Regulatory Natural Killer Cell Expression in Atopic Children with Respiratory Allergy

Thesis

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First, thanks to **ALLAH** *to whom I relate any success in achieving any work in my life.*

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Every challenging work needs self efforts as well as guidance of elders especially those who were very close to our hearts.

I dedicate my work to my sweet and loving Father&Mother,

Whose affection, love, encouragement and prays of day and night make me able to move on.

And To my dear Husband who was and still supporting me in all my hard times.

And to my brother and my lovely Sisters.

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List of Abbreviations

Abb.	Full term
AHR	*Airway hyper responsiveness*
AD	*Atopic dermatitis*
ADCC	*Antibody dependent cell mediated cytotoxicity*
AEC	*Absolute eosinophilic count*
ALC	*Absolute lymphocyte count*
ANC	*Absolute neutrophilic count*
APC	*Allophycocyanin*
AR	*Allergic rhinitis*
BA	*Bronchial asthma*
CBC	*Complete blood count*
CCR7	*Chemokine receptor 7*
CD	*Cluster of differentiation*
CFC	*Chlorofluorocarbon*
CKJOT	*Cho-kyung-jong-ok-tang*
Clr	*C-type lectin related*
CXCL8	*Interleukin 8*
DAP1	*Adapter protein*
DCs	*Dendritic cells*
DNAM	*DNAX Accessory Molecule-1*
FEV1	*Forced expiratory volume in 1st second*
FGFR	*Fibroblast growth factor receptors*
FITC	*Fluorescein isothiocyanate*
flt-3 ligand	*Fms-related tyrosine kinase ligand 3*
GATA3	*Transcription factor belong to GATA family*
GM-CSF	*Granulocyte Macrophage Colony -Stimulating Factor*
HDM	*House dust mites*
HFA	*Hydrofluoroalkane*
HLA	*Human leucocytic antigen*
ICAM1	*Intercellular Adhesion Molecule 1*
ICS	*Inhaled corticosteroids*
IFN-ɤ	*Interferon*
IgE	*Immunoglobulin E*
IL	*Interleukin*
IL2Rα	*Interleukin 2 receptor alpha*
IL2Rβ	*Interleukin 2 receptor beta*
ILT-1	*Ig-like transcript*
ILT2	*Immunoglobulin like transcript 2*
INS	*Intranasal corticosteroids*
ITAM	*Immune receptor tyrosine-based activation motifs*

List of Abbreviations cont…

Abb.	Full term
ITIMs	*Immune receptor tyrosine-based inhibition motifs*
KIRS	*Human Killer immunoglobulin receptors*
KLG1	*Kinase like gene*
KLRK1	*Killer cell lectin-like receptor 1*
LAB	*Lactic acid bacteria*
LAG-3	*Lymphocyte-activation gene 3*
LFA-1	*Lymphocyte function associated antigen-1*
LGL	*Large granular lymphocytes*
LN	*Lymph nodes*
Ly49 x	*Lectin like transmembrane disulfide bonded homodimers expressed on NK cells*
MHC	*Major histocompatibility complex*
MICA	*MHC class I polypeptide-related sequence A*
MIP-1α	*Macrophage Inflammatory Protein 1α*
N-CAM	*Neural cell adhesion molecule*
NCR	*Natural killer cell receptor*
NK reg	*Regulatory natural killer cell*
NK	*Natural killer*
NKG2C	*Natural killer group 2C*
NKTs	*Natural killer T cells*
OVA	*Ovalbumin*
PB	*Peripheral blood.*
PE	*Phycoerythrin*
PEF	*Peak Expiratory Flow*
PGE	Prostaglandin E
pMDI	*Metered-dose inhaler"Puffer"*
PPD	Purified protein derivative
RANTES	*Regulated on activation, normal T cell expressed and secreted*
RSV	*Respiratory syncytial virus*
SPT	*Skin prick test*
STAT6	*Signal transducer and activator of transcription 6*
TCR	*t cell recptor*
TGF	*Transforming growth factor.*
TH1	*t helper 1*
TH2	*t helper 2*
TLC	*Total leucocyte count.*
TLR	*Toll-like receptor*
Tregs	*t regulatory*
ULBPs	*Cell surface glycoprotein encoded by ULBP gene located on chromosome 6*
XCL1	*Chemokin (c motif) ligand belonging to XC chemokine family (Lymphotactin)*

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INTRODUCTION AND AIM OF THE WORK

Natural killer (NK) cells are large granular lymphocytes of the innate immune system that exert a potent function against infected and tumor cells. Although NK cells were originally defined by their capacity to lyse target cells and produce interferon-γ without prior activation, recent studies showed that NK cells also display a potent regulatory function ***(Erten et al., 2008)***.

In *2008, Deniz et al* characterized a regulatory subset of NK cell subsets that are characterized by their IL-10 secretion. These IL-10-secreting NK cells were found to suppress Ag-specific T cell proliferation in response to bee venom major allergen, phospholipase A_2 and purified protein derivative of *Mycobacterium bovis* (PPD), in addition to suppression of IgE production ***(Deniz et al., 2008).*** Few studies addressed its role in fetomaternal tolerance and prevention of fetal rejection ***(Saito et al., 2008).*** Little is known about role of regulatory NK cell in allergy.

Aim of the Work

We sought to investigate the regulatory natural killer cell percentage and counts in atopic children with respiratory allergy in relation to disease severity and control. The ultimate objective is to outline the value of these cells as biomarkers of disease severity and/or control and may pave the way for using non-conventional therapeutic modalities.

NATURAL KILLER CELL

Natural killer cells (NK) are one of cytotoxic lymphocyte which are crucial to the innate immune system. NK cells are able to provide a rapid response to viral-infected cells in only 3 days after infection. They can uniquely recognize stressed cells in the absence of antibodies and major histocompatibility complex (MHC) unlike other immune cells and react much faster. They were named "natural killers" as they easily recognize and kill cells missing "self" markers of MHC 1 class without prior activation (*figure1*). This role is more important because infected cells that are missing MHC 1 markers cannot be detected or destroyed by other immune cells including T lymphocytes ***(Vivier et al., 2011).***

Origin and development of NK cell

NK cells are defined as large granular lymphocytes (LGL). They constitute the third kind of cells differentiated from the common lymphoid progenitor-generating T and B lymphocytes ***(Yang et al., 2011).*** NK cells differentiate and mature in the bone marrow, spleen, tonsils, lymph nodes and thymus, then they enter into the circulation ***(Iannello et al., 2008).***

Peripheral mature NK cells are inactive and undergo low levels of proliferation ***(Dokun et al., 2001)***. Their half-life is

about 7–10 days ***(Koka et al., 2003)***. Mature NK cells migrate to the blood or become resident within tissues. They constitute 5–10% of peripheral blood lymphocytes and are present in high numbers in the lung and liver, and they are also observed in the draining lymph nodes, where they have the ability to interact with dendritic cells (DCs) and activated lymphocytes. NK cells differ from natural killer T cells (NKTs) phenotypically, by origin and by respective effector functions. NKT cell can promote NK cell activity by secreting IFN-γ ***(Mathias et al., 2014)***.

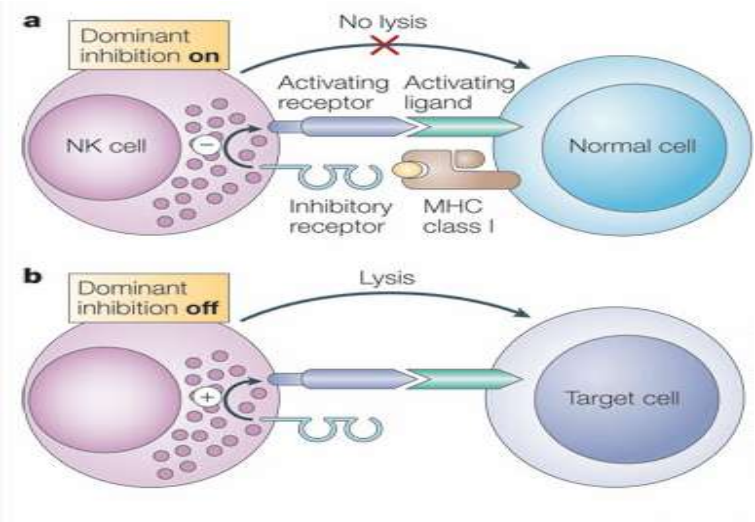

Figure (1): NK cell recognition of target cells *(Fauci et al., 2005).*

NK cell development and maturation is critically dependent on the bone marrow and the production of cytokines such as stem cell factor (c-kit ligand), Fms-related tyrosine kinase ligand 3(flt-3) ligand, Interlukin-7 (IL-7), and IL-15 by

bone marrow stromal cells. IL-15, in particular, is critical for development and survival of mature NK cells in peripheral blood ***(Ranson et al., 2003).*** Compared with T and B cells, NK cell development is self-limited, and NK cell maturation depends on myeloid cells, such as dendritic cells (DCs), monocytes, and neutrophils ***(Narni-Mancinelli et al., 2013).***

Phenotypic characterization of NK cells:

NK cells can be distinguished from other lymphocytes by lack of T-cell receptor and its associated CD3 complex. Although NK cells do not rearrange T-cell receptor subunits, they share a number of features with T cells, including expression of surface molecules and secretion of the same cytokines. The relative expression of the NK cell markers CD16 (the low-affinity receptor for the Fc portion of IgG [FcγRIIIA]) or CD56 (neural cell adhesion molecule) allows definition of several different NK cell subsets ***(Jonges et al., 2001).***

NK CELL SUBSETS:

1- According to expression of surface marker:

Based on the expression of CD56 and CD16, human NK cells in peripheral blood can be divided into at least two

functional subsets (*figure 2*). **$CD56^{dim}$ $CD16^{+}$ NK cells** constitute about 90% of total blood NK cells. This NK cell subset has a great ability to kill target cells, and they secrete only low levels of cytokines. In contrast, **$CD56^{bright}$ $CD16^{-}$ NK cells** constitute <10% of total blood NK cells, but are abundant in secondary lymphoid tissues ***(Ferlazzo et al., 2004).*** In contrast to the $CD56^{dim}$ $CD16^{+}$ NK-cell subset, activated $CD56^{bright}$ $CD16^{-}$ cells produce many cytokines, including TNF, IFN-γ and GM-CSF, but acquire cytotoxicity only after prolonged activation. Therefore, cytotoxic effector functions and cytokine secretion are separated in human NK-cell subpopulations ***(Strowig et al., 2008)***.

$CD94^{high}CD56^{dim}$ NK cells express higher levels of granzyme B and perforin. Their CD94-mediated redirected killing is higher than that of $CD56^{bright}$ NK cells but lower than that of $CD94^{low}CD56^{dim}$ NK cells. The density of CD94 surface expression on $CD56^{dim}$ NK cells might identify an intermediary developmental stage between $CD56^{bright}$ and $CD94^{low}$ $CD56^{dim}$ NK cells. Furthermore, $CD56^{dim}$ NK cells carry homing markers for inflamed peripheral sites. $CD56^{bright}$ NK cells home to sites of chronic inflammation, whereas