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Immune dysregulation in allergic respiratory disease: the role of T regulatory cells

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Abstract

Although earlier research focused on the role of the polarity of T helper cell signalling as the defining factor in immune responses, it is now recognised that other cells with regulatory properties have a more key role. It has been recently proposed that allergic disease may result from an inappropriate balance between regulatory cells (including but not limited to CD4+ CD25+ T regulatory cells) and T helper type 2 (Th2) effector cells. In the airways, a number of other cells also have important regulatory effects on local immune responses, including epithelial cells and airway dendritic cells (DC). Allergic respiratory disease appears to be the culmination of both local epithelial dysfunction and generalised immune dysregulation resulting in Th2 propensity (atopic predisposition). Although these processes are related they also appear to occur independently. This review examines evolving models of allergy pathogenesis, including the newly recognised role of diverse groups of regulatory cells. Increasing rates of allergic disease (and other immune diseases) suggest that environmental changes may be having fundamental effects on common regulatory pathways. Understanding these influences and their mechanism of action could lead to strategies to prevent disease.

Keywords: Immune dysregulation; Atopy; Allergic disease; T regulatory cells; Th1/Th2 cytokines; IL-10, TGFβ

1. Allergic disease and the epidemic of immune dysregulation

Recently reaching epidemic proportions, allergic diseases are now among the most common chronic debilitating conditions affecting industrialised societies. These heterogeneous inflammatory conditions are associated with both a systemic propensity for allergic immune responses (atopy) and local manifestations of allergic inflammation, typically at cutaneous and mucosal surfaces in contact with the environment. Although these parallel processes are clearly linked, they appear to be controlled through independent mechanisms [1]. The local immune events which lead to disease associated inflammation, such as that seen in the respiratory mucosa in asthma and allergic rhinitis, are still poorly understood. As knowledge of underlying immunological processes has evolved, so have working models of pathogenesis and aetiology.

1.1. Humoral dysregulation:

Allergic disease was initially recognised as a disorder of humoral immune responses to environmental allergens. Inappropriate production of IgE antibodies (discovered in the 1960s [2,3]) was associated with the atopic state. In the following decade allergic disease was largely regarded as an 'excessive' inappropriate reactions to the environment, and the search for causal pathways focused largely on the role of allergen exposure. IgE antibodies are associated with many end-organ manifestations of disease and remain a principal diagnostic tool.

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1.2. T cell dysregulation and the emergence of the "hygienehypothesis"

In the 1980s the role of T cell signalling in regulating antibody production was recognised with the discovery of dichotomous T helper (Th) cell subsets in rodents [4]. Although less distinct in humans [5], the increased propensity for Type 2 helper T cell (Th2) responses in allergic individuals is well established. There is also accumulating evidence that most Th2 cytokines ([interleukin] IL-4, IL-5, IL-9, IL-13) are implicated in the expression and development of airways inflammation and hyperactivity (AHR) [6–8]. Reciprocal inhibition between interferon γ(IFNγ) producing Th1 cells and IgE promoting 'pro-allergic' Th2 lead to the proposal that allergic responses were the result of a 'skewing' of T cell responses. Accordingly, efforts to identify environmental causes of the rise in allergic diseases focused on candidate factors with potential 'pro-Th2' or 'anti-Th1' properties.

During the following 10 years there was growing recognition that allergens may not cause allergy, and the "hygiene hypothesis" gathered momentum. While the original hypothesis was based on epidemiological associations [9], the well recognised 'pro-Th1' effects of bacteria provided a biological basis for the proposal that a reduction in 'microbial burden' may be implicated in the dramatic rise in allergic disease. It also became increasingly apparent that these effects were likely to be most relevant in early life when immune response are first initiated. Th1 responses normally mature gradually during the first years of life, not consolidating until after 18 months of age [10], so that responses during this early period are relatively skewed towards the Th2 pattern which normally characterised allergic disease [11,12]. However, despite this, the majority of infants do not go on to develop atopy. There has been longstanding speculation that delayed development of Th1 function in infancy is an important contributing factor in the development of Th2 immune disease [13]. Bacterial exposure during this period arguably provides the strongest signal for maturation of Th1 immune function (which is logically involved in defence against these same organisms). A reduction in the level and variety of early microbial burden is an obvious candidate in the search for culprits in the spiralling levels of allergic disease. Thus the 'hygiene hypothesis' could readily be explained within the Th1/Th2 paradigm, and there is still a body of evidence to support this (as recently reviewed by Romagnani [14]).

However, the advent of molecular technology failed to confirm a primary defect in either the IgE or T cell pathways in allergic or asthmatic individuals. Although numerous polymorphisms have been associated with these immune pathways, susceptibility appears to be determined by multiple genes, and different genes appear to feature in different populations. Of interest, however, is the recent association between serum IgE levels and polymorphisms in CD14 pathway, which is involved in the recognition of bacterial 'pathogen associated molecular patterns' (PAMPs) [15]. Although still speculative, it is possible that functional polymorphisms in these pathways confer altered susceptibility to Th2 allergy responses secondary to altered sensitivity to Th1-inducing bacterial products such as lipopolysaccharide (LPS) [16]. More recently, other genes involved in PAMP recognition (such as Toll like receptor [TLR]2 have also been associated with asthma [17] and TLR4 polymorphisms have been associated with endotoxin hyporesponsiveness [18].

Collectively, these observations provided powerful support for the 'hygiene hypothesis' and a highly plausible mechanism although there is still no definitive proof of this.

1.3. Antigen presenting cell (APC) dysregulation

As the key cells involved in programming T-cell responses, antigen presenting cells (APC) became of central interest in explaining the polarisation of T cell responses in allergic disease. As aptly coined by Solbach et al. in 1991, 'Lymphocytes play the music but the macrophage calls the tune' [19]. These and other APC dictate the pattern of T cell activation and Th1/Th2 polarisation. Mature APC provide pro-Th1 cytokine signalling in the form of IL-12 (and other cytokines IFNα, IL-18, IL-23) which influence the cytokine profile of a T cell once activated. The relative absence of IL-12 signalling, appears to favour the development of default Th2 cytokine profile. Because these cells are all strongly simulated by microbial products, notions that reduced bacterial exposure may delay maturation of the pro-Th1 APC functions integrated well into the 'hygiene hypothesis'. During early life when APC function is known to be less mature [20], less efficient pro-Th1 cytokine production and costimulation is likely to contribute to the observed increased susceptibility to both tolerance [21], and Th2 responses [22]. Although there is some preliminary evidence that atopic heredity, was associated with reduced numbers of IL-12 producing cells [23] and that APC IL-12 signalling in the neonatal period is inversely related to Th2 responses [24], it is still not clear how variations in APC function influence the development of atopy. The role of specific APC (particularly dendritic cells [DC]) in local airways inflammation is discussed further below.

1.4. The emerging role of regulatory pathways

Although the association between allergy and Th2 immune responses remain conclusive, other paradoxical observations challenge the concept that disease states arise from simple polarisation of responses (away from 'normal' Th1 responses). At a population level, there has been a parallel rise in both Th1-mediated autoimmune diseases (such as type 1 diabetes, inflammatory bowel disease, multiple sclerosis) and Th2-mediated allergic diseases [25],

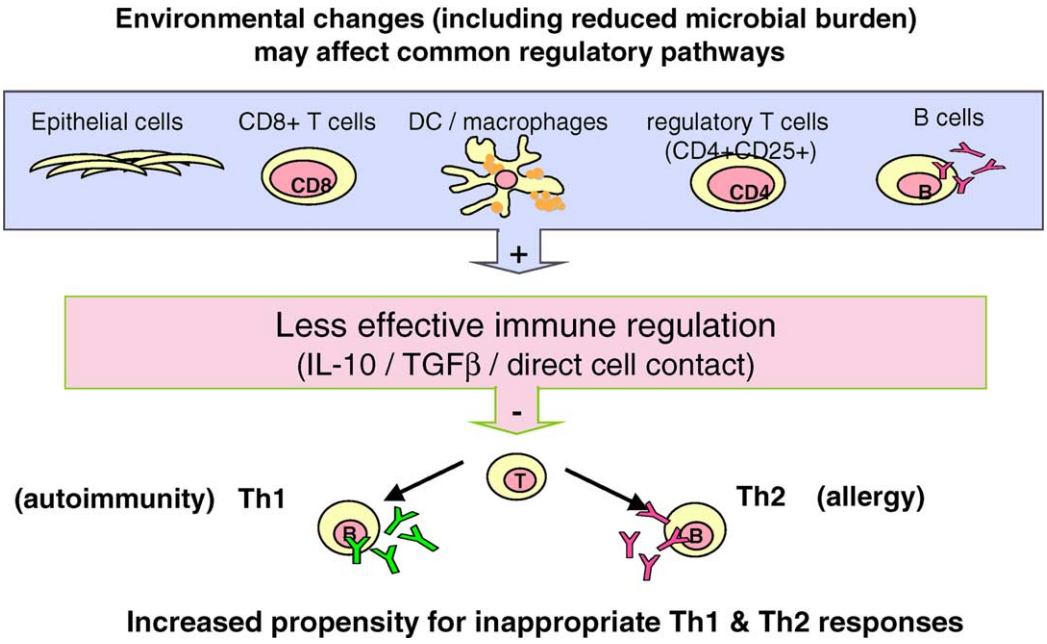

Fig. 1. New models of allergy: Impaired immune regulation.

although this has also been debated [14]. At the individual level, there is accumulating evidence that atopy is associated with an increase in both Th1 and Th2 responses [26–28]. Furthermore, Th1 cells also appear to play a role in allergic inflammation in local tissues, failing to counter-balance Th2 responses in airways inflammation [29]. These observations lead to the notion that the atopic response may develop as a result of a more fundamental failure of underlying immune regulation, rather than a simple skewing of immune response along a Th1–Th2 continuum as previously thought.

In an emerging model of allergy pathogenesis [25,30], it has been proposed that environmental changes (including reduced microbial burden) have lead to impaired development of regulatory pathways such that they allow inappropriate responses (both Th1 and Th2) go unchecked (Fig. 1). Variations in genetic susceptibility determine the pattern of disease expression at the individual level. At a population level, this 'counter-regulatory' model has been used to explain how the same environmental changes could lead to increased rates of both allergy and autoimmunity immune during the same period [25]. In this model impaired immune regulation could have clear consequences for both local and systemic immune development. While it has been well known that mycobacteria can reduce airways inflammation in animal models, recent studies demonstrated that this effect is mediated by T cells producing TGFβ and IL-10 regulatory cytokines [31]. This strongly supports the notion that in addition to their Th1 effects, microbial elements also induce important regulatory pathways (reviewed in [30]). Thus, while the 'hygiene hypothesis' may yet hold true, the proposed mechanisms are probably more complex than originally proposed.

2. The key cells involved in immune regulation

In any situation the immune system must tread a narrow path between adequate, defensive responses and inappropriate (pathological) immune responses (as in allergic disease and autoimmunity). This 'immune homeostasis' is achieved by a diverse group of cells with important regulatory functions (including but not limited to CD4+ CD25+ T regulatory cells, CD8+ T cells, epithelial cells, dendritic cells [DC] and other antigen presenting cells [APC]) (reviewed by [30]) (Fig. 1).

In the most simple terms, a major role of DC is to ensure that an appropriate type and level of immune response is initiated. Typically when these cells are activated during infections, they orchestrate both innate and cognate responses. Cells with regulatory properties (including both T regulatory cells and DC) are also activated by the same pathways but provide additional regulatory signals by direct cell contact (CD4+ CD25+ T regulatory cells) [32,33] (Fig. 2) or by the production of cytokines (IL-10 and TGFβ) [34–36] which inhibit inappropriate or excessive responses. Activation by microbial exposure appears to be an important stimulus for the maturation of these regulatory cells (above).

2.1. Dendritic cells

DC play a critical role in programming T cell responses, following their migration-induced maturation in regional nodes [37]. Animal studies suggest that resting DC stimulate Th2 immune development unless they receive obligatory Th1-trophic signals during antigen processing [38]. These signals may occur under conditions of infection or other local stress [39,40], which evoke protective Th1 effector T

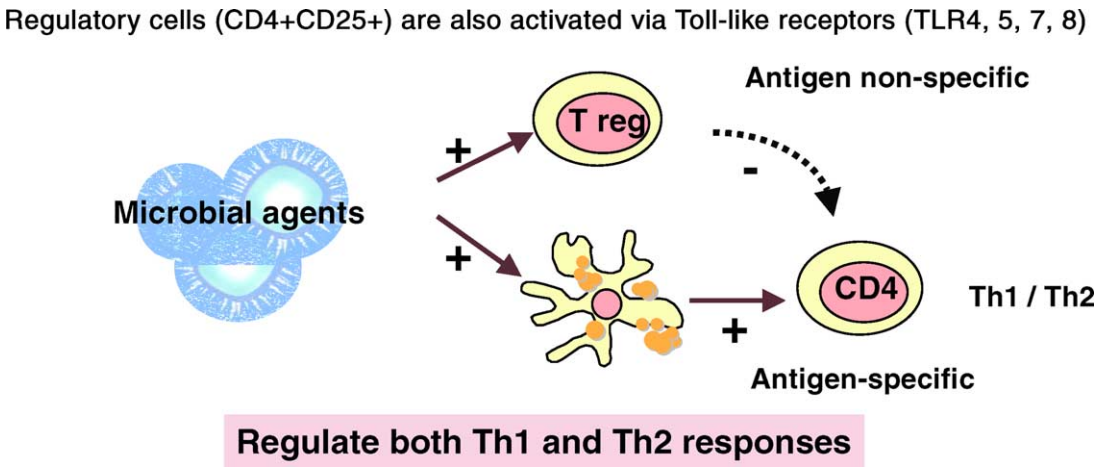

Fig. 2. Counter-regulation by T-regulatory cells.

cell responses. Foreseeably, variations in local inflammation (such as with infection) could have a key role in determining DC maturation and the subsequent pattern of local T cell responses. Animal studies confirm that local airway DC networks are less developed in infant animals, and display markedly attenuated responses to inflammatory triggers [41,42]. Similarly human infants do not typically show DC in the airways in the absence of inflammation [43]. However, mature DC do appear in association with severe respiratory infection even at this age [43]. This suggests that local tissue events in infancy can influence the maturation of DC and modify downstream T cell programming in early life. This has renewed interest in the role of infection in early life in the aetiology of allergic asthma.

2.2. T regulatory cells

Although there were early studies suggesting a role of regulatory T cells (namely CD8+ T cells expressing the of γδT cell receptor) in the development of tolerance in the respiratory tract [44], these were controversial and interest waned. More recently there has been renewed interest with the recognition that there are heterogeneous groups of CD4+ and CD8+ T cells with suppressive or regulatory characteristics (Fig. 3). Although the events leading to the generation of T regulatory populations are still incompletely understood, there is accumulating evidence that the activity of these cells can be influenced by microbial factors.

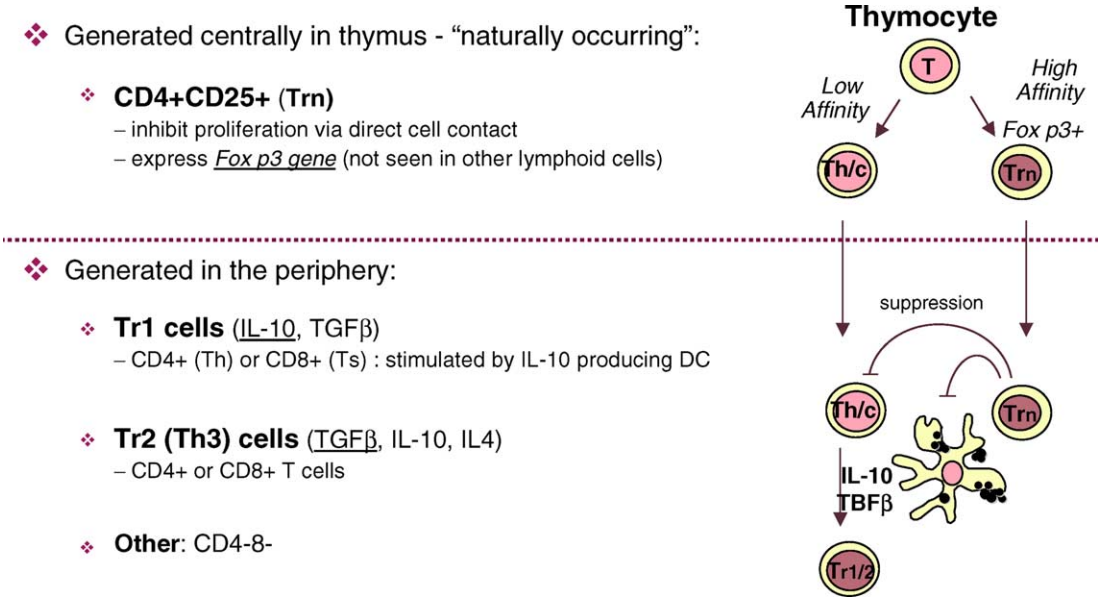

Fig. 3. Diversity of regulatory T cell populations.

'Naturally occurring' CD4+ T regulatory cells (Trn) are derived centrally in the thymus and constitutively express CD25 (the α chain of the IL-2 receptor) and other suppressive molecules including CTLA-4 [32,33]. This subgroup of T regulatory cells generally appears to exert suppressive effects by direct cell contact rather than cytokine production. The *Foxp3* gene appears to be a critical regulator of the development of this subgroup of CD4+ CD25+ Trn cells [45]. The activity of these cells in the periphery can be influenced by microbial exposure and can signal through pathogen recognition receptors known to be on the cell surface (below). These cells have been characterised on the basis of their functional characteristics (as other activated T cells also express CD4 and CD25). However, neurophilin-1 has recently been identified as a possible 'marker' of CD4+ CD25+ regulatory cells [46].

T regulatory cells can also be generated in the periphery from either CD4+ or CD8+ T cells under specific conditions (Fig. 3). These conditions are dictated by ambient cytokine production by other cells including DC, the activity of which can be modified by bacterial encounter (as discussed previously). T regulatory type 1 (Tr1) can be derived from naïve CD4+ [34] or CD8+ [35] stimulated by IL-10 producing DC, and have suppressive effects via high levels of IL-10 (and moderate amounts of TGFβ). Tr1 can also be derived experimentally from CD4+ cells (ex vivo) using dexamethosone and vitamin D3 [47] or other methods. These regulatory cells inhibit the development of both Th1 and Th2 cells. In other animal models Tr1 cells prevent IgE and Th2 differentiation, but produced both IL-5 and IFNγ [48,49]. Other subgroups of T regulatory cells exert potent immunoregulatory properties through the production of large amounts of TGFβ (reviewed in [36]). These Type 2 regulatory T cells (Tr2) and can be derived from CD8+ or CD4+ (also referred to as Th3) T cells.

Thus, although their role is not yet fully understood (discussed further below) there is an emerging role for various populations of T regulatory cells in immune differentiation.

3. Regulatory pathways: Mechanisms of activation (Toll like receptors)

Microbial elements have the capacity to influence DC and T cells during initial signalling events and modify the resulting effector T cell and regulatory T cell phenotypes (Fig. 2). These elements appear to be a key influence on the maturation of these diverse regulatory pathways. Innate immune cells involved in the 'first line' of defence (neutrophils, NK T cells, DC and other APC) are 'hard-wired' to recognise bacterial elements through pathogen recognition receptors (Toll-like receptors [TLR]). Collectively, TLR recognise a broad range of PAMPs [50], although each TLR signals the presence of different microbial components and TLR expression varies with each cell type. Gram-negative bacteria (lipopolysaccharide [LPS]) are largely recognized by TLR4 (in association with CD14). Gram-positive bacterial products (such as peptidoglycan, lipoproteins and lipotechoic acid) are recognized by TLR2. The main ligand for bacterial DNA (CpG oligodeoxynucleotides [ODN]) is TLR9. Other TLR recognise bacterial flagellin (TLR5) and viruses (TLR7). A more detailed discussion of these and other ligands can be found elsewhere [51].

CD4+CD25+ T regulatory cells express both TLR 4 and 9 (but also TLR5 and 7) [52]. Subclasses of dendritic cells also show differential expression of TLR. Myeloid DC (CD14-, CD11c+. CD1a+), found mainly in peripheral tissues, express all TLR except TLR9. Conversely, plasmacytoid DC (CD123+CD45RA+) which are found in circulation blood do express TLR9 (along with TLR1, TLR6, and TLR7) (reviewed in [53]).

Variation in the pattern and strength of TLR activation influence T cell responses. While low levels of LPS appear to favour Th2 differentiation [54], high doses favour Th1 differentiation (via TLR4 activation) [55,56].

Once activated via these pathways, DC and other APC show enhanced expression of costimulatory molecules and cytokines (including IL-12) which appear powerful enough to overcome suppressive properties of CD4+CD25+ T regulatory cells to promote Th1 responses [55,56].

This, together with enhanced regulatory function may reduce the risk of Th2 mediated allergic responses ([57,58] and others) during early life when immune maturation is critical. The importance of 'timing' is supported by animal studies, demonstrating that LPS exposure before allergic responses are established can prevent allergic sensitisation [59]. These effects may be of greater significance in genetically 'allergy prone' individuals who appear to have weaker Th1 responses in the perinatal period ([13] and others).

4. The functional significance of regulatory cells in vivo

Mutations in the *Fox p3* gene have provided valuable information about the normal function of *Fox p3*-expressing regulatory T cells. 'Scurfy' mice with *Fox p3* gene mutations develop fatal lymphoproliferative disease [60]. In further murine studies, *Fox p3* knockout animals do not express regulatory T cells, where as gene transfection to CD4+CD25- cells induces regulatory activity [61–63]. In humans, *Fox p3* mutations are associated with profound immune dysregulation, polyendocrinopathy, enteropathy with X-linked inheritance (IPEX syndrome) [64]. Although eosinophilia and elevated IgE levels are a frequent feature the autoimmune aspects appear more prominent, suggesting that naturally occurring regulatory T cells may have more selective inhibition on Th1 than Th2 cells. Supporting this, Cosmi et al. recently observed that Th2 cells are less

susceptible than Th1 cells to the suppressive influence of CD25+ regulatory T cells [65].

5. Evidence for a role of regulatory cells in allergic respiratory disease

Many cell populations in the airways appear to have regulatory properties, including airway DC and epithelial cells as well as T regulatory cells. However, as yet there is only very limited information about the individual roles of these various cell populations in the human airway, and only marginally more information about the cytokines they produce.

5.1. Role of regulatory cytokines in airways inflammation

It has been proposed that 'normal' exposure to allergen results in development of regulatory T cells and subsequent tolerance, but that inadequate production of IL-10 (or excessive IL-4 or IL-13 production) may instead result in the production of aberrant Th2 clones [66]. Local airway epithelial cells and epithelial–mesenchymal interactions also appear to play a critical role in these events and in asthma pathogenesis [67]. Epithelial cells produce many chemokines, cytokines and growth factors, and are essential for expression of Th2-induced disease in animal asthma models [8,68]. Recent studies suggest that under certain conditions epithelial cells also produce thymic stromal lymphopoietin (TSLP) which may promote Th2 differentiation [69] in favour of T regulatory cell function. The factors, which favour TSLP production in vivo are not yet clearly defined.

Although initially classified as a Th2 cytokine, IL-10 is produced by a wide range of other cell types in the airways (dendritic cells, monocytes, macrophages, CD4+ and CD8+ T cells, natural killer cells, neutrophils and epithelial cells) and is now viewed more as a potent immunoregulatory cytokine. As IL-10 is produced by epithelial cells it may be an important mediating factor in many of the interactions between systemic immune responses and local airways disease. There is some evidence that IL-10 responses are attenuated in atopy, but this is not conclusive in either animal models or humans. Allergic patients demonstrate reduced IL-10 detected in nasal lavage fluid after viral infections [70] and following in-vitro PBMC stimulation [71]. This suggests impaired ability to increase production of this regulatory cytokine after an inflammatory stimulus. Another recent study described reduced IL-10 production by allergen-specific CD8+ T cells from atopic individuals [72]. However, IL-10 responses by viral specific CD8+ T cells were unimpaired compared to non-atopic controls, suggesting that effects could be stimulus specific. IL-10 also appears to mediate the suppressive activity of CD4+CD25+ T cells to corticosteroids, when these cells are subsequently added to allergen-stimulated cultures [73,74]. In animal models, the effects of IL-10 and TGFβ appear to depend on the models used. A number of IL-10 'knock out' murine models demonstrate increased AHR, and eosinophilia exaggerated Th2 responses compared with wild type mice ([75] and others), although other reports are conflicting [76]. IL-10 gene transfer to animal airways inhibits airway inflammation and AHR [77], as does the generation of IL-10 and TGFβ producing CD4+ cells [78,79]. Collectively, most studies support significant regulatory properties of these cytokines in the airway. Not surprisingly, IL-10 deficient animals also develop spontaneous inflammatory bowel disease [80].

The role of IL-10 expression in human asthmatic airways is less clear. Lower IL-10 (protein) levels have been measured in BAL from human asthmatics [81], who also show reduced IL-10 responses by their PBMC (in response to LPS) compared with control subjects [81]. However, other studies demonstrate higher IL-10 mRNA expression in asthmatic airways [82] and gut mucosa [83]. It has been proposed that this may reflect IL-10 dysregulation in the presence of chronic inflammation [30] with inadequate effector mechanisms [84]. Of note, inhaled corticosteroids increase IL-10 responses by alveolar macrophages [85], suggesting that this may contribute to the anti-inflammatory action of these medications. Less is known about the role of TGFβ in the development of airways pathology in humans. Both IL-10 and TGFβ gene polymorphisms have been noted in association with asthma and atopy [86] supporting a role of regulatory pathways in human disease.

5.2. Evidence for T regulatory cells in allergic airway disease (and other allergic states)

The relationship between T regulatory cells and allergic diseases is still unclear, mainly because of the paucity of data. The first study to show clear differences in CD4+ CD25+ T cells in allergic airway disease, reported that CD4+CD25+ T cells derived from patients with symptomatic rhinitis had significantly reduced capacity to inhibit allergen-stimulated lymphoproliferative and IL-5 responses by their own CD4+CD25- T cells, compared with nonatopic individuals [87]. In this study, Ling et al. demonstrated that CD4+CD25+ T cells had suppressive characteristics in vitro and increased expression of the *Fox p3* gene which is selectively expressed by T regulatory cells (distinguishing them from activated Th2 cells expressing CD25) [87]. It is not clear why CD4+CD25+ T cells derived from donors with allergic rhinitis during the pollen season had more attenuated suppressive properties than the same fraction of cells derived from patients with hayfever out-of-season. However, CD4+CD25+ T cells from asymptomatic atopic donors also had reduced suppressive activity compared with nonatopic donors. Some have argued that because these effects were allergen-specific rather than polyclonal, this is not in keeping with the 'hygiene hypothesis' which proposes that resulting impaired

immune regulation should have more general effect on immune function which should not theoretically be limited to specific allergen responses [14]). It has also been argued that expansion of activated Th2 effector cells (also expressing CD25) during pollen exposure may be an explanation for the 'diluted' regulatory activity of CD4+ CD25+ fractions [14]. An alternative explanation is that allergen exposure activates and expands effector T cells to a degree that overcomes regulation by CD4+CD25+ T cells. The study by Ling et al. does suggest possible inherent defect in suppression in atopics, and that allergen exposure may also activate CD4+CD25- T cells (possibly also defective) such that they become refractory to regulation. This also suggests a 'threshold effect' whereby activation versus suppression may vary with allergen exposure and intensity of antigenic stimulus [87].

Differences in T regulatory cells have not been clearly defined in asthma, but there are a number of preliminary studies examining CD4+CD25+ T cells in other allergic conditions. In food allergy, the development of tolerance has been associated with in increase in regulatory T cells [88], however in adults with persistent cows milk allergy no differences in regulatory cells were noted [89] suggesting different mechanisms. In one recent study atopic dermatitis was actually associated with an increase in CD4+CD25+ fractions (expressing *Fox p3* and with suppressive properties), but the notable finding in this study was that staphylococcal enterotoxin B (SEB) inhibited the suppressive properties of these cells [90]. This study suggests a novel mechanism by which staphylococcal superantigens could augment T-cell activation in patients with atopic dermatitis.

The role of regulatory cells in other inflammatory lung conditions is not clear, but it is also possible that chronic conditions such as chronic obstructive pulmonary disease may also arise from imbalance between local immune regulation and cellular responses, as a result of chronic noxious exposures such as cigarette smoke exposure or infection. This remains to be determined.

6. Therapeutic strategies to promote immune regulatory pathways

Developing strategies to enhance immune regulation is a logical approach to suppressing allergic responses therapeutically. There is already evidence that existing strategies (such as immunotherapy) promote immune regulation, adding further support for a role of these cells in disease control.

6.1. Immunotherapy

During immunotherapy, the administration of high dose allergen initially leads to suppression of allergen-specific T cell responses within 60 days [91,92]. Both Th1 and Th2 cells are actively inhibited, and this is associated with increased CD25 expression as well as increased IL-10 and IL-12 signalling from tissue macrophages and other antigen presenting cells [93]. Although T cell anergy is transient, subsequent clinical tolerance is associated with increased activity of IL-10 producing CD4+CD25+ regulatory T cells as noted more recently [94]. Further studies in animal have also shown that induction of tolerance (reduced allergen specific IgE and bronchial eosinophilia) is associated with increased expression of IL-10 and TGFβ in bronchiolar lavage cells [95]. In humans, IL-10 producing cells were also recently increased in cutaneous biopsies of patients undergoing bee venom immunotherapy [96]. IL-10 is believed to play an important role in inducing and maintaining T cell anergy (reviewed in [91]) in immunotherapy. Collectively, these data suggest that the immune tolerance induced by immunotherapy is associated with enhanced activity of a number of regulatory immune cells including APC and regulatory T cell populations. The role of regulatory populations in newer methods of immunotherapy (such as peptide immunotherapy or allergen–gene immunotherapy) remains to be determined.

6.2. Other immune modulation

Other methods of enhancing immune regulation are more experimental. Many of these strategies utilise the well defined immunomodulatory properties of bacteria (discussed above). Early approaches (as early as 1955) included subcutaneous injection of bacteria (including various mixtures of *Staphylococcus, Pneumococcus, Streptococcus, Neisseria* and *Haemophilus* species) with variable clinical effects on 'infectious asthma' (recently reviewed in [97]). More recently, purified bacterial products have been used more successfully in combination with allergen immunotherapy extracts [98].

As potent Th1 immunostimulants, mycobacteria antigens have also been considered as therapeutic agents for treatment of allergic disease (reviewed in [99]). In sensitised animals, administration of mycobacteria results in improved pulmonary function [100], and reduced airways inflammation [101–103]. There is also evidence that these effects are mediated by allergen specific CD4+CD45B-low regulatory cells which produce TGFβ and IL-10 [31]. In humans, BCG vaccination has also been associated with reduced total and specific IgE in allergic individuals [104–106], and improved lung function in asthmatics [107]. Intradermal administration of mycobacteria has previously been used therapeutically in children with existing allergic eczema with some success [108].

Many groups are also investigating the capacity of probiotic bacteria (non-pathogenic gut microflora) to modify allergic disease. The potential pathways by which probiotics influence T cell differentiation are still not clear but could include activation of APC (via CD14 and TLR pathways) and innate pro-Th1 immune responses

[109–111]. There is also preliminary data to suggest that the effects are associated with increasing IL-10 activity [111], again suggesting that regulatory pathways may be involved. Although there is convincing evidence of a role in atopic dermatitis, studies of probiotics in allergic airways disease are limited. In established asthma probiotics failed to show any clinical improvements [112]. In allergic rhinitis, one study found no effect [113], while another (larger study) reported that probiotics were associated with improved quality of life in patients with allergic rhinitis [114]. Attention is now turning to a role of these and other bacterial products in the primary prevention of disease, as it is likely that these have greater therapeutic potential before allergic disease is established.

7. The future: defining early events for disease prevention

It is now generally accepted that events during early life when systems and responses are developing are likely to have more formative effects [115]. This also appears to be true of the immune system. Ultimately a better understanding of these early events is essential for developing safe and effective strategies to prevent disease. Although idealistic, logical future strategies could involve methods of safely promoting normal immune regulation.

7.1. Evidence of immune dysregulation in early life

There is still a great deal of uncertainty surrounding the early immune events that may lead to atopy, but there is accumulating evidence of early pre-symptomatic differences between atopic and nonatopic individuals at birth [11,116–123]. These differences seem to affect a number of different 'read outs' of immune activity at birth, including the magnitude [117,124,125] and pattern on cellular responses in vitro [116–121], circulating neonatal levels of cytokines [122] or cytokine producing cells [126] in vivo, and activity of progenitors that give rise to pro-allergic inflammatory cells (eosinophil progenitors) [123]. It is not clear if these early differences are merely a detectable measure of increased genetic predisposition, or whether they are indicative of early (in utero) environment influences that are already promoting the development of the allergic phenotype. It seems increasingly likely that both are true, particularly as (a) disease is increasing and, (b) these conditions (such as atopic dermatitis and food allergies) may be manifest within months of life. It is thus intuitive that the processes that promote allergic inflammation are initiated in this very early period.

Although allergen specific responses have been detected in fetal life [127] there has been ongoing speculation about whether allergen-responsive fetal T cells have been primed by antigen exposure in vivo or if these reflect some other poorly understood process (reviewed in [128]). Although environmental antigens induce MHC class II dependent cord blood lymphoproliferative responses this is associated with unusually high levels of apoptosis [129]. Furthermore, the surviving cell populations appear to have 'suppressive' or 'regulatory' properties in culture. These cells also express markers which are common (but not exclusive) to T regulatory cells, including CD4, CD25, and CTLA4 [129]. This may be an early manifestation of T cell driven immune regulation. The significance of this is still unclear, and further studies are needed. There is obvious interest given the growing focus on the role of regulatory T cells in early immune development (discussed below).

Although anatomically distinct, mucosal events in the infant gut are of increasing interest in the development of respiratory tolerance. The gut is believed to be central in the development of counter-regulatory immune function. The gastrointestinal tract and associated mucosal immune system is an IL-10 and TGFβ dominant environment, promoting mucosal immune response and systemic immune tolerance (reviewed in [30]). It is of note that germ-free animals that do not achieve normal gut colonisation have profound disorders of immune tolerance including both autoimmunity and allergy [130]. A better understanding of the mucosal events that lead to oral tolerance and normal immune regulation is essential in understanding the pathogenesis of allergic diseases, and may offer avenues of prevention (such as probiotics).

7.2. Modifying early immune responses to prevent disease

Immune interventions have been highly successful in preventing infectious diseases in childhood. Vaccination programs need to evolve with changing disease profiles, and allergen-vaccines are now being investigated for preventing allergic disease. The current approaches involve the mucosal (sublingual) administration of high doses of common aeroallergen to atopic children prior to the development of aeroallergen sensitisation or respiratory disease. The outcomes of these studies will be a number of years away. Other future approaches could include the delivery of allergens with bacterial adjuvants. This remains largely theoretical at present, and there is ongoing concern about unpredictable effects on the developing immune system. Recent studies suggests that 'Th1 adjuvants' (such as complete Freund's adjuvant) have paradoxical effects in neonatal rodents [131] with inhibition of Th1 responses (IgG2a, IL-2, and IFNγ responses) and enhanced Th2 (IL-5) responses. In human neonatal mononuclear cell cultures, we recently observed that bacterial CpG motifs not only enhance Th1 IFNγ responses (to allergens and other antigens) but also result in significantly enhanced Th2 responses to these proteins (not yet published). Together these observations suggest that bacterial antigens may not have the same Th1 polarising effects in neonates, and needs to be investigated further.

A number of other less invasive strategies are also being investigated for prevention including the use of 'natural products' with immune modulating properties including omega-3 fatty acids [132,133] and probiotics [134,135] with interesting preliminary results. There are ongoing studies to address these and other avenues of prevention.

8. Conclusions

Although the specific processes are still not well understood, allergic respiratory disease appears to arise from (1) early immune dysregulation leading to an increased propensity for 'systemic' allergic responses, and (2) additional local immune dysfunction in the airway leading to tissue inflammation and clinical disease. There is ongoing debate as to whether Th2 responses arise from insufficient Th1 stimulation in early life, or from inadequate immune regulation. Probably, both are true to some extent. It is currently proposed [87] that allergic disease results from an inappropriate balance between regulatory CD4+CD25+ T cells and effector Th2 cells. This imbalance could result from a either a deficiency in suppressive activity of regulatory T cells or strong activation signals which overcome regulation.

It is self-evident that environmental changes must be responsible for the epidemic rise of allergic disease in 'westernised' countries. The urgent search for causal associations is driven by the need to reverse this trend either by modifying the environmental changes responsible or developing safe and effective strategies to overcome their effects. While there is a virtually endless list of environmental changes, which could be implicated, the focus is now on factors which could have plausible influences through known immunological effects, particularly on immune regulation. Although the capacity for microbial antigens to activate immunology regulatory pathways and Th1 immune responses is well recognised, the exact contribution to the escalating rates of allergic disease is not clear. While the debate about the 'hygiene hypothesis' continues, the therapeutic potential of various microbial agents is a promising ongoing avenue of investigation for both treatment and prevention of allergic diseases.

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