Induction of Mucosal B-Cell Memory by Intramuscular Inoculation of Mice with Rotavirus

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We investigated the capacity of intramuscular (i.m.) immunization with heterologous-host rotavirus (simian strain RRV) to induce mucosal virus-specific memory B cells in mice. We found that prior i.m. immunization enhanced the magnitude of mucosal virus-specific immunoglobulin A (IgA) production but did not alter the site and timing of induction of virus-specific IgA responses after challenge.

Despite significant efforts, the development of vaccines against mucosal pathogens has been slow. Previous studies have identified several obstacles to the successful development of mucosal vaccines. First, effector B- and T-cell responses at mucosal surfaces are relatively short-lived; mucosal immunoglobulin A (IgA) responses usually wane 4 to 6 months after a primary infection (3, 9, 17, 31), and effector cytotoxic T cells rarely persist at mucosal surfaces for longer than 1 month (4, 14, 22). Second, mucosal pathogens generally have brief incubation periods, often as short as 1 to 3 days. Therefore, a successful mucosal vaccine must either (i) induce a durable effector B- or T-cell response at the mucosal surface or (ii) induce memory B or T cells capable of undergoing rapid expansion and differentiation to mucosal effector cells upon re-exposure.

Prior studies have demonstrated that parenteral immunization can induce mucosal immune responses (6, 7, 22) and protection from mucosal infections (5, 7, 11, 13, 20). In addition, parenteral immunization has been shown to enhance mucosal antibody responses following challenge (5, 18, 24, 25, 30). We recently found that intramuscular (i.m.) immunization of mice with heterologous-host rotavirus (simian strain RRV) induced partial protection against challenge with homologous-host rotavirus (murine strain EDIM) (5). In these studies, partial protection was characterized by early resolution of viral shedding. In addition, production of virus-specific IgA by lamina propria (LP) lymphocytes in i.m.-immunized mice was enhanced compared to that in unimmunized mice 6 days after challenge. These findings suggest that i.m. immunization may induce rotavirus-specific memory B cells that protect against challenge. In this report, we extend our earlier observations and examine the capacity of i.m. immunization with live rotavirus to induce memory B-cell responses in gut-associated lymphoid tissue (GALT).

First, we evaluated the ability of a primary i.m. rotavirus inoculation to induce virus-specific antibody production by peripheral lymph node and GALT lymphocytes. Conventionally reared 6- to 8-week-old female BALB/c mice (Taconic Breeding Laboratories, Germantown, N.Y.) were inoculated i.m. (in the quadriceps femoris muscle) with 2.0 × 10^6 PFU of simian rotavirus strain RRV (obtained from N. Schmidt, Viral and Rickettsial Disease Laboratory, University of California, Berkeley). Serum collected from these mice prior to inoculation did not contain rotavirus-specific antibodies, as determined by enzyme-linked immunosorbent assay (ELISA). Intestinal and inguinal lymph node (ILN) lymphoid cultures were established 0, 2, 4, 6, 8, 11, 14, and 18 days after i.m. immunization. Using three to four mice per time point, lymphoid cultures of LP fragments, mesenteric lymph node (MLN) fragments, Peyer’s patches (PP), and ILN were established as previously described (2, 6). Supernatant fluids from cultures of 22 to 24 LP fragments, 5 to 6 MLN fragments, 16 to 24 PP, and 5 to 6 ILN per group per time point were tested for the presence of rotavirus-specific and total immunoglobulins (IgA and IgG) by ELISA as described previously (19). Screening dilutions of supernatants from all fragments were tested for the production of total IgA and IgG to ensure tissue viability. The mean quantities of IgA and IgG and the ratio of virus-specific to total IgA or IgG produced by each tissue at each time point were calculated.

Transient production of virus-specific IgA by GALT induc- tive sites was observed after parenteral immunization. Eleven days after i.m. inoculation, small quantities of virus-specific IgA were produced by lymphocytes in PP and MLN (2.1 and 6.9 ng/ml, respectively). Trace quantities of virus-specific IgA were produced by LP and MLN lymphocytes 14 and 18 days, respectively, after primary i.m. inoculation (data not shown). No virus-specific IgA was produced by LP or ILN lymphocytes after primary i.m. immunization. Six weeks after i.m. immunization, virus-specific IgA production was not detected in intestinal lymphoid cultures (Fig. 1, day 0). However, primary i.m. immunization induced long-lived production of virus-specific IgG by GALT. Virus-specific IgG was first produced by PP and MLN 6 days after primary i.m. immunization (0.6 and 0.2 μg/ml, respectively) and by LP 8 days after primary i.m. immunization (1.0 μg/ml). Production of virus-specific IgG by GALT persisted for at least 6 weeks (Fig. 2, day 0).

Next, we examined the ability of i.m. inoculation to induce virus-specific memory B cells committed to IgA secretion in GALT. Six weeks after i.m. inoculation with RRV, naive or previously i.m.-immunized mice were orally inoculated with murine rotavirus strain EDIM (initially obtained from Richard Ward, Children’s Hospital Research Foundation, Cincinnati, Ohio, and propagated as previously described [5]). Mice were orally inoculated (by proximal esophageal intubation) with EDIM at a dose of 60,000 50% shedding doses (by Reed and Muench calculation). Using three to four mice per time point,
FIG. 1. Kinetics of virus-specific IgA production by PP (A), MLN (B), and LP (C) from i.m.-immunized and unimmunized animals after oral challenge. Adult BALB/c mice were inoculated i.m. with simian rotavirus strain RRV (i.m. primed). Six weeks after primary i.m. inoculation, naïve (unprimed) and i.m.-primed mice were inoculated orally with EDIM. Lymphoid cultures of systemic and gut-associated tissues were performed 0, 2, 4, 6, and 8 days after oral inoculation. Supernatant fluids were tested for the presence of rotavirus-specific and total IgA by ELISA. Virus-specific antibodies were not detected by ELISA at concentrations of <2 ng/ml. Ratios are rotavirus-specific IgA/total IgA (in nanograms per ml). ND, not done.
FIG. 2. Kinetics of virus-specific IgG production by PP (A), MLN (B), and LP (C) from i.m.-immunized and unimmunized animals after oral challenge. Adult BALB/c mice were inoculated i.m. with simian rotavirus strain RRV (i.m. primed). Six weeks after primary i.m. inoculation, naive (unprimed) and i.m.-primed mice were inoculated orally with EDIM. Lymphoid cultures of systemic and gut-associated tissues were performed 0, 2, 4, 6, and 8 days after oral inoculation. Supernatant fluids were tested for the presence of rotavirus-specific and total IgG by ELISA. Virus-specific IgG was not detected by ELISA at concentrations of <2 ng/ml. Ratios are rotavirus-specific IgG/total IgG (in nanograms per ml). ND, not done.
intestinal and ILN lymphoid cultures were established 0, 2, 4, 6, and 8 days after oral challenge as described above.

We found that i.m. immunization enhanced the magnitude of virus-specific IgA responses by LP lymphocytes after challenge. Six and 8 days after oral challenge, LP lymphocytes from mice previously immunized i.m. produced larger quantities of virus-specific IgA as well as a larger proportion of virus-specific IgA to total IgA than that produced by lymphocytes from unimmunized animals ($P < 0.005$) (Fig. 1C). However, i.m. immunization did not hasten the onset of virus-specific IgA production by GALT lymphocytes after oral challenge. In both immunized and unimmunized animals, production of virus-specific IgA by PP and MLN first occurred 2 days after challenge while that by LP lymphocytes first occurred 4 days after challenge (Fig. 1). Enhanced production of virus-specific IgA production was also observed in GALT inductive sites. Following challenge, larger quantities of rotavirus-specific IgA were produced by PP (days 4 and 6; $P < 0.05$) and MLN (days 4, 6, and 8; $P < 0.01$) lymphocytes from i.m.-immunized than by those from unimmunized mice.

These data suggest that virus-specific memory B cells committed to IgA production were resident in the inductive sites of GALT 6 weeks after i.m. inoculation. Following oral challenge, lymphocytes producing virus-specific IgA were detected initially in PP and MLN. Several days later, lymphocytes producing virus-specific IgA were detected in LP. We hypothesize that memory B cells were induced by parenteral inoculation and homed preferentially to GALT inductive sites (i.e., PP and MLN) compared with effector sites (i.e., LP). Thus, upon oral challenge, the site and time to onset of virus-specific effector B-cell responses were not altered by prior i.m. immunization.

Pierce and Gowans also found that parenteral immunization induced memory B cells resident in inductive sites of GALT (23). Antibody-containing cells were first detected in thoracic duct lymph of parenterally immunized rats 2 days after intraduodenal challenge with cholera toxin. However, antibody-containing cells were not detected in LP of parenterally immunized animals until 4 to 8 days after intestinal challenge. In addition, drainage of thoracic duct lymphocytes after mucosal challenge resulted in a marked reduction of antibody-containing cells in the LP. Similarly, Fuhrman and Cebra found that memory B cells resided in GALT inductive sites after parenteral immunization (10). They demonstrated that comparable quantities of antigen-specific B-cell precursors were induced in PP after intraperitoneal and intraduodenal inoculations. Additionally, 50% of the clonal progeny derived from PP B cells of parenterally immunized animals secreted some IgA. Our studies extend these earlier observations by demonstrating that i.m. immunization induces IgA-committed memory B cells in GALT inductive sites which may undergo differentiation and migration upon mucosal challenge and result in enhanced production of virus-specific IgA by effector cells in the LP of the small intestine.

Recent studies of the expression of homing receptors on lymphocytes and vascular addressins on endothelial cells support the hypothesis that the anatomic location of antigenic stimulation may influence the homing pattern of B cells and, therefore, the location of memory B cells (8, 16, 27, 32). Although 40 to 50% of circulating antigen-specific B cells induced by parenteral inoculation express the mucosal homing receptor α4β7, virtually all express L-selectin (16, 27). The vascular addressin ligand for L-selectin, PNA↓d, is expressed by high endothelial venules (HEV) in PP and MLN, as well as peripheral lymph nodes (21, 29). PNA↓d is not expressed by HEV in LP (1, 21). In addition, the level of α4β7 expression by B cells may be lower after i.m. immunization than after oral immunization (12). Therefore, after i.m. immunization, activated B cells, including memory cells committed to IgA production, may preferentially home to tissues which express PNA↓d, such as PP, MLN, and peripheral lymph nodes. Additional studies examining the homing potential of activated B cells generated by i.m. inoculation are under way.

Finally, we examined the ability of i.m. inoculation to induce virus-specific memory B cells committed to IgG secretion in GALT. Intestinal and ILN lymphoid cultures were established 0, 2, 4, 6, and 8 days after oral challenge of either i.m.-immunized or naive mice, as described above. We found that virus-specific IgG production by LP lymphocytes after oral challenge of i.m.-immunized mice was enhanced compared with that of unimmunized mice. Enhanced production of virus-specific IgG was first evident in PP of i.m.-immunized animals 2 days after challenge ($P < 0.05$) (Fig. 2A) and in LP 6 days after challenge ($P < 0.0005$) (Fig. 2C). Thus, similar to virus-specific IgA responses, enhanced intestinal virus-specific IgG production was first detected in GALT inductive sites and subsequently was found in effector sites. The role of IgG in protection of mucosal surfaces, however, remains unclear. IgG has been shown to migrate across epithelial cells when it is cross-linked to polymeric IgA through a multivalent antigen (15), suggesting that IgG may contribute to mucosal protection through intracellular association with antigen and subsequent activation of complement. IgG may also protect mucosal surfaces through direct neutralization of viral infectivity (26). 

Anamnestic B-cell responses have traditionally been thought to be quicker in onset, larger in magnitude, and higher in affinity than primary B-cell responses (28). We found that although i.m. immunization enhanced production of virus-specific IgA by LP lymphocytes upon oral challenge, the period of time from activation and differentiation of memory B cells in PP and MLN to that of effector B cells in LP was not shortened. Because i.m. inoculation cannot hasten the onset of virus-specific IgA production by effector B cells in the LP, it is unlikely to provide complete protection against mucosal infections characterized by short incubation periods (e.g., rotavirus). Additional studies are required to define the immunologic mechanisms by which parenteral immunization induces protection from mucosal pathogens.

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REFERENCES


