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Research paper

Mimicking microbial strategies for the design of mucus-permeating nanoparticles for oral immunization

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ABSTRACT

Dealing with mucosal delivery systems means dealing with mucus. The name mucosa comes from mucus, a dense fluid enriched in glycoproteins, such as mucin, which main function is to protect the delicate mucosal epithelium. Mucus provides a barrier against physiological chemical and physical aggressors (i.e., host secreted digestive products such as bile acids and enzymes, food particles) but also against the potentially noxious microbiota and their products. Intestinal mucosa covers 400 m^2 in the human host, and, as a consequence, is the major portal of entry of the majority of known pathogens. But, in turn, some microorganisms have evolved many different approaches to circumvent this barrier, a direct consequence of natural co-evolution. The understanding of these mechanisms (known as virulence factors) used to interact and/or disrupt mucosal barriers should instruct us to a rational design of nanoparticulate delivery systems intended for oral vaccination and immunotherapy. This review deals with this mimetic approach to obtain nanocarriers capable to reach the epithelial cells after oral delivery and, in parallel, induce strong and long-lasting immune and protective responses.

1. Introduction

The oral administration of bioactive products (i.e., drugs, antigens or immunomodulators) is an attractive and desirable option under diverse points of view: economic, safety (needle-free), easiness and efficiency, particularly for vaccine delivery, taking into account that oral vaccination can induce a systemic, including mucosal, immune response [1]. However, this practice has to face with a hard and very well organized frontier, the mucosa: a mucus secreting epithelium that lines the internal parts of the body. The intestinal mucosa is made up of epithelium, lamina propria, and *muscularis mucosae*. The epithelium is constituted by cells that are held together by tight junctions, which effectively form a seal against the external environment. In addition, there are two extra levels of protection against the outer milieu, the secreted mucus layer and the apical glycocalyx (Fig. 1). Globally considered these layers constitute the mucus that covers the tips of microvilli on the apical surfaces of intestinal enterocytes [2]. Mucus provides a barrier against physical and chemical aggressors, such as food residues, host secreted digestive products (e.g. bile acids and enzymes), but also against the potentially noxious microbiota and their products. Not surprisingly, pathogens have evolved many ways of evading the mucosal barrier. In fact, mucosae cover 400 m^2 in the human host, and as a consequence is the major portal of entry of the majority of known pathogens [3,4].

This review will deal with the generation of nanocarriers, based on microorganism-mimicking approaches, for the oral delivery of either antigens or allergens for vaccination and immunotherapy purposes.

2. Structure and topology of mucus matrix

Mucus is a complex viscous secretion basically formed by water (approx. 95%), salts, lipids and various kinds of macromolecules including the so-called mucins [5,6]. Mucins, secreted by goblet cells, are densely glycosylated proteins in which the protein backbone (apomucin) is linked to a number of carbohydrate chains (50–90% by weight) [7,8]. In addition the carbohydrate structures themselves can be either linear or branched, and can be acidic (containing sialic acid or sulphate groups) or neutral in nature [7,8]. The degree and type of glycosylation differs depending on the type of mucin and its localization throughout the gut [9]. These glycoproteins can be found as oligomers or non-oligomers, and are

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initially classified into three subfamilies: soluble (3–10 nm long), membrane-bound (100–500 nm), and, gel-forming mucins (up to several micrometres). Gel-forming mucins are the major constituent of mucus and responsible for its viscoelastic properties [10].

From a functional point of view, mucus appears as a dense fluid matrix that requires to be ineludibly porous, as a gel, since it needs to allow the diffusion of molecules to both orientations, into the cells (absorption of nutrients) and from the cells (secretion). However, at the same time, it needs to provide an effective physical barrier to foreign particulate matter, including microorganisms. To achieve successfully both functions, mucus is disposed in an arrangement that comprise two different layers: the external, which is named mucus layer, and the internal one or glycocalyx, that corresponds with the glycoproteins attached to the epithelial cell surface [11].

The mucus layer constitutes then the first line of defence against epithelia damage by physical, chemical or biological aggression. It is thick (100–400 μm in the small intestine, 700 μm in the large intestine) and constantly renewed by the host (approx. 5 L/day) [12,13]. Topographically comprise two layers: (i) the outer layer (70–100 μm diameter), which is loosely attached with large functional pores that allow the residence of normal microbiota, and (ii) the inner layer attached to the subjacent glycocalyx and, therefore, densely packed, with a very small functional pore that impede microbial and particle penetration. The predicted model for the physical mucin pore at this level is around 100 nm, although native mucin fibres may aggregate under certain circumstances to create larger pores which allow larger particles to transit [12].

The glycocalyx consists in long filaments of diverse glycoproteins and glycolipids well attached to the cell surface of enterocytes as a thin but very robust and compact layer (15–30 μm thick in the small intestine and around 100 μm in the large intestine). In fact, this layer would be able to detain any macromolecule above 30 nm [14]. The glycocalyx is renewed every 6–24 h, being then release to the lumen, where is trapped and concentrated at the mucus layers. In addition, epithelial cells actively secrete mucins to block microorganisms in the lumen, before reaching the epithelial cells. Fig. 1 shows a schematic representation of the intestinal mucosa.

Summing up, the structure of a mucin fibre contains hydrophobic domains alternating with hydrophilic glycosidic regions that allow interactions with empathic areas on adjacent mucins or even on other molecules. Consequently, mucin fibres are flexible and sticky. The energy invested by mucosal tissues in the production of mucins, and the finely tuned modulation in response to chemical physical or biological challenges, such as infections, reflects the importance of these glycoproteins. In fact, changes in mucin glycosylation are considered as mechanisms of the innate immune response to mucosal infections [10]. In any case, mucus layers are not insurmountable for the microbial world. Motility and degradative enzymes are main strategies used by many microbial pathogens to penetrate the mucus layers that we will considered in the following section.

3. Strategies of microorganisms to colonize mucosal surfaces

The harsh conditions of the gastrointestinal tract as well as the presence of intense peristaltic wave forces compromise the viability and survival of microorganisms within the gut. Many of them have developed different tricks to interact and even penetrate through the mucus layer in order to adhere to and colonize the

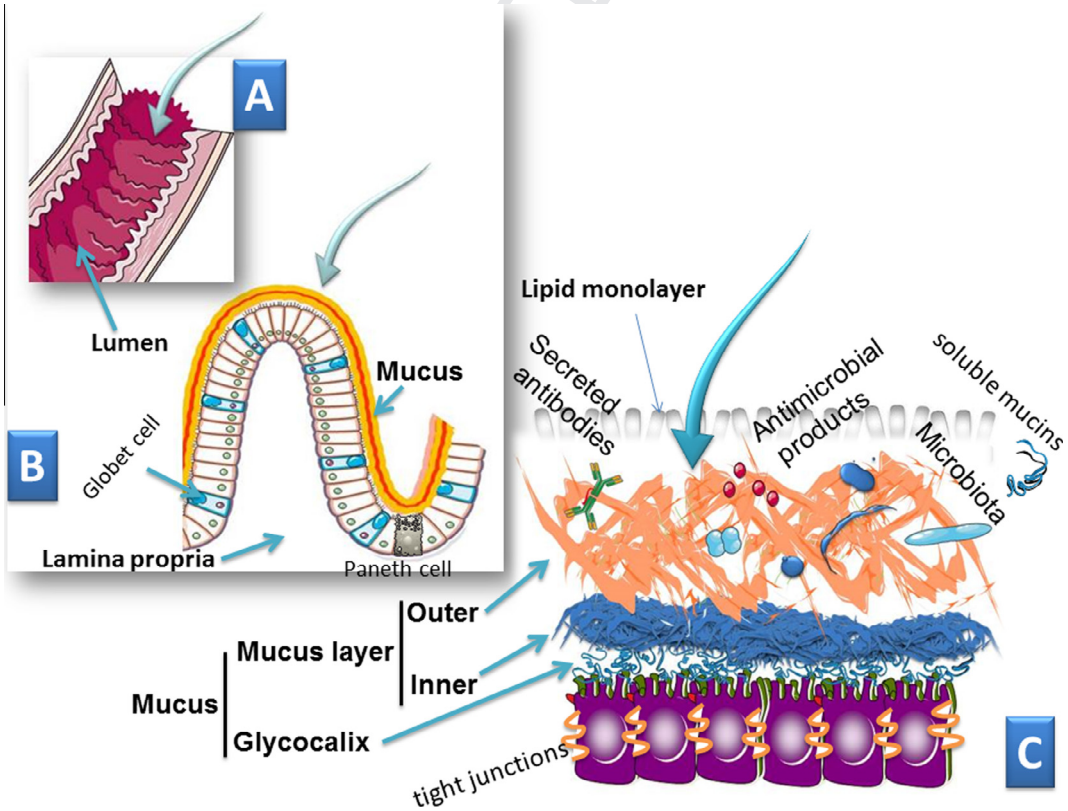

Fig. 1. Schematic representation of intestinal tissues and cell types. The intestinal epithelium is conformed as villi (A). Enterocytes and goblet cells cover most of the villi surface (B). Mucus protects intestinal epithelium presenting a mucus layer (outer layer and inner layer) and the glycocalyx on the cellular membranes (C). The figure displays significant mucosal products and components such as antimicrobial peptides released by Paneth cells, soluble mucins, microbiota and secreted antibodies. (For the interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

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