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Evolution of the Immune System

Immunoglobulin A1 (IgA1) Protease as a Tool for Infection in Humans and Chimpanzees

Introduction

The mucous membranes of the respiratory system in humans are a site for infection by various types of microbes such as *Neisseria meningitides*, *Neisseria gonorrhoeae*, *Streptococcus pneumoniae* and *Haemophilus influenzae*. These four species of bacteria have long plagued humans and been behind many deadly epidemics [1]. Once they get into respiratory system, they attach to the epithelial cells lining the mucous membranes and enter the body. The bacteria produce polysaccharides that coat them in a protective capsule. To counter the infection, the human body develops antibodies against the bacteria [1]. The major class of antibodies on the mucous membranes is immunoglobulin A (IgA). It is believed that after coating the polysaccharide capsule with IgA the bacteria are no longer capable of binding to epithelial cells, which ultimately decreases their ability to infect [1].

Immunoglobulin A has two different forms: IgA1 and IgA2 [2], IgA2 has a deletion of proline residue in the hinge region [1]. Both immunoglobulins function in the same way by binding to the polysaccharide capsule of bacteria. They are produced in plasma cells that underlie the mucosal epithelium, exported first into the epithelium cells and then exocytosed onto the outer surface of the cells [1]. Yet the difference in composition between the two immunoglobulins turns out to have important consequences.

In what could be described as an evolutionary game of one-upmanship, bacteria developed a way to counter the effects of IgA1 by producing a protease that specifically cleaves IgA1. Yet the struggle is far from over. The bacteria actually can use the cleaved form of IgA1 to increase their ability to infect the epithelial cells. The binding of the IgA1 to the capsular polysaccharide (PnPS) that coats the bacteria stimulates the release of the IgA1 proteases from the bacteria [2]. The proteases target the IgA1 molecules that are bound to the capsule and cleave it at the hinge region. The Fab fragment remains attached to the capsule while the cleaved constant region floats off [3] (See Fig. 1). The binding of the bacteria with antibodies disrupts the integrity of the capsule since the Fab fragments have a residual cationic charge neutralizing the negative charge of the capsule, causing an opening at that location [3]. The absence of the capsule exposes a phosphocholine ligand (ChoP) on the membrane surface of the bacteria [4]. The ChoP will subsequently bind to the platelet activating receptor (rPAF) on surface of the host epithelial cells [2]. When this bond is formed, the bacteria become securely attached to the epithelial cells and their ability to infect is greatly enhanced.

The discovery that *Streptococcus pneumoniae* uses the antibodies of host organisms to enhance its infectivity is the first report where bacteria exploit antibodies to work against the immune system [2]. Yet evolution has favored the side of humanity by the introduction of IgA2 that is resistant to the IgA1 protease because of the lack of proline in the hinge region [1]. Although IgA1 is produced in greater quantities, IgA2 production will be unregulated when bacteria start cleaving IgA1 and infecting the epithelial cells [3]. Eventually an IgA2-specific protease may evolve in bacteria. This game of evolutionary one-upmanship, co-evolution, and what could be called counter-evolution may be more common in human-bacteria relationships than previously thought.

A second molecule of interest is human lactoferrin, which is produced in breast milk. When the IgA1 protease precursor is exposed to lactoferrin, it is proteolytically cleaved into an inactive form [5]. A second function of lactoferrin is to cleave Hap, the adhesin that microorganisms use to adhere to host epithelial cells [5]. These observations suggest that humans have long been dealing with the IgA1 protease and possible homologues.

IgA1 proteases produced by the bacteria target not only human IgA1 [3] but also gorillas and chimpanzees [6] and rats [7]. However, the equivalent to human IgA1 in Rhesus monkeys is resistant to proteolytic cleavage by the IgA1 protease produced by bacteria [6]. Author's explanation for this occurrence is that the sugar composition in Rhesus monkey IgA1 is not recognized by the protease. It is unknown if the Rhesus monkey evolved this different sugar composition of IgA1 specifically to counter infection.

I propose to design an experiment that will look for analogues of IgA1 protease-aided infection in other mammals.

Experimental Design

The initial step would be to consult genetic databases to look for similarities between IgA in humans and chimpanzees. Although only the sequence of the constant region is needed for such comparisons, it might be interesting to compare the variable regions for homologies. Similarities, especially in the hinge regions that are cleaved by the IgA1 protease, would suggest an analogy between humans and monkeys. These comparisons could be expanded to include other species, depending on the available genetic sequences.

The second major step in the study would be to determine whether female chimpanzees produce lactoferrin. It is extremely likely that they do because of the usefulness of lactoferrin in developing infants [1]. Collecting milk from females that have recently given birth, to ensure that protein production is at a maximum level, can be done based upon the procedure outlined by Qui in [8]. A culture of *H. influenzae* in exponential growth phase would be exposed to the lactoferrin and incubated 37° C for an hour.

Following the described procedure [5], a Western blot can be used to visualize cleavage of the IgA1 protease precursor by the lactoferrin. Controls are vital to ensure that there are no unknown factors that lead to protease degradation. If cleavage of the precursor occurred, this indicates that like humans, chimpanzees have a vulnerability to the IgA1 protease and developed lactoferrin as a way to combat this protease.

Evidence for the cleavage of the IgA1 protease precursor by lactoferrin does not conclusively determine if the protease has the same role in chimpanzee infection as it does in humans. A second experiment must be undertaken to determine any physiological similarities. Other researches used human B cells after subjects received a pneumococcal vaccine to generate IgA [3]. The established procedure requires the use of an ELISA assay using labeled IgA and exposing it to the IgA1 protease [9]. Because Weiser et al. [3] only recently discovered how the IgA1 protease cleaved bound IgA1, his protocol, especially the controls, should be used initially to investigate the role of IgA in chimpanzees. It is unknown if a chimpanzee equivalent of IgA2 exists, although if it does, it must be removed from IgA1 sample to ensure the quality of the data.

After purified IgA is available, the next step is to determine whether it influences the ability of *S. pneumoniae* to infect epithelial cells. The control would be bacteria without the IgA1 protease. It is difficult to measure infectivity *in vivo* because there are many other factors that could come into play. Fortunately there is a relatively easy solution. As previously mentioned, the bacteria's infectivity increases because it can use ChoP to bind to another receptor on epithelial cells. It can be rationalized that if adding IgA1 to the bacteria increases its ability to bind beads in a column that present the rPAF or homologous receptor, its infectivity inside the body would correspondingly increase. By repeatedly washing the columns with increasing levels of an ionic solution, it is possible to determine the strength of the bonds between ChoP and rPAF. The effects of IgA1 from chimpanzees will be compared against that of human IgA1. If this procedure for measuring changes in infectivity does not work, the more-complicated procedure outline in [3] would be more than adequate. No matter the case, years of study are needed to gain knowledge and insight into the interaction between bacteria and Immunoglobulin A.

References

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