5] [26]-[28]. Further studies are needed to elucidate the molecular mechanism of HIV-1 infection-induced NK dysfunction.

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Authors Contributions

Conceived and designed of experiment: MM, VL, SW, SO; performed the experiment: MM, NL, PS, SH; analyzed the data: MM, KV, SO; wrote the paper: MM, KV, SO; reviewed the manuscript: VL, SW.

Ethical Approval

Ethical approval for the main study was obtained from ethical review committee for Khon Kaen Hospital, Kumamoto University and Khon Kaen University. Written informed consent was obtained from each participant prior to the study initiation.

Competing Interest

The authors declare that there is no financial or personal relationship(s) that may have inappropriately influenced the writing of this article.
References


Nalpha and Ribavirin. *Clinical Immunology*, 128, 46-56. [http://dx.doi.org/10.1016/j.clim.2008.03.521](http://dx.doi.org/10.1016/j.clim.2008.03.521)


**Abbreviations**

Acquired Immune Deficiency Syndrome (AIDS)
Combination Antiretroviral Therapy (cART)
Fetal Bovine Serum (FBS)
Human Immunodeficiency Virus-1 (HIV-1)
Peripheral Blood Mononuclear Cells (PBMC)
Phosphate Buffered Saline (PBS)
White Blood Cell Counts (WBC)
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