CHARACTERIZATION OF THE ADAPTIVE NKG2C+ NK CELL IN HUMAN CYTOMEGALOVIRUS INFECTED INDIVIDUALS: A SYSTEMATIC REVIEW

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Abstract

The significance of adaptive natural killer (NK) cells in the subject pertaining to human cytomegalovirus (HCMV) infection is emphasized by the preferential expansion of a specific subset, termed NKG2C+ which can recognize an array of HCMV-encoded proteins. Until today, the subject of adaptive or 'memory-like' NK cells remains controversial, but more emerging evidence shows the cells' ability to 'remember'. The purpose of this systematic review was to appraise recent literature that centered on the expansion of adaptive NKG2C+ NK cells driven by HCMV infection, specific to 'human'. Databases including PubMed, Scopus, and JURN were systematically searched to source relevant journal articles published between 2011 and 2021, in a period of 10 years. Full-text terms such as 'NKG2C+ adaptive NK cells' AND 'human cytomegalovirus' were applied to retrieve related articles. This systematic review is comprised of continuous data on the expansion of NKG2C+ NK cells driven by HCMV and other HCMV-affiliated receptors, immune cells, and diseases with appropriate implementation of distinct inclusion and exclusion criteria. The authors evaluated the risk of bias and quality evidence of the data independently. The study is beneficial by gathering the evidence to conclude the occurrence of adaptive or 'memory-like' properties in human NK cells.

Keywords: *adaptive. human cytomegalovirus, NK cells, NKG2C, memory*

Introduction

Human cytomegalovirus (HCMV), also interchangeably termed 'CMV', is a herpesvirus that belongs to the Herpesviridae family that causes the expansion of certain immune cells in the infected individuals (1). HCMV infection manifests itself asymptomatically in immunocompetent individuals, which is in contrast to the exhibition of symptoms in the immunocompromised such as patients with human immunodeficiency virus (HIV) infection and recipients of allogeneic hematopoietic stem cell transplantation (allo-HSCT) (2). This virus will continue to replicate in the host cells and prevent apoptosis of infected cells by the production of inclusion bodies (3). HCMV has a broad cellular tropism that includes but is not limited to monocytes, fibroblasts, epithelial cells, and endothelial cells. HCMV can be transmitted through body fluids via the mucous membrane, sexual contact, blood, or organ transplants, as well as to the fetus or child through breast milk and transplacental penetration. HCMV is known to cause secondary infections such as hepatitis, splenomegaly, and colitis, especially in immunocompromised individuals. The incubation period of HCMV is thought to extend to 2 months, hence presenting itself as asymptomatic in immunocompromised individuals that have been exposed to the virus (4).

Upon HCMV infection, the immune system produces antibodies for protection and control of HCMV replication in the cells. As established previously in infections, Immunoglobulin M (IgM) is first produced following primary infection, as seen in individuals after the first encounter with HCMV, and lasting up to 4 months (5), while Immunoglobulin G (IgG) is produced after the class switching of B-cells and is longer lasting. As centered by Tang et al. (2019), the Glycoprotein B variant can disturb HCMV transmission and pathogenicity through its fusogenicity properties which assist in viral

admission, stimulation of cell fusion, and genome stability distress (6). HCMV infection typically induces long-lasting immunity that governs the reactivation of the virus in the host cell and decreases the possibility of unrestrained replication and CMV chronic disease. CD8+ T lymphocytes that bind to major histocompatibility complex (MHC) class I and CD4+ T lymphocytes, that recognize the MHC class II, also play pivotal roles in facilitating the immune system and providing prolonged immunity (7). HCMV is commonly excreted in the ductal epithelial cells of the salivary glands as horizontal transmission (8), renal epithelial and glandular cells of genital organs during viremia (9), and hence allowing CMV infection to be diagnosed by the detection of CMV DNA, cell culture, antigen detection using early markers such as phosphoprotein pp65, and IgG and IgM antibody detection.

Intriguingly, Nauclér, et al. (2019) has reported that HCMV-encoded proteins can elude recognition by the immune system by downregulating both innate and adaptive immunity pathways (10). HCMV produces viral lytic glycoprotein that can affect the removal of CD8+ cytotoxic T-cell, while activating the CD4+ T-cell, leading to the downregulation of HLA class I and II, and upregulation of non-classical HLA proteins, with the former prompting the activation of natural killer (NK) cells. Furthermore, HCMV infection will also initiate the rearrangement of the NK cell receptor repertoire, and it is believed that the expansion of the NK cell subset, NKG2C is its hallmark (11). Changes in the receptor configuration are due to epigenetic rearrangement that occurs after NK cell activation (12, 13). The activation and inhibition of NK cells are dependent on the net inhibitory and activating signals received by a series of receptors such as those from NKG2C, NKG2A, and LILBR1 amongst many others.

In the general sense, NK cells are solely distinguished by the absence of the cell surface marker CD3 and can be further sub-grouped based on their cell-surface density of surface markers CD56 and CD16, as CD56bright CD16neg and CD56dim CD16positive, with the latter being more functionally mature than the first subset (14). Albeit most scriptures use only CD56 to distinguish NK cells, CD16 is another surface receptor of note. It is expressed by essentially all CD56dim NK cells (15). CD16 is a low-affinity Fc receptor for the binding of IgG and is said to prompt either cytotoxicity or cytokine secretion on naïve NK cells (16). As aforementioned, the immune response conferred by the CD56bright, and CD56dim NK cells are distinct from one another, where, upon monokine (monocyte-derived cytokines) stimulation involving IL-15, IL-18, and IL-1β, the CD56bright subtype, unlike CD56dim cells, proliferates and secretes vast amounts of IFN-γ and TNF-β (17,18). CD56dim cells, on the other hand, exhibit antibody-dependent cell cytotoxicity (ADCC), due to the coupling of this population with the expression of CD16 ((17), with production IFN-γ preferentially only after recognition of target cells (19). However, unlike its relatively less mature counterpart, CD56dim NK cells do not proliferate in response to IL-(18).

CD56dim NK cells emerge from the differentiation of CD56bright cells (20, 21), and this progression is an indicator of cell maturity (21, 22). This deduction is based on the longer telomeric region that is found in CD56bright compared to CD56dim, suggesting a low number of events of cell propagation (23). As NK cells progress in their various stages of maturity through their transition from CD56bright CD16negative to CD56dim CD16positive, it eventually gains an additional CD57 surface marker. CD57 is an indicator of cellular maturity in NK and T-cells (21) where it is often described as a terminal differentiation marker. NK cells expressing CD57 are known to have a lower proliferation capacity than CD57-devoid cells, however, they exhibit stronger CD16-associated cytotoxicity than CD57negative NK cells (21). A majority of CD57positive cells belong to the CD56dim CD16positive population.

Initially, NK cells were exclusively thought to be constituents of innate immunity. However, in recent years, they are seen to have features of the adaptive immune, coining the term ‘adaptive’ or ‘memory-like’ NK cells. Adaptive NK cells can be defined as a specialized set of cells that are likened to adaptive immune cells in terms of immunological memory, long-term persistence, and augmented functional responses (24). A distinctive subset of NK cells expressing NKG2C undergo clonal expansion in individuals with HCMV infection or reactivation (25–27). The expansion of these cells is brought upon by the interaction with HLA-E presenting an HCMV viral peptide, glycoprotein UL40 (gpUL40) with NKG2C (28) on CD56bright NK cells. The gpUL40 peptide partly mimics the MHC class I leader peptides, the natural stabilizing ligand of HLA-E (29), whose initial intent was the inhibition of NK cells through interaction with NKG2A.

These adaptive NK cells have additional features in terms of expression of the killer immunoglobulin-like receptors (KIRs), downregulation of NKG2A and signaling molecules such as FcεRIγ chain, and the upregulation of the late differentiation marker of cytotoxic T-cell, CD57 (30,31), which are due to epigenetic changes in methylation profiles (12,13). Davis et al., (2015) iterate in their study that the expansion of this subset of adaptive NK cells is localized to HCMV seropositivity, with the highest frequencies being present in individuals with HCMV reactivation (30). Hence, the purpose of this systematic review was to appraise recent literature that centered on the expansion of the human adaptive NKG2C+ NK cell driven by HCMV infection.

Materials and Methods

Literature search

This systematic review was executed based on journal

articles scoured from several databases such as PubMed, Scopus, and JURN that highlighted the effect of HCMV infection on the expansion of NKG2C+ adaptive NK cells. The search strategy employed to retrieve relevant articles to utilize full-text terms such as 'NKG2C+ adaptive NK cells' AND 'Human cytomegalovirus'. The Boolean keyword 'AND' was used to ensure that the articles included 'human' cytomegalovirus regarding NKG2C+, and the medical subject heading (MeSH) terms were used to warrant an effective searching process. The research period was set for the last 10 years, thus only articles that were published between 2011 to 2021 were selected to be included in this review.

Inclusion and exclusion criteria

This systematic review was designed to establish the NKG2C+ adaptive NK cell profiles that are driven by HCMV infection. The title, abstract, and methodology were screened based on the following criteria by the three authors (SO, FH, and NH): (i) the journals are based upon original research that has been published or peer-reviewed; (ii) the studies specifically examine NKG2C+ adaptive NK cells; (iii) the journals are published in English with the inclusion of free full-text publications; (iv) studies included the method to screen for NKG2C+ cells to achieve the research objectives; and (v) studies were published between 2011 and 2021 to ensure relevance. Studies were excluded if the following criteria were in the journal: (i) the non-human studies which relate to HCMV either in-vitro or in-vivo are of low level of evidence; (ii) only one keyword either HCMV or NKG2C+ was present in the title or abstract; and (iii) the journals are incomplete, provide no access to full-text article and published in other languages.

Screening of the articles and data extraction

The collected articles from some of the databases were imported to Windows for storage. The selection of the articles was based on the inclusion and exclusion criteria. The full-text articles were screened for their eligibility based on the guidelines provided by PRISMA (Supplementary Table 1). The data from the articles such as year of publication, name of the first author, number and age of the subjects, type of samples, and main outcome of the research was extracted and recorded independently by using MS Excel 2010.

Search strategy validation

All articles searched were validated independently by the authors in December 2021. No additional papers were included during validation.

Data analysis

Continuous data on the expansion of NKG2C+ NK cells due to HCMV were extracted from the chosen articles. Similarly, other primary outcomes such as receptors, cells, and diseases affiliated with HCMV were also extracted and analyzed. The methodology, risk of bias, and quality of the data were independently evaluated by all the authors.

Quality assessment

The papers were selected independently by the authors to avoid the risk of selection bias. The chosen articles were assessed for quality and bias (performed by SO, FH, and NH) using the Joanna Briggs Institute (JBI) Checklist for case controls, cross-sectional, and cohort studies based on the study design of each article. All data were recorded in three different tables based on their study design as n supplementary materials (Supplementary Tables 2 to 4). The quality requirements comprised aspects such as appropriate sampling methods, approaches to handle confounders, outcome measure validity, and statistical analysis. The quality scores were calculated according to the checklist answers. If "Yes," the response will be recorded as +1, if "No" or "Non-applicable", the response will be recorded as 0 and if "Unclear", it will be recorded as -1. The "non-applicable" response was due to the item being irrelevant to the study. If the quality score has an overall total of more than 80%, the articles are classified as high-quality studies, while articles with quality scores lesser than 50% are considered low in quality, and are excluded.

Results

Search results

A total of 167 papers were identified from PubMed (n=40), JURN (n=64), and Scopus (n=63). Out of 167 articles, 81 were selected after subsequent screening and removal of duplicates, which were strictly done based on the pre-defined inclusion and exclusion criteria. Following the

full-text screening for eligibility, 25 papers had satisfied the inclusion criteria, whereas 56 publications were excluded for a variety of reasons, including not meeting the criteria for systematic review reporting as outlined in the PRISMA guidelines and the lack of data for methodological quality assessment, animal research, failure to meet PICO (population, intervention, comparison, and outcome) requirements and inability to comprehend text. After quality assessment, only 21 articles were left to be reviewed. Figure 1 summarizes the results of the literature search based on PRISMA guidelines.

Overview of papers

Figure 1 shows the PRISMA flow diagram, comprising information on the publications that were screened, excluded, and incorporated. Tables 1 and 2 summarizes the particulars and major outcomes of the 21 studies included in this review. Scientific articles were taken from several study designs such as observational case-control, cohort, and cross-sectional studies that focused on the expansion of NKG2C+ adaptive NK cells as a result of HCMV infection. Four papers were removed from this review after quality assessment. The authors noticed no conflicts at the conclusion of the examination and screening of the publications.

Participants and Study Characteristics

The mean number of participants across all papers is 193 (ranging from 8 to 674 people). Specifically, the mean number of healthy controls is 89 while the mean number of participants that have other diseases in addition to HCMV is 161.

Table 1 shows a total of sixteen studies that utilized PBMCs, where five studies used peripheral blood or whole blood and six studies used both PBMCs and DNA samples. The major results highlighted in these papers include NK cell expression and repertoire in people of different ages who were infected with HCMV, observed with downregulation of FcεRγ expression, a divergence in NKG2C genotype, function of KIR polymorphism, and the effect of other diseases alongside HCMV on NKG2C+ NK cells. Six studies investigated NK and T-cell expression in people of different ages that were infected with HCMV, another four examined the downregulation of FcεRγ expression, three examined the divergence of NKG2C genotype, six examined the function of KIR polymorphism, and 15 studies examined the effect of other diseases alongside HCMV on NKG2C+ NK cells.

Literature reporting changes in NK cell expression in people of different ages

All 21 publications reported a significant increase in NK cell expression in individuals with HCMV infection. Of these papers, six specifically reported NK cell expression in people with HCMV of different age ranges (32–37). Muccio, et al. (2016) reported the development of NK cells in pediatric patients with hematological malignancies (n=7) while another five investigated NK cell receptor repertoire in adults and elderly subjects with HCMV, stipulating significant expansion in the level of NKG2C+ NK cells in the elderly compared to young adults (36). However, Bayard, et al. (2016) alluded that the expansion of NKG2C+CD57+ NK cells amongst the elderly was independent of HCMV serostatus (32).

According to Juárez-Vega, et al. (2017), besides NKG2C+ NK cells, a subset of NK cells termed CD16 was also found to be elevated in the elderly (33). Furthermore, Muccio, et al. (2016) also reported that HCMV-reactivating individuals had higher frequencies of these distinct NK cell subgroups than non-reactivating individuals in the pediatric sample (36). However, the percentage of these NK cell subgroups experienced a notable decline due to the capability of expanded NKG2C+ NK cell subsets to detect HLA-E, a particular ligand of NKG2C, in αβ+ T/B cell-depleted cohort of HCMV-reactivating patients in that study.

Juárez-Vega, et al. (2017) also reported an increased proportion of T lymphocytes, KIR, and CD161 among the elderly (33). Reed, et al. (2019) outlined that older people with high-stress levels who have been infected with HCMV portray a high proportion of late-differentiated T-cells (37). One study reported that HCMV infection in the elderly had caused the expansion of NKG2C+, thereby increasing the risk of carotid atherosclerotic plaque (CAP) and the expansion of CD8+ T cells (35). Similarly, Bayard et al. (2016) also detailed an increase in CD8+ CD57+ memory T-cells HCMV seropositive individuals (32). In contrast, Bengnér, et al. (2014), had reported that the expansion of CD8+ T cells posed no correlation with NK cells, aging, and HCMV (38).

Literature reporting changes in NK cell signaling molecules, FcεRIγ– chain (FcRγ) expression

Four papers by Moreira, et al., (2019), Kim, et al., (2019), Reed, et al., (2019), and Muntasell, et al., (2016) examined the expression of FcRγ signaling molecules in individuals with HCMV infection (34,37,39,40). Loss of FcRγ expression was a common marker for NK cell response to HCMV and is often associated with the expansion of NKG2C cells. Muntasell, et al. (2016) reported the downregulation of FcRγ expression in the NKG2C+CD57+ CD56dim NK cell subset which was often in the NKG2C+/+ genotype (40). According to Kim, et al. (2019) and Reed, et al. (2019), a significant positive correlation was detected between the frequency of FcεRIγ− and NKG2C+ NK cells that caused a clonal expansion in KIR repertoire (34,37). Besides, NKG2C+ and FcεRIγ− on NK cells were also associated with perceived stress (37). These FcεRIγ− NKG2C+ NK cells boosted the mediated function by CD16 engagement but reduced the production of cytokines in terms of IFN-γ and TNF-α, due to the weakened K562 effector cell (34).

Literature reporting the divergence of NKG2C genotype

Three of the included papers investigated the divergence of the NKG2C genotype as it was believed to give an impact on NKG2C+ NK cell numbers and affect the burden of CMV. According to these studies, the NKG2C+/+ genotype in HCMV+ individuals was found to be related to heightened frequencies and absolute quantities of NKG2C+ NK cells (40,41). As supported by Martinez-Rodriguez, et al. (2016), NKG2C+/+ homozygous individuals with HCMV+ showed high expression of the NKG2C receptor (26).

Literature reporting changes in KIR polymorphism

Six papers by Manser, et al. (2019), Davis, et al., (2015), Muntasell, et al. (2016), Horowitz, et al., (2013), Kim, et al. (2013) and Juárez-Vega, et al. (2017) examined changes in KIR polymorphisms that affect the expansion of NKG2C+ adaptive NK cells due to HCMV infection (30, 33, 40, 43, 51, 52). Manser, et al. (2019), Davis, et al. (2015), and Muntasell, et al. (2016) stated that the co-expression of HLA-C-specific KIR described the expansion of NKG2C+ NK cells (30, 40, 43). To be specific, cognate ligand HLA-C2 would accompany the expansion of NKG2C+KIR2DL1+ NK cells and KIR2DLI expression which was impacted due to HCMV infection (43). In contrast, three other studies revealed that NKG2C+ NK cells expressed a high frequency of KIR2DL2/3 in individuals with CMV (30, 34, 51).

Table 1: Summary of study and participant characteristics

Authors (year)	Journal	Sample	Sample size			
			Diagnosis (Age mean/range)	**HCMV status**	**Healthy donor (Age mean/range)**	**HCMV status**
Pascual-Guardia, et al. (2020) (42)	Respiratory Research	Peripheral blood	COPD (n=66) (Mean: 70±8 years)	Seropositive (n=35)	n=13 (NA)	NA
Moreira, et al. (2019) (39)	Frontiers in Immunology	PBMC	MS (n=151) (Mean: 50.1±11.4 years)	Seropositive (n=103)	n=47 (Mean: 46.6±13.3 years)	Seropositive (n=37)
Kim, et al., (2019)(34)	Frontiers in Immunology	PBMC	NA	NA	n=127 (Range: 20 to 81 years)	Seropositive (n=123)
Manser, et al. (2019) (43)	The Journal of Immunology	PBMC DNA	NA	NA	n=276 (NA)	Seropositive (n=139)
Reed, et al. (2019)(37)	Brain, Behavior, and Immunity	PBMC	NA	NA	n=149 (Mean: 77 years, range: 64–92 years)	Seropositive (n=106)
Ataya, et al. (2019) (44)	The American Society of Transplantation and the American Society of Transplant Surgeons	PBMC	KTR, (n=145) (56 ± 12.8 years)	Seropositive (n=124)	NA	NA
Peppa, et al. (2018) (45)	Frontiers in Immunology	PBMC DNA	HIV-1 (n=21) NA	Seropositive (n=20) (26 to 57 years)	n=20 (NA)	Seropositive (n=10) (28 to 55 years)
Ariyanto, et al. (2018) (41)	Immunological Investigations	PBMC DNA	HIV (n=78) (18 to 40 years)	Seropositive (n=78)	n=17 (Age-matched)	Seropositive (n=17) (age-matched)
Juárez-Vega, et al. (2017) (33)	Human Immunology	PBMC	NA	NA	n=90 (22 to 98 years)	Seropositive (n=90)
Redondo-Pachón, et al. (2017) (31)	The Journal of Immunology	Peripheral blood	KTR: Retrospective study, (n=253) Prospective study, (n=122) (54.5 ± 14.4 years)	Seropositive (n=109)	n=313 (NA)	NA
Muntasell, et al. (2016) (40)	The Journal of Immunology	PBMC DNA	NA	NA	n=81 (Median age: 32 years)	NA
Bayard, et al. (2016) (32)	European Journal of Immunology	Whole blood	Yatec patients, (n=61) (Median age: 23; range: 18 to 29 years)	Seropositive (n=33)	n=10 (Median age: 36, range: 23 to 55 years)	Seropositive (n=5)

Cichocki, et al. (2016) (46)	Leukemia	PBMC	allogeneic HCT, (n=674) (NA)	CMV reactivation, (n=190) (Median: 45; range: 1 to 71 years) Seropositive, (n=214), (Median; 37 , Range: 1 to 74)	n=5 (NA)	Seropositive, (n=5)
Heath, et al. (2016) (47)	Journal of Immunology Research	PBMC	HIV, (n=164) (NA)	Seropositive, (n=138) (Median: 49, range: 45 to 55 years)	n=47 (NA)	Seropositive, (n=25) (Median: 48, range: 39 to 61)
Muccio, et al. (2016) (36)	Hematologica	PBMC DNA	Hematological malignancies HLA-haploidentical HSCT, (n=27) (NA)	Seropositive, (n=13)	n=12 (NA)	Seropositive (n=7)
Martínez-Rodríguez, et al. (2016) (26)	Multiple Sclerosis Journal	PBMC	MS, (n=246) (Older than 18 years)	Seropositive (n=159) (Mean: 47.1±11. 2 years)	n=50 (Age-matched)	NA
Davis, et al. (2015) (30)	Biology of Blood and Bone Marrow Transplant	PBMC	Allogeneic HCT, (n=292) (0 to 65 years)	Seropositive, (n = 152) CMV reactivation, (n=69) no reactivation, (n=83)	NA	NA
Horowitz, et al. (2015) (48)	The Journal of Immunology	PBMC DNA	Allogeneic HCT patients with leukemia, (n=8) (28 to 21 years)	CMV reactivation, (n=4)	n=64 (NA)	NA
Martínez-Rodríguez, et al. (2013) (35)	Arteriosclerosis, Thrombosis, and Vascular Biology	PBMC	CAP (n=39) [high risk, n=16; non–high-risk, n=23] (Mean; high risk:71.4 years; non-high risk; 69.8 years)	All seropositive	n=11 (Mean: 67.7 years)	NA
Bengnér, et al. (2014) (38)	Age	Peripheral blood	NA	NA	n=151 (66 years)	Seropositive, (n=118)

Romo, et al. (2011) (49)	Arteriosclerosis, Thrombosis and Vascular Biology	Peripheral blood	AMI, (n=70) (34 to 87 years)	Seropositive, (n=61)	n=209 (57 years)	Seropositive, (n=167)

PBMC—peripheral blood mononuclear cells, HIV—human immunodeficiency virus, ART—antiretroviral therapy, YATEC—young adults thymectomized during early childhood, LTP—lung transplant patients, PI—primary infection, HCMV—human cytomegalovirus, AMI—acute myocardial infarction, TAP—transporter associated with antigen presentation, HCT—hematopoietic cell transplantation, MS—multiple sclerosis, COPD—chronic obstructive pulmonary disease, CAP—Carotid atherosclerotic plaque, KTR—Kidney transplant recipients, NA—Not available.

Table 2: Summary of primary outcome results

Author (year)	Method	NKG2C expression/genotype	P value	Antibodies and markers used
Pascual-Guardia, et al. (2020) (42)	Flow cytometry	No differences of NKG2C+ NK cells % in COPD as compared to controls. NKG2C+ NK cells % higher in frequent vs occasional exacerbations COPD and normal body composition vs low fat free mass index (FFMI) COPD	NS p<0.05	CD3, CD45, CD56, NKG2C and NKG2A
Moreira, et al. (2019) (39)	Flow cytometry	High % of NKG2C+ CD56dim NK cells in HCMV positive MS and HCMV positive healthy donor	p<0.0001(MS) p<0.01(HD)	NKG2A, CD3, CD56, CD16, NKG2C, FcRγ, PLZF
Kim, et al. (2019) (34)	Flow cytometry	High frequencies of FcεRIγ-NKG2C+ than FcεRIγ+NKG2C+ CD56dim NK cells	p< 0.0001	CD3, CD56, CD158b, CD14, CD19, NKG2C CD158a, CD158e1 Bcl-2, Ki-67, CD57, FcεRIγ, Bcl-2,
Manser, et al. (2019) (43)	Flow cytometry PCR	High % (13.4 6 16.6 %) of NKG2C^{+} CD56^{dim} NK cells in HCMV positive healthy people NKG2C+ KIR2DL1+ co-expression on NK cells NKG2C gene deletion +/+, +/- and -/-	p<0.0001 p=0.008 p=0.032	KIR3DL1, IgG1, NKG2A, KIR2DL1/S1, KIR3DL1/2, KIR2DS4, KIR2DL1, KIR2DL3, NKG2C, KIR2DL2/3/S2, NKG2A, KIR3DL1, CD56, CD16, CD57 and CD3
Reed, et al (2019) (37)	Flow cytometry	NKG2C+, FcεRIγ- and CD57+ on CD3-CD16+CD56dim NK cells association with perceived stress	NS	CD3, CD16, CD56, CD57, KIR CD158b, KIR, CD158a, KIR CD158e/k, NKG2C, FcεRIγ
Ataya, et al. (2019) (44)	Flow cytometry	Independent inverse relation between posttransplant infection and the proportions of total NKG2C+ NKG2C+ NK cells bearing adaptive markers were specifically associated with a reduced incidence of posttransplant symptomatic CMV infection	p=0.022 p=0.016	CD3, CD45, CD56, CD3, TCR Vdelta2, NKG2C, NKG2A, ILT2, CD57, CD4, CD8, FcεRIγ, PLZF
Peppa, et al. (2018)(45)	Flow cytometry DNA methylation	HIV-1 infected HCMV positive with high CD56dim with NKG2C+CD57+ CD85j+, NKG2A-NK cells	p<0.05, p<0.01, p<0.0001	CD14, CD19, CD56, CD3, CD16, CD38, NKG2D, PD1, NKp30, NKp46, CRACC, CD2, Siglec7, 2B4, CD85j, Streptavidin, CD4, CD8, NKG2A, KIR2DL2, CD158b1/b2.j, NKG2C, KIR2DL 1/2DS5, IgG1 (CD158a), KIR3DL2, CD57 , KIR3DL1

				(CD158e1), CD3ζ, Syk, Perforin, IFN-γ, TNF-α, FcεRI-γ, PLZF
Ariyanto, et al. (2018) (41)	Flow cytometry PCR	High level of NKG2C+ NK cells in in HIV patients with the presence of CMV DNA and NKG2C heterozygous deletion.	p<0.05	CD3, Perforin, NKp30, CD56, LIR1, NKG2C and CD16
Juárez-Vega, et al. (2017) (33)	Flow cytometry	Increased NKG2C+ NK and T cells in elderly individuals compared to young adults	p<0.005	NKG2A, NKG2C, ILT2, CD161, KIR, KIR2DL1, KIR3DL1/3DL2, KIR3DL, CD45
Redondo-Pachón, et al. (2017) (31)	Flow cytometry	Retrospective study: the proportions of NKG2C+ NK cells were significantly higher in KTRs who had suffered posttransplant symptomatic CMV infection Prospective study: Increase in the proportions of NKG2C+ cells were perceived in KTRs with CMV viremia NKG2C genotype distribution was comparable in KTR and healthy controls, and greater proportions of NKG2C+ cells were detected in NKG2C+/+ than in NKG2C+/del patients	p=0.038 p=0.028 p=0.006	CD3, CD45, CD56, NKG2C, NKG2A, CD161, KIR
Muntasell, et al. (2016) (40)	Flow cytometry PCR	FcRγ loss observed in the NKG2Cbright NK cell subset in subjects with *NKG2C+/+* whereas NKG2C$^-$FcRγ$^-$NK cell subpopulations were more frequently detected in *NKG2C+/-* and *NKG2C-/-*	p<0.0001	NKG2A, NKG2C, FcRγ, CD56, CD3, LILRB1, NKG2D, NKp30, NKp46, CD161, CD16, CD57, KIR2DL1/S1, KIR2DL2/S2/L3, KIR3DL1/L2, and KIR2DS4, NKG2D, NKp46, NKp30
Bayard, et al. (2016) (32)	Flow cytometry	Persistence of CD57+, CD16+ CD56dim NK and CD8 T-cell phenotypes healthy donors, thymectomized individuals, pregnant women with primary CMV infection and lung transplant patients HCMV-seropositive elderly had also an increase of late NKG2C+ or late NKG2C+CD57+ cells compared to younger HCMV-seropositive individuals	p<0.001 p=0.003	CD4, CD8, TNF-α, IFN-α CD40L, IL-2, CD16, CD56, CD3, CD45, NKG2A, CD45RA, CD57, CD3, CD8, CD27, NKG2C and CD107a
Cichocki, et al. (2016) (46)	Flow cytometry	Reduce leukemia relapse in CMV reactivation allo-SCT after 1 year after reduced intensity conditioning (RIC)	p=0.05	CD3, CD56, CD57, NKG2C, CD107a, TNF and IFN-γ,
Heath, et al. (2016) (47)	Flow cytometry	NK and CD8+ T cell responses against HCMV were significantly greater in the HCMV/HIV coinfected group than the other group	p<0.0164	CD3, CD56, NKG2C, CD57

		The fraction of CMV-specific CD8+ T cells expressing CD28 correlated inversely with NKG2C+CD57+ NK expansion in HIV infection		
Muccio, et al. (2016) (36)	Flow cytometry	NKG2C+CD57+ NK cells detectable after CMV reactivation HSCT, characterized by high KIR, LIR-1 and low Siglec-7, NKG2A and Interleukin-18Rα expression	p<0.5, p<0.01	CD16, NKp30, NKp46, NKp44, NKG2D, CD226, CD244, CD56, Siglec-7, KIR2DL1, KIR2DS1, KIR2DL2/L3/S2, KIR2DS4, KIR3DL1/S1, KIR2DL1/S1/L2/L3/S2/S5, KIR3DL1/S1/L2, KIR2DL3, -NKG2A, LIR-1, CD3, CD20, CD14, CD57
Martínez-Rodríguez, et al. (2016) (26)	Flow cytometry	NKG2C NK cells expression was higher in HCMV (+) NKG2C+/+ homozygous individuals, unrelated to disease-modifying drugs in multiple sclerosis	p<0.05, p<0.001	LILRB1, CD94/ NKG2A, NKG2C, KIRs: (KIR3DL1/L2/S4, KIR2DL2/S2/L3, KIR3DL1, KIR2DL1/S1/S3 (HP-3E4). CD3, CD56, CD14, CD16
Davis, et al. (2015) (30)	Flow cytometry	Increased in KIR-expressing (KIR2DL2/3+) 'adaptive' NK cells phenotype (NKG2C+CD57+) in CMV positive/reactivation allo-HSCT patients.	p<0.05, p<0.1, p<0.001	CD3, CD56, CD158b, CD158a, NKB1, NKG2A, NKG2C, CD57
Horowitz, et al. (2015) (48)	Mass cytometry	Allo-SCT recipients had higher HLA-C expression on CD56−CD16+ NK cells, B cells, CD33bright myeloid cells and CD4 and CD8 T cells The CD57+NKG2C+ NK cells were at highest frequency in CMV-positive HCT recipients (mean ~60%) but infrequent in the controls (mean < 3%).	p <0.0001, p<0.005 P<0.0001	34 markers (50) in supplementary
Martínez-Rodríguez, et al. (2013) (35)	Immunofluorescence Flow cytometry	High-risk carotid atherosclerotic plaque (CAP) had higher % NKG2C+ NK cells compared to non-high-risk CAP, with reduction in NKG2A+ NK cell subset	p<0.026	LILRB1, CD94/NKG2A, NKG2C, KIR3DL1/L2/S4, KIR2DL2/S2/L3, KIR3DL1, KIR2DL1/S1/S3, CD3, CD56, CD69, CD4, CD14, CD8
Bengnér, et al. (2014) (38)	Flow cytometry	The occurrence of NK cell expansions (frequency of NKG2C + NK cells in conjunction with high expression of CD57 and/or ILT-2) was independent of the CD4/CD8 ratio	NS	CD3, CD4, CD8, CD27, CD28, CD45, CCR7, CD56, NKG2A, NKG2C, CD57, ILT-2, CCR7, CD27, CD28, CD45RA, CCR7, CD27, CD28, CD45RA, CCR7, CD27, CD28, CD45RA
Romo, et al. (2011) (49)	Flow cytometry	Proportions of NKG2C+ NK cells and LILRB1+ NK and T-cells were higher in samples from HCMV+ individuals No significant correlation between NKG2C+ NK cells and LILRB1+ NK cells	NS	CD3, CD56, NKG2A, NKG2C, LILRB1

KIR—killer-cell immunoglobulin-like receptors, NK—natural killer, CMV—cytomegalovirus, PCR—polymerase chain reaction, IFN—interferon, NS—not significant

In addition, the study by Davis et al. (2015) mentioned that the increase in KIR2DL1 expression was not significantly associated with reactivated CMV infection (30). Besides, one study reported that single KIR clones could produce resilient expanded NKG2C+ cells better than polyclonal KIR (43). According to Juárez-Vega, et al. (2017), KIR genotype-2 amongst the elderly was significantly lower than in young people with HCMV infection (33).

Literature reporting the effect of other diseases alongside HCMV on the expansion of NKG2C+ adaptive NK cells

Based on the diagnosis in Table 1, there were 15 papers that reported on several diseases that are usually associated with CMV infection such as HIV, atherosclerosis, Chronic Obstructive Pulmonary Disease (COPD), multiple sclerosis (MS), and post-transplant recipient patients. Thirteen of the 15 papers reported significant expansion of NKG2C+ NK cells associated with the diseases and HCMV. Three of the 15 papers agreed that the percentage of NKG2C+ NK cells increased only if the HIV patient was infected with CMV. According to Ariyanto, et al. (2018), patients reportedly respond better to antiretroviral therapy (ART) when the proportion of NKG2C+CD57+ NK cells was heightened (41). This study centered that CMV DNA+ patients with HIV and expanded NKG2C+ had a low level of CMV reactive antibodies, while Heath, et al. (2016) reported that the antibodies were independent of the expansion of NKG2C+ NK cells (47). One of the studies reported that HIV and HCMV unveiled adaptive-like NK cell subsets that enhanced IFN-γ production (45).

Martínez-Rodríguez, et al. (2013) reported that the expansion of NKG2C+ in atherosclerosis patients would amplify the possibility of plagues destabilization in some patients (35). In addition, one of the 15 papers by Romo, et al. (2011), reported no significant relationship between the expansion of NKG2C+ and coronary heart disease (49). Likewise, a paper published by Guardia, et al. (2020), described no significant correlation between HCMV+ and NKG2C+ in chronic obstructive pulmonary disease patients (42). In contrast, two studies regarding MS patients and NKG2C+ NK cell expansion reported an advantageous role played by these cells in immunoregulation and a subsequent decrease in disability development in MS (26,39). In addition, Muccio and colleagues (2016) also disclosed an enhanced degranulation of NK cells in post-transplant patients due to the expansion of NKG2C+ NK cells (36). One of the seven papers reporting on the expansion of NKG2C+ NK cells in post-transplant recipients' patients written by Cichocki, et al. (2016) mentioned that the expansion of these cells increases patient protection and reduces leukemia relapse by controlling cancer reactivation (46). In addition, two studies written by Ataya, et al. (2019), and Redondo-Pachón, et al. (2017) agreed that the expansion of adaptive NKG2C+, controlled and reduced CMV infection in kidney-transplant recipients (31,44).

Quality assessment of papers

JBI checklists for three different study designs were used to evaluate the quality and bias of 25 chosen papers by two authors independently (Supplementary materials in Appendix B). JBI checklist was used because of its specificity on specific study design. From 25 papers, 4 papers were excluded because of low-quality scores which were less than 50% of the overall score (22, 27, 53, 54) which left only 21 papers to be studied. All 21 papers met the JBI criteria for outcomes measured in a valid and reliable way. All 12 cross-sectional studies met the JBI criteria for exposure assessment in a standard, valid, and reliable way. Besides, all 6 cohort studies met the JBI criteria for (i) criteria used for recruiting subjects from the same population; (ii) follow-up time was sufficient for an outcome to occur, and (iii) appropriate statistical analysis was used. All three case-control studies met JBI's criteria for (i) groups that were comparable; (ii) exposure was measured in a standard and valid way; (iii) confounding factors were identified and had detailed strategies to deal with it; and (iv) appropriate statistical analysis was used.

Discussion

This systematic review presents the summary of findings on adaptive NKG2C+ NK cells that expand due to CMV infection in humans. The virus has induced adaptive differentiation of NK cells to a varying level and caused these cells to be capable of aiding the immune system against HCMV and other pathogens as they are able to facilitate ADCC and cytokine release, in terms of TNF-α (39). HCMV infection was found to be able to affect the arrangement of NK cell repertoires, specifically the NKG2C+ cells that are vital in detecting altered cells in an individual.

The persistence of the adaptive NK can be viewed through the stability of the expanded population for at least a few years post-activation (43). As a general rule of thumb, in the aftermath of conducting effector function on tumor cells, neoplasms induce programmed cell death (apoptosis) of NK cells via the engagement of natural cytotoxicity receptors (NCR); NKp30, NKp44, and NKp46, upon which vast quantities of Fas ligand (FasL) are transcribed, translated and released, whom in turn interact with Fas receptors on the surface of NK cells, triggering their 'suicide' (55).

NKp30, NKp44, and NKp46 are all shown to be downregulated in the adaptive NK population (12, 36, 40, 41, 45), thus, enabling their resistance to apoptosis. Additionally, Lee et al., (2015) iterated that ZBTB38, a Broad-complex, Tramtrack and Bric-à-brac - zinc finger (BTB-ZF) transcriptional factor that participates in the negative regulation of apoptosis, is upregulated due to hypomethylation of the gene (12). On another note, Manser, et al. (2019) stated that the NK cell compartment in individuals infected with HCMV would

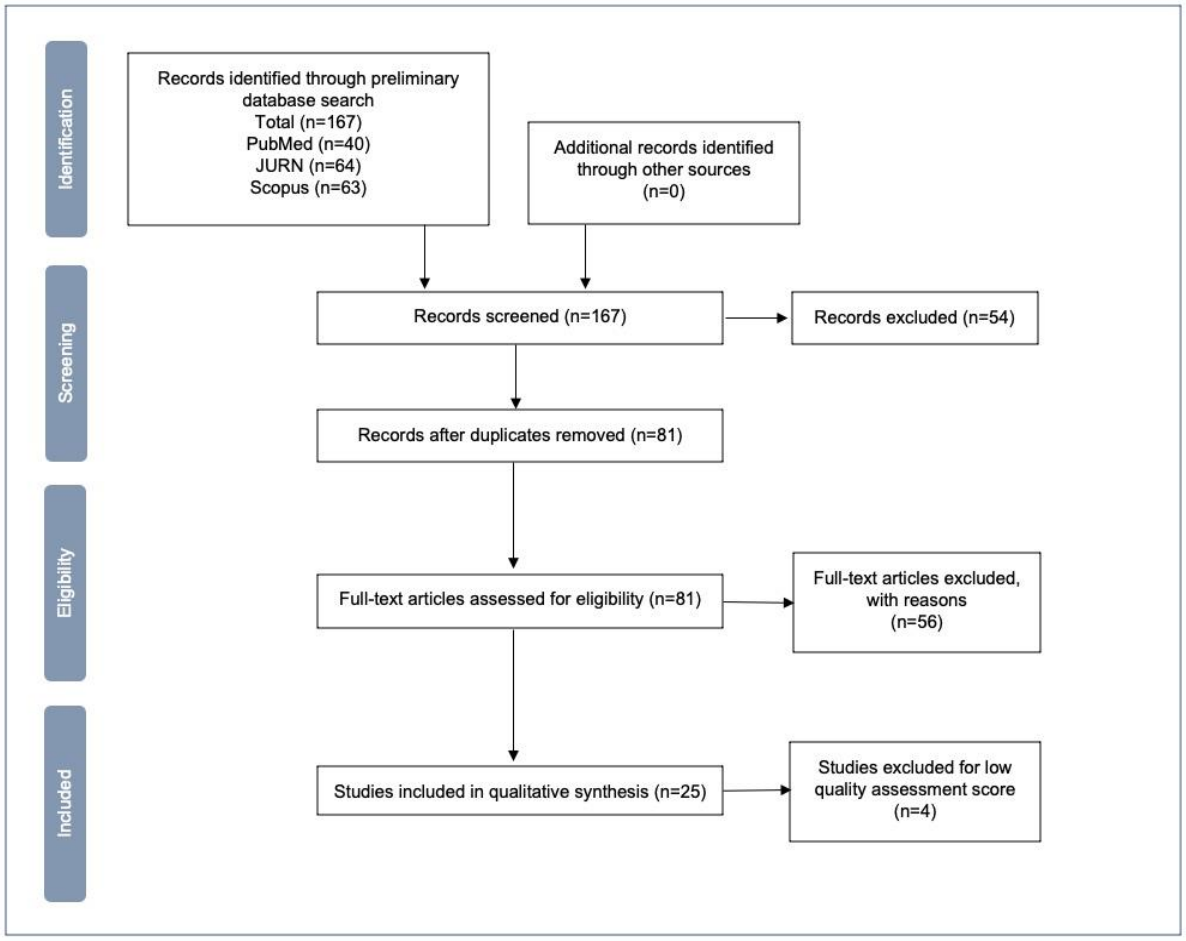

Figure 1: PRISMA flow diagram of literature search displaying selection and exclusion of publications.

have more than 50% greater clonal expansion of NKG2C+ NK cells (43).

In addition, the killer-cell immunoglobulin-like receptors (KIRs), that are found on the plasma membrane of NK cells were observed to be affected by long-term HCMV infection. Manser et al. (2019) and Beziat et al. (2015) stipulated that the downregulated expression of HLA class I-specific KIR was linked to the selective expansion of NKG2C+ NK cells, due to the evasion of CMV from adaptive immunological responses (43,53). In addition, FcRγ downregulation was also a marker in HCMV infection and correlated with NKG2C+ expansion as it is associated with the activating receptors that lead the adaptive NK cell functions (40). In short, the expansion of the NKG2C+ NK cell is dependent on activating KIR and deficiency of FcRγ expression in an infected HCMV individual.

Muntasell, et al. (2013) observed that in the circumstances of a poor T-cell response due to HCMV infection, NKG2C+ NK cells might govern viral replication better in NKG2C+/+ subjects than in the NKG2C+/- (27). It is believed that NKG2C+/+ individuals have a greater biosynthetic rate of NKG2C to return to the steady-state surface levels of the receptor than in other genotypes (27). Additionally, CMV infection also gives rise to the accumulation of late differentiated memory T-cells, as well as an increase in lymphocyte count. According to Bayard, et al. (2016) the increase in the number of NKG2C+ CD57+ NK cells has assisted in governing the recurrent HCMV replication by controlling T and B-cell responses by enhancing their functions (32).

In post-transplant, HCMV could trigger the release of TNF-α and IFN-γ due to the expansion of NK cells in response to tumor-targeting cells. This also assisted in lowering the possibility of leukemia relapse even without firm specificity for CMV antigen (46). In leukemic patients, NK cells are vital in primary immunity after HSCT, and it is important in the clinical health welfare of the patient. Memory-like NK cells are induced to assist in governing infections and supporting anti-leukemic effects in patients that have been infected with HCMV prior to transplantation (36).

Despite the evident correlation, the mechanism of NKG2C+ NK cell expansion due to HCMV infection that induces virus-specific antibody production with the help of T-cells remains unclear. This is because the expansion of NKG2C+ NK cells alone is unable to assist in clearing up viral infections (33). There are also reports that elucidate the expansion of NKG2C+ NK cells due to past infection from other viruses. For example, Muccio, et al (2016)

stated that Hantavirus infection could also induce the expansion of NKG2C+ and these NK cells would remain as long-lived 'memory-like NK cells' capable of fighting against new infections (36). In other words, the supporting nature of NK cells and their aptitude to overcome the limitation of adaptive immunity is unknown at this time. It is believed that additional research with the implementation of a bigger cohort is required to obtain a more specific and less biased result that could establish the benefits of HCMV reactivation against other infections and diseases. Nevertheless, more research should be conducted on the mechanism and potential benefits of the expansion of adaptive NK cells specifically on the expansion of NKG2C+ NK cells due to HCMV infection.

Quality assessment

Quality assessment was based on the study design of each article. All papers that were chosen were from high-impact journals. However, the checklists were still needed to cross-check the qualification of the specific study designs. Most of the publications used flow cytometry to measure the outcome, as it is a gold-standard technique for the detection of cellular populations. Only one out of 21 papers (33) did not provide valid statistical analysis as they did not deliver detailed information to conduct multiple comparisons when the ANOVA test was significant. Most of the papers mentioned confounding factors by making them exclusion criteria or through matching. In addition, based on the cohort studies checklist, none of them detailed the strategies to avoid incomplete follow-up. In short, it is believed that of the 21 articles that were included, the articles still have flaws but are not classified as serious constraints, they are unlikely to cast doubt on the validity of the conclusions reported by the authors. However, in future works in this field, it is suggested that information on the justification of the reported sample size should be included to avoid inadequate representation of populations and time constraints.

Conclusion

The purpose of this systematic review was to study recent literature that reported on the expansion of adaptive NKG2C+ NK cells due to CMV infection in humans. The integration of KIR repertoires, HLA-C, FcRγ, and T-cells was also studied due to their interrelation with NK cells. All the chosen articles have exhibited evidence of the expansion of NK cells in individuals with HCMV and provided sufficient affirmation that NKG2C is indeed one of the markers of HCMV infection. However, the function of NKG2C+ NK cell expansion in combating other infections remains inconsistent between studies, hence requiring additional research in the area.

Acknowledgments

We would like to thank Dr. Nor Shuhada Murad for helping with the guidelines of the JBI checklist. This project is supported by USM Short Term Grant (304.CIPPT.6315515).

Conflict of Interest

The authors have no conflict of interest to declare.

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