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Role of Epigenetics in the Pathogenesis of Asthma

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ABSTRACT

Asthma is a complex, heterogeneous and chronic airway inflammatory disease with different clinical phenotypes caused by diverse triggers and pathophysiological mechanisms. Asthma heritability has been established in many genetic studies but it is evident that only genetic elements are not responsible for the development of asthma. Increasing rate of asthma incidence during past decades has implicated the role of epigenetics in development of asthma.

Environmental factors perform as initiator signals through epigenetic mechanisms. Three epigenetic mechanisms have been identified, including DNA methylation, histone modifications, and small noncoding RNAs.

These mechanisms regulate the immune responses and inflammatory genes expression in asthma and allergy. This review explains the role of epigenetic modifications in controlling Th2 response and IgE production in asthma and also briefly overviews the role of environmental factors such as pollutions, allergens, prenatal exposures and diet in developing asthma.

Recognizing environmental risk factors and their effects on epigenetic mechanisms would be of great interest for prognostic and preventive aspect in treatment of asthma.

Key words: Asthma; DNA methylation; Epigenetics; Histone modification; miRNA; Environmental factors

INTRODUCTION

Asthma is a complex, heterogeneous and chronic airway inflammatory disease with different clinical phenotypes caused by diverse triggers and pathophysiological mechanisms. Asthma is believed to be a heritable disease based on many genetic studies ;h however, increasing incidence of asthma is not only related to genetic elements, especially in recent years.[1] Epigenetics is considered as a major contributor of asthma pathogenesis.[2] There is ample evidence that genes and environment interact to develop complex

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diseases such as cancers, endocrine syndromes, several brain disorders, sleep disorder, depression and pulmonary diseases.[3-9] Environmental factors are initiator signals of epigenetic mechanisms. Epigenetics indicates any changes in gene expression and cellular phenotype without any variation in DNA sequences. The most studied epigenetic mechanisms in asthma are DNA methylation, histone modifications , and small noncoding RNAs.[2] Epigenetic marks can change DNA or chromatin structure through any changes in histone proteins, these changes can be sustained and inherited by epigenetic profile.[10] Meanwhile, a genetic polymorphism may facilitate a methylation quantitative loci, for instance if GA sequences are substituted by CG sequences, these would allow methylation and can generate a methylation site.[10]

DNA methylation is the major modification of DNA, specific DNA methyltransferases (DNMT) add methyl group to the $_{5'}$CpG dinucleotides. Around 75% of CpG islands are methylated but single CpGs are not methylated. DNA methylation can affect gene transcription directly by blocking the binding of transcription factors or enhancers and indirectly by binding to methyl-CpG-binding domain proteins (MBDs) and engaging other proteins such as histone deacetylases (HDACs); therefore, making condensed chromatin ,which prevents transcription.[11-13] Histone acetylation is the most studied histone modification ever, acetylation of lysine residues is achieved by histone acetyltransferases (HATs), which is particularly important in the regulation of gene expression. Histone hyperacetylation is in the favor of gene expression, while deacetylation by histone deacetylases (HDACs) causes gene silencing.[14,15] Imbalance of HATs/HDACs lead to inappropriate gene expression and might be the reason of developing diseases such as cancers and chronic lung diseases like asthma.[2] MicroRNAs (miRNAs) are short (19-25 nucleotides), noncoding RNAs that regulate gene expression by binding to the specific sequences, which results in mRNA degradation or prevention of protein translation.[9,16]

Epigenetics has a great impact on development of immune related lung diseases including idiopathic pulmonary fibrosis, tubercolosis, sarcoidosis, silicosis, chronic obstructive pulmonary disease (COPD) and asthma.[2,9] Although each disease is distinct but the fundamental intracellular processes are the same. Some selectivity occurs in gene expression of the receptors in different cell types in response to environmental exposures.[2] A schematic picture shows the role of epigenetics in pathogenesis of asthma (Figure 1).

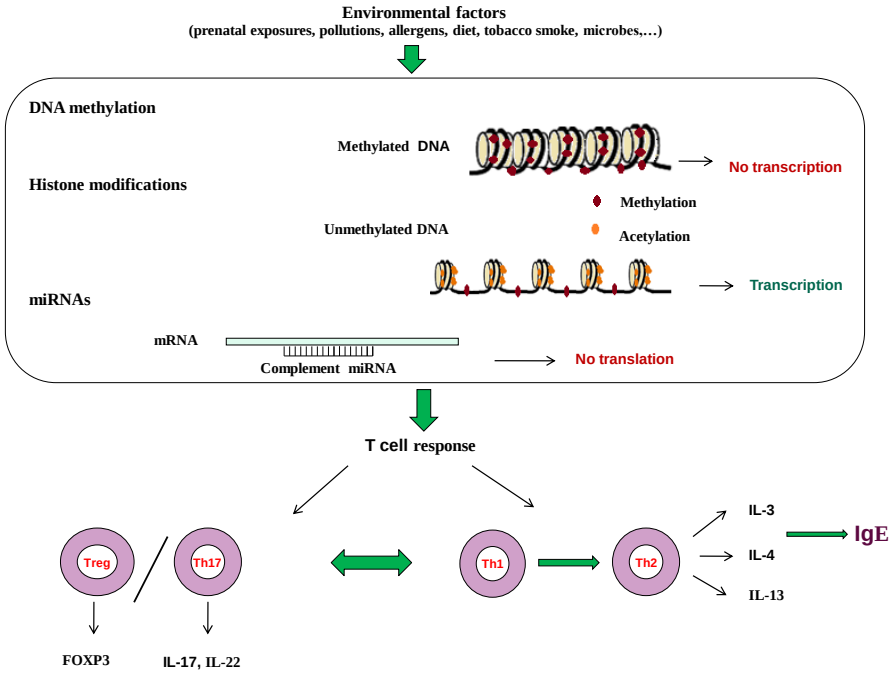

Figure 1. A schematic picture showing the role of epigenetics in pathogenesis of asthma

Epigenetic Regulation and Immune Response

After contact of antigens with T cells, CD4+ T cells differentiate into T helper 1 (Th1) producing IFN-γ and T helper 2 (Th2) subsets, secreting interleukin 4 (IL-4), IL-5 and IL-13,.[17] Each subset is involved in different types of immune response; Th2 response is the main response in the allergic diseases.[18] In naïve CD4+ Tcells, the CpG sites in the promoter of IL-4 and IFN-γ genes are methylated.[19] After exposure to allergens and phytohemaglutinin, demethylated IL-4 promoter in allergen specific T cells is correlated to the levels of IL-4 in asthmatic patients and nonallergic controls.[19] The result of this study shows that the -80 location of IL-4 CpGs is important in its gene expression.[19] Cytokines gene expression in CD4+ Tcells is under control of distinct transcription factors and epigenetic events. Phosphorylation of STAT6 and expression of the master regulator of Th2 cells "GATA3" are involved in Th2 differentiation.[20] GATA3 binds to a specific DNase-1 hypersensitive site 2 (HS2), which is in the second intron of the IL-4 gene. GATA3 binding to HS2 would induce H3K4me3 and H3K9/K14 acetylation and IL-4 expression.[2] Hyperacetylation and H3K4 methylation have a significant effect on gene activation, while H3k9 and H3K27 methylations cause gene repression.[18] Genome wide studies have shown that silent genes with H3K4me3 are mainly activated through sequences of acetylation and deacetylation by HATs and HDACs in human CD4+ Tcells and silent genes without any H3K4me marks have no binding interaction with HDACs.[21]

Next subsets of Tcells which are important in inflammatory response are Th17 and T regulatory (Treg) cells and their imbalance is crucial in allergic airway inflammation.[20,22] Allergic asthmatic patients have higher number of Th17 in their peripheral blood compared to controls and the significance of IL-17 elevation has been shown in the late phase asthmatic response compared to the early responders to allergen challenge.[22] Tregs which express FOXP3 have suppressive function and prevent high inflammatory responses.[20] The absolute demethylation of Treg-specific demethylated region (TSDR) in Tregs was observed while the TSDR of conventional CD4+ T cells is extremely methylated. Demethylation of this region is necessary for constant expression of FOXP3 in Tregs. Also, 10%-45% of the CpG sites in the FOXP3 promoter are methylated in CD4+ T cells compared to total demthylation of these CpG sites in Tregs.[23] Besides TGF-β binding to SMAD3 at CNS1 induces FOXP3 transcription in CD4+ T cells.[20] Induced Tregs by TGF- β could be expanded by addition of retinoic acid. It has been shown that retineoic acid induces histone acetylation at the CNS1 region.[24] These findings indicate the epigenetic regulation of the FOXP3 in CD4+ T cells and Tregs. The role of miRNAs (miR-24 and miR27) in regulation of Th2 response has been demonstrated in Tcells and IL-4 production.[25] The regulatory function of miR-17 ~ 92 clusters has been established to promote T cell proliferation and differentiation to Th1, Th17, T follicular helper (TFH) and Treg cells. One of a potent Th2 modulators is miR-19, which induces PI(3) K, JAK, STAT and NF- κ B signaling.[26]

Role of Epigenetic Events in Asthma

Diverse clinical phenotypes of asthma may be caused by different inflammatory responses.[27] Different phenotypes of adult asthma were defined by cluster analysis, ranging from mild to severe asthma. Analyzing gene expression patterns including expression of miRNAs could be considered as a biomarker for asthma phenotypes.[28] Distinguishing eosinophilic from noneosinophilic phenotypes is possible by gene expression pattern which contains Th2 associated genes.[29] Endotyping of asthma is an imperative approach to define different types of asthma. The integration of cluster analysis, phatophysiological, genetic, transcriptomic, lipidomic, proteomic and mitochondriomics data will provide a precise assessment of defining asthma endotypes.[27,30] Moreover, epigenetic mechanisms reflect the effect of environmental factors on the development of asthma.[27] So, the assessment of epigenomics would be a valuable approach to define sub-phenotype of asthma and may present new therapeutic targets for the course of severe asthma. Table 1 briefly shows selected genes which are regulated by epigenetic modifications and discussed in this review.

Based on the ample evidence about influence of environmental factors on epigenetic compartments and regulatory function of epigenetic in T cell differentiation, epigenetic modification seems to play an important role in increasing asthma prevalence in recent decades.[13] Recognizing environmental risk factors and their effects on epigenetic mechanisms would be of great interest for prognostic and preventive

aspects in asthma management. Moreover, targeting epigenetic mechanisms using epigenetic modifying tools would be a secondary preventive step to diminish disease development.[31]

DNA Methylation in Asthma

Most studies regarding epigenetic and asthma have focused on DNA methylation because of practical and biological points.[10] Altered DNA methylation status may cause differential gene expression of cytokines and their transcription factor.[9] Unmethylated IL-4 correlates with increased IL-4 expression in *Dp/Df* sensitized patients with bronchial asthma.[19] Higher methylation level of IL-2 promoter (site 1IL-2) is associated with increased development of severe asthma in children who were followed to the age of 8 years.[32] Methylation of this IL-2 CpG site is necessary for gene regulation as IL-2 only transcribes when CpG site 1 is demethylated.[32] Increased methylation of CpG sites in FOXP3 and IFN-γ may result in a lower gene expression in Tregs and T effecter cells and impairment of Tcell function in asthmatic patients.[33] The inverse correlation of zona pellucida binding protein 2 (ZPBP2) exon 1 methylation and its RNA expression was shown in lymphoblastoid cell line. ZABP2 is located on 17Q12-Q21 region that harbors asthma associated genes.[34] The hypomethylation of arachidonate 12-lipoxygenase (*ALOX12*) CpG sites may be associated with higher risk of persistent wheezing in children and it could be considered as a biomarker of susceptibility to asthma in early childhood.[35] Analyzing methylation pattern of B cells in house dust mite (HDM) allergy and aspirin-intolerant asthma has shown different methylation pattern in CYP26A1 gene and its expression level compared to control subjects.[36] *CYP26A1* is involved in retinoic acid catabolism, which is a main regulator of immune response engaged in allergic diseases.[37] In a study by Somineni et al., the association of hypomethylation of a CpG site in the TET1 (Ten-eleven translocation 1) promoter with asthma was shown in children born in air polluted area compared to

Table 1. Selected genes which are regulated by epigenetic modifications in asthma

	Genes	Modification	Outcome
	IL-4	Hypomethylation	Asthma
	IFN-γ	Hypermethylation	Asthma
DNA	*IL-2*	Hypermethyalation	Severe asthma
Methylation	*FOXP3*	Hypermethyalatiom	Asthma
	ZABP2	Hypomethylation	Asthma
	ALOX12	Hypomethylation	Wheezing
	CYP26A1	Hypermethylation	Allergy and aspirin-intolerant asthma
	TET1	Hypomethylation	Asthma
	IL1R2	Hypermethylation	Asthma
Histone	*Notch-1*	Hyperacetylation of	Asthma
Modification		H3K9/K14/K18/K27 and trimethylation of H3K4/K79	
	Glucocorticoid receptor (GRα)	HDAC2 down regulation	Severe asthma
	SOX2	HDAC1 up regulation	Severe asthma
	IFN-γ, IL-4, IL-17A, IL-17F, ROR(γ)t, Foxp3	H3K4 trimethylation	Allergic diseases
	ΔNP63, EGFR and STAT6, CXCL8	Increased H3K18 acetylation	Asthma
	CCR4,CCL5	H3K4 dimethylation	Asthma
miRNAs	*IL-12, IL-3,*	miR-21	Asthma
Expression	*IL-5*		
	IL-22	miR323-3p	Asthma
	IL-12p35	miR-21	Severe asthma
	TLR7	miR-150,152,375	Severe asthma

their healthy siblings and controls.[38]

TET1 induces DNA demethylation by converting 5-methycytosine to 5-hydroxymethylcytosine through oxidation followed by decarboxylation.[38,39] IL-1 receptor 2 (*IL1R2*) hypermethylation is shown to be associated with asthma and its methylation conversely related to its expression in asthmatic subjects.[40] In a study on asthma-discordant monozygotic twins by Murphy et al., different methylation pattern is shown to be associated with childhood asthma. The top ranked CpGs that have different methylation pattern are in the downstream of the HLX gene, which has been indicated in asthma pathogenesis.[41]

Histone Modifications in Asthma

Abnormal expression and activity of HAT and HDAC has been indicated in asthma and inflammatory responses.[42] HDACs activity has been reduced in asthmatic smokers and this is correlated with severity of asthma and resistance to steroids in these patients.[2] Increased activity of HAT and hyperacetylation of H3K9, H3K14, H3K27, H3K18 and H3K16, trimethylation of H3K4 and H3K79 in *Notch-1* promoter result in dysregulation of *Notch-1* signaling in T cells.[43] Restore *Notch-1* acethylation by using HATs inhibitor can suppress *Notch-1* expression which result in reduce production of IL-4, IL-5 and IL-13 in CD4+ Tcells in asthmatic rats.[43] Patients with severe asthma are steroid-resistant and HDAC2 deficient cells are insensitive to corticosteroid, increasing HDAC2 expression could retrieve the corticosteroid sensitivity.[44] Deacetylation of glucocorticoid receptors by HDAC2 may suppress inflammatory genes in asthma.[45] HDAC1 is necessary for remodeling of airway epithelium and HDAC1 inhibition could prevent epithelial cell growth through *SOX2* failure expression.[46] Air way remodeling is related to severe asthma and up regulation of HDAC1 has been observed in severe asthma compared with normal patients.[31] Increased acetylation of H3K18 in transcription start site of *ΔNP63, EGFR* and *STAT6* has been found in airway epithelial cells of asthmatic patients.[47] Air way smooth muscle (ASM) cells of asthmatic patients secret more CXCL8 chemokine due to H3K18 acetylation in comparison with healthy individuals.[48] Histone methylation is another histone modification, which related to CD4+ T cell differentiation. H3K4 trimethylation (H3K4me3) is associated with increased expression of IFN-γ, IL-4, IL-17A, and IL-17F, and the transcription factors ROR(γ)t and Foxp3, which are predominantly expressed in Th17 and Treg cells, respectively.[49] The imbalance of Tregs and Th17 cells is important in allergic diseases.[22] H3K4me2 augmentation at SNPs of CCR4 and CCL5 asthma-associated genes, promote Th2 differentiation.[31] Different H3K4me enrichment in enhancer regions has been identified in Th1 and Th2 cells of asthmatic patients compared to healthy controls.[50]

Role of miRNAs in Asthma

Many studies have been conducted in mouse models of asthma to define role of miRNAs in asthma. Recently increasing evidence has been reported in human as well. In experimental model of asthma, miR-21 has prevented expression of IL-12, IL-3 and IL-5. Removal of miR-21 has been reported to induce IL-12 and IFN-γ production in dendritic cells ,while it induces less IFN-γ production in CD4+ Tells.[51] In a study, the differential expression of 217 miRNAs has been shown in bronchial epithelium of asthmatic patients and healthy controls.[31] Expression of C-Abl in human airway smooth muscle (HASM) is negatively regulated by miR-203, C-Abl control HASM cell proliferation and ERK1/2 phosphorylation.[52] High expression of miR323-3p has been inversely correlated with IL-22 production in PBMCs of asthmatics. Increased miR323-3p in IL-17 and IL-22 expressing cells result in TGFβ suppression pathway and diminished IL-22 production in T cells.[53] Higher expression of miR-21 is associated with asthma in children. Also, inverse correlation of miR-21 with IL-12 p35 serum level has been shown in steroid-resistant asthmatic children.[54] The important regulatory role of miR-24 and miR-27 on Th2 cells has been demonstrated in mouse model of asthma.[25] Individual transfected HASM cells with miR-708 and miR -140-3p have dissimilar influence on inflammatory genes. Inhibition of CXCL12, CXCL10, CCL5 and CXCL8 expression has occurred in miR-140-3p transfected cells while miR-708 inhibits PARPES, CD44 and ADAM33 expression.[55] Significant reduction of TLR7 expression has been shown in alveolar macrophages of severe asthmatic patients, who are susceptible to rhinovirus infection. Low production of TLR7 in these patients is under control of 3 miRNAs, miR-150, miR-152 and miR-375.[56] Assessments of circulating

miRNAs can be used as a biomarker in diagnosis of allergic diseases and asthma and in therapeutic intervention as well.[57,58]

Asthma is a syndrome including versatile phenotypes, epigenetic biomarkers, molecular, immunological and functional endotypes which would guide to personalized and phenotype-specific therapies.[59] A number of drugs that target epigenetic marks are successfully developing in mouse models including HDAC and HAT inhibitors, DNMT and DNA demethylase inhibitors and miRNA antagonist and miRNA mimics.[60] All biological and immunological data from various experiments and clinical trials and developing mathematical analysis models lead to computer modeling which will guide to the health information technologies (HITs) and this would be useful for self management of diseases.[61,62]

Role of Epigentics in IgE Release

Immunoglobulin E (IgE) has a significant role in allergic reactions such as asthma, rhinitis and eczema. Atopy is recognized by positive skin prick test or high level production of total IgE or existence of specific IgE against inhalant allergens.[63,64] In allergic reaction and atopic asthma, Th2 cells with distinctive IL-4 and IL-13 cytokine pattern, produce high levels of IgE in response to specific allergens.[64,65] The high level of IgE is related to increased risk and severity of asthma.[63] The level of IgE in cord blood was used to predict asthma and other allergic diseases.[66,67] Furthermore, recent clinical trials using omalizumab (IgE inhibitor) and inhibitors of Th2 cytokines have been shown to be impotant in Th2 response and IgE production and susceptibility to asthma exacerbation.[68] Histone acetylation of H3K4 and H3K27 are important in asthma pathogenesis. Histone acetylation of H3K4 and H3K27 can also regulate IgE production through controlling IL-4/STAT6 pathway.[69]

STAT6 is one of the most principal genes associated with IgE production in allergic asthma. Hypomethylation of *STAT6* promoter has been shown to have a great role in STAT6 gene expression comparing to its polymorphism.[70] Moreover, hypomethylation of IL-4, IL-13 and RunX3 were confirmed in asthmatic children.[71] In a recent study, Yang *et al*. have demonstrated differential methylated regions, which are associated with higher total serum IgE concentration in nine asthmatic children.[71] Also, Everson *et al*. have identified 13 novel epigenetic loci including *ZFM1*, *PRG2*, *EPX* and *COPA*, which are associated with high IgE production.[64]

Environmental Factors and Their Effects on Epigenetic of Asthma

Environmental factors, such as allergens, maternal cigarette smoke, diet, stress, microbes, air pollutions, heavy metals, and pesticides can influence epigenetic modifications; therefore, the interaction of environmental factors on gene transcription through epigenetic mechanisms could be considered for developing complex diseases like asthma and allergy.[20,72,73]

Few studies have implicated the influence of allergens on epigenetic modifications. The effect of HDM on DNA methylation and expression of cytochrom450 26A1 (*CYP26 A1*) has been shown in B cells of asthmatic patients compared to control subjects. CYP26A1 is involved in metabolism of steroids and lipids.[36] Also, global changes in DNA methylation have been demonstated in HDM-treated mouse.[74] Expression of miR-145 has been elevated in HDM-induced allergic mice, as a previous study has been indicated that mi-145 is involved in airway smooth muscle function.[75] Hypermethylation of IFN-γ and hypomethylation of IL-4 have been shown in CD4+ T cells of experimental allergic mouse model.[76,77]

The effects of tobacco smoking in prenatal stage and early childhood have been established in a number of studies as a major risk factor for asthma. Recently, a genome-wide study of 13 cohorts in newborns with the history of maternal smoking has revealed differential DNA methylation pattern in many loci including genes that are involved in asthma.[78] The effect of nicotine exposure and induction of asthma has been demonstrated to be transmitted to the next generation in rat through epigenetic alterations including DNA methylation and H3 and H4 acetylation.[79] Down regulation of HDAC2 has been associated with cigarette smoke in lung biopsies and bronchoalveolar lavage fluid macrophages of smokers compared to nonsmokers. Low expression of HDAC2 results in up regulation of GM-CSF, IL-8 and TNF.[80,81]

Exposure to the particulate matter ($PM_{2.5}$) has been related to severe asthma and hospitalization in children. $PM_{2.5}$ and PM_{10} exposure can cause iNOS hypomethylation and airway inflammation.[73] Exhaust particulate matter (DEP) exposure has been shown to increase methylation of three CpGs in IFN-γ promoter

and a CpG site in IL-4 promoter in mice, these changes are responsible for high level of IgE.[77] Also, DEP exposure has altered DNA methylation and gene expression of FOXP3 in saliva samples of children.[82] Polycyclic aromatic hydrocarbons (PAHs) exposure results in hypermethylation of FOXP3 and changing Treg to Th2 phenotype.[9]

Environmental microbes have an important role in modulating immune response, especially in early childhood.[81] Children who grow up in farming area have lower risk of allergic disease and asthma. More efficient Treg function has been implicated in children whose mothers were in farming environment. Prenatal administration of *Acinetobacter lwoffii* F78 could prevent experimental asthma phenotype in mice. This is related to H4 acetylation at IFN-γ promoter and deacetylation at IL-4 promoter in CD4+ T cells, which results in down-regulation of IL-4 expression and Th2 response.[83]

The role of diet and supplements such as vitamin D, folic acid and fish oil and antibiotics on epigenetic modification have been studied in a number of studies in prenatal stage.[81,84,85] Hypomethylation of *ZFP57* (zinc finger protein57) transcripts, H3 and H4 acetylation at the *ZFP57* and *GATA3* promoter has been shown in CD4+ T cells.[81] Hyperacetylation of H3 at the IL9 promoter has been reported in a group with high folate in their serum compared to those with low level folate serum. There is some controversy in different studies about this issue; hence, no change in taking folic acid is recommended during pregnancy.[86]

Epigenetic mechanisms including DNA methylation, histone modifications and miRNAs expression are involved in immune response and regulation of different cellular functions. Epigenetic studies would be a helpful approach to explain increasing risk of asthma in recent years and ascertain theeffect of environmental factors on development of allergic disease and asthma. Some of these epigenetic alterations are reversible, so they could be considered for novel therapeutic interventions of asthma. To obtain this goal, more clinical, epidemiological, interventional and environmental studies should be conducted to evaluate the endotype of asthma and the epigenetic biomarkers. Distinguishing epigenetic markers could be helpful in endotyping and diagnosis of asthma phenotypes, personalizing treatment and primary prevention of asthma and allergies. Further studies are needed to clarify the inheritance of epigenetic changes and downstream regulation of gene-specific epigenetic modifications. Furthermore, proper study designs and data analyzing should be established to achieve consistent and reliable results in different laboratories.

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