REVIEW ARTICLE

A Review: DNA Methylation of the GATA-3 Gene to Balance T Cells Population During Helminth Infection

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ABSTRACT

Helminth infection has been a severe health issue mostly in the developing countries, where sanitation is not well maintained. The immune mechanism during helminth infection is well understood. The Th2 response is the primary weapon from the immune system to fight against helminth infection. The level of Th2 cells and cytokines are elevated during helminth infection. However, a prolonged Th2 response can cause liver fibrosis and reduce host survival. In the process of T cells differentiation, GATA-3 has a crucial role. It defines the population of Th1 and Th2. The expression of GATA-3 can be regulated through DNA methylation in the CGI sites of the gene. Hence, GATA-3 is able to co-express Th1 and Th2 in one cell, which would give less inflammation effect. This review aims to summarize research about the impact of DNA methylation of the GATA-3 genes to balance T cells population during helminth infection.

Keywords: DNA methylation, GATA-3, Helminth infection, Immune response

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INTRODUCTION

Helminths are parasitic worms that cause severe diseases in mammals. Most parasitic worms are included in the phylum of Platyhelminths (i.e. trematodes, nematodes, and cestode) (1). Helminths cause infection in one-quarter of the human population. Poor sanitation is the most predominant cause of helminth's infection. The intermediate hosts of helminths are various, such as schistosomes that infect snails or filarial worms that infect flies. The transfer mode to humans can be oral, direct penetration of the skin or via a mosquitoes bite. Also, they can infect humans in various stages of their life cycle, either in the form of eggs, larvae or adult worms (2). During helminth infection, helminths can modulate the immune system of the host by downregulating and diverting the immune system, which leads to chronic infection (3).

Helminth infection is known to induce a T helper cells 2 (Th2) response. During infection, helminth antigens will be represented by the dendritic cell. The signal from the dendritic cell as antigen presenting cell (APC) to CD4+ T cells results in the development of Th2. Furthermore, Th2 secretes three kinds of cytokines (Interleukin (IL)-4, IL-5 and IL 13) that generate the activation of eosinophils, basophil and mast cells, which play a major role in killing helminths (4). During helminth infection, the alternative macrophage is also activated, and production of IgE antibodies is elevated. Helminths have some strategies to manipulate the immune system. Helminths are known to be able to penetrate the host cell with mimic host molecules to prevent the formation of the membrane attack complex (MAC). Helminths also secrete a protein that can help to avoid opsonization by antibodies or deactivation of host immune system. In addition, these worms are also able to move through tissue and compartments of the host body and hide in the nodule, which make them survive for a long time and cause chronic infection. Somehow, helminths can also induce regulatory T-cells (T-reg) which will express immunosuppressive cytokines such as tumor growth factor β (TGF-β) or IL-10. In regards to this issue, mechanism to balance Th1 and Th2 in immune responses is a crucial issue during helminth infection regarding host survival.

GATA-3 is the primary transcription factor in Th2 differentiation, which is induced by IL-4 and IL-2 (5). Meanwhile, Th1 differentiation is induced by T-bet as the transcription factor (6). However, GATA-3 is also needed for CD4+ T-cell development in the thymus, unlike T-bet, which is not expressed in naive CD4+. Research shows that the differentiation of Th1 and Th2 is mutually exclusive, but there are some ideas challenge this and state the possibility to co-induce the expression of both cells. The theory is that the Th1 and

Th2 levels are maintained through DNA methylation. DNA methylation mechanism in the gene promoter can induce transcriptional silencing which will be the key to the expression of each transcription factor of Th1 and Th2 response (7).

The objective of this review is to summarize research about the impact of DNA methylation of the GATA-3 genes to balance T cells population during helminth infection. Studies both in animal and human cells. The main body of this review will emphasize on:

1. Th2 induction during helminth infection
2. The overview of the role of GATA3 in CD4 development; to give the reader background of the general CD4 differentiation into Th1 and Th2
3. DNA Methylation of GATA3 to balance the immune system

This discussion is important as research to develop drugs to treat helminth infection is still required and molecular perspective is needed to broaden the possibility of treatment. The aimed audience of this review is the student of molecular life science, medical biotechnology or related science, who are currently interested in the development of the drug for helminth infection.

TH2 INDUCTION DURING HELMINTH INFECTION

Microbes or parasites infection can elicit different immune response. Antigen presenting cells (APC i.e dendritic cells – DC) took up and processed antigen which then presented to naıve T cells. From that point, the expression of surface ligand and cytokines secretion are upregulated. This process direct naıve T cells to differentiate into specific T cells, it can be Th1 or Th2 dominant. Eosinophils, mast cells, and basophils have been proven as the innate sources of IL-4, a Th2-related cytokine that helps to produce and polarize Th2 response (12). The cytokine-inducing process is considered essential. Polarization cytokines at the initial CD4+ T cell activation are the critical factor that influences the Th phenotype (13).

Helminth secretes several antigens which can modulate the immune system in different pathway. One of essential compound from helminth is the egg state of helminth or soluble egg antigens (SEA) (8,9). Studies conducted in human and mice DCs shows Th2 upregulation when cultured with SEA from Schistosoma mansoni (10,11). In depth proteomic study about S. mansoni egg antigens (SmEA) revealed that it consists of potent antigens such as IL-4-inducing principle of S. mansoni eggs (IPSE)/ alpha-1, Omega 1, Sm-p40 or other glycoproteins (12). Different compounds might induce Th2 polarization in different manners. Study in human basophils demonstrated that natural and recombinant IPSE/alpha-1 plays a key role in basophil activation and therefore increase the expression of IL-4 and IL-13. IPSE also has strong binding with IgE, suggesting that there is a cross-linking with IgE on basophils (13). Similarly, research in mice shows that IPSE/alpha-1 is a major antigen identified in the early state of S. mansoni infection (14). To confirm that production of IL-4 is not only occurred ex vivoëfurther investigation in mice was done. The result revealed that IPSE/alpha-1 in SmEA could induce IL-4 production by murine basophils, which mostly exist in the mice liver (15). Alternatively, IPSE/alpha-1 can also induce Th2 polarization via activation of alternatively activated macrophage (AAM) (16).

Another glycoprotein from helminth egg that dominates the initiation of Th2 differentiation is omega-1 (9). Study in human DC cells demonstrates that Omega – 1 is as potent as SEA in regards to Th2 induction. Omega – 1 is able to inhibit the production of IL-12, which is pivotal to Th2 induction. This result is also in line with in vivo research which demonstrate that Omega-1 is essential for DC to produce IL-4 and consequently differentiation of Th2 (8,9). Deeper research also revealed that Omega 1 is a glycoprotein that has ribonuclease activity. Theoretically, the ribonuclease in this protein contributes to the Th polarization and its catalytic activity is essential. The inhibition of ribonuclease would affect the other structural domain in Omega-1 and thus halt the polarization of Th2. To conclude, ribonuclease activity is related with cytotoxicity of omega-1. However, the exact role of the enzymatic activity of omega-1 in Th2 response still yet to be discovered (9,17).

Glycoprotein and lipids are abundant in the helminth eggs. It can interact with pattern recognition receptors (PRR) such as C-type lectins and TLRs on the DCs. Specific DCs can recognize carbohydrate derives the helminth eggs. DC-SIGN, mannose receptor, and macrophage galactose-type lectin-dependent are special and important DC in this pathway. Glycans from T.canis can bind to the DC-SIGN and it leads to induce immune response. Another example is glycans from Schistosoma mansoni, lewis-x. Immature DCs was stimulated with Lewis –x, and binding of TLR-4 with this glycan can trigger Th2 response (18).

The other immunomodulatory from helminth is cysteine proteases. Many allergens from helminths are known as the cysteine proteases. It modulates immune system by downregulating Th1 response. It has been known that the cysteine from helminth prime DC for Th2 polarization. However, detail mechanism on how it affects DC in the way to induce Th2 polarization has not been found in the research (19).

THE ROLE OF GATA3 IN CD4 DIFFERENTIATION

Activation of Th2 Cell Differentiation

CD4 cells have a central role in the immune system. They respond to the different signals that result in their differentiation into Th1 or Th2. Different kind of cytokine signals during activation by antigen can lead to various

differentiation pathways of T cells activation. IL-4 and IL-2 cytokines can induce the expression of the GATA-3 transcription factor, that results in Th2 differentiation. Furthermore, Th2 cells secrete IL-4, IL-5, and IL-3, which regulate protective immunity and inflammation during helminth infections. The GATA-3 expression is essential in the early process of T cell differentiation (20). It is a transcription factor that is located in hemopoietic cells (21). This gene can act as an inducer of Th2 expression and suppressor of Th1 expression. The expression of GATA-3 always exists in the process of Th1 and Th2 activation; the difference is in whether it is up-regulated or down-regulated. The expression of GATA-3 can be activated through a T cell receptor or/and IL-4 mediated signalling (22).

In general, GATA-3 has two primary mechanisms to regulate Th2 expression, which are; chromatin remodeling through DNA methylation in the loci, and direct activation in transcription site. In the first mechanism, protein-histone modification is generated at the Th2 cytokines locus (IL-4, IL-13, and IL-5) (23). Histone modification alters the chromatin structure, therefore make it attainable to transcription factors. GATA-3 might bind to several sites of the Th2 cytokines locus such as conserved non-coding sequence (CNS)-1, IL-5 promoter, the DNase hypersensitive (HS) site, the conserved GATA response element (CGRE), and HSII in intron 2 of the IL-4 gene . The most influencing cytokine during Th2 differentiation is IL-4. Among the Th2 cell cytokine locus, CNS-1 influences the expression of IL-4 to the greatest extend. Recent research has shown that GATA-3 binding to CNS region is not significant for Il-4 expression, it needs other transcription factors. This finding implies that binding also occurred in the different parts of the locus, such as HSVa and HSII. These two regions are also critical for IL-4 expression (24).

As for IL-13 expression, the chromatin remodeling is located at CGRE binding region. CGRE contribute to the histone hyperacetylation region in the Th2 cytokines gene locus and the RNA polymerase II-containing histone acetyltransferase binding. These two have an important role in the IL-13 locus and remodeling chromatin. GATA-3 also strongly binds to the other sites in the locus control region (LCR) in the cytokine locus. This mechanism plays a crucial role in DNA looping and therefore also in Th2 cytokine transcription (25).

The second mechanism is through direct binding in the transcription pathway. As for IL-5 and IL-13 expression, GATA-3 can directly bind to the promoter of the cytokines genes (Figure 1). This mechanism allows GATA-3 to regulate directly in the mRNAs transcription mechanism. In the explanation above, GATA-3 is continuously needed for the cytokines to be expressed, this accounts for IL-5 and IL-13 in particular (25). During a helminth infection, these two mechanisms dominated the immune system, thus the infection would enhance

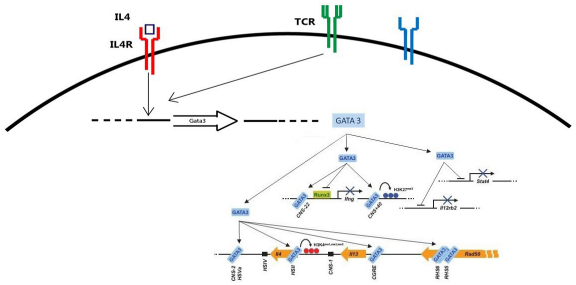

Figure 1: Molecular mechanism pathways of GATA-3 regulation of Th1 and Th2 differentiation (5)

the production of Th2.

GATA-3 is not only crucial for Th2 development, but it also plays a significant role in suppressing Th1 development. Some mechanisms can explain how GATA-3 works to inhibit Th1 development. The inhibition of Th2 differentiation by GATA3 also render IFN-γ down regulation. IL-4 is one of essential cytokines which is normally needed to inhibit IFN-γ production. However, in vivo study in mice indicated that GATA 3 supress IFN-γ independently, without IL-4 induction. Because in the absence of IL-4 suppression of IFN-γ by GATA 3 is still significant (26,27). To date, there are several studies which can explain the mechanism of IFN-γ inhibition (28,29).

The first hypothesis is GATA 3 inhibit IFN-γ through suppression of IL-12. However, this notion is not supported by both *in vitro* and *in vivo* studies which depicted that mice bearing a transgenic IL-12Rβ2 chain expression (which cells always express IL-12Rβ2) are still sensitive to Th2 development (30,31). Thus, further study using mice with transgene-expressing IL-12Rβ2 chain was conducted. In the further study, the focus was shifted to another important event of Th2 development, which is downregulation of Stat4. Downregulation of Stat4 is needed during Th2 process, and these two process are mutually exclusive. This result corroborated with the fact that in Stat4 transgenic mice the Th1 response is significantly enhanced. However, the detail mechanism on how GATA 3 down regulate Stat4 still remains elusive. GATA-3 might act as negative trans-factor which directly bind to the Stat4 promoter (28). But again, the idea needs to be further confirmed. In addition to this specific issue, Stat4 role in IFN-γ induction is also still elusive. Some research demonstrate that Stat4 is not main transcription factor for IFN-γ secretion. Alternatively, it acts as a primary transcription factor to outgrowth Th1 cells which then produce IFN-γ (32).

The second mechanism is by neutralizing Runx3 as the significant transcription factor in IFNγ production. Runx3 is an important IFNγ production in both CD4 and CD8 T cells , without depending on IL-12. ChIPseq analysis shows that Runx3 binds to the IFNγ gene

promoter and several sites along the IFN-γ gene locus to generate the expression. In addition, Runx3 can induce the production with or without coordination of the T-bet group (29,33). In respect to the interaction with GATA-3, it can bind to Runx3. The ratio and balance of this binding determine the amount of the IFNγ that will be produced (29).

In addition to the mechanisms explained above, GATA3 can directly inhibit the transcription factors of Th1 specific genes or related proteins. For instance, direct binding at the *Ifng* gene transcription start site or downstream part of the gene render the alteration of histone protein and thus inactivate the *Ifng* (24,34).

DNA METHYLATION OF GATA-3 TO REGULATE THE BALANCE AMOUNT OF TH1 AND TH2

Long infection with helminths results in cytokine production that can lead to severe inflammation. The discussion of balancing the amount of Th1 and Th2 or integrating Th1 and Th2 signals to prevent excessive inflammation has risen. The new idea came up based on the discussion about the important role of GATA-3. More understanding about the molecular characteristic of this gene can bring new sight to the regulation of T cell plasticity. As stated in the introduction, GATA -3 expressions can be silenced through DNA methylation in CpG sites that will be explained in more detail in this section

What is CpG Methylation?

In vertebrates, CpG sites (CpG island: CGI) are a critical part of a DNA to undergo methylation. CpG site is a short term for 5′-C-phosphate-G-3′ (5′→3′). It is a DNA region where a cytosine base is followed by a guanine base, consecutively. This site is distinctive from normal DNA, because of the lack of DNA methylation, which separates them from bulk genomic DNA. CGI is correlated with gene promoters, which is usually located at TSS (35). However, the recent research has proven that large part of CGI although it is away from annotated TSS, still show a function as a promoter. This part is called "orphan CGI." In the immune system, even in the absence of annotated promoters, these orphan CGIs are marked by H3K4me3 and RNA polymerase II. This mark will make the CGI as detectable to start the transcription (36).

DNA methylation has a primary impact on the epigenetic signal (a genetic signal that can alter transcription or expression of the gene), chromatin structure, gene regulation. Epigenetic signals, such as methylation, phosphorylation, and acetylation, can contribute to modifying histone proteins. The integration of various forms of histone protein and noncoding RNA can modulate chromatin structure. The reby, it can influence transcription activity. DNA methylation is key in the epigenetic process, as it affects the control of gene expression and regulation of parental imprinting (37). Most DNA methylation takes place on cytosine residues, at the C5 position in the CpG site, and only sometimes at nonCG-sites. Normally, in the active genes, there is not much methylation in the transcriptional start site (TSS) and high rate of methylation usually happened in the body of the gene, to increase the gene expression (38).

The mechanism of methylation starts when there is an enzyme called DNA methyltransferases (DNA Mtases) as a catalyst. This enzyme works to catalyze methyl removal from co-factor molecule (such as S-adenosyl-L-methionine) to the 5C site in the DNA sequence. This mechanism creates 5-methylsytosine and releases the co-factor residue from the enzyme (37).

Possibility of DNA methylation to Regulate T cells level

GATA-3, as stated before, plays a crucial role in the differentiation of CD4 cells. This gene is involved in determining the level of Th1 and Th2. It can upregulate Th2 differentiation and on the other hand, can suppress the differentiation of Th1. However, during helminth infection, excessive inflammation could happen. The idea is by balancing the level Th1 and Th2 in the immune system to limit the inflammation. Balance level of these two cells during infection is a critical issue. In the early stage of infection, Th2 response is necessary to limit the disease and infection. However, the prolonged response of Th2 (mediated by IL-13) can cause liver fibrosis and reduce the survival rate (7). One possible idea is by looking deep into one of the crucial genes, which is GATA-3. The absence of a GATA-3 signal would drive the cell differentiation to co-produce Th1 along with Th2, which will help to prevent excessive inflammation, caused by helminth infection. As stated in the previous explanation, DNA methylation can act as a silencing gene signal. Some research has been done, to silence the GATA-3 expression or to co-express Th1 and Th2 in the same time. In this part of review, the writer would like to emphasize the significance of the experiment.

The plasticity of T cell has been discussed and demonstrated by many studies. Th2 that is generated ¬*in vitro* can be re-programmed by promoting Th1 via lymphocytic choriomeningitis virus to produce a GATA-3+T-bet+ phenotype (6). Also, Th1 cells can be altered to Th2 cells that produce IL-4 cytokines (39). Furthermore, the molecular experiments through DNA methylation give new insight to limit inflammation effect of helminth infection. The research has been done mostly through *in vitro* experiments whereby the impact of DNA methylation on T cells balance was monitored. Generally, during Th2 differentiation, the IL4/IL5/Il13 locus lacks DNA methylation. In the other hand, DNA methylation in the IFNγ locus decreasing as the Th1 differentiation goes through.

Screening of genome-wide DNA methylation at CGI sites of the immune system shows that there is only

one CGI methylation different between Th1 and Th2 differentiation *in vitro*. It occurs in the GATA-3 gene (36). The experiment was done *in vitro*. However, the in vivo setting was also mimicked. CD4+ T cells were taken from mice infected with *S. mansoni* for 8 weeks. Cell isolation and population count are used in the experiments. Isolation of pure primary immune cells was done by fluorescence activated cell sorting (FACS) (40).

In the initial stage of infection, CD4+ T cells exhibit the properties of both Th1 and Th2 cells (both phenotype in one cell), which produce both IFNγ and IL-4. Besides, a single cell of Th1 and Th2 also present. In the following explanation, the terms of cells will be used such as IFN-γ+IL-4- for Th1 expression, IFN-γ-IL-4+ for Th2 expression, and IFN-γ+IL-4+ (double positive cell) to show that both of Th1 and Th2 cytokines are expressed. The methylation process is done by adding sodium bisulfite into DNA (gene), which will change unmethylated cytosines base into uracil but did not change the methylated cytosines. Analysis of the DNA methylation was done at the cytokine gene (*Ifng* and Il4) locus, at promoter CGI of GATA-3 gene loci, and also at the body CGI of GATA-3 gene. The analysis in these three sites is done to compare in which site DNA methylation give significant impact. The analysis of the cytokines locus shows a different clear signature for IFN-γ+IL-4+ compare to the single cells (only Th1 or Th2 cells). These cells have significant decreasing of methylation in the of *Il4* and *Ifng* promoter. Thus, it can express both of the cytokines. However, Th1 and Th2 single cells show a normal response, which IFN-γ+IL-4- (Th1) cells lack of methylation when expressed IFN-γ. Reciprocally, DNA methylation in IFN-γ-IL-4+ (Th2) increased (7).

Methylation of GATA-3 in the CGI for Th1 and Th2 differentiation overlap in the same site, more accurate in the third exon of the gene (41). A very low level of methylation of GATA-3 CGI is observed in IL-4 producing IFN-γ-IL-4+ (Th2) cells. In the other hand, Th1 cells have a high degree of methylation at the same CGI site. Meanwhile, IFN-γ+IL-4+ has an intermediate level of methylation, between Th1 and Th2 cells. This analysis underlines the difference cell population and molecular mechanism during helminth infection. The unique DNA methylation enables the flexibility of the immune system to generate double positive cells (IFN-γ+IL-4+) (39) .

The last analysis is the methylation in the body (not the promoter) of CGI GATA-3. Methylation of GATA-3 in the CGI body contributed to the decreasing expression of this gene. In the experiment, CGI in Th1 cells was highly methylated during IFN-γ expression, however only low-level GATA-3 detected. It supports the previous report that DNA methylation in the CGI promoter contributes to the gene silencing . In contrary, GATA-3 is highly expressed in Th2, as the CGI sites of this gene remain demethylated (42). As for GATA-3 gene in IFN-γ+IL-4+ is also at the intermediate level. This result based on the research using cells in spleen isolated from *S. mansoni* infection (Figure 2) (7). In summary, methylation in the CGI GATA-3 body is more potent to silence the expression of the gene. It acts more effectively than methylation in the promoter site.

CONCLUSION

DNA methylation can be applied in GATA-3 gene to co-express Th1 and Th2 in a cell, which will decrease the inflammation during helminth infection. Methylation during helminth infection enhances the flexibility of the immune cells. As the double positive cells (IFN-γ+IL-4+) appears. This cell has less inflammation impact because the level of both Th1 and Th2 cells are intermediate. From the three methylation analysis: in the cytokines locus, CGI promoter of GATA-3 and CGI body of GATA-3; the methylation at the CGI body of GATA-3 have more impact in silencing the GATA-3 gene in the immune cells. The molecular level research opened new possibility to understand helminth infection. However, further research is needed to apply this mechanism as a treatment in helminth infection needs. Suggestion for the next experiment is implement the research of the DNA methylation *in-vivo* level.

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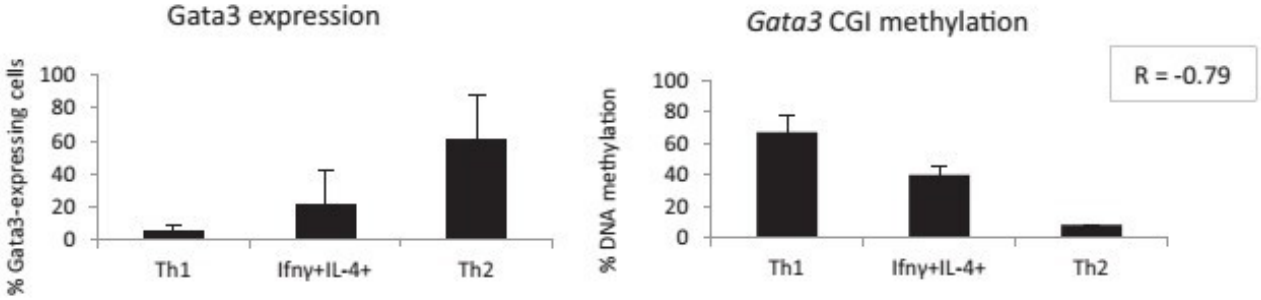

Figure 2: Percentage of GATA-3 expression by the side of GATA-3 CGI methylation level. Research have been done in cells isolated from *S.mansoni* infection (7)

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