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Colonic antigen administration induces significantly higher humoral levels of colonic and vaginal IgA, and serum IgG compared to oral administration

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Summary It was hypothesised that different immune responses would be obtained following oral and colonic antigen administration, due to the significant differences in the immune environments of the colon and that of the small intestine. Antigen administration to the mouse colon (via the rectum) was found to generate different profiles of immune responses compared to oral administration (by gavage). Serum IgG and IgA levels in faecal and colonic extracts and in the vaginal wash were significantly higher following colonic administration of soluble (plus cholera toxin B subunit adjuvant) or encapsulated (in microspheres) antigen while smaller differences were seen in the small intestinal IgA levels. This reflects the compartmentalisation within the common mucosal immune system and suggests that the colon may be an appropriate vaccination target for diseases of the colon, and for sexually and vertically transmitted diseases. Antigen was also administered rectally and intramuscularly as controls. Colonic administration was superior to rectal administration, possibly due to the greater amounts of lymphoid tissue in the colon, although the immune response profiles were similar.

Introduction

Currently, most vaccines are given by injection, the main disadvantage of which is that it does not generally give rise to antibodies on the mucosal surfaces—the main site of pathogen entry into the body [1,2]. To obtain antibodies on the mucosal surfaces, vaccines have to be delivered to the common mucosal immune system (CMIS), for example orally, nasally, rectally. The oral route is especially desirable for vaccine administration, due to its convenience, and oral vaccination is being extensively researched. Following oral administration (generally by gavage in research), the vaccine is expected to be taken up largely by the first lymphoid tissue encountered, i.e. that present in the small intestine.

In our laboratories, we are investigating vaccination targeted to the colon. The latter contains an abundance of lymphoid tissue, thought to be analogous to Peyer's Patches in the small intestine [3–5]. It has so far largely been neglected as a potential target, and we have found only one

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publication where vaccine was administered to the distal colon in patients with a surgical colostomy via the colostomy opening [6]. Advantages of targeting vaccines to the colon (compared to the small intestine) include its lower levels of proteolytic enzymes and a longer residence time (often in excess of 24 h versus 3—4 h [7—9]) which could result in increased antigen uptake.

More importantly, colonic vaccination may turn out to have different applications to the more commonly studied oral vaccines, due to the significant differences between the immune environments in the colon and in the small intestine. The smaller lymphoid follicles in the colon exist largely as individual follicles, and the cell turnover may be more rapid [10]. Significant differences between small intestinal and colonic intraepithelial and lamina propria lymphocytes, in terms of number, phenotype and function, have been reported [11—15]. For example, small intestinal intraepithelial lymphocytes (IELs) are predominantly $CD8^+$ and $\gamma\delta$ TCR^+ while colonic IELs are predominantly $CD4^+$ and $\alpha\beta TCR^+$. A preponderance of IgA2 cells over IgA1 cells is seen in the colon (as in the rectum and in the vagina [16]) in contrast to a predominance of IgA1 cells in the small intestine [17]. The similar predominance of IgA2 cells in the rectum and in the female genital tract has led to the suggestion that the rectal lymphoid tissues may serve as a source of IgA precursor cells destined for the genital tract [16,17]. We suggest that the colon could also serve the same purpose. In addition, the induction of immune responses to bacterial antigens is thought to preferentially occur in the colon [10]. Thus, the colon might prove to be a more appropriate site of vaccination against enteric bacteria, sexually and vertically transmitted diseases and colorectal tumours.

Our hypothesis was therefore that significantly different immune responses would be generated following oral or colonic administration of an antigen, due to the different immunological environments encountered by the antigen. The latter was therefore administered orally or colonically. In addition, intramuscular and rectal administrations were used as controls. Mice were used as experimental animals as the presence of abundant lymphoid tissue in mouse colon has been established [18]. Ovalbumin was used as the model antigen, administered in the presence of commonly used adjuvants (alum for intramuscular administration and cholera toxin B subunit for mucosal administration) or encapsulated in polylactide-*co*-glycolide (PLGA) particles. Following antigen administration, the antibody levels in the serum, faeces, small and large intestines, and vaginal wash were quantified by enzyme-linked immunosorbent assay (ELISA).

Materials and methods

Animals

Female BALB/c mice (6—8 weeks old) were obtained from Harlan Olac Ltd., UK). All procedures had been approved by The School of Pharmacy's Ethical Review Committee and were conducted in accordance with the Home Office standards under the Animals (Scientific Procedures) Act, 1986.

Materials

Ovalbumin (Grade V), methylene blue, cholera toxin subunit B (CTB), alum, sodium dodecyl sulphate (SDS), sodium hydroxide, (2,2′azino-bis) 3-ethylbenzthiazoline-6-sulphonic acid (ABTS), citric acid, disodium hydrogen orthophosphate, ethylenediamine tetraacetic acid (EDTA), sodium azide, trypsin inhibitor, iodoacetic acid phenylmethylsulphonyl fluoride (PMSF) and hydrogen peroxide were from Sigma Aldrich Ltd, UK. Poly(lactic-*co*-glycolide) was from Lakeshore Biomaterials, USA. Polyvinyl alcohol (degree of hydrolysis >85%) was from BDH Ltd. Bicinchonic acid (BCA) assay kit was purchased from Pierce Ltd., UK. The tubing for colonic delivery was supplied by VetTech Solutions, UK. Phosphate buffered saline (PBS) tablets were obtained from Dulbecco, UK. Immulon 2B 96-well plates were from Fisher Scientific Ltd. Coating solution concentrate (phosphate buffer concentrate) and bovine serum albumin (BSA, 10% solution) were from KPL Inc., Maryland, USA. Anti IgG and anti IgA goat anti-mouse horse-radish peroxidase (HRP) conjugates were from AbD Serotec, Oxford, UK.

Preparation of free (adjuvanted) antigen solutions

Ovalbumin (OVA) was dissolved in phosphate buffered saline pH 7.3, at different concentrations. Each animal received the same antigen dose (250 μg). However, due to the different volumes required for the different routes, the antigen concentrations in the solutions were different. Cholera toxin B subunit or alum were added for their immunomodulating and adjuvant effects for mucosal and parenteral antigen administration, respectively, as shown in Table 1. Alum was incubated with the ovalbumin solution overnight in the refrigerator.

Preparation and characterisation of blank and ovalbumin loaded PLGA particles

A double emulsion solvent evaporation method was used. Five millilitres of 5% (w/v) PLGA in dichloromethane were emulsified with 1 ml of 5% (w/v) polyvinyl alcohol (PVA) in deionised water containing 20 mg of OVA, by homogenisation (UltraTurrax T-25 Basic IKA) for 3 min at 24,500 rpm. This primary water-in-oil emulsion was added dropwise to 40 ml of a 5% (w/v) PVA solution, with homogenisation (Silverson 4LRT Homogeniser) for 5 min at 10,000 rpm. The resulting double water-in-oil-in-water emulsion was stirred overnight, to allow solvent evaporation, and hardening of the nanoparticles. The latter were then harvested by centrifugation at 15,000 rpm for 10 min. The pellet was collected, resuspended in water and centrifuged once more and the process was repeated to remove any remaining PVA. The nanoparticles were then lyophilised (Virtis-Advantage Freeze Drying Apparatus, Virtis, UK) and stored at 2—8 °C. Scanning Electron Microscopy (SEM, using a Philips XL20 microscope) revealed the particles to be spherical with a smooth surface, non-aggregated and of fairly uniform size (Fig. 1). The particle diameter, determined using a Zetasizer (Zetamaster S), was 510 ± 41.4 nm (polydispersity index 0.12 ± 0.07). The protein content of the ovalbumin-loaded particles was determined following particle degradation by adding 5 mg