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Epigenetic aspects of the allergic diseases

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1. ABSTRACT

Several studies have proven the important influence that environmental exposures have in the individual's susceptibility to suffer allergy and other related diseases, mostly during embryonic or early life. Although the relationship between the environment and allergic diseases had been previously reported, one interesting attempt to describe this relationship was Strachan's hygiene hypothesis, proposed almost two decades ago. Since then, several studies have identified new environmental factors related to an increased risk of allergic disease. In this context, epigenetic modifications appear as a possible link between the environment and genome, providing a plausible mechanism for explaining how the recent changes in lifestyle can modify gene expression, and thus, lead to a disease state. Here, we will focus on the environmental modifiers that have been described and the possible role of epigenetic modifications.

2. INTRODUCTION

The term atopy involves different diseases such as allergic asthma, allergic rhinitis and atopic dermatitis. In a certain sense, atopy can be considered as a systemic inflammatory disorder derived from an unbalanced T helper 2 (Th2) response to allergens. Characteristically, Th2 responses involve an increase of interleukin (IL) 4, IL-5, IL-9 and IL-13. These responses result in an increase in the total serum IgE, the production of allergen-specific IgE and usually, the apparition of peripheral and tissue eosinophilia.

During the last decades there has been a constant increase in both the frequency and severity of atopic disorders, particularly in westernized countries (1,2). In 1989 Strachan proposed a hypothesis based on the inverse relation between the prevalence of allergic diseases and the family size and position in the household during childhood.

That could reflect the degree of cross-infections among siblings (3). This hypothesis, known as "Hygiene Hypothesis", was initially received with skepticism, but since then, different studies have elucidated new environmental factors contributing to the predisposition of suffering atopic diseases (4).

In the early 90's a plausible mechanism to explain these observations arose from the distinction between Th1 and Th2 lymphocyte populations (5). These two populations of cells are mutually counterbalanced and the reduction in one of them might involve an increase in the other population (6). The definition of the different T cell populations gave space to the hypothesis that lower exposure to microbial products, that normally induce Th1 responses, can induce an increase in Th2 responses, raising the individual susceptibility to asthma (4). This would imply that Th1-mediated disorders should decrease in general population, but some epidemiological studies have demonstrated that disorders mediated by Th1 responses (like multiple sclerosis or type 1 diabetes) have also increased during the last decades, suggesting that the problem could be more complicated than initially expected (7). This apparent paradox has been partially solved with the discovery of T regulatory cells, that are able to regulate both types of responses and could be implicated in the pathogenesis of allergic disorders, but this subject is beyond the scope of this review and has been reviewed elsewhere (8,9).

Although several years have passed since the first mention of the Hygiene Hypothesis, the controversy around still continues, suggesting that it remains far from being solved. The increase in atopic diseases has occurred in such a short period of time that it becomes difficult to be attributed only to genetic factors, which usually need more prolonged time periods to manifest (10). During the last decade, the science of epigenetics has increasingly developed offering new perspectives and opening a new challenging research area. Epigenetics may be defined as the study of any potentially stable and heritable but reversible change in gene expression or cellular phenotype that occurs without changes in the genotype. In this sense, epigenetics could provide another possible explanation for solving the puzzle.

3. EPIGENETICS

3.1 Definition of epigenetics

It is largely known that genetic information is encoded in DNA. In eukaryotic cells, small basic proteins named histones pack the DNA located in the nucleus. There are five different classes of histones: H1, H2A, H2B, H3 and H4. Eight of these molecules conform an octameric nucleosome complex by wrapping the DNA around their surface. The histones conforming these octamers are H2A, H2B, H3 and H4. The linker histone H1 binds the nucleosome and the entry and exit sites of the DNA. The interaction of DNA with the histones conforms the chromatin, a higher structure that can appear as euchromatin, for the transcriptionally active sites, or as heterochromatin, for those inactive regions.

As previously stated, epigenetics may be considered as the study of heritable changes in gene expression or cellular phenotype that occurs without changes in Watson and Crick base pairing of DNA. The role of these modifications is clearly depicted in the case of cellular differentiation: a multipotent stem cell can become many different cell types, each genetically identical but unique in its cellular phenotype (11). The gene expression differences are peculiar to each cell type and their differentiation stage, giving them special and characteristic features.

The epigenetic modifications include a myriad of mechanisms that involve DNA and chromatin changes. DNA methylation, histone modifications, the Polycomb tritorax complex or the non-coding RNAs are some of those mechanisms. Among these, the best-known epigenetic modification is DNA methylation. This chemical event occurs in all eukaryotic cells, ranging from animals to plants, at the 5-position (C5) of cytosine in CpG dinucleotides due to a family of enzymes known as DNA methyltransferases (DNMTs). In humans, five DNMTs (DNMT1, 2, 3a, 3b, and 3L) have been described to be involved in maintenance or in *de novo* methylation. Knockout studies in mice of any of those genes are lethal, implying that these genes are essential for the correct embryonic development (12). CpG dinucleotides are present in a lower frequency than expected across the human genome (13), probably because the spontaneous deamination of methylated cytosines into thymine makes them unusually susceptible to mutation and depletion (14) and have a specific species clustering, revealing a certain degree of conservation among species (15).

At gene promoters, cytosine methylation usually involves transcriptional silencing (Figure 1) and abnormalities in this mechanism can increase genomic instability leading to pathological conditions, like cancer. Even though DNA methylation is perhaps the most extensively studied epigenetic mark, how DNA methylation changes occur in a tissue and in a time specific manner or whether the demethylation process in mammals is active or passive, still remains to be fully elucidated.

Another important epigenetic mechanism of regulation is histone modification. This mechanism of control include acetylation/deacetylation, phosphorylation, ubiquitination and methylation of the histone tails, leading to different chromatin states, which can be open (transcriptionally active) or closed (inactive), depending on the accessibility for the transcription machinery. Although this fact is generally accepted, some studies reveal that it may not occur always in the same way. Depending on the specific lysine or arginine that is modified, either gene activation or repression may result (16,17). Histone acetylation is the best-understood mechanism, reflecting the balance of the histone acetyltranseferases (HATs) and deacetylases (HDACs) activity. 10 HDACs and 16 HATs have been described so far (18), confirming their importance in the regulation of the gene expression. More investigations are needed to fully understand this process.

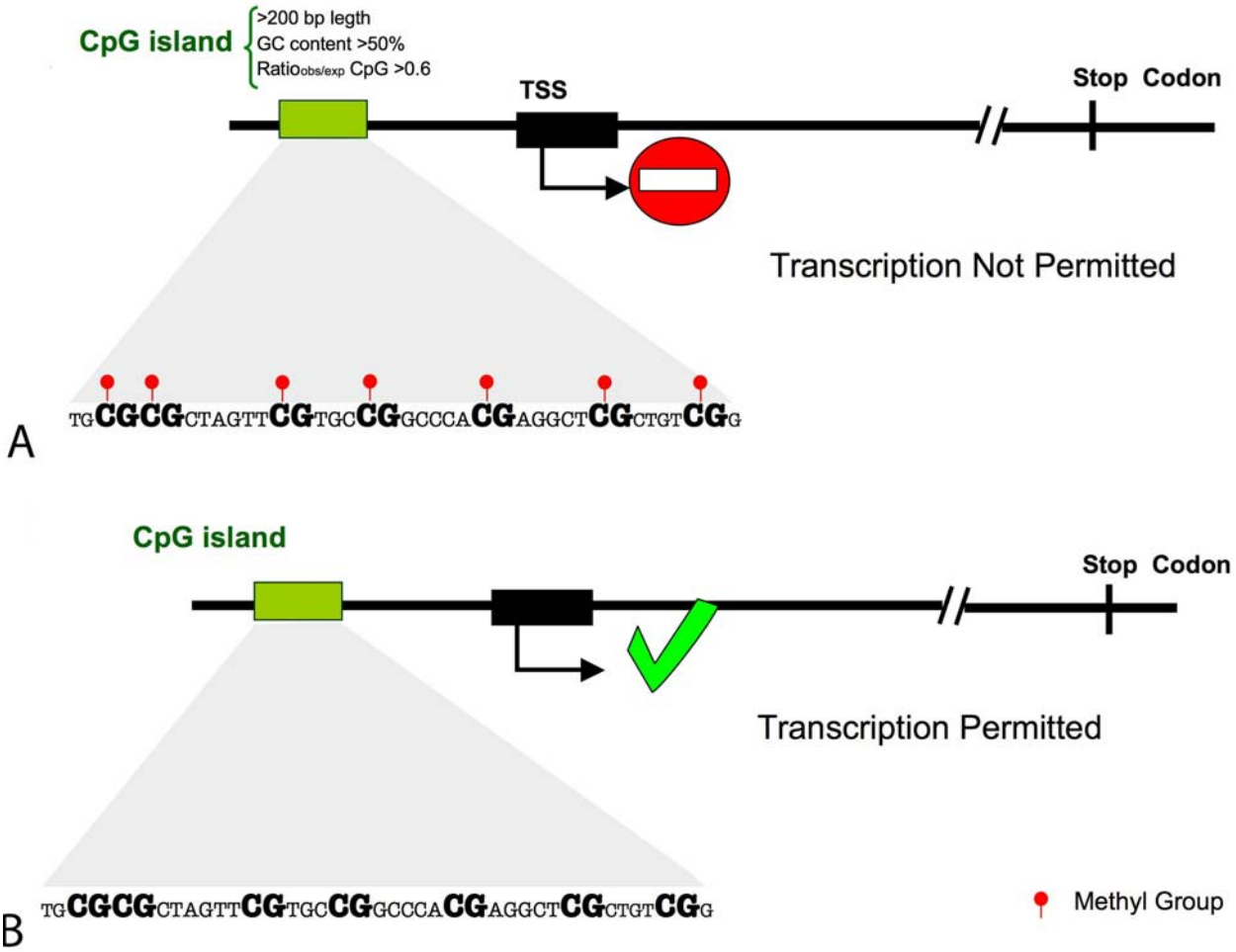

Figure 1. Promoters and DNA methylation. In eukaryotes, from plants to humans, the addition of a methyl group occurs exclusively at cytosines located in a CpG context. Surprisingly, this CpG dinucleotide is depleted in vertebrate genomes, with the exception of some regions, known as CpG islands, usually located at the promoters of the genes. These CpG islands are around 1000 bp long (always more than 200 bp), and are GC-rich (~65%) when compared with the whole genome (~40%). DNA methylation contributes to gene silencing (A), whereas DNA demethylation is related to gene expression (B), as represented in the illustration. TSS: Transcription Start Site.

4. ENVIRONMENT, EPIGENETICS AND ALLERGY

In order to consider the epigenome as a real candidate influencing the etiology of complex disorders, three characteristics have been proposed (19, 20): (i) it has to influence human phenotypes. In this sense, monozygous twins share the same genetic information but phenotypic discordance is observed in 25% of the cases. Epigenetic modifications could bring an explanation to this observation, as well as to sex effects, severity of the disease or age of disease onset (21). (ii) It has to be heritable, i.e. some epigenetic signals can be transmitted along with DNA sequence across germ line generations (22). In cloned human T cells, the acetylation histone pattern is retained through 20 cell divisions in the absence of external stimuli. This is of particular importance when considering the immune memory, because the histone modification profile at a given gene locus can be inherited through mitosis (23,24). And (iii) it should be influenced by "environmental" factors. Several studies have shown that the epigenetic status of genes is more dynamic in comparison to DNA sequence and can be altered by developmental programs and the environment of the organism.

4.1. Environmental tobacco exposure (ETS)

It is well documented that cigarette smoke exposure is the major cause of chronic and irreversible airway inflammation in lungs and, thus, airflow limitation, the hallmark of chronic obstructive pulmonary disease (COPD) (25). The macrophage number grows concomitantly with the severity of the disease in these patients, and the amount of proinflammatory cytokines released by macrophages has been shown to be higher in smokers (26). Interestingly, it has been stated that upon exposure to cigarette smoke extract, both HDAC activity and HDAC1, HDAC2, HDAC3 levels decreased in a macrophage cell line (27). A similar observation has been

done in rat lungs, where an inflammatory response is observed after exposure to cigarette smoke. In this study the authors report a decrease of HDAC2 expression caused directly by components of cigarette smoke (28). Taking into account the main importance of these enzymes in the maintenance of the correct epigenetic balance is not surprisingly to find a deregulation in the expression of some of the major proinflammatory cytokines.

Active and passive tobacco exposures have also been shown to predispose allergic sensitization in mice by disrupting the normal tolerance to innocuous allergens. A possible mechanism proposed, as stated for other substances (29), could be that cigarette smoke extracts improve the antigen presentation either by adsorption of the allergen or by structural modifications in the allergen itself (30). In addition, several studies have shown that smoking can down-regulate gene expression by hypermethylating the promoter of some genes normally expressed by a healthy lung (31, 32).

Interestingly, it has been described that not only maternal, but also grand maternal smoking during pregnancy may increase the risk of childhood asthma, suggesting that epigenetic changes that occur in response to ETS exposure can be transmitted to future generations (33).

4.2. Gender

Differences between male and female fetus appear around the sixth week of gestation, and they will persist throughout life. Despite the fact that female and male are physiological different and can be distinguished by endocrinology differences, it has been reported that the epigenetic pattern might be different for the same loci, reflecting sex differences (34). Epidemiological studies of asthma have shown that asthma is more prevalent in boys during childhood. However, during the puberty the ratio is inversed, and the largest percentage of women are affected during adulthood (35). In addition, it has been reported that the asthma onset may have a "parent-of-origin" effect, giving a possible explanation to the greater risk of developing asthma in children with a maternal history of asthma (36).

4.3. Diet

One factor that influences the epigenetic changes is diet. Deficiencies in folate intake, a common diet supplement, leads to a genome wide cytosine hypomethylation (37). Different studies have shown that supplementation of the mother's diet with single carbon donors, as vitamin B12 or folic acid has a color phenotypic effect in the offspring in mice (38, 39) by altering the DNA methylation and affecting the genetic expression. In this sense, it has been shown that *in utero* exposure to a diet rich in these supplements can enhance the risk of developing allergic airway disease in mice, and this trait is inherited transgenerationally (40). Diet fiber is fermented to short chain fatty acids including butyrate by endogenous bacteria in the large intestine. It is thought that butyric acid acts as a histone deacetylase inhibitor and might protect against colorectal neoplasia (41), although some controversy has arisen. Furthermore, the green tea extract EGCG (Epigallocatechin gallate) is known to be a competitive inhibitor of DNMT (DNA cytosine-5-methyltransferase), showing the influence of common dietary compounds in the epigenetic patterns (42). In a recent large North-European multi-centre study, it was shown that a minimum level of weekly fish intake is associated with protection against asthma, while subjects who never ate fish in childhood were at an increased risk for the disease (43). Moreover, recent data suggest that fish intake during pregnancy has a protective effect on the risk of atopy related outcomes (44). Whether unbalanced maternal nutrition during pregnancy alters epigenetic regulation and whether this is associated with increased disease risk in adulthood are questions pending to be solved. The possibilities to discover novel early biomarkers and risk factors that influence the onset of diseases, like asthma, have focused the attention of the researchers and the field is advancing rapidly (45).

4.4. Lifestyle

Several epidemiological studies have reported an increase in the prevalence of asthma and atopy among developed and developing countries (46), with a higher prevalence in subjects living in urban areas compared to those living in a farming environment (47-49). As stated previously, the short period of time for this increase in prevalence is unlikely due to genetic reasons, and many studies have tried to elucidate whether the farming environment exerts any protective role in the onset of atopic disorders. Contact with livestock or poultry (49), exposure to unpasteurized milk (50), or raw vegetable consumption (more frequent in farming families) (51) have been proposed as protective to lower incidence of the atopic diseases among children raised in farms.

Shaub and colleagues (52) investigated whether the maternal farm exposure during pregnancy exerts any effect in the regulatory T cells from cord blood, when compared with mothers living in a non-farming environment. A decrease in Th2 responses in the offspring from farm-exposed mothers has been reported, suggesting a natural model of immunomodulation that could prevent allergic disease development during adult life (1, 2)

It has been shown that farm milk consumption during pregnancy is significantly associated with lower level of methylation in a conserved element of FOXP3, which plays a major role in T regulatory cell differentiation and function (52). Improved public health, reduction in childhood infections (due to the widespread use of vaccines and antibiotics) (53), alteration in gut flora or reduction in endotoxin exposure, number of siblings (54), farm living and animal exposure (55) or age of day care attendance in infancy (56) have been related to this phenomenon, suggesting that the environment could play a decisive role in the onset of the disease. Whether the farming lifestyle is able to modify the epigenetic pattern and confer protection against atopic disorders or there are other mechanisms beyond this phenomenon is still waiting to be elucidated.

In another study, the investigators sought to asses whether the exposure of pregnant mothers to Airbone Polycyclic Aromatic Hydrocarbons (PAH), the main derivate of traffic related air pollutants, predispose the offspring to asthma related symptoms during childhood (57). The authors studied the DNA methylation patterns in umbilical cord white

blood cells from mothers with low or high exposure to PAH, and found that the promoter of ACSL3 was differentially methylated between groups. Methylation of this gene was significantly associated with PAH exposure and with parental report of childhood asthma. The finding supports the theory of 'fetal origins of adult disease' (58), which proposed that any aberration during *in utero* or early life can precede the development of diseases during adult life. Although the mechanisms that could be involved in this observation are not clear, alterations in the normal epigenetic patterns may cause a disease state.

Another possible explanation proposed to elucidate the "epidemic" increase in allergic diseases is a reduction in parasitic infections. Approximately two billion of world population is infected with various parasitic worms (59). However, whereas parasitic infections are highly prevalent in developing countries, they have been dramatically reduced or eradicated in developed countries, slightly before the increase in allergic disorders. In addition, epidemiological studies of subtropical populations with intensive chronic intestinal helminth infections have demonstrated a reduction in the prevalence of allergic disorders (60). Several studies have shown that the treatment of helminthic infection increases, at least partially, the rate of developing skin reactivity to aeroallergens (61) in humans and animal models, providing evidence that allergic and autoinmune diseases may be suppressed by helminth infections (62). Furthermore, it is known that helminth can induce a reduction in Th2 responses (63), providing a pathogenic mechanism that could explain the recent increase in Th2-driven allergic diseases in developed countries. Nevertheless, the effect has not been described for all parasites or worldwide (64).

In spite of the previous discussion about environmental factors, it is clear that atopic diseases have an important genetic component, as was demonstrated from the pioneering work of Coca and Cooke. Although nobody doubts the importance of the genetic background in the individual predisposition to suffer these diseases, genetic alone is not sufficient to cover all their pathogenic aspects. They are considered complex diseases (asthma and atopy among them) due to the great amount of genes involved in the onset, in addition to environmental agent contributions. It has been reported that more than 35 different genes are involved in the development of asthma, however, none of them have been found to be ultimately responsible for the development of the disease, reinforcing the fact that asthma is the result of complex interactions between genes and environmental factors that have not been fully described (32).

5. Th1/Th2 EPIGENETIC REGULATION

As above mentioned, atopic disorders are typically associated with Th2 responses, characterized by the production of IL-4, IL-5 and IL-13 cytokines involved in the IgE production or eosinophil activation (65). There are some evidences that highlight the importance of the epigenetic regulation in the activation of Th1/Th2 responses (66). STAT4 (Signal Transducer and Activator of Transcription 4) is a transcription factor involved in the regulation of interferon-gamma (INFgamma) expression, which plays a pivotal role in Th1/Th2 balance. STAT4-/- deficient mice appear to be more resistant to autoimmune diseases and have decreased airway hyperreactivity and eosinophil accumulation in asthma model (67). Some studies suggest that epigenetic control, such as DNA methylation in the promoter region, could be the major cause of the transcription and protein expression of STAT4 (68), although some controversy has arisen around this fact, suggesting that it could be more than one mechanism involved in controlling the expression of this transcription factor (69).

The role of INFgamma in asthma is controversial, probably due to the pleiotropic effects that this cytokine exerts. During childhood, the INFgamma response is initially reduced in subjects who subsequently develop atopic sensitization (70, 71). A diminished INFgamma feedback during initial immune responses against allergens may favor the outgrowth of atopy-associated Th2-memory cells (72).

The mechanisms by which this cytokine exerts its function remain unclear. However, some studies suggest that INFgamma is developmentally regulated by epigenetic mechanisms (73), showing that methytation levels were higher in CD4+ CD45RA+ T cells obtained from neonates than in those obtained from adults. During *in vitro* stimulation of CD4+ T cells towards Th1 response, the INFgamma promoter appears to be progressively demethylated, suggesting a possible control mechanism. However, the authors suggest that more studies are necessary to elucidate how the methylation patterns are able to regulate the INFgamma expression and how this expression is connected to the predisposition to the asthma onset (74).

As atopy has been associated with a reduced production of Th1 cytokines, it seems clear that the kinetics of post-natal maturation of INFgamma response plays an important role in determining susceptibility to allergic and infectious diseases. Currently, the balance between INFgamma and IL-4 production is believed to be an important determinant of risk for inflammatory diseases, in particular atopy (75).

A study performed in mice sensitized to *Aspergillus fumigatus* revealed that the combined exposure to *A. fumigatus* and Diesel Exhaust Particles (DEP) in these animals significantly increased their IgE production when compared with saline, diesel or *A. fumigatus* exposure alone. In this study the authors described a tendency of hypermethylation in several CpG located at the promoter of INFgamma and a hypomethylation tendency in IL-4 promoter, that also correlates with changes in IgE levels(76).

Similarly, it has been shown that after stimulation of CD4+ lymphocytes from sensitized human hosts versus controls with two of the major dust mite allergens, the levels of methylation in the promoter region of INFgamma and IL-4 are subject to change. The authors observed a tendency of demethylation in the IL-4 promoter after the stimulation in the cells from sensitized subjects. In contrast the level of methylation increased after stimulation in the INFgamma promoter in this population, which correlates with the expression levels of both cytokines. Besides the small number of subjects in this study, the data suggest that epigenetic

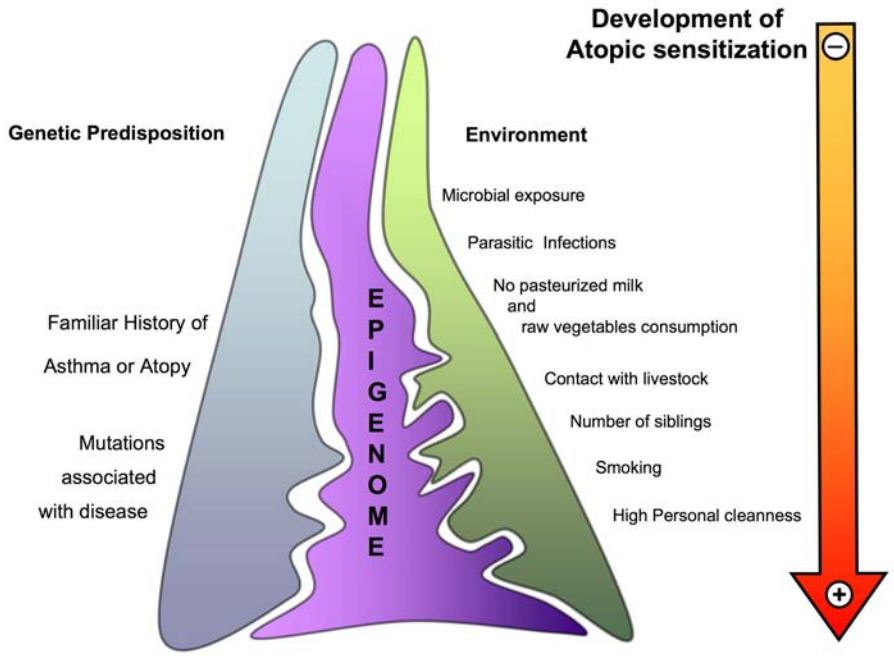

Figure 2. Interactions between individual Genetic Predisposition and Environment through Epigenome. How the environment influences the genetic predisposition of an individual to develop a disease like allergy or asthma is becoming an interesting field for a growing number of researchers. Here we illustrate how the epigenome is influenced by different environmental conditions, some of them "protective" as the microbial exposure and others predisposing to the disease, like smoking. The genetic component of the disease, the interaction with the environment and the influence of the epigenome are represented in this illustration, with an arrow showing the disease susceptibility, from top where the predisposition is low, to bottom, where the predisposition is higher.

control might be exerting an important regulation that need to be further investigated (77).

A study that evidences the potential role of epigenetics and atopy related diseases showed that lower levels of DNMT1 mRNA correlated with the onset of atopic dermatitis (AD) with extremely high levels of IgE (78). The fact that mRNA levels of DNMT-1 in this study did not correlate with all AD cases may suggest that DNMT-1 is not the only factor influencing serum IgE levels, and further investigations may be necessary to clarify this process.

6. QUESTIONS AND FUTURE DIRECTIONS

Several decades have passed since the first mentions of environmental factors influencing the individual's predisposition of developing a disease. Whether these theories and hypothesis are just speculations or real facts is subject to an intense debate and deep investigation. What cannot be denied is the tremendous impact that some environmental factors have on the onset of specific diseases. The field of epigenetics has now emerged as a candidate to integrate external environmental aggressions and disease progression, and deep knowledge of this intricate network is developing and becoming largely available.

With the promising insights previously described, many questions and challenges have arisen. Unraveling the different environmental factors that are influencing the individual predisposition to suffer asthma or other related diseases and whether they are related to changes in the epigenetic patterns in an *in vivo* experimental model could bring some light to the problem. When these modifications occur, whether they are inherited or whether they are able to being modified during a lifetime are questions waiting for an answer.

Should an epigenetic mechanism underlay this complex disease, could it be possible to distinguish healthy subjects to those that are suffering the disease simply by their epigenetic patterns? Will those hypothetical epigenetic patterns, differ for different phenotypes of the disease? For answering those questions we should be able to first define a 'normal' epigenetic pattern because, unlike the genome, the epigenome is different between cell types and fluctuates according to the environment or the phase in the cell cycle.

If we could correlate the epigenetic status of cell types with the associated disease we might be able to speak about new epigenetic biomarkers, perform epigenome association studies and provide new tools for a better diagnosis and prognosis of disease.

A deeper knowledge of the close interactions between genes, epigenetics and environmental factors in diseases like asthma or atopy will lead us to a better comprehension of the pathological process (Figure 2), bringing new targets for drugs discovery and the possibility not only to treat, but to prevent atopy related diseases.

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Abbreviations: Th1: T helper 1; Th2: T helper 2; IL: interleukin; IgE: Immunoglobulin E; DNMT: DNA methyltransferase; HAT: Histone acetyltransferase; HDAC: Histone deacetylase; EGCG: Epigallocatechin gallate; IFNgamma: Interferon gamma; STAT4: Signal Trasducer and Activator of Transcription 4; TSS: Transcription Start Site; PAH: Airbone Polycyclic Aromatic Hidrocarbons; COPD: Chronic Obstructive Pulmonary Disease; DEP: Diesel Exhaust Particles

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