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Veterinary Immunology and Immunopathology 109 (2006) 209–217
Veterinary immunology and immunopathology
www.elsevier.com/locate/vetimm

Immune responses after local administration of IgY loaded-PLGA microspheres in gut-associated lymphoid tissue in pigs

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Received 3 January 2005; received in revised form 25 July 2005; accepted 11 August 2005

Abstract

Oral vaccination of large animals using PLGA MS (poly(D,L-lactide-co-glycolide)microspheres) appeared to be more challenging than immunization of mice. The purpose of this study was to deliver to GALT an immunogenic model protein (IgY), free or encapsulated by spray-drying in PLGA MS, and to evaluate systemic immune response in SPF Large White pigs. Pigs were surgically processed for local administration of IgY in three sets of experiments. In two sets of experiments, administration was locally performed in temporary ligatured intestinal segments, in jejunal Peyer's patches and in mesenteric lymph nodes. In the third experiment, pigs received IgY via an intestinal cannula. Total IgY-specific antibodies were detected in the sera of pigs after a single local immunization, but not in the sera of cannulated pigs. The study of IgG1 and IgG2 isotypes indicated that PLGA MS are able to elicit a combined serum IgG2/G1 response with a predominance of IgG1 response when locally administered. PLGA MS can be a potential oral delivery system for antigen but our results underlined the difficulty to immunize large animals like pigs. Transposition of data between small and large animals appears to be complex and suggests that physiological features need to be considered to increase intestinal availability of oral encapsulated vaccines.

Keywords: Pig; Gut-associated lymphoid tissue; Local administration; PLGA microspheres; Immune response

Abbreviations: GALT, gut-associated lymphoid tissue; PP, Peyer's patches; PLGA MS, poly(D,L-lactide-co-glycolide)microspheres; Specific Ig, total anti-IgY antibodies; IgY, avian immunoglobulin Y; IM, intramuscular; jPP, jejunal Peyer's patches; jPP-E, administration in jPP after enterectomy; jPP-IW, administration in jPP through intestinal wall; MLN, mesenteric lymph nodes; SPF, specified pathogen-free

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doi:10.1016/j.vetimm.2005.08.016

1. Introduction

Mucosal tissues of the gastrointestinal, respiratory and urogenital tracts play a key role in the organism since they are both, the gateway and the first line of defence against invasion by microorganisms. Protection requires effective vaccination that stimulates immune responses at the mucosal tissue (Brandtzaeg et al., 1997). Thus, there is a great deal of interest in the development of mucosal vaccine strategies (Chen, 2000). For this purpose, poly(D,L-lactide-co-glycolide)microspheres (PLGA MS) designed for the encapsulation of soluble antigens (Ag) are intensively investigated.

Most of the studies focus on oral administration of vaccines since: (i) the oral route appears to be easy to handle in humans and animals; (ii) the gut-associated lymphoid tissues (GALT) represent the largest mucosal area. GALT is characterized by lymphoid follicles, named Peyer's patches (PP). Pig presents isolated PP in the jejunum (jPP) and a continuous PP in the terminal ileum (iPP) (Pabst et al., 1988). jPP have a preferential function as sites for antigen sampling, frequency of Ig secreting cells and induction of intestinal immune response (Barman et al., 1997; Pabst and Rothkotter, 1999; Bianchi et al., 1999). PP are specifically covered by specialized epithelial cells, M cells. These cells allow the transport of Ag across the follicle-associated epithelium (FAE) to the antigen-processing cells of the underlying dome region with subsequent stimulation of B and T lymphocytes (Gebert, 1997; Kraehenbuhl et al., 1997; Neutra, 1999). Transcytosis activity has been demonstrated for various polymeric nano- or microparticles (Jepson et al., 1993a; Ermark et al., 1995; Smith et al., 1995) in many species including pig (Torché et al., 2000). Different attempts were performed to demonstrate the potentiality of PLGA MS as vaccine adjuvant systems in order to induce both systemic and local immune responses following oral immunization. Experiments with antigens entrapped in PLGA microspheres have demonstrated that immunization boosts the systemic antibody response, enhances mucosal IgA responses in mice following oral or intragastric administration (Conway et al., 2001; Jung et al., 2001; Fattal et al., 2002; Yeh et al., 2002; Carcaboso et al., 2003) and is able of priming cellular (cytotoxic T lymphocyte) immune responses in vivo (Maloy et al., 1994; Partidos et al., 1999).

Most vaccination experiments were successfully performed on small animals like mice. Even if the number of published data is smaller, it appeared clearly more difficult to obtain the same results with large animals. To date, these delivery systems remain unsuccessful (Felder et al., 2000) or are largely untried for oral vaccine delivery in large animals (Mutwiri et al., 2002; Lin et al., 2003). The apparent discrepancy between results from small and large animals raises the problem of transposition and suggests that additional barrier may impede the local trafficking of MS throughout the GALT.

In order to challenge this assumption, we have investigated the systemic immune response (total immunoglobulins, IgA, IgG1 and IgG2 isotypes) after administration of a model antigen (IgY) to pigs, as solution or encapsulated in PLGA MS, at different steps of Ag trafficking in the GALT. A surgical experimental model was set up to locally deliver IgY at different GALT locations, i.e. in intestinal lumen, in mesenteric lymph nodes or within Peyer's patches.

2. Materials and methods

2.1. Preparation of PLGA microspheres containing IgY

Avian immunoglobulin Y (IgY 160 kD) was isolated from chicken egg yolks by sodium precipitation. Two milliliters of aqueous solution containing IgY was emulsified by an ultrasonic probe with a 3% (w/v) solution of PLGA dissolved in dichloromethan (40 ml, RPE-ACS, Carlo Erba Reactifs) in a thermostated beaker (2 °C) to produce a stable W/O nanoemulsion. PLGA MS were subsequently formed by spray-drying the nanoemulsion at a rate of 2 ml/min under the following conditions: inlet air temperature 50 °C; aspiration setting 100%; pump control 15%; air flow rate 500 NL/h (Mini-Büchi 190).

Two formulations of PLGA MS (A and B) were prepared by a spray-drying method. Formulation A was prepared using an organic phase containing Phusis® 75:25 poly(L-lactide-co-glycolide) and 0.05% (w/v) phosphatidylcholine. IgY was diluted in PBS at a concentration of 4.5 mg/ml (w/v).