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The plasmacytoid dendritic cell: a cell at the cross-roads in asthma

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Abstract

The onset, progression and exacerbations of asthma are frequently associated with virus infections of the lower respiratory tract. An emerging paradigm suggests that this relationship may be underpinned by a defect in the host's antiviral response, typified by the impaired production of type I and type III IFNs. The failure to control viral burden likely causes damage to the lung architecture and contributes to an aberrant immune response, which together, compromise lung function. Although a relatively rare cell type, the plasmacytoid dendritic cell dedicates much of its transcriptome to the synthesis of IFNs and is pre-armed with virus-sensing pattern recognition receptors. Thus, pDCs are specialised to ensure early viral detection and the rapid induction of the antiviral state to block viral replication and spread. In addition, pDC can also limit immunopathology, and promote peripheral tolerance to prevent allergic sensitisation to harmless antigens, possibly through the induction of regulatory T cells. Thus, this enigmatic cell may lie at an important intersection; orchestrating the immediate phase of antiviral immunity to effect viral clearance while regulating tolerance. Here we review the evidence to support the hypothesis that a primary defect in pDC function may underlie the development of asthma.

Introduction

Asthma is characterised by airway hyperreactivity (AHR) to non-specific spasmogens, structural alterations to the airway wall, and chronic inflammation. The inflammatory response is typically associated with the expression of the T_H2-type cytokines interleukin (IL)-4, IL-5, IL-9 and IL-13 [1], which can induce all of the cardinal pathologic features of disease [2, 3]. Indeed, the molecular and cellular aspects of the asthmatic reaction, particularly in response to classical allergen provocation, are now fairly well defined, although the emergence of the type-2 innate lymphoid cell (or 'nuocytes') has reinforced the concept that innate cells also provide a rich source of Th2 type cytokines [4], in addition to classical Th2 lymphocytes. Despite such progress, our understanding of the processes that promote a Th2-inducing microenvironment and break tolerance to innocuous antigens remains rudimentary, and as a consequence a scarcity of new immunomodulatory therapies has emerged [5].

It is now appreciated that the great majority of exacerbations of asthma are associated with both respiratory virus infections and evidence of Th2 immunity [6-10]. Moreover, epidemiological studies have implicated frequent/severe wheezy lower respiratory tract (LRT) infections as a major risk factor for the onset and progression of asthma in early life [11-15]. Largely determined by a lack of type I interferons (IFN-α/β; IFN-I) production in responses to virus infection, a new paradigm has emerged in the field linking defective innate antiviral responses in both haematopoietic cells and non-haematopoietic cells to increased fragility and damage of the airway epithelium. This defect may also contribute to the development of Th2 immunity, although this concept requires further support. Here we focus on the plasmacytoid dendritic cell (pDC) and present evidence supporting the hypothesis that a primary defect in the host's 'natural type I IFN-producing cell' may underlie the development of asthma. With the capacity to rapidly secrete large amounts of IFN-I and present antigen to naive and memory CD4+ and CD8+ cytotoxic T lympocytes, pDCs are at the interface of innate and adaptive antiviral immunity. Curiously, pDC have also been implicated in mediating tolerance to prevent the induction of allergic asthma [16, 17]. Thus this rare and enigmatic cell may lie at an important intersection; orchestrating the immediate phase of antiviral immunity to effect viral clearance while regulating tolerance to self and non-self antigens.

pDCs and their known PRR systems

pDCs are a relatively rare type of DC that reside predominantly in lymphoid tissues. Both human and murine pDC express the surface antigen CD45RA and lack the myeloid marker CD11b, although subtle differences exist since human, and not murine, pDC express the surface markers blood DC antigen-2 (BDCA-2/CD303), BDCA-4 (CD304), immunoglobulin-like transcript 7 (ILT7), and the IL-3 receptor-α chain (CD123). In contrast murine, but not human, pDC express Siglec H, B220 (CD45R), bone marrow stromal cell antigen 2 (BST2/CD317) and CD11c. As with other antigen presenting cells, pDC can acquire and present antigen to T lymphocytes, although they must first be licensed to do so e.g. via pattern recognition receptor (PRR) activation [18, 19]. pDC also provide help to T cells through the provision of co-stimulatory molecules and soluble factors [20]. Indeed, both human and murine pDCs produce prolific amounts of IFN-I (including -α, −β, −κ and -ω) dedicating 60% of their transcriptome to IFN production [21] and can release 100-fold more IFN than any other known cell type (3-10 pg of IFN-α/cell) [22-25]. pDC also produce type III IFNs [26] to induce a similar transcriptome in the target cell as IFN-I. Strikingly, pDC possess the necessary PRRs and signalling intermediaries (e.g. interferon regulatory factor 7; IRF7) to recognize viral-derived motifs, and are thus uniquely placed to rapidly sense and respond to viral infections [27-29], even in the absence of cellular infection or viral replication [29, 30]. This first wave of IFN stimulatory genes establishes the antiviral state, blocks viral replication, and facilitates the targeted lysis of infected cells. Amongst the PRR families, pDC are most widely acknowledged to express toll-like receptor (TLR)7 and TLR9, which recognise single stranded (ss)RNA and unmethylated CpG-DNA respectively. Both of these receptors signal via the adaptor protein MyD88, which through a signalling cascade, activates transcription of IFN-I, pro-inflammatory cytokines and co-stimulatory molecules. Murine pDC also express TLR8 which can recognise DNA as well as RNA [31], although it is less clear whether human pDC express and respond to TLR8 ligands [32, 33]. Activation of the cytosolic RNA-sensor RIG-I (retinoic acid-like receptor-I), MDA-5 (melanoma differentiation-associated protein 5) or NOD-2 (nucleotide-binding oligomerization domain-containing protein 2), all of which recognise ssRNA (as well as other microbial motifs), can induce IFN-I production by pDCs [32, 34, 35]. RIG-I has been shown to be functional in pDC but only in the absence of TLR responsiveness [35], however, RIG-I deficiency does not affect IFN-I or IL-6 production in response to infection with ssRNA viruses, suggesting a compensatory mechanism [36]. Of note, pDC also express the receptor for advanced glycation end products (RAGE), which has been associated in two genome wide association studies as a risk factor for poor lung function [37, 38]. In an elegant study, the RAGE ligand – high mobility group box 1 protein (HMGB1) – was shown to facilitate viral nucleic acid recognition and optimal IFN-I production following activation of TLRs and RIG-I-like receptors (RIG-I, MDA-5) alike [39, 40]. TLR9-induced responses are diminished in RAGE- or HMGB-null pDC, which may relate to altered trafficking and a lack of retention of the PRR ligand in the endosome [40]. Whether the RAGE/HMGB1 axis contributes to the activation of TLR7 remains unresolved. Exogenous HMGB1 can inhibit TLR9-mediated IFN-I secretion by pDC [41], although others have reported that HMGB1 blockade decreases

CpG-induced IFN-I production [42]. These conflicting reports may relate to post-translational modifications of HMGB1 which can change its functional activity [43]. In addition to TLR9, pDC can also detect microbial DNA via the cytosolic helicase DHX36 (aspartate-glutamate-any amino acid-aspartate/ histidine (DExD/H)-box helicase 36) which like TLR9 employs the MyD88-IRF7 signal transduction cascade to induce IFN-I production [44]. Less is known with respect to the expression of other PRR such as the NOD-like receptors (NLR) family, although the ability to secrete mature IL-1β and IL-18 suggests pDC are capable of forming an active inflammasome [45-47]; further studies are warranted to investigate the nature of the inflammasome(s) in pDC.

Alterations in peripheral pDC in allergic disorders and effect of allergen challenge.

The earliest studies that sought to determine whether pDC numbers are altered in atopic or asthmatic individuals were performed after observations in the late 1990s suggested that the DC1 subtype of DCs promoted Th1 responses and the DC2 subtype (with phenotypic characteristics of pDC i.e. HLA-DR^{+}CD11c^{-}CD123^{+}) promoted Th2 responses, especially when cultured with IL-3 [48, 49]. Congruent with this, Uchida and colleagues reported that the number of HLA-DR^{+}CD11c^{-}CD123^{+} 'DC2' cells was approximately twice as high in peripheral blood of atopic as compared to healthy subjects [50]. These findings were later confirmed in both atopic and nonatopic asthmatics as compared to healthy controls [51, 52]. With the advent of reagents to detect BDCA antigens, the use of CD123^{high}/CD11c^{-} to identify pDC declined, and DC subsets were increasingly redefined as DC1 (BDCA-1/CD1c+), DC2 (BDCA-3/CD141+), and pDC (BDCA-2/CD303). Nevertheless, this new phenotyping strategy once again confirmed that circulating pDC are significantly greater in asthmatics as compared to healthy controls [52].

In response to allergen challenge, pDC numbers increase moderately in the lung and decrease in the periphery. When the bronchial mucosa is sampled 6 hour post allergen challenge, mDC ($CD1c^{+}HLA\text{-}DR^{+}$) are increased, but pDC numbers are unchanged [53]; however, analysis of bronchiolar lavage fluid (BALF) and sputum at 24 h post-segmental allergen challenge found an increase in pDC [54, 55]. Even after accounting for the different methodologies employed across these studies, the overall picture from asthmatic adults suggests that pDC are elevated in the periphery and are recruited into the airways in response to allergen challenge. At present, the mechanistic basis of this observation is unclear, however, one possibility is that the inability of asthmatics to produce sufficient IFN-I in the airways in response to infection may fail to activate the negative regulatory feedback loop that exists to homeostatically regulate pDC numbers [56].

In contrast to adults, children with allergic asthma tend to have lower numbers of pDC in the periphery [57]. Analysis of frozen peripheral blood mononuclear cells collected from children age 6 or 7 who had had a wheezing episode associated with a severe RSV infection necessitating hospitalisation in the first year of life found that BDCA-2+ pDCs were approximately 50% lower in the children who were subsequently diagnosed with asthma [58]. In light of the association between wheezy LRT infections in early life and later diagnosis of asthma, Upham and colleagues prospectively examined pDC numbers in peripheral blood in infancy [59]. Intriguingly, pDC numbers were inversely associated with LRT infections and physician-diagnosed asthma at age 5 years [59]. Thus, higher pDC numbers in infancy appear to be protective against asthma inception. Whether this observation relates to a defect in the development and maturation of pDC, or is reflective of a greater infiltration into the airways in response to respiratory allergen challenge or virus infection to lower circulating pDC [54, 60-62] remains to be determined. However, it is important to note that blood sampling was deferred for two weeks in those children who were unwell, suggesting this did not account for the lower pDC count.

Confirmation of these findings together with a greater understanding of the molecular basis of this defect is now of paramount importance. pDC numbers and RV-stimulated IFN-I responses of peripheral blood mononuclear cells (PBMCs) are greater at 1 month of age and again by 6 months of age as compared to data obtained 'at birth' [63, 64], raising the possibility that an ontogenic defect may exist in 'at risk of asthma' infants. Do low pDC numbers arise from a maturation defect? Or alternatively, perhaps there is greater differentiation of pDC to cDC [65, 66]? It will also be important to determine the influence of environmental factors, such as maternal smoking, gut bacterial colonisation, and diet, on the ontogeny of pDC in early life. Although not in a neonatal setting, it was recently shown that antibiotic treatment of mice (which typically increases the magnitude of allergic inflammation in mouse models of asthma) increases the susceptibility to influenza virus infection-associated damage in the airways [67], an effect linked to lower IRF7 expression in lung macrophages. Should alterations to the microbiota affect IRF7 expression in pDC, then this would profoundly affect the induction of anti-viral immunity given the importance of pDC in the initiation of the immediate IFN-I response (see below).

pDC from allergic or asthmatics subjects generate aberrant IFN-I responses

To evaluate whether the innate antiviral response is impaired in allergic or asthmatic subjects, multiple laboratories have measured the release of IFN-I and III, or biomarkers thereof, from peripheral blood leukocytes in response to various TLR stimuli or virus infection. Newcastle disease virus-induced IFN-I release is impaired from PBMCs (which include pDC) of allergic asthmatic as compared to nonallergic asthmatic children [68] and adults [69]. Intriguingly TLR7-, but not TLR3-induced transcription of antiviral molecules and release of the chemokine IP-10 from PBMCs is reduced in atopic adolescents with mild to moderate asthma as compared to healthy controls [70]. Although it was not directly established that the defect was intrinsic to pDC, others have shown that the capacity of allergic or asthmatic donor pDC to produce IFN-I (and where examined, IFN-III) is impaired in response to TLR9/CpG or virus (influenza or rhinovirus) stimulation [71-75]. Defects in pDC responsiveness may also arise from genetic abnormalities such as single nucleotide polymorphisms. Recent studies have demonstrated that TLR7 and TLR8 SNPs have a strong association with asthma across diverse populations [76, 77], although how these impact upon expression and protein function is unknown at present. Collectively, these findings suggest that the defect in asthma may relate to the TLR7 and TLR9 pathway (which both signal via MyD88-IRF7) and not the TLR3 pathway (which preferentially signals via IRF3-TIR-domain-containing adapter-inducing interferon-β).

The high-affinity Fc receptor for IgE is a negative regulator of pDC-derived IFN-I production

An impressive body of work now suggests that (i) expression of the high-affinity IgE receptor, FcεRIα, on pDC is greater in allergic and/or asthmatic subjects [75], (ii) FcεRIα expression on pDC is inversely proportional to IFN-I/III production [72, 73, 75] and, (iii) cross-linking of FcεRIα impedes the capacity of pDCs to release IFN-I and IFN-III [74, 75]. Segmental allergen challenge in human subjects reduces the production of IFN-I in BDCA-4+ pDC purified from PBMCs, supporting the notion that IgE-mediated signalling pathways operate *in vivo* to modulate pDC function [78]. Mechanistically, anti-IgE has been shown to down-regulate TLR9 expression by inducing TNF production from pDC [79]. Moreover, cross-linking of FcεRI on pDC can activate ILT7, an inhibitory receptor bearing an immunoreceptor tyrosine based activation motif, to negatively regulate IFN-I production by pDC [80](see Figure 1). The IFN-stimulated antigen BST2 has since been identified as a ligand of ILT7, suggesting a negative feedback loop to prevent excessive IFN-I production

[81]. Indeed, BST2 ligation of ILT7 suppresses influenza virus (TLR7) or CpG (TLR9) triggered release of IFN-I by pDC [81]. Similarly activation of either human BDCA-2 or murine Siglec H induces an inhibitory signal through Syk kinase to attenuate IFN-I production [81]. A recent report found that Hepatitis C viral glycoprotein e2 ligates BDCA-2 to inhibit pDC production of IFN-I and III [82], while elevated phosphorylation of Syk is associated with attenuated IFN-I production by HIV-stimulated pDC [83]. It will be important to determine whether respiratory viruses, and in particular those associated with the onset of asthma, are able to engage BDCA-2 or ILT7 to evoke inhibitory signals that suppress IFN-I release. The pharmaceutical industry has long sought to develop Syk kinase inhibitors to prevent IgE-mediated mast cell degranulation, and in a stroke of serendipity, it now seems evident that this strategy may also remove the negative tonic on pDC, thus promoting the release of IFNs to induce antiviral immunity, and feasibly dampening aberrant Th2 responses.

In light of these experimental data, therapies aimed at decreasing FcεRI expression might also enhance antiviral immunity. Encouragingly, immunoneutralisation of circulating IgE with anti-IgE therapy (omalizumab) has been shown to decrease the expression of FcεRI on human pDC in severe asthma [84], although an important and unresolved question is whether this would increase IFN-I production in response to virus stimulation. It is also worth noting that subcutaneous allergen immunotherapy has been found to heighten IFN-I production by CpG-stimulated pDCs [85], although this was not associated with a fall in pDC FcεRI expression or serum IgE. Blockade of Th2 responses may also be beneficial since both IL-4 and IL-13 promote B cell class switching to IgE. Moreover, IL-4 promotes apoptosis of human pDC, down-regulates MHC class I expression [86], and both IL-4 and IL-13 can diminish CpG-induced IFN-I production. The molecular mechanism(s) by which activation of the IL-4 receptor-α attenuates TLR signalling remains to be determined. Collectively, these data suggest that Th2 immune responses dampen the effectiveness of pDC to produce anti-viral cytokines, and may help to explain the sizeable increase in asthma risk in those subjects who are both sensitised in early life and experience severe or frequent LRT infections [87].

Although pDC numbers appear to be greater in asthmatics in later life and lower in high risk infants in early life, more studies are required to substantiate these important findings. The available data support the notion that IFN-I production by pDC is impaired in subjects with atopic dermatitis, allergic rhinitis, and allergic and non-allergic asthma, irrespective of age. Despite recent evidence of heightened production of T-cell derived Th2 cytokines to allergen- or RV-stimulated PBMCs in the absence of pDC (or presence of an IFNAR antagonist)[88, 89], it remains an open question *in vivo* as to whether defective pDCs directly contribute to the development of Th2 responses or merely fail to constrain them.

Regulation of TLR7-mediated responses

Hyper- and hypo-IFN responses may underlie a number of pathologies including microbial infections, tumour development, autoimmune diseases, and chronic inflammatory diseases, including asthma, and as a consequence rapid advances have occurred with regard to our understanding of the molecular processes by which endosomal TLRs are regulated (reviewed elsewhere [90]). For example, it is now appreciated that TLR7 and TLR9 translocate from the endoplasmic reticulum to a specialised lysosome-like organelle prior to IFN-I synthesis. This process is in part orchestrated by the ER-associated molecule UNC93b [91], and involves a number of lysosome-related organelle trafficking and biogenesis proteins including adapter-related protein complex-3 (AP-3), Hermansky-Pudlak syndrome proteins BLOC-1 and BLOC-2 and the solute channel protein Slc15a4 [92, 93]. While evidence is emerging in systemic lupus erythematosus to suggest that defect(s) in the lysosomal machinery contributes to dysregulated IFN-I responses [94, 95], no data are yet available with regard to asthma, which is perhaps surprising in light of the substantial evidence showing that both TLR7 and TLR9 responses of pDC are blunted.

In an elegant study, activation of RIG-I-like receptor-activated IRF3 was found to interfere with TLR-induced transcription factor complexes, impairing gene expression of *IL-12b* (which encodes the p40 subunit) [96]. The authors suggest that this has important consequences with respect to poly-microbial infections, although it may operate during single pathogen exposures which can independently activate multiple PRRs. Of note, IRF3 is employed by several PRRs including MDA-5, NOD2, RIG-I and TLR3, all expressed by pDC and activated by viral RNA. We have recently observed that infection of IRF3-deficient mice with pneumonia virus of mice (PVM) led to a hyper IFN–I response (Phipps, unpublished findings), while NOD2 activation by the bacterial ligand muramyl dipeptide can suppress CpG stimulated IFN-I secretion by liver pDC [34], suggesting that IRF3 might also impair TLR7/TLR9/IRF7-mediated responses by pDC. It remains to be formally determined that cross-interference occurs in pDC, but it is tantalising to speculate that microbiota of the gut or the lung, or an existing pathogenic infection, may affect the functional responses of pDC to a respiratory viral infection. Finally, control of IRF7 gene expression via the translational repressors 4E-BP1 and 4E-BP2, can dramatically alter the magnitude of IFN-I production and consequently viral clearance [97]. It will be fascinating to learn whether modulation of these influential repressors contributes to the IFN-I defect in asthma.

Viral subversion of TLR7 responses.

TLR7 activation and induction of IFN-I expression can occur in the absence of viral replication [98, 99]. In addition to the classical delivery of material via the endocytic and micropinocytic pathways to endosomes, it is now recognised that cytosolic viral RNA intermediates can be delivered to endosome-sequestered TLRs via the process of autophagy

[100], which can be accelerated via HMGB1 ligation of RAGE [43, 101]. Thus, diminished cellular function that typically occurs following UV-inactivation of virus may not simply reflect the requirement for virus replication *per se*; rather inactivation may affect the delivery of the cargo, and hence the PRRs by which it is first recognised [102]. This may in part explain why some investigators have reported UV-inactivation of RSV to diminish IFN-I production by pDC [103, 104]. The ability of clinical isolates of RSV (and metapneumovirus) to attenuate TLR7/TLR9-induced IFN-I responses in pDC has been consistently shown in human and murine models [104-106]. The infection of pDC by RSV was implied when cell surface expression of the viral F protein was observed, which was later confirmed using GFP-labelled RSV; although the fraction of infected pDC was extremely low [103, 107].

Somewhat surprisingly, few studies have investigated pDC-Rhinovirus interactions irrespective of the context of asthma. Pritchard and colleagues demonstrated that RV16 induced IFN-I release is dramatically reduced when pDC are depleted from healthy PBMC cultures [88], inferring that pDC recognise RV. Similar to studies with RSV, TLR7-induced IFN-I production from PBMC or cord blood pDC is reduced by ~50% when cultured with RV strain type 1b prior to stimulation [63]. In light of the important role of respiratory viruses in the onset, progression and exacerbations of asthma, a priority for the future will be to unravel the mechanisms by which pDC responses can be subverted by RSV, RV, MPV (and other viruses) and to determine whether this process is enhanced in allergen sensitised or other at risk of asthma infants.

Evidence from mouse models that pDC contribute to host defense against respiratory virus infection

Recently generated pDC ‘knock-in’ mice harbouring the diphtheria toxin receptor (DTR) have allowed for the inducible depletion of pDC via the administration of diphtheria toxin [108, 109]. Using these novel transgenic mice, it was definitively shown that pDC provide the immediate source of IFN-I in the very early phase of infection to slow viral propagation and contribute to cytotoxic T lymphocyte responses [108, 109]. At present, these strains have not been employed in conjunction with a respiratory viral infection. However two studies have investigated the effect of antibody-mediated pDC depletion on the course of RSV infection, and both reported an attenuated early IFN-I response and increased viral burden in the absence of pDC [110, 111]. Of note, Th2 responses and the magnitude of immunopathology to primary human RSV infection are enhanced following infection of pDC-depleted or TLR7-deficient mice [110, 112]. These early studies may require verification since it is now apparent that the use of less than three antigens (e.g. reliance on CD11c and B220 alone) is insufficient to discriminate pDC using flow cytometry. Additionally, BST-2 (also known as PDCA-1) is up-regulated on various cell types in response to infection [113], potentially confounding some analyses or studies where the depleting antibody has been used for protracted periods of time. We have elected to use the rodent-specific pneumovirus (PVM), which propagates in mice, allowing low inoculums of 5 PFU to be used (in contrast to human RSV where typically between 10^5 and 10^7 PFU are administered). Importantly, the use of a physiologically relevant low dose of inoculum more likely ensures that the pertinent PRRs and cell types are activated in a spatio-temporal manner akin to a natural infection. This concept is supported by the observation that pDC-mediated control of viral load only occurs to low dose virus [108]. We have demonstrated that the immediate response to low dose PVM is TLR7-dependent, and that TLR7 on pDC mediates the early IFN response to control viral spread (Figure 2)[114]. In the absence of an early antiviral response, TLR7 deficiency leads to airway epithelial cell sloughing and denudation of the basement membrane. Moreover this was associated with increased expression of the tissue alarmin and Th2-instructive cytokine IL-33, the infiltration of type 2 innate lymphoid cells and elevated IL-13 production. Virus challenge at 7 weeks of age induced all of the hallmark features of asthma including AHR and increases in airway smooth muscle mass. Furthermore, sensitisation with the cockroach antigen during primary infection of TLR7-deficient mice markedly increased the magnitude of allergic airways inflammation (Phipps *et al*, *unpublished observations*). Our data suggest that TLR7, Pneumovirus infection and a clinically relevant allergen interact to establish an aberrant adaptive response that underlies virus-induced asthma exacerbations in later life.

pDC-derived paracrine support for structural cells

A central question that remains to be addressed is how the absence of pDC-derived paracrine signals (in particular IFN-I and III) impacts on the airway epithelium, the underlying mesenchyme and other resident cells within the airway wall. Pathological analyses of the airway wall reveal the airway epithelium of asthmatics to be highly disorganised, with evidence of sloughing and denudation of the basement membrane, mucus cell metaplasia, AEC hyper proliferation and an impaired ability to undergo re-epithelisation [5]. This phenotype may in part be mediated by a combination of genetic and environmental factors affecting epithelial barrier function [115], immunopatholgy caused by an aberrant host response or an inability to clear microbial infections. Although some investigators have shown the airway epithelium itself is unable to produce a robust IFN-I response [116, 117], this phenotype has not been reproduced by others [118, 119]. We speculate that it is the absence of a robust pDC response that primarily underlies the antiviral defect in asthma. The unique ability of pDCs to rapidly secrete large amounts of IFN-I suggests they orchestrate the early phase response, whereby the antiviral state is primed in non-specialised cells such as airway epithelial cells (AEC)(Figure 2). Thus, in the absence of paracrine signals produced by pDC, the AEC will be ill-equipped to (i) recognise the invading pathogen, (ii) recruit and signal its deletion by cytotoxic immune cells, and (iii) produce IFN-I to amplify the antiviral response in neighbouring cells.

Additionally, the attenuated IFN-I response may impede the repair process. Using a skin model of reepithelisation, pDC-derived IFN-I was recently found to be critical for the effective repair of the epidermis [120]. Thus, it is highly conceivable that defects in the pDC pool will affect not only susceptibility to infection but also the ability of the epithelial-mesenchymal trophic unit to repair itself. Additionally, changes to the epithelium might promote the development of Th2 immunity by modulating the underlying network of DC cells [121, 122]. For example, house dust mite-induced epithelial-derived IL-1α can activate an autocrine loop to induce the Th2-instructive cytokines IL-33 and GM-CSF, which license local DCs to promote Th2 lung inflammation [123]. Thus, damage to the epithelium caused by the proteolytic activity of allergens, excessive immunopathology, and/or respiratory viral infection-associated cytopathology induces the release of tissue alarmins - IL-1α, HMGB1, and IL-33 –which appear to promote the development of Th2 immunity (Figure 2). Teleologically, this response may have developed to initiate tissue repair, but following repeated environmental insult may lead to tissue remodelling [124-126]. We postulate that a defect in the pDC compartment may establish a pro-Th2 microenvironment as a consequence of increased tissue damage and release of alarmins by structural cells. In this paradigm, one can envisage how a viral infection and encounter with an allergen in a susceptible host (e.g. with a defect in the pDC compartment) may collude to establish allergic-specific Th2-type immunity.

pDCs and 'non-infectious' asthma

Mouse models have decisively demonstrated the requirement for conventional DC to elicit both allergic sensitisation and the effector/challenge phase [127]. However, in a seminal study, Lambrecht and colleagues demonstrated that pDC contribute to peripheral tolerance [16]. Whereas inhalation of the model allergen ovalbumin in the absence of an adjuvant leads to tolerance, prior depletion of pDC leads to the development of allergic sensitisation and pathologic features of asthma. Conversely, the adoptive transfer of OVA-pulsed pDC prior to a fully immunogenic asthma protocol suppresses the magnitude of the allergic response [16], an effect subsequently shown to be independent of IFN-I, and instead mediated via the co-inhibitory receptor programmed death-1 and its cognate ligand programmed death ligand 1 [128]. Intriguingly, immunoneutralisation of the cytokine osteopontin during i.p. OVA/alum sensitisation also attenuates allergic sensitisation through a mechanism involving pDC, apparently though a reduction of the regulatory capacity of pDC [17]. However, the molecular pathway that underpins this response requires further elucidation. While pDC are able to acquire antigen and traffic to the draining lymph nodes they appear less able to prime naive T cells to proliferate [16, 17]. This led De Heer and colleagues to hint that pDC may mediate their tolerogenic properties through the induction of Tregs, although this was not directly demonstrated [16]. However, a recent report showed specific subsets of pDC ($CD8\alpha^+\beta^+$ or $CD8\alpha^+\beta^-$ but not $CD8\alpha^-\beta^-$) are able to induce the differentiation of Tregs to abrogate inflammation and block AHR [129]. The tolerogenic pDC subsets expressed aldehyde dehydrogenase which in part catalyses retinol to retinoic acid, which together with TGF-β, supports the development of FoxP3+ CD4+ T regs. It is noteworthy that the adoptive transfer of the non-tolerogenic pDC subset (pulsed with OVA) is able to initiate allergic airways inflammation and AHR. It will be important to determine whether human equivalents to these subsets exist and whether they exhibit similar functions. Indeed, other pDC subsets have been proposed based on the expression of CCR9 [130], CX3CR1 [131], CD9 [132] or the balance of IFN-I:IFN-III production [133]. Further work is now needed to confirm their distinct functional repertoires and to relate these to normal and pathogenic process.

Conclusions

In summary, evidence from clinical and murine studies suggests that TLR7 deficiency or defects in the number of circulating pDCs is associated with the development of Th2-associated lung inflammation in response to virus infection. In adults, pDCs are twice as prevalent in the circulation of individuals with atopic dermatitis, allergic rhinitis, or asthma as compared to healthy controls. Moreover, these pDC are refractory to TLR ligand or virus stimulation, a phenotype that may relate to the activation of FcεR1and other surface receptors which activate Syk kinase. Targeting the pathways that establish a state of pDC hypo-responsiveness should now be a priority for the treatment of asthma since restoration of pDC function may help redress the defective antiviral immune response, reduce immunopathology and increase tolerance to harmless antigens.

Figure legends

Figure 1. Negative regulation of plasmacytoid dendritic cell antiviral function by inhaled allergens and virus-infected cells. Allergen-induced crosslinking of FcεRIα and/or engagement of ILT7 by BST2 on pDC activates the B-cell receptor (BCR)- like pathway via FcεRIγ which contains a transmembrane ITAM (immunoreceptor tyrosine-based activating motif) domain. FcεRIγ-ILT7 and –FcεRIα complexes drive the BCR- like signal transduction cascade which involves Lyn kinase, B-lymphoid tyrosine kinase (BLK) and spleen tyrosine kinase (Syk), and the adaptor proteins B-cell-specific adaptors BLNK (B-cell linker), and BCAP (B-cell adaptor protein). Activation of the BCR-like pathway inhibits type-I IFN (IFN-I) and cytokine production in response to DNA or RNA virus activation of the TLR7/9-MyD88 signaling cascade.

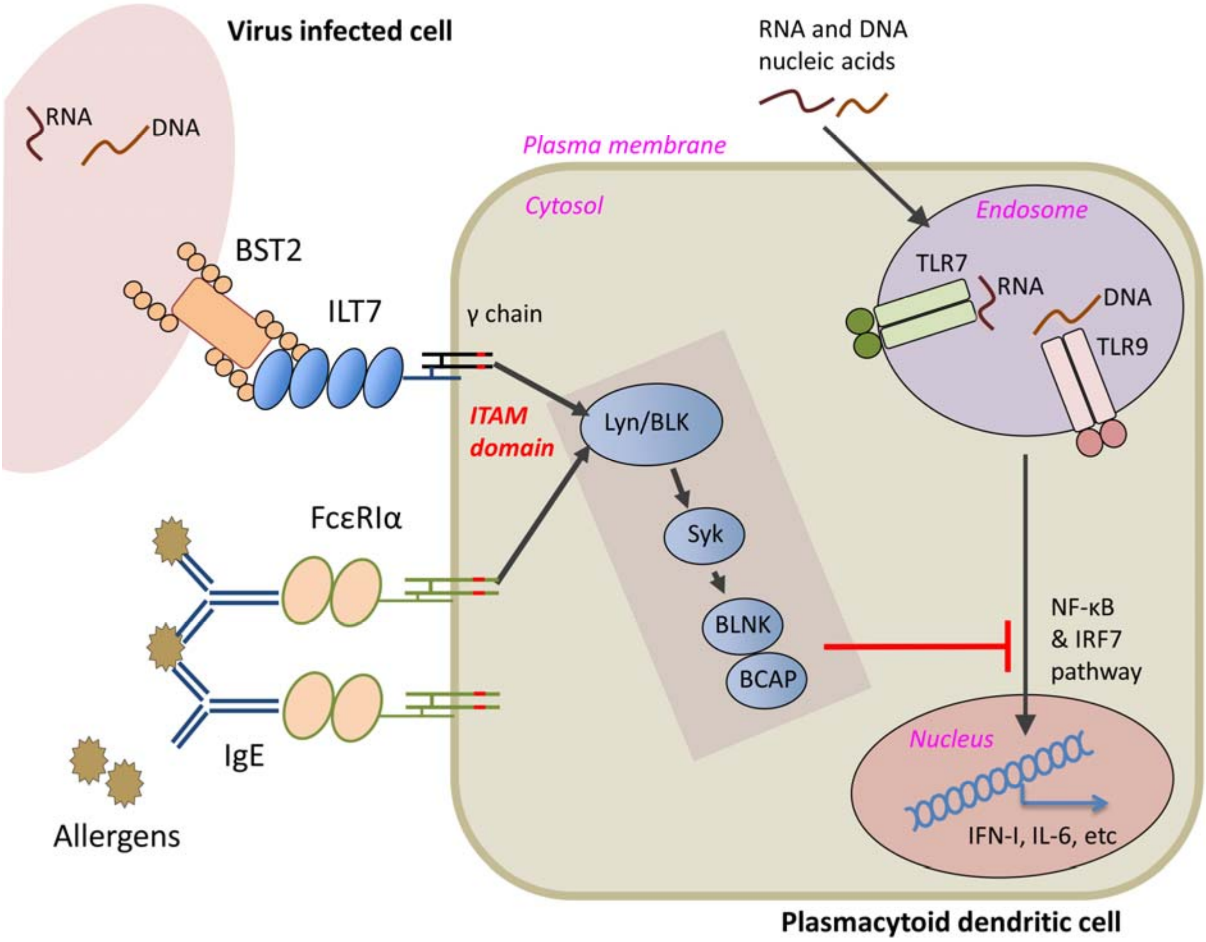
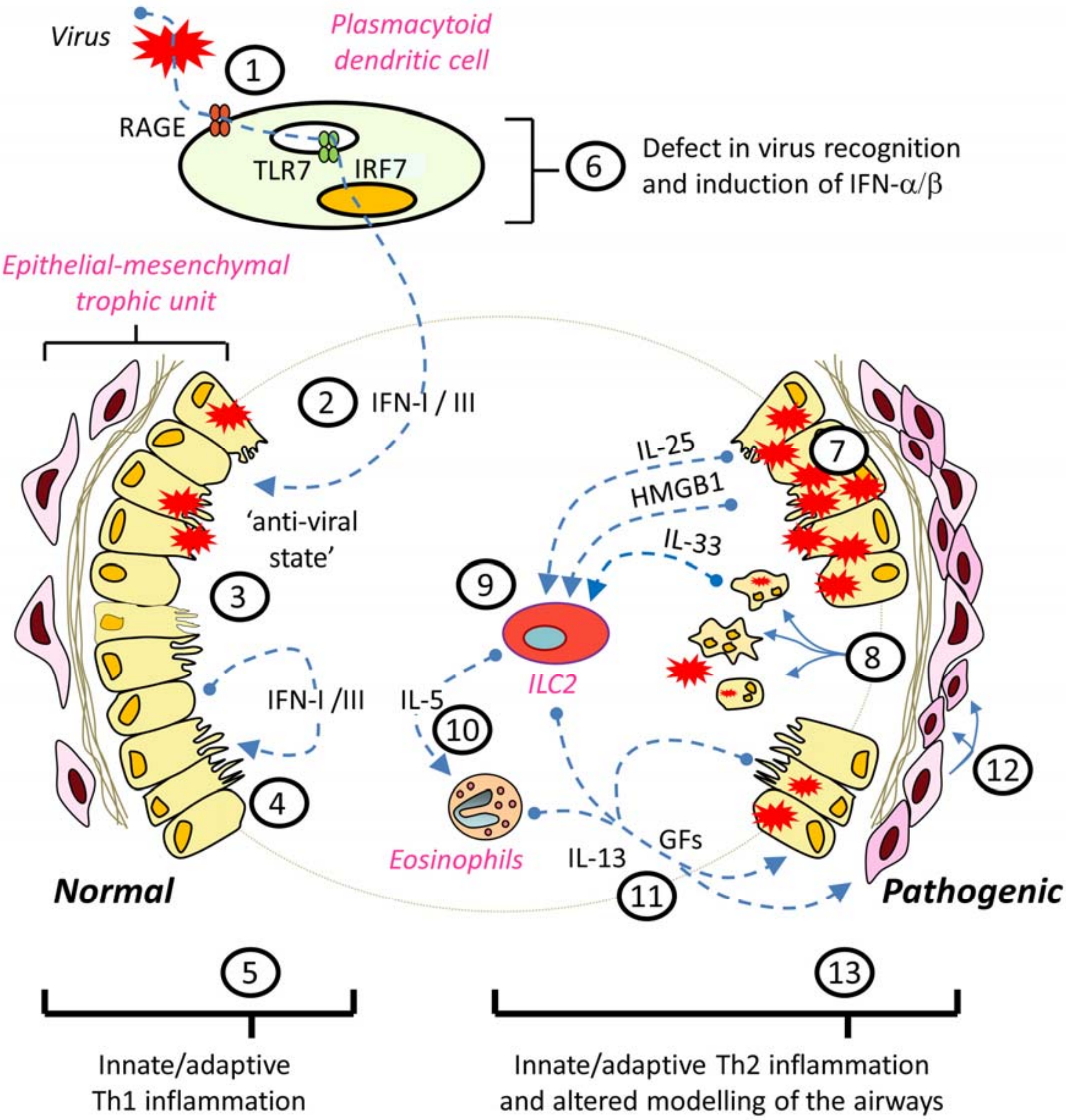


Figure 2. Impaired type I and type III IFN production consequent to a genetic/functional defect(s) in virus-sensing PRRs expressed by pDCs promotes a type-2 immune response and airway remodelling. In healthy individuals, pDC recognise an invading respiratory virus (e.g. RSV/RV) through a RAGE-TLR7-IRF7 axis (step 1), and rapidly produce vast amounts of type I and III IFNs (step 2). IFN secretion by pDC acts in a paracrine manner to establish the immediate phase of the antiviral state in non-specialised antiviral cells (step 3). The airway epithelium fortifies itself against the virus by various means, including the production of IFNs, which act locally to support neighbouring cells (step 4). An appropriate Th1 response is generated, the virus is cleared, and tissue homeostasis is restored (step 5). In contrast, a genetic/functional defect(s) in virus-sensing PRRs expressed by pDCs (step 6) fails to induce the antiviral state in airway epithelial cells,

increasing viral burden (step 7) and injuring the airway epithelium, which becomes necrotic leading to the formation of 'Creola bodies' in the lumen (step 8). The damaged epithelium releases alarmins and pro-Th2 instructive cytokines to promote the recruitment and expansion of type-2 innate lymphoid cells (step 9), which support eosinophil survival through the production of IL-5 (step 10). These Th2-type effector cells, together with the injured epithelium, promote wound repair through the secretion of various growth factors (step 11). In susceptible individuals, this cycle is repeated upon subsequent infections, leading to airway remodelling (step 12) and chronic Th2-type inflammation (step 13). GFs, growth factors; IFN-I/III, type I and type III interferon, IRF7, interferon regulatory factor 7; ILC2, type-2 innate lymphoid cell (or 'nuocyte'); HMGB1, high-mobility group box 1 protein; RAGE, receptor for advanced glycation endproducts; TLR7, toll-like receptor 7.



Virus
Plasmacytoid dendritic cell
1
RAGE
TLR7
IRF7
6
Defect in virus recognition and induction of IFN-α/β
Epithelial-mesenchymal trophic unit
2
IFN-I / III
'anti-viral state'
3
IFN-I /III
4
IL-25
HMGB1
IL-33
7
9
ILC2
8
IL-5
10
Eosinophils
IL-13
GFs
11
12
Normal
Pathogenic
5
Innate/adaptive Th1 inflammation
13
Innate/adaptive Th2 inflammation and altered modelling of the airways

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