CHAPTER 5

DNA METHYLATION AND B-CELL AUTOREACTIVITY

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Abstract: Although not exclusive, mounting evidence supports the fact that DNA methylation at CpG dinucleotides controls B-cell development and the progressive elimination or inactivation of autoreactive B cell. Indeed, the expression of different B cell specific factors, including Pax5, rearrangement of the B-cell receptor (BCR) and cytokine production are tightly controlled by DNA methylation. Among normal B cells, the autoreactive $CD5^+$ B cell sub-population presents a reduced capacity to methylate its DNA that leads to the expression of normally repressed genes, such as the human endogenous retrovirus (HERV). In systemic lupus erythematosus (SLE) patients, the archetype of autoimmune disease, autoreactive B cells are characterized by their inability to induce DNA methylation that prolongs their survival. Finally, treating B cells with demethylating drugs increased their autoreactivity. Altogether this suggests that a deeper comprehension of DNA methylation in B cells may offer opportunities to develop new therapeutics to control autoreactive B cells.

INTRODUCTION

Epigenetics is defined as heritable changes in gene expression that did not affect the DNA sequence of the genome. Epigenetic modifications involve either methylation of cytosine in CpG dinucleotides or covalent posttranslational modifications of the histones. In B cells, like in other cells, CpG dinucleotides are globally methylated with the exception of the CpG rich regions called CpG islands. CpG islands are important for control of gene expression and it has been estimated that 50% of human RNA polymerase II-transcribed genes possess CpG islands. Using a high resolution technique to map the entire B-cell CpG methylome, it was observed that 10% of the promoters were repressed by DNA

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methylation when analyzing peripheral blood B cells.[1] The CpG methylation process is controlled by DNA methyltransferases (DNMTs), a family that encompasses five members: DNMT1, DNMT3a, DNMT3b, DNMT3L and DNMT2. DNMT1 preferentially methylates hemi-methylated DNA, such as appear during cellular division, whereas DNMT3a and DNMT3b are involved in the de novo introduction of methyl groups on unmethylated DNA. Methylated CpG can limit transcription directly or indirectly via the recruitment of transcriptional repressors, such as the methyl-CpG binding domain proteins (MBD) a group that includes MBD1-4 and MECP. In their turn, MBDs can recruit other repressors like the histone deacetylases (HDAC). HDACs are histone posttranslational modifying enzymes that introduce positive charges in the histone amino terminal protruding tail and permit interactions with the negatively charged DNA, leading to DNA compaction and preventing transcription. Other histone posttranslational modifications have been described, such as methylation (me), ubiquitination, phosphorylation, sumoylation, deimination/ citrullinisation, ADP ribosylation and proline isomerisation.[2] Among them, some are repressive (H3K9me2/3, H3K27me3 and H4K20me2/3) while others have been associated with active transcription (H3K4me2, H3K36me2/3 and H3K79me2). As a consequence, DNA methylation and histone modifications control chromatin density which can be divided into dense regions referred to as heterochromatin (increased DNA methylation, histone deacetylation and histone hypermethylation) and into less dense regions referred to as euchromatin (decreased CpG DNA methylation, increased histone acetylation and decreased histone methylation). In lymphocytes, including B cells, epigenetic mechanisms may be reversed and these changes can be rapid, particularly during the cell cycle, in response to a stimulus or after exposure to environmental factors.

B CELLS AND DNA METHYLATION

B-Cell Development

As represented in Figure 1, B-cell development can be divided into three steps. First step, in the bone marrow, B-cell maturation starts from a lymphoid stem cell that differentiates to a progenitor B (pro-B) cell, to a precursor B (pre-B) cell and to an immature B cell. Bone marrow B-cell differentiation is concomitant with the progressive rearrangement of the B-cell receptor (BCR). Second step, immature B cells migrate to the spleen wherein they differentiate through a transitional stage into follicular B cells or marginal zone B cells according to the antigen stimulation if it is T-cell dependant or not, respectively. The transitional B-cell stage is crucial to the acquisition of the B-cell repertoire (positive selection) and to the elimination of autoreactive B cells (negative selection). In humans, circulating transitional B cells have been characterized; they are $CD10^{+}$, $CD24^{+}$, $CD38^{++}$ and $CD5^{+}$.[3] Third step, follicular B cells proliferate in the germinal center (GC) of lymphoid follicles and differentiate into GC B cells that express high affinity BCR and class-switch isotypes. B cells that leave the GC can develop into memory B cells or plasma cells.

The transcription factor Pax5 plays a crucial role in B-cell development by controlling the commitment of lymphoid progenitors into the B-cell lineage and later by controlling the evolution from pro-B cells to mature B cells at different check points. Pax5 expression is controlled by DNA methylation. Indeed, analysis of the CpG methylation status at the Pax5 locus by Decker et al has revealed that Pax5 regulatory elements are progressively

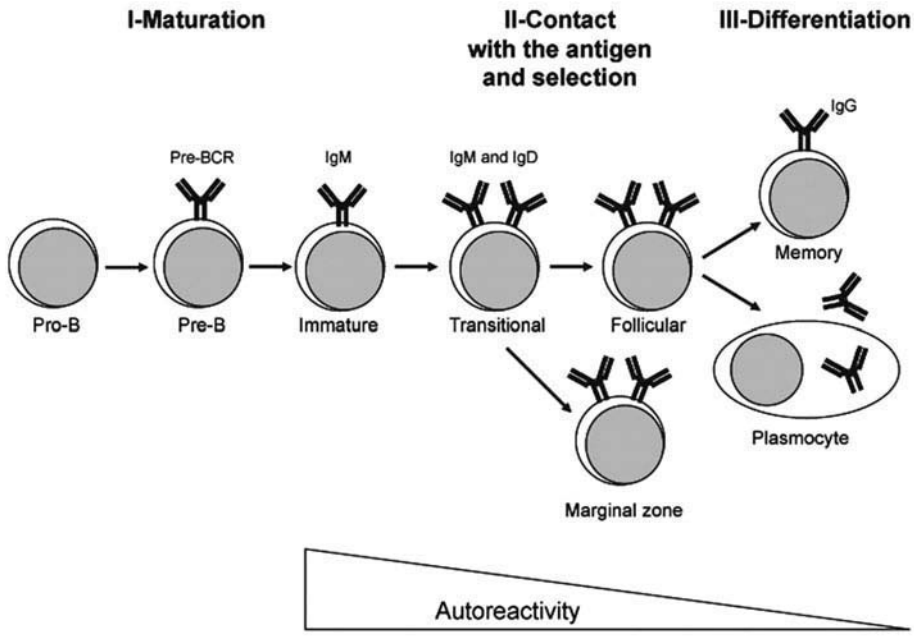

Figure 1. Model of B-cell development. B-cell development occurs in both the bone marrow and peripheral lymphoid tissues such as the spleen and can be divided into three stages. In bone marrow, the first stage, rearrangement of the immunoglobulin locus results in the generation and surface expression of the pre-B cell receptor (pre-BCR) in pre-B cells and finally a mature BCR in immature B cells. In peripheral lymphoid tissues, the second stage, B cells undergo a process of positive and negative selection to eliminate autoreactive cells. Both receptor editing/revision and clonal deletion are important at this stage. Cells completing selection will mature into follicular B cells (or marginal zone B cells). In the germinal center of lymphoid follicles, the third stage and following an immune response, antigen selected B cells develop into either plasmocyte (antibody-secreting B cell) or memory B cells.

demethylated.[4] In lymphoid stem cells, the enhancer but not the promoter starts to be demethylated and from the pro-B cell to the mature B cells both elements are demethylated. At the plasma cell stage, the promoter is remethylated and Pax5 is not expressed. In addition to a direct influence of DNA methylation on Pax5 expression, DNA methylation controls Pax5 binding and transcription factor activity. Thus, explaining that CD79a/Igα expression starts in pro-B cell, CD19 in preB cell and the human telomerase reverse transcriptase in mature B cell.[5]

B-Cell Receptor

As demonstrated by Sakano et al, sequential rearrangements of the immunoglobulin (Ig) genes are necessary to produce the BCR.[6] In the first step, the rearrangement concerns the D_H to J_H Ig heavy chain in pro-B cells, followed by V_H to D_H-J_H rearrangement at the pre-B cell stage forming the pre-BCR. When Ig_H is completed, the Ig light chain starts its V_L to J_L rearrangement which also proceeds in a stepwise manner since kappa chain rearrangement precedes lambda chain rearrangement. The rearrangement is initiated by two enzymes, Rag1 and Rag2, which form a complex with the well conserved

recombination sequence signal (RSS). In GC, the expression of the activation-induced cytidine deaminase (AID) is critical for somatic hypermutation of Ig V-region genes and class-switch recombination of C-region genes.

Since BCR rearrangement may be mutagenic or may produce autoreactive Ig, such a mechanism needs to be hightly regulated. The accessibility hypothesis was proposed to explain that external factors (e.g., IL-7 and IL-4) and/or B cell specific factors (e.g., Pax5) control chromatin accessibility through DNA methylation and histone posttranslational modifications. Several observations support this hypothesis: (1) the Ig locus is highly methylated before V(D)J recombination and undergoes demethylation during gene rearrangement.[7] (2) Demethylation on the Ig light chain occurs after demethylation on the Ig heavy chains.[8] (3) DNA binding and subsequent recombination by Rag1 and Rag2 enzymes is affected when the RSS sequences are methylated.[9] (4) The methylation status of the RSS sequences influences the rearrangement between the frequently and the infrequently rearranged V_H genes.[10] (5) Somatic hypermutations are influenced by DNA methylation since CpG methylation represses AID expression and protects deamination of cytosine to uracil by AID.[11-12]

Cytokine Production

In addition to antibody (Ab) production and antigen presentation, B cells are able to produce cytokines. According to Harris et al and following antigen stimulation, naïve B cells differentiate themselves into effector B cells of Type 1 (Be1) or Type 2 (Be2).[13] On one hand, Be1 cells produce Type 1 cytokines like IFN-γ that provide protection against intracellular pathogens and cancer and, on the other hand, Be2 cells produce Type 2 cytokines like IL-4, IL-5 and IL-13 that are involved in host defense against parasites. In T cells the choice between Type I (IFNγ, IL-2) and Type 2 cytokines (IL-4, IL-5, IL-6 and IL-13) is orchestrated by DNA methylation and histone acetylation.[14] A demonstration that DNA methylation controls cytokine production in B cells has not been provided, but may be suspected.

$CD5^+$ B CELLS

CD5, first described as a T-cell marker, is detected in a subset of B cells referred to as B1 cells as opposed to conventional B2 cells that did not express CD5. B1 cells are further divided into B1a and B1b B cells, the latter sharing all the properties of B1a except the cell surface expression of CD5.[15] Different functions were ascribed for $CD5^+$ B cells: (1) $CD5^+$ B cells produce polyspecific Abs and they are believed to constitute the main source of natural Abs; (2) Repeated BCR stimulation of B cells leads to CD5 expression that controls B-cell activation, maintains transitional and mature B cells in anergy and contributes to the re-expression of Rag1/Rag2 and BCR Ig light chain revision;[3,16-18] (3) $CD5^+$ B cells may also exert their effect through the production of interleukin (IL)-10, which is an immunoregulatory cytokine. Il-10 production is related to the expression of CD5 since transfection of B cells with CD5 is associated with IL-10 production.[19] The role of IL-10 on autoimunity is controversial, on the one hand, IL-10 is exacerbated in SLE patients and, on the other hand, a protective role for IL-10 has been demonstrated in vivo.[20-21]

CD5+ B Cells Are Hypomethylated

The human CD5 gene, which possesses 11 exons, is located on chromosome 11 at position 11q12.2 adjacent to CD6 (Fig. 2). The CD5 and CD6 genes derive from a common ancestor present in fish that has been duplicated after the divergence with birds around 150-200 million years ago, explaining that CD5 and CD6 are highly conserved in avian, bovine, rodent and human. In addition, at the divergence between monkeys from the old world and the new world, around 25 million years ago, a human endogenous retrovirus (HERV) has integrated the 5′ portion of the CD5 locus.[22] HERV-CD5 retrovirus is 5,254 bp long and possesses two long terminal repeats (LTR) plus nonfunctional gag-pol and env elements.

In B1a and B1b B cells isolated from humans two transcripts are expressed,[23] a classical one called CD5-E1A and a fusion transcript, called CD5-E1B, that contains the 5' LTR part of the HERV-CD5 element (exon 1B) and the CD5 gene (exons 2-11). Transcription of the fusion transcript CD5-E1B is controlled by DNA methylation and its expression is restricted to B cells.[24] DNA methylation dependence was demonstrated first by testing the activity of the DNMT1 which is reduced in CD5-E1B positive cells (B1a and B1b) in comparison to B2 cells (Fig. 3). In a second instance, it was observed that B cells treated with DNMT inhibitors like procainamide, 5-azacytidine and PD98059 leads to CD5-E1B overexpression. Finally, analysis of the CpG sites in the HERV-CD5 5'LTR using methylation sensitive endonuclease assays followed by PCR and bisulfite sequencing revealed that the methylation status of the U3 promoter present in the 5'LTR is inversely proportional to CD5-E1B expression in B cells (techniques are summarized in Fig. 4).

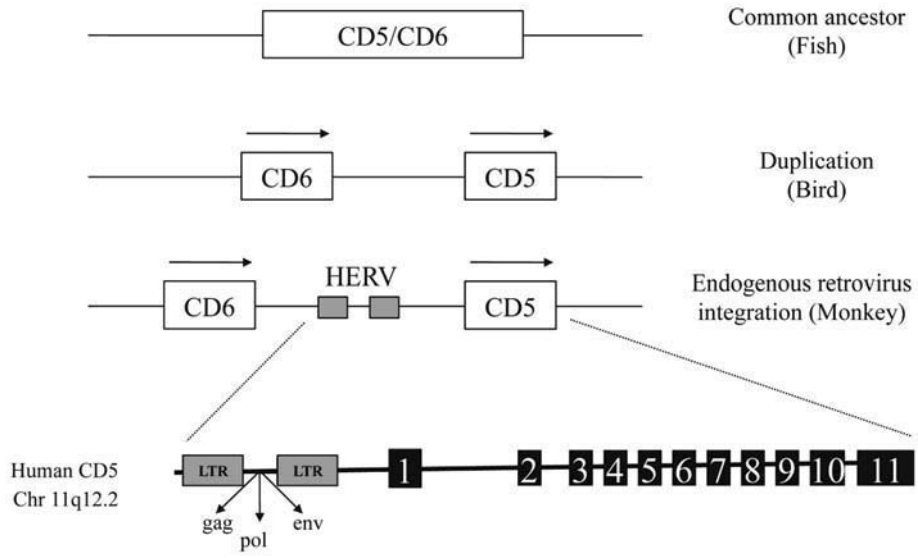

Figure 2. Characterization of CD5 gene. CD5 and CD6 genes have evolved from the duplication of a common ancestral gene. The human CD5 gene maps to the chromosome (Chr) 11q12.2 region, 82kb downstream from the human CD6 gene. A human endogenous retrovirus (HERV) element is integrated in the 5′ region of human CD5.

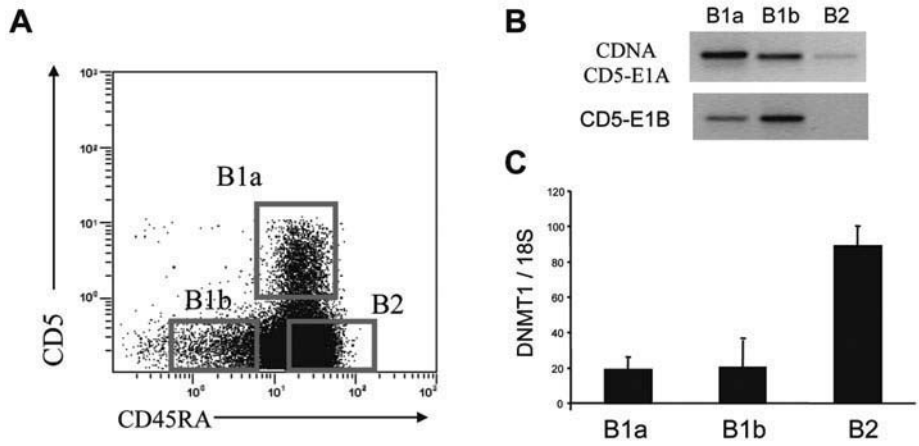

Figure 3. B1 cells are characterized by a reduced capacity to methylate their DNA. A) B1 cells can be discriminated based on their membrane cell surface expression of CD5 and CD45RA.[23] B) The CD5 gene generates two transcripts CD5-E1A and CD5-E1B that are expressed at the cell surface or intracellularly, respectively. CD5-E1A predominates in B1a cells in contrast to CD5-E1B that predominates in B1b B cells. C) Expression of CD5-E1B is controlled by DNA methylation and its expression is inversely proportional to the expression of DNA methyl transferase 1 (DNMT1) as determined by real time PCR.

Endogenous Retrovirus Expression Is Impaired in CD5$^+$ B Cells

In addition to CD5-E1B up-regulation, DNA hypomethylation observed in CD5$^+$ B cells influences other genes and particularly HERV elements. Such an assertion is based on the observation that HRES-1, another HERV element, is repressed by DNA methylation in B2 cells while the other genes tested (Pax5, CD70, CD19 and Syk) were demethylated.[25] HERVs represent 8% of human chromatin and their contribution to lymphocyte autoreactivity is strongly suspected. Several mechanisms have been proposed to explain how HERV elements contribute to autoreactivity (see review 26 for references). (1) HERV-encoded proteins are considered as foreign antigens that stimulate B cells to produce Abs. Among anti-HERV Abs some of them might cross-react with self proteins by molecular mimicry. For example, Abs against HRES-1 p30gag that cross-react with the nuclear autoantigen U1-snRNP are detected in up to 50% in SLE compared to less than 5% in controls. (2) HERV proteins may act as superantigens which could induce expansion of autoreactive T cells. (3) HERVs may induce immune response dysregulation through their capacity to modulate T-cell activation, cytokine expression and they can activate innate immunity by pattern recognition receptors. (4) HERVs may act as insertional mutagens causing activation, inhibition or alternative splicing of genes involved in immune regulation. One example is the insertion of a HERV element in the CD5 gene that generates the fusion transcript CD5-E1B (see above). CD5-E1B encodes a truncated cytoplasmic protein able to interact with the classical form of CD5, CD5-E1A, forming intracellular aggregates when co-expressed in the same cell.[24] Such an interaction is suspected to contribute to B-cell autoreactivity by downregulating a BCR dampener.[15]

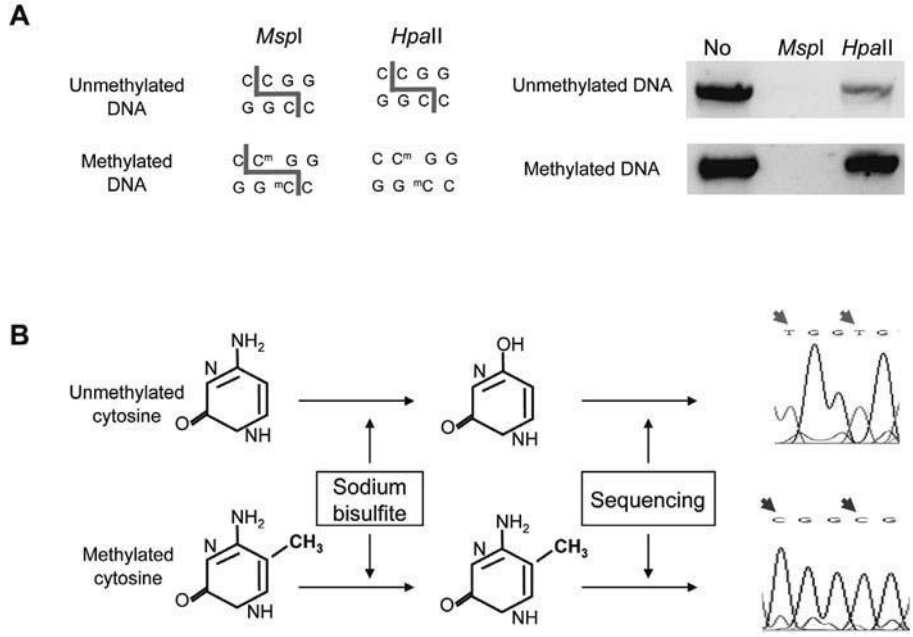

Figure 4. Methods of DNA methylation analysis. A) Methylation-sensitive endonucleases are used to assess the methylation status of CpG sites within a CpG island. This assay is based on the inability of a methylation sensitive restriction enzyme (*Hpa II*) to digest methylated CpG sites, in contrast to a methylation insensitive restriction enzyme (*Msp I*). Digested DNA is analysed by polymerase chain reaction (PCR) showing whether the CpG sequence is preserved, if methylated, or not in the amplified region. B) Bisulfite sequencing is used to determine the pattern of methylation after treatment of DNA with bisulfite that converts unmethylated-cytosine residues to uracil, methylated-cytosine remains unaffected.

AUTOREACTIVE B CELLS

Animal Models

Observing that prolonged treatment with isoniazid, an antituberculous drug, was associated with the development of an SLE-like disease in humans, the influence of long-term oral administration was tested in normal mice.[27] After several weeks murine B cells became autoreactive, antinuclear Abs appeared and the mice developed an SLE-like disease that disappeared after the drug was removed (Fig. 5). Such an effect has been reproduced with procainamide and 5-azacytidine, two other DNA methylation inhibitors and the effects were more pronounced. The direct implication of B cells was confirmed more recently.[28] B cells were purified from mice, treated ex vivo with DNA methylation inhibitors and subsequently reintroduced in syngenic mice by adoptive transfer. This led to the detection of antinuclear Abs in the recipient. Of note, the effect is not restricted to B cells, since Richardson's group has also demonstrated that adoptive transfer of DNA hypomethylated $CD4^+$ T cells, not $CD8^+$ T cells, induced an SLE-like disease with glomerulonephritis and anti-dsDNA Ab deposition in the kidney.[29]

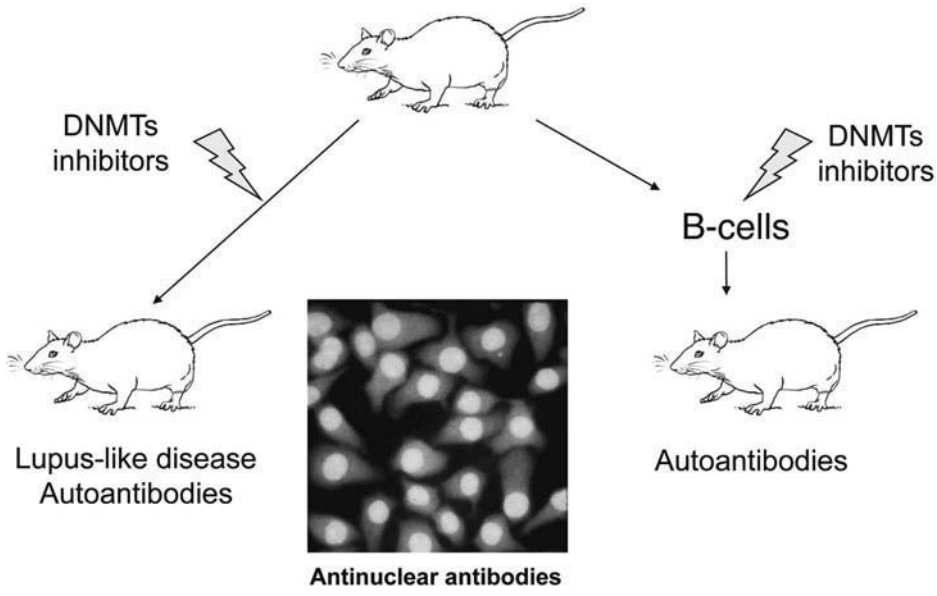

Figure 5. Epigenetic alterations in B cells contribute to the pathogenesis of lupus. Both, the long-term oral administration of DNMT inhibitors like hydralazine or isoniazide on one hand (left), or adoptive transfer of bone marrow B cells pretreated with the same drugs to naïve syngeneic mice on the other hand (right), resulted in autoantibody production and a lupus-like disease.

DNA Methylation Is Impaired in SLE B Cells

In SLE, peripheral blood mononuclear cells (PBMC) are characterized by a global DNA hypomethylation status. This was elegantly demonstrated by Javierre and colleagues who compared pairs of monozygotic twins discordant for SLE, rheumatoid arthritis (RA) and dermatomyositis (DM).[30] Indeed, in comparison to the corresponding healthy sibling, PBMC isolated from SLE patients, but neither RA nor DM patients, present a profound DNA methylation defect associated with DNMT1 and DNMT3b reduction and histone acetylation. Among PBMC from SLE patients, $CD4^+$ T cells were first reported to present DNA methylation abnormalities and recently we have also observed that SLE B cells were defective in their capacity to methylate DNA.[25,31] Such a defect has been related to a blockage in the PKC delta/Erk pathway that regulates DNMTs expression.[32] The reason for the initial defect is currently unknown but transgenic mice defective for PKC delta developed an SLE-like disease with B-cell expansion, autoantibody production, IL-6 overexpression and had the constitution of ectopic GC in the absence of stimulation.[33]

IL-6 is a multifunctional cytokine involved in B-cell differentiation/maturation, Ig secretion and T-cell functions. A relation between the IL-6 level detected in the sera and lupus disease activity has been reported, thus providing a clue implicating IL-6 in B-cell autoreactivity.[34] In mice, the treatment of lupus prone mice with an

anti-IL-6 mAb prevents the development of an SLE-like disease and the production of anti-dsDNA Abs.[35] The impact of IL-6 in autoreactivity is not completely understood but we have recently proposed that IL-6, by blocking the cell cycle progression at the G0/G1 interface in B cells, controls DNMT1 expression.[25,36,37] Such assertion is based on the observation, in one hand, that addition of IL-6 to normal B cells is associated with a reduction of DNA methylation and DNMT1, while, on the other hand, blocking abnormal IL-6 production with an anti-IL-6-receptor mAb restores DNA methylation in SLE B cells. Interestingly, DNA methylation plays an essential role in IL-6 silencing, suggesting that an amplification loop occurs in SLE B cells.[38,39]

ICF Syndrome and Autoreactivity

The immunodeficiency, centromeric region instability and facial anomalies syndrome (ICF) is a rare genetic disease, less than 50 cases have been reported world wide, that displays DNA hypomethylation.[40] In the majority of cases, ICF is related to mutations in the catalytic domain of the DNMT3b gene. ICF diagnosis is associated with hypogamma-globulinemia or agamma-globulinemia, normal peripheral blood B cell number and cytogenetic abnormalities involving chromosomes 1, 16 and sometimes 9 in mitogen-stimulated lymphocytes. Analysing B cells from ICF patients, BlancoBetancourt, et al have observed that peripheral B cell express autoreactive BCRs, and that terminal differentiation is blocked at the transitional stage.[40] Thus, it could be suspected that DNA methylation controls the negative selection of transitional B cells through an unknown mechanism.

CONCLUSION

Evidence for a role for DNA methylation in the pathogenesis of SLE and common autoimmune diseases has emerged. Patients with SLE have global hypomethylation of DNA with a decrease in the activity of the DNMTs that affects primarily lymphocytes. However and particularly in B cells, the pathways that control DNA methylation and the pathways that are controlled by DNA methylation are poorly understood. Consequently, a better understanding of these pathways may constitutes a revolution in our comprehension of autoreactive B cells.[41]

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REFERENCES

1. Rauch TA, Wu X, Zhong X et al. A human B-cell methylome at 100-base pair resolution. Proc Natl Acad Sci USA 2009; 106:671-8.
2. Dieker J, Muller S. Epigenetic histone code and autoimmunity. Clin Rev Allergy Immunol 2009; DOI: 10-1007/s12016-009-8173-7.
3. Sims GP, Ettinger R, Shirota Y et al. Identification and characterization of circulating human transitional B-cells. Blood 2005; 105:4390-8.
4. Decker T, Pasca di Magliano M, McManus S et al. M. Stepwise activation of enhancer and promoter regions of the B-cell commitment gene Pax5 in early lymphopoiesis. Immunity 2009; 30:508-20.
5. Renaudineau Y, Garaud S, Le Dantec C et al. Autoreactive B-cells and Epigenetics. Clin Rev Allergy Immunol 2009; DOI: 10.1007/s12016-009-8174-6.
6. Sakano H, Maki R, Kurosawa Y et al. Two types of somatic recombination are necessary for the generation of complete immunoglobulin heavy-chain genes. Nature 1980; 286:676-83.
7. Storb U, Arp B. Methylation patterns of immunoglobulin genes in lymphoid cells: correlation of expression and differentiation with undermethylation. Proc Natl Acad Sci USA 1983; 80:6642-6.
8. Xu CR, Feeney AJ. The epigenetic profile of Ig genes is dynamically regulated during B-cell differentiation and is modulated by pre-B-cell receptor signaling. J Immunol 2009; 182:1362-9.
9. Nakase H, Takahama Y, Akamatsu Y. Effect of CpG methylation on RAG1/RAG2 reactivity: implications of direct and indirect mechanisms for controlling V(D)J cleavage. EMBO 2003; 4:774-80.
10. Espinoza CR, Feeney AJ. Chromatin accessibility and epigenetic modifications differ between frequently and infrequently rearranging VH genes. Mol Immunol 2007; 44:2675-85.
11. Fujimura S, Matsui T, Kuwahara K et al. Germinal center B-cell-associated DNA hypomethylation at transcriptional regions of the AID gene. Mol Immunol 2008; 45:1712-9.
12. Larijani M, Frieder D, Sonbuchner TM et al. Methylation protects cytidines from AID-mediated deamination. Mol Immunol 2005; 42:599-604.
13. Harris DP, Haynes L, Sayles PC et al. Reciprocal regulation of polarized cytokine production by effector B and T-cells. Nat Immunol 2000; 1:475-82.
14. Bowen H, Kelly A, Lee T et al. Control of cytokine gene transcription in Th1 and Th2 cells. Clin Exp Allergy 2008; 38:1422-31.
15. Youinou P, Renaudineau Y. The paradox of CD5-expressing B-cells in systemic lupus erythematosus. Autoimmun Rev 2007; 7:149-54.
16. Qian Y, Santiago C, Borrero M et al. Lupus-specific antiribonucleoprotein B-cell tolerance in nonautoimmune mice is maintained by differentiation to B-1 and governed by B-cell receptor signaling thresholds. J Immunol 2001; 166:2412-9.
17. Hippen KL, Tze LE, Behrens TW. CD5 maintains tolerance in anergic B-cells. J Exp Med 2000; 191:883-8.
18. Hillion S, Rochas C, Youinou P et al. Expression and reexpression of recombination activating genes: relevance to the development of autoimmune states. Ann NY Acad Sci 2005; 1050:10-8.
19. Garaud S, Le Dantec C, de Mendoza AR et al. IL-10 production by B-cells expressing CD5 with the alternative exon 1B. Ann NY Acad Sci 2009; 1173:280-5.
20. Gunnarsson I, Nordmark B, Hassan Bakri A et al. Development of lupus-related side-effects in patients with early RA during sulphasalazine treatment-the role of IL-10 and HLA. Rheumatology (Oxford) 2000; 39:886-93.
21. Fillatreau S, Sweenie CH, McGeachy MJ et al. B-cells regulate autoimmunity by provision of IL-10. Nat Immunol 2002; 3:944-50.
22. Renaudineau Y, Vallet S, Le Dantec C et al. Characterization of the human CD5 endogenous retrovirus-E in B lymphocytes. Genes Immun 2005; 6:663-71.
23. Renaudineau Y, Hillion S, Saraux A et al. An alternative exon 1 of the CD5 gene regulates CD5 expression in human B lymphocytes. Blood 2005; 106:2781-9.
24. Garaud S, Le Dantec C, Berthou C et al. Selection of the alternative exon 1 from the CD5 gene down-regulates membrane level of the protein in B lymphocytes. J Immunol 2008; 181:2010-8.
25. Garaud S, Le Dantec C, Jousse-Joulin S et al. IL-6 modulates CD5 expression in B-cells from patients with lupus by regulating DNA methylation. J Immunol 2009; 182:5623-32.
26. Brooks WH, Le Dantec C, Pers JO et al. Epigenetics and autoimmunity. J Autoimmun 2010; 34:J207-19.
27. Cannat A, Seligmann M. Induction by isoniazid and hydrallazine of antinuclear factors in mice. Clin Exp Immunol 1968; 3:99-105.
28. Mazari L, Ouarzane M, Zouali M. Subversion of B lymphocyte tolerance by hydralazine, a potential mechanism for drug-induced lupus. Proc Natl Acad Sci U SA 2007; 104:6317-22.

29. Quddus J, Johnson KJ, Gavalchin J et al. Treating activated CD4+ T-cells with either of two distinct DNA methyltransferase inhibitors, 5-azacytidine or procainamide, is sufficient to cause a lupus-like disease in syngeneic mice. J Clin Invest 1993; 92:38-53.
30. Javierre BM, Fernandez AF, Richter J et al. Changes in the pattern of DNA methylation associate with twin discordance in systemic lupus erythematosus. Genome Res 2010; 20:170-9.
31. Zhao S, Long H, Lu Q. Epigenetic perspectives in systemic lupus erythematosus: Pathogenesis, biomarkers and therapeutic potentials. Clin Rev Allergy Immunol 2009; DOI:10-1007/s12016-009-8165-7.
32. Gorelik G, Fang JY, Wu A et al. Impaired T-cell protein kinase C delta activation decreases ERK pathway signaling in idiopathic and hydralazine-induced lupus. J Immunol 2007; 179:5553-63.
33. Miyamoto A, Nakayama K, Imaki H et al. Increased proliferation of B-cells and auto-immunity in mice lacking protein kinase Cdelta. Nature 2002; 416:865-9.
34. Youinou P, Jamin C. The weight of interleukin-6 in B-cell-related autoimmune disorders. J Autoimmun 2009; 32:206-10.
35. Liang B, Gardner DB, Griswold DE et al. Anti-interleukin-6 monoclonal antibody inhibits autoimmune responses in a murine model of systemic lupus erythematosus. Immunology 2006; 119:296-305.
36. Hillion S, Garaud S, Devauchelle V et al. Interleukin-6 is responsible for aberrant B-cell receptor-mediated regulation of RAG expression in systemic lupus erythematosus. Immunology 2007; 122:371-80.
37. Brown SE, Fraga MF, Weaver IC et al. Variations in DNA methylation patterns during the cell cycle of HeLa cells. Epigenetics 2007; 2:54-65.
38. Dandrea M, Donadelli M, Costanzo C et al. MeCP2/H3meK9 are involved in IL-6 gene silencing in pancreatic adenocarcinoma cell lines. Nucleic Acids Res 2009; 37:6681-90.
39. Mi XB, Zeng FQ. Hypomethylation of interleukin-4 and -6 promoters in T-cells from systemic lupus erythematosus patients. Acta Pharmacol Sin 2008; 29:105-12.
40. BlancoBetancourt CE, Moncla A, Milili M et al. Defective B-cell-negative selection and terminal differentiation in the ICF syndrome. Blood 2004; 103:2683-90.
41. Renaudineau Y. The Revolution of Epigenetics in the Field of Autoimmunity. Clin Rev Allergy Immunol 2009; DOI: 10-1007/s12016-009-8171-9.