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Key role of ERK pathway signaling in lupus

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Abstract

Systemic lupus erythematosus is a poorly understood autoimmune disease, characterized by autoantibodies to nuclear antigens and immune complex deposition in organs like the kidney. Current evidence indicates that a pathologic CD4+T cell subset, characterized by impaired extracellular signal-regulated kinase (ERK) pathway signaling, DNA hypomethylation, and consequent aberrant gene expression contributes to disease pathogenesis. Hydralazine is a lupus-inducing drug that also decreases T cell DNA methylation by inhibiting the ERK signaling pathway, replicating the defect found in lupus T cells. These observations suggest that defective ERK pathway signaling alters gene expression in T cells by inhibiting DNA methylation, contributing to lupus pathogenesis. The signaling defect in hydralazine-treated and lupus T cells has now been mapped to protein kinase C δ . Understanding the mechanism causing decreased ERK pathway signaling in lupus may shed light on mechanisms contributing to disease development in genetically predisposed people.

Keywords

Lupus T cells; epigenetics; DNA methylation; extracellular signal-regulated kinase pathway signaling; Protein Kinase δ

Introduction

Systemic lupus erythematosus (SLE) is an autoimmune disease characterized by autoantibodies to nuclear components with immune complex formation and deposition in tissues including the kidney. The mechanisms causing lupus are not clearly understood. However, current evidence indicates that predisposing genetic factors, combined with exposure to incompletely characterized environmental factors, or to drugs like procainamide and hydralazine, plays an important role in disease development.

T cells are clearly involved in exacerbating the autoimmune response and autoantibody formation in lupus [1]. A failure to maintain T cell epigenetic homeostasis, and in particular DNA methylation patterns, is implicated in the development of idiopathic and drug-induced lupus, and may represent a mechanism by which the environment can alter immune responses in SLE. These epigenetic T cell abnormalities result in the loss of self-tolerance, abnormal

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cytokine production, the acquisition of cytotoxic responses, and overexpression of other genes that together result in the development of autoimmunity.

Abnormal T lymphocyte signal transduction also characterizes this disorder. Defective T cell extra-cellular signal-regulated kinase (ERK) pathway signaling occurs and has been directly implicated in causing epigenetic abnormalities that result in lupus-like autoimmunity. This review will focus on mechanisms causing defective ERK signaling pathway in lupus, and how it relates to disease development in idiopathic and hydralazine-induced lupus.

The mitogen-activated protein kinase (MAPK) signaling pathway

The mitogen-activated protein kinase/ERK (MAP-K/ERK) pathway is a kinase signaling cascade ubiquitously expressed in eukaryotic cells, and links receptors on the cell surface with the genetic response. The molecules involved are part of a large family of serine/threonine kinases that play a key role in immune-mediated inflammatory responses. The MAP kinase pathways include three major well-characterized subfamilies, namely the ERK, c-Jun N-terminal kinase/stress-activated protein kinases (JNK/SAPKs), and *p*-38-MAPK. More than 20 MAPK isoforms have been reported but ERK is the best characterized regarding function and regulation [Reviewed in Ref. 2]. The ERK pathway has been related to cell division, growth, and differentiation while the JNK/SAPK and *p*-38 MAPK pathways play an important role in stress responses and apoptosis [Reviewed in Ref. 3].

The ERK pathway is a cascade consisting of at least three families of protein kinases, including Raf (MAPKKK or MEKK), MAPKKs (MEK 1 and MEK 2), and MAPK (ERK 1 and ERK 2 or p42/p44 MAPKs). Each molecule acquires protein kinase activity after phosphorylation, and is subsequently able to phosphorylate and then activate the next member of the signaling cascade.

Raf is a product of the *c-ras* oncogene, and Raf-1 is the most thoroughly studied protein of this family. Raf-1 is ubiquitously expressed and is usually phosphorylated by Ras, although activation of ERK1/2 has been described independent of Ras [4]. Raf-1 activates MAPK kinases by phosphorylation of two serine residues. In turn, the MAPK kinases activate ERK 1 and ERK 2. These are the only known downstream effectors of these kinases, and are activated by dual phosphorylation of a threonine and a tyrosine residue. Once activated, ERK1/2 translocates to the nucleus where it activates transcription factors such as NF- κ B and activating protein 1 (AP-1), a family of transcription factors that includes c-Jun and c-Fos. The fact that Raf is much less abundant than MEK or ERK, and the specific dual phosphorylation mechanism, generates a system with a high degree of signal amplification [5].

Ras activation uses a different mechanism since it is a small G-protein that can bind either GTP or GDP. When Ras-bound GDP is exchanged with GTP by guanyl-nucleotide exchange factors (GEF), Ras becomes active. Ras conformation then changes, permitting interactions with Raf and sequestering Raf to the plasma membrane where it is accessible to its activators. This initiates the kinase cascade.

MAPK and T lymphocytes

ERK1/2 mediated signaling was initially believed to primarily regulate cell growth and proliferation, but is now believed to mediate inflammatory responses as well. ERK1/2 is critical for T cell activation, mediated by activation of the AP-1 family of transcription factors. In T lymphocytes, the ERK pathway is activated by T cell receptor (TCR)-dependent and independent responses.

Ligation of TCRs by antigen–MHC complexes on antigen presenting cells triggers T cell activation and induces activation of the Ras–MAPK pathway [6]. The mechanism by which this pathway is activated is not completely understood. ERK is activated through Ras in T cells after TCR/CD3 complex formation, but is also activated by other molecules such as the IL-2 receptor [7], and by phorbol myristate acetate (PMA) [8]. PMA directly induces activation of protein kinase C (PKC), mimicking the effects of diacylglycerol, a second messenger produced from inositol phosphates by phospholipase C γ 1 following TCR ligation. Early reports suggested that PKC directly activates Ras, which then transmits a signal activating the downstream pathway that culminates with ERK.

However, later studies showed conflicting results since there is evidence for PKC-dependent and independent ERK activation [9]. A dominant negative Ras blocks the PKC-induced ERK activation in some cell systems [10]. However, in T cells, several reports demonstrated that phosphorylation and activation of Raf-1 and ERK following TCR ligation is PKC-dependent, although the participation of Ras is controversial [11]. T cells deficient in PKC θ , a PKC isoform highly expressed in T cells, have normal ERK responses [12]. However, others authors have demonstrated that PKC θ , PKC α , and PKC ϵ were able to activate the ERK pathway in T cells [13].

PKC δ and PKC ζ might also be involved in the TCR-mediated regulation of ERK, suggesting a complex mechanism regulating ERK and different and specific roles for the various PKC isoforms [14]. The differences in ERK activation observed in different T cell subpopulations following different stimuli, and in other experimental systems, were attributed to the existence of different pathways driven by different guanine exchange factors for Ras, such as RasGRP and Sos. RasGRP1, a RasGEF protein expressed almost exclusively in T cells, may predominate in some subsets of lymphocytes and respond to certain stimuli [15] and may also be the link between PKC and ERK [16].

T cells and lupus

T cells are involved in the production of lupus autoantibodies, which are a hallmark of the disease. T cells from patients with active lupus have multiple signaling abnormalities that contribute to the abnormal immune response. The signaling defects include diminished TCR ζ -chain expression, that was recently reported to be due to increased lysosomal degradation caused by NO-induced mammalian target of rapamycin (mTOR) activation [17]. This defect in TCR plays a critical role in calcium fluxing, causing a large and rapid increase in cytoplasmic and mitochondrial Ca²⁺. These impairments are related to the persistent mitochondrial hyperpolarization characteristic of lupus T cells. The signaling abnormalities conclude with the altered activation of transcription factors and gene expression. These T cell signaling aberrancies together drive altered cytokine production, apoptosis, oxidative stress responses, and overproduction of anti-dsDNA antibodies, as some of the defects characterizing lupus [Reviewed in Ref. 18].

Our group has described a defect in lupus T cell ERK pathway signaling, and reported that this defect can cause epigenetic abnormalities in T cells including genome-wide decreases in DNA methylation, a repressive DNA modification. The abnormal lupus T-cell DNA methylation may be responsible in part for the autoreactivity, and directly [19,20] or indirectly [21] for the altered secretion of cytokines. The hypomethylation of regulatory DNA sequences appears to contribute to the pathogenesis of both drug-induced and idiopathic human lupus by causing overexpression of genes promoting disease development and progression [Reviewed in Ref. 22].

Extracellular signal-regulated Kinase (ERK) pathway signaling and lupus

Preliminary results from our group showed that treating cells with a DNA methyltransferase (Dnmt) inhibitor increases transcription of genes regulated by DNA methylation, and that human T cell Dnmt levels can be decreased by inhibiting signaling through the Ras–MAPK pathway [23]. These results are in accordance with other reports indicating that Dnmt1 levels are regulated through the ras–MAPK signaling pathway by interacting with AP-1 sites in an intronic promoter, and that inhibiting this signaling pathway can inhibit DNA methylation in actively proliferating cells [24,25]. It is important to note that methylation of regulatory elements inhibits gene expression, while the regulatory elements of transcriptionally active genes are typically unmethylated.

Procainamide and hydralazine are two drugs that have been implicated in inducing lupus-like diseases. Both drugs induce autoreactivity by inhibiting T cell DNA methylation [26] but through different mechanisms. The former is a competitive DNA methyltransferase inhibitor causing a decrease in its enzyme activity [27]. In contrast, hydralazine does not directly inhibit Dnmt activity, but decreases T cell Dnmt mRNA expression *in vivo* [28]. Similar results were observed in T cells following treatment with the MEK inhibitors PD98059 and U0126. These results suggest that hydralazine is acting through the ERK pathway. This hypothesis was tested by measuring ERK phosphorylation in PMA-stimulated T cells pre-treated with hydralazine. The ERK phosphorylation inhibition induced by hydralazine was similar to that caused by PD98059, while JNK and p38 pathways were unaffected.

These results demonstrated that hydralazine selectively inhibits signaling through the ERK pathway, causing downregulation of Dnmt1 and Dnmt3a in stimulated T cells, with subsequent DNA hypomethylation. To confirm a pathologic role for T cell ERK pathway impairment in the development of autoimmunity, murine CD4+T cell clones were treated with hydralazine or the ERK inhibitor U0126. Both treatments caused overexpression of the leukocyte function-associated antigen 1 (LFA-1) and the cells became autoreactive *in vitro*. Injecting the treated cells into syngeneic mice induced anti dsDNA antibodies [28], supporting the concept that hydralazine is acting through inhibition of the ERK pathway to cause a lupus-like disease.

Since a lupus-like disease can be induced by inhibiting the ERK pathway, and lupus T cells have multiple signaling abnormalities, evidence for impaired ERK pathway signaling was sought in lupus T cells. Patients with active lupus were found to have impaired T cell ERK1/2 phosphorylation, similar to hydralazine-treated T cells, and the degree of impairment was proportional to disease activity [29]. The same studies demonstrated that Dnmt mRNA levels in T cells from patients with active lupus were ~50% lower than those observed in healthy donors, consistent with the ~50% decrease in Dnmt enzyme activity found in active lupus T cells [30]. It is important to note that the impaired ERK pathway was reproduced in purified CD4+T cells from patients with active lupus, same subpopulation that becomes autoreactive in the presence of DNA methylation inhibitors and MEK inhibitors [31]. In contrast, CD8+T cells did not become autoreactive in this system.

Other authors have reported defective MAPK signaling in lupus. Abnormalities of Ras activation and signaling was attributed in the development of lupus [Reviewed in Ref. 32]. Defective ERK-1 and ERK-2 activation was also reported in lupus T cells following TCR activation, due to altered coupling of Ras to adapter proteins [33]. Also, inhibition of AP-1 transcriptional regulators, that normally induce proinflammatory proteins such as IL-2, was observed in lupus T cells and attributed to defective PKC and Ras–ERK pathway [34]. However, the observation that T cells treated with ERK inhibitors can cause a lupus-like disease in model systems suggests that abnormalities in this pathway may induce the disease rather than being a consequence of the disease pathogenesis.

Together, these observations indicate that impaired ERK pathway signaling may contribute to human lupus by decreasing DNA methylation, causing gene dysregulation and autoreactivity. This hypothesis was tested by comparing the effects of ERK pathway inhibitors, DNA methyltransferase inhibitors, and lupus on the methylation and expression of methylation-sensitive genes. CD70 is a methylation-sensitive gene initially identified by oligonucleotide array-based approaches and is a member of the tumor necrosis factor (TNF) family. CD70 is a B cell costimulatory molecule expressed on activated CD4+ and CD8+ T cells and B cells [35].

Human T cells were treated with the ERK pathway inhibitors U0126, PD98059, and hydralazine or the DNA methyltransferase inhibitors, 5-azaC or procainamide. The ERK pathway and DNA methyltransferase inhibitors all caused overexpression of CD70 at the mRNA and protein levels. The increase was only observed in CD4+ T cell subset, while CD8+ T cells did not show significant increases in CD70 expression.

Next, B cells cultured with Dnmt inhibitor or ERK inhibitor-treated T cells were shown to secrete greater amounts of IgG than B cells incubated with untreated T cells, and pretreatment of the T cells with anti-CD70 antibody abrogated cell IgG production. Furthermore, a similar pattern of CD70 overexpression was seen in lupus T cells as in the drug-treated T cells, and lupus T cells were also able to stimulate IgG synthesis by autologous B cells in the absence of added antigen or mitogen. Pretreatment of the lupus T cells with anti-CD70 similarly inhibited IgG production, indicating that CD70 may contribute to lupus pathogenesis by increasing B cell stimulation [36]. Finally, CD4+ T cells from lupus patients were also found to have hypomethylation of the same CD70 promoter sequences demethylated by ERK inhibitors and Dnmt inhibitors [37]. Because T cells from patients with active lupus have impaired ERK pathway signaling and decreased Dnmt levels, these studies indicate identical effect of ERK inhibitors, Dnmt inhibitors, and lupus on the methylation of the TNFSF7 (CD70) promoter.

A causative relationship between decreased ERK pathway signaling and autoimmunity is further supported by a transgenic mouse model that inducibly expresses a dominant negative MEK in T cells. These mice demonstrate inducible decreases in T cell ERK pathway signaling accompanied by decreased DNA methyltransferase expression and overexpression of the methylation-sensitive genes CD11a and CD70. These transgenic animals also develop anti ds-DNA antibodies and an “interferon signature”, strongly supporting the hypothesis that T cell ERK pathway signaling defects contribute to the development of autoimmunity [38].

Based on these observations, efforts were made to elucidate the mechanism causing decreased ERK pathway signaling in idiopathic and drug-induced lupus T cells. T cells from patients with active lupus or hydralazine-treated T cells were stimulated with PMA, then ERK, MEK, and Raf phosphorylation were compared. All three signaling molecules displayed decreases in phosphorylation in both lupus and hydralazine-treated T cells. PKC isoforms were next studied as upstream signaling molecules. Only PKC δ showed impaired phosphorylation in response to PMA stimulation of lupus and hydralazine-treated T cells. In contrast, no effects were observed on PKC α or PKC θ . Next, PMA-stimulated human CD4+ T cells were treated with Rottlerin, a selective PKC δ inhibitor.

The treated cells demonstrated identical decreases in ERK phosphorylation with concomitant CD70 overexpression and demethylation of the TNFSF7 (CD70) promoter, similar to lupus and hydralazine-treated T cells. The same results were seen in CD4+ T cells transfected with a dominant negative PKC δ , strongly indicating a direct signaling link between PKC δ and ERK in T cells [39]. Together, these results strongly suggest that impairment of ERK pathway signaling, due to defective PKC δ activation, may play a role in the development of human and drug-induced lupus mediated by demethylation of T cell DNA. Importantly, mice genetically

deficient in PKC δ also develop a lupus-like disease [40], strongly supporting this hypothesis. Further experiments are necessary to characterize the mechanisms causing impaired PKC δ activation.

Summary

A series of reports extending over the past 23 years demonstrate that impaired T cell DNA methylation contributes to the development of a lupus-like disease in murine models, that the lupus inducing drugs procainamide and hydralazine are DNA methylation inhibitors, and that 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. Current evidence points to impaired ERK pathway signaling, due to impaired PKC δ phosphorylation, as the mechanism responsible for the demethylation. Further studies are needed to characterize mechanisms causing the PKC δ activation defect in hydralazine-treated and lupus T cells. Such studies may reveal new therapeutic targets.

Conclusions

- T cell DNA methylation contributes to the development of lupus.
- Inhibition of ERK pathway signaling may be responsible for T cell DNA demethylation in idiopathic and hydralazine-induced lupus T cells.
- Defective T cell PKC δ activation contributes to impaired ERK signaling in SLE and hydralazine-treated T cells.

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