Immunobiology 219 (2014) 671–679

Contents lists available at ScienceDirect

Immunobiology

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

Expression and regulation of complement receptors by human natural killer cells

Xiaoyun Min[a,1], Chengfei Liu[a,1], Yang Wei[a], Na Wang[a], Guolian Yuan[a], Dan Liu[a,c], Zongfang Li[a,d], Wuding Zhou[b,2], Ke Li[a,*,2]

[a] Core Research Laboratory, The Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China
[b] Medical Research Council (MRC) Centre for Transplantation, King's College London, Guy's Hospital, London, UK
[c] The Fifth Hospital of Xi'an, China
[d] Department of General Surgery, The Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an, China

ARTICLE INFO

Article history:
Received 8 January 2014
Received in revised form 27 March 2014
Accepted 29 March 2014
Available online 8 April 2014

Keywords:
Complement receptors
NK cells
Subsets of NK cells
Receptor expression

ABSTRACT

Integration of cellular and humoral arms of the innate immune response is fundamental to the development of powerful effector functions in host defence as well as aberrant immune responses. Here, we provide evidence in support of the relationship between complement activation and NK cell functional modulation. We demonstrate that human NK cells and both $CD56^{bright}CD16^{-}$ and $CD56^{dim}CD16^{+}$ populations express receptors known to detect the biologically active peptides C3a and C5a (i.e. C3aR, C5aR, C5L2) and the covalently-bound fragments C3b and metabolites iC3b and C3d which serve in immune adhesion (e.g. CR3, CR4). We also show that several pathogen- or tumour/inflammation-related stimuli differentially regulated those complement receptor expression. Furthermore, our results suggest that C3 fragments (C3a, iC3b) have a negative regulatory effect on IFN-γ production in NK cells. This work provides extensive information of human complement receptors relevant to the integrated actions of complement and NK cells which has been suggested by animal studies. The observations may act as a resource that allows further understanding and exploitation of role of complement in human health and immune mediated diseases.

Introduction

Natural killer (NK) cells are lymphocytes of the innate immune system. They can mediate spontaneous killing of certain cells (e.g. tumour, foreign cells, viral-infected and aberrant host cells) and produce a number of cytokines/chemokines (e.g. IFN-γ, TNF-α, MIP-1α, MIP-1β, IL-8), thus playing important roles in immune surveillance and immune regulation (Di Santo, 2006; Vivier et al., 2008), NK cells express an array of receptors including the activating receptors (e.g. NKp46, NKG2D), and inhibitory receptors (e.g. CD94/NKG2A, KIR-L). NK cell function is regulated by a dynamic balance between these activating and inhibitory receptor signals. In addition, NK cells also express many other receptors such as the receptors for cytokine/chemokine, hormones and adhesion molecules. Signalling through these receptors can also regulate NK cell function; this suggests that endogenous molecules may play important roles in NK cell functional modulation under both physiological and pathological conditions (Vivier et al., 2011). In humans, NK cells can be divided into $CD56^{bright}$ and $CD56^{dim}$ subsets. $CD56^{dim}$ subset are predominant in peripheral blood, express high levels of CD16 and mainly responsible for cytotoxic function. The minor $CD56^{bright}$ subset is virtually negative for CD16, and exhibit low cytotoxicity, while secreting large amounts of Th1 cytokines (Lanier et al., 1986).

The complement system is an important part of the humoral response in innate immunity. It consists of a set of soluble and membrane-bound proteins, including pathway components, receptors and regulators. Complement activation occurs rapidly in response to infection and other conditions including physical and chemical stresses. In response, complement activation generates a set of effector molecules which have diverse biological functions. The terminal product C5b-9 (also called membrane attack complex) mediates direct killing of certain pathogens and can also cause host tissue damage; the large fragments C3b/iC3b/C3d through interaction with their respective receptors (e.g. CR1, CR2,

* Corresponding author at: Core Research Laboratory, The Second Affiliated Hospital, School of Medicine, Xi'an Jiaotong University, Xi'an 710004, China.
Tel.: +86 29 8767 8329.
[1] Co-first authors.
[2] Co-last authors.

http://dx.doi.org/10.1016/j.imbio.2014.03.018
0171-2985/

CR3, CR4) mediate oposonophagocytosis of pathogens/pathogenic particles; while the small fragments C3a and C5a (also called anaphylatoxin) through interaction with their specific receptors C3aR and C5aR on endothelial cells and inflammatory cells induce local inflammation. All of these biological functions mediated by complement effector molecules are critical for fighting infection. While complement receptors are expressed on certain types of cells to detect/interact with complement effect molecules, membrane complement regulators such as CD55 (also known as DAF), CD46 (also known as MCP), CD59 have a wide cellular distribution to protect cells from autologous complement attack (Walport, 2001).

In addition to its traditional roles in innate immunity, recent research has revealed that complement also plays important roles in immune regulation through engagement of complement receptors expressed on immune cells to modulate the cell functions. It has been shown that engagement of complement receptors C3aR and C5aR on bone marrow-derived DCs or monocyte-derived DCs causes cell activation and subsequent enhances the DC function in T cell stimulation (Peng et al., 2008, 2009; Li et al., 2008, 2012). Cross-linking of CR2 and the B-cell receptors through complement-opsonised antigens decreases the threshold necessary for B-cell activation and enhances antibody class switching (Dempsey et al., 1996), while clustering CR1 by its natural ligand causes a dose-dependent inhibition of proliferation, plasmablast differentiation and antibody secretion of B cells (Kremlitzka et al., 2013). Cross-linking of the TCR and CD46 on naive CD4T cells induces the development of IL-10 producing regulatory T cells (Kemper et al., 2003). While these findings have greatly advanced our knowledge about the role of complement and complement receptors in regulation of antigen presenting cells, T cells and B cells, such information in NK cells is limited. Although it is known that human NK cells express C3b receptor CR3, it is unknown whether human NK cells also express anaphylatoxin receptors (C3aR, C5aR, C5L2) and CR4, whether different subsets of the NK cells ($CD56^{bright}$ and $CD56^{dim}$), have the same characteristics or exhibit different capacities to express complement receptors, and whether expression of these receptors is regulated by pathogen- or tumour/inflammation-related stimuli or C3 fragments. Addressing these questions could improve our understanding of the interactions between cellular and molecular components of the innate immune system.

In the present study, we thoroughly investigated gene and protein expression of complement receptors (CR3, CR4, C3aR, C5aR, C5L2) by human $CD56^{+}CD3^{-}$ NK cells and $CD56^{bright}$ and $CD56^{dim}$ NK cell subsets. We assessed whether expression of complement receptors by NK cells is regulated by different stimuli, including LPS, IL-15, TNF-α, Poly(I:C) and serum. We also examined the effects of C3 fragments on CR3 and C3aR expression and IFN-γ production in NK cells.

Materials and methods

Isolation and of human PBMCs and NK cells

Human peripheral blood samples were obtained with informed consent from a panel of Seven healthy donors (male:female, 4:3) aged between 20 and 41 from the department. The study had been approved by the School Human Studies Ethics Committee. Peripheral blood mononuclear cells (PBMC) were obtained by Ficoll-Hypaque gradient centrifugation (Lymphoprep, PAA laboratories). Total NK cells were isolated from PBMCs using NK isolation kit (Myltenyi Biotec, Germany) according to the manufacturer's instructions. After the isolation, the purity of the $CD56^{+}CD3^{-}$ NK cell preparation was routinely more than 90%, as determined by flow cytometry. In some experiments, NK cells were further sorted using a BD FACSAria II (Becton Dickinson, San Jose, USA). The purity of sorted cells was consistently more than 97%.

Cell culture

PBMCs or NK cells (at a concentration of 10^6/ml) were either used directly, or cultured for 24 h in complete RPMI-1640 (10% heat-inactivated FCS, 50 μM 2-mecaptoethanol, 2 mM glutamine, 50 U/ml penicillin and 50 μg/ml streptomycin, all from Invitrogen China Limited, Beijing, China) supplemented with 50 pg/ml recombinant human IL-2 (Peprotech China, Suzhou, China) at 37 °C in a humidified atmosphere of 5%CO_2. In some experiments, PBMCs or NK cells were incubated with culture medium containing 10 ng/ml IL-15 (Peprotech) or 10 ng/ml TNF-α (Peprotech) or 100 ng/ml LPS (Sigma-Aldrich, Shanghai, China) or 50 ng/ml PolyI:C (Sigma) or 10% human serum (Sigma) (i.e. normal serum [NS], C3-depleted serum [C3DS]) or normal serum pre-treated with methylamine [MA-NS] or human C3 fragments (Sigma) (i.e. C3a [100 nM], iC3b [10 μg/ml]), for 24 h. MA-NS was prepared by incubating serum with 0.675% reagent for 2.5 h at 37 °C, followed by extensive dialysis against PBS as previously described (Sandor et al., 2009).

Conventional RT-PCR

Total RNA was extracted from FACS sorted $CD56^{+}CD3^{-}$ cells using Ambion RNA isolation kit and subsequently used for cDNA synthesis. cDNA synthesis was carried out with 2 μg of total RNA, 160 ng of oligo$(dT)_{12-18}$, 500 μM of each dNTP, and 200U Moloney murine leukemia virus reverse transcriptase in 20 μl of solution (50 mM Tris–HCl pH 8.3, 75 mM KCl, 10 mM DDT, 3 mM MgCl2, 1.5 U/ml RNasin) at 37 °C for 45 min. At the end of the reaction, cDNA was stored at −20 °C until further use. PCR was carried out with 1 μl diluted cDNA (reflecting 0.1 μg of total RNA), 12.5 pmol of each 3′ and 5′ primer pair for each testing gene (the information for primer sequences are shown in Table 1) in 25 μl of reaction buffer (Promega, Southampton, UK). The PCR cycle consisted of 1 min at 94 °C, 1 min at 62 °C, and 1 min at 72 °C, with 35 cycles as above without ramping, and a final elongation at 72 °C for 10 min. Amplified PCR products were visualized after electrophoresis on 1.5% or 2% agarose gel containing ethidium bromide.

ELISA for C3a and iC3b

The supernatants collected from 24 h PBMC cultures in the presence of NS or C3DS. Sandwich ELISA was performed using human C3a OptEIA ELISA kit (BD Biosciences) and Microvue iC3b EIA kit (Quidel Corporation) according to the manufacturer's instructions.

Flow cytometric analysis

For three-colour flow cytometric analysis, aliquots of $0.5–1 \times 10^6$ fresh cells or cultured PBMCs were centrifuged for 10 min at $300 \times g$ and re-suspended in 100 μl PBS containing pairs of monoclonal anti-human antibodies: allophycocyanin (APC) conjugated anti-CD56 (BD Pharmingen™) and fluorescein (FITC) conjugated anti-CD3e (BD Pharmingen™) at a dilution of 1 in 50. The following phycoerythrin (PE) conjugated monoclonal antibodies against human complement receptor/regulator (i.e. anti-CR1, -CR2, -CR3, -CR4, -C3aR, -C5aR, and -C5L2) or IFN-γ (Biolegend, San Diego, USA) were used. Appropriate isotype controls were also used: PE-IgG1, -IgG2a and -IgG2b (Biolegend). After incubation for 30 min at 4 °C in the dark, labelled cells were washed twice with cold PBS. In some experiments, cells were permeabilised with Cytofix/Cytoperm and Perm/Wash buffers (BD Biosciences) before being stained for IFN-γ, C5aR and C5L2.