ORIGINAL ARTICLE

Allergic diseases, gene–environment interactions

H. Renz, M. Conrad, S. Brand, R. Teich, H. Garn & P. I. Pfefferle

Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, University Hospital Giessen and Marburg Faculty of Medicine, Philipps University Marburg, Germany

To cite this article: Renz H, Conrad M, Brand S, Teich R, Garn H, Pfefferle PI. Allergic diseases, gene–environment interactions. *Allergy* 2011; **66** (Suppl. 95): 10–12.

Keywords
Allergic disease; gene–environment interaction; early intervention; epigenetics.

Correspondence
Harald Renz, Institute of Laboratory Medicine and Pathobiochemistry, Molecular Diagnostics, University Hospital Giessen and Marburg Faculty of Medicine, Philipps University Marburg, Germany.
E-mail: renzh@med.uni-marburg.de

Accepted for publication 17 March 2011

DOI:10.1111/j.1398-9995.2011.02622.x

Abstract

Allergic asthma develops in part from dysregulation of the innate and adaptive immune functions, particularly an imbalance in the Th2-driven adaptive immune response. This dysregulation is the result of complex interactions between genes and environment. These interactions occur both pre- and postnatally, providing opportunities for early interventions in immunological programming.

Development of the inflammatory component of allergy and asthma depends on intimate interactions between the innate immune system, for example dendritic cells (DCs), and the adaptive immune system, particularly T lymphocytes. This interaction determines the type of T-effector cells, such as Th1/Th2, Th9, Th17, and Th22, that emerge. This model of innate and adaptive communication has been validated by research results over the last 20 years. It depends on the plasticity of the immune system, which determines whether a high Th2 response occurs with an array of inflammatory cytokines, such as interleukin (IL)-4, IL-5, and IL-13, which are the major drivers in the inflammatory immune response. On the other side, Th1 cells produce interferon (IFN)-γ, the signature cytokine of the counterregulator, and exert negative effects on Th2 immune responses. Allergy and asthma phenotypes develop from dysregulation and imbalance between the innate and adaptive immune response, which is perturbed in the allergic patient and is the result of intricate gene–environment interactions.

Environmental–maternal–fetal communication provides an intervention opportunity

Programming events in this system occur very early in life. The mother plays an important role in instructing the development of the fetal immune system. The mother receives information from the environment, and in turn, the fetus receives input about immune development from the mother (1). This close environment–maternal–fetal communication provides an important early window of opportunity for programming to occur early in life.

Thus, genetic disposition is not the only driving force in allergy, as demonstrated by a major analysis of monozygotic twins (2), comparing atopic phenotypes such as self-reported asthma and hyperresponsiveness to house dust mites as measured by skin prick test or lung function parameters. The results showed that a certain contribution of genes is necessary and required, but does not completely explain occurrence and development of allergic phenotypes. The search for additional environmental components responsible for allergy development showed changing results over the last two decades. For example, after the reunification of Germany, in the short time span of 20 years, the incidence of allergic phenotypes changed dramatically in the former East Germany in spite of very stable genetic dispositions (3).

Epigenetic contributions to allergy

Investigation of how the environment communicates to the genes has opened the new field of epigenetics to both basic and clinical science. Epigenetics is the inheritance of gene function without altering the genetic code. Our genome has

Table 1 Epigenetic regulation of IFN-γ production

Many CpG sites in Th genes are highly conserved	Young et al. (13)
Methylation of -53 CpG regulates IFN-γ production in TH1/TH2 development	Jones et al. (14)
Hypermethylation of IFN-γ CpG sites in cord blood CD4+ T cells	White et al. (7)
Inhaled diesel exposure induced hypermethylation of IFN-γ promoter	Liu et al. (6)

several main mechanisms of epigenetic control. DNA methylation can occur at the 30 million CpG islands found in the human genome. Histone modification can occur on the 30 million nucleosomes that are found on the DNA. Close nucleosome packing prevents gene expression, while opening of chromatin makes genes accessible to transcription and subsequent translation by making gene promoters accessible to transcription factors that bind and activate mRNA production. This step is prevented if CpG motifs in the promoter are methylated, which prevents transcription factor binding, but can be reversed through demethylation. Methylation patterns are not stable throughout life, with different patterns occurring in different cell types and organs. Patterns also change with time and in response to environmental pressures (Table 1).

Epigenetics affects allergy and asthma, as shown by dietary supplementation of mice with high or low amounts of methyl donors such as vitamin B12, folic acid, choline, or L-methionine during gestation. When F1 offspring were exposed to a protocol for experimental asthma, those from mothers receiving high levels of supplements showed a higher level of the asthma phenotype than offspring whose mothers received the low supplementation levels (4). This should not send the message that dietary supplementation with folic acid in pregnancy should be discontinued, because the results are still controversial and under investigation. Nonetheless, they demonstrate that changing the epigenetic codes during pregnancy can change the phenotype in the next generation.

This has implications for the development of the Th1–Th2 pattern later in life (5). The characteristics of Th2 cells include high production of IL-4, which requires opening of the *IL-4* gene promoter to allow binding of the transcription factor GATA-3. A high Th2-type immune response also requires silencing of the gene for the counterregulatory cytokine IFN-γ. On the other hand, high amounts of IFN-γ require an accessible *IFN*-γ promoter while silencing *IL-4*. Thus, the promoter status of these genes determines whether a Th1 or Th2 immune response develops. In turn, promoter status is under epigenetic control, as demonstrated by a study (6) investigating exposure to diesel exhaust particles, which is an epidemiological risk factor for asthma development. Exposure to diesel exhaust particles promoted high levels of IL-4 production and high amounts of allergen-specific IgE in an animal model of asthma. This was regulated by the degree of *IL-4* promoter methylation, which correlated with IgE production. Thus, environmental factors can modify gene expression and hence the risk of asthma. Another example is tobacco smoke, which has a profound effect on demethylation of many genes including some involved in the development of Th1 and Th2 cells.

IFN-γ gene regulation changes over life, with promoter methylation status starting at approximately 100% methylation. Therefore, at birth and at a young age, we make little IFN-γ. Studies in mice (7) showed that demethylation and opening of the *IFN*-γ promoter occurs later in life, demonstrating a high degree of plasticity in the regulation of this gene. Thus, the *IFN*-γ and *IL-4* genes are under close epigenetic control. In addition, many CpG sites and motifs in *IFN* genes are conserved and susceptible to methylation and demethylation, which change in response to environmental signals.

In addition, regulatory T-cell development is epigenetically regulated through the FOXP3 transcription factor, which is required to make T-regulatory cells that are critical for immunological tolerance (8, 9). This in turn is highly dependent on *FOXP3* expression, which is under epigenetic control (10). This has been shown in studies on hemotological conditions, but is not yet appreciated in the fields of immunology and asthma. The generation of regulatory T cells starts in the fetal stage and is under close control of maternal cells. So regulation occurs early in fetal life and is under control of maternal events that are dependent on environmental factors.

Protective effects of *Acinetobacter lwoffii*

A model for natural exposure to environmental factors, in particular naturally occurring microbes, is the traditional farming environment. Epidemiologically, this environment is strongly associated with an immunoprotective effect (11). Animal studies indicate that a variety of microbial compounds and strains exhibit an asthma-protective effect. These effects are bacterial strain dependent, and together with our collaborators, we have identified a bacterium with a highly protective effect: the Gram-negative bacterium *Acinetobacter lwoffii*. This bacterium protects both adult animals and gives transmaternal protection that is dependent on the innate immune system in the mother.

We asked whether this protective effect had an epigenetic component in a proof-of-principle study (12). *A. lwoffii* was administered to pregnant mice by inhalation, and the pups were introduced to a protocol for experimental asthma. Maternal exposure to the bacterium had an asthma-preventative effect on the offspring, as measured by mucus production, airway inflammation, and prevention of hyperresponsiveness. This effect was dependent on IFN-γ production in the offspring, as shown using IFN-γ blocking antibodies. The protective effect from maternal exposure to the bacterium was blocked when the offspring were depleted for IFN-γ activity.

To determine whether the effect involved epigenetic regulation, modification of the *IFN*-γ promoter was examined (H. Renz, in preparation). No changes in promoter methylation were observed, but histone acetylation showed a marked increase at the *IFN*-γ promoter. Inhibition of the histone

acetyltransferase with garcinol blocked the protective effect of the bacterium. This demonstrated that bacterial exposure during pregnancy might in part work by epigenetic modification, and this modification has a broad impact on the balance of Th2 and Th1 maturation and development. This in turn is important for the development of the clinical phenotype in the model animals.

Additional studies are ongoing, but the results open the field of epigenetic influence to more than just nutritional exposure. We can also consider the effects of microbial compounds, strains, and interference. These are all candidates of how we might modulate the programming of immune modulation. The proof-of-principle results from animal model studies must be now be taken into patient cohort studies to see whether the changes seen in animals are relevant to humans.

Conflict of interest

Harald Renz has received research grants from Allergopharma and Mead Johnson Nutritionals, consultancy fees from Allergopharma and lecture fees from Allergopharma, Bencard, and ALK-Abelló.

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