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# Transmethylation in immunity and autoimmunity

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## Abstract

The activation of immune cells is mediated by a network of signaling proteins that can undergo post-translational modifications critical for their activity. Methylation of nucleic acids or proteins can have major effects on gene expression as well as protein repertoire diversity and function. Emerging data indicate that indeed many immunologic functions, particularly those of T cells, including thymic education, differentiation and effector function are highly dependent on methylation events. The critical role of methylation in immunocyte biology is further documented by evidence that autoimmune phenomena may be curtailed by methylation inhibitors. Additionally, epigenetic alterations imprinted by methylation can also exert effects on normal and abnormal immune responses. Further work in defining methylation effects in the immune system is likely to lead to a more detailed understanding of the immune system and may point to the development of novel therapeutic approaches.

### Keywords

Autoimmunity; Transmethylation; T cells; Lupus; EAE

## 1. Introduction

Nucleic acids and proteins can be fine-tuned by a wide spectrum of modifications, which are essential for regulating gene transcription and amplifying protein repertoires. Major post-translational modifications include phosphorylation, methylation, acetylation, nitration, citrullination and glycosylation. Phosphorylation, because of its ubiquitous role in cell signaling, has been the most studied with regard to immunity, but accumulating evidence indicates that methylation also plays an important role in immune cell function. Interestingly, post-translational modifications may trigger a break in tolerance and provoke an anti-self response [1], suggesting that inhibition of methylation may be a means to intervene in inflammatory or autoimmune processes. A past hurdle in such an approach was that transmethylation inhibitors were irreversible and somewhat toxic, but less toxic reversible inhibitors have now been developed, and their applicability to a variety of abnormal immunological responses has been tested. We summarize herein recent findings pertaining to methylation and its role in immune system function, and discuss the potential therapeutic utility of methylation inhibitors in immune-related diseases.

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**10. Conflict of interest statement**
The author(s) declare that there are no conflicts of interest.

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## 2. The transmethylation pathway

The major players controlling transmethylation are S-adenosylmethionine (SAM) and the methyltransferase (MT) superfamily, which includes DNA methyltransferases (DNMTs), protein arginine methyltransferases (PRMTs), and protein lysine methyltransferases (PKMT), among others. The terms methylation and transmethylation are used interchangeably to describe the general donation of methyl groups from SAM to various macromolecules [2–4]. MTs remove a methyl group from SAM, thereby converting it to S-adenosyl-L-homocysteine (SAH), which can act as a potent feedback inhibitor of upstream transmethylation reactions by blocking most MT activity (Fig. 1). Since the reactions are reversible, the ratio of SAM to SAH influences whether SAH is converted back into SAM or, more likely, hydrolyzed by S-adenosyl-L-homocysteine hydrolase (SAHase) into adenosine and homocysteine. Thus, blockade of SAHase activity raises intracellular SAH levels, reduces MT activity, and indirectly inhibits transmethylation reactions.

In mice with the non-agouti mouse $a^x$-mutation lacking the gene (*Ahcy*) encoding SAHase [5], the failure of embryo implantation suggests a critical role for SAHase in some cellular functions. Indeed, exposure of the inner cell mass of non-mutant embryos to an irreversible SAHase inhibitor *in vitro* results in abnormal proliferation and differentiation (Table 1). These findings are consistent with the observed high *Ahcy* mRNA expression throughout embryonic development [GNF SymAtlas database (http://symatlas.gnf.org)]. Correspondingly, in humans with decreased SAHase activity levels (5%–20% of normal) associated with elevated levels of serum creatine kinase, SAH, and SAM, severe defects, including myopathy and developmental delay, are seen [6–8]. Interestingly, dietary restriction of methionine and supplementation with creatine and phosphatidylcholine appreciably reduced such manifestations, suggesting that a minimum threshold of SAHase activity is sufficient to sustain normal cellular function.

## 3. Protein arginine methyltransferases

As mentioned above, the large family of MTs that catalyze the transfer of methyl-groups from SAM (~250 members) includes a sub-family of protein arginine methyltransferases (PRMTs) that have recently been shown to play key roles in many biological processes. The PRMT-mediated transfer of methyl groups yields three products: monomethyl, asymmetric dimethyl, and symmetric dimethyl arginine (Fig. 2). The eight PRMT members currently characterized (PRMT1–8) all generate monomethylarginine residues. PRMTs are further classified as type I (PRMT 1, 3, 4, 6, and 8) if they produce asymmetric dimethylarginines or type II (PRMT 5 and 7) if they produce symmetric dimethylarginines (46); PRMT 2 has not yet been classified. PRMT1 is thought to exert more than 90% of all type I PRMT activity. Three additional enzymes show PRMT activity and have been denoted as PRMTs 9, 10, and 11, but their classification and substrates have not yet been defined [9].

Arginine methylation can increase steric hindrance and hydrophobicity but, in contrast to phosphorylation, does not affect protein charge. Little is known about PRMT regulation, but homodimerization [10] and association with other cellular proteins appear to augment and/or inhibit their activity [11–13]. Arginine methylation occurs in a wide variety of substrates, including histones, transcription factors, cytoskeletal proteins, cytoplasmic signaling proteins, and apoptosis proteins (reviewed in [14] and [15]). Additional substrates of PRMT methylation have recently been identified and include the estrogen receptor ERα [16], splicing and elongation factors SAP49, UIC, and CA150 [17], FGF-2 [18], and Ewing Sarcoma Oncoprotein [19,20]. Further work is needed to define the sub-cellular distribution, potential redundancy, and expression profiles of these enzymes in specific cell types, particularly immunocytes.

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## 4. The role of transmethylation in immunity

Initial studies showed that transmethylation in monocytes was necessary for chemotactic responsiveness, as well as the morphologic changes, arachidonic acid release from membrane phospholipids, and second messenger signaling via phosphoinositide metabolism [21–23]. Subsequently, when the turnover rate of SAM was measured in many cell types, the amount of SAM consumed was found to be 4–10 times higher in activated vs. resting lymphocytes, and 3–5 times higher in resting lymphocytes vs. most other cell types, suggesting that lymphocytes may be more sensitive than most other cells to transmethylation inhibition (Table 2) [24].

As a corollary, adenosine deaminase deficiency, an inborn error of purine metabolism that results in severe combined immunodeficiency with characteristic T cell dysfunction, is associated with a 57% reduction in cellular SAHase activity [25] and accumulation of deoxyATP [26–28]. In this condition, adenosine is not deaminated to inosine, but rejoins with endogenous homocysteine to resynthesize SAH. As the SAH accumulates, it indirectly inhibits transmethylation reactions. Combined with the above findings, the adenosine deaminase deficiency phenotype led to the hypothesis that SAHase is required for proper immune cell function.

Indeed, when Wolos and co-workers studied the effects of the irreversible SAHase inhibitor (Z)-5′-fluoro-4′,5′-didehydro-5′-deoxyadenosine (MDL 28842) in murine systems, they found that it blocked generation of cytotoxic T cells *in vitro* and significantly prolonged skin allograft survival *in vivo* (Table 1) [29]. When they stimulated T cells with concanavalin A and B cells with lipopolysaccharide in the presence and absence of this compound, they found that it inhibited IL-2 production and the proliferation of T, but not B, cells despite SAH accumulation in both cell types (Table 2). Moreover, mice treated with this SAHase inhibitor showed reduced OVA-specific T cell responses and anti-OVA antibody levels [30].

A possible mechanistic explanation for the T cell-specific effects following SAHase blockade was provided by Cohen and colleagues. Working with a murine adenosine deaminase model, they showed that treatment with MDL 28842 arrested thymocyte development at the $CD8^{lo}$ and $CD4^{+}CD8^{+}$ double-positive stages, an effect not due to increased apoptosis, but to T cell-specific inhibition of mRNA for the CD4 and CD8 co-receptor molecules (Fig. 3 and Table 2) [31]. These results suggest that a SAM-mediated methylation event(s) is required for the signal that regulates transcription of the T cell-specific molecules during intrathymic T cell development. Interestingly, mice deficient in the protein arginine methyltransferase CARM1 (PRMT4) exhibited partial developmental arrest of $CD4^{-}CD8^{-}$ double-negative thymocyte progenitors (Fig. 3) [32]. Finally, mouse peritoneal macrophages treated with MDL 28842 and subsequently stimulated with LPS showed normal antigen processing and presentation, but significantly reduced TNF-α production, suggesting that SAHase inhibitors perhaps preferentially block proinflammatory responses (Table 2) [33].

We recently published a series of papers detailing the immunosuppressive characteristics of a novel, potent, and reversible SAHase inhibitor, methyl 4-(adenine-9-yl)-2-hydroxy-butanoate (DZ2002) [34–37], which unlike traditional irreversible inhibitors is not cytotoxic at working concentrations, thus allowing treatment for up to 9 months. DZ2002 blocked neither concanavalin A-induced T cell proliferative responses nor IL-2 production, but in monocytes inhibited IL-12p40 and TNF-α production as well as expression of costimulatory molecules (Table 2) [37]. Moreover, DZ2002 reduced delayed-type hypersensitivity reactions, pointing to potentially important roles for SAHase in both macrophages and T cells.

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We also examined the role of DZ2002 in T-dependent antigen responses [34] in DZ2002-treated mice and found suppression of OVA-specific lymphocyte proliferation, anti-OVA IgG levels, and IL-2 and IFN-γ production. However, IL-4 production and anti-OVA $IgG_1$ antibody levels were less affected, suggesting that SAHase inhibition preferentially targets type 1 helper T cell (Th1)-specific responses. Mice treated with SAH alone showed similar reductions in T cell activity and OVA-specific responses, confirming that, at least in part, the mode of action of DZ2002 involves increases in intracellular SAH levels. These data indicate that *in vivo* transmethylation inhibition has a profound suppressive effect on T cells.

## 5. The role of transmethylation in cell signaling

One of the first reported observations of the direct involvement of methyltransferases in receptor-mediated signaling pathways was the specific binding of PRMT1 to interferon-α receptor I (IFNAR1) in a two-hybrid screening analysis (Table 3) [38]. Binding of type I IFNs to its receptor (IFNAR1) signals through the Jak/Stat family of kinases and transcription factors, which leads to anti-viral, antiproliferative, and immunoregulatory activities. Indeed, when PRMT1 expression was blocked by antisense oligonucleotides in a myeloma cell line, these cells become less responsive IFN-β. This novel and unexpected observations led to a reassessment of the role of protein arginine methylation in cellular signaling. Further evidence for the importance of transmethylation in this pathway comes from a study detailing the requirement of STAT1 arginine methylation by PRMT1 for type I IFN signaling [39]. Although STAT1 phosphorylation was unaffected by a transmethylation inhibitor, its ability to bind to DNA response elements was reported to be severely compromised. This finding, however, has not been replicated in other studies, perhaps due to technical reasons [40–42].

Interestingly, another protein methyltransferase, SETD6, has been implicated in NF-κB signaling (Table 3). This methyltransferase is a PKMT that allows monomethylation of RelA at lysine 310 [43]. This form of monomethylated RelA (RelAK310me1) can be found bound to chromatin under basal conditions, and its binding is mediated *via* recognition of its GLP domain (ankyrin domain), which anchors RelA310me1 to the dimethylated lysine 9 of H3. Phosphorylation by protein kinase Cζ of RelAK310me1 at serine position 311 blocks GLP-RelAK310me1 recognition, thereby leading to chromatin relaxation and expression of RelA target genes, including IL-8, IL-1A, MYC and CCND1. This mechanism appears to be a protective cellular event that illustrates synergistic cross-talk between phosphorylation and methylation reactions, which are often studied separately.

Recently, it was shown that Vav1, a guanine nucleotide exchange factor important in T cell signaling, is methylated following CD28 ligation [44]. In CD28-activated cells, methylated Vav1 translocates to the nucleus, while unmethylated Vav1 remains in the cytoplasm. Moreover, indirect inhibition of transmethylation reactions by irreversible blocking of SAHase activity demonstrates a functional defect in IL-2 production that directly links methylation with T cell effector function. These findings suggest that methylation either directs Vav1 to the nucleus or, following phosphorylation, allows it to act as a transcription factor [45]. We recently confirmed and extended those observations [36] by showing that in the presence of DZ2002, activated CD4, but not CD8, T cells had reduced Vav1 methylation, suggesting that the two cell types differ in their post-activation methylation requirements (Table 3). CD4 T cells treated with DZ2002 also showed reduced phosphorylation of several key signaling molecules, including Akt, Erk1/2, and NF-κB, which led to a downstream reduction in calcium mobilization.

Similar CD4 T cell signaling abnormalities are also seen in mice carrying an arginine-to-glycine mutation in the pleckstrin homology domain of Vav1 [46], suggesting that

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methylation at that particular arginine residue is essential for Vav1 function in this subset. Furthermore, Vav1-deficient mice showed defective positive and negative selection in the DP thymocytes and reduced T cell receptor-mediated proliferation and IL-2 secretion, demonstrating the broader role of Vav1 in T cell function (Fig. 3) [47–49]. No clear explanation for the lineage-specific effect of arginine methylation of the pleckstrin homology domain was provided but, in conjunction with our findings, it appears that CD4 and CD8 T cells have differing requirements of arginine methylation in this particular domain. The findings suggest that agents that block or inhibit methylation reactions may be exploited when devising treatments for CD4-mediated autoimmune diseases.

## 6. SAHase inhibition and autoimmune models

Initially, Wolos and colleagues reported a dramatic block in the development of type II collagen-induced arthritis in MDL 28842-treated mice, directly demonstrating that transmethylation inhibition could be an effective treatment for autoimmune diseases [50]. At the highest dosage, none of the treated mice developed disease, while 87% of untreated controls did. Moreover, this treatment was associated with reduced anti-collagen type II antibody levels, decreased T cell proliferative responses to this antigen, and fewer bone erosions (Table 1). Even more striking was the amelioration of established disease. In addition, Saso et al., using a separate irreversible SAHase inhibitor, DHCaA, showed *in vitro* inhibition of T cell proliferation and *in vivo* inhibition of delayed type hypersensitivity reactions as well as a reduction in severity of peptidoglycan polysaccharide-induced arthritis [51]. Of interest, disease in this arthritis model is mediated by IL-1β, suggesting that methylation inhibitors may interfere with IL-1β processing, which encompasses an initial TLR-mediated induction of pro-IL-1β followed by inflammasome-mediated conversion and secretion of mature IL-1β [52,53]. Indeed, our preliminary data support the idea that methylation inhibitors broadly block TLR-signaling and production of proinflammatory cytokines (unpublished data).

In light of our previous findings with DZ2002, we used a similar approach to establish the efficacy of transmethylation inhibition in the treatment of experimental autoimmune encephalomyelitis (EAE), a classic Th1-type autoimmune disease [35]. EAE can be induced in mice by immunization with several myelin antigens, such as peptide 35–55 of myelin oligodendrocyte glycoprotein (MOG), which leads to chronic paralysis and demyelination of the central nervous system, and peptide 139–151 of proteolipid protein (PLP), which results in a relapsing/remitting type of disease [54]. Administration of the reversible SAHase inhibitor DZ2002 reduced the incidence and severity of $MOG_{35–55}$-induced EAE in the C57BL/6 mouse, and also decreased anti-$MOG_{35–55}$ T cell responses as well as other Th1 cytokine production. Inhibition of proliferation was associated with downregulation of several cyclins and cyclin-dependent kinases (CDKs) (e.g., cyclin D3, CDK4, and CDK6), as well as upregulation of the CDK inhibitor p27 (Table 1).

In a more recent study, we reexamined the effects of DZ2002 in the $PLP_{139–151}$-induced relapsing/remitting model of EAE, which closely reflects the course of human MS. Impressively, we not only blocked induction of disease, but reduced the severity and incidence of relapses [36]. Moreover, the effectiveness of this treatment appears to be primarily mediated by its inhibitory effects on autoreactive T cells, since the ability of encephalitogenic $CD4^+$ T cells to induce disease upon adoptive transfer was greatly reduced in DZ2002-treated mice (Table 1).

Finally, it should be noted that global transmethylation inhibitors, reversible or otherwise, likely exert many unanticipated off-target effects on a diverse array of genes/ proteins since there are >250 known methyltransferases. These unintended effects could potentially lead to

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adverse events. Therefore, efforts to develop more specific transmethylase inhibitors, such as PRMT1-specific inhibitors, are highly warranted.

## 7. Post-translational protein modifications and autoimmunity

On a different note, tolerance to self-proteins can be compromised by post-translational protein modifications. This possibility has been supported by several experiments, particularly those conducted by Mamula and colleagues, which showed that 1) intracellular isoaspartyl (isoAsp) residues accumulate with age in T cells from autoimmune mice and are elevated in hyperproliferating T cells [55], 2) immunization with *in vitro* isoAsp-modified tyrosinase-related protein (TRP)-2 rendered it immunogenic and a target for CD8 T cells [56], 3) transfer of bone marrow from mice lacking protein carboxyl methyltransferase (PCMT), the isoAsp repair enzyme, into wild-type recipients resulted in development of lupus-like disease associated with anti-DNA autoantibodies and kidney disease (Table 1) [57], and 4) mice immunized with the isoAsp form of mouse cytochrome c mounted a strong B and T cell response, and anti-isoAsp cytochrome c antibodies cross-reacted with native cytochrome c [1]. Other post-translational protein modifications have also been implicated as playing a role in the pathogenesis of autoimmune diseases, for example, in RA, citrullination (conversion of arginine to citrulline) [58–63] and glycosylation [64–66]. Finally, DNA methylation was demonstrated to indirectly interfere with post-translational modifications implicated in autoimmunity. For example, promoter regions of peptidyl arginine deiminase type II (PAD2), an enzyme involved in the citrullination of myelin basic protein (MBP), have been shown to be hypomethylated and overexpressed in white matter from MS patients (Table 1). The citrullination of MBP by PAD2 is thought to promote protein autocleavage, resulting in the potential creation of neoepitopes for which tolerance has not been established [67]. Thus, epigenetic events (as detailed below) and post-translational protein modifications appear intimately intertwined.

## 8. Epigenetics, immunity, and autoimmunity

Because DNA can also be transmethylated similar to proteins, as noted above, blockade of transmethylation should significantly affect the epigenetics of immunocytes. Epigenetic modifications (heritable changes in gene expression that leave DNA sequences unaffected) are fundamental to tissue differentiation [68], gene imprinting [69], X chromosome inactivation [70], and suppression of foreign DNA acquired by mammalian cells during evolution [71]. DNA methylation and histone modifications alter chromatin structure in a way that affects DNA accessibility to transcription factors and gene expression [72,73].

DNA methylation, which occurs on cytosine bases in CpG-dinucleotides widely distributed in the genome, are mediated by three DNA methyltransferases (DNMT1, responsible for heritable maintenance of CpG-methylations during replication; DNMT3a and DNMT3b, responsible for *de novo* methylation). These enzymes catalyze the transfer of methyl groups from SAM to the 5 position of the cytosine ring. Methylation of CpG islands in or near a promoter region contributes to the silencing of genes by promoting chromatin remodeling and preventing the binding of transcriptional factors.

Gene expression can also be controlled by methylation of certain lysine or arginine residues within the histone tail. For example, methylation of lysine 9 or 27 on histone H3 (H3K9 or H3K27), or lysine 20 on histone H4 (H4K20), is associated with transcriptional repression by formation of heterochromatin (dense form of chromatin) [74]. In fact, the presence of both H3K9me3/H3K9me2 and H3K27me3 in the same region of the genome marks the local CpG islands for DNMT-mediated methylation [75]. In contrast, di- and trimethylation of lysine 4 on histone H3 (H3K4me3 or H3K4me2) is found in actively transcribed euchromatin (less compact form of chromatin) and may promote transcription, notably by

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association with RNApol II in actively transcribed genes [74,76]. Indeed, methylation of arginine residue 3 on histone H4 (H4R3) or arginine 17 on histone H3 (H3R17) was associated with gene expression [67]. Epigenetic regulation bequeaths gene expression changes to subsequent cell generations. Thus, it is not surprising that the mechanism is ubiquitous in the immune system [77,78] wherein the preservation of adaptations developed in various cell subsets permits and transmits a rapid and robust defense against specific pathogens.

Hematopoietic cell lineages nicely illustrate epigenetic regulation mediated either by selective demethylation or *de novo* methylation at the DNA level or by histone modifications as a pre-priming mark for lineage specific differentiation (reviewed in [79]). For example, demethylation of the *Lck* gene (encoding a Src family kinase essential in TCR receptor signaling) or POU domain class 2-associating factor *(Pou2af1* encoding a B-cell specific co-activator) has been demonstrated in T and B cells, respectively (Table 2). Similar demethylation events were observed in *CD8*α and *CD8*β genes during thymocyte transition from the double-negative (DN) to the double-positive (DP) stage (Fig. 3 and Table 2) (reviewed in [80]). Moreover, during lineage development, many genes are marked with H3K4me2 either close to their transcription start points or in transcription factor binding regions. In some cell types, activation of these genes is characterized by an additional methylation event (H3K4me3), while other genes are silenced by demethylation (H3K4). The latter is the case for recombination activating gene-2 (*Rag*-2) and GATA-binding protein-1 (*GATA-1*), which both exhibit the H3K4me2 dimethylation priming mark in hematopoietic stem cells (HSC), but are either trimethylated (H2K4me3), as is the case with Rag2, or demethylated, as is the case with GATA-1, upon differentiation to B cells and inversely in erythroid cells (Table 2) (reviewed in [79]).

V(D)J rearrangement of B and T cell receptor genes is also regulated by epigenetic events that promote the accessibility of certain gene loci, such as *Ig*κ and *TCR*β (Table 2) [81]. For example, a single methylated CpG island in the heptamer of the Dβ element recombination signal sequence (RSS) can block RAG-mediated cleavage, suggesting methylation plays a role in recombinant regulation [82]. Indeed, hypomethylation of CpG dinucleotides may be induced by the action of a V(D)J element promoter (Pβ1), since hypermethylation of CpG dinucleotides across Dβ1–Jβ1 clusters was demonstrated after Pβ1 deletion [83]. Furthermore, as previously discussed, histone modifications can also predict a V(D)J rearrangement. The D element of either the *IgH* or *Ig*κ loci can be targeted for histone modifications (acetylation and methylation) that later induce rearrangement. In this context, the Rag-2 enzyme recognizes the H3K4me3 motif *via* its specific plant homeodomain (PHD) finger, thus directly activating its cleavage activity (Table 2). Therefore, it is likely that epigenetic modifications are necessary in orchestrating recombination events during immunoglobulin or TCR gene rearrangement [79,82].

Naïve $CD4^+$ T cell differentiation is also a well-studied example of epigenetic regulation primarily mediated by two transcriptional factors, T-bet and GATA-3, which, through chromatin modifications, enable access of transcription factors to the IFN-γ gene or to the IL-4 gene and subsequent differentiation into Th1 or Th2 $CD4^+$ T cells, respectively (Table 2) [84,85]. This is accomplished by demethylation of the IFN-γ gene and methylation of the IL-4 gene for Th1 cells [86], and the converse for Th2 cells (Fig. 4) [80,87].

Epigenetic modifications play similarly significant roles in the differentiation of naïve $CD4^+$ T cells into Th17 and regulatory T cells. Th17 cells are produced in the presence of IL-6 and TGF-β [88], which induce histone H3 acetylation and H3K4me3 in the IL-17 and IL-17F promoter regions, allowing transcription of the respective cytokines (Fig. 4 and Table 2) [89]. Natural regulatory T cells of the $CD4^+CD25^+FOXP3^+$ phenotype are characterized by

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complete DNA demethylation in the FOXP3 promoter and increased histone acetylation and H3K4me3 in associated chromatin. Of note, complete demethylation of FOXP3 is required for a permanent regulatory functional status [90]. Considering the effects of methylation on several $CD4^+$ T cell subtypes, it can be surmised that epigenetic changes likely play a significant role in autoimmunity.

Indeed, the role of DNA methylation has been extensively investigated in autoimmunity, especially in lupus. Early studies showed that $CD4^+$ T cells treated *in vitro* with the DNMT inhibitor 5-azadeoxycytidine acquired autoreactive potential, as indicated by the ability of these cells to induce a lupus-like disease upon adoptive transfer (Table 1) [91]. In further support of the role of epigenetic changes in lupus, it was found that T cells from patients with active SLE had decreased deoxymethylcytosine content and DNMT1 mRNA (Table 1) [92], suggesting that abnormal demethylation may lead to overexpression of certain genes leading to a break in tolerance. In this regard, particular emphasis has been placed on genes affecting adhesion molecules (LFA-1), apoptosis-related proteins (perforin), and costimulatory molecules (CD70 and CD154). Of interest, female-derived $CD4^+$ T cells treated with 5-azadeoxycytidine demethylate the normally silenced copy of methylated CD154 (CD40L) on the X-chromosome, leading to a double-dose of this cost-imulatory molecule and thus providing a potential role for methylation in the female predominance of this disease [93]. These susceptibility genes are found overexpressed in $CD4^+$ T and B cells, but not in $CD8^+$ T cells from SLE patients. Further investigations using 5-azadeoxycytidine defined a putative model in which drug-induced autoreactive $CD4^+$ T cells eliminated macrophages by perforin secretion or signal delivered through T cell FasL, TWEAK and TRAIL promote B cell activity by overexpression of CD70 and/or IFN-γ and IL-4. The release of nucleosomal material following macrophage apoptosis may induce anti-nucleosomal T-cell responses, leading to an anti-nucleic acid antibody response [91,94].

Erk signaling pathways have also been implicated in DNA methylation modifications by regulating DNMT activity. Inhibition of Erk was investigated in T cells by using hydralazine, an Erk-inhibiting drug, or T cells from transgenic mice that inducibly express a dominant negative form of mitogen-activated protein kinase (MEK) (Table 3). Both of these approaches led to decreased DNA methylation associated with overexpression of LFA-1 and CD70 [67,95,96]. Interestingly, this decreased Erk signaling was also observed in T cells from SLE patients and appeared related to impaired protein kinase C δ activation [97]. However, this remains a matter of conjecture derived from in vitro studies and requires further work. Epigenetic changes have also been considered a potential explanation for the incomplete concordance of SLE in monozygotic twins, although this “incompleteness” may be explained by many other stochastically-imposed factors, including, in particular, environmental factors such as viral infections that can act as precipitating events in genetically-predisposed individuals. Interestingly, in contrast to lupus, T cells from RA patients show no defects in DNMT1 or LFA-1 activity, but instead show defects in global DNA hypomethylation in synovial fibroblasts, especially in CpG islands in long interspersed nuclear element-1 (LINE-1, an intragenomic parasitic DNA), and in monocytes, within the IL-6 promoter and the death receptor-3 inducing hyper-inflammation and apoptosis resistance gene (Table 1) [67,94].

## 9. Conclusions

Transmethylation is a key pathway common to both post-translational protein modification and gene regulation. By modifying protein functionality or generating novel structures, post-translational modifications can contribute to the pathogenesis of autoimmunity. Additionally, epigenetic mechanisms that disturb immune system gene expression can profoundly influence self-tolerance. Intervention in those processes with either small-

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molecule chemical inhibitors or agonist/antagonist biologicals could ultimately lead to novel autoimmune disease therapies.

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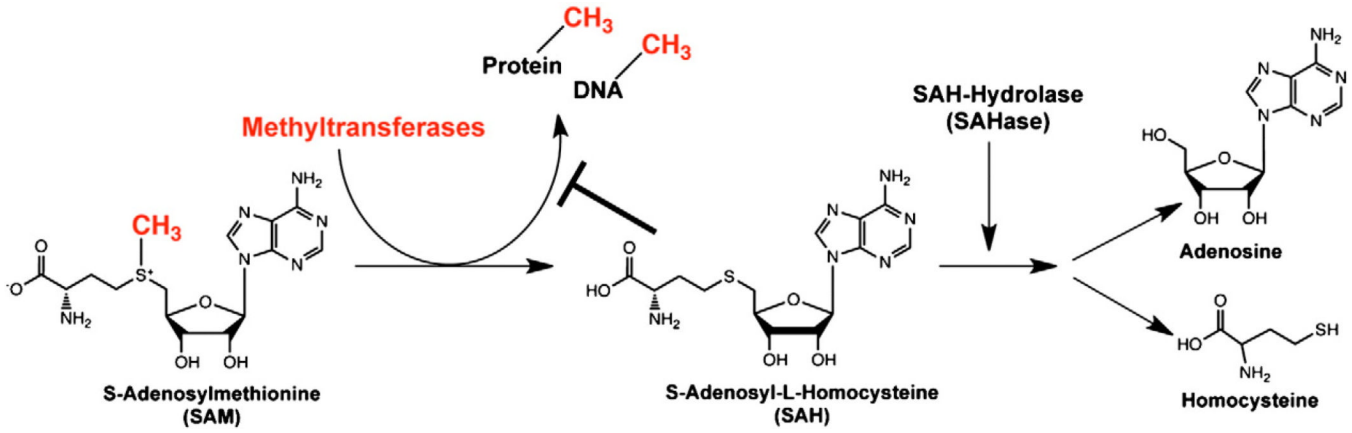


**Figure 1.**

Biochemical pathway of protein and DNA transmethylation. Methyltransferases (MT) catalyze the transfer of methyl groups from SAM to either proteins or DNA, resulting in conversion of SAM to SAH. SAH is then hydrolyzed into adenosine and homo-cysteine by SAHase. If the balance between SAM and SAH concentrations favor SAH, SAH can then provide negative feedback inhibition of transmethylation activity allowing restoration of SAM concentrations. Thus, pharmacological blockade of SAHase increases intracellular levels of SAH, thereby indirectly blocking MT activity.

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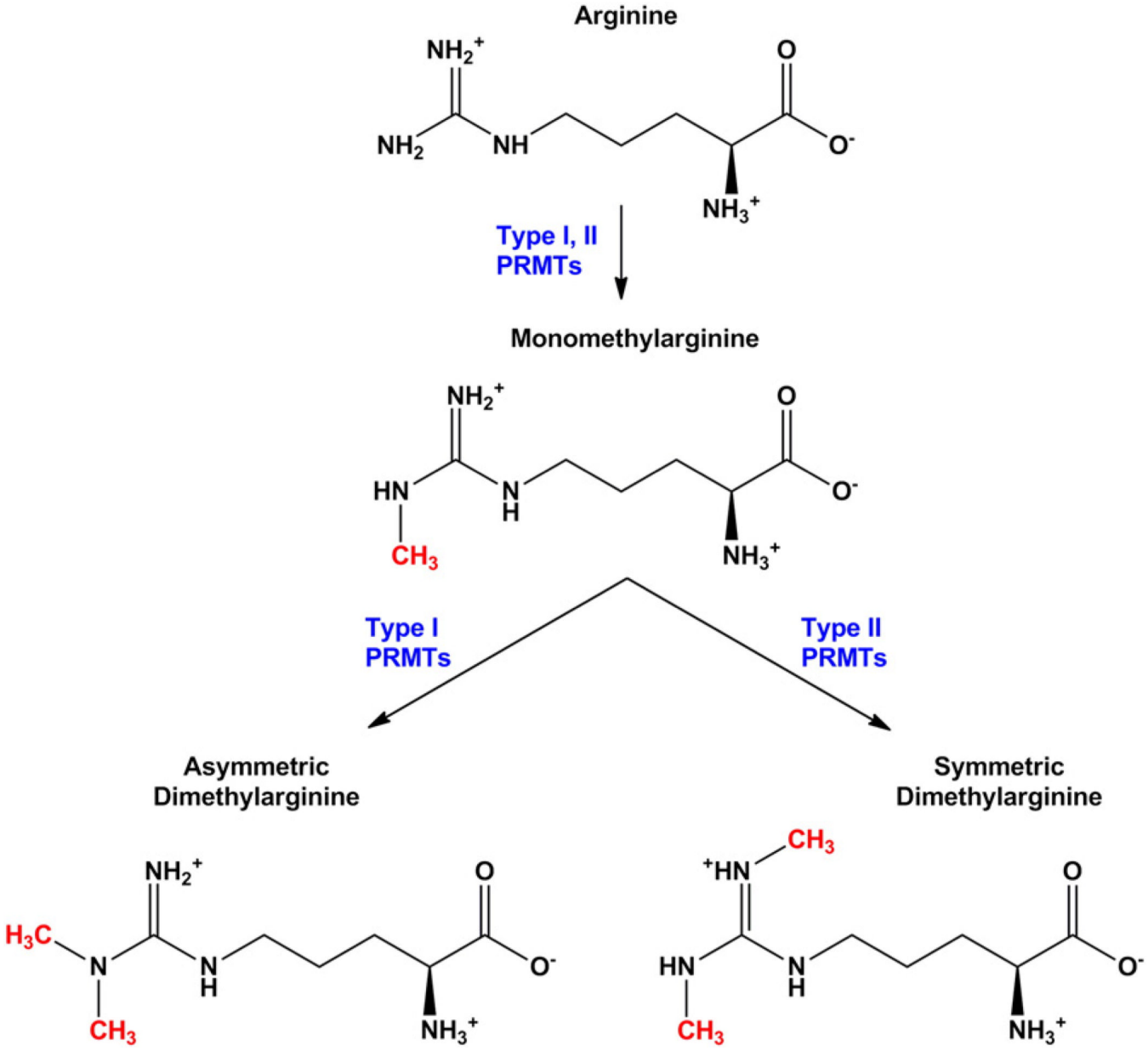


**Figure 2.**

Arginine methylation. Monomethylarginine is generated upon transfer of a methyl group from SAM to the guanidino amino group of arginine. Type I protein arginine methyltransferases (PRMTs 1, 3, 4, 6 and 8) then transfer a second methyl group to the same nitrogen, resulting in an asymmetric dimethylarginine. Type II PRMTs (PRMTs 5 and 7) transfer the additional methyl group to the opposite terminal nitrogen, creating symmetric dimethylarginine. PRMT2 is not yet classified.

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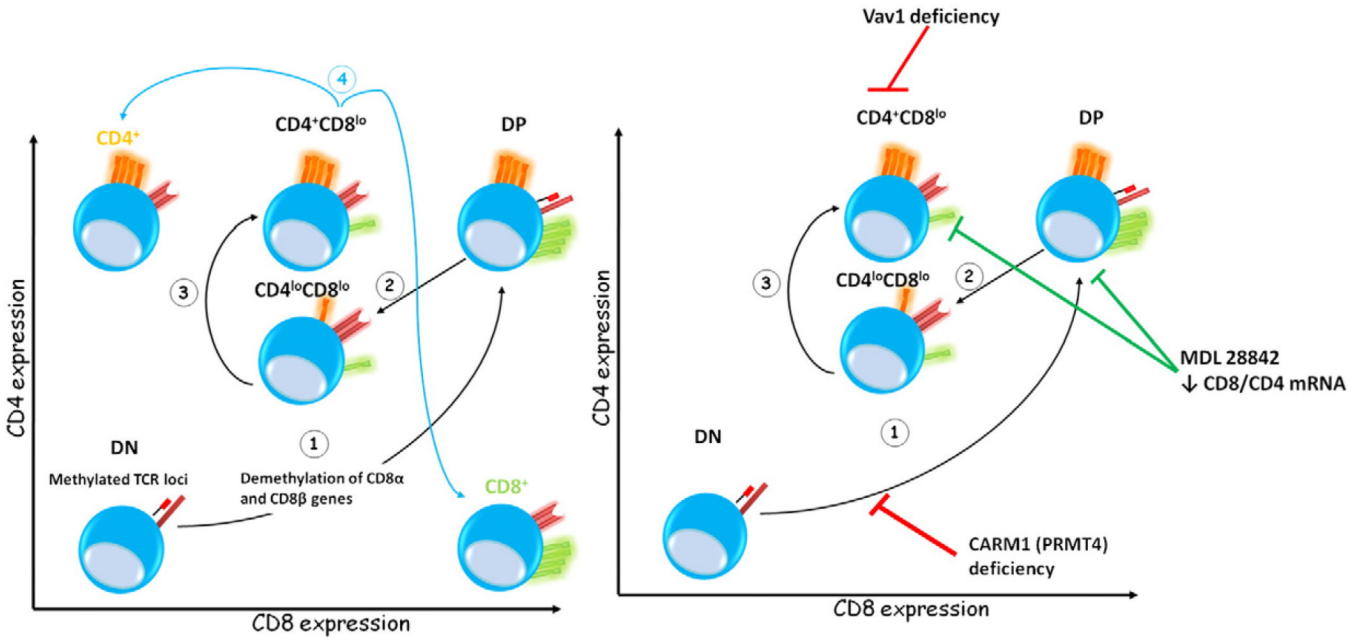


**Figure 3.**

Methylation events during thymocyte development. Gene methylation status fluctuates during thymocyte differentiation. Double-negative (DN) thymocytes exhibit methylated TCR loci and expression of TCRβ is closely associated with signaling through the IL-7Rα and demethylation of the TCRβ locus, leading to locus accessibility and VDJ recombination. The first step (1) in thymocyte differentiation is the transition from DN to double-positive (DP) $CD4^+CD8^+$ cells and is accompanied by demethylation of CD8α and CD8β. The second (2) and third (3) steps lead to intermediate thymocyte subpopulations, e.g., $CD4^{lo}CD8^{lo}$ and $CD4^+CD8^{lo}$, and correspond to acquisition of a functionally rearranged TCR, again implying DNA and protein methylation modifications. Finally, $CD4^+$ or $CD8^+$ T cells are produced (4), but whether methylation status affects CD4 or CD8 expression remains to be clearly elucidated. However, retention of demethylated CD8 genes in $CD4^+$ single cells was demonstrated, and *de novo* remethylation of CD8α genes was associated with apoptosis of misselected thymic $CD8^+$ T cell emigrants that do not correctly recognize MHC class I in the periphery, inhibiting development of autoimmune disease. Interestingly, inhibition of SAHase activity by MDL28842 leads to arrested thymocyte differentiation at the $CD8^{lo}$ and $CD4^+CD8^+$ DP stages associated with decreased CD8 and CD4 mRNA. This effect may be a result of MDL28842 inhibition of PRMT4 and/or Vav1 activity, as both have been implicated in thymocyte developmental arrest.

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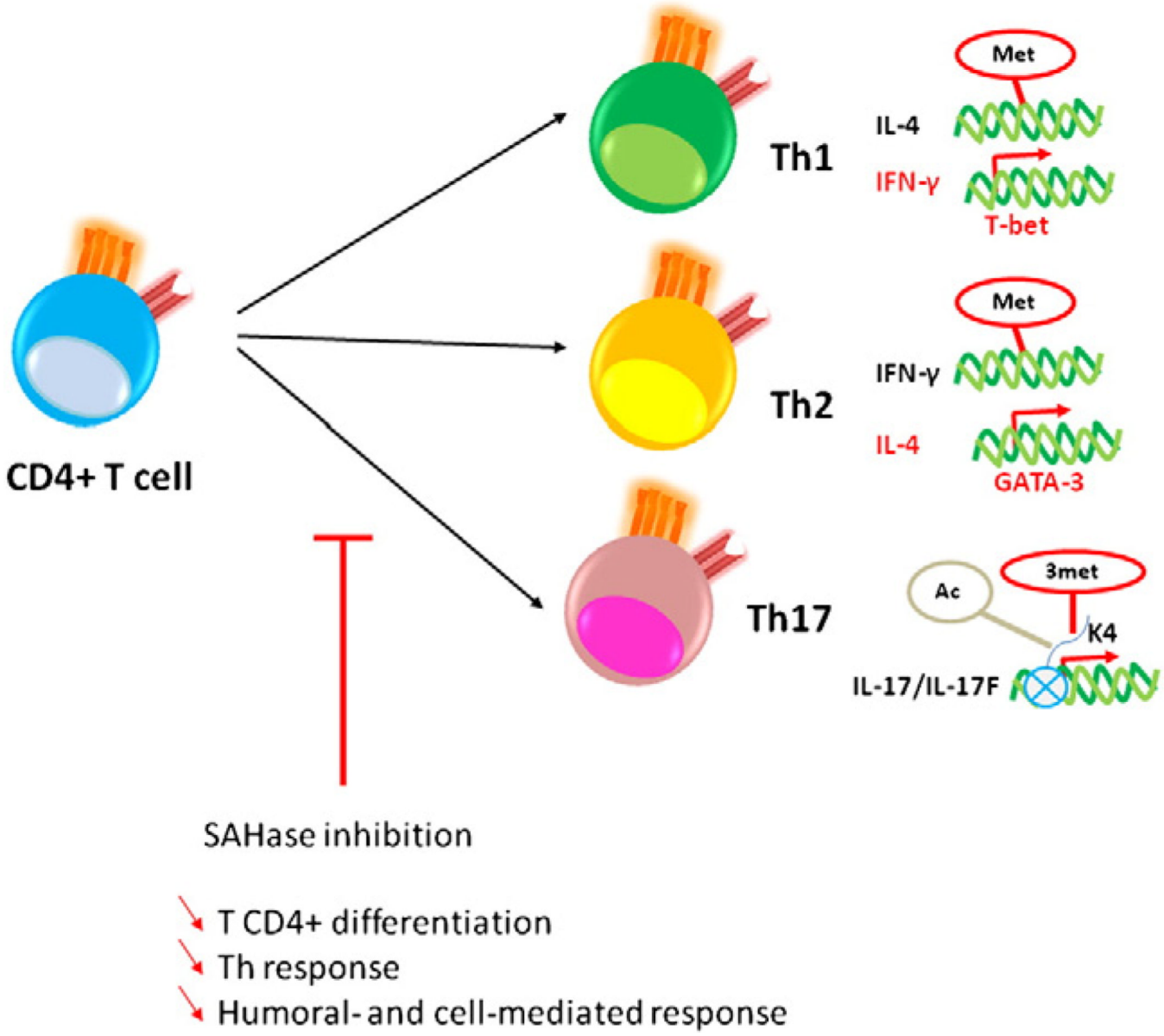


**Figure 4.**

Putative role of methylation during $CD4^+$ T cell and global immune responses. DNA or histone methylation allows DNA structural changes and transcription factor accessibility. These events occur during $CD4^+$ T cell differentiation and direct $CD4^+$-mediated helper responses. Th1 and Th2 differentiationismediatedbymethylationofthe IL-4 gene and demethylationofthe IFN-γ gene,ormethylation of the IFN-γ gene and demethylation of the IL-4 gene, respectively. Moreover, histone modifications in the IL-17 and IL-17F promoter regions (e.g. H3K4 trimethylation and H3 acetylation) lead to the expression of these cytokines and a Th17 response. Thus, SAHase inhibition may block T helper responses by ineffective epigenetic events (DNA or protein) leading to defective humoral- and cell-mediated responses, [38,51,52]. H3K4, lysine 4 of histone 3; 3met, trimethylation; ac, acetylation; met, metylation).

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## Table 1

Impact of transmethylation pathway on embryonic and disease development.

| Pathology/affected field | Treatment/model | Observation | References |
|---|---|---|---|
| *Embryonic development* | | | |
| Mouse model | *Ahcy* (encoding SAHase) lacking mice | -Embryo implantation failure<br>-High *Ahcy* mRNA expression during embryonic development | [5] |
| | SAHase inhibition | -Abnormal proliferation and differentiation of embryo inner cell mass | |
| Human deficiency | Gene encoding SAHase 2 missense mutations | -Myopathy-developmental delay<br>-5–20% of normal SAHase activity level<br>-High levels of creatine kinase, SAH and SAM | [6–8] |
| | Methionine dietary restriction | -Reduced symptoms | |
| | Adenosine deaminase deficiency | -57% reduction of cellular SAHase<br>-DeoxyATP accumulation<br>-T cell dysfunction as a hallmark | [25–28] |
| *Autoimmunity* | | | |
| Rheumatoid arthritis | | | |
| | T cells | -Normal level of DNA methyltransferase activity and DNMT1 mRNA | [67,94] |
| | Synovial fibroblasts | -Decrease in global DNA methylation<br>-Hypomethylation of CpG island in LINE-1 promoter | |
| | Monocytes | -Unmethylated CpG island within IL-6 promoter<br>-Methylation changes in CpG island promoter of DR-3 | |
| Type II collagen-induced | SAHase inhibition | -Reduced disease development<br>-Reduced anti-collagen type II antibody level<br>-Reduced T cell proliferative response<br>-Fewer bone erosions<br>-Amelioration of established disease | [50] |
| Peptidoglycan-polysaccharide-induced | SAHase inhibition | -Reduced disease severity<br>-Inhibition of IL-1β production (spleen+joint) | [49] |
| Systemic lupus erythematosus (SLE) | Active SLE | -Global DNA hypomethylation<br>-Reduced DNMT-1 mRNA levels | [67,94,98] |
| | $CD4^+$ T cells | -CD70–CD40L (in women)–CD11a–perforin–IL-6–IL-4 demethylation→genes overexpressed | |
| | B cells | -CD70–perforin–CD40L–CD6 demethylation | |
| Multiple sclerosis | White matter cells | -30% reduction in CpG island methylation<br>-Hypomethylation of PAD2 region promoter | [67] |
| | $CD4^+$ T cells | -No consistent variation in epigenetics between twins | |
| EAE ($MOG_{35–55}$) | SAHase inhibition | -Reduced disease incidence and severity<br>-Reduced anti-$MOG_{35–55}$ T cell proliferative response<br>-Reduced Th1 cytokines response<br>-Downregulation of cyclin D3 and cyclin-dependent kinases CDK4–CDK6<br>-Upregulation of p27 | [35,36] |
| EAE ($PLP^{139–151}$) | SAHase inhibition | -Blockade of disease induction<br>-Reduced relapse incidence and severity<br>-Inhibition of autoreactive $CD4^+$ T cells | |
| *Epigenetic modification* | | | |
| Mouse lupus | DNA methyltransferase inhibitor | -Lupus-like disease development<br>-Autoreactive potential acquired by CD4+ T cells | [91] |

| Pathology/affected field | Treatment/model | Observation | References |
|---|---|---|---|
| | | -Possible role of CD4+ T cells in B cell IgG production and macrophage killing | |
| Human SLE | DNA methyltransferase inhibitor | -Demethylation of the silenced *CD154* (*CD40L*) gene on the X-chromosome<br>-Potential role for methylation in female predisposition to SLE | [92,93] |
| *Post-translational modifications* | Bone marrow transfer from PCMT-KO mice | -Lupus-like disease development<br>-Anti-DNA autoantibody production<br>-Kidney disease<br>-Isoaspartyl protein modification- associated pathogenesis | [57] |
| *Allergy* | | | |
| Delayed hypersensitivity reaction | SAHase inhibition | -Significant reduction in ear swelling | [37,51] |
| *Transplantation* | | | |
| Mouse skin allograft | SAHase inhibition | -Significantly prolonged graft survival<br>-Blockade of *in vitro* cytotoxic<br>-T cell generation | [29] |



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## Table 2

Transmethylation effects in immune cell types.

| Type of immunocytes | Studied aspect | Consequences | References |
|---|---|---|---|
| Immunocyte differentiation | -Selective demethylation or *de novo* methylation of genes in tissues or lineage-specific manner<br>-Demethylation of *Lck* gene in T cells<br>-Demethylation of *Pou2af1* in B cells<br>-Methylation of dachshund homologue 1 (Dach1) in common lymphoid progenitors and DN thymocytes<br>-Histone modification as pre-priming marks of lineage differentiation | -Myeloid/lymphoid commitment<br>-T cell/B cell commitment | [79] |
| Thymocyte development | -Demethylation of CD8α and CD8β genes | -Transition of double negative to double positive stage | [80,81] |
| | -Retention of demethylated CD8 genes | -Single-positive $CD4^+$ T cells | |
| | -SAHase inhibition | -Arrested development ($CD8^{lo}$ and $CD4^+CD8^+$ double positive stages)<br>-Not due to increased apoptosis<br>-T cell-specific inhibition of co-receptor CD4 and CD8 mRNA | [31] |
| B cell/T cell | -Demethylation of *TCR*β and Igκ loci<br>-*Ig*H locus: preference for D element pre-marked with histone modification<br>-Plant homeodomain (PHD) of Rag2 | -Increased gene accessibility allowing V(D)J rearrangement | [79–82] |
| CD4+ T cells | | | |
| Th1 | -Demethylation of the IFN-γ gene<br>-Methylation of the IL-4 gene | -Control of IFN-γ gene accessibility by transcription factor T-bet | [80,84–87] |
| Th2 | -Methylation of the IFN-γ gene<br>-Demethylation of the IL-4 gene | -Control of IL-4 gene accessibility by transcription factor GATA-4 | |
| Th17 | -Acetylation of histone H3 in the IL-17/IL-17F promoter region<br>-Trimethylation of H3K4 in IL-17/IL-17F promoter region | -Transcription of IL-17 and IL-17F cytokines | [88,89] |
| nTreg | -Increased acetylation of histone H3 in FOXP3 promoter region<br>-Trimethylation of H3K4 in FOXP3 promoter region<br>-Complete DNA demethylation in the FOXP3 promoter | -nTreg phenotype<br>-Requires complete DNA demethylation of FOXP3 for a permanent regulatory state | [90] |
| Human monocytes | -Adenosine deaminase inhibitor | -Increased intracellular SAH levels<br>-Decreased chemotactic responsiveness and attendant morphologic changes | [21,22] |
| | -SAHase inhibition | -Decreased arachidonic acid release from membrane phospholipids<br>-Decreased second messenger activation via phosphoinositide metabolism | |
| Macrophages | -SAHase inhibition | -Normal antigen processing and presentation<br>-Significant reduction of TNF-α | [33] |
| B cells | -SAHase inhibition | -No inhibition of B cell proliferation | [29] |
| T cells Mouse | -S-adenosyl-L-methionine burst | -High sensitivity of transmethylation inhibition | [24] |
| | -Irreversible SAHase inhibition | -Inhibition of conA stimulated-T cell proliferation and IL-2 production<br>-Reduced OVA-specific T cell responses<br>-Reduced anti-OVA antibody levels | [29,30] |
| | -Reversible SAHase inhibition | -No inhibition of conA stimulated-T cell proliferation or IL-2 production | [37] |

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| Type of immunocytes | Studied aspect | Consequences | References |
|---|---|---|---|
| | | -Did inhibit IL-12p40 and TNF-α from monocytes | |

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## Table 3

Signaling pathways of methylation and transmethylation.

| | Treatment/model | Signaling pathway | References |
|---|---|---|---|
| *Immunocytes* | | | |
| T cells | -CD28-activated | -Vav1 R-methylation and translocation into the nucleus | [44,45] |
| $CD4^+$ T cells (but not $CD8^+$ T cells) | -SAHase inhibition | -Reduced Vav R-methylation<br>-Reduced Akt, Erk 1/2, NF-κB phosphorylation | [36] |
| *Protein methyltransferase* | | | |
| PRMT-1 | -Arginine methyltransferase | -Binding to IFNAR1<br>-Inhibition of antiproliferative effects of IFN-β by blocking PRMT-1<br>-STAT1 R-methylation by PRMT1? | [38]<br>[39–42] |
| SETD6 | -Lysine methyltransferase | -Monomethylation of RelA at Lysine 310<br>-Basal condition: RelAK310me1 bound to H3K9me2 by recognition of the GLP domain of SETD6<br>-Pro-inflammatory stimulus: PKCζ-mediated phosphorylation of RelAK310me1 blocks GLP-RelAK310me1 recognition→chromatin relaxation and RelA target genes expression | [43] |
| *Autoimmunity* | | | |
| Lupus | -Transgenic mouse that inducibly expresses a dominant-negative MEK in T cells<br>-Hydralazine/ Erk inhibitor-treated murine T cells<br>-Human T cells from SLE patient | -Decreased Erk 1/2 pathway signaling<br>-Impaired PKCδ<br>-Decreases DNA methylation<br>-Modification of gene expression rendering T cells autoreactive | [95–97] |