

Immunopathogenic Interaction of Environmental Triggers and Genetic Susceptibility in Diabetes

Is Epigenetics the Missing Link?

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Autoimmune type 1 diabetes is caused by an interactive combination of genetic and environmental factors, most of which remain unknown. Each individual appears to have a unique combination of these factors that allow for their susceptibility to disease. Investigators have long searched for physiological mechanisms that could link diverse environmental events to inheritable genetic traits and the aberrant gene expression in immune cells. Much of this search has focused on either uncovering elusive gene polymorphisms found in at-risk populations or on finding common elements in the lives of susceptible individuals that “trigger” their immune system toward a self-destructive path (1,2). To date, only a few gene polymorphisms have linked to susceptibility, with none so far proven both essential and sufficient to promote disease (1). The search for environmental etiological agents in this multifactorial disease has been no less difficult, since each individual afflicted appears to have a unique set of factors promoting autoimmunity rather than a single or few common triggers (2). So how can such a diverse set of environmental stimulators rapidly and reversibly trigger a variety of undefined genetic regions to promote the loss of something as basic to normal physiology as tolerance of self? Is there a common mechanism even when the end targets are elusive and environmental slings and arrows are so diverse? Enter epigenetic gene regulation into the tableau, adding a new meaning to the term genetic susceptibility (Fig. 1).

The Human Genome Project gave us the revelation that most of the genome is made up of noncoding regions that are responsible in some way for the preservation and regulation of the small portion of the genome that encodes gene products (3). Chromatin shape changes in these nontranscribed regions that promote DNA accessibility play a pivotal role in control of genes in both local and distant regions along the chromosome (4). Inheritable and reversible changes in chromatin topology are actively promoted by reversible enzymatic modification of histones and DNA with small molecules (e.g., acetylation, ubiquiti-

nation, sumolation, and methylation) in response to signal transduction, allowing gene expression, DNA repair, and DNA replication enzymes to have access to DNA within large regions of chromatin (5–8). Acetylation/deacetylation of histones allows for temporary open and closing chromatin topology, whereas histone methylation modifications yield more stable change in chromatin accessibility. These epigenetic modifications occur in rapid response to environmental triggers such as viral infection and endotoxin promulgated through cytokine, hormonal, and antigen signaling, which all have the potential to promote both establishment and loss of immune tolerance (8).

Animal and human genetic studies (9–12) of type 1 diabetes have defined genetic regions associated with disease susceptibility and resistance. Often, studies of these regions have uncovered that the polymorphisms associated with disease susceptibility or resistance are found in nontranscribed regulatory regions rather than within the gene-coding regions. Congenic breeding analysis of *Idd* loci in nonobese diabetic (NOD) mice has brought to light phenomenon of gene expression clustering in autoimmune susceptibility, such as *Idd* loci containing multiple members in a single pathway (e.g., interleukin-2 signaling components on Chr3/*Idd3* and *Idd10* [10] and the major histocompatibility complex Chr17/*Idd1* [11]) or genes whose products support similar functions (e.g., cytokine gene cluster on Chr 11/*Idd4.3* [12]). These findings suggest that aberration of a regional regulatory control mechanism may be underlying the association of the entire locus with type 1 diabetes. Epigenetic modifications of histones and DNA that regulate chromatin structure and accessibility of proteins to transcription regulatory regions would be likely candidates for such a regional control mechanism.

Changes in Lys9 methylation in islet stem cells are associated with insulin secretion potential (13). Recent compelling evidence (14) suggests that microsatellite RNA involved in autoimmune susceptibility are located in epigenetic hot spots and implicate their dysregulation in the pathology of rheumatoid arthritis. STAT proteins, which are activated by cytokines and hormones to act as adaptor proteins for histone modification acetylases and deacetylases, have linked epigenetic dysfunction with autoimmunity including STAT3 dysregulation in B-cells in lupus (15), STAT6 function in B-cell dysfunction in Sjögren’s disease (16), and STAT5 dysfunction with aberrant cytokine signaling in inflammation, regulatory T-cell activation, and myeloid cell functional maturation in type 1 diabetes (17,18).

The work of Maio et al. (19) in this issue of *Diabetes* provides further evidence that dysregulation of epigenetic modification is associated with type 1 diabetes. These

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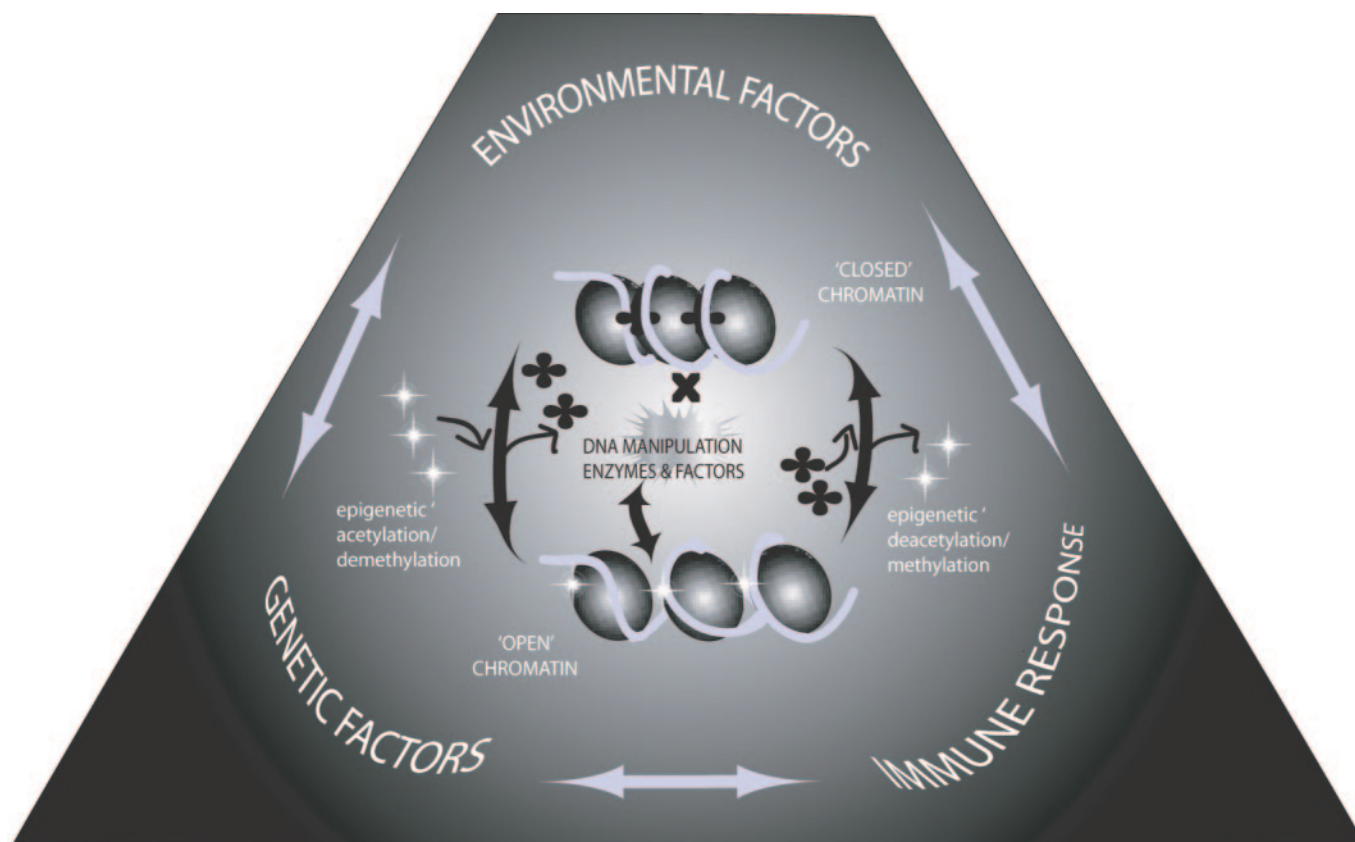


FIG. 1. A vicious cycle: interactions of environmental triggers stimulate genetic regulation, which in turn promotes a proinflammatory microenvironment and immune dysfunction. Epigenetic regulation of genes in a chromosomal region through their nontranscribed regulatory sequences both affects and is affected by environmental and genetic variations that confer susceptibility to disease. This sets the stage for interactions that self-perpetuate and feedback to exacerbate immunopathology and suggests a central role for epigenetic mechanisms in the etiology of autoimmune diabetes.

studies show that histone methylation is significantly increased in nontranscribed regulatory regions near or within human type 1 diabetes susceptibility regions, including loci containing the gene for *CLTA4* and genes in inflammation-associated pathways. These elegant bioinformatic ChIP-on-CHIP-based studies shed needed light on the importance of regional chromatin control in response to environmental stimuli and highlight the pathways most responsive in type 1 diabetes susceptibility, including T-cell energy, inflammation, and innate response signaling networks. However, the ChIP selection here is limited to one, albeit important, modification—Lys9—and was not functionally tested. Still, these intriguing data parallel the 2005 studies by Wren and Garner (20), which found that epigenetic changes in proinflammatory and cytokine genes were among the highest statistical scoring target hits in their analysis of type 2 diabetes pathogenesis. Both these studies fall short of making the functional link between these modifications and the genes they may affect. However, together with the phenotypic studies that proceeded them, these findings suggest pathway candidates and give a starting point for investigation of the potential role for epigenetic gene regulation in insulin signaling and inflammation in the shared etiology for both type 1 and type 2 diabetes. In addition, these genome-wide scans provide a roadmap for future functional studies linking environmental triggers to genetic dysregulation in autoimmunity. Defining dysfunctional epigenetic gene regulation's potential as a more global mechanism for susceptibility may lead to a shift in the way we approach

treatment and prevention of autoimmune pathologies and other multifactorial diseases. For diabetes, the discovery of defects in epigenetic modification in control of susceptibility loci makes it one of the strongest candidates for that elusive “missing link” of autoimmune disease research: a mechanism common to and potentially linking both environment stimulation and genetic susceptibility in immunopathogenesis.

REFERENCES

1. Jahromi MM, Eisenbarth GS: Cellular and molecular pathogenesis of type 1A diabetes. *Cell Mol Life Sci* 64:865–872, 2007
2. Peng H, Hagopian W: Environmental factors in the development of type 1 diabetes. *Rev Endocr Metab Disorders* 7:149–162, 2006
3. Adams MD, Kelley JM, Gocayne JD, Dubnick M, Polymeropoulos MH, Xiao H, Merril CR, Wu A, Olde B, Moreno RF, et al.: Complementary DNA sequencing: expressed sequence tags and human genome project. *Science* 252:1651–1656, 1991
4. Jenuwein T, Allis CD: Translating the histone code. *Science* 293:1074–1080, 2001
5. Ito K, Barnes PJ, Adcock IM: Glucocorticoid receptor recruitment of histone deacetylase 2 inhibits interleukin-1b-induced histone H4 acetylation on lysines 8 and 12. *Mol Cell Biol* 20:6891–6903, 2000
6. Chen X, Wang J, Woltring D, Gerondakis S, Shannon MF: Histone dynamics on the interleukin-2 gene in response to T-cell activation. *Mol Cell Biol* 25:3209–3219, 2005
7. Zhang Y, Reinberg D: Transcription regulation by histone methylation: interplay between different covalent modifications of the core histone tails. *Genes Dev* 15:2343–2360, 2001
8. Sivolob A, Prunell A: Nucleosome conformational flexibility and implications for chromatin dynamics. *Phil Trans R Soc Lond A* 362:1519–1547, 2004
9. Yui MA, Muralidharan K, Moreno-Altamirano B, Perrin G, Chestnut K,

- Wakeland EK: Production of congenic mouse strains carrying NOD-derived diabetogenic genetic intervals: an approach for the genetic dissection of complex traits. *Mamm Genome* 7:331–334, 1996
10. Dendrou CA, Wicker LS: The IL-2/CD25 pathway determines susceptibility to T1D in humans and NOD mice. *J Clin Immunol*. 9 September 2008 [Epub ahead of print]
 11. Wicker LS, Todd JA, Prins JB, Podolin PL, Ranjilian RJ, Peterson LB: Resistance alleles at two non-major histocompatibility complex-linked insulin-dependent diabetes loci on chromosome 3, *Idd3* and *Idd10*, protect nonobese diabetic mice from diabetes *J Exp Med* 180:1705–1713, 1994
 12. Litherland SA, Grebe KM, Belkin NS, Paek E, Elf J, Atkinson M, Morel L, Clare-Salzler MJ, McDuffie M: Nonobese diabetic mouse congenic analysis reveals chromosome 11 locus contributing to diabetes susceptibility, macrophage STAT5 dysfunction, and granulocyte-macrophage colony-stimulating factor overproduction. *J Immunol* 175:4561–4565, 2005
 13. Mutskov V, Raaka BM, Felsenfeld G, Gershengorn MC: The human insulin gene displays transcriptionally active epigenetic marks in islet-derived mesenchymal precursor cells in the absence of insulin expression. *Stem Cells* 25:3223–3233, 2007
 14. Tili E, Michaille JJ, Costinean S, Croce CM: MicroRNAs, the immune system and rheumatic disease. *Nat Clin Pract Rheumatol* 4:534–541, 2008
 15. Liu K, Liang C, Liang Z, Tus K, Wakeland EK: Sle1ab mediates the aberrant activation of STAT3 and Ras-ERK signaling pathways in B lymphocytes. *J Immunol* 174:1630–1637, 2005
 16. Nguyen CQ, Gao JH, Kim H, Saban DR, Cornelius JG, Peck AB: IL-4-STAT6 signal transduction-dependent induction of the clinical phase of Sjögren's syndrome-like disease of the nonobese diabetic mouse. *J Immunol* 179:382–390, 2007
 17. Seydel F, Garrigan E, Stutevoss B, Belkin N, Makadia B, Carter J, Shi JD, Davoodi-Semiromi A, McDuffie M, Litherland SA: GM-CSF induces STAT5 binding at epigenetic regulatory sites within the *Csf2* promoter of nonobese diabetic (NOD) mouse myeloid cells. *J Autoimmun*. In press
 18. Tsuji-Takayama K, Suzuki M, Yamamoto M, Harashima A, Okochi A, Otani T, Inoue T, Sugimoto A, Toraya T, Takeuchi M, Yamasaki F, Makamura S, Kibata M: The production of IL-10 by human regulatory T cells is enhanced by IL-2 through a STAT5-responsive intronic enhancer in the IL-10 locus. *J Immunol* 181:3897–3905, 2008
 19. Miao F, Smith DD, Zhang L, Min A, Feng W, Natarajan R: Lymphocytes from patients with type 1 diabetes display a distinct profile of chromatin histone H3 lysine 9 dimethylation: an epigenetic study in diabetes. *Diabetes* 57:3189–3198, 2008
 20. Wren JD, Garner HR: Data-mining analysis suggests an epigenetic pathogenesis for type 2 diabetes. *J Biomed Biotech* 2:104–112, 2005