Advanced Drug Delivery Reviews 106 (2016) 402–409

Contents lists available at ScienceDirect

Advanced Drug Delivery Reviews

journal homepage: www.elsevier.com/locate/addr

The immune system of the gut and potential adverse effects of oral nanocarriers on its function☆

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ARTICLE INFO

Available online 29 September 2016

Keywords:
Oral peptides
Intestinal permeation enhancers
Therapeutic peptides
Peptide clinical trials
Oral nanotechnology
Gut microbiome

ABSTRACT

There is substantial effort in modern pharmacotherapy to use nanoparticle-based drug delivery systems (nDDS) for improving the oral absorption of drugs. An often neglected circumstance regarding this approach is that the gut is a major part of the immune system that may be vulnerable for immune-cell toxicity, or mediate humoral immune response against various components of nDDS, recognized as foreign. This review recapitulates the structure and function of gut-associated lymphoid tissue (GALT), i.e., the enteral section of mucosa-associated lymphoid tissue (MALT) and reminds how virus-like nDDS may potentially induce immunogenicity just as attenuated or killed viruses do in oral vaccines. Furthermore, we present examples for immune toxicities of emulsifiers and polymer-containing micelles, manifested in complement activation-related pseudoallergy (CARPA). A major message of the review is that early testing of immunogenicity or other adverse immune effects of nDDS in appropriate test systems or models may be prudent to recognize the risk of rare immune problems that may surface in late-stage clinical trials or after marketing of nDDS.

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Abbreviations: ADDR, Advanced Drug Delivery Reviews; APC, antigen presenting cell; API, active pharmaceutical ingredient; CARPA, complement activation-related pseudoallergy; CrEL, Cremophor EL; DDS, drug delivery system; FDC, follicular dendritic cells; GALT, gut-associated lymphoid tissue; GI, gastrointestinal system; GP2, glycoprotein-2; HSR, hypersensitivity reaction; MALT, mucosa-associated lymphoid tissue; nDDS, nano-structured drug delivery system; NPs, nanoparticles; OVA, ovalbumin; PrP, prion protein; R&D, research and development; SC, secretory component of IgA; sIgA, secretory IgA; SLN, solid lipid nanoparticle; Tc lymphocyte, cytotoxic lymphocyte; Th lymphocyte, helper lymphocyte.

☆ This review is part of the *Advanced Drug Delivery Reviews* theme issue on "SI: Oral delivery of peptides".

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http://dx.doi.org/10.1016/j.addr.2016.09.009
0169-409X/

1. Introduction

A major goal in nanomedicine R&D is to find nano-structured drug delivery systems (nDDS) that improve the gastrointestinal (GI) absorption of drugs of low oral bioavailability. Nano-DDS with and without penetration enhancers in preclinical and/or clinical trials include a variety of lipid or polymeric or other types of nDDS (see Aguirre et al., in this volume), and water soluble or insoluble payloads, among which this topical volume of Advanced Drug Delivery Reviews (ADDR) focuses on peptides and proteins. A few oral peptide formulations have already reached clinical trials (Aguirre et al., in this volume), however, there are many difficulties of this approach, including the low and hardly controllable intestinal absorption and/or degradation of nDDS by intestinal enzymes. These problems represent the greatest challenge in this field at present, however, there is still another potential barrier to the clinical use of oral nDDS which has got no, or little attention to date; immune side effects manifested in either activation or suppression of the immune system by nDDS. The goal of the present short, "targeted" review is limited to address this potential safety risk of nDDS, highlighting reasons why immune side effects might occur on the basis of available experimental and preclinical information. In particular, we draw attention to two potential adverse immune effects of nDDS; immunogenicity and immune reactivity leading to hypersensitivity reactions (HSRs). Considering that the gut is a major immune organ, another goal of the present review is to introduce the essentials of gut-associated lymphoid tissue (GALT) to all interested in oral drug delivery without being initiated in immunology. We start with the latter subject.

2. Structure and function of mucosa-associated lymphoid tissue (MALT)

2.1. The gut as an immune organ

The immune functions of the GI system has been intensely studied over the past 40 years leading to the recognition of two major roles for this virtual immune organ; one is the defense against orally entering pathogens, and second, the maintenance of the equilibrium of the enteral bacterial flora [1–14]. While these functions of the GI is not relevant to most small molecular-weight drugs, nDDS and nanomedicines are different, as they are much larger and may engage the immune system in some action even if they do not have any intrinsic effect on immune cells. Considering that the GI immune system contains >50% of all lymphocytes in the body [15], the impact may be significant.

Reiterating some basic information, the immune system in the gut is part of the mucosa-associated lymphoid tissue (MALT), a "secondary" lymphoid tissue that is dispersed in the submucosa of the respiratory, GI and urogenital tracts and glands that feed into these tracts (e.g. salivary, pancreatic and biliary). The lymphocytes in other glands, e.g., the lacrimal, mammary and sweat glands also belong to the MALT [15]. These lymphoid structures in the mucosa may be temporary focal lymphocytic infiltrates or demarcated structures with direct contact with the epithelium, called lymphoid follicle. The location of MALT structures enables their categorization as listed in Table 1.

Although the MALT sites listed above are anatomically separated, they are functionally connected in what is known as "common mucosal immune system". This means information sharing, i.e., antigen presentation and B-cell activation at one mucosal site can result in IgA secretion at mucosal sites of other organs [16–19]. MALT can also be functionally divided into inductive and effector sites [20,21]. At inductive sites immunogenesis proceeds via APC-lymphocyte interactions, IgA class switching and clonal expansion of B-cells in a limited, defined area (within and around the follicle). The effector sites, where immune attacks occur, are those where antibody secretion and binding proceeds.

Table 1
Categorization of MALT according to anatomical localization.

Anatomical location	Abbreviations
bronchus-associated LT	B-ALT, BALT
conjunctiva-ALT	C-ALT, CALT
diffuse mucosa-ALT	DM-ALT, DMALT
gastric mucosa-ALT	G-MALT, GMALT
gut-ALT	GALT, Peyer's patches
lacrimal drainage-ALT	LAD-ALT, LADALT
larynx-ALT	L-ALT, LALT
mammary gland-ALT	MG-ALT, MGALT
organized mucosa-ALT	OM-ALT Waldeyer's ring (tonsils)
pharynx-ALT	P-ALT, PALT
salivary duct-ALT	SD-ALT, SDALT
salivary gland-ALT	SG-ALT, SGALT
skin-ALT	S-ALT, SALT

-ALT, — associated lymphoid tissue.

2.2. Structure and function of lymphoid follicles

Here we focus on the follicles of GALT, that may occur as single (isolated) lymphoid nodules or as larger aggregates of several lymphoid nodules, like the Peyer's patches and the appendix. Fig. 1A is a schematic presentation of a GALT follicle, formed from a group of submucosal lymphocytes closely associated with follicular dendritic cells (FDC) and macrophages. These lymphocytes show CD41 and CD81 phenotypes. The nodules consist of germinal centers loosely surrounded by free T and B lymphocytes in the inter-nodular space. T lymphocytes may represent up to 80% of MALT [22,23]. Panel B shows further details of GALT structure and function in the case of Peyer's patches, i.e. aggregates of solitary nodules in the ileum, the primary sites of antigen uptake and presentation via the function of microfold (M) cells.

2.3. Microfold (M) cells

The "M" in the name of these cells refers to "membranous", since the apical surface of these cells express only small, irregular, disorganized microvilli with a poorly developed brush border without glycocalyx, so they appear among the enterocytes as smooth membrane-based pits. These cells are difficult to distinguish with light microscope, and they have no specific histochemical or immunohistochemical markers. They can be indirectly localized at the clusters of lymphocytes in the follicle-associated epithelium. The name "microfold" is due to the presence of microfolds in non-human primates. As shown in panel B and C, M cells are among the epithelial cells directly situated above the Peyer's patches. Their basal membrane is invaginated, forming a pocket that typically contains one or more lymphocytes (T-cells or B-cells) and antigen presenting cells (APCs), such as follicular dendritic cells (FDCs) or macrophages. The role of M cells is "antigen sampling", i.e., transfer of pathogens and pathogenic antigens from the gut lumen to their basolateral pockets or the subepithelial dome region, where APCs take them up and process them for antigen presentation.

The physical proximity of APC and lymphocytes in the dome region and in the pocket of M cells (Fig. 1C) ensures effective antigen presentation and cooperative immunogenicity, a complex interaction of immune cells with the aim to educate B and T cells for accelerated and specific humoral and cellular responses against the antigens. Panel B also illustrates that the inter-nodular cells include regulatory T cells and plasma cells, the former securing long-term memory of the antigen, while the latter cells secrete IgA which passes through the epithelial wall into the gut lumen to form secretory IgA (sIgA). Fig. 1D and E show the macro- and microscopic images of the Peyer's patches, and panel E shows the apical surface of M cells. The latter does not display glycocalyx or microvilli, but express, among other bacterium-binding molecules, glycoprotein-2 (GP2) and prion protein (PrP).