**TEMPLATE FOR PROJECT OUTLINE**

**(please type!)**

Student’s name: Anik Muhuri

Topic chosen: Epigenetic markers associated with Asthma

SPECIFIC QUESTION: Can certain epigenetic modifications be associated with higher risk for asthma incidence?

HYPOTHESIS: DNA and histone methylation changes within genes or loci associated with T cell differentiation into Th2 cells can be associated with higher risk for asthma incidence.

EVIDENCE ON WHICH THE HYPOTHESIS IS BASED (INCLUDE REFERENCES):

Asthma has been associated with an irregular shift in the proportions of Th1-Th2 cell populations. Abnormally high number of Th2 cells and their associated cytokines (IL-4, IL-5, IL-13) are thought to drive inflammation in the airways (Lloyd & Hessel, 2010). Naive T cells (CD4+ T lymphocytes) require activation of the IL-4 and IFN-γ genes in order to differentiate into Th2 cells (Begin & Nadeau, 2014). CD4+ T lymphocyte cultures from hosts with bronchial asthma exhibited a strong degree of demethylation at the IL-4 promoter when treated with an allergen relative to cultures not treated with allergenic stimulation (Kwon *et al*., 2008). In addition, epigenetic modifications such as H3K4me3 are found at IL-4-containing regions of the genome in Th2 cells but not in Th1 cells (Wei *et al*., 2009).

PREDICTION(S): Cultured naive T cell lymphocytes exposed to allergenic stimulation will exhibit a greater degree of demethylation at genes necessary for Th2 differentiation (IL-5, IL-13).

Genes which encode transcription factors that regulate the Th2 cell differentiation mechanism such as STAT6, GATA-3, TBET, and EZH2 will also display a strong demethylation pattern when treated with allergenic stimulation.

EXPERIMENTAL APPROACH TO TEST PREDICTION (INCLUDE ANY DETAILS THAT YOU HAVE WORKED OUT SO FAR):

Experimental approach #1: Obtain a WT mouse strain. Treat these mice to a diet which contains a compound which can act as a strong methyl donor. In theory, the methyl donor can affect the methylation/demethylation pattern of the genome as a whole. Determine if the mouse strain develops higher asthmatic incidence over several generations. If the mice do develop asthma, then methylation has a role to play. Compare the methylation pattern in these mice to genes in methylation profile databases such as DevMouse.

Experimental approach #2: Obtain a WT mouse strain as well as a mouse model for acute and chronic asthma. Harvest T lymphocyte populations from both strains and treat these cultures to allergenic stimulation. Observe any methylation pattern changes that may arise between WT, acute asthma model, or chronic asthma model for any of the genes mentioned in the prediction section.

Experimental approach #3: Use CRISPR-CAS system in mice to introduce substitutions or deletions for known methylation sites in the regulatory regions for genes such as GATA-3 and EZH2. Determine if these substitutions or deletions have an impact on Th2 cell differentiation or even whether the mice develop asthmatic symptoms.

LIST OF RELEVANT PRIMARY AND REVIEW ARTICLES READ, AND SUMMARY OF RELEVANT INFORMATION FROM EACH (this is the start of an annotated bibliography):

Lloyd, C.M., Hessel, E.M. (2010). Function of T cells in asthma: more than just T(H)2 cells. *Nature Reviews Immunology*, *12*, 838-848. doi:10.1038/nri2870

Scientists have classically thought that a shift in the balance of Th1-Th2 cells help aid allergic airway inflmmation; high numbers of Th2 cells along with their associated cytokines drive inflmmation and hyperresponsiveness. This review article summarizes previous work on Th2 cells and relates it to work done on other T-cell subsets, which shows that a complex interaction mechanism within T cell subsets may drive asthmatic symptoms.

Begin, P., Nadeau, K.C. (2014). Epigenetic regulation of asthma and allergic disease. *Allergy, Asthma, and Clinical Immunology, 10*, 10-27. doi:10.1186/1710-1492-10-27

Th2 cells have always been thought to be a primary player in driving allergic hyperresponsiveness and inflammation but the mechanisms are yet to be fully elucidated. This review article describes common epigenetic inheritance patterns as well as the current knowledge on how epigenetic inheritance can be possibly responsible for abnormal Th2 cell differentiation from naive T cell lymphocytes.

Kwon, N.H., Kim, J.S., Lee, J.Y., Oh, M.J., Choi, D.C. (2008). DNA methylation and the expression of IL-4 and IFN-gamma promoter genes in patients with bronchial asthma. *Journal of Clinical Immunology, 28*, 139-146. doi:10.1007/s10875-007-9148-1

DNA methylation is a common and well-studied epigenetic inheritance mechanism with widespread effects throughout a genome. The IL-4 cytokine gene is critical for naive CD4+ T lymphocyte differentiation into Th2 cells, which are thought to drive allergic airway inflammation. CD4+ T cell cultures from patients with bronchial asthma exhibited a strong degree of IL-4 promoter demethylation after allergenic stimulation. CD4+ T cell cultures from control, non-asthmatic subjects exhibited less IL-4 promoter demethylation. The degree of demethylation corresponded with the concentration of IL-4 produced by the cultures. This article suggests that demethylation of regulatory regions of genes involved in Th2 differentiation is a possible mechanism behind allergic asthma.

Wei, G., Wei, L., Zhu, J.,… Zhao, K. (2009). Global mapping of H3K4me3 and H3K27me3 reveals specificity and plasticity in lineage fate determination of differentiating CD4+ T cells. *Immunity, 30*, 155-167. doi: 10.1016/j.immuni.2008.12.009.

As cells progress further down a lineage pathway, their transcriptome profiles become distinct from other cells derived from the same precursor cell. CD4+ T lymphocytes differentiate into several T cell subsets; it's possible that the distinct transcriptome profiles each T cell subset expresses is due to heritable epigenetic mechanisms. Mapping H3K4me3 and H3K27me3 in different T cell subsets revealed that signature cytokine genes (Th2 – IL-4, Th17 – IL-17) showed certain methylation/demethylation patterns in only the specific T cell subsets they are associated with, and not any other subset. For example, the regulatory region of the IL-4 gene showed distinct methylation patterns in Th2 cells which were not present in Th1 cells. This suggests that epigenetics has a role to play in T cell differentiation, and by extension, asthma.

HOW DOES THE QUESTION FIT INTO THE BROADER PICTURE, AND WHAT IS ITS IMPACT?

Research has indicated that there are a lot of genes and mechanisms that contribute to asthma. The complex interactions between multiple components makes it difficult to pinpoint one therapeutic solution that will address every problem with asthma. It's easier to break asthma into several smaller problems and tackle them individually. One of these smaller problems is the rapid airway epithelial inflammation observed in asthma patients. This is thought to be driven by too much Th2 cell differentiation as compared to non-asthmatic patients. My project would seek to address why too much Th2 cell differentiation occurs and may possibly lead to a therapeutic solution. It can go a long way into developing drugs that are able to treat asthma at its root rather than just treating the symptoms.

POTENTIAL WAYS TO MAKE YOUR QUESTION KNOWN TO THE PUBLIC AT LARGE (OR TO YOUR NON-BIOLOGIST FAMILY AND FRIENDS):

Social media has really changed how we reach out to globally; promoting this research question online on social media sites or forums like facebook or reddit can provide some attention from the general public. It would be nice if this research question was also promoted on television or even scientific journals to really generate attention. The only way to reach out to close family and friends would be to talk to them and try to explain why it is so important to address this question scientifically.

ANY OTHER PARTS OF THE PROJECT COMPLETED SO FAR:

As far as I am aware, only the methylation/demethylation pattern of IL-4 has been looked at in terms of Th2 differentiation. Other cytokines and transcription factors are also involved and the methylation/demethylation patterns regarding these genes hasn't been fully explored. I believe that needs to be done fully to tie this story together.

ANYTHING YOU WOULD LIKE SPECIFIC FEEDBACK ON:

I am not sure whether my experimental proposals are viable, makes logical sense, or even the best possible experiments to propose for my specific question. I would like to know if I'm heading down the right direction for this matter.