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Epigenetics in human autoimmunity:

(Epigenetics in autoimmunity—DNA methylation in systemic lupus erythematosus and beyond)

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Abstract

Epigenetic mechanisms are essential for normal development and function of the immune system. Similarly, a failure to maintain epigenetic homeostasis in the immune response due to factors including environmental influences, leads to aberrant gene expression, contributing to immune dysfunction and in some cases the development of autoimmunity in genetically predisposed individuals. This is exemplified by systemic lupus erythematosus, where environmentally induced epigenetic changes contribute to disease pathogenesis in those genetically predisposed. Similar interactions between genetically determined susceptibility and environmental factors are implicated in other systemic autoimmune diseases such as rheumatoid arthritis and scleroderma, as well as in organ specific autoimmunity. The skin is exposed to a wide variety of environmental agents, including UV radiation, and is prone to the development of autoimmune conditions such as atopic dermatitis, psoriasis and some forms of vitiligo, depending on environmental and genetic influences. Herein we review how disruption of epigenetic mechanisms can alter immune function using lupus as an example, and summarize how similar mechanisms may contribute to other human autoimmune rheumatic and skin diseases.

Keywords

Epigenetics; DNA methylation; lupus; psoriasis; atopic dermatitis; vitiligo

Epigenetics and systemic autoimmunity

Systemic lupus erythematosus

Human lupus is a systemic autoimmune disease primarily affecting women. Lupus is characterized by autoantibody formation to nuclear antigens and immune complex deposition in tissues such as the kidney and blood vessels. Studies of familial systemic lupus erythematosus (SLE) indicate that multiple genetic loci are involved in determining SLE-susceptibility [1]. However, incomplete concordance in monozygotic twins, by definition carrying the same SLE-susceptibility genes, suggests that environmental factors are also important [2,3]. Established examples of exogenous agents affecting lupus include drugs like

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procainamide and hydralazine that cause a lupus-like disease, and UV radiation, which triggers lupus flares [4]. As discussed elsewhere in this issue, reactivation of latent retroviral and Epstein–Barr genes is also proposed as a mechanism. Epigenetic changes, and in particular altered DNA methylation, are likely to be involved in these environment–host interactions. Insights into the mechanisms derive from studies on the role of DNA methylation in regulating gene expression in mature T cells.

DNA methylation and T-cell autoreactivity—Early studies used the DNA methyltransferase (Dnmt) inhibitor 5-azacytidine (5-azaC) to probe for functional changes caused by DNA methylation inhibition in mature T cells. One observation was that CD4 + T cells become autoreactive following 5-azaC treatment. CD4 + T cells normally respond to peptides presented in the antigen binding cleft of “self” class II major histocompatibility antigen (MHC) molecules on antigen presenting cells. Following 5-azaC treatment, antigen specific CD4 + T cells lose the requirement for specific antigen and respond to antigen presenting cells without added antigen. The response is specific for self class II MHC molecules and is reversible, in that antigen responsiveness recovers 1–2 weeks after the Dnmt inhibitor is removed [5]. The autoreactivity has been demonstrated with cloned and polyclonal human and murine CD4 + T cells. CD8 + T cells do not become autoreactive; the reason is unknown [5,6].

Mechanistic studies revealed that development of autoreactivity correlates with increased expression of the adhesion molecule LFA-1 (CD11a/CD18), caused by increased levels of CD11a (*ITGAL*) transcripts [7]. The increase in *ITGAL* expression is due to demethylation of *alu* elements 5' to the *ITGAL* promoter [8]. LFA-1 overexpression caused by transfection results in identical autoreactivity in human [9] and murine [10] T cells. LFA-1 surrounds the T-cell antigen receptor (TCR) to form the “immunologic synapse” providing both stability to the TCR–MHC interaction as well as co-stimulatory signals that activate T cells [11]. Overstabilizing the lower affinity interaction between the TCR and “self” class II MHC molecules bearing inappropriate peptide fragments, together with increased costimulatory signaling, may be responsible for initiating the T-cell response to self MHC molecules without the appropriate antigen.

T-cell DNA demethylation and autoimmunity—The response of demethylated CD4 + cells to self class II MHC molecules demonstrates that normal, antigen reactive T cells can be modified by exogenous agents to become autoreactive, potentially contributing to an autoimmune disease. In animal models, CD4 + T cells responding directly to class II MHC molecules cause chronic graft-vs.-host disease, with many features of human lupus, including anti-nuclear antibodies (ANAs) and an immune complex kidney disease [12]. This suggests that 5-azaC modified, class II responsive cells may also cause a disease resembling SLE. Pathogenicity of the autoreactive cells was demonstrated by injecting cloned or polyclonal 5-azaC treated CD4 + T cells intravenously into syngeneic mice. The recipients developed anti-DNA antibodies and an immune complex glomerulonephritis as well as other histologic features of autoimmunity, depending on the T cells treated and/or the strain [13]. LFA-1 transfected CD4 + T cells caused a similar lupus-like disease in the same system, indicating that LFA-1 overexpression contributes to the autoimmunity induced by demethylated T cells [10].

Another consequence of increased adhesion between T cells and antigen presenting cells is that demethylated or LFA-1-transfected CD4 + T cells kill autologous or syngeneic macrophages (M ϕ) without specific antigen, also in an MHC restricted fashion [7,10]. The killing utilizes conventional mechanisms of apoptosis involving the death receptor Fas, TRAIL and TWEAK, [14] but demethylated CD4 + T cells also acquire perforin expression through demethylation of *PRF1* [15], and perforin inhibitors prevent the killing [16]. The autoreactive

killing of Mø and perhaps other cells by hypomethylated T cells could stimulate ANAs by increasing antigenic apoptotic material. Injection of apoptotic cells into mice causes anti-DNA antibodies, as does impairment of molecules involved in clearing apoptotic debris. Mø killing would result in the release of apoptotic material. Further, since Mø remove apoptotic debris, the material will not be cleared effectively, amplifying the effect. A role for Mø apoptosis in lupus-like autoimmunity is supported by studies demonstrating that causing Mø apoptosis *in vivo* with clodronate-filled vesicles, selectively phagocytosed by Mø to cause their death, induces anti-DNA and antinucleosome antibodies in normal mice and accelerates autoimmunity in lupus-prone mice [17].

Anti-DNA antibody production may be augmented by B-cell overstimulation, due to overexpression of cytokines and cell surface costimulatory molecules by demethylated T cells. Co-culture of demethylated T cells with autologous B cells results in IgG hypersecretion [6], due in part to increased expression of Th1 and Th2 cytokines including IFN- γ , IL-4 and IL-6 [13] and in part to overexpression of B-cell costimulatory molecules including CD70 and CD40L [18-20]. IL-4 and IL-6 are suppressed by methylation in Th1 cells, and IFN- γ in Th2 cells, and expression is induced by 5-azaC [21]. Similarly, T cells treated with DNA methylation inhibitors also overexpress the B-cell costimulatory molecule CD70 (*TNFSF7*), and overexpression is due to demethylation of a region flanking the *TNFSF7* promoter. Co-culture of B cells with autologous CD4 + T cells treated with DNA methylation inhibitors results in IgG overproduction that is reversed with anti-CD70 antibodies [18,22]. CD40L is another methylation sensitive B-cell costimulatory molecule expressed on CD4 + T cells. However, in contrast to CD70, CD40L (*CD40LG*) is encoded on the X chromosome, so men have one unmethylated gene in CD4 + T cells, while women have one methylated and one unmethylated gene. 5-azaC doubles CD40L expression on CD4 + T cells from women, due to demethylation of the gene on the inactive X, but has only minimal effects on CD40L in CD4 + T cells from men [19].

Together, these studies suggest that demethylated, autoreactive T cells could interact with Mø *in vivo* to cause apoptosis and release of antigenic nucleosomes, resulting in anti-DNA antibodies, and overstimulate B cells, increasing autoantibody production. Such cells might be more potent inducers of autoimmunity than cells made autoreactive by LFA-1 transfection alone. This was tested by injecting mice with LFA-1 overexpressing T cells caused by transfection, or T cells demethylated with a Dnmt inhibitor. Demethylated T cells were more potent in inducing autoimmunity than transfected cells [10].

DNA methylation and drug induced lupus—As noted above, procainamide and hydralazine are lupus-inducing drugs. Both cause ANAs in a majority of people and a lupus-like disease in a subset [23]. Development of the systemic manifestations presumably requires the presence of lupus susceptibility genes [24]. Interestingly, both are DNA methylation inhibitors. Procainamide is a competitive inhibitor of Dnmt 1 enzymatic activity, reducing the affinity of the enzyme for its substrates, hemimethylated DNA and S-adenosylmethionine [25]. It has no effect on intracellular S-adenosylmethionine or S-adenosylhomocysteine pools [26]. In contrast, hydralazine selectively inhibits T- and B-cell extracellular signal-regulated kinase (ERK) pathway signaling, preventing upregulation of T-cell Dnmt 1 and 3a during mitosis, resulting in hypomethylation of the daughter cells [27], as well as receptor editing in B cells [28]. Pathogenicity of decreased ERK pathway signaling was demonstrated by treating CD4 + T cells with U0126, a selective mitogen-activated protein kinase (MAPK) inhibitor that decreases ERK pathway signaling, and injecting the cells into syngeneic mice. The treated T cells overexpressed LFA-1 and became autoreactive, and mice receiving the treated cells developed anti-DNA antibodies, similar to procainamide treated cells [29]. More recent studies mapped the hydralazine-induced ERK pathway defect to protein kinase C- δ (PCK- δ), and confirmed that transfection of T cells with a dominant negative PKC- δ demethylates T-cell

DNA and causes CD70 overexpression [30,31]. Interestingly, the PKC- δ knockout mouse develops lupus [32].

DNA methylation and idiopathic lupus—Similar mechanisms contribute to idiopathic human lupus. T cells from patients with active lupus have decreased total deoxymethylcytosine content and decreased Dnmt 1 transcripts in lupus T cells relative to patients with inactive lupus and normal controls [33]. Since lupus T cells have multiple signaling abnormalities [34], and Dnmt 1 expression is regulated by the ERK and Jun N-terminal kinase (JNK) pathways [27], T-cell signaling was examined in human lupus. Patients with active but not inactive lupus had decreased ERK phosphorylation in response to stimulation that was identical to hydralazine treated cells [29], while signaling through the JNK and p38 pathways was intact [35]. Interestingly, the lupus ERK pathway defect maps to PKC- δ , also inhibited by hydralazine [30].

Other studies revealed additional functional and epigenetic similarities between lupus and experimentally demethylated T cells. Functional studies demonstrated that lupus T cells overstimulate autologous B-cell antibody production, similar to 5-azaC treated T cells, and that the overstimulation is inhibited with anti-CD70 or anti-CD40L [18,36]. A subset of lupus T cells overexpresses LFA-1, and this subset spontaneously kills autologous M ϕ in an MHC restricted, autoreactive fashion identical to experimentally demethylated cells [7]. Further, patients with active lupus have circulating, apoptotic monocytes in their peripheral blood, suggesting that a similar killing occurs *in vivo* [37].

Epigenetic studies demonstrated demethylation of the same *ITGAL* promoter sequences in CD4 + lupus T cells as in 5-azaC treated T cells. The degree of demethylation was proportional to disease activity [38]. Similarly, CD4 + T cells from patients with active lupus demethylated the same *PRF1* sequences and aberrantly expressed perforin [16]. Concanamycin, a perforin antagonist, prevented the autoreactive M ϕ killing by lupus T cells, implicating perforin in this phenomenon [17].

Also similar to the *in vitro* model, bisulfite sequencing revealed that the core *TNFSF7* promoter is normally demethylated in CD4 + T cells, and that 5-azaC extends the demethylated region upstream by ~300 bp. Cassette methylation confirmed transcriptional suppression when the region is methylated. Importantly, other DNA methylation inhibitors including procainamide, hydralazine, and the MEK inhibitor U0126 all demethylated the same sequence and increased CD70 expression as 5-azaC. CD4 + T cells from lupus patients overexpressed CD70 and demethylated the same region [18,22]. Further, CD4 + T cells from women with active lupus also demethylated the *CD40LG* gene on the inactive X and overexpressed CD40L, while men with the same degree of lupus activity did not overexpress CD40L [19]. This suggests that demethylation of the inactive X may contribute to increased incidence of lupus in women. Thus, for at least four genes (*ITGAL*, *TNFSF7*, *PRF1* and *CD40LG*), identical changes in methylation, expression and function are found in experimentally demethylated and lupus T cells.

Histone modifications in lupus—The role of histone modifications in lupus is less well understood. Treating lupus T cells with histone deacetylase inhibitors including trichostatin A and suberoylanilide hydroxamic acid restores aberrant expression of some genes [39]. However, these drugs also modify acetylation of transcription factors, nuclear transport proteins, and cytoskeleton proteins [40], and confirmatory studies at the chromatin level still need to be performed in lupus T cells.

Summary—Identical epigenetic effects occur in experimentally demethylated and lupus T cells at the DNA, mRNA, protein, and functional levels for at least four genes contributing to

T-cell autoreactivity, B-cell overstimulation and macrophage killing (*ITGAL*, *TNFSF7*, *CD40LG* and *PRF1*, respectively). Experimentally demethylated T cells cause a lupus-like disease in animal models. At least two lupus-inducing drugs are DNA methylation inhibitors with effects on T cells identical to those caused by 5-azaC and found in idiopathic lupus. In idiopathic and hydralazine-induced lupus, DNA demethylation appears to be caused by a failure to upregulate Dnmt 1 during mitosis, due to a defect in ERK pathway signaling that maps to PCK- δ , and the PKC- δ knockout mouse develops lupus. It seems reasonable to propose that defective T-cell DNA methylation may contribute to the pathogenesis of lupus in genetically predisposed individuals.

Epigenetic changes in other systemic autoimmune disorders

Rheumatoid arthritis—Rheumatoid arthritis (RA), a systemic autoimmune disease causing a destructive arthritis, is also associated with genome-wide T-cell DNA hypomethylation [33]. However, the genes affected appear to be different than those in lupus [22,38], and the mechanism causing the demethylation is unknown. Methotrexate, used to treat RA, increases d^mC levels in RA T cells, which may contribute to its therapeutic benefits [41]. However, methotrexate is a folate antagonist, so this effect is paradoxical and may be secondary to other effects on the disease process. None-the-less, the increased methylation is interesting and deserves further study.

Progressive systemic sclerosis—Progressive systemic sclerosis (PSS), or scleroderma, is a rare and poorly understood condition characterized by excessive collagen deposition in skin as well as other organs. PSS is considered an autoimmune disease because of the presence of autoantibodies to nuclear and other autoantigens, and frequent overlap with other autoimmune diseases. Aberrant fibroblast activation and collagen secretion contributes to the fibrosis in PSS. The *FLII* gene, encoding a regulatory protein that suppresses collagen synthesis, is aberrantly methylated in PSS fibroblasts, which could result in increased collagen synthesis. This raises the possibility that fibroblast DNA methylation abnormalities may contribute to this disorder [42].

X chromosome skewing and autoimmunity—Women develop autoimmune disease more frequently than men. The mechanisms causing this sexual dimorphism are unknown, but skewed X chromosome inactivation may contribute, particularly in scleroderma [43] and autoimmune thyroiditis [44]. This is discussed elsewhere in this issue of *autoimmunity*.

Epigenetics in autoimmune skin disorders other than lupus

Although lupus has a cutaneous component typified by the characteristic “butterfly” rash, it is fundamentally a systemic disorder, affecting multiple organs. The following sections summarize evidence for epigenetic influences in autoimmune diseases predominantly of the skin, including atopic dermatitis (AD), psoriasis and vitiligo. Epigenetic regulation via genomic imprinting, in which genes from one parent are silenced by methylation, plays a role in several dermatologic diseases but will not be discussed here. The reader is referred to a recent review of this topic [45]. Unlike lupus, the study of epigenetics in skin disease is still in its infancy. Further, many of the susceptibility genes for autoimmune skin disorders are still unknown. However, incomplete disease concordance between monozygous twins with AD, psoriasis and vitiligo indicate that environmental factors likely play an important role in the etiology of autoimmune skin disease similar to that in lupus, and may involve epigenetic mechanisms.

Skin is not only the largest organ in the body; it is an immune organ as well. The skin is host to T cells, Langerhans cells (epithelial dendritic cells), tissue M ϕ , monocytes, granulocytes, mast cells, and lymphatic endothelial cells [46]. Langerhans cells are the major resident antigen

presenting cell of the skin, and form an extensive network in the epidermis. Similar to “professional” immune cells, keratinocytes are also capable of producing an extensive list of cytokines including IL1 α/β , IL6, IL7, IL10, IL12, IL13, IL15, TNF- α , TGF- β and growth factors such as SCF and GM-CSF. Many of these are proinflammatory and directly influence the immune response [47]. Genetic and epigenetic factors that influence keratinocyte functions, both barrier and immunologic, can have a profound impact on both innate and adaptive immune responses. Thus, the skin environment is an orchestra of cells and factors that direct immune and inflammatory responses to infectious agents, chemicals and self antigens.

Atopic dermatitis—AD (eczema) is a common skin disease that can occur at any age, but the majority of cases arise before the age of five [48]. Epidemiologic studies show a slight but reproducible prevalence in girls. AD patients with high levels of IgE are especially susceptible to viral infections (herpes simplex, Molluscum contagiosum, verrucae) and superficial fungal infections (*Trichophyton rubrum*, *Pityrosporum ovale*), suggesting a defect in delayed type hypersensitivity. In the acute phase of AD, activated CD25 + HLA-DR + CD4 + T cells with enhanced IL2R, selectin, CD40L and perforin expression appear in the dermis [48,49]. Both CD40L and perforin expression have been shown to be important in lupus autoimmunity and are upregulated by demethylation [15,19]. However, the mechanism by which CD40L and perforin expression is enhanced in AD has not been determined. Epidermal and dermal Langerhans cells bear elevated CD1a, CD1b, CD36 and CD32 molecules, suggesting that they are in a heightened state of antigen presenting activity for autoreactive T cells [50]. Barrier-disrupted skin in AD may enhance sensitivity to environmental agents such as allergens and bacterial products, as well as skew the Th1/Th2 response induced by these agents. Percutaneous sensitization with allergens through barrier disrupted skin induces a predominantly Th2 response, shown by elevated IL-4 and decreased IL2 and IFN- γ Th1 responses [51].

The genetic basis of AD susceptibility is complex. A loss of function of the gene *FLG*, encoding filaggrin, an important stratum corneum precursor protein, was recently reported in 30–40% of AD patients with European descent [52]. Filaggrin is a 37 kD protein that aggregates the keratin cytoskeletal system to form a dense protein–lipid matrix. When crosslinked by transglutaminases the matrix forms the cornified cell envelope of keratinocytes, providing a barrier that prevents both water loss and entry of allergens into the epidermis. The identification of two *FLG* mutations, R501X and 2282del4, which result in the complete loss of the processed protein, and the association of the null alleles with AD and associated asthma in AD patients, represents a major breakthrough in understanding this disease [52]. The genetic basis of AD is probably the strongest of the major dermatologic autoimmune diseases, with a concordance of ~72% in monozygotic twins [53]. Thus, environmental factors, perhaps acting through epigenetic mechanisms, may contribute to disease pathogenesis. In support of this, Dnmt 1 transcripts in peripheral blood mononuclear cells of AD patients with high IgE levels are significantly lower than controls [54]. The effect of reduced Dnmt 1 levels on IgE may be indirect, with DNA hypomethylation of T cells resulting in their increased production of IL-4, which then stimulates IgE production by B cells [55,56]. Enhanced immune responses at epidermal surfaces, caused by DNA hypomethylation of methylation-sensitive immune genes, together with the activation of genes critical to T/B interactions and inflammation, could initiate a sustained autoimmune response.

Psoriasis—Psoriasis is a chronic, relapsing T-cell-mediated disease of the epidermis characterized by erythematous plaques of various sizes, often covered by a silvery scaling [57]. Psoriasis occurs equally in men and women, and exhibits enhanced keratinocyte proliferation caused by exuberant inflammatory infiltrates into the epidermis and dermis [58]. Although genes predisposing to psoriasis are still under intense investigation, environmental/epigenetic factors likely play a role in disease pathogenesis, as the concordance in monozygous twins is only 35–56% [59,60]. One candidate gene for epigenetic regulation is *SHP-1* (or

PTPN6), a non-receptor tyrosine phosphatase and possible tumor suppressor that regulates growth and proliferation in a variety of tissues [61-63]. *SHP-1* activation is also thought to play a role in this psoriasis. For example, the *SHP-1* promoter is demethylated in psoriatic skin but not in AD or normal skin [63]. Interestingly, STAT3 together with Dnmt 1 and histone deacetylase mediates epigenetic silencing of *SHP-1*, contributing to malignant transformation of T cells [61]. In a mouse model of psoriasis, constitutively active Stat3 targeted to murine keratinocytes induces lesions closely resembling psoriasis, demonstrating an important role for STAT3 in disease pathogenesis, although the specific effects on target genes and downstream signaling pathways depends upon the cell type and the range of transcription factors available [63,64].

Besides defects in T-cell function, abnormalities in B cells, neutrophils, and the cytokine environment occur in psoriasis. Zhang et al. [65] reported that the *P16* gene was demethylated in hematopoietic stem cells from psoriasis patients, decreasing their proliferative activity and colony forming capacity *in vitro*. Increased p16 protein could disrupt formation of active kinase complexes, preventing transition of cells from G1 to S phases of the cell cycle. Increased *P16* transcription may reflect the effect of cytokines on the hematopoietic environment or indicate a defect in the hematopoietic stem cell itself. A defect in hematopoietic stem cells serving as a susceptibility factor in psoriasis may be inferred from transplantation studies, where bone marrow from donors with psoriasis administered to patients without psoriasis resulted in disease development [66]. In contrast, transplantation of non-psoriatic bone marrow into a psoriasis patient resulted in long-term disease remission [67]. Methylation sensitive T cells genes implicated in the pathogenesis of lupus are also expressed in T cells from patients with psoriasis. For example, demethylation of the perforin promoter by 5-azaC treatment or in lupus results in aberrant upregulation of the gene and enhanced cytotoxic activity by CD4 + T cells, and enhances perforin expression in CD8 + T cells [15,16]. High numbers of perforin-positive CD4 + and CD8 + lymphocytes are found in psoriatic plaques and sites of inflammation in allergic contact dermatitis [49,68], although the methylation status of the perforin gene was not examined in those studies.

A second methylation sensitive gene product overexpressed in both lupus and psoriasis is LFA-1 (CD11a/CD18). 5-azaC increases T-cell LFA-1 expression by demethylating elements 5' to the CD11a (*ITGAL*) promoter [8]. LFA-1 expression is similarly elevated in psoriasis, and has been successfully used as a target for biologics based therapy in that disease (reviewed by Gibling and Lemieux, [69]). Again, the methylation status of *ITGAL* has not been investigated in psoriasis.

Genetic imprinting, a phenomenon involving selective epigenetic inactivation of the maternal or paternal allele of certain genes, was suggested as a mechanism for preferential paternal transmission of psoriatic arthritis. Psoriatic arthritis occurs in 10–40% of patients with psoriasis [70]. Although occurring equally in men and women, the transmission of psoriatic arthritis appears to be dependent on the gender of the affected parent, with a paternal transmission rate significantly higher than maternal [71]. A genetic locus on chromosome 16q was identified as both a psoriasis susceptibility gene and a locus potentially involved in paternal transmission of psoriatic arthritis [72]. A susceptibility gene in which a mutation confers increased risk of disease, could be preferentially transmitted by one parent in a non-Mendelian fashion by imprinting.

Vitiligo—Vitiligo is the most common depigmenting disorder, and affects approximately 0.38% of the population worldwide [73,74]. It occurs with similar frequency in Caucasians and other ethnic groups, and affects men and women equally. Vitiligo is clinically characterized by progressive, often bilateral patchy loss of pigmentation of the skin, overlying hair and mucous membranes [75]. Vitiligo vulgaris (or generalized vitiligo), acrofacial vitiligo (which

can progress to more generalized vitiligo), and vitiligo universalis (which progresses to complete or near complete loss of cutaneous melanocytes), are associated with other autoimmune diseases including autoimmune thyroiditis, RA, psoriasis and lupus [76,77].

Vitiligo is thought to be a disorder in which genetic and environmental factors contribute to the autoimmune destruction of melanocytes [75,78]. While most cases of generalized vitiligo are sporadic, 15–20% of cases have an affected first degree relative, and the inheritance pattern is suggestive of a polygenic, multifactorial disease [78]. A linkage between a locus at chromosome 17p13 and multiple autoimmune diseases associated with vitiligo was recently described [78,79]. This locus contains genes involved in immune recognition and inflammation, including the lupus-associated gene *SLEVI*, and has been proposed as an autoimmune susceptibility locus [80]. *NALP1* is another major susceptibility gene for generalized vitiligo and other vitiligo-associated autoimmune diseases [79]. *NALP1* encodes NATCH, the leucine-rich-repeat protein-1. *NALP1* is thought to direct the assembly of the NALP1-inflammasome, which regulates the activation of caspases that convert proinflammatory cytokines such as IL-1 β to their active forms [81].

Despite the importance of genetic susceptibility in vitiligo, environmental factors may be even more important, since the concordance of vitiligo among genetically susceptible monozygous twins is only 23% [76]. Onset of vitiligo has been described in association with pregnancy, severe sunburn, and physical trauma to an area that subsequently depigments. However, the mechanism by which these factors contribute is unclear. Circulating autoantibodies to melanocyte proteins and skin-homing, melanocyte-specific cytotoxic T cells have been described, but the lesions are not usually associated with inflammation. One exception that may provide insight into the role of an anti-melanocyte immune response is that therapeutic strategies designed to enhance immune responses to melanocyte antigens on melanoma tumor cells by decreasing naturally occurring regulatory T cells, results in depigmentation in a pattern reminiscent of generalized vitiligo [82].

Unfortunately there are few good animal models to study mechanisms involved in vitiligo and its relationship to other autoimmune diseases. However, treatment of a vitiligo-susceptible chicken strain with the DNA methylation inhibitor 5-azaC causes feather depigmentation due to loss of skin melanocytes [83]. In this model, both T cells and B cells are involved in the disease, and the chickens develop antibodies to the melanocyte-specific protein TRP-1 that cross-reacts with mouse and human melanocytes. Chronic low dose administration of 5-azaC also increases the incidence of autoimmune thyroiditis in a chicken model for Hashimoto's autoimmune thyroiditis [84]. These results support the interrelationship between vitiligo and autoimmune thyroiditis observed in humans and suggest a role for demethylation of immune genes in the pathogenesis of these autoimmune diseases in genetically susceptible individuals.

Conclusions

T lymphocytes differentiate throughout life, and epigenetic mechanisms play an essential role in the regulation of subset specific genes. The expression of some of these genes, such as IL-4 in Th1 cells, IFN- γ in Th2 cells, and perforin in CD4 + cells, involve changes in DNA methylation, and pharmacologic demethylation of these genes can lead to their reexpression. The level of expression of some genes, like *ITGAL* and *TNFSF7*, is also modified by the methylation status of regions flanking their promoters. Current models indicate that failure to maintain these methylation patterns can modify T-cell gene expression and hence immune function, contributing to the development of lupus-like diseases and perhaps other forms of autoimmunity. Further studies are needed to extend these observations and identify ways to correct these abnormalities.

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