Veterinary Immunology and Immunopathology 150 (2012) 69–78

Contents lists available at SciVerse ScienceDirect

Veterinary Immunology and Immunopathology

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

Research paper

Natural killer cells: Frequency, phenotype and function in healthy cats

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ARTICLE INFO

Article history:
Received 20 June 2012
Received in revised form 13 August 2012
Accepted 27 August 2012

Keywords:
Natural killer cells
Cat
Phenotype
Quantification
Innate immunity
K562

ABSTRACT

Natural killer (NK) cells play a central role in innate immunity and have been shown to influence adaptive immune responses as well. This study aimed to provide a general NK cell quantification and phenotyping in several compartments of healthy cats and assess their functional properties. The results indicated that NK numbers, both absolute and relative, and phenotype mostly correspond with those found in bovine, ovine, human and murine immunology. However, there were also distinct differences, especially with regard to the expression of the integrin CD11b and the selectin CD62L (between 10 and 30% of feline NK cells stain positive for these markers) and the relative frequencies in lymph nodes (6.7%), which stand central in NK cell development. Caution should be taken when extrapolating findings on NK cell properties over species, notwithstanding the generally accepted evolutionary conservation of NK cells and their subtypes. It was also shown that K562 cells, the 'golden' target cell line for NK functionality tests did not work for feline cells. The feline kidney cell line CRFK proved to be very responsive to NK- and NKT-mediated lysis and therefore, represents an ideal alternative target. This study is a good reference for NK cell numbers, both absolute and relative, phenotype and function in several anatomical compartments of healthy cats and for cat-specific cytotoxic assays involving both NK and NKT cells.

1. Introduction

Since their description by Govaerts (1960), NK cells have been found to be major players in tumor, anti-viral and host–graft immunology (Yu et al., 1992; Diefenbach and Raulet, 2002; Brandstadter and Yang, 2011). NK cells fight infection by directly lysing the infected cells. In addition they can modulate the immune response through the secretion of pro-inflammatory cytokines and interaction with antigen-presenting cells (Jonjic et al., 2008). NK cells have been found in blood, lymphoid tissues, several organs and inflammatory sites (Cooper et al., 2001; Fehniger et al., 2003; Dalbeth et al., 2004). In human immunology, NK cells are characterized by the expression of CD56, CD16 and the absence of CD3. Early research confirmed the existence of feline NK cells but was somewhat hindered by the lack of monoclonal antibodies against specific NK markers. Feline NK cells were found in blood and lymphoid tissue and were determined to be non-adherent, to bear Fc receptors, to possess cytoplasmatic granules and to be activated by IL-2 (Mccarty and Grant, 1983; Tompkins et al., 1983, 1989). Crucial roles for NK cells

Abbreviations: CFSE, carboxyfluorescein succinimidyl ester; FIV, feline immunodeficiency virus; LN, mesenteric lymph node; NKT cell, natural killer T cell; PI, propidium iodide.

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0165-2427/$ – see front matter © 2012 Elsevier B.V. All rights reserved.
http://dx.doi.org/10.1016/j.vetimm.2012.08.010

have been described for human viruses such as human immunodeficiency virus, cytomegalovirus and herpesvirus (Brandstadter and Yang, 2011). Indeed, NK cell deficiencies cause severe recurrent viral diseases (Wood et al., 2011).

NK cells play, without a doubt, a major role in innate defences against pathogens. Some viruses however, have evolved immune evasive mechanisms by disrupting NK function. In cats for example, infection with feline immunodeficiency virus (FIV) leads to strongly reduced NK cell activity and frequency (Hanlon et al., 1993; Zaccaro et al., 1995; Howard et al., 2010). Still, there is very limited information concerning the general distribution and phenotype of NK cells in cats. Recent findings in human NK biology showing major differences in NK cell distribution, phenotype and function between blood, lymphoid organs and tissue, incited us to deepen our knowledge of NK cell biology in cats. This could contribute to a better understanding of cellular immunity toward feline pathogens and shed light on how pathogens may escape from NK-mediated lysis of infected cells.

In this study, general frequency, phenotype and functionality of feline NK cells were investigated. In a first part, NK cell quantification, both absolute and relative was performed in blood, mesenteric lymph node (LN), spleen, mesentery and kidney. In a second part, general NK cell phenotyping was done on mononuclear cells derived from blood, LN and spleen from of healthy cats. To this end, several surface markers (CD56, CD3, CD8, CD11b, CD16, CD25 and CD62L) and appropriate isotype controls were used. In the last part, a functionality assay was optimized and used for feline NK and NKT cells originating from blood, LN and spleen, since the 'golden' NK target cell line K562 was unresponsive to feline NK cell-induced lysis.

2. Materials and methods

2.1. Antibodies

Monoclonal antibodies against the epsilon chain of feline CD3 (NZM1) and against feline CD56 (SZK1) were kindly provided by Dr. Yorihiro Nishimura (Tokyo University, Japan) (Shimojima et al., 2003). Monoclonal antibodies recognizing feline CD25 were produced by the cell line 9F23.3, which was purchased from North Carolina State University (Raleigh, North Carolina, USA). Monoclonal antibodies FE5.4D2, CA16.3E10YFC120.5 and CA2.1D6 recognizing feline CD8β, canine CD11b, human CD16 and canine CD21 respectively, were purchased from AbD Serotec (Dusseldorf, Germany). A monoclonal antibody DREG56, cross reactive with feline CD62L (Kaname et al., 2002), was purchased from Acris antibodies (Hereford, Germany). Conjugated secondary antibodies [Molecular Probes (Invitrogen, Carlsbad, USA)] were goat anti-rat Alexa Fluor 488, goat anti-mouse IgG2a Alexa Fluor 488, goat anti-mouse IgG1 Alexa Fluor 647 and goat anti-mouse IgG3 fluorescein isothiocyanate (FITC). When primary antibodies from the same IgG1 isotype were used, one primary antibody was labeled with Zenon Alexa Fluor 488 Mouse IgG1 (Invitrogen, Carlsbad, USA).

2.2. Target cell lines

Crandell feline kidney cells were cultured in Minimal Esential Media (GlutaMAX) supplemented with 5% fetal calf serum (FCS) (Greiner Bio-one, Kremsmuenster, Germany), 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin, 0.1 mg ml^{-1} kanamycin and 2% lactalbumin (Invitrogen). After two days of culture, CRFK cells were detached through incubation with Accutase (Sigma–Aldrich) for 5 min at 37 °C. The human K562 cell line was cultured in Iscove's Modified Dulbecco's medium (GlutaMAX) (Invitrogen) supplemented with 10% FCS, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin and 0.1 mg ml^{-1} gentamycin (Invitrogen). Before being used as target cells, cells in suspension (1×10^6 ml^{-1}) were stained with 2 μM carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen) for 10 min at 37 °C, followed by several washing steps.

2.3. Cell-isolation from blood and tissue

Six healthy, conventionally housed, feline leukemia virus- and feline immunodeficiency virus-negative cats were used in this study, all originating from private owners (Table 1). Blood from all cats was collected from *the vena jugularis* in heparin (15 U ml^{-1}) (Leo, Zaventem, Belgium) and mononuclear cells were separated on Ficoll-Paque (GE Healthcare, Little Chalfont, Buckinghamshire, United Kingdom). Cells in tissue were isolated by grinding small pieces of tissues through a tissue grinder (250 μM mesh) (Sigma–Aldrich, St. Louis, MO, USA) and subsequently through a smaller cell strainer (70 μM mesh) (Becton, Dickinson and Company, New Yersey, USA). After isolation, cells were counted and frozen. Briefly, a maximum 2×10^7 cells ml^{-1} were resuspended in RPMI supplemented with 30% fetal bovine serum (FBS), 100 U penicillin ml^{-1}, 0.1 mg streptomycin ml^{-1}, and 10% dimethyl sulfoxide (DMSO). Subsequently, cells were frozen by lowering the temperature with 1 °C min^{-1} until −30 °C, followed by a 15 min incubation period at −30 °C and finally lowering the temperature to −150 °C at a rate of 1 °C s^{-1} (PTLPD81, Orthodyne, Alleur, Belgium). After freezing, cells were stored in liquid nitrogen.

2.4. NK cell phenotyping

Phenotyping of cells from all compartments was performed simultaneously. All analyzed cells were first stored in liquid nitrogen, facilitating analysis workflow. Several precautions were taken in order to preserve

Table 1
Detailed overview of the characteristics of cats used in this study.

Cat	Age	Gender	Breed
11GK5	8 years	♀	European shorthair
11GK12	11 years	♀	European shorthair
11GK13	2 years	♂	European shorthair
11GK18	9 years	♂	European shorthair
11GK24	5 years	♂	European shorthair
11GK25	7 years	♀	Persian