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Fcgbp – A Potential Viral Trap in RV144

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Abstract: Years of extensive research have yielded much knowledge in many aspects of HIV-1 infection, treatments, and education. However, without a vaccine, the number of people infected worldwide continues to grow. The partial success of the Thai RV144 vaccine trial provides hope that a method of protection is indeed possible. Understanding the mechanism behind the protection is critical if we hope to achieve our goal of inhibiting new infections of HIV-1. We hypothesize that the Fc of IgG binding protein (Fcgbp) is associated with the protection observed in the RV144 vaccine trial. It has the ability to trap viral-antibody complexes in the mucosa by binding the Fc of IgG to Fcgbp. This property could be used in the form of a microbicide containing antibodies to a variety of HIV-1 epitopes to prevent sexual transmission of HIV-1. The aim of this paper is to stimulate further research into Fcgbp and its role in innate immunity.

Keywords: Fcgbp, HIV-1, mechanism, microbicide, vaccine.

INTRODUCTION

After decades of intense global effort to stop the spread of HIV-1, the number of people infected worldwide continues to grow. Although progress has been made in drug therapies, education, and social supports, a vaccine remains elusive. It may not be possible to develop a vaccine to elicit broadly neutralizing antibodies to the HIV-1 virus in a timely manner. The very cells selected by HIV-1 for infection are involved in the process of acquired immunity. Infected antigen presenting cells travel to the lymph nodes to engage B cells and trigger humoral immune responses while simultaneously allowing dissemination of the virus. Therefore, it is imperative to stop infection at mucosal barriers to reduce, and hopefully one day to eliminate, the scourge of HIV-1. The partial success of the Thai RV144 vaccine trial suggests that the ability of antibody to bind to the virus, without actually having the capacity to neutralize it, may offer some temporary protection [1]. We hypothesize that the Fc fragment of IgG binding protein's (Fcgbp) ability to trap HIV-1-antibody complexes at mucosal surfaces is involved in the mechanism of efficacy observed in the RV144 vaccine trial. Together with mucins, it functions as a protective barrier overlaying the epithelia of the cervix and complete digestive tract [2]. In the RV144 vaccine trial, the production of IgG antibodies with the ability to bind HIV-1 may have enhanced the protective capacity of these mucosal barriers by trapping the antibody-HIV-1 complex through binding of the IgG Fc domain to Fcgbp.

FCGBP

Fcgbp was first isolated from intestinal mucosa by Harada *et al*. [3]. Examination of its amino acid sequence and its tissue distribution led them to conclude that it "is an important component of mucosal immunological defences" [2]. It is a large protein (>500kDa) which resides in the mucosa of endodermal derived tissue, including the complete digestive tract and cervix [2, 4]. Fcgbp is composed of many repeated domains, including thirteen Von Willebrand factor D domains, and twelve each of cysteine rich (Cys-rich) and trypsin inhibitor-like domains. At the gene level, the size and repetition of corresponding nucleotide sequences comprising these domains would likely make this region of the genome highly susceptible to meiotic mis-alignment events resulting in copy number variants. The cys-rich domains permit the formation of many disulfide bridges with like molecules, plausibly resulting in a net-like scaffold within the mucosal barrier. The fact that Fcgbp has been identified as a protein which is down-regulated or lost in many cancers [5-7] suggests that it may be important in the maintenance of homeostasis.

CLUES FROM THE CERVICAL MUCOSA

We know that normal cervical and colonic mucosa is fairly efficient at limiting viral entrance because most sexually transmitted HIV-1 infections are caused by a single virus particle [8]. The RV144 vaccine efficacy was reported to be greater for female subjects (38.6%) than for males (25.8%) [1]. There are a number of factors which can account for this difference. Fcgbp is known to be an estrogen-responsive gene [9]. Therefore, women may naturally express more Fcgbp protein than men. If Fcgbp is indeed protective in cervical and rectal tissues, greater concentrations of the protein would presumably provide a superior barrier against male to female sexual transmission of HIV-1. Secondly, the difference in efficacy could reflect differences in the vulnerability of cervicovaginal and rectal tissues. Although Fcgbp is present in both environments, there is greater potential for tissue damage in rectal tissue and therefore greater risk of infection. The transmission probability per exposure event from HIV-1 infected semen *via* the female genital tract is estimated to be 1:200 – 1:2,000 whereas the estimate of transmission probability increases to

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1:20 -1:300 per exposure event from HIV-1 infected semen *via* the rectum. Female to male sexual transmission of HIV-1 *via* cervicovaginal and rectal secretions carries a lower risk with transmission probability per exposure event reported to be 1:700 – 1:3,000. This risk can be reduced by 60% if the male partner is circumcised [8]. The following evidence comes from studies of the cervical mucosa but may be applicable to the rectal mucosa as well.

There is a group of HIV-exposed, seronegative (HESN) women in Kenya that have been studied for many years for clues to the mechanism of their resistance to HIV-1 [10]. It is believed that the answer lies within their cervicovaginal mucosa and their innate immune system [11]. Compared to controls, these HESN women are described as having a "quiescent" innate immune phenotype [12]. It is unclear whether this can be attributed to some genetic factor(s) or if it is a sign that their innate immune systems have not been challenged. In fact, it could be both. Copy number variants for the Fcgbp gene have been reported in sub-Saharan populations [13]. If the Kenyan cohort of HESN women expresses a copy number variant of Fcgbp, it could explain their resistance to HIV-1 infection. A copy number variant of Fcgbp could result in the translation of a larger protein, or more protein, which in turn could provide a superior shield from pathogens. This would result in less frequent challenge to the innate immune system which could be perceived as a quiescent phenotype. Greater expression of proteins that are encoded by linked genes may be evidence for a copy number variant. The second most significantly up-regulated gene in this cohort identified by microarray analysis on blood samples, compared to HIV negative controls is ZNF 146 (p=1.17E-07) [14]. It maps to chromosome 19q13.1, the same region as the Fcgbp gene. Additionally, the genes for 4 out of 34 proteins identified by mass spectrometry as being abundantly over-expressed in the cervicovaginal mucosa of these HIV-1-resistant sex workers map to 19q13.1 – 19q13.2 [15].

Another observation to support the existence of a protective network within the cervical mucosa comes from a study of SIV transmission in rhesus macaques. Non-human primate studies have been validated as useful models to study mucosal immune events in SIV infection because symptoms and outcomes parallel what has been observed for HIV-1 infection in humans [16,17] It was discovered that "systemic or intravaginal treatment with estrogen efficiently protects female rhesus macaques against the transmission of SIV, likely by enhancing the natural protective properties of the lower genital tract mucosal tissue" [18]. Estrogen is known to enhance expression of Fcgbp [9].

The Thai RV144 vaccine trial has been viewed as a partial success because it was able to provide those receiving the vaccine with almost one third greater protection over that of the control group [1]. We know that Fcgbp is part of a net-like scaffold within the mucosa of the cervix and colon, and that it is an important component of the innate immune system with the ability to bind the Fc of IgG antibodies. Examination of the data collected from the Thai RV144 vaccine trial can be interpreted as support for Fcgbp involvement. The IgG antibodies secreted into the mucosal environment can catch HIV-1 with their ligand binding domain and anchor their Fc domain to Fcgbp, preventing the virus particles from infecting the tissue beneath this barrier. This was plausibly due to "protective, non-neutralizing antibody" (pnnAb) production, a term applied to antibodies which exert their protective effect through their Fc domain [19]. This is usually accomplished *via* Fc receptors on cells of the innate immune system. In this instance the protective effect is also through the Fc domain. However, we propose that it is accomplished *via* attachment to Fcgbp. The antibody's ability to tag the virus was enough to offer some protection. The effectiveness of this mechanism would likely reflect the avidity of antibody – antigen binding; greater avidity would result in better protection. Avidity of antibody binding to antigen is an important factor in effective pnnAb function [19]. Furthermore, it has been shown that avidity of IgG to SIV correlates with protection [20]. In his **Nature Medicine** article "*To neutralize or not, a key HIV vaccine question*", Thomas Hope suggests several mechanisms by which a pnnAb may be able to inhibit viral infection [21]. He theorizes that one mechanism of pnnAb protection may be that pnnAb-viral complexes are trapped by the mucosal barrier. The ability of Fcgbp to bind the Fc fragment of IgG would prevent viral particles that are bound to IgG antibodies from reaching the epithelial tissue, essentially trapping them in the mucosa. These IgG antibodies need not have the capacity to neutralize the virus. It would be sufficient to tag the viral particle.

IgA production is an important component of mucosal innate immunity and therefore may provide additional clues for protective mechanisms. Data from the Thai trial suggests that protection correlated inversely with monomeric IgA in the serum [22]. It is assumed that this correlation is true for dimeric SIgA and HIV-1 infection rates at mucosal surfaces, although lavage samples were not collected at these sites. It has been proposed that SIgA to HIV-1 interferes with effector functions of IgG [23, 24]. Another explanation for this inverse relationship is that it is a consequence of the IgG response to HIV-1. A robust, effective IgG response would prevent virions from reaching the epithelium to elicit a sustained SIgA response by tethering the virus-IgG complex to Fcgbp. An inadequate or ineffective IgG response would allow virions to reach the epithelium where a SIgA response is elicited. Furthermore, it is known that SIgA is able to transport bound antigen across intestinal epithelium to dendritic cells in the gut-associated lymphoid tissue, increasing the possibility of infection [25]. This could explain why higher monomeric IgA antibodies in the serum correlated with risk of infection in the Thai RV144 vaccine trial.

Why did the RV144 vaccine provide limited protection when previous vaccines showed no efficacy at all? There were some unique features in the design of the RV144 trial that may account for its partial success. It was the first to employ a prime/boost regimen and the canarypox vector it used expressed Env, Gag, and Pol viral proteins [26]. None of the previous trials included Env as an immunogen. IgG directed to the V1V2 regions of the Env significantly correlated with protection [22,24]. Antibodies produced by previous vaccine trials may not have had adequate immunogen binding avidity to trap virions in the mucosal barrier. The Fc region of IgGs would still be able to bind to Fcgbp but they would not demonstrate any benefit to the vaccine recipient. Also, most of the vaccine recipients in the

RV144 trial were low-risk heterosexuals and vaccine efficacy decreased with increased risk [27]. Still, the reported 31.2% efficacy might have been masked if not for the large number of participants in this trial [1].

FUTURE DIRECTIONS

Despite the progress made in the identification of broadly neutralizing antibodies (Bnabs) to HIV-1, attempts to design a vaccine to elicit these Bnabs have not been successful. An alternative to developing a vaccine that can elicit Bnabs is to exploit the interaction of Fcgbp with the Fc region of IgG to inhibit infection by trapping HIV-1 at the mucosal barrier. A microbicide containing antibody to HIV-1 could help reduce sexual transmission of the virus. Presumably, adequate antibody-antigen binding avidity would be the most critical feature required. Perhaps the addition of low levels of estrogen to such a product would enhance Fcgbp expression, providing extra protection.

The function of Fcgbp is one of protection. Once a person becomes infected, they can be given highly active antiretroviral therapy, or HAART, to prevent progression of infection to AIDS, but they cannot yet be cured. This fact emphasizes the importance of coming up with novel strategies to reduce the number of new infections. A microbicide containing antibodies to HIV-1 is, perhaps, less complicated to manufacture and test compared to vaccine studies and therefore worthy of consideration.

CONCLUSION

Although there is obviously something within the mucosal barriers of the cervix and rectum preventing the majority of HIV-1 from reaching the epithelium, the extensive search has not yielded anything that could be responsible for the observed bottleneck. The size and structure of the Fcgbp protein makes it an attractive candidate. It likely forms a net-like structure within the mucosa which the virus must pass through. Whether or not it is able to hinder movement of free virus particles is not known. However, the ability to trap IgG antibody-HIV-1 complexes is a possible function of Fcgbp that requires further exploration.

The possible involvement of Fcgbp in the partial success of the Thai RV144 vaccine trial is intriguing. Further investigation is necessary before we can confirm how the vaccine influences HIV-1 infection. Knowledge gained from these studies could unveil new strategies to prevent sexual transmission of the virus. Mechanistic studies to understand the interaction of the Fc portion of IgGs and the Fcgbp protein are warranted.

CONFLICT OF INTEREST

The author confirms that this article content has no conflict of interest.

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