Published in final edited form as:
Autoimmun Rev. 2009 January ; 8(3): 196–198. doi:10.1016/j.autrev.2008.07.043.

Aberrant T cell ERK pathway signaling and chromatin structure in lupus

Gabriela Gorelik[*] and **Bruce Richardson**[*,†]

[*] *Dept. of Medicine, University of Michigan, Ann Arbor MI*

† *Ann Arbor VA Medical Center, Ann Arbor MI*

Abstract

Human systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibodies to nuclear components with subsequent immune complex formation and deposition in multiple organs. A combination of genetic and environmental factors is required for disease development, but how the environment interacts with the immune system in genetically predisposed hosts to cause lupus is unclear. Recent evidence suggests that environmental agents may alter T cell chromatin structure and gene expression through effects on DNA methylation, a repressive epigenetic mechanism promoting chromatin inactivation, to cause lupus in people with the appropriate genetic background. DNA methylation is regulated by ERK pathway signaling, and abnormalities in ERK pathway signaling may contribute to immune dysfunction in lupus through epigenetic effects on gene expression. This article reviews current evidence for epigenetic abnormalities, and in particular DNA demethylation, in the pathogenesis of idiopathic and some forms of drug induced lupus, and how impaired ERK pathway signaling may contribute to the development of human lupus through effects on T cell DNA methylation.

Keywords

Lupus T cells; Epigenetics; DNA methylation; ERK pathway signaling; PKCδ

Take-Home messages

- Identical changes in T cell DNA methylation patterns, T cell gene expression, and T cell function are found in experimentally demethylated and lupus T cells.
- Impaired ERK pathway signaling, due to impaired PKCδ phosphorylation has been proposed as the mechanism responsible for T cell demethylation.
- A detailed analysis of PKCδ activation in hydralazine treated and lupus T cells may provide further insights into the pathogenesis of this puzzling disease.

Address correspondence to: Bruce Richardson MD PhD, 3007 BSRB, Ann Arbor, MI 48109-2200 Phone: 734 936-5560 Fax: 734 936-9220.

Introduction

T cells do not play a direct role in lupus tissue damage. Rather, they promote the autoimmune response and are important for autoantibody production [1]. T cells from patients with active lupus have multiple biochemical abnormalities, resulting in functional aberrations that cause a lupus-like disease in animal models, and may contribute to the development of autoimmunity in humans. Recent evidence suggests that abnormal T cell ERK pathway (PKC → Ras→ Raf→ MEK→ ERK) signal transduction may be fundamental to some of these abnormalities through effects on DNA methylation [2]. This review summarizes current understanding of DNA methylation and gene expression, and how abnormal ERK pathway signaling can alter T cell DNA methylation, and consequently gene expression, to promote lupus development in genetically predisposed hosts.

DNA methylation and gene expression

DNA methylation refers to the post-synthetic methylation of deoxycytosine (dC) to form deoxymethylcytosine (d^mC). d^mC occurs primarily in CpG pairs. In general, methylation of regulatory elements suppresses gene expression, while regulatory elements of active genes are typically unmethylated. Methylation of regulatory elements tethers chromatin inactivation complexes that stabilize nearby chromatin in an inactive configuration [3]. DNA methylation patterns are established during development by the de novo DNA methyltransferases (Dnmt) 3a and 3b, then are replicated during mitosis by the maintenance methyltransferase Dnmt1 [3].

Mitotically inactive T cells express relatively low levels of Dnmt1 [4]. However, during mitosis Dnmt1 levels are increased in part by signals transmitted through the JNK and Ras-ERK pathways and interacting with AP-1 sites in an intronic promoter. Inhibiting either pathway inhibits DNA methylation in proliferating cells [5]. This suggests that environmental agents may affect the replication of DNA methylation patterns during mitosis by inhibiting these pathways, with the potential of altering gene expression.

DNA methlyation and lupus

Early studies demonstrated that inhibiting DNA methylation with 5-azacytidine (5-azaC) caused CD4+ T cell autoreactivity. The autoreactivity correlated with overexpression of the adhesion molecule LFA-1 (CD11a/CD18) [6] due to demethylation of sequences 5′ to the CD11a promoter [7], and overexpressing LFA-1 by transfection caused an identical autoreactivity [8]. Demethylated, autoreactive CD4+ T cells overstimulate antibody production by B cells and kill macrophages (Mø) [3], releasing apoptotic nuclear material that stimulates lupus-like autoantibodies [9]. Further, injecting experimentally demethylated CD4+ T cells into syngeneic mice causes anti-DNA antibodies and a lupus-like disease [8].

The obsevation that a DNA methylation inhibitor can cause a lupus-like disease suggests that drugs which cause a lupus-like disease might be DNA methylation inhibitors. Hydralazine and procainamide cause anti-nuclear antibodies in a majority of people receiving these drugs, and a lupus-like disease in a subset [10]. Both were found to inhibit T cell DNA methylation and cause LFA-1 overexpression and autoreactivity [11], and the demethylated T cells caused a lupus-like disease in mice like 5-azaC [8]. Procainamide is a competitive Dnmt1 inhibitor [12] while hydralazine decreases Dnmt1 levels by inhibiting ERK pathway signaling [13].

T cells from lupus patients similarly have decreased Dnmt1 and d^mC levels (14). Lupus T cells also overexpress LFA-1 [6], due to demethylation of the same sequences affected by 5-azaC [7], and kill Mø [6], like the demethylation model. Identical demethylation and overexpression

of perforin, CD70 and CD40L is also seen in experimentally demethylated and lupus T cells [3].

Patients with active lupus have impaired T cell ERK1/2 phosphorylation, similar to hydralazine treated T cells. The degree of impairment is proportional to disease activity [15]. Further, inhibiting T cell ERK1/2 phosphorylation with MEK inhibitors decreases Dnmt1 expression and d^mC content, causes LFA-1 overexpression, makes T cells autoreactive, and the treated T cells induce anti-DNA antibodies in animal models [3]. CD4+ T cells treated with ERK pathway and Dnmt inhibitors also demethylate and overexpress *TNFSF7*, encoding the B cell costimulatory molecule CD70, and identical demethylation and overexpression of CD70 is found in lupus T cells [16,17]. These latter studies demonstrate identical effects of Dnmt inhibitors, ERK pathway inhibitors, and lupus, on *TNFSF7* promoter methylation and gene expression.

Mapping the lupus ERK pathway signaling defect

Since T cell ERK pathway signaling is decreased in lupus [15], hydralazine inhibits ERK pathway signaling [13], and ERK pathway signaling regulates Dnmt1 levels [15], decreased ERK pathway signaling may be fundamental to human SLE through effects on DNA methylation. The ERK pathway defect was isolated by stimulating lupus or hydralazine treated T cells with PMA and comparing PKC, ERK, MEK, and Raf phosphorylation. Identical phosphorylation decreases were observed in ERK, MEK and Raf. No effects were seen on PKCα or PKCθ. However, PKCδ phosphorylation was diminished in lupus and hydralazine treated T cells. Further, PMA stimulated, primary human CD4+ T cells treated with Rottlerin, a selective PKCδ inhibitor, and CD4+ T cells transfected with a dominant negative PKCδ, demonstrated identical decreases in ERK phosphorylation with concomitant *TNFSF7* promoter demethylation and CD70 overexpression, similar to lupus and hydralazine treated-T cells.

This supports a direct link between PKCδ and ERK [18] and suggests that abnormalities in the PKC-ERK pathway, due to impaired PKCδ activation, may contribute to human lupus through effects on DNA methylation in T cells and perhaps other cells (Fig 1). This is supported by reports that mice genetically deficient in PKCδ develop a lupus-like disease [19]. The mechanisms causing impaired PKCδ activation at present are unknown.

Summary

Current evidence indicates that lupus is an epigenetic disease caused by inhibiting T cell DNA methylation in a genetically predisposed host. The methylation defect traces to impaired PKCδ activation, causing decreased ERK pathway signaling and a failure to upregulate Dnmt1 during mitosis. Better understanding of the PKCδ defect may suggest ways to prevent lupus in predisposed hosts.

Acknowledgements

This work was supported by PHS grants AR42525, AG25877 and ES015214, and a Merit grant from the Dept. of Veterans Affairs.

References

1. Kotzin BL. Systemic lupus erythematosus. Cell 1996;85:303–306. [PubMed: 8616885]
2. Kammer GM, Perl A, Richardson BC, Tsokos GC. Abnormal T cell signal transduction in systemic lupus erythematosus. Arthritis Rheum 2002;46:1139–1154. [PubMed: 12115215]
3. Richardson B. Primer: epigenetics of autoimmunity. Nat Clin Pract Rheumatol 2007;3:521–527. [PubMed: 17762851]

4. Yang J, Deng C, Hemati N, Hanash SM, Richardson BC. Effect of mitogenic stimulation and DNA methylation on human T cell DNA methyltransferase expression and activity. J Immunol 1997;159:1303–1309. [PubMed: 9233626]
5. MacLeod AR, Rouleau J, Szyf M. Regulation of DNA methylation by the Ras signaling pathway. J Biol Chem 1995;270:11327–11337. [PubMed: 7744770]
6. Richardson BC, Strahler JR, Pivirotto TS, Quddus J, Bayliss GE, Gross LA, O'Rourke KS, Powers D, Hanash SM, Johnson MA. Phenotypic and functional similarities between 5-azacytidine-treated T cells and a T cell subset in patients with active systemic lupus erythematosus. Arthritis Rheum 1992;35:647–662. [PubMed: 1376122]
7. Lu Q, Kaplan M, Ray D, Ray D, Zacharek S, Gutsch D, Richardson B. Demethylation of ITGAL (CD11a) regulatory sequences in systemic lupus erythematosus. Arthritis Rheum 2002;46:1282–1291. [PubMed: 12115234]
8. Yung R, Powers D, Johnson K, Amento E, Carr D, Laing T, Yang J, Chang S, Hemati N, Richardson B. Mechanisms of drug-induced lupus. II. T cells overexpressing lymphocyte function-associated antigen 1 become autoreactive and cause a lupus-like disease in syngeneic mice. J Clin Invest 1996;97:2866–2871. [PubMed: 8675699]
9. Denny MF, Chandaroy P, Killen PD, Caricchio R, Lewis EE, Richardson BC, Lee KD, Gavalchin J, Kaplan MJ. Accelerated macrophage apoptosis induces autoantibody formation and organ damage in systemic lupus erythematosus. J Immunol 2006;176:2095–2104. [PubMed: 16455965]
10. Yung RL, Richardson BC. Drug-induced lupus. Rheum Dis Clin North Am 1994;20:61–86. [PubMed: 7512273]
11. Cornacchia E, Golbus J, Maybaum J, Strahler J, Hanash S, Richardson B. Hydralazine and procainamide inhibit T cell DNA methylation and induce autoreactivity. J Immunol 1988;140:2197–2200. [PubMed: 3258330]
12. Lee BH, Yegnasubramanian S, Lin X, Nelson WG. Procainamide is a specific inhibitor of DNA methyltransferase 1. J Biol Chem 2005;280:40749–40756. [PubMed: 16230360]
13. Deng C, Lu Q, Zhang Z, Rao T, Attwood J, Yung R, Richardson B. Hydralazine may induce autoimmunity by inhibiting extracellular signal-regulated kinase pathway signaling. Arthritis Rheum 2003;48:746–756. [PubMed: 12632429]
14. Richardson B, Scheinbart L, Strahler J, Gross L, Hanash S, Johnson M. Evidence for impaired T cell DNA methylation in systemic lupus erythematosus and rheumatoid arthritis. Arthritis Rheum 1990;33:1665–1673. [PubMed: 2242063]
15. Deng C, Kaplan MJ, Yang J, Ray D, Zhang Z, McCune WJ, Hanash SM, Richardson BC. Decreased Ras-mitogen-activated protein kinase signaling may cause DNA hypomethylation in T lymphocytes from lupus patients. Arthritis Rheum 2001;44:397–407. [PubMed: 11229472]
16. Lu Q, Wu A, Richardson BC. Demethylation of the same promoter sequence increases CD70 expression in lupus T cells and T cells treated with lupus-inducing drugs. J Immunol 2005;174:6212–6219. [PubMed: 15879118]
17. Oelke K, Lu Q, Richardson D, Wu A, Deng C, Hanash S, Richardson B. Overexpression of CD70 and overstimulation of IgG synthesis by lupus T cells and T cells treated with DNA methylation inhibitors. Arthritis Rheum 2004;50:1850–1860. [PubMed: 15188362]
18. Gorelik G, Fang JY, Wu A, Sawalha AH, Richardson B. Impaired T cell protein kinase C delta activation decreases ERK pathway signaling in idiopathic and hydralazine-induced lupus. J Immunol 2007;179:5553–5563. [PubMed: 17911642]
19. Miyamoto A, Nakayama K, Imaki H, Hirose S, Jiang Y, Abe M, Tsukiyama T, Nagahama H, Ohno S, Hatakeyama S, et al. Increased proliferation of B cells and auto-immunity in mice lacking protein kinase Cdelta. Nature 2002;416:865–869. [PubMed: 11976687]

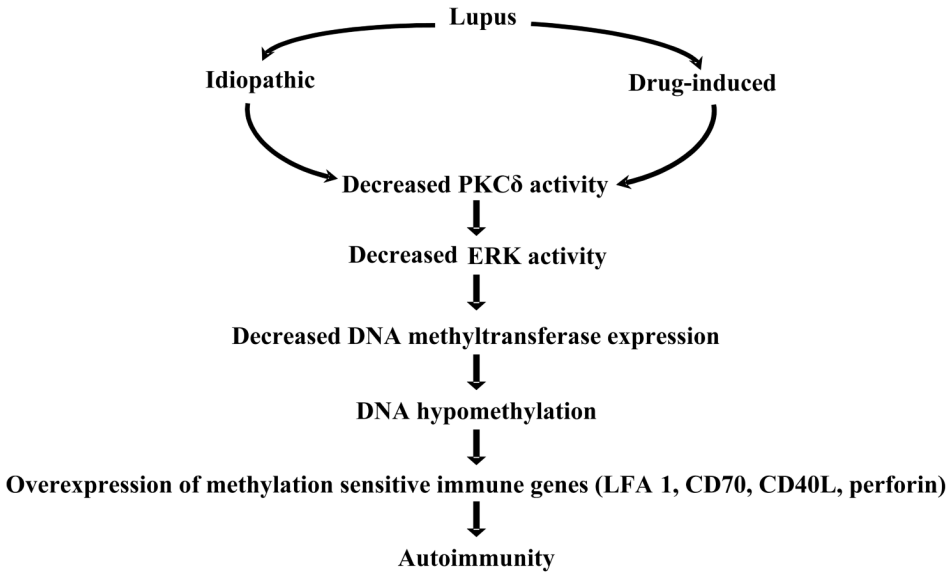

Figure 1.
Proposed mechanism by which epigenetic modifications in T cells may induce idiopathic and drug-induced lupus. Decreased ERK pathway signaling due to impaired PKCδ decreases DNA methylation what modifies gene expression making T cells autoreactive.