Methylation status of B cells of those afflicted with Transient Hypogammaglobulinemia

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Research Hypothesis and Experimental Proposal

1 Background

1.1 THI

Immunoglobulins are a vital component of the adaptive immune system[1]. The B cells which produce antibodies (active immunoglobulin) in adults are not fully mature in young infants, resulting in a decrease in serum immunoglobulin levels after birth[2,3]. Physiologic hypogammaglobulinemia refers to the point when serum immunoglobulin reaches its lowest point, commonly at 4-6 months of age[4]. In some individuals, immunoglobulin levels are extremely low, due to defects in the immune system[5]. Transient hypogammaglobulinemia (THI) is one such disorder whereby affected persons have a prolongation or exacerbation of regular hypogammaglobulinemia, followed by spontaneous recovery[4–12]. The mechanism causing low serum immunoglobulin in THI patients has not been elucidated.

Recurrent infections:[7]

1.2 THI Causes

Immunoglobulin deficiency can result from B cell precursors failing to either mature into B cells or further fail to differentiate into antibody secreting plasma cells[13]. Studies investigating THI have found that levels of circulating B cells are normal, and subpopulations of B cells are intact, including the level of memory and class-switched B cells[4,8,9,13,14]. With no obvious B cell deficiency, the cause of THI remains unknown[5]. The following mechanisms have been proposed for THI. Fudenberg and Fudenberg[15] proposed that the fetal IgG molecules produced during pregnancy were enough to stimulate an immune response from the mother. The immune response was thought to produce maternal antibodies which caused transient suppression of fetal immunoglobulin production. Rosen and Janeway[7] were unable to find agglutinators in four mothers of infants with THI, furthermore Nathenson et al.[16] conducted a prospective study to test the hypothesis and found no supporting evidence. Soothill[17] proposed THI

as a manifestation of genetic heterozygosity for other immunodeficiency diseases, noting the high number of patients who had relatives with immunodeficiencies. While it remains a possibility as noted by McGeady[10], no studies have shown supporting evidence[8,13,18] Siegel et al.[14] hypothesised that a T cell deficiency was the cause after the observation of low T cell numbers in THI patients. Low T cell numbers have not been observed in proceeding studies. McGeady[10] mentioned a suggestion that frequent antibiotic treatment could induce hypogammaglobulinemia by diminishing bacteria gut flora. The explanation was not described in any other THI literature, and was indeed described by McGeady[10] to be probable due to the mostly brief courses of antibiotic treatments given to THI patients. In summary, a wealth of theories have been proposed for the symptoms defining THI, Dalal et al.[11] suggests that THI is not a simple disorder, but instead is the manifestation of a heterogeneous group of errors in the immune system. The most intriguing feature of THI is its self-limited nature; that gradually recurrent infections subside and serum IgG levels increase with no obvious cause generally between 30-40 months of age[4,8,10,11,14,17,19].

2 Happies

2.0.1 B cells in hypogammaglobulinemia - Fiorilli et al. 1986

- Immunoglobulin deficiency can be the result of (1) a failure of pre-B cells to differentiate into B cells, as in X-linked hypogammaglobulinemia, (2) a defect of isotype switch or (3) a failure of B cells to differentiate terminally into antibody-secreting plasma cells.
- (3) is commonly seen in patients with common variable immunodeficiency, which may present with low, normal or increased numbers of circulating B cells carrying IgM, IgG or IgA molecules on their surface.
- A number of studies have indicated that distinct mechanisms may prevent patients' B cells from differentiating properly, including the so-called "intrinsic B cell defects", deficiencies, deficiencies of helper T cells, and the presence of activated autosuppressive T cells
- B cells of some patients with CVI have been said to display patterns of membrane immunoglobulin isotypes resembling those of immature B lymphocytes
- Study investigated the presence of immature B cells, ie, cells carrying simultaneously IgG and IgM molecules on their surface, in 12 patients with primary IgG deficiencies.
- In 10 patients (except 2 with CVI) have the majority of the circulating IgG bearing B cells also expressing surface IgM.
- Found that patients with a profound deficiency of serum IgG usually have significant numbers of circulating IgG bearing B cells. - In most cases they resembled immature B lymphocytes in that they express multiple surface immunoglobulin isotypes

- Platts-Mills et al. found that B cells from the majority of CVI patients behaved as functionally immature cells in the sense that they produced IgM but very little IgG or IgA in vitro. The other CVI patients had CVI which was uniformly associated with autoimmune disorders or thymomas as well as increased suppression, suggesting a secondary (acquired) hypogammaglobulinemia. Furthermore, B cells from patients of the latter group responded to polyclonal activation with Epstein-Barr virus in a manner qualitatively similar to that of mature B cells.

2.0.2 Subsets of transient hypogammaglobulinemia of infancy Dalal et al. 1998

- Study aimed to characterise the subsets of THI, in an attempt to define the disorder.
- Used 35 patients, assigned to three categories:
    1. Patients eventually have normal total serum levels with normal IgG subclass division, and normal specific antibody production. The process may have transient phase whereby some IgG subclasses may become unbalanced
    2. Patients continue to have low serum IgG levels, and poor antibody titres
    3. Serum IgG becomes normal, but individuals remain incapable of mounting an adequate antibody response
- The groups are likely to represent a heterogenous group of different genotypes, and it is important to understand the phenotypes, before the genotypes are explored

3 Lineage commitment and methylation status in other cells

Epigenetic changes have been coined as the hallmark of cell differentiation. T cell activation and skewing, a certain type of cell differentiation, is governed in great parts by epigenetic changes which insure that the clone of a T cell will retain its phenotype (Th1/Th2 etc).

As said by Choukrallah and Matthias[20] the same TFs can be equally expressed in different cell types, and yet have different binding profiles. So, addition to the DNA sequence recognition, TF binding strongly depends on chromatin structure and epigenetic modification.

3.1 Methylation

- DNA methylation was the first epigenetic mechanism recognised, and the one that is most extensively studied.[21]
- *De novo* methylation occurs in response to various cellular stressors, and results in the addition of a methyl group to position 5 of a cytosine residue[21].

- CpG islands have clusters at promoters and enhancers. Up to 90% of genome CpG sites are methylated, with most unmethylated CpG islands being in active genes[21]
- Schmidl et al.[22]: Methylation of cytosine residues in genomic DNA is an important epigenetic mark that is essential for normal embryonic development in mammals, imprinting, X inactivation and silencing of potential hazardous genetic elements such as transposons
- DNA methylation provides an additional mechanism for gene regulation, it can occur at the fifth position of cytosine and can repress via the following mechanisms: (1) Inhibit protein binding (like TFs). (2) Recruit proteins containing domains which interfere with transcription by recruiting repressors[20,21].

3.2 T cells

3.2.1 Bégin and Nadeau2014: T cell methylation in development

- The Th1/Th2 balance of T cells can be affected by environmental exposures which change epigenetic controls.
- In resting $CD4^+$ cells, the IL-4 and IFN-γ genes are methylated
- Upon allergic sensitisation, the IL-4 promoter in allergen-specific T cells is demethylated, correlating with IL-4 expression.
- The IL-4 locus of Th2 cells is also marked with permissive histone modifications (H3K4me3) which are absent in Th1 or naïve T cells.
- At the main Th2 gene locus on chromosome 5, a chromatin hub that interacts with GATA-3 is formed.
- GATA-3 interacts with HAT enzyme p300 and Chd to induce permissive histone and chromatin changes.
- GATA-3/Chd complex binds HDAC to repress the locus encoding TBET, the master regulator which activates Th1 and suppresses Th2 genes
- Further suppression of Th1 cytokines is achieved by the increase of their DNA methylation from naïve state.
- Th2 genes are demethylated - mechanism is still incompletely understood.
- Epigenetics is complicated: the GATA-3 promoter has been shown to keep its repressive histone modification despite Th2 activation. It presents a bivalent state with repressive and activating histone modifications - positive feedback is important to insure stable expression.

- In the thymus, iTregs are induced by TCR engagement, subsequent NF-κB signalling induces permissive histone modifications and potentially initiates chromatin remodelling in the Foxp3 locus.
- DNA demethylation of the CNS2, also called the Treg-specific demethylated region (TSDR) is a major event in tTreg differentiation, and carries an important function in Foxp3 stabilising Foxp3 expression. CNS2 is the site at which Foxp3 binds to its own gene to maintain expression in a positive feedback mechanism allowing for a persistent phenotype and suppressive function
- Activated, Non Treg T cells also express Foxp3 upon TCR engagement, however the expression is transient owing to the continued methylation of the CNS2.
- Comparing tTregs to Foxp3$^+$ activated effector T cells, there are hundreds of loci throughout the genome which show demethylation and correspond to binding sites for Foxp3. The methylation changes are not induced by Foxp3, but rather, allow Foxp3 to access its targets and exert its function.
- In activated T cells, the loci are methylated, which could explain the difference in function despite the expression of Foxp3 in activated T cells.

3.2.2 Schmidl et al. 2009: Differentially methylated regions that may be enhancers

Study used conventional CD4$^+$ T cells to compare to CD4$^+$ CD25$^+$ T regs. Differentially methylated regions were found and thought to indicate methylation sensitive enhancers

- It has been shown that the functional program of Treg cells is at least partially controlled by the miRNA pathways
- For continuous expression of the lineage-directing transcription factor Foxp3, the methylation status of a methylation-sensitive, Treg cell-specific enhancer in intron I is important.
- The restriction of cell type-specific enhancers is a key function of DNA methylation.
- STAT5:
  - Regions specifically demethylated in Treg cells were enriched for STAT5 consensus sites.
  - TF STAT5 is activated by IL-2
  - Treg cell survival requires IL-2
  - STAT5 also has an essential role in Treg homeostasis and is known to regulate Foxp3 through an intronic, methylation-sensitive enhancer.
  - It would make sense that STAT5 bind at this region, but it has not been confirmed *in vivo*

- In T-reg cells, A functionally important intronic enhancer of the Foxp3 gene was shown to be methylation sensitive properties.
- In the study, conventional $CD4^+$ T cells were compared to $CD4^+$ $CD25^+$ T regs
- Half of the tested DMRs (differentially methylated regions) significantly enhanced the activity of a heterologous promoter in transient reporter gene assays performed in a T cell leukaemia line.
- All regions lost enhancer activity upon CpG methylation.
  - Eg. Found methylation sensitive enhancer in intron 4 of CD40LG in Tconv ($CD4^+$ cells). CD40L is important in regulating B cell function through interaction with CD40 on B cells and Dendritic cells.

4 B cell development and differentiation

4.1 Stages and markers of B cell differentiation:

- Pro-B cells express B220, which coincides with entry into B cell lineage
- pre-BI cells express CD19 and complete recombination of heavy chain IgH D to J segments.
- Next stage sees generation of IgH V(D)J alleles, allowing heavy chain expression which assembles with the surrogate light chain to form the pre-B cell receptor.
- Cells need to pass functional tests here
- small pre-BII cells rearrange the light chain allowing formation and exposure of a functional Ig molecule (BCR)
- immature cells can leave the bone marrow and enter the periphery[20]]

4.2 Transcription factors in B cell development

4.2.1 Pu.1

- Very upstream TF, essential for the development of lymphoid cells as well as macrophages and neutrophils.
- Disruption of PU.1 in mouse was shown to prevent commitment of MPPs towards lymphoid lineage

4.2.2 IKAROS

- Also upstream
- Mutational disruption of Ikaros DNA-binding domain leads to an early block in lymphopoiesis before commitment to lymphoid restrictd stages.
- Also involved in later stages of B cell development, where it promotes heavy chain gene rearrangement by inducing expression of RAG1/2 genes.
- Also required for differentiation of large pre-B cells to small pre-B cells and for transcription and rearrangement of the IgL locus.

4.2.3 E2A

- Required for Ebf1 and FoxO1 expression at the Common Lymphoid Progenitor stage
- E2A mutant mice lack B cells

4.2.4 EBF1

- Essential for B cell specification and commitmnt.
- Regulates expression of genes required for B cell development including FoxO1 and Pax5

4.2.5 Pax5

- Essential for B cell commitment and maintenance of B cell identity through activation of B cell specific genes and repression of lineage inappropriate genes
- Deletion of Pax5 in mature B cells leads to de-differentiation to lymphoid progenitors, which can differentiate into functional T cells

4.2.6 FoxO1**

- Early deletion of FoxO1 causes substantial block at pro-B cell stage due to failure to express IL-7 receptor alpha chain
- Inactivation of FoxO1 in late pro-B cells results in arrest at pre-B cell stage due to impaired expression of RAG1/2 (direct targets of FoxO1)
- Deletion in peripheral B cells leads to reduced number of LN B cells, due to down regulation of L-selectin and defect in class-switch recombination

4.2.7 c-Myb and Runx

- Deletion of c-Myb in mice leads to a block at the pre-pro B cell stage which is accompanied with impaired expression of the α chain receptor and Ebf1
- Deletion of Runx1 also causes a developmental block at the the pro-B cell stage accompanied by reduced expression of E2A, Ebf1 and Pax5.
- Runx1-deficient pro-B cells were shown to harbour excessive amounds of the repressive histone mark H3K23me3 in the Ebf1 proximal promoter
- Retroviral transduction of Ebf1, not Pax5, into Runx1-deficient progenitors restores B cell development.

4.3 Transcription factors in B cell differentiation - Li et al. 2013

- Resting B cells display genome wide DNA hypomethylation
- Genes crucial for the maintenance of B cell identity (*Pax5, Spib, Ebf1*) and B cell marker genes (*CD19*), display active epigenetic state
- Chromatin in transcribed *Igh* V_HDJ_H regions, the intronic μ enhancer and the *Igh* 3' locus control region contains hypomethylated DNA and activating histone modifications
- The epigenetic marks were likely introduced during B cell development, because the open chromatin state of these regions is required for V(D)J recombination
- Active epigenetic marks in the *Igh* locus and in the *Pax5, Spib, Ebf1* and *CD19* loci persist during naïve B cell activation
- Upon activation by antigens, B cells undergo DNA demethylation and histone modifications, and express a specific set of miRNAs.
- Repression of the *Aicda* gene in naïve B cells is mediated by promoter hypermethylation, during B cell activation, *Aicda* DNA is demethylated and the locus becomes enriched in active histone modifications
- DNA hypomethylation seems to be important, as B cells carrying identical pre-rearranged Igκ alleles, only the hypomethylated allele is hypermutated despite comparable transcription of both alleles.
- S regions: the genes which contain IgG, IgA etc genes. They are acted upon when undergoing Class Switch Recombination
- Active epigenetic state is found in even naïve B cells, indicating that Sμ is in a constitutively open state, poised for switching.
- For plasma cell differentiation: Blimp-1 (encoded by *Prdm1.* Epigenetic induction of Blimp-1 causes events which drives plasma cell differentiation and possibly maintains plasma cell identity

- Differentiating into memory cells not likely a problem
- Overall DNA hypomethylation has been associated with systemic autoimmune diseases

Region/gene	Epigenetic mark	function of epigenetic mark
V(D)J	DNA hypomethylation	Increases region accessibility
Igh 3' LCR	DNA hypomethylation	Mediates germline VDJ and I_H-S-C_H transcription

4.4 DNA methylation in B cells during maturation and differentiation

4.4.1 Why epigenetics?

- Lara-Astiaso et al.[24] noted that mutations in loss of chromatin factors lead to haematopoiesis defects and disease
- Tagoh et al.[25] said that even before the onset of gene expression and stable TF binding, specific chromatin alterations are observed (including methylation changes). Hence the idea that epigenetic programs guiding blood cell differentiation are engraved into the chromatin of lineage-specific genes, and such chromatin changes are implemented **before cell lineage specification**; Epigenetic programs are engraved into the chromatin of lineage-specific genes before cell lineage specification and the onset of detectable gene expression
- Differentiation and lineage commitment are associated with specific methylation or demethylation events[22]

4.4.2 Methylation status in B cells

- Methylation loss is observed as B cells mature[26].
- Hypomethylation is enriched in enhancer/promoter regions
- The TF families which show hypomethylation are AP-1, EBF, RUNX, OCT, IFF and NFκB
- The cell subtypes which show the most pronounced methylation changes in comparison to the preceding stage are germinal centre B cells, memory B cells and BM plasma cells[27]
- It is possible to accurately classify B cells into their maturation stage by the methylation state of 5 CpGs in genes important to B cell differentiation[27].
- Transition from HPCs to pre-B1 cells has an inverse corelation between the expression of TFs and the methylation of their binding sites; High methylation status occurs with low expression of TFs[27].

5 Proposal

- Bisulfite sequencing of enhancers or transcription factors that are important to B cell development
- Take B cells from infants with CVID and THI, as well as controls. B cells should be taken at different stages, up until age 5

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