

Epigenetic changes in childhood asthma

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Childhood asthma is linked strongly to atopy and is characterised by a T helper 2 (Th2)-polarised immunological response. Epidemiological studies implicate severe lower respiratory tract viral infections, especially in early childhood, and repeated inhalational exposure to allergens as important synergistic factors in the development of asthma. The way in which these and other environmental factors induce stable alterations in phenotype is poorly understood, but may be explained on the basis of epigenetic changes, which are now recognised to underlie the establishment and maintenance of a Th2 response. Furthermore, ongoing asthmatic inflammation of the airways may be driven by alterations in the expression profile of regulatory microRNA genes, to which epigenetic mechanisms may also contribute. Thus, an understanding of epigenetic mechanisms in asthma has the potential to reveal new approaches for primary prevention or therapeutic intervention in childhood asthma.

Two novel conceptual frameworks have captured the imagination of biomedical scientists who are interested in the pathogenesis of chronic and complex diseases. One is epigenetics, which may explain how environmental factors can cause stable alterations in phenotype without changes in genotype. The other is transcriptional regulation by microRNA. In this Commentary, we examine the relevance of epigenetics and the role of microRNA in the induction and progression of asthma in childhood, and propose a model to link these mechanisms.

Childhood asthma

Asthma is one of the most common chronic diseases affecting children, especially in economically developed nations. For example, in Australia the prevalence of asthma is 9-11% in the 2-4-year-old age group and 12-16% in the 5-17-year-old age group, with males affected more commonly than females (Ampon et al., 2007). Childhood asthma is linked strongly to atopy, which is a

genetic predisposition to produce immunoglobulin E (IgE) antibodies in response to ordinary exposures to environmental allergens, and is often associated with symptoms of asthma, rhinoconjunctivitis or eczema (Johansson et al., 2004). In turn, atopy is associated characteristically with a T helper 2 (Th2)-biased immunological response (Bacharier et al., 2008). However, the pathogenesis of this illness remains incompletely understood. Both genetic predisposition and various environmental factors clearly play important roles in the development of allergic asthma (Sly et al., 2008), but the relative importance of these various contributory mechanisms and the ways in which they might interact are difficult to assess.

Epidemiological studies strongly suggest that the most important environmental factors that predispose to the development of asthma are lower respiratory tract viral infections associated with wheezing, especially in early childhood, and repeated inhalation exposure to allergens, which appear to be synergistic (Holt and Sly, 2007; Sly et al., 2008). The role of early-life viral infections is somewhat controversial because the so-called 'hygiene hypothesis' proposed that infections in early childhood, which are acquired by contact with older siblings or other children, might protect against subsequent development of asthma and other allergic diseases (reviewed by Wong and Chow, 2008). However, a recent longitudinal study involving prospective follow-up to 8 years of age strongly suggests that this is unlikely to be a valid notion (Caudri et al., 2009). Among the early-life viral infections, respiratory syncytial virus (RSV) and rhinovirus are of particular interest, because wheezing lower respiratory tract infections in the first year of life are associated strongly with subsequent development of asthma (Kristjansson et al., 2005; Sigurs et al., 2005; Kusel et al., 2007; Jackson et al., 2008; Mohapatra and Boyapalle, 2008). Other clearly defined risk factors are exposure to airborne environmental irritants such as tobacco smoke and traffic pollutants: evidence for their role comes from numerous large population-based studies, mostly involving retrospective analyses but also including some prospective cohort studies, which have been reviewed comprehensively by Wigle et al. (Wigle et al., 2008). Additional factors that may play a significant contributory role include exposure to chemicals and various therapeutic drugs, such as paracetamol, and the availability of specific nutrients (Dietert and Zelikoff, 2008; Kim et al., 2009). For some of these risk factors, prenatal (i.e. maternal) exposure appears to be at least as important as exposure during the neonatal period or early childhood (Miller, 2008; Schaub et al., 2009).

The observation that early-life exposure to environmental agents such as infections, pollutants and drugs can contribute to the development of allergic asthma raises fundamental questions about the underlying mechanisms. If atopy and a Th2-polarised immunological response are indeed precursors to the development

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of childhood asthma (Sly et al., 2008), how does an infection by a respiratory virus (that does not infect CD4⁺ T lymphocytes) elicit the required stable change in the phenotype of T-helper cells? Epigenetics may provide the answer.

Epigenetics, honeybees and T-helper cells

Epigenetics is the study of mitotically heritable changes in gene expression that occur without directly altering the DNA sequence. It includes methylation of DNA by the covalent addition of a methyl group to a cytosine residue in a CpG site (Klose and Bird, 2006), as well as the post-translational modification of the amino acid tails of histones by acetylation, phosphorylation, methylation, sumoylation or ubiquitylation (Kouzarides, 2007). As summarised in Fig. 1, such modifications collectively activate or silence genes by influencing chromatin structure and stability, and/or by altering DNA interaction with transcription factors. Methylation of CpG islands that span the promoters of genes results in stable gene silencing. CpG methylation is accompanied invariably by repressive modifications of histone tails, which classically include trimethylation of histone 3 (H3) lysine (K) 9 and H3-K27. Histone modifications that are associated with active chromatin and unmethylated DNA include acetylation of H3-K9 and trimethylation of H3-K4. Both types of modifications may co-localise in embryonic and somatic stem cells, or in other populations of cells that retain the potential for alternative functional differentiation. These 'bivalent' epigenetic marks are most commonly found at CpG-rich promoters of developmentally regulated genes that are inactive, but poised for induction or silencing on differentiation, suggesting that lack of expression in this context is an active process (Bernstein et al., 2006; Rowell et al., 2008; Locksley, 2009).

Epigenetic mechanisms are thus able to govern phenotypic plasticity with respect to individual cells and even the developmental fate of an entire organism. This has been demonstrated spectacularly in terms of the control of reproductive status in honeybees: the effect of feeding royal jelly to larvae, which leads to the development of fertile queen bees, could be replicated substantially by silencing the expression of the DNA methyltransferase Dnmt3 in newly hatched larvae. Remarkably, the majority of Dnmt3 siRNA-treated larvae emerged as queens (Kucharski et al., 2008). Furthermore, these studies established that environmental stimuli (in this case, nutritional stimuli) could influence DNA methylation if delivered at the appropriate time.

What relevance does this have to Th2 cells and asthma in children? Quite simply, epigenetic modifications are responsible for the establishment, maintenance and inheritance of the Th2 phenotype in CD4⁺ T cells (for reviews, see Bowen et al., 2008; Janson et al., 2008). The expression of Th2 cytokines and the accompanying upregulation of the transcription factor GATA3 are associated with epigenetic changes in chromatin structure, which are inherited by each of the daughter cells as they proliferate and recirculate. This 'heritable chromatin remodelling' (van Panhuys et al., 2008) eventually endows the body with a pool of allergen-specific memory Th2 cells. In these cells, epigenetic changes associated with lineage commitment are observed characteristically in the region of the Th2 cytokine gene cluster, with accumulation of active chromatin marks reflecting the transcriptional

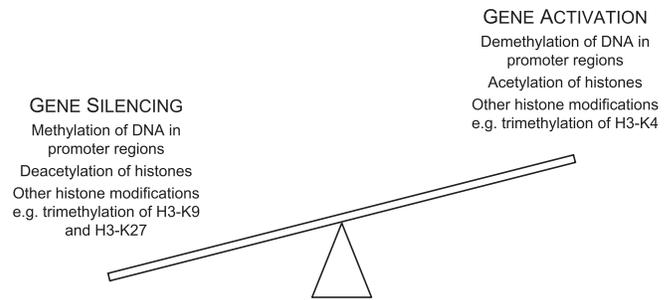


Fig. 1. Epigenetic regulation of gene expression involves shifting the balance between changes that cause gene silencing or activation.

Epigenetic changes are observed across the IL-4 locus in cells exhibiting Th2 differentiation. Stem cells, or cells that retain phenotypic plasticity, may exhibit both types of marks at key loci. Additional regulation is provided by control mechanisms such as microRNA.

upregulation of the cytokines encoded therein. These changes initially include the loss of methylation at the CpG sites flanking the GATA3 transcription factor binding sites within the first intron of the interleukin (IL)-4 gene, which is accompanied by increased H3-K9 acetylation and trimethylation of H3-K4 across the IL-4 locus. CpG demethylation spreads further upstream and downstream with lineage commitment (Lee et al., 2002). By contrast, naïve CD4⁺ T cells, as well as Th1-differentiated cells, retain the repressive epigenetic marks of dense CpG methylation and trimethylation of H3-K27 at the corresponding sites within the IL-4 locus (Fields et al., 2002; Lee et al., 2002; Tykocinski et al., 2005; Zhu and Paul, 2008; Wei et al., 2009). Epigenetic changes at the interferon (IFN)- γ locus also occur during lineage commitment, with increased H3-K9 acetylation and H3-K4 trimethylation in Th1 cells mirroring IFN- γ activation; by contrast, naïve CD4⁺ and Th2 cells retain repressive marks at the IFN- γ locus (Fields et al., 2002; Wei et al., 2009).

Linking environmental stimuli and asthma

Epigenetic regulation provides an attractive mechanistic explanation for some of the molecular events linking early-life environmental exposures with the subsequent development of disease (Miller and Ho, 2008). Most epigenetic alterations are believed to occur prenatally and shortly after birth, thus coinciding with the specific time periods when individuals are apparently most susceptible to the effects of environmental exposures and other triggers that induce asthma. However, there is limited evidence of a direct link between childhood asthma and epigenetic mechanisms. Nevertheless, environmental exposures (e.g. to airborne particulates) do have the potential to alter DNA methylation status (Baccarelli et al., 2009). Furthermore, in a recent study, Perera et al. (Perera et al., 2009) have correlated high-level maternal exposure to traffic-related polycyclic aromatic hydrocarbons with methylation of the CpG island of acyl-CoA synthetase long-chain family member 3 (ACSL3) and with the development of asthma symptoms in children before 5 years of age.

Some data are available regarding the induction, by environmental triggers, of epigenetic changes that might alter the expression of cytokines by CD4⁺ T cells. A study of the interaction

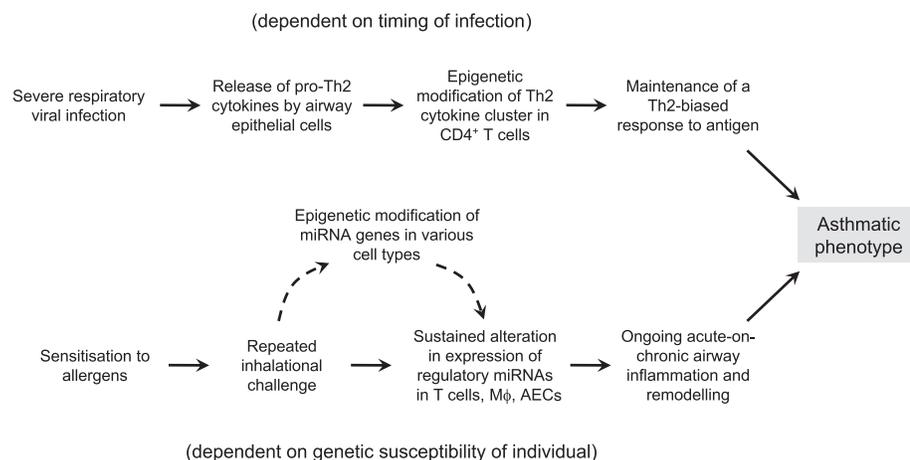


Fig. 2. A model of possible molecular mechanisms underlying the synergistic interaction between respiratory viral infection and chronic inhalation exposure to allergens in the pathogenesis of childhood asthma. AECs, airway epithelial cells; M ϕ , macrophages.

between inhalation of diesel exhaust particles and intranasal challenge with *Aspergillus fumigatus* demonstrated induction of hypermethylation at CpG sites of the IFN- γ promoter and hypomethylation of the IL-4 promoter, which correlated significantly with changes in IgE levels (Liu et al., 2008).

There is, however, currently no information about whether early-life respiratory viral infection, which is the best-recognised risk factor for childhood asthma, is associated with epigenetic changes that might predispose to the development of a Th2-polarised immunological response following exposure to allergens. Assessment of this relationship is complicated by the lack of suitable animal models of respiratory infection (RSV and human rhinovirus are species-specific and generally fail to replicate in mice) and of appropriate models of chronic allergen challenge. We have developed realistic mouse models of mild chronic asthma and allergen-induced acute exacerbations (Kumar and Foster, 2002; Siegle et al., 2006), and have established a model of early-life infection using pneumonia virus of mice (PVM), a paramyxovirus of the genus Pneumovirus that does induce a productive lower respiratory tract infection, simulating severe RSV disease in human infants (Domachowske et al., 2004; Rosenberg and Domachowske, 2008). Together, these models have, for the first time, provided direct experimental evidence that early-life pneumovirus infection synergises with allergen sensitisation and chronic challenge to induce development of a Th2-biased asthmatic phenotype (Siegle et al., 2009). Current studies in our laboratories focus on whether this phenotype is associated with epigenetic changes involved in the transcriptional regulation of T-helper cell differentiation, which would provide a crucial link in our understanding of the pathogenesis of asthma.

A possible mechanism for driving such changes might be the production of cytokines by airway epithelial cells (AECs) in response to viral infection/injury. A particularly relevant candidate in this context is IL-25, which is very effective in promoting a Th2 response (Fort et al., 2001; Sharkhuu et al., 2006; Angkasekwina et al., 2007). This concept is illustrated in Fig. 2, which shows our working model for the molecular events that might explain the epidemiological data indicating synergy between wheezing lower respiratory tract viral infection in early childhood and chronic inhalation exposure to allergens in the pathogenesis of asthma.

Micromanagement by RNA and methylation mimicry

Although the induction of a Th2-polarised pulmonary immunological response may be a precursor to childhood asthma, it does not lead inevitably to development of the disease. The central characteristic of asthma is chronic inflammation of the airways, on the background of which are superimposed episodes of acute inflammation, but atopy alone does not drive the development of such airway inflammation. Thus, another important question about disease mechanisms arises, namely, what sustains airway inflammation as asthma is established?

Recent attention has focused on the role of small non-coding RNA transcripts, designated microRNAs (miRNAs). These are short non-coding RNAs that regulate gene expression post-transcriptionally, usually by binding to the 3' untranslated region of their target mRNAs and repressing protein production by destabilising the mRNA. Because miRNAs are not perfectly complementary to their targets, each is capable of regulating a large number of mRNAs, and thus miRNAs can control entire 'transcriptional programs', although key individual targets may also be crucially important (Flynt and Lai, 2008). In asthma, miRNAs may be crucial in regulating the phenotypic programming of, for example, T cells, macrophages or AECs, to enhance the production of relevant cytokines and/or other mediators that promote the development of lesions (Yang et al., 2006; Mattes et al., 2007; Mattes et al., 2008).

Thus, it is plausible that airway inflammation in asthma may be sustained as a consequence of altered profiles of miRNA expression. Recently, we have obtained evidence that asthmatic inflammation is associated with characteristic patterns of miRNA expression and miRNA-mediated regulation of the host response (Mattes et al., 2009; P.S.F., A. Collison and R.K.K., unpublished). Moreover, targeting these upregulated miRNAs by treatment with antagomirs (chemically modified single-stranded RNA analogues, which are complementary to the target miRNA and are capable of long-lasting silencing) reveals their crucial functional roles in regulating airway inflammation (Mattes et al., 2009). However, it is unknown whether similar (or other) miRNAs are upregulated selectively following exposure to environmental triggers of childhood asthma, such as early-life respiratory viral infection, or following chronic allergen challenge after recovery from such infection. Identification of miRNA targets that regulate either host

defence responses to viral infection, or the subsequent allergic inflammation of the airways, offers considerable potential for novel approaches to therapy.

A further intriguing question is whether viral infection, or the subsequent allergen-induced chronic inflammation, generates a cellular environment in which inflammation becomes self-sustaining. It is noteworthy that, in chronic inflammation, epigenetic reprogramming may occur (Backdahl et al., 2008). Inflammation itself might be able to cause cytosine damage that alters methylation status. For example, hydroxymethylcytosine, generated as a result of oxidative stress, may lead to loss of methylation. Conversely, halogenated cytosine residues, which can form by reacting with hypochlorous acid (HOCl) from neutrophils or hypobromous acid (HOBr) from eosinophils, can lead to methylation mimicry (Valinluck and Sowers, 2007). Altered methylation status could result in activation or silencing of key genes that regulate the inflammatory response. These might include miRNA genes, which could thus lead to sustained alteration of regulatory networks or of the levels of expression of miRNA genes themselves. Such changes have been demonstrated in the setting of malignant neoplasia and the development of metastases (Lujambio et al., 2008; Meng et al., 2008), but have not yet been investigated in the setting of progression from a Th2-biased state to ongoing airway inflammation.

The concepts outlined above are also illustrated in Fig. 2. The possibility that epigenetic changes also drive ongoing airway inflammation and remodelling in asthma, through altered regulation of miRNA genes, provides a unifying hypothesis that integrates DNA methylation, modification of histones, and changes in miRNA as mechanisms that might contribute collectively to the synergy between key triggers in the pathogenesis of childhood asthma.

Meddling with epigenetics

Can epigenetic mechanisms that contribute to asthma be harnessed for primary prevention or therapeutic application? The notion is not as implausible as it might first seem. In a recent study using a mouse model, Hollingsworth et al. (Hollingsworth et al., 2008) showed that a maternal diet that is rich in methyl donors enhanced the severity of allergic airway inflammation in the offspring. Presumably there is some merit in the inverse inference, namely that modification of the diet of a pregnant mother might, through epigenetic mechanisms, be able to diminish the severity or likelihood of development of asthma in her child. The impact of dietary modification, both prenatally and during early childhood, is a potentially fruitful area for further research.

There is evidence that acetylation of histones is relevant to the maintenance of T-cell differentiation and that skewing of the cytokine profile of T cells towards a Th2 bias is crucially dependent on the activity of histone deacetylase (HDAC) (Su et al., 2008). The importance of HDAC as a potential therapeutic target has recently been demonstrated elegantly in a mouse model of Huntington's disease, in which chronic administration of HDAC inhibitors ameliorated disease manifestations (Thomas et al., 2008). The use of HDAC inhibitors to suppress immunologically driven inflammation, through the induction of FoxP3⁺ regulatory T cells, is currently an area of active investigation (Szyf, 2009; Wang et al., 2009), and could also have relevance to asthma.

Little is yet known about approaches to modify miRNA expression, although at least some miRNA may be regulated hormonally or nutritionally (Cheung et al., 2009). Whether some such intervention might prove to be relevant to suppressing the induction or progression of childhood asthma remains an intriguing possibility.

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COMPETING INTERESTS

The authors declare no competing financial interests.

REFERENCES

- Ampon, R. D., Correll, P., Marks, G. B., Poulos, L. and Waters, A. M. (2007). *Asthma in Australia: findings from the 2004-05 National Health Survey*. Canberra: Australian Institute of Health & Welfare.
- Angkasekwinai, P., Park, H., Wang, Y. H., Wang, Y. H., Chang, S. H., Corry, D. B., Liu, Y. J., Zhu, Z. and Dong, C. (2007). Interleukin 25 promotes the initiation of proallergic type 2 responses. *J. Exp. Med.* **204**, 1509-1517.
- Baccarelli, A., Wright, R. O., Bollati, V., Tarantini, L., Litonjua, A. A., Suh, H. H., Zanobetti, A., Sparrow, D., Vokonas, P. S. and Schwartz, J. (2009). Rapid DNA methylation changes after exposure to traffic particles. *Am. J. Respir. Crit. Care Med.* **179**, 572-578.
- Bacharier, L. B., Boner, A., Carlsen, K. H., Eigenmann, P. A., Frischer, T., Gotz, M., Helms, P. J., Hunt, J., Liu, A., Papadopoulos, N. et al. (2008). Diagnosis and treatment of asthma in childhood: a PRACTALL consensus report. *Allergy* **63**, 5-34.
- Backdahl, L., Bushell, A. and Beck, S. (2008). Inflammatory signalling as mediator of epigenetic modulation in tissue-specific chronic inflammation. *Int. J. Biochem. Cell Biol.* **41**, 176-184.
- Bernstein, B. E., Mikkelsen, T. S., Xie, X., Kamal, M., Huebert, D. J., Cuff, J., Fry, B., Meissner, A., Wernig, M., Plath, K. et al. (2006). A bivalent chromatin structure marks key developmental genes in embryonic stem cells. *Cell* **125**, 315-326.
- Bowen, H., Kelly, A., Lee, T. and Lavender, P. (2008). Control of cytokine gene transcription in Th1 and Th2 cells. *Clin. Exp. Allergy* **38**, 1422-1431.
- Caudri, D., Wijga, A., Scholtens, S., Kerkhof, M., Gerritsen, J., Ruskamp, J. M., Brunekreef, B., Smit, H. A. and De Jongste, J. C. (2009). Early daycare is associated with an increase in airway symptoms in early childhood but is no protection against asthma or atopy at 8 years. *Am. J. Respir. Crit. Care Med.* **180**, 491-498.
- Cheung, L., Gustavsson, C., Norstedt, G. and Tollet-Egnell, P. (2009). Sex-different and growth hormone-regulated expression of microRNA in rat liver. *BMC Mol. Biol.* **10**, 13.
- Dietert, R. R. and Zelikoff, J. T. (2008). Early-life environment, developmental immunotoxicology, and the risk of pediatric allergic disease including asthma. *Birth Defects Res. B. Dev. Reprod. Toxicol.* **83**, 547-560.
- Domachowske, J. B., Bonville, C. A. and Rosenberg, H. F. (2004). Animal models for studying respiratory syncytial virus infection and its long term effects on lung function. *Pediatr. Infect. Dis. J.* **23**, S228-S234.
- Fields, P. E., Kim, S. T. and Flavell, R. A. (2002). Cutting edge: changes in histone acetylation at the IL-4 and IFN-gamma loci accompany Th1/Th2 differentiation. *J. Immunol.* **169**, 647-650.
- Flynt, A. S. and Lai, E. C. (2008). Biological principles of microRNA-mediated regulation: shared themes amid diversity. *Nat. Rev. Genet.* **9**, 831-842.
- Fort, M. M., Cheung, J., Yen, D., Li, J., Zurawski, S. M., Lo, S., Menon, S., Clifford, T., Hunte, B., Lesley, R. et al. (2001). IL-25 induces IL-4, IL-5, and IL-13 and Th2-associated pathologies in vivo. *Immunity* **15**, 985-995.
- Hollingsworth, J. W., Maruoka, S., Boon, K., Garantzotis, S., Li, Z., Tomfohr, J., Bailey, N., Potts, E. N., Whitehead, G., Brass, D. M. et al. (2008). In utero supplementation with methyl donors enhances allergic airway disease in mice. *J. Clin. Invest.* **118**, 3462-3469.
- Holt, P. G. and Sly, P. D. (2007). Prevention of allergic respiratory disease in infants: current aspects and future perspectives. *Curr. Opin. Allergy Clin. Immunol.* **7**, 547-555.
- Jackson, D. J., Gangnon, R. E., Evans, M. D., Roberg, K. A., Anderson, E. L., Pappas, T. E., Printz, M. C., Lee, W. M., Shult, P. A., Reisdorf, E. et al. (2008). Wheezing rhinovirus illnesses in early life predict asthma development in high-risk children. *Am. J. Respir. Crit. Care Med.* **178**, 667-672.
- Janson, P. C., Winerdal, M. E. and Winqvist, O. (2008). At the crossroads of T helper lineage commitment-epigenetics points the way. *Biochim. Biophys. Acta.* **1790**, 906-919.
- Johansson, S. G., Bieber, T., Dahl, R., Friedmann, P. S., Lanier, B. Q., Lockey, R. F., Motala, C., Ortega Martell, J. A., Platts-Mills, T. A., Ring, J. et al. (2004). Revised nomenclature for allergy for global use: Report of the Nomenclature Review Committee of the World Allergy Organization, October 2003. *J. Allergy Clin. Immunol.* **113**, 832-836.

- Kim, J. H., Ellwood, P. E. and Asher, M. I. (2009). Diet and asthma: looking back, moving forward. *Respir. Res.* **10**, 49.
- Klose, R. J. and Bird, A. P. (2006). Genomic DNA methylation: the mark and its mediators. *Trends Biochem. Sci.* **31**, 89-97.
- Kouzarides, T. (2007). Chromatin modifications and their function. *Cell* **128**, 693-705.
- Kristjansson, S., Bjarnarson, S. P., Wennergren, G., Palsdottir, A. H., Arnadottir, T., Haraldsson, A. and Jonsdottir, I. (2005). Respiratory syncytial virus and other respiratory viruses during the first 3 months of life promote a local TH2-like response. *J. Allergy Clin. Immunol.* **116**, 805-811.
- Kucharski, R., Maleszka, J., Foret, S. and Maleszka, R. (2008). Nutritional control of reproductive status in honeybees via DNA methylation. *Science* **319**, 1827-1830.
- Kumar, R. K. and Foster, P. S. (2002). Modeling allergic asthma in mice: pitfalls and opportunities. *Am. J. Respir. Cell Mol. Biol.* **27**, 267-272.
- Kusel, M. M., de Klerk, N. H., Kebadze, T., Vohma, V., Holt, P. G., Johnston, S. L. and Sly, P. D. (2007). Early-life respiratory viral infections, atopic sensitization, and risk of subsequent development of persistent asthma. *J. Allergy Clin. Immunol.* **119**, 1105-1110.
- Lee, D. U., Agarwal, S. and Rao, A. (2002). Th2 lineage commitment and efficient IL-4 production involves extended demethylation of the IL-4 gene. *Immunity* **16**, 649-660.
- Liu, J., Ballaney, M., Al-alem, U., Quan, C., Jin, X., Perera, F., Chen, L. C. and Miller, R. L. (2008). Combined inhaled diesel exhaust particles and allergen exposure alter methylation of T helper genes and IgE production in vivo. *Toxicol. Sci.* **102**, 76-81.
- Locksley, R. M. (2009). Nine lives: plasticity among T helper cell subsets. *J. Exp. Med.* **206**, 1643-1646.
- Lujambio, A., Calin, G. A., Villanueva, A., Ropero, S., Sanchez-Cespedes, M., Blanco, D., Montuenga, L. M., Rossi, S., Nicoloso, M. S., Faller, W. J. et al. (2008). A microRNA DNA methylation signature for human cancer metastasis. *Proc. Natl. Acad. Sci. USA* **105**, 13556-13561.
- Mattes, J., Yang, M. and Foster, P. S. (2007). Regulation of microRNA by antagonirs: a new class of pharmacological antagonists for the specific regulation of gene function? *Am. J. Respir. Cell Mol. Biol.* **36**, 8-12.
- Mattes, J., Collison, A. and Foster, P. S. (2008). Emerging role of microRNAs in disease pathogenesis and strategies for therapeutic modulation. *Curr. Opin. Mol. Ther.* **10**, 150-157.
- Mattes, J., Collison, A., Plank, M., Phipps, S. and Foster, P. S. (2009). Antagonism of microRNA-126 suppresses the effector function of TH2 cells and development of allergic airways disease. *Proc. Natl. Acad. Sci. USA* (in press).
- Meng, F., Wehbe-Janeck, H., Henson, R., Smith, H. and Patel, T. (2008). Epigenetic regulation of microRNA-370 by interleukin-6 in malignant human cholangiocytes. *Oncogene* **27**, 378-386.
- Miller, R. L. (2008). Prenatal maternal diet affects asthma risk in offspring. *J. Clin. Invest.* **118**, 3265-3268.
- Miller, R. L. and Ho, S. M. (2008). Environmental epigenetics and asthma: current concepts and call for studies. *Am. J. Respir. Crit. Care Med.* **177**, 567-573.
- Mohapatra, S. S. and Boyapalle, S. (2008). Epidemiologic, experimental, and clinical links between respiratory syncytial virus infection and asthma. *Clin. Microbiol. Rev.* **21**, 495-504.
- Perera, F., Tang, W. Y., Herbstman, J., Tang, D., Levin, L., Miller, R. and Ho, S. M. (2009). Relation of DNA methylation of 5'-CpG island of ACSL3 to transplacental exposure to airborne polycyclic aromatic hydrocarbons and childhood asthma. *PLoS ONE* **4**, e4488.
- Rosenberg, H. F. and Domachowske, J. B. (2008). Pneumonia virus of mice: severe respiratory infection in a natural host. *Immunol. Lett.* **118**, 6-12.
- Rowell, E., Merckenschlager, M. and Wilson, C. B. (2008). Long-range regulation of cytokine gene expression. *Curr. Opin. Immunol.* **20**, 272-280.
- Schaub, B., Liu, J., Hoppler, S., Schleich, I., Huehn, J., Olek, S., Wieczorek, G., Illi, S. and von Mutius, E. (2009). Maternal farm exposure modulates neonatal immune mechanisms through regulatory T cells. *J. Allergy Clin. Immunol.* **123**, 774-782.
- Sharkhuu, T., Matthaei, K. I., Forbes, E., Mahalingam, S., Hogan, S. P., Hansbro, P. M. and Foster, P. S. (2006). Mechanism of interleukin-25 (IL-17E)-induced pulmonary inflammation and airways hyper-reactivity. *Clin. Exp. Allergy* **36**, 1575-1583.
- Siegle, J. S., Hansbro, N., Herbert, C., Yang, M., Foster, P. S. and Kumar, R. K. (2006). Airway hyperreactivity in exacerbation of chronic asthma is independent of eosinophilic inflammation. *Am. J. Respir. Cell Mol. Biol.* **35**, 565-570.
- Siegle, J. S., Hansbro, N., Herbert, C., Rosenberg, H. F., Asquith, K. L., Foster, P. S. and Kumar, R. K. (2009). Interaction between early-life viral infection and allergen exposure induces an asthmatic phenotype in mice. *Respirology* **14**, A16.
- Sigurs, N., Gustafsson, P. M., Bjarnason, R., Lundberg, F., Schmidt, S., Sigurbergsson, F. and Kjellman, B. (2005). Severe respiratory syncytial virus bronchiolitis in infancy and asthma and allergy at age 13. *Am. J. Respir. Crit. Care Med.* **171**, 137-141.
- Sly, P. D., Boner, A. L., Bjorksten, B., Bush, A., Custovic, A., Eigenmann, P. A., Gern, J. E., Gerritsen, J., Hamelmann, E., Helms, P. J. et al. (2008). Early identification of atopy in the prediction of persistent asthma in children. *Lancet* **372**, 1100-1106.
- Su, R. C., Becker, A. B., Kozyrskyj, A. L. and Hayglass, K. T. (2008). Epigenetic regulation of established human type 1 versus type 2 cytokine responses. *J. Allergy Clin. Immunol.* **121**, 57-63.
- Szyf, M. (2009). Epigenetics, DNA methylation, and chromatin modifying drugs. *Annu. Rev. Pharmacol. Toxicol.* **49**, 243-263.
- Thomas, E. A., Coppola, G., Desplats, P. A., Tang, B., Soragni, E., Burnett, R., Gao, F., Fitzgerald, K. M., Borok, J. F., Herman, D. et al. (2008). The HDAC inhibitor 4b ameliorates the disease phenotype and transcriptional abnormalities in Huntington's disease transgenic mice. *Proc. Natl. Acad. Sci. USA* **105**, 15564-15569.
- Tykocinski, L. O., Hajkova, P., Chang, H. D., Stamm, T., Sozeri, O., Lohning, M., Hu-Li, J., Niesner, U., Kreher, S., Friedrich, B. et al. (2005). A critical control element for interleukin-4 memory expression in T helper lymphocytes. *J. Biol. Chem.* **280**, 28177-28185.
- Valinluck, V. and Sowers, L. C. (2007). Endogenous cytosine damage products alter the site selectivity of human DNA maintenance methyltransferase DNMT1. *Cancer Res.* **67**, 946-950.
- van Panhuys, N., Le Gros, G. and McConnell, M. J. (2008). Epigenetic regulation of Th2 cytokine expression in atopic diseases. *Tissue Antigens* **72**, 91-97.
- Wang, L., Tao, R. and Hancock, W. W. (2009). Using histone deacetylase inhibitors to enhance Foxp3(+) regulatory T-cell function and induce allograft tolerance. *Immunol. Cell Biol.* **87**, 195-202.
- Wei, G., Wei, L., Zhu, J., Zang, C., Hu-Li, J., Yao, Z., Cui, K., Kanno, Y., Roh, T. Y., Watford, W. T. et al. (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity* **30**, 155-167.
- Wigle, D. T., Arbuckle, T. E., Turner, M. C., Berube, A., Yang, Q., Liu, S. and Krewski, D. (2008). Epidemiologic evidence of relationships between reproductive and child health outcomes and environmental chemical contaminants. *J. Toxicol. Environ. Health B Crit. Rev.* **11**, 373-517.
- Wong, G. W. and Chow, C. M. (2008). Childhood asthma epidemiology: insights from comparative studies of rural and urban populations. *Pediatr. Pulmonol.* **43**, 107-116.
- Yang, M., Mattes, J., Hansbro, P. and Foster, P. S. (2006). Employment of microRNA profiles and RNA interference and antagonirs for treatment of respiratory disease. *Drug Discov. Today* **3**, 325-332.
- Zhu, J. and Paul, W. E. (2008). CD4 T cells: fates, functions, and faults. *Blood* **112**, 1557-1569.