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Epigenetic regulation of Th1 and Th2 cell development

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Abstract

All cells of the body, regardless of the tissue type, contain the same genetic material, but express this genetic material differently. Epigenetics is one process by which differential gene expression within a cell is regulated. Epigenetic mechanisms involve postsynthetic modifications to DNA and/or DNA-associated histones that do not change the DNA sequence itself, but which remodel chromatin, are passed along at each cell division, and occur during and after early development. The $CD4^+$ T cell best represents a cell in which epigenetic mechanisms are used to affect mature cell physiology. As a naïve $CD4^+$ T cell develops into either a Th1 or Th2 cell that secretes predominantly IFN-γ or IL-4, respectively, the expression of one cytokine gene and the permanent silencing of the other is orchestrated using epigenetic mechanisms. Because there appears to be an association between Th1/Th2 cell immunity, behavior, and/or disease, it is possible that an environmentally induced epigenetic change that occurs during Th1/Th2 cell development could explain how certain Th1/Th2-associated conditions develop. This article will review basic epigenetic mechanisms and what is known about how these mechanisms influence cytokine gene expression in a naïve $CD4^+$ T cell as it develops into a Th1 or Th2 cell.

Keywords: Epigenetics; $CD4^+$ T cell; Th1 cell; Th2 cell; Cytokines; DNA methylation; Histone acetylation

1. Introduction

An epigenetic-induced change in chromatin structure occurs via modifications to DNA and/or DNA-associated histones, e.g., gene silencing via DNA methylation and/or histone deacetylation/methylation. Epigenetic regulation of gene expression operates not only during early development, but also during post-developmental differentiation of mature cells. The environment is known to influence the development of diseases, such as cancer, via mutational effects on the genome. However, the environment is also known to influence the development of disease via epigenetic effects on the genome (Egger et al., 2004; Jaenisch and Bird, 2003; Vercelli, 2004). The role that the environment might play in mediating epigenetic changes in immunity, and how such changes might be associated with changes in immune-related diseases, is unknown at present.

2. Overview of gene expression

Gene expression in eukaryotic cells occurs when a gene encoded by DNA is transcribed into mRNA, and mRNA is translated into a functional protein. However, every gene is not expressed in every cell type. In eukaryotic cells, the ability to express biologically active proteins comes under regulation at several points, although chromatin structure and the initiation of transcription are the primary mechanisms that exist to regulate gene expression within a cell. In eukaryotes, transcription occurs in the context of chromatin, where DNA winds around a group of eight histone proteins to form a structure called a nucleosome. Specific DNA elements within the context of nucleosomal DNA are recognized by transcription factors that bind to the element to cause a disruption of the nucleosomal structure so that promotor or enhancer sequences surrounding the actual coding region of a gene become available for the binding of other transcription factors. The binding of transcription factors to a promoter determines not only when a gene is

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expressed, but also in which cell type the expression will occur.

The first transcription factors that bind to DNA recruit chromatin remodeling complexes that disrupt local chromatin structure to increase the stability of transcription factor–DNA complexes and to increase access to promoter sequences for binding of the transcription machinery. Epigenetic factors, i.e., factors that induce alterations in gene expression that can be passed on with each cell division without changing the DNA sequence of the gene (Wolffe and Matzke, 1999), most often affect accessibility of the chromatin to transcription factors and RNA polymerases. For the purposes of this review, epigenetic factors will be discussed that affect accessibility of DNA to transcription factors specifically. The reader is referred to the following review for a discussion of factors related to RNA polymerases and differential RNA splicing (Herbert, 2004). Mechanisms involved in the epigenetic control of gene expression involve a change to chromatin structure that is initiated by DNA methylation or demethylation, and/or modification of histones by the addition of methyl and/or acetyl groups to change chromatin structure. Such epigenetic mechanisms are used during early development when tight regulation of gene expression is required, particularly with regard to gene silencing when transcriptionally silent DNA is often associated with regions of highly methylated DNA, deacetylated histones, and a condensed chromatin structure (Nakao, 2001; Razin, 1998).

3. Epigenetic mechanisms

3.1. DNA methylation/demethylation

It was first proposed in 1975 that DNA methylation played a role in regulating the level of gene expression in eukaryotic cells (Holliday and Pugh, 1975; Riggs, 1975). Since then, many researchers have studied the role played by epigenetic mechanisms in regulating gene expression at the level of DNA and/or DNA-associated histones (Jiang et al., 2004). One epigenetic mechanism of gene silencing involves the methylation of DNA, specifically at the C5 position of a cytosine that is followed by a guanine in the dinucleotide sequence CpG [Fig. 1 and (Holliday and Pugh, 1975)]. In general, DNA in higher eukaryotes is devoid of the CpG sequence, except within areas termed CpG islands, that are found primarily within gene promotor sites. The CpG islands are defined as regions of at least 500 base pairs containing greater than a 55% G + C content (Takai and Jones, 2002) and are maintained primarily in an unmethylated state when a gene is expressed within a cell.

A CpG island becomes methylated through the action of a DNA methyltransferase (Bestor, 2000). When the island is methylated, gene repression occurs in a manner that can be passed on to each subsequent cell following division, without any change occurring in the DNA sequence itself. The activity of a specific promoter and the rate of gene transcription is often methylation-dependent (Hmadcha et al., 1999; Kang et al., 1999), such that an increased level of DNA methylation inhibits the binding of the transcriptional apparatus to the gene promoter, thus inhibiting gene transcription. Although the majority of gene silencing occurs during early development, further de novo methylation decreases as cells progress to end-stage differentiation. In contrast, de novo methylation occurs frequently in cell lines and clones (Kawai et al., 1994), as well as in cells that become malignant (Baylin and Herman, 2000). As will be discussed later, DNA methylation also occurs in lymphocytes, particularly in naïve $CD4^+$ T cells as they differentiate into effector Th1 and Th2 cells.

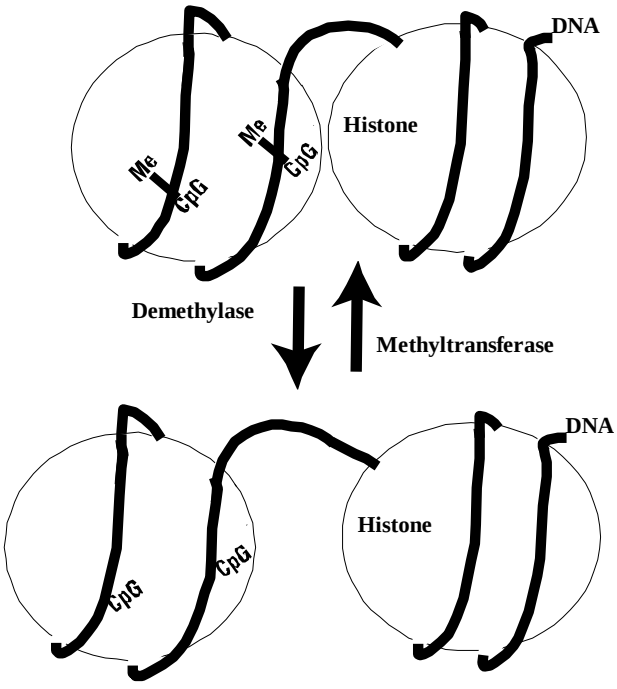

Fig. 1. DNA methylation/demethylation alters the conformation of a nucleosome. Nucleosomes with methylated DNA are more stable and less accessible than demethylated DNA. See text for detailed description.

The first requirement for gene transcription is increased locus accessibility that is achieved when the gene promoter dissociates from histones and when inhibitory methylation that inhibits the initiation of gene transcription is removed. Demethylation of methylated CpG sites is less well characterized, but occurs if either the DNA methyltransferase level is insufficient or the DNA methyltransferase is prevented from acting during replication [Fig. 1 and (Bird, 2002)]. Another proposed mechanism by which demethylation occurs is through the action of a DNA demethylase, which has not been isolated as yet, even tough a number of findings strongly suggest its existence (Bruniquel and Schwartz, 2003; Chen et al., 2003; Li, 2002; Martinowich et al., 2003; Mostoslavsky et al., 1998; Thomassin et al., 2001). One example of how a DNA demethylase may function in immunity comes from a study addressing IL-2 regulation by T cells. DNA demethylation of the promoter-enhancer region of the IL-2 gene regulates the level of IL-2 gene transcription in T cells within 20 min of their activation, with-

out the need for DNA replication (Bruniquel and Schwartz, 2003). The lack of a requirement for cell division to initiate DNA demethylation, coupled with the early time at which DNA demethylation was detected in a specific region of the IL-2 gene enhancer region, indicates that an active enzymatic mechanism, perhaps via DNA demethylase activity, is likely involved in the process.

3.2. Histone acetylation/deacetylation

Histones are structural proteins composed of two each of the following subunits, H2a, H2b, H3, and H4. The eight histone subunits stack to form a barrel-like structure that DNA wraps around. It was proposed in 1964 that an increase in transcription was associated with the acetylation of histones (Allfrey et al., 1964) by a histone acetyltransferase. Histone acetylation promotes the accessibility of transcription factors to promoter and enhancer regions of DNA [Fig. 2 and (Utley et al., 1998)], via a mechanism that involves an alloseteric change in nucleosome structure that is caused by a neutralization of the charge attraction between histones and DNA (Garcia-Ramirez et al., 1995; Lee et al., 1993). In contrast, histone deacetylation is catalyzed by a histone deacetylase that silences gene expression (Taunton et al., 1996), via a mechanism that involves a removal of the charge-neutralizing acetyl group, leading to the tight packing of DNA around histones to impose a physical barrier to the binding of the transcription apparatus and gene expression. Transcription is also regulated by histone methylation via a histone methyltransferase at H3Lys3 and/or H3Lys4 residues, primarily through a mechanism that is not well understood (Noma et al., 2001). Histone demethylation may also play an important role in the repression of transcription and appears to be initiated by a histone demethylase (Shi et al., 2004). Thus, the level of histone acetylation and methylation defines transcriptionally active and/or inactive areas of DNA (Jenuwein and Allis, 2001; Khan and Krishnamurthy, 2005).

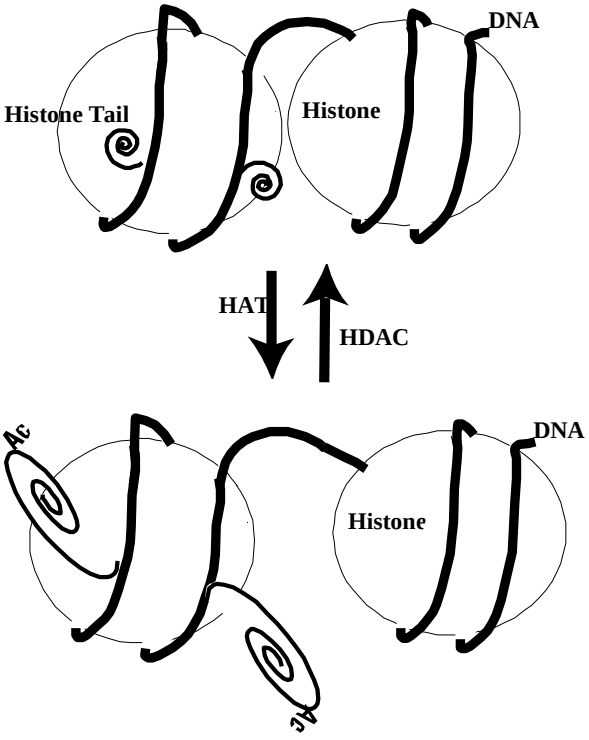

Fig. 2. Histone acetylation/deacetylation alters the conformation of a nucleosome. Nucleosomes with deacetylated histones are more stable and less accessible than acetylated acetylated. HAT, histone acetyltransferase; HDAC, histone deacetylase. See text for detailed description.

A major controversy in the field concerns the order in which epigenetic mechanisms might become activated before a gene can be permanently silenced. One study suggests that histone deacetylation/methylation may precede and trigger de novo methylation of DNA, although exceptions are evident (Bachman et al., 2003), via a mechanism that involves the activation of methyl transferase to initiate DNA methylation. Nonetheless, one thing is clear; namely that some change occurs in DNA that does not affect the DNA sequence itself, but which can be passed along to subsequent generations of cells as they divide and which can affect gene expression during development and post-developmental event.

4. Th1 and Th2 cell development and function

Perhaps one of the most striking examples of how epigenetic mechanisms are used to regulate gene expression post-developmentally involves specific cell types of the immune system. As some immune cells continue to differentiate after early development, they become prime candidates for epigenetically induced genetic changes that can be perpetuated for future generations of cells, without a change in DNA sequence. The immune cell type that best represents how epigenetic mechanisms affect cell function is the $CD4^+$ T cell, which is a family of cells that consists of a naïve T cell precursor and two effector T cell subsets, named Th1 and Th2, that develop from the naïve T cell after antigen exposure and are characterized by the secretion of IFN-γ and IL-4, respectively (Mosmann and Coffman, 1989). Although the polarization of Th1 versus Th2 cytokines and effector functions is readily apparent in murine models of infection and disease, the strict polarization of cytokines in human cells is less apparent, while the polarization of effector functions is clearer (Abbas et al., 1996; Romagnani, 1994; Romagnani, 2000). Th1 and Th2 cells function primarily to control the spread of pathogenic microorganisms. Th1 cells produce IFN-γ to promote a delayed hypersensitivity response (DTH) response (Cher and Mosmann, 1987), promote B cell class switching to complement-fixing antibodies such as IgG_{2a} in mice(Finkelman et al., 1990; Stevens et al., 1988), and provide protective immunity against intracellular pathogens (Mosmann and Coffman, 1989; Seder and Paul, 1994). In contrast, Th2 cells produce IL-4 to promote B cell class switching to neutralizing antibodies such as IgG_1 in mice/IgG_4 in humans and allergy/asthma-associated antibodies

such as IgE (Finkelman et al., 1990; Kaminski and Holsapple, 1987; Mosmann et al., 1986), regulate the intensity of a Th1 response (Hsieh et al., 1993), and provide protective immunity against extracellular pathogens (Seder and Paul, 1994). In addition, both Th1 and Th2 cells function to either control or promote susceptibility and resistance to a variety of clinical diseases. For example, Th1 cells play a crucial role in the development and/or progression of pro-inflammatory autoimmune diseases, such as rheumatoid arthritis and multiple sclerosis (Mosmann and Sad, 1996; Popko et al., 1997), in which increased IFN-γ production is found systemically, as well as locally, at inflammatory sites (Park et al., 2001; Yin et al., 1999). Conversely, the Th2 cytokine IL-4 plays a role in the pathogenesis of allergy and asthma (Mosmann and Sad, 1996). The involvement of Th1/Th2-associated cytokines in the development/progression of certain behavioral conditions is also apparent (Chida et al., 2005; Glaser and Kiecolt-Glaser, 2005; Schwarz et al., 2001). Therefore, a functional balance appears to be needed between IFN-γ- and IL-4-producing Th1 and Th2 subsets so that health can be maintained and clinical disease can be prevented.

The development of Th1 or Th2 cells begins with the activation of a common naïve T cell precursor that develops in the thymus before it populates lymphoid organs. One activation signal to the naïve T cell confers the antigen-specificity of the T cell response and is delivered through the TCR (Germain, 1994; Mitchison, 1971), while another activation signal provides costimulation and is delivered through CD28 (Linsley et al., 1993). A number of other factors affect the differentiation of a naïve T cell to a Th1 or Th2 cell, including the concentration of antigen, level and type of costimulation, physical properties of Ag, and the avidity of peptide/MHC interaction (Constant and Bottomly, 1997). However, the most important factor that determines whether a naïve $CD4^+$ T cell differentiates into a Th1 or a Th2 cell is the presence of a specific cytokine within the microenvironment in which the naïve T cell is activated. More specifically, Th1 development requires the presence of IL-12, while Th2 development requires IL-4 (Abbas et al., 1996). However, at the molecular level, it appears that specific transcription factors play the key roles in determining the effector subset that will develop. Specifically, IFN-γ gene expression is regulated by the transcription factor T-bet either directly at the IFN-γ gene level, or indirectly, via a T-bet-induced upregulation of the IL-12Rβ2 that binds IL-12 to activate STAT4, which maintains T-bet expression and enhances the amount of IFN-γ produced per cell (Szabo et al., 2003). In contrast, Th2 cell development is regulated directly by the transcription factor GATA-3, which is upregulated by STAT6 that is activated by IL-4R stimulation on a naïve T cell (Szabo et al., 2003). Thus, although IL-12/STAT4 and IL-4/STAT6, respectively, are necessary for optimal IFN-γ and IL-4 gene expression in Th1 and Th2 cells, the transcription factors T-bet (Szabo et al., 2000) and GATA-3 (Kurata et al., 1999; Ouyang et al., 2000; Zheng and Flavell, 1997), respectively, are essential for the induction of cytokine gene expression.

Therefore, the balance between Th1 and Th2 cells in a host depends on the expression and activation of key molecules within the microenvironment of an activated naïve $CD4^+$ T cell and within the cell itself. The mechanisms by which these key molecules either promote or repress expression of the appropriate or inappropriate cytokine genes within the naïve cell as it differentiates are being actively investigated and will be discussed below.

5. Regulation of cytokine gene expression during Th1 and Th2 cell development

It is reported that cytokine genes in a naïve T cell are only partially silenced, because a baseline low level of IFN-γ and IL-4 transcription is evident within an hour of naïve T cell activation, independent of T-bet or GATA-3 expression (Grogan et al., 2001). However, to sustain expression of one cytokine and repress the other in these cells, STAT4/T-bet or STAT-6/GATA-3 expression is critical, suggesting that polarized conditions in which IL-12 and IL-4, respectively, were available is also critical (Grogan et al., 2001). Also, the dependency of the differentiative process on cell division suggested that time was required for one cytokine locus to become completely accessible, while one was completely silenced (Grogan et al., 2001; Mullen et al., 2001b). Therefore, an epigenetic modification of cytokine genes was proposed as a mechanism to promote cytokine gene accessibility for one cytokine loci over the other as a naïve T cell differentiates into a Th1 or Th2 cell (Ansel et al., 2003; Szabo et al., 2003; Wilson et al., 2005).

Following the initial activation of a naïve T cell, GATA3 and T-bet mediate many of the chromatin structural changes that occur during naïve T cell differentiation that will render either the IFN-γ or IL-4 loci, respectively, accessible to regulatory enzymes and transcription factors (Ansel et al., 2003; Murphy and Reiner, 2002; Rao and Avni, 2000). Detecting areas of the genome that are associated with regulatory regions of active genes, and which must become accessible for transcription to occur, is possible because these areas are susceptible to DNase I digestion (Agarwal and Rao, 1998; Hardy et al., 1987, 1985). Overexpression of T-bet induces the DNase I hypersensitivity of the IFN-γ gene locus and enhances IFN-gene transcription (Mullen et al., 2001a; Szabo et al., 2000). T-bet induces IFN-γ by inducing a co-factor, HLX (Mullen et al., 2002; Zheng et al., 2004), which together with T-bet, appears to activate the IFN-γ locus synergistically to promote a remodeling of chromatin structure.

A role for DNA methylation in the regulation of IFN-γ in T cells was indicated by a number of findings. Increased IFN-γ gene expression was found in T cells activated in the presence of DNA methylation inhibitors (Young et al., 1986, 1994) and when T cells from DNA methyltransferase-knockout mice were used (Makar and Wilson, 2004). In addition, decreased DNA methylation was found in the IFN-γ promoter region of Th1 cells (Melvin et al., 1995), while an increase in de novo methylation was found in

T cells with reduced expression of IFN-γ (Mikovits et al., 1998). In addition, histone acetylation of the IFN-γ locus occurs in a STAT4/T-bet-dependent manner (Fields et al., 2002). As a result, intronic and distal elements within the IFN-γ gene become accessible to DNase I during Th1 differentiation so that full expression of the IFN-γ gene occurs (Agarwal and Rao, 1998). IL-4 silencing also occurs as a naïve T cell develops into a Th1 cell, and this silencing is associated with DNA methylation/histone deacetylation of the GATA-3 and IL-4 genes (Grogan et al., 2001; Mullen et al., 2001a).

As Th2 cells develop, increased expression of GATA-3 is implicated in the changes that occur within the IL-4 gene locus, including DNA demethylation (Bird et al., 1998), the appearance of DNase I hypersensitive sites (Lee et al., 2005, 2000; Takemoto et al., 2002), and histone acetylation of the IL-4 gene locus (Fields et al., 2002, 2004), all of which increase the accessibility of regulatory enzymes and transcriptional factors within the IL-4 locus. IFN-γ silencing occurs as a naïve T cell develops into a Th2 cell, and this silencing is associated with DNA methylation/histone deacetylation of the T-bet and IFN-γ genes (Grogan et al., 2001; Hewitt et al., 2004; Mullen et al., 2001a). As well, the gene silencing of GATA-3 in Th1 cells and T-bet in Th2 cells is cell cycle-dependent (Grogan et al., 2001; Mullen et al., 2001a). Thus, the role of epigenetic mechanisms in the commitment of a naïve T cell to the Th1 or Th2 lineage expressing distinct patterns of cytokine genes is evident, but much remains unknown concerning the precise mechanisms by which this occurs.

6. Epigenetics and the environment

Th1 and Th2 cell activity is critical for the control of infectious organisms and susceptibility/resistance to disease. But if all cytokine genes in a naïve T cell are initially methylated, but transcriptionally active, then epigenetic mechanisms must commence to either promote cytokine gene accessibility or repress further gene accessibility as a naïve T cell differentiates into a Th1 or Th2 cell. Therefore, any factor that induces a change in the epigenetic process may also induce a change in the effector cell cytokine pattern of an individual, which could subsequently change their susceptibility/resistance to disease. One mechanism by which such changes are precipitated in the epigenetic regulation of gene expression involves changes that occur within the environment of an individual.

One example of how the environment uses epigenetic mechanisms to establish a gene expression pattern in cells is through diet. A key amino acid needed for the de novo methylation process is *S*-adenosylmethionine (Jiang et al., 2004). For this amino acid to be synthesized, methyl groups and co-factors must be available, primarily obtained from the diet in the form of folic acid and vitamin B12. The agouti gene expressed in mice, which causes melanocytes in the hair follicles to switch from producing black to yellow melanin, is the best known example of how maternal diet can affect gene expression (Millar et al., 1995; Wolff et al., 1998). If the agouti mouse diet is rich in methyl donors, then the agouti gene of offspring is repressed via de novo methylation, and the mouse color will appear black (Waterland and Jirtle, 2003). Thus, the nutritional status of these mice in young life may determine the extent of CpG methylation within the agouti gene promoter, and thus influence heritable changes that will occur and persist into adult life. The aging process itself appears to be another way in which epigenetic changes occur to affect disease development and behavioral changes, without a change in the DNA sequence itself (Egger et al., 2004; Jaenisch and Bird, 2003; Jiang et al., 2004; Richardson, 2002; Vercelli, 2004). The latter possibility is supported by findings that the clinical phenotype between identical twins changes as the twins age together, i.e., they show differential expression of the same genes, but that this change is not associated with a change in the primary DNA sequence (Martin, 2005). These findings suggest that epigenetic factors associated with the aging process itself, in combination with specific environments to which the twins are exposed as they age, induce an epigenetic-mediated phenotypic change in one twin, but not the other. Thus, environmental factors, such as diet and aging, may exert an effect on a naïve T cell as it is developing into a Th1 or Th2 cell in a way that will change the effector cell phenotype. Also, environmental factors may affect the differentiated T cell itself to change what cytokines are produced, and passing this change along as the cell divides. What is important to assume, however, is that such environmentally induced epigenetic changes may be either harmful or beneficial to the survival of the host.

Therefore, an epigenetically induced change in the balance between the number of Th1 and Th2 cells, as well as the level produced of their respective cytokines, may be partially responsible for changes that occur in disease susceptibility/resistance, as well as in various behavioral conditions (Chida et al., 2005; Schwarz et al., 2001). Some individual differences in human disease and behavior that cannot be accounted for genetically may be due to epigenetic changes in gene expression, which are propagated at the cellular level, that allow the organism to adapt to changes within a particular environment. Because there appears to be an association between behavior and disease, and because Th1/Th2 immunity is linked closely to disease and behavior, it is probable that environmentally induced epigenetic changes might explain how certain Th1/Th2-associated diseases occur, without a change in DNA sequence. The development of some clinical diseases may involve environmentally induced epigenetic mechanisms (Egger et al., 2004; Jaenisch and Bird, 2003; Jiang et al., 2004; Richardson, 2002; Vercelli, 2004) induced by diet, aging, or the use of certain pharmacological agents. If these diseases/conditions are associated with a slant to a specific Th1 or Th2 cell profile, then the development of such profiles by environmentally induced epigenetic mechanisms during naïve T cell differentiation

is a possible cause. Of course, this is a hypothesis that requires intensive testing, but the rationale to begin testing it is clear.

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