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PERSPECTIVE

Viruses and the origin of microbiome selection and immunity

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The last common metazoan ancestor (LCMA) emerged over half a billion years ago. These complex metazoans provided newly available niche space for viruses and microbes. Modern day contemporaries, such as cnidarians, suggest that the LCMA consisted of two cell layers: a basal endoderm and a mucus-secreting ectoderm, which formed a surface mucus layer (SML). Here we propose a model for the origin of metazoan immunity based on external and internal microbial selection mechanisms. In this model, the SML concentrated bacteria and their associated viruses (phage) through physical dynamics (that is, the slower flow fields near a diffusive boundary layer), which selected for mucin-binding capabilities. The concentration of phage within the SML provided the LCMA with an external microbial selective described by the bacteriophage adherence to mucus (BAM) model. In the BAM model, phage adhere to mucus protecting the metazoan host against invading, potentially pathogenic bacteria. The same fluid dynamics that concentrated phage and bacteria in the SML also concentrated eukaryotic viruses. As eukaryotic viruses competed for host intracellular niche space, those viruses that provided the LCMA with immune protection were maintained. If a resident virus became pathogenic or if a non-beneficial infection occurred, we propose that tumor necrosis factor (TNF)-mediated programmed cell death, as well as other apoptosis mechanisms, were utilized to remove virally infected cells. The ubiquity of the mucosal environment across metazoan phyla suggest that both BAM and TNF-induced apoptosis emerged during the Precambrian era and continue to drive the evolution of metazoan immunity.
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Introduction

Microbial selectives

Most immune components have been discovered within the context of pathogenesis (Tanji and Ip, 2005; Kawai and Akira, 2010). This emphasis has led to the implicit assumption that immunology is the study of host versus pathogen (Casadevall and Pirofski, 2014). This two-dimensional bias is exemplified through the pervasive use of antimicrobial when describing host–microbe interactions (Brogden, 2005; Casadevall and Pirofski, 2014). In nature, host–pathogen interactions occur within the context of an ecological community, that is, a host in symbiosis with its microbial partners, which is called the holobiont (Casadevall and Pirofski, 2014; Bordenstein and Theis, 2015). These symbioses run the gamut of mutualistic to parasitic/pathogenic. Niche exclusion is an essential dynamic for maintaining the holobiont; any microbe, compound or entity that removes a microbe from a particular ecosystem creates novel niche space for another microbe to occupy (Rodriguez-Brito *et al.*, 2010). In this perspective, we will utilize the term microbial selective to describe mechanisms that maintain specific microbes associated with a metazoan host.

The ever-changing, ubiquitous surface mucus layer (SML)

Mucosal environments coat the surfaces of specific epithelial cell types across the spectrum of metazoan life (Bäckhed *et al.*, 2005; Brown and Bythell, 2005). These environments are constructed by mucin macromolecules, which consist of a peptide backbone covalently bonded to variable oligosaccaride side chains (Ferez-Vilar and Hill, 1999; Hang and Bertozzi, 2005; Corfield, 2013). The process of glycosylation is controlled by secondary structural motifs (Julenius *et al.*, 2005), the cellular repertoire

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of glycosyltransferases and their localization within the Golgi apparatus resulting in distinct cellular profiles (Hanisch, 2001). *O*-linked glycosylation has been shown to be integral in immune protection across the animal phyla (Tsuboi and Fukuda, 2001; Bond *et al.*, 2014). Following posttranslational modification, mucins are either tethered to the epithelial cell surface or secreted into the surrounding environment forming the SML. Molecular interactions between mucin molecules via hydrophobic cysteine-rich domains (Silberberg and Meyer, 1982; Bansil *et al.*, 1995) and the formation of disulfide bonds (Roberts, 1976) results in a viscoeleastic material that provides the host with a physiochemical barrier from the surrounding environment (Gendler and Spicer, 1995; Johansson *et al.*, 2013). In addition to providing the host with protection, the SML also concentrates particles from the environment by providing a smooth layer that encourages laminar (versus turbulent) flow, thereby creating an effective particle trap (Wild *et al.*, 2004; Yang *et al.*, 2012; Hill *et al.*, 2014). There is significant turnover of the SML; the mouse gastrointestinal tract is capable of replacing its entire mucin pool in a single day (Faure *et al.*, 2002) and corals release up to 4.8 l of mucus per square meter of reef per day (Wild *et al.*, 2004).

Microbes and viruses in the SML

Despite the high turnover, the SML is inhabited by a diverse and stable assemblage of microbes and their associated viruses, forming the SML microbiome (Bäckhed *et al.*, 2005; Lozupone *et al.*, 2012; Schluter and Foster, 2012; Closek *et al.*, 2014). Individual members of the SML microbiome gain access to energy-rich mucins (Derrien *et al.*, 2010) while providing the metazoan host with a variety of benefits, including immune protection (Cash and Hooper, 2005; Sun and Chang, 2014) and nutrient production (Thompson *et al.*, 2015). Here we focus on SML-associated bacteria and their predators, bacteriophage (a.k.a. phage). To ensure retention within the SML, bacteria and phage have evolved mucus-binding proteins capable of responding to rapid environmental change. For example, *Lactobacillus* sp. express a range of proteins containing mucus-binding domains that exhibit high genetic heterogeneity between strains, suggesting they are adaptive (MacKenzie *et al.*, 2010). Similarly, T4 phage use the immunoglobulin-like (Ig-like) domains of their capsid proteins to promote mucus adherence. (Fraser *et al.*, 2006; Barr *et al.*, 2013). Ig-like domains and related protein folds, such as C-type lectins, contain variable regions, potentially allowing phage to adapt to changes in the mucin pool and maintain specific phage–metazoan associations (Minot *et al.*, 2012; Barr *et al.*, 2013).

Phage drive bacterial evolution

Within the SML, phage outnumber their bacterial hosts by roughly an order of magnitude (Barr *et al.*, 2013). Upon infection, phage replicate via either lytic or lysogenic life cycles. The lytic cycle involves the production of new virus particles, ultimately leading to cell lysis and viral release. Alternatively, in the lysogenic cycle the phage genome integrates into the host genome and becomes a prophage. Temperate phage utilize both lytic and lysogenic strategies and are important drivers of evolution in the SML (De Paepe *et al.*, 2016). Depending on the genetic repertoire of the newly acquired prophage, bacterial physiology can be directly affected through the donation of novel genes, disruption of host genes and manipulation of cellular metabolism (Brüssow *et al.*, 2004). In addition, some temperate phage provide their host with immune protection by preventing the attachment of other phage particles (superinfection exclusion) (Soller and Epstein, 1965) or preventing phage propagation of a secondary infection (superinfection immunity) (West and Scott, 1977; Fogg *et al.*, 2010; Abedon, 2015). These mutualistic temperate phage enhance the competitive fitness of their hosts (Bossi *et al.*, 2003) and drive bacterial evolution (Obeng *et al.*, 2016).

Discussion

Colonization of the SML in the last common metazoan ancestor (LCMA)

Fossil evidence and molecular data suggest the LCMA emerged sometime between the Cryogenian and Ediacaran periods approximately 542–720 million years ago (Davidson and Erwin, 2009). The LCMA most likely consisted of two cell layers: an ectoderm with a SML and an internally facing endoderm (Müller, 2003; Lang *et al.*, 2007). We propose that the first bacteria arrived to the SML through active chemotaxis toward energy-rich mucins (Bansil *et al.*, 1995; Stocker and Seymour, 2012), random sequestration by SML fluid dynamics (Wild *et al.*, 2004; Yang *et al.*, 2012; Hill *et al.*, 2014) or both. The dynamic properties of the SML would have selected for bacteria that could be maintained through the expression of mucus-binding proteins or similar mucus-binding mechanisms (MacKenzie *et al.*, 2010). Once established within the SML, bacteria that provided the metazoan host with a fitness advantage via competitive exclusion of potential pathogens or nutrient production would have been further selected. The arrival of the first phage may have occurred in conjunction with the first bacteria as an integrated prophage or from the environment as a temperate/lytic phage. Prophage associated with the first bacterial colonizers increased host fitness through superinfection exclusion and superinfection immunity mechanisms (Soller and Epstein, 1965; Abedon, 2015). As the first bacterial species continued to propogate, phage capable of binding to mucins (for example, Ig-like domains, among others) were favored by natural selection. Colonization by additional bacterial

species and their associated phage continued in the SML until niche space was filled and a community was formed. Mutualistic bacteria continued to protect the metazoan host through competitive exclusion of potential pathogens (Hibbing *et al.*, 2010) while phage provided immune protection as proposed in the bacteriophage adherence to mucus (BAM) model (Barr *et al.*, 2013, 2015). In the BAM model, phage adhering to mucus provide the host with immune protection against invading pathogens (Figure 1a). For an in depth discussion of where competitive exclusion and lytic dynamics are operating within the SML, the authors point the reader to the following article (Silveira and Rohwer, 2016).

Tumor necrosis factor (TNF)-induced apoptosis—an internal microbial selective

Although the colonization of the SML by specific phage species provided the metazoan host with immune protection, the same fluid dynamics also increased the retention of eukaryotic viruses and subsequent adsorption to the LCMA host. As with

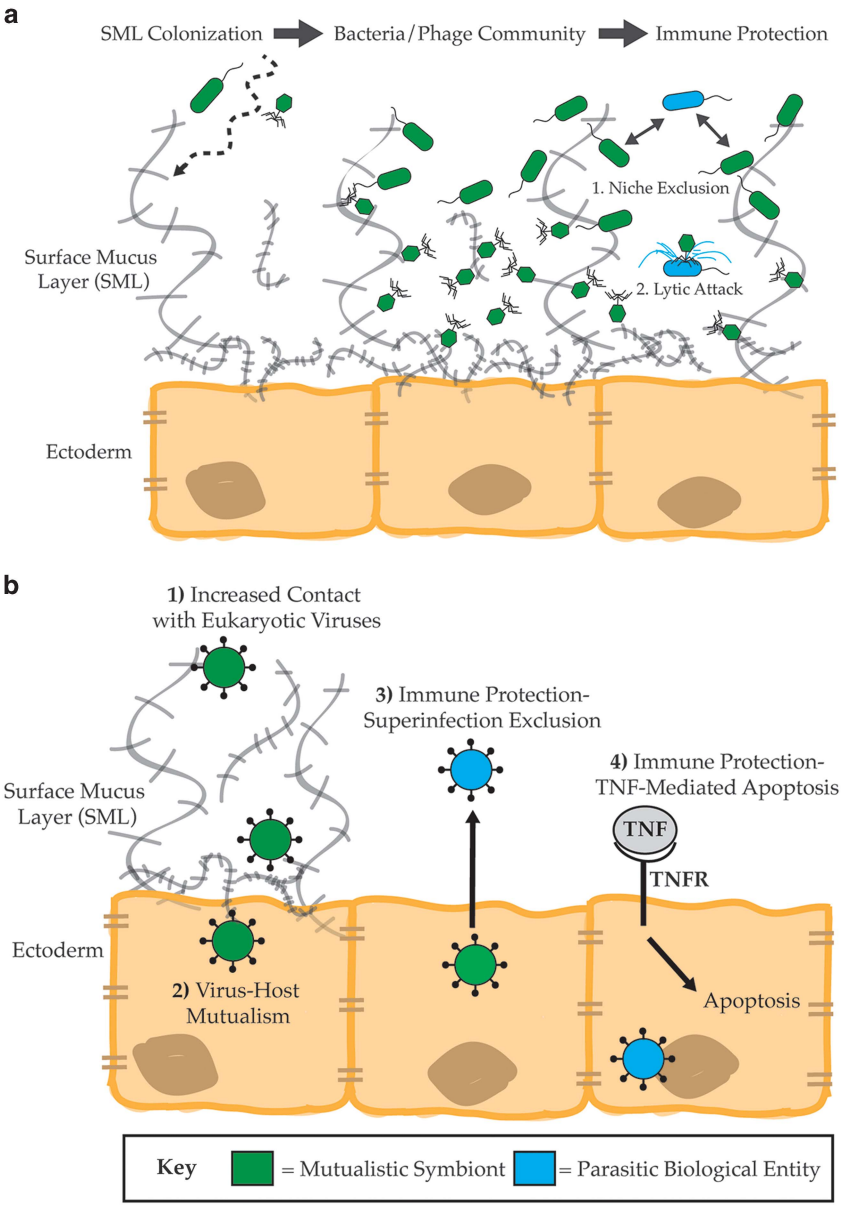

Figure 1 Microbial selective mechanisms in the LCMA. (**a**) BAM. Energy-rich mucin macromolecules secreted from the ectoderm formed a SML that was colonized by bacteria and associated phage. Mutualistic phage provided the LCMA with immune protection from invading bacteria via (1) competitive exclusion and (2) lytic attack. (**b**) TNF-mediated apoptosis. Mucins also increased the rate of contact between eukaryotic viruses and their metazoan hosts leading to the formation of mutualistic relationships. Viruses that provided the LCMA host with immune protection were maintained. If a beneficial virus became pathogenic or if a parasitic virus invaded, the infected cell was removed via TNF-mediated apoptosis and other apoptotic mechanisms.

the phage, these eukaryotic viruses developed mechanisms to bind to mucins and infect host cells (for example, influenza virus today; Wild *et al.*, 2004). Upon infection of a multicellular host, the canonical response is programmed cell death or apoptosis of the infected cell, thus preventing viral dissemination to neighboring cells (Barber, 2001). Apoptosis has been observed across the spectrum of life from bacteria to animals (Lewis, 2000; Bidle *et al.*, 2007). Many versions of apoptosis exist (Holler *et al.*, 2000; Berg *et al.*, 2001; Bratosin *et al.*, 2001) and the general process likely emerged with the origin of multicellularity (Ellis and Horvitz, 1986; Raff, 1992; Steller, 1995; Aravind *et al.*, 2001). However, metazoan apoptosis appears to be unique through its use of TNF receptors (Quistad and Traylor-Knowles, 2016), which are activated by TNF ligands (Aggarwal, 2003). Many of the domains involved with apoptotic signaling via TNF receptors are also present and functional in cnidarians, considered to be among the oldest animal phyla (Lasi *et al.*, 2010; Quistad *et al.*, 2014; Sakamaki *et al.*, 2014, 2015; Lu *et al.*, 2016; Moya *et al.*, 2016).

The targeted destruction of a virally infected cell is the most conservative approach to maintain organismal integrity; however, viruses can also provide the host with a selective advantage. For example, Herpesviruses provide mice with protection from bacterial infection (Barton *et al.*, 2007) and latent dynamics with Herpesviruses and their metazoan hosts have been described from cnidarians (Vega Thurber *et al.*, 2008; Grasis *et al.*, 2014) to humans, suggesting an ancient origin (Steiner, 1996). In addition, similar to temperate phage, metazoan viruses provide their hosts with immune protection via superinfection exclusion (Tscherne *et al.*, 2007; Zou *et al.*, 2009). If the beneficial virus or virally encoded element is transferred to the germline through reverse transcription (RNA viruses) or recombination (DNA viruses), then the trait could be inherited by future generations and drive evolutionary processes (for example, endogenous retroviruses) (Grow *et al.*, 2015). Evidence for past viral co-option events can be found throughout the modern metazoan immune system (Villarreal, 2011) including the canonical response to viral infection: interferon production (Chuong *et al.*, 2016).

We propose that competition between viruses for host niche space led to the formation of mutualistic relationships between the LCMA and its resident viruses. Those associations that provided the host with immune protection were maintained. In those cases where resident viruses were not protective or they developed into a pathogenic infection, the LCMA removed the infected cell via TNF-mediated apoptosis, among other apoptotic mechanisms (Figure 1b). Based on extant animal phyla, the LCMA possessed a large and dynamic stem cell population (Bosch, 2009), therefore, it could rapidly replace any cells deemed to be a risk to organismal integrity without incurring a major fitness cost. Taken together, TNF receptors served as the viral gatekeepers to the LCMA, promoting beneficial chronic infections and eliminating destructive interactions.

Conclusions

Here we have proposed a model for the development of metazoan immunity via external (phage) and internal (TNF-mediated apoptosis) microbial selective mechanisms. The LCMA secreted mucins from epithelial tissue, generating an SML that selected for bacteria and phage with mucin-binding properties. Phage provided the LCMA with an external microbial selective in which phage bound to mucus via hypervariable domains protect the metazoan host from invading bacteria (BAM) (Barr *et al.*, 2013, 2015). In addition to attracting bacteria and phage, mucins also increased the rate of contact with eukaryotic viruses resulting in the development of mutualistic symbiosis that provided the LCMA with immune protection. If a new virus was pathogenic or if a resident virus became parasitic, those cells were eliminated via TNF-mediated apoptosis and other versions of apoptosis. We hypothesize that both microbial selective mechanisms described here evolved during the Precambrian era and continue to drive the evolution of metazoan immunity in modern day phyla.

Conflict of Interest

The authors declare no conflict of interest.

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