IgA ANTIBODY PRODUCTION BY INTRARECTAL IMMUNIZATION OF MICE USING RECOMBINANT MAJOR CAPSID PROTEIN OF HAMSTER POLYOMAVIRUS

K. Messerschmidt¹, S. Hempel², P. Holzlohner¹, R. G. Ulrich³, D. Wagner¹ and K. Heilmann^{1*}

¹Junior Research Group “Antibody Technologies,” Department of Biotechnology, Institute of Biochemistry and Biology, Potsdam University, Potsdam, Germany

²Beuth University of Applied Sciences, Berlin, Germany

³Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute for Novel and Emerging Infectious Diseases, Greifswald – Insel Riems, Germany

Received: June 10, 2012; Accepted: June 14, 2012

Viral proteins are highly antigenic and known as potent stimulators of adaptive immune responses. This mechanism is often used for biotechnological applications in monoclonal antibody production resulting in high-affinity IgG antibodies in most cases. The aim of this study was to increase antigen-specific IgA antibody levels in mice in order to generate monoclonal IgA antibodies by hybridoma technology. For this purpose, hamster polyomavirus (HaPyV) major capsid protein VP1 was used to immunize mice by different routes in order to induce VP1-specific IgA titers. Recombinant HaPyV-VP1 was generated in *Escherichia coli* and administered intraperitoneally, orally, and intrarectally. VP1-specific antibodies were determined by ELISA in sera and organ culture supernatants. We found a significant increase of HaPyV-VP1-specific IgAs in spleen organ cultures after rectal immunization of mice but not in cultures of mesenteric lymph nodes, colon, or Peyer’s patches. In contrast, oral and intraperitoneal immunization did not provide an appropriate specific IgA induction at all. These results show that specific IgA antibodies can be induced by intrarectal immunization in the spleen. The generation of monoclonal IgA antibodies with well-defined properties is a useful tool for the investigation of mucosal immune responses or autoimmune diseases and extends the spectrum of antibodies with specific effector functions.

Keywords: polyomavirus capsid proteins, intrarectal immunization, IgA immune responses, hybridoma technology, monoclonal antibodies

Introduction

Monoclonal antibodies are known as very powerful and highly specific tools for broad applications in biotechnical research and therapeutic approaches. Hybridoma technology which is used to generate monoclonal antibodies results in most cases in IgG antibodies. Although IgG antibodies are sufficient and wanted for most biomedical and biotechnical applications, for certain research, diagnostics and therapy applications antibodies of other isotypes as e.g. IgA are required. IgA antibodies are part of the mucosal immune system and predominate mucosal effector sites as the mammary and salivary glands, the nasal and bronchial mucosa, and the upper digestive tract. In comparison to human IgA with two subclasses (IgA1 and IgA2), mice only have one IgA isoform. The function of IgA is described as high or low affinity system. IgA antibodies with a high affinity are neutralizing toxins and pathogens whereas low affinity IgA antibodies inhibit the adhesion of symbiotic bacteria to the epithelia [1]. In the mucosal system, IgA is secreted by plasma cells as a dimer and is transported into the gut lumen by transcytosis [2]. Secretory IgA is regulating the

bacterial communities in the gut lumen and plays a key role in establishing and maintaining a tolerant non-inflammatory relationship between host and microbes [3]. There is evidence that monoclonal IgA antibodies produced *in vitro* by hybridoma technology occur in a polymeric or dimeric form analogue to *in vivo* produced IgA [4]. The obtained secretory IgA antibodies were used for experimental studies of mucosal surfaces and microfold (M) cells in order to investigate bacterial and viral intestine infections. Further investigations showed that secretory IgAs seem to have a higher functional activity and stability than IgG counterparts [5]. Because of their special effector functions, IgA antibodies are of high clinical interest as they are highly effective in recruiting polymorphonuclear cells for antibody dependent cellular cytotoxicity (ADCC) [6] and in enhancing respiratory burst and phagocytosis of human leukocytes [7]. These data indicate that antibodies with an IgA isotype have interesting properties and potential applications in research. Because of these interesting functions, the present study was designed to find immunization procedures which are able to induce IgA specific immune responses in mice. For this purpose, recombinant HaPyV-VP1 was used as

*Corresponding author: Katja Heilmann; Phone: +49-331-97-75348; Fax: +49-331-97-75061; E-mail: katja.heilmann@uni-potsdam.de

antigen because this viral structure protein is highly immunogenic and induces potent immune responses in mice. Therefore, the administration of viral proteins can be carried out without any additional adjuvant compared to usual immunization strategies [8]. In addition, HaPyV-VP1 is able to assemble *in vivo* and *in vitro* into virus-like particles (VLPs) [9] which can be modified by chemical coupling of entire proteins, protein segments, or peptides or by incorporation of foreign sequences into the VLP-encoding gene in order to induce specific and high-titered antibody responses against the coupled or inserted antigen [10]. Such chimeric VLPs are used successfully for vaccine development in case of e.g. HIV and malaria [11]. Recently, chimeric VLPs have also been exploited for the generation of monoclonal antibodies (mAbs) of desired specificity in mice [10]. In the present study, we tested whether the titer of IgA antibodies can be raised by an unconventional immunization route. Recombinant HaPyV-VP1 was chosen as antigen because it is intended to modify them for further experiments by insertion of foreign peptide sequences. For this purpose, we immunized mice with HaPyV-VP1 by three different routes (oral, intrarectal, and intraperitoneal) in order to enhance the induction of specific antibodies of IgA isotype. Organ cultures of spleen, mesenteric lymph nodes, Peyer's patches, and colon were applied, and the antibody titers were compared to that of the serum. We could clearly demonstrate that only the intrarectal route leads to an efficient induction of antigen-specific antibodies with an IgA subtype within 2 weeks of immunization. The here described immunization procedure will be useful for the highly efficient generation of monoclonal IgA antibodies for biotechnical applications.

Materials and methods

HaPyV-VP1 production

The generation of plasmids encoding the HaPyV-VP1 sequence was described in detail by Siray et al. [12]. For expression in *Escherichia coli*, the sequence encoding the entire amino-terminally prolonged VP1 (of 388 amino acid residues) was subcloned into plasmid pET15b according to standard procedures. The expression plasmid is de-

fined as pKP3. The resulting sequence consists of a hexahistidine tag and additional restriction sites 5' and 3' to the VP1-encoding sequence (Fig. 1). For the production of HaPyV-VP1, 1 l LB medium containing 50 µg/ml carbenicillin was inoculated with 10 ml overnight culture of the pET15b-VP1 transformed *E. coli* strain *RosettaGami* DE3 (Merck KGaA-Novagen, Darmstadt, Germany). The culture was incubated at 37 °C with shaking at 150 rpm until an OD₆₀₀ of about 0.6. The synthesis of HaPyV-VP1 was induced by addition of IPTG (final concentration 1 mM). The cultivation was continued for 15 h at 18 °C with shaking at 100 rpm. Finally, the *E. coli* cells were harvested by centrifugation at 13,000×g over 5 min at 4 °C, and cell pellets were stored at -20 °C until sonification.

Purification of HaPyV-VP1

Cell pellets were resuspended in 20 ml disruption buffer (10 mM MOPS, 300 mM NaCl, 0.5% Sarcosyl, pH 7.4) and disrupted by sonification. For the removal of cell debris, the mixture was centrifuged at 16,000×g and 4 °C for 30 min. The supernatant was used for purification of HaPyV-VP1 by affinity chromatography on a nickel-nitrilotriacetic acid (Ni-NTA) gel matrix (Qiagen GmbH, Hilden, Germany). A column containing 4 ml of Ni-NTA resin was first equilibrated with 10 ml disruption buffer, and thereafter, the supernatant of the *E. coli* lysate was applied to the column. The column was washed three times with 3 column volumes of disruption buffer. Recombinant HaPyV-VP1 was eluted by increasing the imidazole concentration to 250 mM and 500 mM. Fractions containing the recombinant protein were pooled and dialyzed against 10 mM MOPS buffer with 350 mM NaCl (pH=7.4) for 36 h to remove imidazole and sarkosyl. The purity of recombinant HaPyV-VP1 was analyzed by protein gel electrophoresis and Western blot analysis.

Protein gel electrophoresis and Western blot analysis

The purified fractions of HaPyV-VP1 were analyzed in 12.5% polyacrylamide gels and subsequent Coomassie

```
HHHHHHSSGLVPRGSHMRKSMAPKRKSGASSRCANPCGKPCPKPANVPKLI MRGGVGVL D
LVTGEDSITQIEAYLNPRMGQNKPGTGTGQYYGFSQSIKVNSSLTADEVKANQLPYYSM
AKIQLPTLNEDLTCDTLQMW EAVSVKTEVVGVSLLNVHGYGSRSETKDIGISKPEVGT T
HMFVAVGGEPLDLQGLVQNYNANYEAAIVSIKTVTGKAMTSTNQVLDPTAKAKLDKDGRY
PIEIWGDPDSKNENSRYYGNGFTGGTSTPPVMQFTNTLTTLTVLLDENGVGPLCKGDGLYLSA
ADVGMWYIEYNSAGWHWRGLPRYFNVTLRKRWVKNPYPVTSLLASLYNNMLPTIEGQPM E
GEAAQVEEVRIYEGTEAVPGD PDVNRFDKYGQQHTKPPAKPANLEDPANKARKEAELA
AATAEQ
```

Fig. 1. Schematic presentation of the recombinant HaPyV-VP1.

The entire VP1 encoding sequence of pFR36-VP1/2-9 was subcloned into plasmid pET15b resulting in an expected fusion protein containing a hexahistidine (H₆) tag at the amino-terminus. Abbreviations: H₆ – histidine tag, MCS – multiple cloning site, VP1 – major capsid protein VP1

Fig. 2. Detection of purified HaPyV-VP1 in SDS-PAGE (a) and by Western blot analysis (b).

Twenty μ l of the eluate fractions of HaPyV-VP1 (lanes 1–5), a positive control (yeast-expressed purified HaPyV-VP1; lane 6), expression control (lysate of pKP3-transformed *E. coli* cells), and a negative control (lysate of pET15b-transformed *E. coli* cells; lane 8) were loaded into the gel pockets of a 12.5% polyacrylamide gel. The gel was stained by Coomassie brilliant blue (a) or alternatively used for blotting onto a nitrocellulose membrane for 1 h at 400 mA (b). HaPyV-VP1 was detected by using a VP1-specific monoclonal antibody (3D10, diluted 1 to 4000 in PBS/5% NCS) and a horse radish peroxidase-labeled rabbit-anti-mouse IgG secondary antibody (1 to 5000 in 1 $\times$ PBS/5% NCS). Enzyme reactions were performed with DAB solution (500 μ l 1 M Tris-HCl pH 7.5, 25 μ l 80 mg/ml NiCl₂, 100 μ l 40 mg/ml DAB, 1 μ l 30% H₂O₂ add 5 ml)

blue staining as described previously [13] and by Western blot analysis. The separated proteins were blotted onto a nitrocellulose membrane (Roti®-Nitrocellulose, Carl Roth GmbH, Karlsruhe, Germany) using a semi-dry blotter unit (Bio-Rad Laboratories GmbH, München, Germany) for 1 h at 400 mA. The membrane was blocked with 1 $\times$ PBS/5% newborn calf serum (NCS) overnight at 4 °C. Afterwards, the membrane was washed five times with water and then incubated with mouse-anti-HaPyV-VP1 antibody (clone 3D10, [10]), diluted 1 to 4000 in

1 $\times$ PBS/5% NCS for 45 min, at room temperature with continuous shaking. After washing three times, the membrane was treated with horse radish peroxidase-labeled rabbit-anti-mouse IgG antibody (Dianova GmbH, Hamburg, Germany) diluted 1 to 5000 in 1 $\times$ PBS/5% NCS for 45 min at room temperature with continuous shaking. The membrane was washed again three times and incubated with diaminobenzidine (DAB) solution (500 μ l 1 M Tris-HCl pH 7.5, 25 μ l 80 mg/ml NiCl₂, 100 μ l 40 mg/ml DAB, 1 μ l 30% H₂O₂ add 5 ml).

Fig. 3a **Fig. 3.** Detection of HaPyV-VP1-specific IgG (a) and IgA (b) in mice immunized intraperitoneally (i.p.) and intrarectally (i.r.) with purified HaPyV-VP1.

Mice were immunized with recombinant HaPyV-VP1 in 10 mM MOPS buffer + 300 mM NaCl (pH=7.4) 4 times within 2 weeks (i.p. $n=2$; i.r. $n=3$). Non-immunized mice served as negative controls ($n=3$). Organ cultures of spleen, mesenteric lymph nodes, Peyer's patches, and colon were prepared by adding 4 ml cell culture medium (RPMI 1640, 10% fetal calf serum, 2 mM glutamine, 50 μ M mercaptoethanol, 100 μ g/ml penicillin/streptomycin) supplemented with 1 μ g/ml LPS. Blood was removed by heart puncture and centrifuged by 3000 $\times$ g for 15 min in order to obtain serum. The detection of HaPyV-VP1-specific antibodies was performed by ELISA according to standard procedures. The mouse monoclonal HaPyV-VP1-specific antibody 3D10 was used as positive control. A peroxidase-conjugated goat anti-mouse IgG antibody (1 to 5000 in 1 $\times$ PBS/5% NCS) and a biotinylated goat-anti-mouse-IgA antibody (1 to 5000 in 1 $\times$ PBS/5% NCS) with peroxidase-conjugated streptavidin (0.1 U/ml in 1 $\times$ PBS/5% NCS) were used for detection (*** >0.0002 ; ** >0.002).

Animals and immunization

BALB/c mice were bred in the animal facility at Potsdam University (Potsdam, Germany) and used for the different immunization strategies. For intraperitoneal immunization of HaPyV-VP1, 8–12-week-old mice were injected with 50 μ g ammonium sulfate precipitated recombinant antigen in 10 mM MOPS buffer+300 mM NaCl (pH=7.4). The rectal immunization procedure com-

prises first the flushing of the colon with 80% ethanol and the application of 200 μ g HaPyV-VP1 in 10 mM MOPS buffer+300 mM NaCl (pH=7.4) with a catheter. Control mice received the ethanol flushing and the application of 200 μ l 10 mM MOPS buffer+300 mM NaCl (pH=7.4). The oral immunization of mice was performed by applying 50 μ g HaPyV-VP1 in 1 $\times$ PBS by gavage. Non-immunized mice served as negative controls. Immunizations were repeated four times within 2 weeks.

Fig. 3b

For legend see page 234.

Organ cultures

Four days after the last immunization, the mice were sacrificed, and spleen, Peyer's patches, colon, and mesenteric lymph nodes were dissected. For the preparation of sera, the blood was removed by heart puncture in the course of organ preparations and was centrifuged by $3000 \times g$ for 15 min at room temperature (RT). The spleen, Peyer's patches and mesenteric lymph nodes were meshed for single-cell preparation. The colon was cut into pieces of 0.5 cm size. Organ cultures were performed at 37 °C by adding 4 ml medium (RPMI 1640, 10% fetal calf serum, 2 mM glutamine, 50 μ M mercaptoethanol, 100 μ g/ml penicillin/streptomycin) supplemented with 1 μ g/ml lipopolysaccharide (LPS) as a B-cell mitogen. On days 1, 5, 7, 9, and 11, 500 μ l supernatants were collected and used for the detection of specific antibodies.

ELISA analysis

HaPyV-VP1 specific antibodies from serum samples and culture supernatants were determined by indirect ELISA using 96-well plates (Greiner bio-one GmbH, Frickenhausen, Germany). The coating with recombinant HaPyV-VP1 (5 μ g/ml in $1 \times$ PBS, 50 μ l per well) was performed overnight at 4 °C. The wells were washed with tap water and blocked with $1 \times$ PBS containing 5% NCS (Biochrom AG, Berlin, Germany) for 1 h at RT. After each incubation step, the wells were washed three times with tap water. Supernatants and serum samples were added in different dilutions ranging from 1 to 50 till 1 to 50,000 for sera and 1 to 10 till 1 to 100 for culture supernatants (50 μ l per well). The samples were incubated for 1 h at RT. The mouse monoclonal HaPyV-VP1-specific antibody 3D10 was used as positive control. As secondary antibody

for the detection of HaPyV-VP1-specific IgG antibodies, a peroxidase-conjugated goat anti-mouse IgG antibody (Dianova) was added in a dilution of 1 to 5000 (50 μ l in 1 $\times$ PBS/5% NCS per well) and incubated for 1 h at RT. The detection of HaPyV-VP1-specific IgA antibodies was revealed by incubation with a biotinylated goat-anti-mouse-IgA antibody (Southern Biotech, Alabama, USA; 1 to 5,000 in 1 $\times$ iPBS/5% NCS, 50 μ l per well) for 1 h at RT and peroxidase-conjugated streptavidin (0.1 U/ml in 1 $\times$ PBS/5% NCS, 50 μ l per well, Roche, Penzberg, Germany) for 45 min at RT. After a final washing step, 50 μ l substrate solution (1.2 g/l tetramethylbenzidine in ethanol; 0.1% H₂O₂/urea, 0.1 M NaH₂PO₄) was added to the wells. After 5 min, the reaction was stopped by adding 100 μ l 1 M H₂SO₄. The absorbance was measured at 450 nm with a reference wave length of 630 nm using a Paradigm Reader (Beckman Coulter GmbH, Krefeld, Germany).

Results

Detection of purified HaPyV-VP1

To confirm the synthesis of HaPyV-VP1 in *E. coli*, we characterized the affinity chromatography-purified protein by SDS-PAGE (Fig. 2a) and subsequent Western blot analysis (Fig. 2b). As shown in Figure 2b, there is a specific protein band reacting with the mouse monoclonal anti-VP1 antibody 3D10 at a molecular weight of 50 kDa (calculated molecular weight of 46.4 kDa; lanes 1–5 and 7). There was no specific reaction with extracts from control *E. coli* cells transformed with pET15b alone (lane 8). A control using yeast-expressed purified VP1 (lane 6) showed a pronounced band at the same size as the protein expressed here.

Induction of HaPyV-VP1-specific immune responses in mice

In order to determine the induction of VP1-specific antibodies, BALB/c mice were immunized with recombinant HaPyV-VP1 by three different routes. We compared the intensity and the generation of VP1-specific antibodies of subclass IgA and IgG by performing ELISAs with sera and organ culture supernatants. A typical IgG response was observed by intraperitoneal immunization. As shown in Figure 3a, we detected significantly higher levels of VP1-specific IgG antibodies in sera as well as in organ culture supernatants from the spleen, the mesenteric lymph nodes, and the colon. The rectal immunizations did not induce any remarkable IgG titer in serum and in organ culture supernatants in comparison to non-immunized mice (Fig. 3a). Interestingly, the induction of VP1-specific IgA antibodies by rectal immunization could be only obtained in the spleen culture supernatants (Fig. 3b). In organ culture supernatants of Peyer's patches, colon, and mesenteric lymph nodes, no significant induction of VP1-

specific IgA responses was found. The oral immunization route was observed to be very ineffective. We found no VP1-specific responses in sera or spleen culture supernatants (Fig. 4).

Discussion

The generation of mAbs by conventional hybridoma technology is generally performed by intraperitoneal application of antigen-conjugates in order to obtain high-titered IgG responses. The induction of IgA specific responses usable for hybridoma technology is rarely possible by this immunization route. Previous experiments for the generation of mAbs against human chorionic gonadotropin (hCG) with more than 10 fusions had an outcome of 15 monoclonal anti-hCG antibodies out of over thousand growing hybrids [14]. Only one was of the IgA isotype, and this was the only IgA-producing hybridoma in our whole panel (our unpublished data). Here we describe the use of recombinant HaPyV-VP1 to generate VP1-specific IgA immune responses in mice. For this purpose, mice were immunized by three different routes, and the titers

Fig. 4. VP1-specific IgG and IgA responses in serum and spleen cell culture supernatants of mice immunized orally with HaPyV-VP1.

Oral immunization of mice was performed by application of 50 μ g HaPyV-VP1 in 200 μ l 1 $\times$ PBS by gavage. Non-immunized mice received 200 μ l 1 $\times$ PBS as negative control ($n=3$). For measuring VP1-specific immune responses spleen and blood of the mice were isolated and used for HaPyV-VP1-specific IgG and IgA detection as described in Figure 3

of VP1-specific IgG and IgA antibodies were compared in sera and organ culture supernatants. We were able to induce a significant HaPyV-VP1-specific IgA response in the spleen by intrarectal immunization of mice without any further adjuvant. In comparison to other studies, where rectal immunization procedures were performed with rotavirus-derived VLPs [15, 16], an appropriate IgA response could only be induced by supplemental administration of cholera toxin or *E. coli*-derived heat-labile toxins as adjuvants. The oral and intraperitoneal application did not lead to an efficient IgA induction in our studies. This could be due to the fact that the induction of IgA responses after oral delivery requires higher concentrations of antigen than used in this study [17]. Further, the oral immunization route is often accompanied by the induction of tolerance mechanisms as described previously depending on antigen concentrations and exposure [18]. The exclusive identification of VP1-specific IgA antibodies in spleen cultures was astonishing. This may indicate that the spleen is the place where IgA-producing B-cells mature. In that case, they had to be transported from the spleen to the epithelia surrounding tissues to allow the transcytosis into the mucosa. Hitherto, the lymphoid tissues in the mucosal environment are regarded as the sites where IgA antibodies are produced. Nevertheless, the described generation of VP1-specific IgA antibodies seems to work very well and outperforms usually low efficient common immunization procedures. Monoclonal IgA antibodies could be very useful for the investigation of mucosal immune responses to enteric viruses or bacteria. Thus, the administration of *Vibrio cholerae*-specific IgA antibodies was found to protect mice against a lethal oral challenge by hampering epithelial colonization [19]. Also, influenza virus infections could be prevented in mice by the application of an H5 hemagglutinin-specific monoclonal IgA antibody [20]. In conclusion, the present study has established a routine method for the generation of IgA antibodies by intrarectal immunization of mice. Future investigations have to prove if this strategy can be applied for the hybridoma technology-based generation of monoclonal IgA antibodies of defined antigen specificity. Further, the use of chimeric viral proteins and polyomavirus-derived VLPs for the induction of peptide-specific antibody responses will be verified in ongoing experiments.

Acknowledgements

This work was supported by the Federal Ministry of Education and Research (InnoProfile 703). We thank Professor Burkhard Micheel for proofreading the article and providing comments and Katrin Pittasch for helpful technical assistance. Further, we thank Aurelija Zvirbliene and Alma Gedvilaite (Vilnius University Institute of Biotechnology, Vilnius, Lithuania) for kindly providing the anti-HaPyV-VP1-specific antibody 3D10 and purified VP1 for control experiments.

References

1. Cerutti A, Cols M, Gentile M, Cassis L, Barra CM, He B, Puga I, Chen K: Regulation of mucosal IgA responses: lessons from primary immunodeficiencies. *Ann NY Acad Sci* 1238, 132–144 (2011)
2. Cerutti A, Chen K, Chorny A: Immunoglobulin responses at the mucosal interface. *Annu Rev Immunol* 29, 273–293 (2011)
3. Fagarasan S: Evolution, development, mechanism and function of IgA in the gut. *Curr Opin Immunol* 20, 170–177 (2008)
4. Weltzin R, Lucia-Jandris P, Michetti P, Fields BN, Kraehenbuhl JP, Neutra MR: Binding and transepithelial transport of immunoglobulins by intestinal M cells: demonstration using monoclonal IgA antibodies against enteric viral proteins. *J Cell Biol* 108, 1673–1685 (1989)
5. Wycoff KL: Secretory IgA antibodies from plants. *Curr Pharm Des* 11, 2429–2437 (2005)
6. Dechant M, Vidarsson G, Stockmeyer B, Repp R, Glennie MJ, Gramatzki M, van de Winkel JGJ, Valerius T: Chimeric IgA antibodies against HLA class II effectively trigger lymphoma cell killing. *Blood* 15, 4574–4580 (2002)
7. Van Egmond M, Damen CA, van Sriel AB, Vidarsson G, van Garderen E, van de Winkel JGJ: IgA and the IgA Fc receptor. *Trends Immunol* 22, 206–211 (2001)
8. Gedvilaite A, Zvirbliene A, Staniulis J, Sasnauskas K, Krüger DH, Ulrich RG: Segments of Puumala hantavirus nucleocapsid protein inserted into chimeric polyomavirus-derived virus-like particles induce a strong immune response in mice. *Viral Immunol* 17, 51–68 (2003)
9. Ulrich RG, Gedvilaite A, Voronkova T, Kazaks A, Sasnauskas K, John R (2008): Polyomavirus-derived virus-like particles. In: *Medicinal Protein Engineering*, ed. Khudyakov YE, CRC Press, Taylor & Francis Group, pp. 249–269
10. Zvirbliene A, Samonskyte L, Gedvilaite A, Voronkova T, Ulrich R, Sanauskas K: Generation of monoclonal antibodies of desired specificity using chimeric polyomavirus-derived virus-like particles. *J Immunol Methods* 311, 57–70 (2006)
11. Grgacic EVL, Anderson DA: Virus-like particles: Passport to immune recognition. *Methods* 40, 60–65 (2006)
12. Siray H, Özel M, Jandrig B, Voronkova T, Jia W, Zocher R, Arnold W, Scherneck S, Krüger DH, Ulrich R: Capsid protein-encoding genes of hamster polyomavirus and properties of the viral capsid. *Virus Genes* 18, 39–47 (1998)
13. Laemmli UK: Cleavage of structural proteins during the assembly of the head bacteriophage T4. *Nature* 15, 680–685 (1970)
14. Böttger V, Micheel B, Scharte G, Kaiser G, Wolf G, Schmechta H: Monoclonal antibodies to human chorionic gonadotropin (HCG) and their use in two-side binding enzyme immunoassays. *Hybridoma* 12, 81–91 (1993)
15. Parez N, Fourgeux C, Mohamed A, Dubuquoy C, Pillot M, Dehee A, Charpilienne A, Poncet D, Schwartz-Cornil I, Garbarg-Chenon A: Rectal immunization with rotavirus virus-like particles induces systemic and mucosal humoral immune responses and protects mice against rotavirus infection. *J Virol* 80, 1752–1761 (2006)
16. Di Martino C, Basset C, Ogier A, Charpilienne A, Poncet D, Kohli E: Distribution and phenotype of rotavirus-specific B cells induced during the antigen-driven primary response to 2/6 virus-like particles administered by the intrarectal routes. *J Leukoc Biol* 82, 821–828 (2007)

17. Ball JM, Hardy ME, Atmar RL, Conner ME, Estes MK: Oral immunization with recombinant Norwalk virus-like particles induces a systemic and mucosal immune response in mice. *J Virol* 72, 1345–1353 (1998)
18. Weiner HL, da Cunha AP, Quintana F, Wu H: Oral tolerance. *Immunol Rev* 24, 241–259 (2011)
19. Apter FM, Michetti P, Winner III LS, Mack JA, Mekalanos JJ, Neutra MR: Analysis of the roles of antilipopolysaccharide and anti-cholera toxin immunoglobulin (IgA) antibodies in protection against *Vibrio cholerae* and cholera toxin by use of monoclonal IgA antibodies in vivo. *Infect Immun* 61, 5279–5285 (1993)
20. Ye J, Shao H, Hickman D, Angel M, Xu K, Cai Y, Song H, Fouchier RAM, Qin A, Perez DR: Intranasal delivery of an IgA monoclonal antibody effective against sublethal H5N1 influenza virus infection in mice. *Clin Vaccine Immunol* 17, 1363–1370 (2010)