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Early View

Review

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NK cells in the lung: novel insight and future challenge in the airway diseases

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Take home message: Growing evidence cast a spotlight on the pathogenetic role of NK cells on airway diseases. This narrative review gives an overview on NK cells in airway diseases focusing on pathophysiological and clinical implications.

1. NK and the lung

Among numerous innate immune cells that populate the lung, Natural Killer (NK) cells play a crucial role in inflammatory processes as they act as the first line of defence by killing stressed, transformed, or infected cells. Moreover, NK cells play regulatory roles by interacting with other immune cells of both the innate and adaptive immune systems. A wide network of activating and/or inhibitory receptors on their surface are involved in these interactions [1].

Natural Killer (NK) cells are the major cytotoxic effector arm of the innate immune system and are responsible for constant tissue surveillance and destruction of malignant and infected cells. Human NK cells develop in the bone marrow and in secondary lymphoid tissues (SLTs) including tonsils, spleen and lymph nodes [2]. NK cell development proceeds by six steps, each characterized by the differential expression of surface markers [2]. NK cells belong to the family of innate lymphoid cells (ILCs). ILCs are classified as classic cytotoxic cells and lymphoid tissue inducer (LTi) cells and recently described non-cytotoxic ILC populations. ILCs are further categorized into three distinct groups: ILC1, ILC2 and ILC3 [3]. These subsets are analogous to and release the same effector molecules as the helper T cell phenotypes Th1, Th2 and Th17[4].

The ILCs share some functional and phenotypic features with NK cell developmental intermediates, in particular the dependence on the transcription factor ID2 to promote effector function in response to microbial products, cytokine stimulation, and contact with other leukocytes [5]. Moreover, NK cells are related to ILC1 as both produce interferon -γ (IFN-γ) and tumour necrosis factor-α (TNF-α) upon stimulation, although NK cells have cytolytic functions similar to CD8 features [6].

NK cells target cells for destruction based on signalling from numerous germline encoded receptors (summarised in Table 1). The Killer-cell Immunoglobulin-like receptor (KIR), Leukocyte Immunoglobulin-like receptors (LIR) and Natural Cytotoxicity Receptors (NCR) represent some of the main families of receptors. Inhibitory signalling from MHC class I dependent receptors are key to NK cell function, as NK cells remain in a "ready state" of cytotoxic function and require constant signalling to prevent granule release. In the past decade further roles for NK cells have been identified including inflammatory signalling, cellular crosstalk (e.g DC licensing) as well as deeper classification of NK cell phenotypes (e.g tissue residency and inflammation resolution). Whilst the lack of access to human lung tissue remains a limiting factor in understanding the role of NK cells in chronic respiratory diseases, here we aim to summarise the current literature for this field.

Based on the expression of CD56 and CD16 surface markers, human NK cells are commonly divided into two main subsets: $CD56^{dim}$ and $CD56^{bright}$ NK cells [7]. The $CD56^{bright}$ $CD16^{-}$ NK cells are mainly located in human secondary lymphoid organs and have a high-density surface expression of CD56 [8]. $CD56^{bright}CD16^{-}$ represents the immature subset with limited cytotoxic activity and characterized by a greater capacity to produce inflammatory cytokines such as IFN-γ, TNF-α , interleukin (IL)-10 and IL-13 in response to soluble factors like IL-12 and IL-18 [9, 10]. $CD56^{dim}CD16^{+}$ NK cells have a low surface density of CD56 and comprise about 90% of NK cells of the peripheral blood. The $CD56^{dim}CD16^{+}$ subset express KIR and have a greater cytolytic capacity as they contain high levels of perforin, granzymes A and B and they can induce antibody-dependent cellular cytotoxicity (ADCC) [11]. Research on NK cells have been mainly performed in humans on peripheral blood, however, much less is known about NK cells in human lungs.

Human NK cells are present in the lung as both circulating and resident cells. The percentage of circulating lung NK cells ranges from 5 to 20% of the $CD45^{+}$ lymphocytes (Figure 1). About 80% of these cells showed a mature $CD56^{dim}CD16^{+}$ phenotype [12, 13] while resident NK cells, determined by the homing receptor CD69+, are mainly immature. Resident lung NK cells are identified by the expression of CD49a and CD103 [14]. In murine models, NK cells represent about 10% of the lymphocytes in the lung and this percentage is higher than the rate in other tissues like liver, spleen, and thymus [15]. Although the proportions of lung NK cells do not seem to vary throughout life, the impact of environmental factors may affect cell number. Among these factors, cigarette smoking decreases the number of NK cells, while viral infection rapidly increases the number of NK cells [14].

Asthma and Chronic Obstructive Pulmonary Disease (COPD) are very common chronic inflammatory diseases of the lung and NK cells are implicated in both diseases. Chronic inflammation drives the airway obstruction in both asthma and COPD, often resulting in a decline in lung function [16]. Similarly, bronchiectasis is considered a progressive lung damage results from a 'vicious cycle' of recurrent bacterial infection and a poorly regulated inflammatory response. Although the pathogenesis is still unknown, NK cells seem to also play a role in the development of bronchiectasis [17].

2. NK cells and their role in asthma

Asthma is a chronic respiratory disease characterized by airway inflammation and hyperresponsiveness [18]. Worldwide, up to 300 million people are affected [19]. Severe

asthma (SA) is defined as asthma which requires treatment with high dose ICS, plus a second controller (and/or systemic corticosteroids) to prevent it from becoming 'uncontrolled' or which remains 'uncontrolled' despite this therapy [19]. Two different endotypes of asthma were reported in literature, classified based on the type of immunological cells that characterized the airway inflammation, as T2 high and T2 low [20]. Type 2 immunity is driven by the secretion of the cytokines IL-4, IL-5, and IL-13, which is manifested as high IgE antibody titers and/or eosinophilia [21]. Eosinophilic inflammation is characterized by eosinophilic infiltrate promoted by the cytokine IL-5. IL-5 and IL-13 are released by Th2 cells upon contact with allergens or other triggers andby ILC2 in response to the production of alarmins induced by allergens, pathogens, mechanical insults, contaminating agents [22].

Severe asthma is associated with exacerbations characterized by the worsening of clinical conditions which require the use of systemic corticosteroids and/or hospitalization [23, 24]. Often, exacerbations are associated with respiratory tract viral infections but the mechanisms of virus-induced asthma exacerbations are still not yet fully understood [25]. The identification of a specific phenotype of disease can help both to introduce correct therapy and limit the risk of exacerbation [24]. However, as a heterogeneous disease, various actors of innate and adaptive immunity contribute to endotype, clinical control and risk of exacerbation [18]. NK cells are key players of the innate immune response which play a defensive role against bacterial and viral insults [26]. Increasing evidence suggests a key role for the innate immune system and particularly of NK cells in the allergic sensitization and eosinophilic inflammation underlying asthma development [27]. The following studies depict a pleiotropic action of the NK cell, with regulatory and effector action that contributes to the asthmatic complex immune and inflammatory environment. Therefore, translating the role of NK cells into the clinical setting by evaluating their relationship with the therapies currently in use constitutes a very stimulating and pioneering research starting point.

2.1 In vitro and in vivo evidence on blood NK cell in in mouse model of asthma and human samples

The role of NK cells in murine models of asthma has been widely investigated in recent years, specifically in models of allergic asthma.

Cannabinoid type 2 (CB2) receptors is crucial in regulating NK cells function. NK cells through CB2 receptors limit ILC2 expansion and subsequent allergic inflammation . [28]. The depletion

of Natural Killer group 2 member D (NKG2D) receptor decreases the level of eosinophils and Th2 cells in mice [29]. The suppression of prostaglandin I2 (IP) receptor also reduced murine lung inflammation induced by allergen sensitization and ILC2 count [30].

Whilst the majority of NK cell research in mice is limited to allergic sensitization models, studies on human cohorts have highlighted that NK cell–mediated regulatory functions contribute to Th1/Th2 T-cell polarization. NK cells may therefore play a key role in the development of type 2 cytokine-biased status that characterize both allergic and eosinophilic asthma phenotypes [31]. NK cells help to promote the appropriate Th1 cell responses through a crosstalk with DCs. NK cells of asthmatic patients lack the ability to produce IFN-γ in response to a dendritic cell (DC)-mediated Th1 stimulus. Moreover, NK cells of patients with allergy appear ineffective in the cellular killing of immature DCs and are incapable of inducing adequate Th1 responses [32].

2.2 NK cells in asthma exacerbations

Natural killer (NK) cells are also important actors of the antimicrobial immune response acting against bacterial and viral insults through the secretion of cytokines and chemokines [26, 33]. Liu et al., evaluated changes of peripheral NK cells and sputum cytokines in paediatric asthma patients at baseline and during acute upper respiratory viral infections. The results showed an upregulation of peripheral $CD56^{+}CD16^{+}$ NK cells as well as significantly increased sputum concentrations of IL-4, IL-5 and IFN-γ during viral infections. During these conditions (viral infection and acute exacerbations) the increase in NK cells therefore induces a shift in the Th1/Th2 balance, favouring the prevalence of Th1 immune response, essential for host defence toward intracellular pathogens and for the resolution of the clinical relapse [34]. However, changes in human NK cell phenotype subsets and functions have been observed in asthma [35]. Starting from the hypothesis that impaired innate immunity can lead to the exacerbation of severe virus-induced asthma, Devulder et al. evaluated the responses of NK cells from severe asthmatic patients after an in vitro stimulation of toll-like receptors (TLR)-3 and 7/8 and exposure to Rhinovirus (RV)-A9. The capsid of the virus is recognised by TLR2, single stranded RNA and double stranded RNA from rhinoviruses are recognised by TLR3 and TLR7/8. This study demonstrated that NK cells from severe asthma patients were less cytotoxic and expressed less IFN-γ than those from healthy donors in response to IL-12 and IL-15, typical cytokines produced upon viral infection occurring[36]. Those observations emphasized the prominent role of NK cells in defending against infectious triggers underlying clinical exacerbations. Aberrant NK cell functions may therefore contribute to the frequency, severity,

and virus-induced exacerbation of asthma [37]. The restoration of NK cell cytotoxicity and immunoregulatory functions could improve the clinical outcome in asthma patients who remain clinically uncontrolled despite maximal therapy.

2.3 NK Cells and airway inflammation in asthma

NK cells can also interact with eosinophils in the lungs of people with asthma, inducing apoptosis of the eosinophils and the resolution of inflammation [38]. NK-mediated eosinophil depletion has also been demonstrated to prevent the chronicity of bronchial inflammation and so the airways remodelling which often characterizes severe forms of asthma [39]. The pro-apoptotic action of NK cells was mainly carried out by the $CD56^{dim}$ phenotype of NK cells [38]. Phenotypic analysis of NK cells in asthma has shown contradictory results. In severe asthma, a reduction of peripheral $CD56^{dim}$ NK cells was reported [38, 40]. Duvall et al found that NK cells from BAL samples shifted towards $CD56^{dim}$ cytotoxic NK cells. In severe asthma , NK cells showed impaired killing abilities [35, 38], linked to a dysregulation of their metabolic pathways [41]. An in vitro co-culture model of NK cells and the K562 myeloid line demonstrated lower cell-mediated killing of NK cells derived from asthmatic patients when compared with healthy controls (HC) [35].

LipoxinA4 has also been demonstrated to be involved in NK cell cytotoxicity. This protein is the natural pro-resolving ligand for formyl peptide receptor Type 2/ lipoxin A4 (ALX/FPR2) receptors, and it determines the effect on NK cell cytotoxic activity against both eosinophils and neutrophils [38]. This intriguing role of endogenously produced LipoxinA4 to potentiate NK-mediated bronchial clearance, could pave the way for novel therapeutic approaches (see Figure 2).

3. NK and COPD

3.1 COPD pathophysiology

COPD is a chronic inflammatory disorder of the lung, characterized by progressive irreversible lung obstruction and is the third leading cause of death worldwide [42]. 80% of COPD deaths are related to smoking, representing the largest risk factor for disease development [43]. Air pollution, exposure to biomass fuels and chemical irritants are additional risk factors for COPD, linking environmental insult to dysregulated lung immunity and function [44]. COPD is a

clinically heterogeneous syndrome that can manifest as emphysema, chronic bronchitis and small airways disease and is primarily diagnosed by reduced lung function [44]. Dysregulated immunity, epithelial apoptosis, and microbiome dysbiosis are all hallmarks of this disease [45–47]. Although primarily a disease associated with neutrophilia, recent evidence also suggest a role for eosinophils in driving COPD symptoms [48].

COPD symptoms undergo periodic exacerbation that, similarly to asthma, are often viral in origin, particularly during winter seasons [49]. Exacerbations are defined as an acute deterioration of symptoms and reduction in lung function and can result in hospitalization and death [49]. Furthermore, the exacerbation process accelerates overall lung function decline, which does not recover to baseline after the infection [49, 50]. Thus exacerbation is considered one of the major costs of COPD patient care [51]. A better understanding of the role of the major innate anti-viral NK cell in these processes in the COPD lung is therefore clearly warranted.

3.2 Blood NK Cells in COPD

Until relatively recently, the majority of our understanding of human NK cell phenotype and function in COPD comes from studies using peripheral blood. Despite this limitation, Osterburg et al. (2020) demonstrated changes in surface expression of NK cell activating/inhibitory receptors and a decreasing size of NK cell subpopulations associated with smoking, COPD and exacerbation. [52]. More recent work in the much larger COPDGene and ECLIPSE cohorts further support an association of decreased circulating NK cells with increased susceptibility to COPD exacerbations [53]. In the human lung, NK cells are predominantly stage 5 $CD56^{dim}CD16^{+}$ cells, similar to the peripheral blood [12, 54, 55]. Marquardt et al reported that the majority of NK cells in the lung are circulating NK cells [12]. NK cells make up 5–15% of peripheral blood mononuclear cells (PBMCs) and thus are constantly passing through lung tissue during steady state [10, 12]. However, steady state lung tissue expresses chemokines, such as C-X-C chemokine ligand (CXCL)-2, C-X-C3 chemokine ligand (CXC3CL)-1, C-C chemokine ligand (CCL)-4, CXCL9, CXCL10 and CXCL12, capable of recruiting $CD56^{dim}$ and $CD56^{bright}$ NK cells suggesting that NK cells may be recruited to the lung parenchyma from the periphery [54]. Marquardt et al also identified CD69 expression on approximately 75% of $CD56^{bright}$ lung cells, which could indicate tissue residency [12].

3.3 Lung-resident NK cells in COPD

NK cell tissue residency has been demonstrated based on transcriptional signatures similar to those expressed by resident memory T cells as well as cell-surface expression of CD49a, CD69 and CD103 [14, 56–58]. CD49a is a dominant marker of lung tissue-resident (tr)-NK cell phenotype, with ~13% of human lung NK cells expressing this integrin [14, 57]. Although the function of these lung-resident NK cells remains to be fully elucidated, lung trNK cells were hyper-responsive to influenza infection in an ex vivo lung model of infection [14, 58]. In a murine experimental model of COPD, the proportion of $CD49a^+$ trNK cells was like that observed in human lung and CD49a was expressed on predominantly immature NK cell subsets in both species [14, 59].

In COPD patients, these lung NK cells exhibit increased cytotoxicity against lung epithelial cells, implicating NK cells in the lung tissue destruction and emphysema observed in COPD [55, 60]. Finch et al demonstrated that increased lung NK cell cytotoxicity was mediated by IL-15 stimulated priming of NK cells by DCs isolated from the COPD lung [60]. These investigators suggested that the distorted DC activity may originate from inflammatory epithelial signalling following damage and stress, but this has not yet been shown [60].

In addition to soluble factors, NK cell receptors and ligands have also been shown to be dysregulated in COPD. Mkorombindo et al have recently described polymorphisms of both Human Leukocyte Antigen (HLA)-C and KIRs that influence risk of developing COPD [61], further suggesting a role for NK cells in COPD development. Additionally, MIC-A/B, a "stress" ligand recognised by the NKG2D receptor, is increased on the COPD lung epithelium, with a greater expression correlating with reduced lung function [55]. Furthermore, cigarette smoke exposure has been shown to upregulate NKG2D ligands on epithelial cells in mice, which may prime NKG2D-mediated NK cell activation to virus. Therefore, increased cytokine priming of lung NK cells in COPD may facilitate greater responsiveness and destruction of stressed structural cells [55, 60] (Figure 2).

This cell destruction may be further exacerbated by viral infections such as influenza which may enhance NK cell activation through NKG2D-dependent detection of cellular stress [62]. A recent study looking at the phenotype of circulating NK cells in COPD patients seropositive for cytomegalovirus (CMV) lends further weight to this cellular stress driven by viral infection hypothesis [63]. CMV seropositivity was associated with increased expression of CD57 and NKG2C by NK cells and this association was enhanced in cells from heavy smokers and COPD patients [63].

In the lung, more severe COPD correlated with a significant enhancement in the proportion of $CD56^{bright}$ cells, with a corresponding reduction in $CD56^{dim}CD16^+$ NK cells in lung tissue

from COPD patients [59]. $CD56^{dim}$ NK cells demonstrate significant cytotoxicity in other areas of the body and therefore may be strongly activated during COPD [26]. Sustained NK cell activation ultimately results in NK cell apoptosis which might explain the distorted $CD56^{bright}$:$CD56^{dim}$ ratio seen in COPD lung tissue [55, 60]. In addition, a higher proportion of $CD49a^{+}$ expression in the lungs also correlated with increased disease severity as well as development of COPD in the experimental mouse model [59].

Overall, there are complex effects of both smoking and COPD on the phenotype and function of circulating and lung-resident NK cells. Given the potential of NK cells to respond to both systemic and local insults and mediate heightened inflammatory responses, understanding how this innate immune cell is dysregulated in COPD is needed. Such understanding may lead to the identification of targets to prevent disease development as well as reduce susceptibility to COPD exacerbations.

4. NK cells and their role in bronchiectasis and lung infections

Bronchiectasis is a chronic inflammatory lung condition characterised by a vicious vortex of chronic infection, mucociliary dysfunction, airway inflammation and airway structural damage [64]. The complex interplay between these pathophysiological factors contributes to the development, progression, and exacerbation punctuations of this multifaceted disease process. Bronchiectasis is a heterogeneous condition resulting from a wide variety of aetiological causes associated with immune dysregulation, ranging from immunodeficiencies to immune excess with inflammatory hyperactivation in response to severe viral, bacterial, or fungal infections or autoimmune disorders.

Natural killer (NK) cells play a critical role at the innate–adaptive interface during lung infection and are implicated in immunity to many of the key pathogens implicated in bronchiectasis [65]. NK cells can either enhance or inhibit immune responses. In the airway, they can promote neutrophil survival following stimulation by pro-inflammatory cytokines or promote neutrophil apoptosis following stimulation with anti-inflammatory cytokines. Despite the high prevalence of bronchiectasis, there is a paucity of data about lung immunology, particularly the role of NK cells, in this patient group.

Bronchiectasis has long been accepted as a predominantly neutrophil-driven disease. Neutrophils migrate to the airway under the action of pro-inflammatory cytokines such as CXCL8, IL-1β, TNF-α and neutrophil serine proteases such as neutrophil elastase (NE), all of which are increased in the airway sputum or bronchoalveolar lavage (BAL) of patients with

bronchiectasis [66, 67]. Neutrophils are crucial elements of the innate immune system, which assure host defence via a range of effector functions, such as phagocytosis, degranulation, and neutrophil extracellular trap (NET) formation. In bronchiectasis, bacterial infection persists in the airway despite large numbers of neutrophils that would be expected to phagocytose and kill pathogens under normal circumstances.

Evidence suggests that neutrophils are disabled by multiple mechanisms including cleavage of phagocytic receptors by neutrophil elastase and inhibition of phagocytosis by neutrophil peptides. Organisms also evade clearance by adapting to chronic infection. The formation of biofilms, reduced motility and the downregulation of virulence factors are among the strategies used to subvert innate immune mechanisms. NETs [68]. Immunogenetic evidence suggests that there may be a link between the level of NK cell activation and disease susceptibility, implicating a predisposing role for innate immune mechanisms. A role for adaptive immune mechanisms is suggested by the genetic association of HLA-DR1, DQ5 with increased susceptibility to idiopathic bronchiectasis [69].

In a cohort of patients with idiopathic bronchiectasis, Boyton et al. showed that there was HLA-C group 1 homozygosity with analysis of the relationship between HLA-C and KIR genes suggested a shift to activatory NK cell function [70]. However, a subsequent study demonstrated a lack of association between KIR and HLA-C type and susceptibility to idiopathic bronchiectasis [65]. The diverging conclusions in these two studies could potentially be due to the use of a different control group but given bronchiectasis is a heterogeneous disease, inconsistent results across studies are not unusual and we speculate that neutrophil dysfunction may be limited to subsets of bronchiectasis patients. Abundant data argue for a role of NK cells in viral infections and bacterial sepsis, including immunity to *Pseudomonas*, *Haemophilus* and *non-tuberculous Mycobacteria* (NTM) [71]. Both protective and pathogenic roles have been ascribed to the NK cell response in these settings. NK cell activation in these infections may be driven largely by innate cytokines and may involve modulatory effects on T cells and other cell types, rather than being limited to direct effects on infection.

Despite recent breakthroughs in bronchiectasis, there is still a pressing need to look for new treatments. The recent WILLOW study has given a strong indication of the importance of neutrophil serine proteases in bronchiectasis. WILLOW is a Phase 2 clinical study for the drug brensocatib (INS1007), a dipeptidyl peptidase-1 (DPP1) which prevents activation of NE, proteinase 3 and cathepsin G in neutrophil granules during maturation in the bone marrow. By doing so, it greatly inhibits neutrophil serine protease activity in sputum in bronchiectasis

patients and is found to prolong the time to next exacerbation at two doses compared with a placebo [72, 73].

Manipulating the immune response in bronchiectasis may potentially have therapeutic potential. Other lung-inflammatory diseases such as asthma and COPD are benefitting from new biologics that impact pathogenic cytokine polarization [74]. Therapeutic monoclonal antibodies against various proinflammatory cytokines have been successful in treating asthma; RA; psoriasis and many other autoimmune associated conditions. A major challenge in bronchiectasis is the application of emerging phenotyping and endotyping techniques to identify the patient populations who would most benefit from a specific treatment, with the goal of better targeting existing and emerging treatments and achieving better outcomes. Although many potential interactions of NK cells are likely, their location, timing and importance during different phases of ongoing respiratory infection or inflammatory response in bronchiectasis are still largely unknown, as is the role of different NK subsets. To utilize NK cells as a potential therapeutic target in chronic lung disease, it is necessary to establish a therapeutic strategy for controlling their functions in consideration of the basic functions of NK cells in each disease.

5. NK cells and steroid treatment

Systemic glucocorticoids are the cornerstone of treatment for exacerbations of airway diseases, including asthma and COPD [75]. Inhaled corticosteroids (ICS) are considered as first-line therapy in all asthma treatment guidelines and are a mainstay of COPD treatment for patients with a history of exacerbations [76]. The role of systemic or inhaled corticosteroids in patients with bronchiectasis is still a matter of debate [77, 78] but steroid treatment is largely used particularly during exacerbation of disease. Blood eosinophils are a clinically reliable biomarker of ICS response in asthma and COPD [79, 80]. More recent evidence also highlight the potential value of blood eosinophils in also predicting an ICS response bronchiectasis [81, 82].

It is well known that corticosteroids can deplete NK cells [83], although this interaction has been poorly explored in asthma. According to the literature, the anti-inflammatory action of steroids can interfere with NK cell activity and with their eosinophilic killing in airways [35, 84]. Di Lorenzo et al. evaluated the effects of fluticasone propionate (FP) treatment on NK activity in peripheral blood of healthy volunteers and from asthmatic atopic subjects. FP

significantly reduced NK cytotoxic activity against myeloid cell lines in both groups [85]. Duvall et al. evaluated the impact of systemic steroid treatment on NK cells from broncho alveolar lavage (BAL), before treatment and after 3 to 6 weeks of follow-up, showing an increase in NK cell immature phenotypes $CD56^{bright}$ and a reduction in mature $CD56^{dim}$ NK cells [35]. In the same study, exposure of NK cells to dexamethasone reduced cell lysis by 40% in HC and asthmatic patients. A reduction of cytotoxic mediators (granzymes, perforin, granulysin, sFas and sFasL) was reported only in blood NK cells derived from asthmatic patients. The alteration of the cytotoxic activity of blood NK cells induced by steroids was associated with cellular modifications in terms of functional and maturational changes with a numerical reduction of both $CD56^{dim}$ and $CD56^{bright}$ cells [35].

The link between NK cell activity and the use of corticosteroids remains a challenge of research in the field of respiratory disease, particularly asthma. The worsening of NK responses, already compromised in severe asthma, could lead to the persistence of bronchial eosinophilia and to the chronicity of airway inflammation and chronic bacterial colonisation. Moreover, considering the pathogenetic and clinical role of eosinophils and bacterial colonisation in COPD and bronchiectasis [86, 87], the impairment of NK cell activity by steroids may assume paramount importance in these airway diseases.

6. NK cells and monoclonal antibodies treatments

Considering the countless side effects of systemic steroid therapy, biological treatment is emerging, in severe asthma, as a preferential alternative to oral glucocorticoids [88, 89]. In recent years, several monoclonal antibodies have been developed to target allergic and eosinophilic inflammation of severe asthma. Such treatments include monoclonal antibodies not only against the IL-5 cytokine (mepolizumab, reslizumab) and the IL-5 receptor alpha (IL5Rα – benralizumab) but also against the IL-13/IL-4 receptor alpha (IL-4Rα - dupilumab). By targeting membrane bound receptors these latter two modalities may lead to NK cell mediated ADCC of the cells expressing these receptors [40] [90].

The better understanding of the pathological mechanisms of COPD and bronchiectasis has cast a spotlight on the underlying eosinophilic inflammation allowing a better definition and characterization of disease endotypes [91, 92]. Recent scientific inquiries evaluated the effect of the blockade of type 2 cytokines in COPD with conflicting results. Anti-IL-5 and IL-5Rα targeting agents failed to reduce the annual rate of moderate or severe exacerbations in COPD

[93] while dupilumab reduced exacerbations and improved lung function and quality of life in COPD patients with elevated blood eosinophil counts [94]. The identification of a T2-high endotype in bronchiectasis may pave the way for the design of randomized controlled trials on biological treatments targeting the key cytokines of T2 inflammation[87]. However, at the current time, no biological drugs are approved for the treatment of bronchiectasis and COPD [95].

As suggested above, benralizumab directly interacts with NK cells through the binding of the CD16 receptor expressed on NK cells. After this interaction, NK cells activate ADCC leading to the apoptosis of eosinophils. An immunological analysis of peripheral blood of severe eosinophilic asthma patients highlighted the impact of benralizumab on NK cell proliferation, maturation and activation [40]. Benralizumab therapy enhanced the total NK cell count and a maturation towards a more cytotoxic phenotype $CD56^{dim}$. An increase in the proliferative potential and activation state of NK cells, assessed through expression of CD137 was also observed. NK cells also play a crucial role in non-eosinophilic responses in patients with asthma. An analysis of sputum from patients with sub-optimal response to benralizumab showed that exacerbations were largely non-eosinophilic. Circulating NK cells and particularly the $CD56^{dim}$ population were reduced in this sub-optimal responder population. Failure to restore the NK axis could lead to the increased infection frequency and therefore to poor clinical response to benralizumab [96].

There is poor evidence regarding the immunological effect on NK cells in response to mepolizumab. Exhausted NK cells, determined by the expression of PD-1 and CTLA4 showed a rebalancing of these cell subsets after 24 months of treatment [97]. The same was established for benralizumab. However, to better define mepolizumab effects on NK cells, further studies are needed.

The relationship between other biologics licenced for asthma treatment, namely omalizumab (targeting free IgE) or dupilumab, and NK cells has not been explored before in literature in the context of respiratory disease. However, dupilumab had significant impacts on NK cell proliferation and maturation in atopic dermatitis, a chronic inflammatory disease with similar pathogenetic features to allergic asthma [90].

Conclusions

NK cells are involved in the development of airway diseases. COPD and asthma share similar molecular and cellular mechanisms (Figure 3). Among these, $CD56^{dim}CD16^{+}$ are decreased in lung tissue of both diseases, following decrease cytotoxicity mediated by limited release of IFN-γ. NKG2D-mediated NK cell activation also plays a key role in response to viral infection. In both diseases, interactions with other innate immune cells such as DCs have been reported. Increased expression of CD57 and NKG2C was observed in both pathologies. Resident lung NK cells found in both asthma and COPD patients expressed the same markers of tissue residency (CD49a, CD103 and CD69), although, to date, no data regarding the comparison between asthma and COPD patients is available.

Several interesting issues remain to be solved regarding bronchiectasis, although as for the other airway diseases, impaired NK cells correlate with disease severity and play a modulatory effect on T cells. In bronchiectasis the role of NK cells was reported in relationship with neutrophil survival

Given the predominant anti-viral and anti-eosinophil roles of NK cells outlined, further experimental studies that unify methodological criteria that influence NK action appear necessary to clearly define the roles, both common and specific, of the NK cell in the development of airway diseases. Novel molecular and cellular technologies and tools, including single cell 'omics analysis in the future will help the better understanding of NK cells biology and possible implications as to therapeutical approaches.

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RECEPTORS	LIGANDS
CD56 (NCAM)	Unknown
CD16 (FcgRIIIA)	IgG
Natural Cytotoxicity Receptors (NCR)	
NKp46 (NCR1)	Viral hemagglutinin
NKp44 (NCR2)	Viral hemagglutinin
NKp30 (NCR3)	B7h6, HCMV-pp56
Killer-cell Immunoglobulin-like receptor (KIR)	
KIR2DL1 (CD158a)	HLA-C
KIR2DL2/3 (CD158b)	HLA-C
KIR3DL1	HLA-B
KIR3DL2	HLA-A
Leukocyte Immunoglobulin-like receptors (LIR)	
LILRA-2	HLA-I
NKG2 (CD159)	
NKG2D	MICA, MICB, ULBP-1, ULBP-2, ULBP-3, ULBP-4, ULBP-5, ULBP-6
CD94/NKG2C	HLA-E
NKG2A (CD94/CD159a)	HLA-E
Other receptors	
ALX/FPR2 receptor	Lipoxin A4
CB2 receptors	Arachidonyl ethanolamide, 2-arachidonoyl-glycerol (2-AG)
IP receptor	PGI2
Homing receptors	
CD49a (αEβ7 integrin)	Collagen I, VI
CD103 (ITGAE)	β7-ITGB7
CD69	Gal-1

Table 01. Summary of receptors expressed by NK cells and the ligands they recognise if known.

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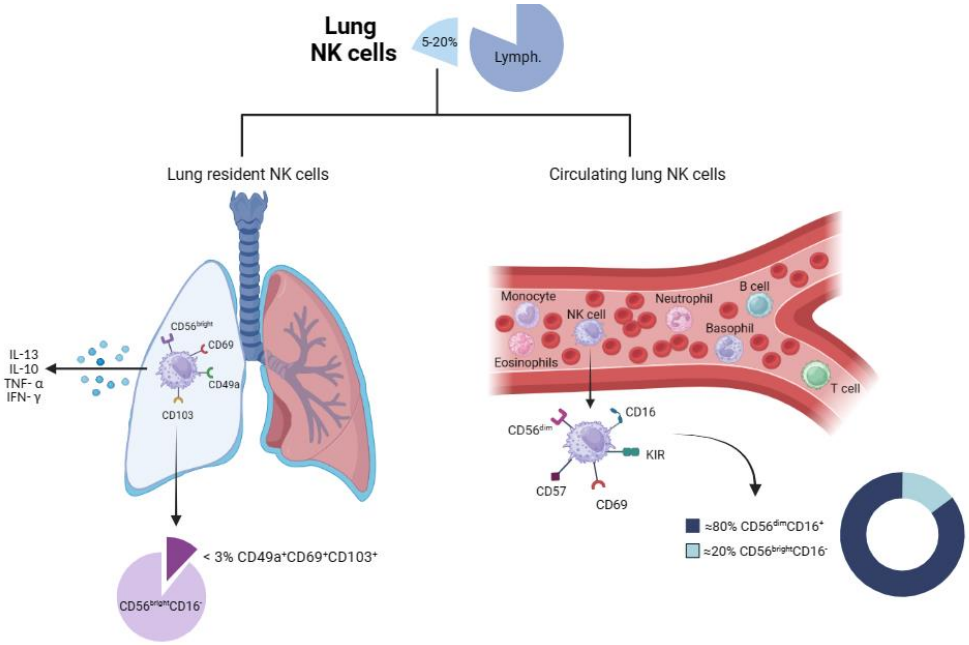

Figure 1. NK cell phenotype and distribution. Among all lymphocytes, the proportion of lung NK cells ranges from 5 to 20%. Of these cells, it is possible to distinguish the circulating NK cells and the resident NK cells. Most circulating NK cells belong to the mature $CD56^{dim}CD16^{+}$ phenotype. In contrast, tissue-resident lung NK cells are mostly composed of $CD56^{bright}CD16^{-}$ and are characterized by the expression of three markers: CD69, CD49a and CD103.

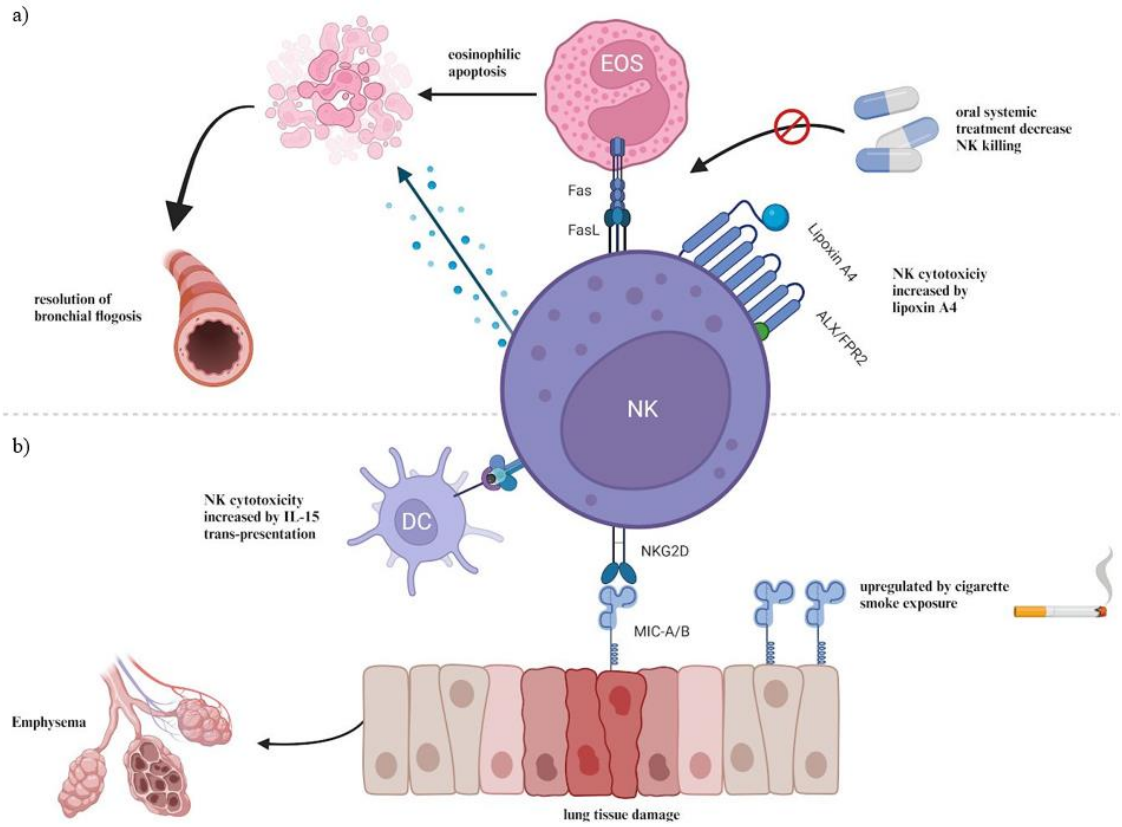

Figure 2. Proposed model of natural killer (NK) cells cytotoxic activity in asthma and COPD. In asthma, NK cells interact with airway eosinophils inducing the extrinsic pathway of apoptosis. Apoptosis is mainly carried out by Fas/FasL signaling and by the release of Granzyme B and perforin. NK-mediated eosinophil apoptosis leads to the resolution of airway inflammation. NK cytotoxicity against eosinophils is weakened by systemic steroid treatment and increased by the interaction between Lipoxin A4 and ALX/FPR2 receptors. In COPD, the expression of MIC-A/B cellular stress by the lung epithelium, enhanced by smoke exposure, induces the activation status of natural killer (NK) cells through the binding to NKG2D. Lung resident NK cells exhibit increased cytotoxicity against lung epithelial cells. NK cytotoxic responses contribute to lung tissue destruction and emphysema. NK cytotoxic responses are boosted by DCs IL-15 trans-presentation.

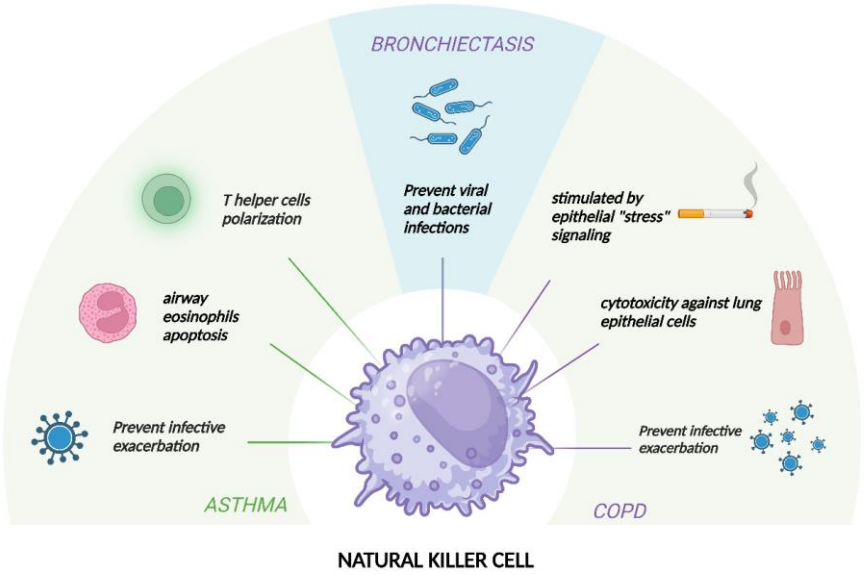

Figure 3. Graphical summary of the main natural killer (NK) cells functions in the different airway diseases.