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Review

A new role for mucins in immunity: Insights from gastrointestinal nematode infection

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ABSTRACT

The body's mucosal surfaces are protected from pathogens and physical and chemical attack by the gel-like extracellular matrix, mucus. The framework of this barrier is provided by polymeric, gel-forming mucins. These enormous O-linked glycoproteins are synthesised, stored and secreted by goblet cells that are also the source of other protective factors. Immune regulation of goblet cells during the course of infection impacts on mucin production and properties and ultimately upon barrier function. The barrier function of mucins in protection of the host is well accepted as an important aspect of innate defence. However, it is becoming increasingly clear that mucins have a much more direct role in combating pathogens and parasites and are an important part of the coordinated immune response to infection. Of particular relevance to this review is the finding that mucins are essential anti-parasitic effector molecules. The current understanding of the roles of these multifunctional glycoproteins, and other goblet cell products, in mucosal defence against intestinal dwelling nematodes is discussed.

Contents

1. Introduction

The mucosal barrier is the dynamic first line of defence that has evolved to be responsive to environmental, physiological and immunological stimuli. For example, the intestinal tract is able to readily absorb nutrients and provide a niche for commensal bacteria without leaving the host open to attack by invading pathogenic organisms. The ability of the host to provide for such complex homeostatic requirements is aided, in large part, by the mucus layer. Mucus is a dynamic and complex network that allows the

Abbreviations: ER, endoplasmic reticulum; ECM, extracellular matrix; CK, cysteine knot; IBD, inflammatory bowel disease; IFN, interferon; Tff, trefoil factor; STP-domain, serine, threonine, proline domain; vWF, von Willebrand factor; UC, ulcerative colitis.

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diffusion of small molecules from the lumen to the surface of the epithelial cells but traps bacteria and slows the diffusion of large viruses (Lieleg et al., 2012). Moreover, as one of the first arms of innate immune defence the mucus barrier holds within it a variety of host-derived molecules, for example, immunoglobulin A (IgA) and anti-microbial peptides, that interact with and aid clearance of pathogenic organisms (Strugnell and Wijburg, 2010; Phalipon et al., 2002; Bruno et al., 2005; Iontcheva et al., 1997).

The mucus layer is dominated by carbohydrate-rich macromolecules (gel-forming mucins) that provide the molecular framework of a highly organised extracellular matrix (ECM), which prevents direct contact of particulates, toxins, pathogens and commensal flora with the epithelium (Thornton et al., 2008; McGuckin et al., 2011). Thus, the role of mucins in preventing disease has traditionally been viewed as that of a barrier. While this is undoubtedly true in part, it is now clear, that mucins have a much more direct role in combating viral, bacterial and fungal pathogens (Gururaja et al., 1999; Kawakubo et al., 2004) as well as parasites (Hasnain et al., 2011a) and can be viewed as an additional important but under-appreciated component of the immune response.

In this review we will discuss the role of the mucus barrier, and in particular the gel-forming mucins, in the protection against intestinal dwelling nematode worms and how this is regulated by the immune system. Intestinal nematode worms are a major cause of human morbidity. In 2010 it was estimated that 5.3 billion people worldwide live in areas that support the stable transmission of at least one soil-transmitted nematode. This figure includes 1 billion school-aged children, for which infection with nematodes is particularly detrimental, affecting physical and cognitive development and causing general malaise and weakness due to the malabsorption of nutrients (Pullan and Brooker, 2012). The main species of nematode responsible are the whipworm, the roundworm and the hookworm.

Gastrointestinal nematodes are large multicellular pathogens and successful expulsion of these worms from the host is reliant on CD4+ Th2 responses. The cytokines produced as part of the Th2 response, in particular IL-4, IL-9 and IL-13 control various host effector mechanisms, which aid the expulsion of these nematodes. Along with inhibiting the induction of Th1 response, in particular the production of the Th1 cytokine IFNγ; the Th2 response has been shown to increase smooth muscle hypercontractility and promote the rate of epithelial cell turnover which displaces the worm from its niche (Khan et al., 2003; Cliffe et al., 2005). Finally and most recently, there are novel and exciting data suggesting that the Th2-regulated changes in the mucosal barrier, and various components of it, are essential for expulsion of these worms (Hasnain et al., 2010, 2011a,b).

This review will provide an overview of the role of the mucus barrier and in particular, the gel-forming mucins in defence against intestinal dwelling nematodes. Understanding the intricacies of mucin structure, synthesis and gel-formation are pre-requisites to gaining a coherent picture of barrier organisation and function.

2. The mucus barrier

The intestinal epithelium is protected by a glycoconjugate-rich extracellular matrix. Directly above the epithelium is the glycocalyx, a carbohydrate-rich network made up of glycolipids and glycoproteins, anchored to the epithelial cell membrane. In addition to providing an increased surface area for absorption and enzymatic digestion of complex nutrients, the glycocalyx can prevent bacterial cells or virus particles, which may have diffused through the mucus layer, from binding to the epithelial cells. The mucus barrier, lying above the glycocalyx, varies in thickness along the length of the intestinal tract. Measurements made in rats have shown the thickness of the barrier ranges from 120 μm in the duodenum and jejunum to up to 1000 μm in the colon, with the thickness of the mucus layer positively correlating with the number of commensal bacteria found at each site (Atuma et al., 2001). In humans, barrier thickness is reduced in inflammatory bowel diseases (IBD) such as ulcerative colitis and Crohn's disease and likely results in ineffective protection of the underlying epithelium from luminal contents and bacteria (Sheng et al., 2012).

Mucus is a mixture of organic (proteins, glycoproteins, lipids and nucleic acids) and inorganic components (water and salts). The major structural components of the barrier, the gel-forming mucins, are secreted by goblet cells that are found interspersed within the epithelial monolayer. In addition to forming a physical barrier, the disulphide-linked mucin polymers act as lubricants, prevent dehydration of the epithelial surface and present specific ligands to bind pathogens. Although the full network of interactions within mucus are not yet specified, it is clear that other proteins in the secretion can interact with the mucin network (Kesimer et al., 2009; Bruno et al., 2005; Iontcheva et al., 1997). Such interactions may modulate the physical properties of the barrier and also, have the potential to localise protective molecules at the site of an infection (Vaishnava et al., 2011).

To date at least 17 mucin (MUC) genes have been identified, which are highly conserved across species. Throughout the review, MUC refers to the human gene and protein, whereas Muc refers to the mouse counterparts. Mucins have been classified into two major groups: cell surface mucins (MUC1, MUC3A, MUC3B, MUC4, MUC12, MUC13, MUC15, MUC16, MUC17 and MUC20; Thornton et al., 2008), which are a dominant glycoconjugate component of the glycocalyx; and secreted mucins that are the major macromolecular component of the secreted mucus layer (Thornton and Sheehan, 2004). Of the secreted mucins, MUC2, MUC5AC, MUC5B, MUC6 and MUC19 in the human and Muc2, Muc5ac, Muc5b, Muc6 and Muc19 in the mouse are classed as polymeric, gel-forming mucins (Escande et al., 2004; Thornton et al., 2008; Thornton and Sheehan, 2004). Two other secreted mucins, MUC7 (Muc10 in the mouse) and MUC8, are non-polymeric mucins (Thornton et al., 2008). Gel-forming mucins share a common evolutionary ancestor and, with the exception of MUC19, are encoded by genes on chromosome 11p15.5 in humans and on chromosome 7 in mice (Escande et al., 2004; Pigny et al., 1996).

The composition, properties and functions of the mucus barrier differ between epithelial tissues. Indeed, particular mucins are known to predominate at different mucosal surfaces. For example, MUC5AC and MUC7 are the major secreted mucins present in the ocular mucosa (Gipson and Inatomi, 1998; Jumblatt et al., 2003); MUC5B, MUC7 and MUC19 are expressed in the oral mucosa (Troxler et al., 1997; Bobek et al., 1993; Zhu et al., 2011). In the surface epithelium of the airways MUC5AC and low levels of MUC2 are expressed, whereas MUC5B and MUC19 are expressed mostly in the submucosal glands (Buisine et al., 1999; Chen et al., 2004). In the stomach, MUC5AC is expressed by goblet cells at the epithelial surface and MUC6 expression is restricted to the lower gastric glands (De Bolos et al., 1995; Ho et al., 1995). MUC2 is the predominant mucin expressed in the normal adult intestine; however, MUC5AC is produced in the intestine during foetal development (Buisine et al., 1998) and in adenocarcinoma (Forgue-Lafitte et al., 2007). How mucin composition relates to the functional properties of mucus is not yet specified in most cases. However, defined roles for the gastric mucins, MUC5AC and MUC6, have been identified in protection against *Helicobacter pylori* infection (Linden et al., 2002; Kawakubo et al., 2004).

Currently, our understanding of how polymeric, gel-forming mucins form mucus gels is incomplete; although entanglement of mucin chains is important (Thornton et al., 2008). Many other factors will influence the physical properties of the barrier including