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Innate Immunity Post-Hematopoietic Stem Cell Transplantation: Focus on Epigenetics

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Abstract

Epigenetic regulation of gene expression is important for normal biological processes like immune cell development, immune responses, and differentiation from hematopoietic stem cells. Furthermore, it is well understood that epigenetic mechanisms can include methylation, histone modification, and more recently, microRNAs. Interestingly, aberrant epigenetic modification can also promote pathology in many diseases like cancer. The effects of methylation on gene expression and its resulting phenotype have been extensively studied. In this review, we discuss the inhibition of innate immunity that occurs in humans and animal models post-stem cell transplant. In addition, we highlight the changes methylation and microRNA profiles have on regulating pulmonary innate immune responses in the context of hematopoietic stem cell transplantation in experimental animal models.

Keywords

Alveolar macrophage; hematopoietic stem cell transplantation; epigenetics; methylation; microRNA; lung

INTRODUCTION TO HEMATOPOIETIC STEM CELL TRANSPLANTATION (HSCT)

HSCT is now considered a standard of care for many malignant and non-malignant diseases. Depending on the underlying disease, a patient will receive either an autologous [self donation of hematopoietic stem cells (HSC)] or allogeneic [human leukocyte antigen (HLA)-matched related or unrelated hematopoietic stem cell donor] transplant [1]. Initially, sources of stem cells were limited to the bone marrow. However, knowledge of HSCs and HSCT has expanded options in stem cell sources. Now HSCs can be harvested from peripheral blood and cord blood, in addition to bone marrow [1, 2]. HSCs collected from the

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periphery must first be mobilized to the periphery through the use of the growth factor, granulocyte colony-stimulating factor (G-CSF). Peripheral blood HSC harvests contain higher amounts of mature T cells than bone marrow sources of HSCs. The presence of mature T cells promotes engraftment and augments the desired graft-versus-leukemia (GvL) reactions; however, the likelihood of graft-versus-host disease (GvHD) similarly increases. Each source of HSCs for HSCT harbors advantages and disadvantages to their utilization and these are reviewed elsewhere [3].

Prior to receiving the HSC graft, transplant patients undergo a conditioning regimen (i.e. total body irradiation, chemotherapy) to remove any malignant or self-reactive “diseased” cells and to create a niche to replace the hematopoietic system with healthy cells from the graft. Under myeloablative conditions, the hematopoietic compartment is reconstituted from the graft; however, under nonmyeloablative conditions, the reconstitution can originate from the host and/or the graft. A retrospective analysis conducted on a cohort of patients over the age of 50 who received either a myeloablative or nonmyeloablative conditioning regimen prior to an allogeneic HSCT found both advantages and disadvantages to each regimen. This study reported that patients receiving nonmyeloablative therapy were protected from non-infectious pulmonary complications whereas 40% of patients receiving myeloablative therapy developed these lung problems [4]. Other studies report up to 60% of HSCT patients receiving myeloablative therapy develop either infectious or noninfectious pulmonary complications [5–7]. While Alyea and colleagues saw a reduction in non-infectious complications with nonmyeloablative conditioning, they still noted that infection remained a major cause for nonrelapse mortality in both the myeloablative and nonmyeloablative groups. Furthermore, disease relapse was significantly higher in patients receiving nonmyeloablative conditioning regimens than those receiving myeloablative regimens. Thus, although reduced conditioning therapy does offer some lung protection and therapeutic advantage to a group of older individuals, myeloablation remains more effective in preventing disease relapse over time.

Infectious pulmonary complications of HSCT

Both infectious and noninfectious pulmonary complications can afflict HSCT patients regardless of the type of transplant (allogeneic or autologous) [1, 8, 9]. Studies focused on understanding the mechanisms behind the increased susceptibility are extensive. This susceptibility may be due to phenotypic changes that occur in the alveolar environment. The alveolus is composed primarily of type I and type II epithelial cells (structural cells), as well as alveolar macrophages [10]. Alveolar epithelial cells are vital for proper lung function (i.e. gas exchange, secretion of surfactant) [10] while alveolar macrophages are the sentinel phagocytes in the alveolus, highlighting their importance in initiating an immune response against an invading pathogen in the lower airways [11]. Infectious complications arise throughout the different phases of reconstitution. In the context of transplantation, there are three phases: 1) the pre-engraftment (0–30 days post-transplant), 2) the early post-engraftment (30–100 days post-transplant), and 3) the late post-engraftment phase (beyond 100 days post-transplant) [8, 12–14]. Not surprisingly, transplant patients in the pre-engraftment phase are afflicted with pulmonary bacterial and viral infections. At this stage, incomplete reconstitution of the immune system is likely the cause for inefficient immune

responses against invading pathogens as this phase has been reported to contain decreased numbers of neutrophils and defective function of circulating neutrophils [15]. Similarly, human alveolar macrophages from allogeneic transplant patients exhibit defective chemotaxis, phagocytosis and bacterial killing [16]. Interestingly, patients in the late post-engraftment phase continue to exhibit increased susceptibility to infection indicating that the immune system remains impaired in function even when reconstituted in numbers.

As alveolar macrophages mediate an early immune response to pathogens invading the lower airways and their function has been shown to be impaired post-HSCT, studies have been focused on understanding the mechanisms driving the defects in this cell population. These studies have largely been conducted using animal models of HSCT.

Animal models of innate immunity post-stem cell transplant

In order to study mechanistic alterations in innate immune cells post-stem cell transplant, laboratories have employed mouse models of bone marrow transplant (BMT). To model autologous transplants, syngeneic (genetically identical littermate) bone marrow donors are used. To model allogeneic transplants, mice of different genetic backgrounds are used as donors and hosts. Mice can be conditioned with either chemotherapy or total body irradiation (TBI) [17], but TBI is most common. Turnover of murine lung alveolar macrophages require radiation levels of at least 9 Gy [17]. Our laboratory has routinely used 13 Gy split dose irradiation to myeloablate C57Bl/6 mice [17–19]. Additionally, we have demonstrated that 5 weeks post-BMT is the optimal time point for reconstitution of the alveolar macrophage compartment from donor marrow. Neutrophils are replaced from donor marrow much faster and turnover of peripheral leukocytes from donor marrow can occur at lower TBI doses as well. Using a model of syngeneic BMT, we have previously demonstrated that alveolar macrophages and neutrophils from BMT mice have defective functions [18, 20]. Neutrophils were shown to have bacterial killing defects whereas alveolar macrophages had both phagocytic and bacterial killing defects. In both cases, the defects were associated with elevated expression and activity of the cyclooxygenase (COX) pathway [18, 19]. There are two isoforms of the COX enzymes: COX-1 and COX-2. COX-2 is the inducible form of COX and has been associated with inhibitory effects on immune responses [21]. Interestingly, alveolar macrophages and neutrophils from BMT mice over-expressed COX-2 and, as a result, also overproduced prostaglandin E₂ (PGE₂), an end product of COX-2 activity [18]. Inhibition of the COX pathway by giving indomethacin either *in vivo* or *in vitro* could restore innate immune function against *Pseudomonas aeruginosa*. Similarly, inhibition of PGE₂ signaling via antagonism of the EP2 receptor also restored function of alveolar macrophages from BMT mice *in vitro* [18]. These results and other noted alterations to be discussed are summarized in Fig. 1. While these results demonstrated the importance of COX-2 overexpression and PGE₂ signaling in the inhibition of innate immunity post-BMT, they did not explain why COX-2 levels were elevated post-transplant. The fact that this was a stable phenotype of alveolar macrophages even when removed from the BMT lung suggested that the results could be due to epigenetic effects.

EPIGENETIC REGULATION

Epigenetics can be defined as potentially heritable changes to gene expression that are independent of changes to the DNA sequence [22]. Epigenetic regulation can involve methylation, histone modification, and microRNA (miRNA). For the purposes of this review, discussion of epigenetic changes in HSCT will be restricted to DNA methylation and miRNA which are the best studied epigenetic alterations in this context.

Methylation

Epigenetic regulation of gene expression via methylation has been studied extensively due to its importance in regulating diverse biological processes. Though dispensable and nonexistent in some organisms like *Caenorhabditis elegans* (*C. elegans*), methylation is widely conserved across prokaryotes and most eukaryotes [23]. Studies in bacteria suggest methylation as an important mechanism for distinguishing self/host DNA from invading phage DNA [24]. In mammals, methylation has been extensively studied in natural processes (e.g. embryogenesis) as well as disease processes (e.g. cancer). We now know that in mammals, methylation occurs on DNA whereby a cytosine base is converted to a 5-methylcytosine via *de novo* (DNMT3a/3b) or maintenance (DNMT1) DNA methyltransferases (DNMTs) [25]. Although methylation in mammals is primarily regulated by DNMT1, DNMT3a, and DNMT3b, two separate DNMTs have also been identified known as DNMT3L and DNMT2. DNMT3L is implicated in the regulation of DNMT 3a and 3b while DNMT2 has been renamed tRNA aspartic acid methyltransferase for its role in methylated tRNA rather than DNA [25, 26].

Maintenance DNMTs are vital for conserving inherited methylation patterns following cellular replication while *de novo* DNMTs function to create methylation at new or previously unmethylated CpG sites. DNMT1 has traditionally been highlighted as a maintenance DNMT. This was primarily due to studies indicating that *de novo* methylation remained intact despite DNMT1 knockout in embryonic stem (ES) cells [27]. A separate study had similarly observed that deletion of DNMT1 in these same cells, resulted in a significant reduction in methylated cytosines [28]. DNMT3a and DNMT3b, were coined *de novo* DNMTs due to intact inherited methylation patterns in ES cells [29] and overexpression of DNMT3a resulted in increased *de novo* methylation [30]. However, other data suggest that these *de novo* DNMTs may exhibit properties of maintenance DNMTs under particular circumstances as in a DNMT1 knockout adenocarcinoma cell line exhibiting intact methylation patterns [31]. It is also possible, that other maintenance DNMTs have not yet been identified.

Though DNA is scattered with cytosine bases, the importance of the methylation of cytosines on gene expression localizes to regions of the DNA that are more highly concentrated with cytosine-phosphate-guanine (CpG) dinucleotides, also known as CpG islands. The mechanism by which DNMTs target certain CpG islands within genes is not entirely understood.

Methylation-regulated gene expression has also been studied in the context of disease. ICF (immunodeficiency, centromeric region instability, and facial anomalies) syndrome is a

recessive autosomal disease characterized by mutated DNMT3b, resulting in targeted chromosome breakage and decreased serum immunoglobulin levels despite normal B cell counts [32]. In idiopathic pulmonary fibrosis, some fibroblasts lose responsiveness to inhibitory effects of PGE₂ signaling via increased methylation of the EP2 receptor [33]. In addition, various studies have pointed to dysregulated methylation patterns as cofactors for development of cancer [34]. Global hypomethylation and hypermethylation of specific genes, like tumor suppressor genes, were previously observed in tumor cells [35, 36]. Hypomethylation of CpG islands in the promoter of COX-2 has been previously associated with increased expression of COX-2 mRNA and contributes to progression of many human cancers [37–39]. In gastric cancer, increased expression of COX-2 directly correlated with increased risk of death from gastric cancer compared to patients that did not exhibit induced expression of COX-2 [40]. That aberrant COX-2 expression is associated with altered methylation status of CpG regions in the promoter suggests an important role in epigenetic regulation of this gene. Taken together, the above data prompted us to examine the methylation patterns of COX-2 post-BMT.

Aberrant methylation of COX-2 in HSCT

As discussed above, HSCT patients are at higher risk for developing bacterial pneumonias compared to untransplanted individuals. This increased susceptibility can be independent of immunosuppressive therapy as autologous patients, in contrast to allogeneic patients, do not receive immunosuppressive therapy and still develop infectious pulmonary complications [9, 41]. These complications arise despite successful reconstitution of the immune system. Thus, these observations suggest that impaired immune function promotes increased susceptibility in this patient population.

Epigenetic regulation by methylation is important for multiple differentiation processes. Differentiation of distinct lineages from the hematopoietic stem cell is a process that is tightly regulated by methylation. The “on” and “off” expression of many genes ensure tissue- and lineage-specificity [42]. For example, T helper cell differentiation is supported by DNA methylation profiles [43]. Similarly, alteration or dysregulation of methylation has been implicated in promoting pathology in diseases like rheumatoid arthritis, lupus, diabetes, and cancer. As cytokines have previously been shown to induce signaling pathways in a variety of cells, it is possible that cytokines may be influencing epigenetics to promote cellular activation or inactivation. A study conducted by Hashimoto and colleagues demonstrated that inflammatory cytokines like IL-1 β can, in fact, regulate DNA methylation states [44]. As environmental factors have been suggested to regulate epigenetic modifications which influence many lung diseases, an interest in understanding the underlying mechanisms by which microenvironments affect epigenetic regulation continues to grow [45].

Our previous studies using a murine model of BMT reported defective host defense against both Gram negative and Gram positive pathogens [18, 19, 46]. As mentioned above, alveolar macrophage function is impaired in BMT mice and this defect directly correlated with elevated COX-2 expression and increased production of PGE₂ by alveolar macrophages post-BMT [18, 46]. Furthermore, we showed that inhibition of the COX

pathway with indomethacin post-BMT rescued the functional impairment [18]. However, the mechanisms driving the upregulation of COX-2 in this model of BMT were unclear. Recently, bisulfite conversion and pyrosequencing analysis revealed that aberrant COX-2 expression levels observed previously were associated with decreased methylation within the promoter and near the first exon of the COX-2 gene [47]. One obvious question was why COX-2 was hypomethylated in the BMT alveolar macrophages. One possibility was that increased levels of transforming growth factor- β (TGF β) synthesis from alveolar epithelial cells post-BMT [48] may be impacting the alveolar macrophages which differentiate in the lung post-BMT. In support of this hypothesis, TGF- β was shown to induce COX-2-driven luciferase expression in transfected primary alveolar macrophages despite extensive *in vitro* methylation of the COX-2 construct [47]. This observation was supported by *in vivo* data showing that alveolar macrophages derived from the marrow of a mouse expressing a CD11c-driven dominant negative TGF- β RII exhibited restored methylation of the COX-2 CpG sites post-BMT [47]. These data suggest that TGF- β induces COX-2 expression post-BMT via epigenetic hypomethylation; thus, it is likely that TGF- β may regulate COX-2 expression through inhibition of DNMT activity. In fact, we have unpublished observations that TGF β stimulation of alveolar macrophages *in vitro* does reduce expression of all 3 DNMT genes (data not shown). Our results in alveolar macrophages are in contrast to a previous study which reported that TGF- β induces DNMT expression in an aggressive prostate cancer cell line [26]. Thus, it may be that the effects of TGF- β on DNMTs may be context or even cell type-dependent.

MiRNAs

MiRNAs are small, noncoding RNAs that, like methylation, can regulate protein-encoding genes. However, miRNAs primarily modulate expression post-transcriptionally, affecting mRNA stability as well as inhibiting protein translation. Originally discovered in *C. elegans* [49], the presence of miRNA has been described in both animal and plant cells. Since the initial description of miRNA, many studies have tried to understand how miRNAs are processed and the mechanism behind RNA interference (RNAi) as a form of regulation of gene expression. In humans, it is estimated that about a quarter of miRNA genes are encoded in intronic regions while others are found clustered throughout the genome [50, 51]. We now know that miRNAs arise from noncoding RNA, primarily transcribed by RNA polymerase II. These long noncoding RNA, also known as pri-miRNA, contain stem-loop structures, and go through various processing steps to ultimately yield mature miRNA, roughly about 22 nucleotides in length. Interestingly, multiple miRNA can arise from a single pri-miRNA [52, 53]. Cleavage and further processing of these stem-loop structures can occur in one of two identified pathways: the canonical Drosha pathway or a Drosha-independent pathway. The latter pathway has been investigated primarily in *Drosophila* and *C. elegans*, and these studies suggest that some intron-originating miRNA can form pre-miRNA from pri-miRNA via a Drosha-independent splicing mechanism [54, 55]. In contrast, the canonical Drosha pathway involves cleavage via a complex containing DiGeorge syndrome critical region gene 8 (DGCR8) and the RNase type III, Drosha. Also known as the microprocessor complex, DGCR8 and Drosha are responsible for mediating the formation of pre-miRNAs from pri-miRNA, the primary miRNA transcript [50, 56]. Interaction with exportin-5-Ran-GTP supports transportation of the pre-miRNA to the

cytoplasm from the nucleus, where it can be further processed by Dicer, a dsRNase type III, to yield a double stranded miRNA at its mature length. This double stranded miRNA contains a functional strand, (the mature miRNA), which is loaded onto the RNA-induced silencing complex (RISC) together with Argonaute proteins, and a nonfunctional (passenger) strand, which is degraded.

Unlike small interfering RNA (siRNA) which form approximately 21–23 base-paired duplexes that are complementary to any region of the target mRNA and trigger degradation via Argonaute proteins [57], mature miRNA guide the RISC complex via identification of target sequences in 3' untranslated regions [3'UTR] of mRNA to affect mRNA stability and/or inhibit translation [56, 58]. It remains the general rule that recognition of a target mRNA by a miRNA requires complete complementarity between the target sequence in the 3'UTR of the mRNA and the 'seed' region, defined as nucleotides 2–8 of the 5' end of the miRNA. However, exceptions to this rule do exist and have been summarized elsewhere [57]. Although such exceptions can complicate studies focused on understanding the effects of miRNA on gene expression, it is important to remember that when performing such studies, targeting of the miRNA to the gene sequence under investigation should be verified.

MiRNA and immune responses

The importance of miRNA on developmental, cellular, and physiological processes was previously underappreciated. However, multiple studies have now shown that miRNA are important for basic biological processes like hematopoiesis as well as regulating immune cell development and immunological responses [59]. Initiation of innate immune responses are important not just for the immediate attack on invading pathogens, but also for the further activation of the adaptive immune response, which can confer protective and long-lasting immunity. One way that innate immune cells sense pathogens is through membrane-bound Toll-like receptors [60]. Binding to these receptors triggers the activation of intracellular signaling cascades that result in a variety of responses, e.g. activation of pro-inflammatory genes. These pathways have been studied extensively. However, what has been more recently observed is that miRNA can exert regulatory functions that can either support or antagonize these innate immune responses. MiR-146a/b, for example, has been shown to be induced by TLRs expressed on the cell surface and not TLRs bound to intracellular membranes [60, 61]. Interestingly, miR-146 targets adaptor proteins important for TLR signaling suggesting that miR-146 functions as a negative regulator of TLR-initiated immune responses [61]. MiRNA-mediated gene regulation is extremely complex because of the broad and various mRNA targets. Studies focused on miR-155 regulation of immune responses highlight the complexity of miRNA-mediated regulation. Both bacterial and viral products, through recognition primarily mediated by TLRs, as well as various proinflammatory cytokines (e.g. IFN- β , TNF α) can induce miR-155 expression in macrophages [61, 62]. Furthermore, miR-155 can stabilize TNF α , and thus promote translation of TNF α and support proinflammatory responses, particularly in macrophages [63]. Studies on macrophage polarization have shown a role for miRNA regulation [33]. In one study, overexpression of miR-155 on tumor-associated macrophages, which typically resemble alternatively activated macrophages and exhibit anti-inflammatory functions, resulted in a switch in polarization to a classically activated macrophage [20]. However,

similar to miR-146a/b, miR-155 can also serve as a negative regulator of inflammatory responses, as genes involved in LPS-induced signaling have been identified as targets of miR-155 [64]. Thus, miRNA can have multifunctional roles and harbor both positive and negative effects on immune responses.

MiRNA regulation in the setting of stem cell transplantation

As mentioned above, HSCT patients often suffer from a variety of pulmonary complications that can arise long after transplantation. Unlike autologous transplant patients, allogeneic transplant patients often suffer from GvHD, whereby the donor cells mount an immune response against host tissues. There are several degrees of GvHD and much effort has been put into predicting the development of GvHD. Recently, a study from Beelen and colleagues [65] showed that changes in miRNA-146, 21, and let7 expression in whole blood of patients receiving an allograft correlated with the development of acute GvHD. This study further proposed the possibility of using miRNA expression profiling as a way to predict the various degrees of GvHD.

In studies of innate immune function post-BMT, a role for miR-155 was demonstrated [46]. Following BMT, miR-155 was the only miRNA in alveolar macrophages with decreased expression. As miR-155 has been shown to play a role in both inducing and inhibiting immune responses, this study examined whether miR-155 affected alveolar macrophage phagocytosis via regulation of scavenger receptors. Previous studies had demonstrated that phagocytosis of *P. aeruginosa* was impaired by alveolar macrophages post-BMT [18, 20]. This finding is due to PGE₂-mediated loss of the macrophage receptor with collagenase structure (MARCO) which is the primary receptor for non-opsonized uptake of *P. aeruginosa* [46]. Thus, we were surprised to note that following myeloablative conditioning, alveolar macrophages from BMT mice exhibited enhanced phagocytosis of *Staphylococcus aureus* [46]. This enhanced phagocytosis was due to increased expression of the Class A scavenger receptor, SR-A. Interestingly, SR-A contained a target sequence for miR-155 in its 3'UTR. Thus, this study showed that loss of miR-155 promoted upregulation of SR-A expression post-BMT. Although these cells exhibited enhanced phagocytosis of the SR-A utilizing pathogen *S. aureus*, defective intracellular killing of the bacteria was still present, explaining why there is increased susceptibility of BMT mice to *S. aureus* infection. Interestingly, the decrease in miR-155 observed in this BMT model was directly mediated by the overproduction of PGE₂ in the lung [46], which is a direct consequence of the hypomethylation pattern discussed earlier [47]. Thus, taken together, our data demonstrate that epigenetic modifications result in multiple functional alterations in innate immune function post-BMT.

TARGETING EPIGENETICS AS A THERAPY

As our knowledge on how epigenetic alterations regulate normal biological processes and disease states grows, so does the desire for targeting this form of genetic regulation for the benefit of a variety of patients. As methylation has been characterized to be dysregulated in certain cancers, it has become an area of focus for drug development. Azacitidine, or 5-azacytidine, is a pyrimidine nucleoside analogue of cytidine that incorporates itself into DNA or RNA, and upon incorporation into DNA, it functions to disrupt DNA

methyltransferase activity [66]. Azacitidine can also incorporate into RNA and inhibit the translation of oncogenic proteins by inducing disassembly of ribosomes [67]. Thus, because of its effective mechanism of action against features promoting carcinogenesis, the refinement of Azacitidine as an effective drug was exciting. Currently, Azacitidine is an approved therapeutic for the treatment of myelodysplastic syndromes (MDS) [68]. Additional methylation-targeting drugs in the developmental and testing stages are reviewed elsewhere [68].

MiRNAs comprise a new class of RNAi that contain attractive properties as targets for therapeutic development [69]. Effective targeting and delivery of miRNAs remain a concern for the development of miRNAs as a therapy, but there is promise in this area as highlighted by the development of Miravirsen (SPC3649), an anti-miR drug candidate that inhibits miR-122. Miravirsen is currently in clinical trials for the treatment of hepatitis C virus [70]. Of course, such therapies will need to be rigorously tested since a single miRNA can target many host genes.

While this review explores the contribution of epigenetic-mediated changes, primarily via miRNA and DNA methylation, in HSCT, it should be noted that post-translational modification of histones (not discussed) is another pathway for epigenetic regulation that may yet be shown to play an important role in promoting immunosuppression in lungs of transplant mouse models and patients. With the ability to be modified at the amino-terminal tails or within the globular core, histones can be modified multiple ways [34, 48]. The most commonly known are histone acetylation, methylation, and phosphorylation, however, many modifications exist and are less well known, for example, sumoylation, deimination, and ADP ribosylation [34, 45]. This non-exclusive list provides appreciation for the variety of ways histones can be regulated. The consequence of these modifications can result in changes to the histone structure or histone-DNA interactions that can subsequently alter chromatin structure, as well as the regulation of chromatin binding factors specific to certain histone modifications [34]. Thus, these modification-induced changes to chromatin structure/interactions ultimately affect gene expression.

Epigenetic research has expanded significantly, as has our understanding of the importance of epigenetic regulation in promoting normal/healthy or disease pathologies. As a result, the potential of reversing disease states through targeting different forms of epigenetic regulators, or using epigenetic profiles as biomarkers or diagnostic tools is an evolving and exciting field.

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Fig. 1.

Summary of epigenetic changes in BMT AMs. Increased production of TGF-β by type II alveolar epithelial cells (AEC) signals to alveolar macrophages, resulting in decreased expression of DNMTs (DNMT 1, 3a, 3b). Methylation in CpG islands on COX-2 was decreased, inducing expression of COX-2. Overexpression of COX-2 in alveolar macrophages enhances PGE₂ production, which can autocrine signal back via the EP2 receptor. Signaling through EP2 results in inhibition of miR-155. With the loss of its negatively regulating miRNA (miR-155), SR-A expression increases, allowing enhanced non-opsonized phagocytosis of *S. aureus* (S.a.) through this receptor. Signaling through the EP2 receptor also results in inhibition of another Class A scavenger receptor, MARCO. Decreased MARCO inhibits effective uptake of *P. aeruginosa* (P.a). Finally, PGE₂ via EP2 inhibits phagocytosis of *P. aeruginosa* and killing of *P. aeruginosa* and *S. aureus*.