Signs of molecular arms races in immunity genes

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Introduction

Asthma is an autoimmune disease characterized by the inflammation of airway walls and tightening of the smooth muscles around the airway in response to factors such as allergens. This process is diagrammed below. A person's genetics can also affect their chances of developing asthma.

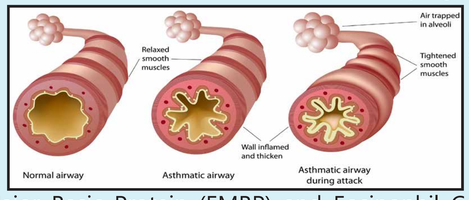


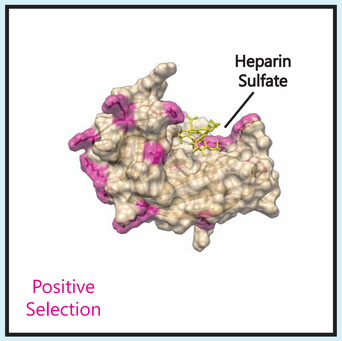
Photo Courtesy of NIH

Eosinophil Major Basic Protein (EMBP) and Eosinophil Cationic Protein (ECP) are found in vacuoles inside eosinophils, a type of white blood cell. They are both important in the innate immune system as a first line of defense against multicellular parasites and pathogenic bacteria. However, scientists have repeatedly linked EMBP and ECP to the pathogenesis of asthma. Because other asthma linked genes are in a molecular arms race with pathogens, we began to wonder whether these two proteins are rapidly evolving in humans and other primates.

Major Questions

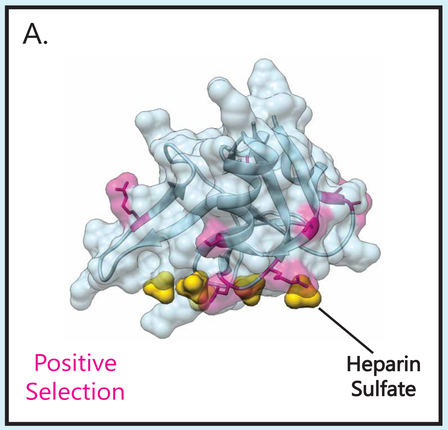
1. Are EMBP and ECP in molecular arms races?
2. Which amino acid residues are under selection?
3. Do amino acid changes in certain sites have a significant effect on protein activity?

ECP Shows Strong Signs of Positive Selection



Crystal structure of ECP bound to heparin sulfate. Pink regions indicate amino acid positions under positive seleciton. I have included heparin sulfate in the figure because researchers have hypothesized that both ECP and EMBP bind to pathogens through surface sugars such as heparin. A number of the sites under selection are near the heparin binding site.

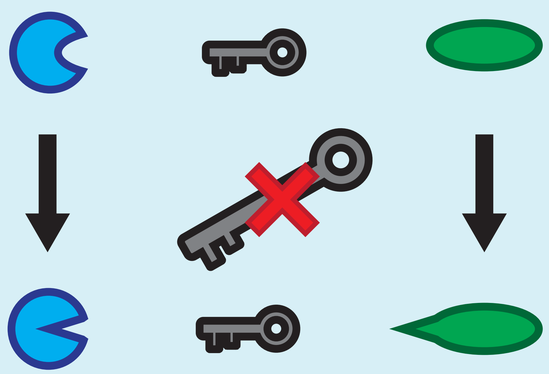
EMBP is Rapidly Evolving in Primates



Crystal structure of EMBP bound to heparin suflate. Pink regions on the protein indicate amino acid positions under positive selection. Similar to ECP, a number of these regions are near the heparin binding site, implying they may be rapidly evolving in response to pathogens.

Molecular Arms Races

In evolutionary biology, coevolution is the process by which two species evolve in response to each other. For pathogens and hosts, this process takes the form of antagonistic coevolution or "molecular arms races" wherein selective pressures modify the pathogen's proteins to better avoid detection by the host and vice versa.



Positive selection is a useful gauge of molecular arms races. We can determine if a gene is under positive selection by examining amino acid substitution rates in the gene.

EMBP Purification

We have encountered considerably difficulty while trying to answer our third question: Do amino acid changes in certain sites have a signficant effect on protein activity?

We successfully transformed EMBP orthologs from a number of different primate species into bacterial expression vectors which add a GST tag to the protein. However, we have found that expressing EMBP in E. coli largely results in the protein being packed into inclusion bodies. We can purify the protein in this form, but the resultant protein is inactive, and unusable for killing assays.

Going forward, we are exploring expressing EMBP in CHO cells, which other labs have done successfullly in the past.

Killing Assays

Once we have successfully purified functional EMBP, we will test its toxicity by subjecting mammalian cells and bacteria to each species's ortholog of EMBP, we can determine the relative toxicity of each protein. Combined with amino acid sequence data, we can determine what specific amino acid changes significantly impact protein function.

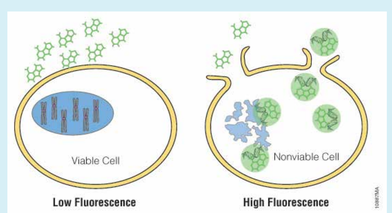
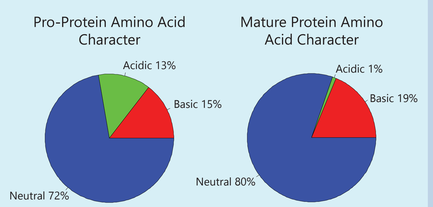


Photo courtesy of promega.com

Additionally, we also plan to use killing assays to answer an unresolved question pertaining to EMBP. Initially, EMBP is expressed as a 222 amino acid pro-protein. The highly acidic "pro domain" is thought to neutralize the basic nature of EMBP in order to protect the eosinophil during protein packaging, however, this has never been proven. Using killing assays, we can compare the toxicity of the pro-protein to that of the mature protein, potentially lending support to this hypothesis.



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