



Defective Respiratory Tract Immune Surveillance in Asthma

A Primary Causal Factor in Disease Onset and Progression

Patrick G. Holt, DSc; Deborah H. Strickland, PhD; Belinda J. Hales, PhD;
and Peter D. Sly, MD

The relative importance of respiratory viral infections vs inhalant allergy in asthma pathogenesis is the subject of ongoing debate. Emerging data from long-term prospective birth cohorts are bringing increasing clarity to this issue, in particular through the demonstration that while both of these factors can contribute independently to asthma initiation and progression, their effects are strongest when they act in synergy to drive cycles of episodic airways inflammation. An important question is whether susceptibility to infection and allergic sensitization in children with asthma arises from common or shared defect(s). We argue here that susceptibility to recurrent respiratory viral infections, failure to generate protective immunologic tolerance to aeroallergens, and ultimately the synergistic interactions between inflammatory pathways triggered by concomitant responses to these agents all result primarily from functional deficiencies within the cells responsible for local surveillance for antigens impinging on airway surfaces: the respiratory mucosal dendritic cell (DC) network. The effects of these defects in DCs from children with asthma are accentuated by parallel attenuation of innate immune functions in adjacent airway epithelial cells that reduce their resistance to the upper respiratory viral infections, which are the harbingers of subsequent inflammatory events at asthma lesion site(s) in the lower airways. An important common factor underpinning the innate immune functions of these unrelated cell types is use of an overlapping series of pattern recognition receptors (exemplified by the Toll-like receptor family), and variations in the highly polymorphic genes encoding these receptors and related molecules in downstream signaling pathways appear likely contributors to these shared defects. Findings implicating recurrent respiratory infections in adult-onset asthma, much of which is nonatopic, suggest a similar role for deficient immune surveillance in this phenotype of the disease.

CHEST 2014; 145(2):370–378

Abbreviations: CTL = cytotoxic T cell; DC = dendritic cell; FcεR1 = high-affinity IgE receptor; IFN = interferon; IL = interleukin; LRI = lower respiratory viral infection; Th = T-helper cell; TNF = tumor necrosis factor; Treg = T-regulatory cell

It has long been recognized that risk for asthma in childhood tracks with intensity of expression of the atopic phenotype,¹ and this association is strongest during the teen years.^{1,2} These findings have been replicated in multiple birth cohort studies, some extending to early adulthood (reviewed in Holt and Sly³). It is also established that sensitization is an independent asthma risk factor in early childhood^{4,5} and that the earlier sensitization occurs the greater the risk of subsequent asthma development.^{6–9} This is thought to reflect the differential sensitivity of rapidly growing lung tissues during early life to the persistent inflammation

accompanying sensitization to perennial aeroallergens, which has potential to perturb normal patterns of lung differentiation.³ However, it has been unclear how atopy-associated inflammation in young children can attain sufficient intensity to severely damage airway tissues.

Early sensitization to aeroallergens in this context implies failure to generate protective immunologic tolerance, which is the normal default response of the healthy immune system to de novo allergen exposures (reviewed in Strickland and Holt¹⁰). Data from birth cohorts exemplified by our findings¹¹ have pinpointed

the first few years of life as the time when sensitization to ubiquitous aeroallergens is most frequently initiated in atopy, implying that immunoregulatory mechanisms underlying tolerogenesis may be developmentally compromised during this period.

RESPIRATORY VIRAL INFECTIONS

The first years of life are also acknowledged as the period of highest risk for respiratory infections, which, in first-world countries, represent the most frequent cause of hospitalization in this age group. The possible link between early respiratory infections and risk for asthma in children has been recognized since the 1970s, but the full impact of this causal pathway on community disease rates has only become evident through long-term follow-ups from the major prospective birth cohorts.^{4-6,12,13} Notably, recurrent symptomatic lower respiratory viral infections (LRIs) have been identified as the strongest independent risk factor for asthma inception and for its persistence through later childhood. Until comparatively recently, the focus of interest was predominantly upon respiratory syncytial virus, particularly in infants,¹⁴ but additional evidence has also demonstrated a major role for rhinovirus,¹⁵ particularly beyond preschool age.

It is pertinent to note that upper respiratory infections, in contrast, are not associated with increased asthma risk,⁹ indicating that initial failure of innate immune defenses to contain primary infections at the upper respiratory infection site is a necessary first step toward unmasking the asthmagenic potential of these pathogens. This may be due in part to an intrinsic defect in the interferon (IFN)-producing capacity of epithelial cells in susceptible subjects.^{16,17} However, it is additionally noteworthy that LRIs per se are not associated with increased asthma risk,⁹ only those events evoking severe symptoms of wheeze and/or fever,^{9,12} suggesting that underlying failure to control the intensity/duration of adaptive immune antiviral responses may also be a key predisposing factor. This hints at deficiencies in T-regulatory cell (Treg) func-

tions, which have been identified as a potential risk factor in asthma pathogenesis,^{10,18,19} including in infants and school children.^{20,21}

DOUBLE JEOPARDY

An important issue arising from these epidemiologic findings concerns the potential effects of comorbidity, given that risk for both sensitization to aeroallergens and for respiratory infections are concomitantly maximal during this early life phase. Evidence (reviewed in Holt and Sly³) argues strongly that recurrent symptomatic LRI during the first 2 to 3 years of life occurring against a background of preexisting sensitization to aeroallergens results in much higher risk for asthma onset than is associated with either of these factors alone,^{4,5,9,15} suggesting synergistic interaction between underlying asthmagenic inflammatory pathways.

There is a wide body of complementary evidence in the literature for similar interactions at later stages of the disease, including (1) increased susceptibility of atopic children with asthma to intensification and spread of viral infections from the upper to lower respiratory tract with attendant loss of asthma control,²² (2) amplification of rhinovirus cold symptoms in atopic adults,²³ (3) rhinovirus-induced triggering of T helper cell (Th) 2-associated immunity in the lower respiratory tract of adult patients with asthma,²⁴ (4) potentiation of Th2-associated airways inflammation in rhinovirus-infected atopic adults by allergen bronchoprovocation,²⁵ and (5) synergism between atopy and recurrent LRI in relation to risk for de novo onset of asthma in adulthood.²⁶ However, the most profound examples relate to hospitalization for severe virus-associated asthma exacerbation: Depending upon study methodologies, up to 90% of affected children beyond preschool age and > 60% of affected adults are sensitized and concomitantly exposed to perennial allergens.^{3,11,27,28} Moreover, this subgroup of atopics typically comes from the severe end of the sensitization spectrum as defined by IgE titers.^{27,28}

AN ADDITIONAL ROLE FOR BACTERIAL INFECTIONS IN ASTHMA PATHOGENESIS?

The question of the role of bacteria, either as independent inducers of airways inflammation or as secondary agents operating in conjunction with viral infections, has been brought sharply into focus by a series of findings. First, in relation to asthma initiation, studies using conventional bacterial culture methodology suggest that nasopharyngeal colonization during infancy with common respiratory pathogens exemplified by *Haemophilus influenzae* and *Streptococcus pneumoniae* is associated with increased risk for early-onset asthma.²⁹ Second, the application of

Manuscript received June 11, 2013; revision accepted July 31, 2013.

Affiliations: From the Telethon Institute for Child Health Research and Centre for Child Health Research (Profs Holt, Strickland, Hales, and Sly), The University of Western Australia, Perth, WA; and QCMRI-Queensland Children's Medical Research Institute and University of Queensland (Profs Holt and Sly), Brisbane, QLD, Australia.

Funding/Support: The authors are supported by the National Health and Medical Research Council of Australia.

Correspondence to: Patrick G. Holt, DSc, Division of Cell Biology, Telethon Institute for Child Health Research, PO Box 855, W Perth, WA 6872, Australia; e-mail: patrick@ichr.uwa.edu.au

© 2014 American College of Chest Physicians. Reproduction of this article is prohibited without written permission from the American College of Chest Physicians. See online for more details.

DOI: 10.1378/chest.13-1341

highly sensitive metagenomic technology to analysis of samples from the lower airways has identified a previously unrecognized and complex resident microbiome.³⁰ There are significant differences in the density and diversity of bacterial species between children with asthma and control subjects,³⁰ but the presence of a broad range of potential pathogens in microbiome samples from healthy individuals indicates that the presence of bacteria per se in this microenvironment is not necessarily associated with disease risk. This suggests that effective mechanisms must normally operate in airway tissues to protect against transepithelial incursions in situations where local homeostasis is temporarily disrupted, for example, during inflammatory episodes (exemplified by relatively common viral LRI) in which compromised barrier function is a recognized feature.

HOST: RESPIRATORY MICROBIOME INTERACTIONS—MULTIPLE LAYERS OF DEFENSE

The characterization of immunologic interactions between the host immune system and the respiratory microbiome is at an early stage, but recent findings underscore the potential significance of this issue in asthma pathogenesis. In particular, our birth cohort data indicate that the postnatal development of IgG1 responses to common microbiome constituents exemplified by *Haemophilus* is attenuated in children who subsequently develop sensitization to ubiquitous aeroallergens, and particularly in those who develop persistent asthma (Fig 1B^{31,32}). The likely role of specific IgG1 antibody is to accelerate phagocytic clearance of organisms that breach mucosal barriers during cycles of episodic airways inflammation, and a relative deficiency in production of this antibody could plausibly be associated with increased accumulation of infection-associated tissue damage over time. It is additionally pertinent to note that susceptibility to severe virus-associated exacerbations in atopic children with asthma is associated with a comparable relative deficiency in bacterial-specific IgG1.³³

In the schema illustrated in Figure 1A, the first line of cell-mediated immune defense against bacterial invasion in the healthy airway is provided by resident airway mucosal macrophage populations. At baseline, these cells normally display the resting phenotype and are capable of presenting antigen-specific inductive signals to Th-memory cells. However, uptake of bacteria via a combination of IgG1- and Toll-like receptor (TLR)-dependent mechanisms constitutes one of the most potent signals known for macrophage activation, which shuts down their T-cell stimulation functions and triggers secretion of chemokines and inflammatory cytokines exemplified by interleukin-1 (IL-1)/IL-6/tumor necrosis factor (TNF)- α . The resultant danger

of bystander tissue damage via this pathway is significant unless bacterial clearance is effected rapidly. As noted previously, the continuous occupancy of lower respiratory epithelial surfaces by bacteria means that low-level breakthrough into underlying tissues is likely to be a relatively frequent occurrence, and hence these dangers are always present. We have described a mechanism that may be central to ongoing homeostatic control of potential bystander damage via this pathway, in the form of Th2-polarized cellular immunity directed at particulate antigens associated with bacterial membranes.³⁴ Notably, Th2 immunity against respiratory mucosal-dwelling bacteria, detectable as bacterial-specific serum IgE, is virtually universal across the population, and the strength of this immunity is inversely associated with risk for asthma (Fig 2A).

It is unlikely that high-affinity IgE receptor (Fc ϵ R1)-mediated reactions per se play any significant role in this context as this bacterial-specific IgE is directed against nonsoluble membrane antigens which are only revealed during intracellular processing by antigen-presenting cells; these antigens are unlikely to be bioavailable as they are not released in the soluble form necessary to crosslink receptor-bound IgE.^{35,36} However, this IgE is also a surrogate marker for underlying populations of bacterial-specific Th2-memory cells secreting the IL-4/IL-13 required for IgE class switching (Fig 2B), and such memory cells have previously been documented.^{37,38} Relevant to this issue, an additional recognized function of IL-4/IL-13 is downregulation of bacterial-induced TLR-dependent macrophage activation and resultant prevention of secretion of proinflammatory IL-1/IL-6/TNF α (reviewed in Hollams et al³⁴) (Fig 2B). The titers of bacterial-specific IgE antibodies in individual subjects should reflect the relative abundance of their circulating populations of bacterial-specific Th2 memory cells, and this may explain the association seen in Figure 2A. Thus, in situations of low-level bacterial breakthrough (which may be relatively common), presentation of processed bacterial antigen to incoming specific Th2-memory cells by airway tissue macrophages actively engaged in bacterial clearance provides an autocrine-like mechanism for avoidance of excessive activation during this process (Fig 2B); at higher bacterial loads, it is likely that this mechanism would be overwhelmed.

ANTIVIRAL AND ATOPIC INFLAMMATORY PATHWAYS: POTENTIAL INTERACTION MECHANISMS

Animal model studies suggest a variety of mechanisms through which these pathways may interact. First, IL-4/IL-13-dependent alternatively activated macrophages have been identified as possible contributors to postviral lung damage, and IL-13-secreting

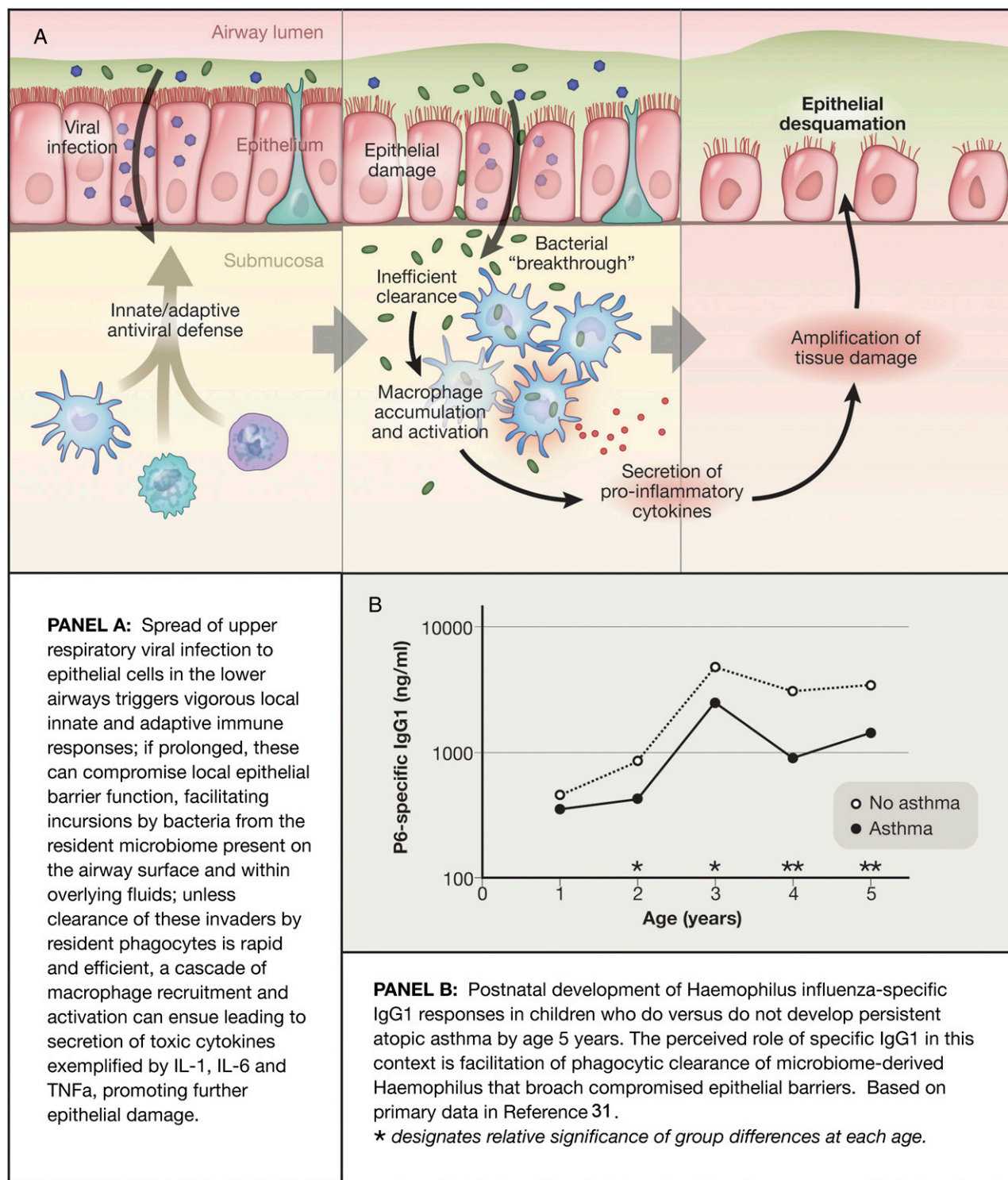


FIGURE 1. Amplification of virus-associated airway epithelial damage via bacterial incursions. IL = interleukin; TNF = tumor necrosis factor. (Illustrations by Haderer & Müller Biomedical Art, LLC.)

invariant natural killer T cells have been implicated in their local induction/activation.³⁹ Second, a potentially important role for viral modulation of Fc ϵ R1 expression, particularly on myeloid dendritic cells (DCs), is suggested via a range of studies. These include the dem-

onstration in a murine parainfluenza model of type1 IFN-dependent Fc ϵ R1 upregulation on lung DCs,⁴⁰ and complimentary findings in a murine influenza model in which Fc ϵ R1 upregulation in infected lung tissue was accompanied by enhanced susceptibility to

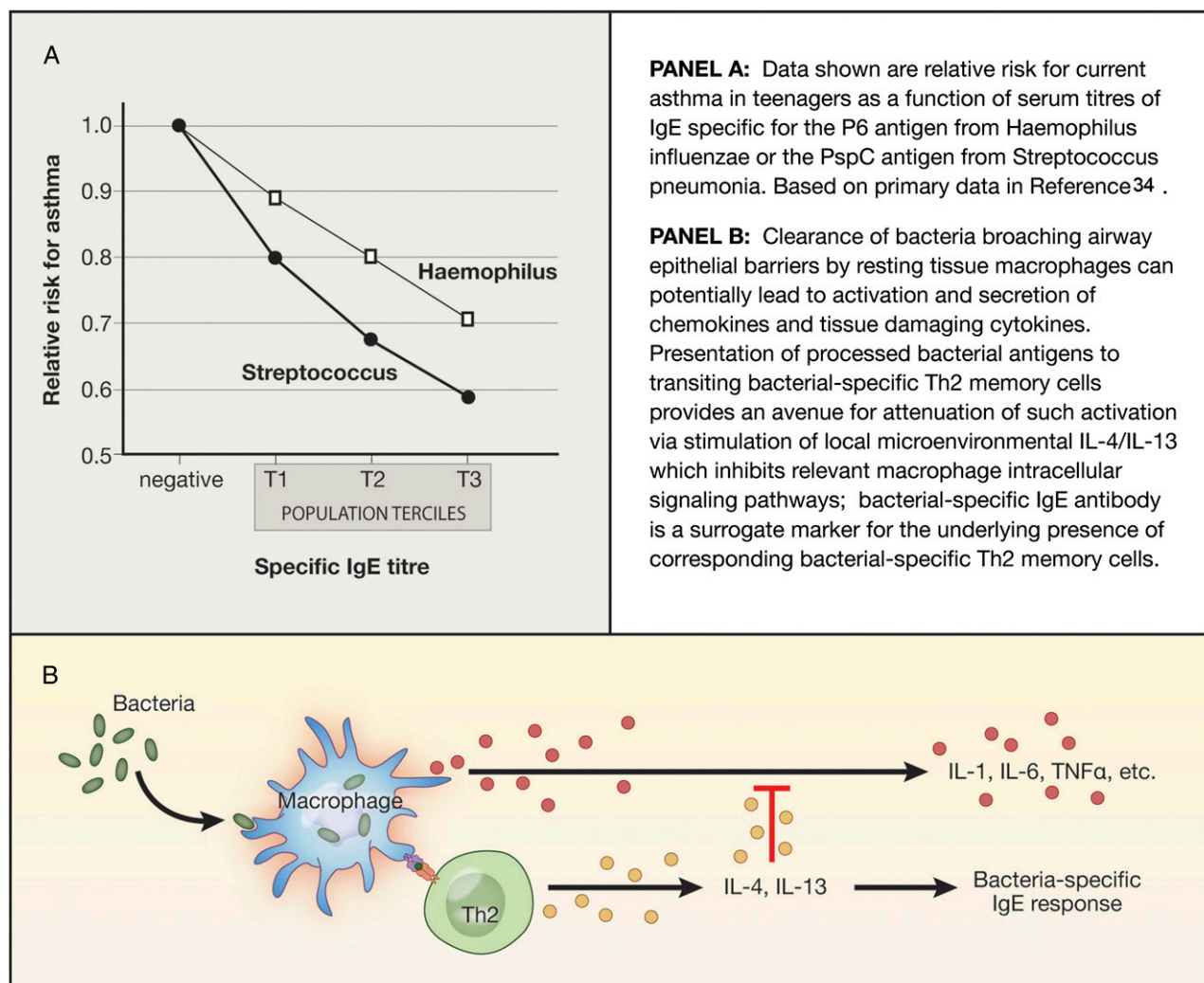


FIGURE 2. Th2-mediated regulation of bacterial-associated inflammation. Th = T-helper cell. See Figure 1 legend for expansion of other abbreviations. (Illustrations by Haderer & Müller Biomedical Art, LLC.)

Th2 cell-associated airways inflammation.⁴¹ FcεR1 upregulation has also been observed on plasmacytoid DCs, and cross-linking of FcεR1 on these cells has been shown to inhibit their type1 IFN responses to viral exposure.^{42,43}

An additional interaction mechanism has been suggested by studies on acute virus-associated asthma exacerbation in children, involving comparative gene expression profiling of circulating cell populations at baseline vs during exacerbation.²⁸ These studies suggest that during the early phase of exacerbations in atopics concomitantly exposed to aeroallergen, initial upregulation of FcεR1 may occur on resident airway mucosal DCs in situ via viral-induced type 1 IFN resulting in stimulation of production of the FcεR1 γ chain, the availability of which limits overall receptor expression.^{3,28} This is followed by recruitment of fresh FcεR1^{hi} myeloid DCs via cytokine signals that are translocated to precursors in bone marrow,^{3,28} presumably carried by

migrating cells initially activated in the airways as demonstrated in earlier bronchial challenge models.⁴⁴ Based on observations relating to atopic dermatitis lesions wherein FcεR1^{hi} DCs use IgE to maximize allergen capture/processing/presentation for Th2-memory activation,⁴⁵ it is plausible that transient FcεR1 hyperexpression on airway DCs of viral-infected atopic asthmatics could recruit allergen-induced Th2 cytokine storms into the inflammatory milieu of the infected airway, contributing directly to tissue damage and also potentially antagonizing Th1-polarized antiviral immunity.²⁸

AIRWAY MUCOSAL DCs AT CENTER STAGE

Since the discovery of an organized network of DCs within and below the epithelium of the conducting airways,⁴⁶ subsequent investigations from multiple groups (reviewed in Holt et al,⁴⁷ Lambrecht and Hammad,⁴⁸ and Wills-Karp⁴⁹) have elucidated many of the functions

of these cells and confirmed predictions that they play a gatekeeper role in local immune surveillance of mucosal surfaces. Of particular note, mobilization of this DC network constitutes the universal default response to inhalation challenge regardless of the class of antigen (viral, bacterial, allergenic) involved, with kinetics equivalent to or exceeding those exhibited by neutrophils.⁴⁷

Consistent with the phenotype of DCs in other tissues, it is evident that their functions in the airway mucosa include initial recognition and classification of incoming foreign antigens via pattern recognition receptors, and subsequent programming of adaptive immunity to meet the specific challenge. The complex hierarchical process orchestrated by airway mucosal DCs during initial exposures, illustrated in the inset in Figure 3, includes (1) discrimination between pathogen-derived vs non-pathogen-derived inhaled antigens, (2) induction of tolerance to the latter via T-cell deletion or generation of specific Treg populations, (3) discrimination between classes of pathogens, and (4) programming of balanced pathogen-specific responses comprising Th/effector memory and/or cytotoxic T-cell (CTL) memory responses appropriate to the pathogen class, together with parallel cohorts of Tregs required to control the intensity of downstream effector responses.

These events occur following the migration of antigen-bearing DCs to regional lymph nodes where they acquire T-cell-activating properties, as the DCs normally remain in quiescent (surveillance only) mode in peripheral tissues. On subsequent reexposure to antigens to which Th-memory has been primed (eg, reinfection with pathogen), local CD40-ligand-dependent interactions with transiting Th memory cells trigger rapid *in situ* functional maturation of antigen-bearing DCs involving uploading of processed peptide onto surface major histocompatibility complex (MHC) and expression of T-cell-docking and costimulator molecules⁵⁰; these armed DCs proceed to activate any incoming memory cells they subsequently encounter leading to intense local expression of T-effector responses (Fig 3); cytokine(s) from these cells may also contribute to local CTL activation. In situations where tolerance mechanisms have previously failed and aeroallergen-specific Th-memory has been primed, a similar sequence of events follows reexposure via inhalation, triggering local Th2-associated inflammation.⁵⁰ However, as illustrated in Figure 3, such responses (as well as Th1/CTL-memory responses directed against pathogens) are subject to an additional level of control in the form of Tregs which modulate the local transition of DCs from antigen surveillance mode into T-cell activation mode; it is important to emphasize that the initial generation of these Tregs is itself DC-dependent (eg, Holt et al⁴⁷ and Strickland et al⁵¹).

The precise relationship between these particular Treg populations and those that normally induce tolerance during initial encounters with inhaled antigens lacking pathogen-associated molecular signatures remains to be established. However, it is pertinent to note that in experimental models the efficiency of DC-dependent sampling of antigen/allergen from airway mucosal surfaces has been directly linked to the efficiency of tolerance induction during initial exposures, and also to the intensity/duration of airway inflammatory responses that follow aeroallergen challenge of presensitized animals. Of note, genetically determined susceptibility to airways inflammation in one such model was circumvented by optimal loading of airway mucosal DCs *ex vivo* with high-dose aeroallergen to compensate for the diminished *in vivo* antigen surveillance capacity of the affected animal strain, resulting in restoration of normal levels of Treg function and reversal of their airways hyperresponsiveness.⁵¹ Several lines of epidemiologic evidence (reviewed in Strickland et al⁵¹) suggest that a comparable human phenotype, defined by an inverse relationship between levels of aeroallergen exposure and susceptibility to development of symptomatic allergic airways inflammation, is relatively common in the population.

CONCLUSIONS

A variety of evidence is thus accumulating which suggests that underlying susceptibility to asthma initiation and progression is a series of related defects in mechanisms that underpin capacity to sample, classify, and respond appropriately to inhaled antigens, regardless of the class of antigens involved. The emphasis in this review has been upon childhood atopic asthma, but it is noteworthy that recurrent respiratory tract infections, one of the key indices of the deficient immune surveillance phenotype described here, is also a marker of risk for adult-onset nonatopic asthma.²⁶ The most prominent manifestations of the immune surveillance defect described here are seen in the functions of the DC population which is at the epicenter of the complex cellular networks that maintain overall immunologic homeostasis in the lung and airways, effectively bridging the gap between the innate and adaptive arms of the immune system. Nevertheless it is pertinent to reemphasize that deficiencies in surveillance-related functions in susceptible populations are also seen in mesenchymal cells^{16,17} and in migratory inflammatory cells present within BAL⁵² and peripheral blood mononuclear cells.⁵³⁻⁵⁵

A key question arising from these findings is whether they point toward new and potentially better asthma treatment strategies for the future. Specifically targeting the DC at center stage in airway mucosal immune surveillance appears a tall order at present, particularly

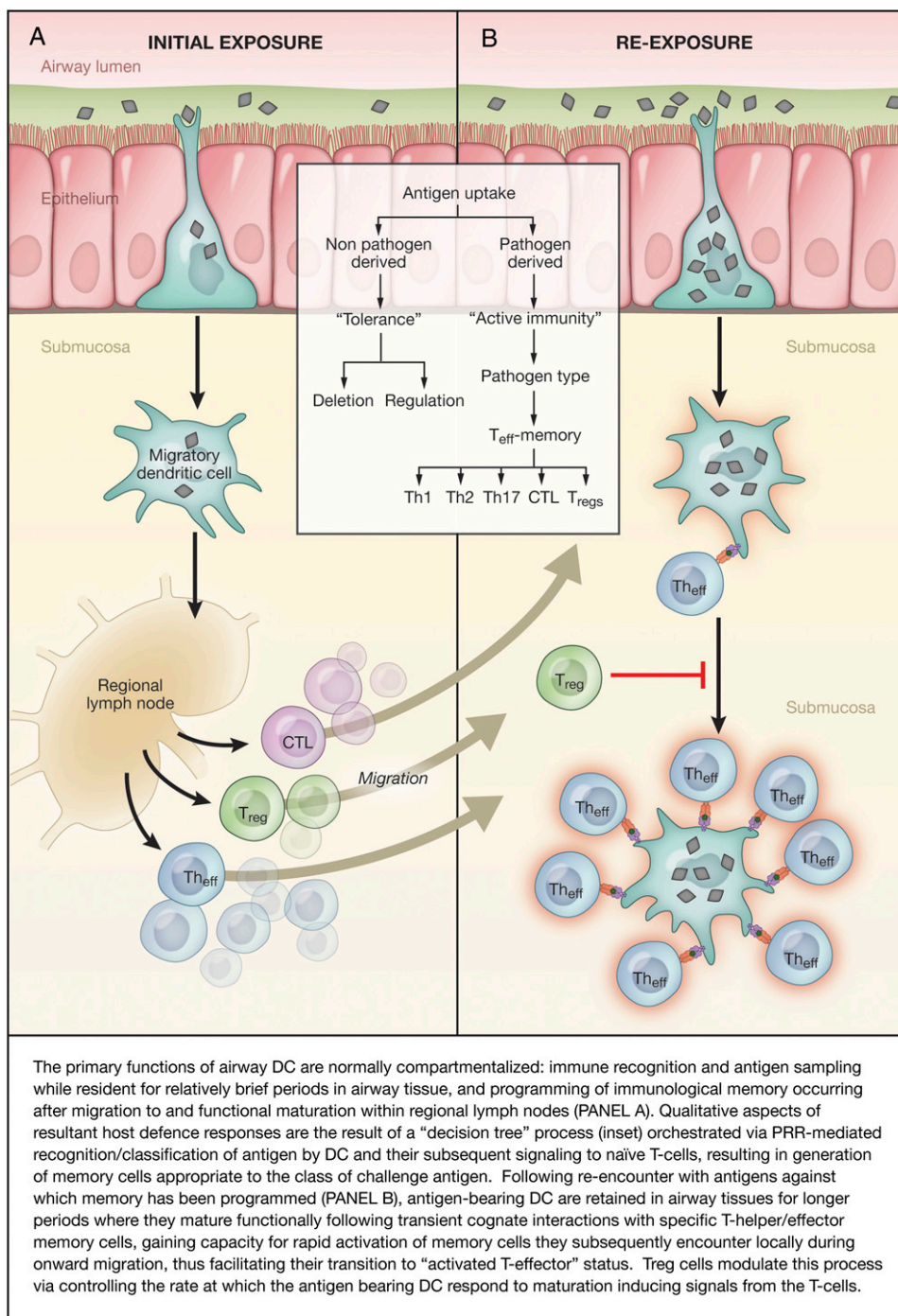


FIGURE 3. Immune surveillance functions of airway mucosal dendritic cell (DC) populations. CTL = cytotoxic T cell; T_{eff} = T-effector cell; Th_{eff} = T-helper/effecter cell; T_{reg} = T-regulatory cell. See Figure 2 legend for expansion of other abbreviations. (Illustrations by Haderer & Müller Biomedical Art, LLC.)

given the overlap between these cells and closely related macrophage population(s) within the myeloid lineage. However, a common factor linking the surveillance functions of virtually all the cell types/lineages implicated here is the TLR family of receptors, the functions of which are central to protection against pathogens, and which also appear to extend to allergen surveillance.^{56,57} There is growing interest in drugs tar-

geting TLR-associated pathways, and the data accumulating are providing increasingly plausible rationales for this approach to asthma control.

ACKNOWLEDGMENTS

Financial/nonfinancial disclosures: The authors have reported to *CHEST* that no potential conflicts of interest exist with any

companies/organizations whose products or services may be discussed in this article.

Role of sponsors: The sponsor had no role in the design of the study, the collection and analysis of the data, or the preparation of the manuscript.

REFERENCES

- Burrows B, Martinez FD, Halonen M, Barbee RA, Cline MG. Association of asthma with serum IgE levels and skin-test reactivity to allergens. *N Engl J Med*. 1989;320(5):271-277.
- Hollams EM, Devereill M, Serralha M, et al. Elucidation of asthma phenotypes in atopic teenagers through parallel immunophenotypic and clinical profiling. *J Allergy Clin Immunol*. 2009;124(3):463-470.
- Holt PG, Sly PD. Viral infections and atopy in asthma pathogenesis: new rationales for asthma prevention and treatment. *Nat Med*. 2012;18(5):726-735.
- Jackson DJ, Evans MD, Gangnon RE, et al. Evidence for a causal relationship between allergic sensitization and rhinovirus wheezing in early life. *Am J Respir Crit Care Med*. 2012;185(3):281-285.
- Oddy WH, de Klerk NH, Sly PD, Holt PG. The effects of respiratory infections, atopy, and breastfeeding on childhood asthma. *Eur Respir J*. 2002;19(5):899-905.
- Illi S, von Mutius E, Lau S, Niggemann B, Grüber C, Wahn U; Multicentre Allergy Study (MAS) group. Perennial allergen sensitisation early in life and chronic asthma in children: a birth cohort study. *Lancet*. 2006;368(9537):763-770.
- Peat JK, Salome CM, Woolcock AJ. Longitudinal changes in atopy during a 4-year period: relation to bronchial hyperresponsiveness and respiratory symptoms in a population sample of Australian schoolchildren. *J Allergy Clin Immunol*. 1990;85(1 pt 1):65-74.
- Sherrill D, Stein R, Kurzius-Spencer M, Martinez F. On early sensitization to allergens and development of respiratory symptoms. *Clin Exp Allergy*. 1999;29(7):905-911.
- Kusel MM, de Klerk NH, Kebadze T, et al. Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J Allergy Clin Immunol*. 2007;119(5):1105-1110.
- Strickland DH, Holt PG. T regulatory cells in childhood asthma. *Trends Immunol*. 2011;32(9):420-427.
- Holt PG, Sly PD. Interaction between adaptive and innate immune pathways in the pathogenesis of atopic asthma: operation of a lung/bone marrow axis. *Chest*. 2011;139(5):1165-1171.
- Kusel MM, Kebadze T, Johnston SL, Holt PG, Sly PD. Febrile respiratory illnesses in infancy and atopy are risk factors for persistent asthma and wheeze. *Eur Respir J*. 2012;39(4):876-882.
- Stern DA, Morgan WJ, Halonen M, Wright AL, Martinez FD. Wheezing and bronchial hyper-responsiveness in early childhood as predictors of newly diagnosed asthma in early adulthood: a longitudinal birth-cohort study. *Lancet*. 2008;372(9643):1058-1064.
- James KM, Gebretsadik T, Escobar GJ, et al. Risk of childhood asthma following infant bronchiolitis during the respiratory syncytial virus season. *J Allergy Clin Immunol*. 2013;132(1):227-229.
- Busse WW, Lemanske RF Jr, Gern JE. Role of viral respiratory infections in asthma and asthma exacerbations. *Lancet*. 2010;376(9743):826-834.
- Contoli M, Message SD, Laza-Stanca V, et al. Role of deficient type III interferon-lambda production in asthma exacerbations. *Nat Med*. 2006;12(9):1023-1026.
- Wark PAB, Johnston SL, Bucchieri F, et al. Asthmatic bronchial epithelial cells have a deficient innate immune response to infection with rhinovirus. *J Exp Med*. 2005;201(6):937-947.
- Lloyd CM, Hawrylowicz CM. Regulatory T cells in asthma. *Immunity*. 2009;31(3):438-449.
- Runyon RS, Cachola LM, Rajeshuni N, et al. Asthma discordance in twins is linked to epigenetic modifications of T cells. *PLoS ONE*. 2012;7(11):e48796.
- Schaub B, Liu J, Hoppler S, et al. Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J Allergy Clin Immunol*. 2009;123(4):774-782.e5.
- Hartl D, Koller B, Mehlhorn AT, et al. Quantitative and functional impairment of pulmonary CD4+CD25hi regulatory T cells in pediatric asthma. *J Allergy Clin Immunol*. 2007;119(5):1258-1266.
- Olenec JP, Kim WK, Lee WM, et al. Weekly monitoring of children with asthma for infections and illness during common cold seasons. *J Allergy Clin Immunol*. 2010;125(5):1001-1006.e1.
- Bardin PG, Fraenkel DJ, Sanderson G, et al. Amplified rhinovirus colds in atopic subjects. *Clin Exp Allergy*. 1994;24(5):457-464.
- Message SD, Laza-Stanca V, Mallia P, et al. Rhinovirus-induced lower respiratory illness is increased in asthma and related to virus load and Th1/2 cytokine and IL-10 production. *Proc Natl Acad Sci U S A*. 2008;105(36):13562-13567.
- Calhoun WJ, Dick EC, Schwartz LB, Busse WW. A common cold virus, rhinovirus 16, potentiates airway inflammation after segmental antigen bronchoprovocation in allergic subjects. *J Clin Invest*. 1994;94(6):2200-2208.
- Rantala A, Jaakkola JJ, Jaakkola MS. Respiratory infections precede adult-onset asthma. *PLoS ONE*. 2011;6(12):e27912.
- Heymann PW, Platts-Mills TA, Johnston SL. Role of viral infections, atopy and antiviral immunity in the etiology of wheezing exacerbations among children and young adults. *Pediatr Infect Dis J*. 2005;24(suppl 11):S217-S222.
- Subrata LS, Bizzintino J, Mamessier E, et al. Interactions between innate antiviral and atopic immunoinflammatory pathways precipitate and sustain asthma exacerbations in children. *J Immunol*. 2009;183(4):2793-2800.
- Bisgaard H, Hermansen MN, Buchvald F, et al. Childhood asthma after bacterial colonization of the airway in neonates. *N Engl J Med*. 2007;357(15):1487-1495.
- Hilty M, Burke C, Pedro H, et al. Disordered microbial communities in asthmatic airways. *PLoS ONE*. 2010;5(1):e8578.
- Hales BJ, Chai LY, Elliot CE, et al. Antibacterial antibody responses associated with the development of asthma in house dust mite-sensitized and non-sensitized children. *Thorax*. 2012;67(4):321-327.
- Hales BJ, Pearce LJ, Kusel MM, Holt PG, Sly PD, Thomas WR. Differences in the antibody response to a mucosal bacterial antigen between allergic and non-allergic subjects. *Thorax*. 2008;63(3):221-227.
- Hales BJ, Martin AC, Pearce LJ, et al. IgE and IgG anti-house dust mite specificities in allergic disease. *J Allergy Clin Immunol*. 2006;118(2):361-367.
- Hollams EM, Hales BJ, Bachert C, et al. Th2-associated immunity to bacteria in teenagers and susceptibility to asthma. *Eur Respir J*. 2010;36(3):509-516.
- Lee EY, Bang JY, Park GW, et al. Global proteomic profiling of native outer membrane vesicles derived from *Escherichia coli*. *Proteomics*. 2007;7(17):3143-3153.
- Kadioglu A, Weiser JN, Paton JC, Andrew PW. The role of *Streptococcus pneumoniae* virulence factors in host respiratory colonization and disease. *Nat Rev Microbiol*. 2008;6(4):288-301.
- Epton MJ, Hales BJ, Thompson PJ, Thomas WR. T cell cytokine responses to outer membrane proteins of *Haemophilus influenzae* and the house dust mite allergens Der p 1 in allergic

- and non-allergic subjects. *Clin Exp Allergy*. 2002;32(11):1589-1595.
38. King PT, Hutchinson PE, Johnson PD, Holmes PW, Freezer NJ, Holdsworth SR. Adaptive immunity to nontypeable *Haemophilus influenzae*. *Am J Respir Crit Care Med*. 2003;167(4):587-592.
 39. Kim EY, Battaile JT, Patel AC, et al. Persistent activation of an innate immune response translates respiratory viral infection into chronic lung disease. *Nat Med*. 2008;14(6):633-640.
 40. Grayson MH, Cheung D, Rohlfing MM, et al. Induction of high-affinity IgE receptor on lung dendritic cells during viral infection leads to mucous cell metaplasia. *J Exp Med*. 2007;204(11):2759-2769.
 41. Al-Garawi A, Husain M, Ilieva D, et al. Shifting of immune responsiveness to house dust mite by influenza A infection: genomic insights. *J Immunol*. 2012;188(2):832-843.
 42. Durrani SR, Montville DJ, Pratt AS, et al. Innate immune responses to rhinovirus are reduced by the high-affinity IgE receptor in allergic asthmatic children. *J Allergy Clin Immunol*. 2012;130(2):489-495.
 43. Gill MA, Bajwa G, George TA, et al. Counterregulation between the FcepsilonRI pathway and antiviral responses in human plasmacytoid dendritic cells. *J Immunol*. 2010;184(11):5999-6006.
 44. Wood LJ, Sehmi R, Dorman S, et al. Allergen-induced increases in bone marrow T lymphocytes and interleukin-5 expression in subjects with asthma. *Am J Respir Crit Care Med*. 2002;166(6):883-889.
 45. Novak N, Kraft S, Bieber T. Unraveling the mission of FcepsilonRI on antigen-presenting cells. *J Allergy Clin Immunol*. 2003;111(1):38-44.
 46. Holt PG, Schon-Hegrad MA, Phillips MJ, McMenamin PG. Ia-positive dendritic cells form a tightly meshed network within the human airway epithelium. *Clin Exp Allergy*. 1989;19(6):597-601.
 47. Holt PG, Strickland DH, Wikström ME, Jahnsen FL. Regulation of immunological homeostasis in the respiratory tract. *Nat Rev Immunol*. 2008;8(2):142-152.
 48. Lambrecht BN, Hammad H. Biology of lung dendritic cells at the origin of asthma. *Immunity*. 2009;31(3):412-424.
 49. Wills-Karp M. Interleukin-13 in asthma pathogenesis. *Immunol Rev*. 2004;202:175-190.
 50. Huh JC, Strickland DH, Jahnsen FL, et al. Bidirectional interactions between antigen-bearing respiratory tract dendritic cells (DCs) and T cells precede the late phase reaction in experimental asthma: DC activation occurs in the airway mucosa but not in the lung parenchyma. *J Exp Med*. 2003;198(1):19-30.
 51. Strickland DH, Judd S, Thomas JA, Larcombe AN, Sly PD, Holt PG. Boosting airway T-regulatory cells by gastrointestinal stimulation as a strategy for asthma control. *Mucosal Immunol*. 2011;4(1):43-52.
 52. Sykes A, Edwards MR, Macintyre J, et al. Rhinovirus 16-induced IFN- α and IFN- β are deficient in bronchoalveolar lavage cells in asthmatic patients. *J Allergy Clin Immunol*. 2012;129(6):1506-1514.e6.
 53. Gehlhar K, Bilitewski C, Reinitz-Rademacher K, Rohde G, Bufe A. Impaired virus-induced interferon-alpha2 release in adult asthmatic patients. *Clin Exp Allergy*. 2006;36(3):331-337.
 54. Papadopoulos NG, Stanciu LA, Papi A, Holgate ST, Johnston SL. A defective type 1 response to rhinovirus in atopic asthma. *Thorax*. 2002;57(4):328-332.
 55. Roponen M, Yerkovich ST, Hollams E, Sly PD, Holt PG, Upham JW. Toll-like receptor 7 function is reduced in adolescents with asthma. *Eur Respir J*. 2010;35(1):64-71.
 56. Hammad H, Chieppa M, Perros F, Willart MA, Germain RN, Lambrecht BN. House dust mite allergen induces asthma via Toll-like receptor 4 triggering of airway structural cells. *Nat Med*. 2009;15(4):410-416.
 57. Wills-Karp M. Allergen-specific pattern recognition receptor pathways. *Curr Opin Immunol*. 2010;22(6):777-782.