The role of dendritic cells and regulatory T cells in the regulation of allergic asthma

Jennifer T. Burchell[a,c], Deborah H. Strickland[b,c], , Philip A. Stumbles[b,c,d]

[a] Division of Clinical Sciences, Telethon Institute for Child Health Research, Perth, Australia

[b] Division of Cell Biology, Telethon Institute for Child Health Research, Perth, Australia

[c] Centre for Child Health Research, University of Western Australia, Perth, Australia

[d] Faculty of Health Sciences, School of Veterinary and Biomedical Sciences, Murdoch University, Perth, Australia

Abstract

Airways hyperresponsiveness (AHR) is one of the major clinical features of allergic airways disease including allergic asthma, however the immunological mechanisms leading to the induction and regulation of this disorder are not fully understood. In this review we will summarise the evidence of a number of studies, principally in murine models of AHR, suggesting a central role for respiratory tract dendritic cells (RTDC) in the induction of AHR through the generation of lung-homing, allergen-specific effector T cells. We will also summarise the evidence supporting a role for regulatory T cells in the attenuation of AHR and will propose that, as a counterpoint to their capacity to induce AHR, RTDC may also play a role in the attenuation of AHR through the generation of regulatory T cells (T_{reg}). A better understanding of the relationship between the physiological and immunological responses to allergen-induced AHR attenuation, and particularly the role of RTDC and T_{reg} in this process, will be essential for the development of new treatments and therapies.

Abbreviations: AHR, airways hyperresponsiveness; AMDC, airway mucosal dendritic cells; EAAD, experimental allergic airways disease; DC, dendritic cell; DLN, draining lymph nodes; mDC, myeloid DC; OVA, ovalbumin; pDC, plasmacytoid DC, RTDC, respiratory tract dendritic cell; Th2, T helper type 2 cell; $T_{reg,}$ regulatory T cell

Keywords: Th2 cell; Regulatory T cell; Dendritic cell; Hyperresponsiveness; Asthma; Allergy

1. Introduction

Allergen exposure in atopic individuals can result in the generation of an aberrant Th2-skewed immune response to the eliciting allergen, often resulting in the development of the major clinical features of atopic asthma, the most prominent and clinically relevant of which is the development of airways hyperresponsiveness (AHR). The development of AHR is a process mediated by a complex series of pathways, however persistent airway inflammation is believed to play an important underlying role. Although a variety of cell types are believed to be involved in the inflammatory response, a number of human and animal studies have highlighted the central role for thymus-derived (T) cells in both the initiation and regulation of allergic airways inflammation. In particular, the interplay between pro-allergic T helper 2 (Th2) cell populations and counterbalancing T regulatory (T_{reg}) cells has recently emerged as an important concept in understanding how allergic airways inflammation may be initiated and regulated. Given that T helper cells require allergen to be processed and presented in order to mediate their effector functions, we believe that a greater knowledge of the cell types involved in allergen capture and processing in the respiratory tract, the most potent of which are dendritic cells (DC), will ultimately lead to new avenues for understanding and manipulating the balance between pro- and anti-inflammatory T cell subsets. In this review, we will focus on the experimental evidence, primarily from murine models, supporting a role for respiratory tract dendritic cells (RTDC) in controlling the expansion of Th2 and T_{reg} cells and explore a role for RTDC in regulating the balance between inflammation and tolerance in the airways.

2. Distribution and function of respiratory tract dendritic cells

In the respiratory tract, RTDC form a dense network within and underneath the submucosa where they are specialised to capture inhaled allergens and particles [reviewed in (Holt and Stumbles, 2000)]. A number of studies have shown that RTDC within or beneath the airway epithelial cell layer can extend their dendrites through the epithelial junctions, presumably to directly sample the luminal microenvironment (Vermaelen et al., 2001; Jahnsen et al., 2006; Blank et al., 2007; Wikstrom & Stumbles, 2007). We have shown that airway RTDC become activated and up-regulate their capacity to capture and process inhaled allergen prior to the onset of Th2-mediated airways inflammation in mice, suggesting that RTDC play an important early role in the activation and recruitment of Th2 cells to the airways (von Garnier et al., 2007). In addition to the airway epithelium, RTDC subsets are also present in the submucosa and lung parenchyma although subset distribution and turnover rates differ at these sites (Holt et al., 1994; von Garnier et al., 2005). We and others have shown that RTDC are capable of capturing inhaled allergens and rapidly transporting these to DLN for presentation to T cells and that the rate of this transport can be modified by microbial products (Vermaelen et al., 2001, 2003; Wikstrom et al., 2006). Furthermore, we have reported that under steady-state conditions, so-called ‘immature’ RTDC have the capacity to preferentially stimulate a Th2-skewed immune response and that this response can be modified by exposure to microbial products (Stumbles et al., 1998; Wikstrom et al., 2006). These studies have lead to the proposal that RTDC demonstrate a ‘default’ Th2 diverting capacity unless instructed otherwise by exposure to environmental stimuli that alter the phenotype and/or subset balance of RTDC entering the lung DLN.

Additional studies have provided further information on the variety of DC subsets located in the respiratory tract. Two main subsets of murine RTDC have been identified — myeloid DC (Lambrecht et al., 2000a,b) and plasmacytoid DC (de Heer et al., 2004), with further subsets evident dependant on anatomical location (von Garnier et al., 2005). Although not yet fully elucidated, RTDC subsets may have differing roles in generating adaptive immunity or tolerance (de Heer et al., 2004 and discussed further in following sections). Airway mucosal DC are thought to derive from myeloid precursors (Lambrecht et al., 2000a,b). After exposure to allergen they express high levels of MHC class II and

CD11c (Wikstrom & Stumbles, 2007) and are functionally immature as shown by low expression of costimulatory molecules CD40, CD80 and CD86 (Stumbles et al., 1998; Huh et al., 2003; de Heer et al., 2005; van Rijt et al., 2005). They are able to capture antigen rapidly in the main conducting airways (Lambrecht, 2001; Vermaelen et al., 2001; von Garnier et al., 2007). This DC subset has a high migratory capacity for trafficking antigen to the draining lymph nodes (Lambrecht, 2001; Vermaelen et al., 2001; von Garnier et al., 2007). CD11c+MHCIIhi DC in the respiratory mucosa are known to exhibit a high rate of turnover (approximate half-life of 12 h) as antigen-bearing DC continuously migrate to the DLN and are replaced by de novo DC from the bone marrow (Holt et al., 1994; Lambrecht et al., 1998; von Garnier et al., 2005). Although outside the scope of this review, DC migration into and from the airways is modulated by the expression of chemokine receptors (Stumbles et al., 2001; Jakubzick et al., 2006) and can be modified by microbial exposure (McWilliam et al., 1994, 1996; Stumbles et al., 2001). Once in the DLN, DC have been reported to stimulate naïve T cells (Lambrecht et al., 1998; Vermaelen et al., 2001).

3. Respiratory tract dendritic cell in airway inflammation and asthma

Mouse and rat models of experimental allergic airways disease (EAAD) have shown that dendritic cells are the most potent antigen-presenting cells in the respiratory tract (Holt et al., 1988; Vermaelen et al., 2001). DC are highly migratory cells and in the upper respiratory tract it is known that DC form a dense network in antigen-exposed tissues such as in the upper airway, bronchial mucosa, lung interstitium and pleura (Holt et al., 1985; Sertl et al., 1986; Holt & Schon-Hegrad, 1987) where they can continuously sample the inhaled microenvironment.

3.1. Dendritic cell–T cell interactions

It is widely accepted that T cell activation is driven by presentation of antigen to T cells by antigen-presenting cells (Holt et al., 1991; Krinzman et al., 1996; Lambrecht, 2001; Swirski et al., 2002; Henrickson et al., 2008). Activation is also dependant on a number of other factors, including antigen dose, the length of presentation of antigen and the strength of the DC–T cell interaction (Jenkins et al.,

2001). In a study by Henrickson et al. (2008) T cells rapidly formed tight stable contacts with DC presenting large numbers of antigen–MHC complexes on their surface, resulting in T cell activation. In contrast, DC presenting low numbers of antigen–MHC complexes formed slow, prolonged and serial interactions with T cells, which did not result in T cell activation until the cells accumulated signals via sufficient antigenic stimulation (Henrickson et al., 2008). These data suggest that antigenic dose can set a threshold for T cell activation. Furthermore, the microenvironment of the cell can play a role in T cell activation. Inhibitory or suppressive cytokines such as IL-10 and TGF-β released into the microenvironment have also been shown to inhibit T cell activation and proliferation via direct actions on T cells (Fox et al., 1993) or via down-regulating the antigen-presentation functions of DC (Moore et al., 2001). Similarly, Strickland and colleagues demonstrated that T cell activation resulting in a Th2 airway allergic response was dependant on bi-directional interactions between airway mucosal DC and memory T cells in the airways (Huh et al., 2003). These data suggested that DC and T cell interactions can occur locally within the airways in addition to within the airway draining lymph nodes.

3.2. Respiratory tract dendritic cell in the development of experimental allergic airways disease

The role of DC in the development of EAAD has been elegantly elucidated using in vivo DC depletion and adoptive transfer protocols in murine models. Using an OVA-driven murine model of the asthmatic response, van Rijt et al. (2005) demonstrated that depletion of $CD11c^+$ DC using diphtheria toxin (DT) in CD11c-DT receptor transgenic mice abolished airway eosinophilia, goblet cell hyperplasia and AHR. In support of the role of DC in the allergic asthmatic response, adoptive transfer of OVA-pulsed DC to naïve mice followed by a respiratory allergen challenge elicited sensitisation and elevated numbers of activated airway OVA-specific $CD4^+$ cells (Lambrecht et al., 2000a,b) . We have demonstrated an increase in the interactions between RTDC and T cells in the airways in rats, and in the activation and allergen-capture status of RTDC in mice, prior to the onset of EAAD and have demonstrated a role for circulating allergen-specific antibody in mediating T cell activation during this response (Huh et al., 2003; von Garnier et al., 2007). Collectively these data suggest that modulation of RTDC function is a key event in the development of EAAD.

3.3. A role for antibody?

Antigen-specific IgE plays a major role in contributing to bronchoconstriction in the early-phase response to allergen challenge in humans (Holgate et al., 1985). The main role of IgE is in immediate hypersensitivity. IgE binds to FceRI (high affinity receptor for IgE) or FceRII (CD23; low affinity receptor for IgE) receptors on a variety of cells including mast cells and basophils (Gilmartin et al., 2008; Kashyap et al., 2008). Cross-linking of Fc receptor-bound IgE results in mast cell or basophil degranulation. Mediators secreted by mast cell degradation have been reported to directly result in, or contribute to, bronchoconstriction as mentioned previously.

In contrast, the role of IgG in atopic asthma is less clear. An association between levels of IgG and IgE to antigens from grass pollen and *Dermatophagoides pteronyssinus* in 69 subjects with atopic dermatitis has been observed with a complete absence of IgG from non-atopic subjects (Chapman et al., 1983). In addition, IgG_4 levels have been associated with IgE levels in atopic subjects (Olsson et al., 2001; Johansson et al., 2004). In a study of atopic and non-atopic farmers exposed to dust mite, IgE and IgG_4 levels were significantly correlated in atopic subjects while IgG_4 was absent in non-atopic subjects despite chronic allergen exposure. Furthermore, IgG_1 was detected regardless of atopic status (Olsson et al., 2001). These data suggest that in humans, IgG_4 may be important in atopy while IgG_1 may be a ubiquitous bystander response. These studies are somewhat limited as humans are often sensitised to more than one allergen which makes it more difficult to determine the precise role of IgG in atopic asthma. The role of IgG in atopic asthma has been more extensively studied using murine models (Nakanishi et al., 1995; Renz et al., 1995; Blaser, 1996; Oshiba et al., 1996; Ravetch & Bolland, 2001; Crosby et al., 2002; Lange et al., 2002; Sehra et al., 2003; Matsui et al., 2004; Strait et al., 2006).

A question arising from these studies is the relative contribution of antibody isotype to allergen handling by DC and the contribution of this to the pathogenesis of allergic airways disease. DC have been shown to capture IgE bound to allergen through the high affinity IgE receptor FceRI on their surface and that mice deficient for the FcRI common γ-chain ($Fc\gamma RI^{-/-}$) showed reduced airways hyperresponsiveness and other inflammatory indicators despite producing comparable levels of IgE

and IgG to wild-type mice (Maurer et al., 1998; Kitamura et al., 2007). Similarly, expression of the high affinity IgG receptor FcγRI on APC populations was crucial during the sensitisation phase for the development of allergic airway inflammation (Mudde et al., 1995; Kitamura et al., 2007). Furthermore, we have recently shown that passive transfer of allergen-specific immunoglobulin can enhance allergen capture and process by RTDC, while others have shown that passive transfer of anti-OVA IgE and IgG1, but not IgG2a and IgG3, resulted in increased airways reactivity and BAL fluid eosinophilia following OVA challenge (Oshiba et al., 1996; von Garnier et al., 2007). Together, these data suggest that both IgG1 and IgE are important contributors to the pathogenesis of airway inflammation, with the potential for both to enhance allergen capture by RTDC and other DC populations and promote allergen-specific T-cell mediated inflammation.

4. Peripheral T cell regulation

Several mechanisms of peripheral tolerance to self and foreign antigens have been proposed including T cell deletion, anergy and more recently via active suppression mediated by T_{reg} populations (Sakaguchi et al., 2001). The role of DC in peripheral (mucosal) T cell tolerance is still under investigation.

While peripheral tolerance has been reported to develop via a number of different mechanisms, it generally results in some form of altered effector Th cell response. Again, the role of DC in T_{reg}conversion in the periphery remains unclear, however tolerogenic mechanisms that may involve DC include:

1.Immature DC that are specialised in capturing antigens but are considered to be relatively poor at processing and presenting these antigens to T cells (Wilson et al., 2004).

2.Immature or semi-mature DC expressing low levels of costimulatory molecules and T cell receptor ligands have been shown to initiate tolerance via incomplete T cell activation, anergy and even T cell deletion (Lutz & Schuler, 2002; Schwartz, 2003).

3.Mature DC are considered to be immunogenic, mainly because of their marked up-regulation of MHCII and costimulatory molecules, thus making them potent inducers of T cell immunity (Lambrecht et al., 2000a,b); van Rijt et al., 2004).

These studies suggest that different stimuli can result in varying states of DC maturation, leading to different T cell effector functions. In addition, molecules derived from bacterial or viral products, as well as pro-inflammatory cytokines (TNFα and IFNγ) and T cell signals such as CD40-ligand (CD40L), can promote the secretion of IL-12p70 and TNFα from DC thereby promoting a Th1-skewed response (Schulz et al., 2000; Vieira et al., 2000). In contrast, anti-inflammatory molecules such as IL-10, prostaglandin E_2 (PGE_2) and corticosteroids can inhibit RTDC maturation and cytokine production and therefore promote a Th2-skewed response (Kalinski et al., 1997; Faries et al., 2001; Kalinski et al., 2001). Therefore RTDC can potentially play a major role in the development of peripheral tolerance or immune suppression to allergen.

4.1. Regulatory T cells

The main role of T_{reg} cells is to control auto-reactive T cells (Chang et al., 2006). Naturally occurring T_{reg} cells were traditionally described to be thymically-derived with the function of maintaining systemic homeostasis to control total lymphocyte numbers in order to protect the integrity of tissues and organs in vivo (Sakaguchi et al., 2001). More recently, T_{reg} populations have been induced in vitro and in vivo via culturing with cytokines or in mouse models via pathogenic or allergenic stimulation (Verhasselt et al., 2004; Kretschmer et al., 2005; Luo et al., 2005; Skapenko et al., 2005; Moo-Young et al., 2006; Nicolson & Wraith, 2006; Strickland et al., 2006). The induced T_{reg} populations also have the ability to control other lymphocytes in vivo however they differ from naturally occurring T_{reg} in a number of ways which will be discussed below. Several subpopulations of $CD4^+$ T_{reg} have been described in the literature, and these are summarised in Table 1.

4.2. Markers of peripherally-induced regulatory T cells

T_{reg} cells are generally classified as $CD4^+$ and express high levels of the CD25 cell surface marker and can also express high levels of the transcription factor FoxP3.

4.2.1. CD25

CD25 is a cell surface marker that is part of the IL-2 receptor (IL-2R) subunit expressed on both activated effector and regulatory T cells (Li et al., 2007). As such both effector and regulatory T cells require IL-2 for growth and differentiation (Le Gros et al., 1990; Papiernik et al., 1998; Cote-Sierra et al., 2004; Fontenot et al., 2005; Yamane et al., 2005). Thus the presence or up-regulation of CD25 cannot unequivocally distinguish T_{reg} from activated effector T cells.

4.2.2. Forkhead box P3

The intracellular nuclear forkhead transcription factor FoxP3 has been suggested to be a unique marker expressed by various populations of T_{reg} including naturally occurring and induced $T_{reg}$and $CD25^{-}$ cells with regulatory activity (Fontenot et al., 2003; Hori et al., 2003; Khattri et al., 2003). Fontenot et al. (2003)recently demonstrated that the FoxP3 marker is required for the development of $CD4^{+}25^{+}$ cells. In addition, this group demonstrated that FoxP3-deficient scurfy mice develop a lethal autoimmune syndrome which results from a deficiency in $CD4^{+}25^{+}$ cells. Furthermore, transfer of $CD4^{+}25^{+}$ cells into neonatal FoxP3-deficient mice results in preferential expansion of transferred cells and rescues disease development. Ectopic expression of FoxP3 confers suppressor function on $CD4^{+}25^{-}$cells (Fontenot et al., 2003). These data suggest that FoxP3 is a critical regulator of $CD4^{+}25^{+}$ regulatory T cell development and function. The FoxP3 marker is known to be critical for the function of T_{reg} as loss of FoxP3 expression renders cells unable to perform their regulatory functions (Williams & Rudensky, 2007). Thus FoxP3 is the most unambiguous marker available to identify naturally occurring and induced T_{reg} populations. Therefore in mice, naturally occurring T_{reg} are often described as $CD4^{+}25^{hi}foxP3^{+}$ cells. One limitation of the use of FoxP3 in T_{reg}identification is that its intracellular location precludes its use as a direct tool for T_{reg} cell isolation.

4.2.3. $CD4^{+}25^{+}foxP3^{+}$ 'natural' T_{reg}

Naturally occurring $CD4^{+}25^{+}foxP3^{+}$ T_{reg} cells are thought to develop in the thymus (Jordan et al., 2001) but can also be induced to differentiate from $CD4^{+}$ naïve T cells in the periphery mediated by

IL-4 and IL-13 (Skapenko et al., 2005). They express the cell surface markers CD4 and high levels of CD25 and foxP3. $CD4^+$ T cells expressing the CD25 marker in the thymus of rats and mice represent 2–5% of the single positive CD4 T cell population which represents 0.1–0.2% of total thymocytes (Itoh et al., 1999; Stephens & Mason, 2000).

The main role of $CD4^+25^+foxP3^+$ T_{reg} appears to be involved in the maintenance of homeostatic mechanisms that control total lymphocyte numbers in the body as they have been shown to suppress T cell proliferation and to promote T cell anergy (Kim, 2006). Hadeiba and Locksley (2003) demonstrated that T_{reg} could suppress an allergic Th2 response in a mouse model of OVA/alum immunisation with no parallel suppression in AHR. However, these studies were carried out in transgenic mice with the generation of OVA-specific T cells and therefore cannot be directly compared to sensitisation and allergen exposure in naïve mice. More recently a number of studies have demonstrated a direct link between $T_{reg}$and suppression of AHR (Strickland et al., 2006; Joetham et al., 2007). The role of induced $CD4^+25^+foxP3^+$ T_{reg} in the regulation of the immune response in atopic asthma will be discussed later in this section.

4.2.4. Interleukin-10

This T_{reg} population is derived in culture and expresses the CD25 cell surface marker due to their activation in vitro in addition to producing IL-10 (Levings et al., 2002; Martin et al., 2003; Zhang et al., 2004; Hawrylowicz, 2005; Hawrylowicz & O'Garra, 2005; MacDonald et al., 2005; Nakagome et al., 2005; Maynard et al., 2007). The in vivo generation of anergy or tolerance to antigen administration can also induce the appearance of IL-10-producing T cells (Buer et al., 1998; Burkhart et al., 1999; Sundstedt et al., 2003). Sundstedt et al. (2003) have reported that IL-10-producing T_{reg} cells can be induced by repeated intranasal instillation of an antigenic peptide of MBP. These T_{reg} were able to inhibit the proliferation of naïve MBP-specific $CD4^+$ T cells both in vitro and in vivo. In support of this data, an early study by Groux et al. (1997) demonstrated that chronic activation of both human and mouse $CD4^+$ cells in the presence of IL-10 gave rise to a clone of antigen-specific $CD4^+$ cells with low proliferative capacity, producing high levels of IL-10, low levels of IL-2 and no IL-4. These antigen-specific T cell clones were able to suppress the proliferation of

$CD4^+$ cells in response to antigen and prevent the development of experimentally-induced colitis in severe combined immuno-deficient (SCID) mice. These data suggest that IL-10 can drive the development of a population of $CD4^+$ cells with the capacity to suppress the antigen-specific immune responses and can actively down-regulate a pathological immune response in vivo.

4.3. Mechanisms of action of regulatory T cells

While little is known of the mechanism(s) of action of T_{reg}-mediated inhibition of T cells several of the proposed mechanisms are considered below:

4.3.1. Surface cytotoxic T lymphocyte antigen-4 expression

T_{reg} cells have been reported to suppress naïve T cells in a contact-dependant manner in vitro (Nakamura et al., 2001; Dieckmann et al., 2002; Gondek et al., 2005). One reported mechanism of cell contact-dependant suppression occurs via binding of cell surface molecules such as cytotoxic T lymphocyte antigen-4 (CTLA-4) on T_{reg} to CD80 and CD86 on the surface of effector T cells, suggesting that T_{reg} transmit a suppressive signal to effectors cells (Takahashi et al., 2000; Paust et al., 2004).

4.3.2. Interleukin-2 sequestration

IL-2 is central to Th2 and T_{reg} cell differentiation and proliferation (Cote-Sierra et al., 2004; de la Rosa et al., 2004). As receptors for IL-2 subunits (e.g. CD25) can be found on both effector and regulatory T cells, it has been postulated that these two cell types may compete for endogenous IL-2. T_{reg} cells express higher levels of CD25 compared with effector cells, suggesting that T_{reg} may competitively consume IL-2 resulting is less IL-2 available for growth and differentiation of effector T cells (de la Rosa et al., 2004).

4.3.3. Interleukin-10 production

IL-10 is produced in relatively high concentrations from IL-10-producing T_{reg}. However, $CD4^+$ cells need to be stimulated with relatively high concentrations of IL-10 in vitro for the generation of this subpopulation. A recent study by Joetham et al. (2007) reported that T_{reg}-mediated suppression of the

allergic response in mice is dependant on IL-10-induced production of TGFβ. These data suggest that while IL-10 can promote the secretion of TGFβ it is the actions of TGFβ which can inhibit an allergic airways response.

4.3.4. Surface/secreted transforming growth factor beta

The transforming growth factor (TGF) subfamily encompasses ~ 30 members in mammals (Camoretti-Mercado & Solway, 2005). In the healthy lung the role of TGFβ involves maintaining homeostasis and homeodynamics. In asthmatic conditions, TGFβ can be produced by resident and inflammatory cell types including epithelial cells (Camoretti-Mercado & Solway, 2005; Alcorn et al., 2007). In addition TGFβ has been shown to be produced by naturally occurring and allergen-induced T_{reg} in vitro (Haneda et al., 1999) suggesting a role for TGFβ in immune suppression.

The precise role of TGFβ in the regulation of the immune response remains unclear. In a study by Nakamura et al. (2001), naturally occurring T_{reg} were shown to produce high levels of TGFβ and IL-10 when stimulated in vitro. In addition, the presence of T_{reg} cells suppressed proliferation of $CD4^{+}25^{-}$ cells, however this suppression was abolished with administration of anti-TGFβ antibody. Furthermore, this group found no evidence of a soluble factor which could mediate suppression in vitro (Nakamura et al., 2001). These data suggest that naturally occurring T_{reg} exert immuno-suppression via cell–cell interaction involving cell surface TGFβ.

In support of these results a number of groups have reported that overexpression of TGFβ (Fu et al., 2006) or administration of $CD4^{+}$ cells engineered to produce latent TGFβ in vivo (Hansen et al., 2000) could attenuate AHR and inflammation in recipient allergen-challenged mice. Collectively these studies support the role of TGFβ in AHR attenuation. These studies were limited in a number of ways including a lack of a mechanistic data of TGFβ-mediated immune suppression and the use of an insensitive technique (BP) to measure lung function in vivo.

The precise mechanism of action of TGFβ in modulating the immune response to allergen is poorly defined. In a study by Heath et al. (2000), $TGF\beta_1$ was shown to down-modulate Th2 development by down-regulating expression of the Th2-specific transcription factor GATA-3. This process also

resulted in impaired IL-4-induced STAT6 activation. These data suggest that TGFb$_1$can down-regulate Th2 development via inhibition of critical aspects of the intracellular signalling pathway to prevent the production of Th2 cytokines.

TGFβ has also been shown to inhibit dendritic cell function via preventing DC maturation (Fogel-Petrovic et al., 2007) and inhibiting MHC classes I and II expression (Geiser et al., 1993). Mice deficient in TGFβ (TGFβ — null) demonstrated increased expression of both MCH I and II on a wide variety of cell types in comparison to wild-type control mice. This may suggest that TGFβ can regulate the immune response via inhibition of antigen presentation to T cells.

A recent study (Marie et al., 2005) demonstrated that TGFβ is required for the maintenance of FoxP3 expression of T_{reg} cells however TGFβ is not required for their thymic development. These data suggest that TGFβ could act in an autocrine or paracrine manner to maintain the suppressive function of T_{reg} cells.

5. T cell mediated regulation of allergic asthma

5.1. Regulatory T cells in allergic asthma

An increasing number of studies have identified and proposed a role for T_{reg} in mediating the suppression of allergic airways disease (Stock et al., 2006; Taylor et al., 2006; Tournoy et al., 2006; Hartl et al., 2007; Joetham et al., 2007; Kocks et al., 2007; Larche, 2007; Mantel et al., 2007; Maynard et al., 2007; Niu et al., 2007; Xystrakis et al., 2007; Holt et al., 2008). The proposed mechanisms of T_{reg}-mediated suppression in atopic asthma are thought to involve the immunomodulatory cytokines IL-10 and TGFβ (Taylor et al., 2006) and are summarised in Fig. 1.

5.1.1. Regulatory T cells and airways hyperresponsiveness attenuation

An induced population of T_{reg} cells ($CD4^+25^{high}$ $foxP3^+$) has been reported to inhibit the development of AHR in animal models of allergic airways disease (Kearley et al., 2005; Strickland et al., 2006; Joetham et al., 2007). Kearley et al. (2005) demonstrated that adoptively transferred $CD4^+25^+$ cells

could reduce AHR in mice. However, a limitation of this study was the use of BP (baromatric plethysmography) which is not a measure of airway resistance. In support of the role of T_{reg} in AHR suppression, Joetham et al. (2007) reported that intra-tracheal but not intravenous transfer of naïve $CD4^{+}25^{+}$ cells could suppress AHR which was dependant on IL-10 and TGFβ. This group used a somewhat more robust method for determining AHR (airway pressure time index) however this technique does not enable the separate analysis of reactivity in the main conducting and peripheral airways. This is important as it is known that in allergic airways diseases such as asthma, altered lung function is predominantly focussed in the main conducting airways and to a lesser extent the lung periphery.

Strickland et al. (2006) utilised a unique rat model of allergic airways disease to show that multiple airway allergen challenges induced a population of $CD4^{+}25^{+}$ regulatory cells in the airways. Adoptive transfer of the $CD4^{+}25^{+}$ cells could suppress Th-mediated up-regulation of airway DC function and AHR in sensitised and airway allergen-challenged recipient rats (Strickland et al., 2006). A major strength of this study was the use of a sensitive and highly accurate technique for determination of airway mechanics separately in the airways and tissue parenchyma (LFOT). However there is a need to validate T_{reg}induced AHR suppression using accurate and highly sensitive measurements of airway mechanics in a mouse model of allergic airways disease.

Our laboratory recently developed a model of allergen-induced attenuation of airway hyperresponsiveness in the mouse (Burchell et al., 2009). We reported a significant increase in T_{reg}($CD4^{+}25^{+}foxP3^{+}$) cell numbers in the main conducting airways which was associated with suppression of AHR in both the airways and parenchymal tissue compartments of the respiratory system using the highly sensitive and accurate forced-oscillation technique to measure AHR as described above. AHR attenuation was also associated with a significant reduction in Th2 cytokines (IL-4, IL-5 and IL-13) in the airways. In addition, adoptive transfer of T_{reg} taken from mice exhibiting attenuated AHR and given to mice exhibiting AHR effectively inhibited AHR in the main conducting airways and parenchymal tissue components of the recipients. Furthermore, partial systemic depletion of T_{reg} using an antibody in AHR attenuated mice restored AHR in the main conducting airways. As

TGFβ1 was elevated concomitant to T_{reg}cell numbers in the airways, we suggested that the attenuation of AHR was mediated by either an autocrine or paracrine induction of TGFβ by T_{reg} in the airways.

5.2. How can regulatory T cells be induced to inhaled allergens?

Despite the current uncertainty of the exact mechanisms mediating T_{reg} generation and function in the periphery, there is a growing body of evidence that T_{reg} can be generated de novo in the periphery from naïve Th cells and that these have the potential to act in an antigen-specific manner (Chen et al., 2003; Kretschmer et al., 2006). In this section we will describe some of the potential mechanisms that may be involved in antigen-specific T_{reg} generation in the periphery and propose a role for RTDC in this process.

5.2.1. Antigen context and dose

Presentation of inhaled antigen to naïve Th cells occurs in the draining lymph nodes (DLN) of the airways and lungs (Lambrecht et al., 2000a,b; Wikstrom et al., 2006) and this is the site where RTDC–T cell interactions are critical in determining the fate of allergen-activated Th cells. We have previously demonstrated that the main RTDC subset involved in antigen presentation in airway DLN in mice are $CD8\alpha^{low}$ and that the outcome of the T cell response induced by this subset of RTDC in the DLN can be modified by exposure to microbial products at mucosal surfaces (Wikstrom et al., 2006). For example, a strong pro-inflammatory Th1 response will develop if mice are exposed to inhaled bacterial endotoxin at the time of allergen exposure, while cholera toxin promotes Th2-mediated allergen-specific responses and this is likely to be due to signals provided by RTDC at the time of allergen presentation to T cells. Thus, allergen context is an important component in determining the fate of the effector T cell response to inhaled allergen. Relatively less well understood, however, is the role of antigen context in the peripheral selection of T_{reg} cells. One possibility is that certain microbial products (e.g. those derived from *Mycobacterium vaccae*) may instruct the development of allergen-specific T_{reg} that can control airway inflammation and this offer exciting opportunities for new drug development (Zuany-Amorim et al., 2002). Alternatively, a lack

of microbial signalling may be important in order to maintain allergen in an “innocuous” context in order to induce T_{reg}-mediated tolerance, such as has been described for self and tumour antigens [reviewed in (Kretschmer et al., 2006)]. This appears to be a particular feature of allergen captured across the mucosal surfaces of the airways and although the mechanisms remain unclear, this form of tolerance is likely to involve a combination of signals from DC and other cells, particularly cytokine signals such as IL-2, IL-10 and TGF-β (Akbari et al., 2001; Knoechel et al., 2005; Kretschmer et al., 2005) and cell–cell contact signals such as those provided by the ICOS–ICOS ligand pathway (Akbari et al., 2002).

In addition to, but not exclusive from, the notion that antigen context is important for T_{reg} selection is also the fundamental role that antigen dose plays in the process of effector and regulator T cell selection. It has been known for decades that low-dose antigen delivery across airway mucosal surfaces can induce antigen-specific immune tolerance mediated by a suppressive population of T cells (Sedgwick & Holt, 1983, 1985). More recently, low-dose antigen delivery has been shown to convert mature effector T cells into $CD4^+CD25^+$ T_{reg} cells that can control subsequent immune responses to antigen challenge (Apostolou & von Boehmer, 2004). Furthermore, the strength of the MHC-peptide interaction presented on the surface of DC affects the nature of the T cell response and that peptide agonist ligands can mediate T_{reg} conversion through a mechanism of low-dose antigen combined with sub-optimal DC activation (Kretschmer et al., 2005; Skokos et al., 2007). We have recently identified a decline in the capacity of RTDC to present inhaled allergen to Th cells during the course of AHR attenuation induced by chronically inhaled low levels of allergen (Burchell, Wikstrom, Turner, Sly, Stumbles, manuscript in preparation), and postulate that this process is, at least in part, mediated by $CD4^+$ $CD25^+$ $FoxP3^+$ T_{reg}cells (Fig. 2).

While we have yet to prove a direct role for RTDC in the selection of T_{reg} following chronic allergen aerosol, we would propose the hypothesis that declining levels of allergen capture, processing and presentation by RTDC would result in a situation that mimics low-dose antigen exposure, resulting in sub-optimal T cell activation and T_{reg} conversion (Fig. 3).

5.2.2. Role for respiratory tract dendritic cell in regulatory T cell induction?

While a clear role for RTDC has been shown in the early stages of the initiation of airways inflammation and AHR, could a complimentary role exist for these cells in the selection of allergen-specific T_{reg} in the periphery? In this context, an evolving role for RTDC may be to promote T_{reg} cells capable of directly and actively suppressing the immune response to antigen once the antigen has been cleared (Jonuleit et al., 2001; Weiner, 2001; Martin et al., 2003; Verhasselt et al., 2004; Chen, 2006; Tarbell et al., 2006; Cools et al., 2007; Fujita et al., 2008). Akbari et al. (2001) demonstrated that respiratory exposure to antigen (OVA) could induce phenotypically mature pulmonary DC that transiently produced IL-10. These DC could stimulate development of $CD4^{+}$T regulatory 1-like cells (Tr1) that also produced high levels of IL-10. In addition, adoptive transfer of pulmonary DC from IL-$10^{+/+}$but not IL-$10^{-/-}$ mice also exposed to respiratory OVA, induced antigen-specific T cell unresponsiveness in recipient mice (Akbari et al., 2001). It is believed that under steady-state conditions, harmless or self antigens are presented to DC resulting in incomplete DC maturation which can further result in an abortive T cell proliferative response or the generation of T_{reg} cells (Akbari et al., 2001; Brimnes et al., 2003; Ostroukhova et al., 2004). Further support of the role of RTDC in regulating atopic asthma, Koya et al. (2007) demonstrated that adoptive transfer of IL-10-treated DC could attenuate AHR. A limitation to this study was the use of measures of total lung resistance that does not enable determination of site of disease in the respiratory system. These data suggest that IL-10 production by DC is critical for the induction of T cell unresponsiveness and that phenotypically mature DC can mediate unresponsiveness induced by respiratory exposure to antigen.

Several studies have also suggested that different RTDC subsets can induce either immunity or tolerance. Myeloid DC (mDC) have been shown to promote immunity whereas plasmacytoid DC (pDC) have been shown to promote tolerance (de Heer et al., 2004; Oriss et al., 2005). In further support of this notion, depletion of pDCs from mice using antibodies resulted in a break in inhalation tolerance to OVA resulting in the development of asthmatic inflammation (de Heer et al., 2004). However the current knowledge of the role of DC subsets in T cell activation in general, and T_{reg} induction in particular, remains unclear. Recently, a population of $CD103^{+}$ DC has been shown to

be important in the T_{reg}-mediated suppression of colitis in mice, as T_{regs} transferred into $CD103^{-/-}$ mice were unable to control the disease (Annacker et al., 2005). In the mouse airways we have identified two populations of RTDC that are $CD103^{+}$ $CD11b^{-}$ and $CD103^{-}$ $CD11b^{+}$ with the former subset more efficient at allergen capture in the airways but both capable of allergen traffic to the DLN (Wikstrom & Stumbles, 2007). Thus, we would postulate that RTDC subsets may also contribute to the selection of allergen-specific T_{reg} in the periphery following chronic exposure to inhaled allergen. In this regard, we would hypothesise that antigen dose and context are of major importance in determining the functionality of RTDC subsets and the ultimate direction that the T cell response will take (Fig. 3).

6. Conclusions

RTDC and T cells have been shown to be important mediators of the induction of allergic airways inflammation and AHR mouse and rat models of this disease. A role is now emerging for T_{reg} in controlling this process, with several mouse and rat studies of EAAD demonstrating the capacity for T_{reg}-mediated immune suppression in vivo via either contact-dependant suppression or the secretion of cytokines such as IL-10 and TGF-β. Although a greater understanding of the peripheral generation of T_{reg} to foreign antigens such as allergens is required, growing evidence supports the concept that dendritic cells can play a role in the peripheral selection of T_{reg} from naïve or mature T cells. We and others have proposed that RTDC are likely to be critical in the generation of T_{reg} cells in the periphery to inhaled allergens, however a greater understanding of the basic nature of T_{reg}selection and the role of antigen dose, context and RTDC subsets in this process is required in order to harness the potential of these cell types for therapeutic purposes.

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Table 1. Regulatory $CD4^+$ T cell populations.

Regulatory cell type	Major cytokines produced	Comments	Refs.
TH3	TGFβ	Develop during oral tolerance induction. Can inhibit autoimmune disease.	Weiner, 2001
Tr1	IL-10	Develop during culture with IL-10 and produce IL-10. Can inhibit experimental colitis.	Groux et al., 1997
$CD25^+FoxP3^+$nTreg	IL-10, TGFβ	Naturally occurring regulatory cells that develop in the thymus. Can inhibit autoimmune disease. Have been shown to inhibit Th2 responses but not AHR.	Saoudi et al., 1996; Curotto de Lafaille and Lafaille, 2009
$CD25^+FoxP3^+$iTreg	IL-10, TGFβ	Induced or adaptive T_{reg} that develop in the periphery under tolerogenic conditions from $CD4^+$ $CD25^-$precursors. Can inhibit Th2 responses and AHR.	Burchell et al., 2009; Curotto de Lafaille and Lafaille, 2009

Adapted from Umetsu et al. (2003).

Fig. 1. Mechanisms of suppression of Th2-mediated features of allergic airways disease through IL-10 and TGF-β production by T_{reg} cells. T_{reg}can potentially modify many of the pathways of allergic airways inflammation mediated by Th2 cells, including IL-4 and IL-13 mediated antibody production by B cells, Th2 cell homing to airways, IL-9 and IL-13 mediated goblet cell hyperplasia and mucous production and cytokine-mediated recruitment of inflammatory cells including mast cells, basophils (baso) and eosinophils (eos). (red lines and crosses = suppression; black lines = stimulation). Adapted from Taylor et al. (2006).

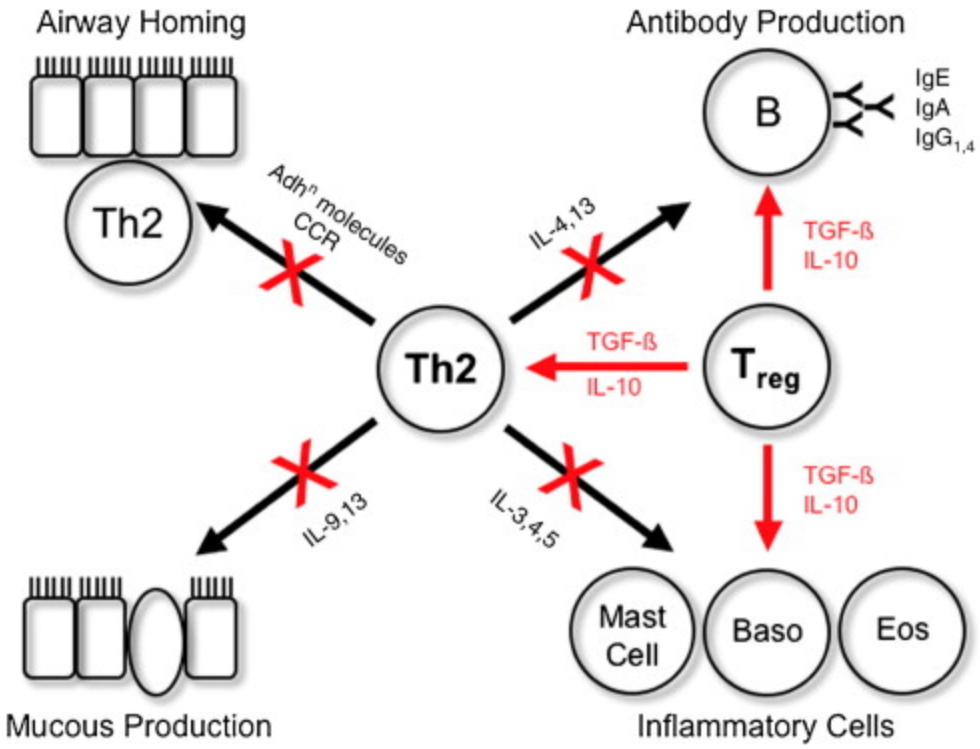
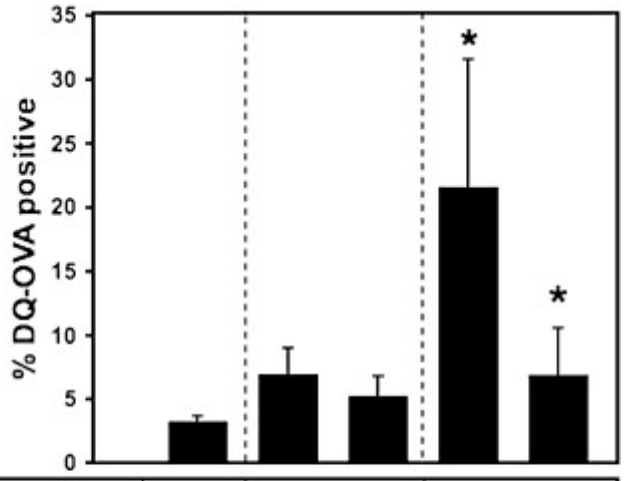


Fig. 2. Antigen processing by airway RTDC is attenuated following multiple aeroallergen challenge. BALB/c mice were systemically sensitised and boosted to ovalbumin (OVA) or phosphate buffered saline (PBS) in aluminium hydroxide adjuvant and then challenged by aerosol with OVA or saline as a control either singly or three times on consecutive days. In order to track allergen processing by airway RTDC, DQ-OVA delivered intranasally was used in place of the final aerosol in the case of 3 challenges or as allergen in case of 1 challenge. Airway RTDC were then isolated and analysed by flow cytometry for the percentage of cells that were DQ-OVA fluorescence-positive: DQ-OVA only fluoresces when processed intracellularly and a positive signal indicates active antigen processing (Data courtesy of C. von Garnier).



Sensitisation	OVA	PBS		OVA	
Challenge	Saline	DQ-OVA		DQ-OVA	
# of challenges	3	1	3	1	3

Fig. 3. Theoretical model for the role of airway mucosal dendritic cells (AMDC) in the initiation and regulation of allergic airways inflammation. During the sensitisation phase, aeroallergen exposure in genetically susceptible individuals leads to the systemic production of allergen-specific antibodies, most notably IgE and IgG. These antibodies are known to sensitise mast cells for histamine and prostaglandin (PG) production and in this model we propose a similar role for sensitisation of AMDC by IgE and IgG, this time for enhanced allergen capture. During the challenge phase, sensitised AMDC readily capture and process inhaled allergens and traffic to the draining lymph nodes (DLN) where they preferentially activate airway-homing Th2 cells. During sensitisation, airway epithelium will also become activated, leading to production of a number of modifying factors such as the cytokine TSLP, which can bias dendritic cells towards Th2 cell stimulation (Zhou et al., 2005). As aeroallergen challenge continues, influxing AMDC become attenuated in terms of allergen capture perhaps in part due to declining IgE and/or IgG production so that they are now relatively poor antigen processors. This reduction in available antigen presentation in DLN, along with the changing mucosal microenvironment occurring during chronic inflammation (along with potential changes in antigen distribution amongst AMDC subsets), will alter AMDC function in the DLN towards that of T_{reg} induction. These T_{reg} may recirculate back to the airways to continue modifying AMDC function, or alternatively T_{reg} may also be converted from effector/memory cells in the airways.