IgA Immunodeficiency Leads to Inadequate Th Cell Priming and Increased Susceptibility to Influenza Virus Infection

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IgA Immunodeficiency Leads to Inadequate Th Cell Priming and Increased Susceptibility to Influenza Virus Infection

Bernard P. Arulanandam, Roberta H. Raeder, John G. Nedrud, Doris J. Bucher, Jianhua Le, and Dennis W. Metzger

IgA is considered to be the principal Ab involved in defense against pathogens in the mucosal compartment. Using mice with a targeted disruption in IgA gene expression (IgA<sup>−/−</sup> mice), we have examined the precise role of IgA in protective anti-influenza responses after intranasal vaccination. IgA<sup>−/−</sup> mice immunized intranasally with soluble hemagglutinin (hemagglutinin subtype 1) and neuraminidase (neuraminidase subtype 1) vaccine in the absence of adjuvant were found to be more susceptible to influenza virus infection than IgA<sup>+/+</sup> mice (13 vs 75% survival after virus challenge). Inclusion of IL-12 during immunization restored the protective efficacy of the vaccine to that seen in IgA<sup>+/+</sup> animals. IgA<sup>−/−</sup> mice had no detectable IgA expression, but displayed enhanced serum and pulmonary IgM and IgG Ab levels after IL-12 treatment. Assessment of T cell function revealed markedly depressed splenic lymphoproliferative responses to PHA in IgA<sup>−/−</sup> animals compared with IgA<sup>+/+</sup> mice. Furthermore, IgA<sup>−/−</sup> animals displayed impaired T cell priming to the H1N1 subunit vaccine, with concomitant reduction in recall memory responses due to a defect in APC function. Collectively, these results provide evidence that a major role of IgA is to facilitate presentation of Ag to mucosal T cells. IL-12 treatment can overcome IgA deficiency by providing adequate T cell priming during vaccination. The Journal of Immunology, 2001, 166: 226–231.

Here is renewed interest in exploiting the mucosal immune system for purposes of vaccination to induce protective immunity. Secretory IgA is the predominant Ig isotype in mucosal tissue and is believed to be involved in inhibition of bacterial attachment and neutralization of viruses at these sites. In addition, IgA, unlike IgG, is translocated across epithelial tissue and can neutralize viruses intracellularly. Together, these findings indicate that IgA is the first line of defense in the mucosal compartment (1).

The precise role of IgA in anti-influenza immunity has recently been investigated using mice with a targeted disruption in IgA gene expression (IgA<sup>−/−</sup> mice) (2). IgA<sup>+/−</sup> and IgA<sup>−/−</sup> mice immunized with an influenza subunit vaccine in the presence of cholera toxin B subunit (CTB)<sup>3</sup> and whole cholera toxin (CT) were similarly protected against subsequent influenza virus infection. Based on these observations, the authors suggested that IgA-mediated protection was not essential for immunity against influenza virus. However, the effects of vaccination in the absence of an added immunostimulatory agent were not investigated in this study. It is possible that the use of CTB and CT, which are potent mucosal adjuvants, masked the requirement for IgA in mediating protective anti-influenza immunity.

It is likely that there is another unrecognized function for IgA at mucosal sites in addition to simple neutralization of pathogenic organisms. Accumulating evidence suggests that cognate IgA/FcγR interactions are an important component of host defense. FcγR have been identified on phagocytic cells and are thought to enable phagocytic cells to augment the protective effects of IgA (3, 4). In fact, IgA-mediated phagocytosis of Streptococcus pneumoniae has been demonstrated with murine alveolar macrophages (5).

Using IgA<sup>−/−</sup> mice, we have now demonstrated a novel role for IgA in mediating protection against influenza virus infection. Our results show that IgA<sup>−/−</sup> mice exhibit an intrinsic defect in Th cell priming and are highly susceptible to influenza virus infection upon immunization in the absence of an adjuvant. Inclusion of IL-12 during vaccination can overcome this deficiency by providing adequate Th cell priming.

Materials and Methods

Mice

C57BL/6 × 129 IgA<sup>−/−</sup> mice were generated as described previously (6) and genetically matched IgA<sup>+/+</sup> mice were used as controls. Mice were bred and housed in the animal facility at Albany Medical College and provided food and water ad libitum. Animal care and experimental procedures were performed in compliance with the Institutional Animal Care and Use Committee guidelines.

Virus infection

Intranasal treatments were performed as described previously (7, 8). Briefly, IgA<sup>−/−</sup> and IgA<sup>+/+</sup> mice were anesthetized i.p. with a combination of ketamine HCl (Fort Dodge Laboratories, Fort Dodge, IO) and xylazine (Bayer, Shawnee Mission, KA). Mice were immunized intranasally (i.n.) on day 0 with 25 μl of sterile PBS containing 1 μg of subunit influenza vaccine which consisted of soluble hemagglutinin subtype 1 (H1) and neuraminidase subtype 1 (N1) purified from influenza virus A/PR8/34. This was followed on days 0, 1, 2, and 3 with i.n. inoculation of 1 μg of recombinant murine IL-12 (Genetics Institute, Cambridge, MA) in PBS containing 1% normal mouse serum (PBS-NMS) or in the case of control mice, with PBS-NMS only. Some mice received only IL-12 in PBS-NMS.
or only PBS-NMS (no H1N1 subunit vaccine). The dose of Ag chosen was based on previous experiments in BALB/c mice (8) and no toxicity was observed with this treatment regimen. Approximately 4–5 wk after priming, BAL fluid was collected essentially as described elsewhere (7, 8). Briefly, mice were sacrificed and their tracheas were intubated using a 0.58-mm OD polyethylene catheter (Becton Dickinson, Sparks, MD). The lungs were then lavaged two to three times with PBS containing 5 mM EDTA. The recovered BAL fluid was centrifuged at 12,000 × g for 5 min at 4°C and the supernatant was stored at −70°C until use.

Analysis of Ab and isotype expression by ELISA

For examination of Ab expression, mice were immunized i.n. on days 0, 1, 14, and 28 with 25 μl of PBS containing 1 μg of H1N1 subunit influenza vaccine. This was accomplished on days 0, 1, 2, 3, and 28 by i.n. inoculation of 1 μg of IL-12 in PBS-NMS or of PBS-NMS only. Anti-H1N1 levels in serum and BAL fluid were determined by ELISA essentially as described previously (8). Microtiter plates (Nalge Nunc International, Rochester, NY) were coated overnight with 1 μg/ml H1N1 in PBS. The plates were washed with PBS containing 0.3% Brij-35 (Sigma, St. Louis, MO) and blocked for 1 h at room temperature with PBS containing 5% FCS (HyClone, Logan, UT) and 0.1% Brij-35. Serial dilutions of serum or undiluted BAL were added and the plates were incubated for 2 h at room temperature. The plates were washed and incubated with goat anti-mouse total Ig, IgG1, IgG2a, IgG2b, IgM, or IgA conjugated to alkaline phosphatase (Southern Biotechnology Associates, Birmingham, AL). After incubation for 1 h, the plates were washed and p-nitrophenyl phosphatase substrate was added to obtain color development. Plates were read at 405 nm with an ELISA microplate reader (Bio-Tek Instruments, Winooski, VT). In all cases, appropriate working dilutions and isotype specificities of the secondary Ab conjugates were determined using purified myeloma proteins of known isotypes (Sigma). Statistical significance was determined using the Mann-Whitney U test. Data were considered statistically significant if p values were < 0.05 using 50% end point titers for sera. Because of the low amounts of Ab in BAL fluids and the large dilution involved in the lavage procedure, titers could not be obtained for BAL and all samples were tested undiluted.

Lymphocyte proliferation assay

For in vitro lymphoproliferative responses, spleen cells (2 × 10^5 cells/well) were stimulated in 96-well microtiter plates with 10 μg/ml PHA with or without IL-12 (5 ng/ml or 50 ng/ml as specified in Results) in RPMI 1640 supplemented with 10% FCS (HyClone, 1 mM sodium pyruvate (Life Technologies, Gaithersburg, MD), 4 mM l-glutamine (Life Technologies), and 10 μg/ml gentamicin (Sigma). The cultures were maintained at 37°C for 72 h, pulsed with [3H]TdR (1 μCi/well; ICN Radiochemicals, Irvine, CA) for 2 h at room temperature. After incubation for 1 h, the plates were washed and reincubated with streptavidin-peroxidase conjugate (Sigma) for 2 h at room temperature. After incubation for 1 h, the plates were washed and 2, 2'-azino-di-(3-ethylbenzthiazoline-6-sulfonate) substrate (Kirkegaard & Perry, Gaithersburg, MD) was added to obtain color development. Plates were read at 630 nm with an ELISA microplate reader (Bio-Tek Instruments).

In vitro recall response to H1N1 subunit vaccine

Six- to 8-wk-old IgA−/− and IgA+/+ mice were immunized i.p. with 10 μg of H1N1 emulsified in CFA (Life Technologies). Two weeks after priming, spleens were removed and single-cell suspensions were prepared. T cells were enriched by depletion of B cells on anti-Ig-coated plates (10). Irradiated (3000 rad) spleen cells from unvaccinated IgA−/− and IgA+/+ mice were used as APCs. Enriched T cells (5 × 10^5 cells/well) and APCs (2.5 × 10^5 cells/well) were cultured along with H1N1 (1 or 10 μg/ml) with or without 5 ng/ml IL-12 in RPMI 1640 supplemented with 10% FCS. After 72 h, the cultures were assayed for proliferation by [3H]TdR incorporation. Only background levels of [3H]TdR incorporation were observed when the enriched T cells were incubated alone, with IL-12 only, or with H1N1 in the absence of added APCs.

Results

IgA−/− mice vaccinated with H1N1 subunit vaccine are not protected against influenza virus infection

It was previously reported that IgA−/− mice have the same susceptibility as IgA+/+ mice to lethal doses of influenza virus after no pretreatment or after vaccination in the presence of CT and CTB as adjuvants (2). We similarly found that unvaccinated IgA−/− and IgA+/+ mice were equally susceptible to infection (Fig. 1; PBS treatment group). However, i.n. vaccination with soluble H1N1 in the absence of adjuvant revealed a major difference between IgA−/− and IgA+/+ animals. Challenge of these mice with influenza virus 5 wk after immunization resulted in 75% survival of IgA−/− animals but only 13% survival among IgA+/+ animals (Fig. 1). Inclusion of IL-12 as a mucosal adjuvant during vaccination (8) restored the protective efficacy of the H1N1 vaccine such that 63% of IgA−/− mice survived the challenge compared with 75% of IgA+/+ mice. In mice that survive virus challenge in this manner, there is typically no detectable virus remaining in the lungs (8). Treatment with IL-12 only did not lead to significant changes in overall survival rates, which is consistent with our previous findings with BALB/c animals (8). These results demonstrate that IgA deficiency results in decreased effectiveness of vaccination and enhanced susceptibility to respiratory infection. Nevertheless, this susceptibility can be overcome by coadministration of appropriate immunostimulatory molecules such as CT or IL-12 during vaccination.
Analysis of Ab profiles after H1N1 subunit vaccination

Protection against influenza challenge in vaccinated animals treated with IL-12 is due to anti-influenza Ab (8). To examine the mechanisms responsible for the observed differences in protection between IgA−/− and IgA+/+ mice, sera and BAL fluids were thus analyzed for expression of anti-H1N1 Abs of defined isotypes. IgA+/+ mice produced serum anti-H1N1 Abs of all isotypes and the titers of these tended to be increased by IL-12 treatment, consistent with our previous findings (Fig. 2) (7–9, 11). A small decrease in serum IgA expression was seen in IgA+/+ mice due to IL-12 treatment, but this decrease was not statistically significant. The same patterns were observed in IgA−/− mice except that, as expected, H1N1-specific IgA was not detected in the sera of IgA−/− mice. Our results are essentially identical to those obtained by Mbawuike et al. (2), including a relatively large variation in responsiveness of individual mice to H1N1 vaccination, which is likely attributable to the mouse strain used.

Respiratory Ab expression in BAL fluids of i.n. immunized mice was also analyzed. In IgA+/+ mice, there were no differences in BAL Ab levels between vaccinated animals with or without IL-12, with the exception of IgA, which was slightly reduced in the presence of IL-12. Similarly, there were no differences in Ab expression between IgA+/+ animals; however, there was an overall increase in total and IgG1 anti-H1N1 production compared with IgA−/− mice (Fig. 3). In addition, IgA−/− mice vaccinated in the presence of IL-12 had enhanced IgM Ab levels compared with IgA+/+ mice treated in the same fashion. Thus, IgA+/+ and IgA−/− mice did not differ dramatically in levels of specific Abs (except for IgA) but IL-12 treatment clearly had a stronger enhancing influence in IgA+/+ animals.

IgA−/− mice exhibit depressed lymphoproliferative responses

We also examined the proliferative responses of spleen cells from naive IgA−/− and IgA+/+ mice to T cell mitogens. It was found
that IgA−/− mice had a significant decrease in the in vitro proliferative response to PHA compared with IgA+/+ mice (Fig. 4A). Addition of IL-12 to IgA−/− spleen cell cultures reconstituted the proliferative responses to wild-type levels. Since IL-12 is a potent inducer of IFN-γ, IFN-γ expression was analyzed after in vitro stimulation. RT-PCR analysis of PHA-stimulated IgA+/+ and IgA−/− cells revealed low levels of IFN-γ expression that was increased by exposure to IL-12 (Fig. 4B). IFN-γ protein levels in the supernatant fluids were comparable between IgA+/+ and IgA−/− PHA-stimulated cultures (Fig. 4C). Of note, this assay revealed that IgA−/− spleen cells were much more responsive to IL-12 than IgA+/+ cells, with a 2-fold difference in IFN-γ production between IL-12-stimulated IgA−/− and IgA+/+ spleen cell cultures.

**Discussion**

Secretory IgA is the principle Ab isotype expressed in mucosal tissues and is thought to play a pivotal role in host defenses at mucosal sites. However, IgA does not efficiently mediate complement fixation; therefore, its precise function in pathogen elimination has remained elusive. Using IgA−/− mice, we have now revealed a surprising requirement for IgA in mucosal APC function and T cell priming to influenza virus.

Unimmunized IgA−/− and IgA+/+ animals had similar susceptibility to influenza virus infection. However, upon i.n. vaccination with soluble H1N1 subunit vaccine in the absence of adjuvant, IgA−/− animals were not protected against influenza virus infection. Inclusion of IL-12 during immunization restored protective immunity to the levels observed in IgA+/+ mice. Mbawuike et al. (2) recently reported that IgA is not necessary for prevention of influenza virus infection. In their study, IgA−/− mice vaccinated with a subunit vaccine in the presence of CTB and CT were found

**FIGURE 5.** IgA−/− mice display impaired recall memory responses to H1N1 subunit vaccine. IgA−/− mice were immunized i.p. with 10 μg of H1N1 subunit vaccine. Two weeks after priming, spleens were removed and single-cell suspensions were prepared. T cells were enriched by depletion of B cells on anti-Ig-coated plates. Irradiated (3000 rad) spleen cells from unvaccinated IgA−/− and IgA+/+ mice were used as APCs. Purified T cells and APCs were cultured with H1N1 with or without IL-12 and assayed after 72 h for [3H]Tdr incorporation. Background proliferation for IgA+/+ T cells cultured with IgA+/+ APCs or IgA−/− APCs was 2100 ± 544 and 1820 ± 492, respectively. The amount of proliferation in cultures of IgA+/+ T cells plus IgA−/− APCs were significantly different (p < 0.05) from all of the other experimental groups.
to be completely protected from subsequent influenza virus challenge. Like the present study, they used an H1N1 influenza virus; therefore, differences in antigenic recognition are unlikely to account for the differing results between the two studies. However, Mbawuike et al. (2) did not examine the protective efficacy of vaccination in the absence of CTB or CT. Both of these immunomodulators are potent mucosal adjuvants and therefore may have masked the need for IgA to achieve appropriate immune cell priming. In our studies, vaccination was performed in the absence of an adjuvant and, under these conditions, IgA−/− mice were found to be highly susceptible to influenza virus infection, demonstrating the need for IgA in protective antiviral immunity.

Others have also examined the role of IgA in protection against viral infection. Renegar and Small (12, 13) reported that IgA Abs in nasal secretions are the sole mediators of immunity to influenza virus infection in immunized mice. However, Gerhard and colleagues (14) found that IgG but not IgA or IgM Abs were able to cure influenza virus pneumonia. IgA was also reported not to be essential in protective immunity against other mucosal pathogens such as Helicobacter pylori and rotavirus (15, 16). However, using a ligated intestinal loop model to evaluate the ability of IgA to neutralize CT toxicity, Lycke et al. (17) found that IgA−/− mice display significant toxicity. Although these animals had elevated levels of anti-CT IgM Abs in the gut, this was not sufficient to provide functional protection.

The basis for increased susceptibility of IgA−/− mice to influenza virus was analyzed by in vitro lymphoproliferative responses. It was found that IgA−/− mice exhibited depressed proliferative responses upon stimulation with PHA, and these responses were reconstituted to normal levels with IL-12. Flow cytometric analysis revealed that IgA−/− mice had similar levels of CD3+ and B220+ lymphocytes in the splenic compartment compared with IgA+/+ animals (B. P. Arulanandam, V. C. Huber and D. W. Metzger, unpublished observations). This would suggest that the decreased proliferative responses observed in the IgA−/− mice were not due to skewed lymphocyte populations within the splenic compartment. However, further assessment of IgA−/− mice revealed a major impairment of APC function. This dysfunction could be overcome with IL-12, suggesting that the APCs in the IgA−/− animals were not sufficiently activated to allow adequate priming of memory T cells. Although IL-12 is known to also activate cytolytic T cells, such T cells are likely to be involved in recovery from infection whereas antiviral Ab is important for protection in immunized animals treated with IL-12 (8).

In humans, IgA deficiency is the most common immunodeficiency and includes both healthy individuals and those with severe disease (18, 19). In the latter group, individuals with IgA deficiency have a greater predisposition to autoimmune and atopic diseases (20–22). Interestingly, T cell immunity can also be affected in IgA-deficient individuals. In fact, Ammann and colleagues (23) found a marked reduction in the proliferative responses of lymphocytes from IgA-deficient children to PHA stimulation. IFN production in response to T cell mitogens was also reported to be depressed in IgA-deficient individuals (24). Our results would strongly suggest that the impairment of T cell immunity observed in IgA-deficient individuals is a result of underlying APC defects. Indeed, it was recently found that the human FcγR (CD89) is a functional receptor for effective Ag presentation (L. Shen, unpublished observation).

It is of interest that splenic lymphocytes from IgA−/− animals produced 2-fold more IFN-γ than IgA+/+ cells in response to IL-12. Furthermore, we have found increased production of NO from peritoneal cells of IgA−/− animals stimulated with IL-12 compared with IgA+/+ mice (B. P. Arulanandam and D. W. Metzger, unpublished observations). The increased production of these molecules may be a compensatory mechanism for the absence of IgA and related to the anti-inflammatory properties of this Ab isotype (1, 25, 26). Experiments are currently in progress to address the role of IgA in the regulation of inflammatory responses.

In summary, our results indicate an important role of IgA in APC function and development of antiviral T cell memory. IgA−/− mice vaccinated i.n. with a soluble influenza subunit vaccine alone were highly susceptible to influenza virus infection and exhibited diminished protective immunity. These animals also displayed impaired Th cell priming due to a surprising defect in APC function that could be overcome by IL-12 treatment. Interestingly, it was recently shown by others (27) that B cells are critical for appropriate development of M cells and organogenesis of mucosal immune barriers.

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References


