Immunoglobulin A Mediation of Murine Nasal Anti-Influenza Virus Immunity

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Most mice which have recovered from influenza virus infection are immune to reinfection with the same influenza virus. This immunity could be abrogated by the intranasal instillation of anti-immunoglobulin A (anti-IgA) but not of anti-IgG or anti-IgM antiserum. Thus, IgA is the major, if not the sole, mediator of nasal immunity to influenza virus in immunocompetent mice.

Since Tomasi et al. first recognized secretory immunoglobulin A (S-IgA) (18), a number of studies have described a correlation between an increased respiratory tract S-IgA level and protection against challenge with a number of pathogens (2, 3, 7, 8, 11, 17). On the basis of this correlation, S-IgA is hypothesized to play a major role in respiratory tract mucosal immunity; however, there is no direct proof that IgA is the cause of that immunity. Other immunoglobulins, such as IgM (13) or transudated IgG (4, 19), as well as nonimmunoglobulin antimicrobial factors, including lysozyme, lactoferrin, interferon, and secretory peroxidases (1, 6), have been postulated to contribute to the observed local immunity. To obtain proof that S-IgA can mediate local immunity, intravenously administered polymeric IgA anti-influenza virus monoclonal antibody was shown to be selectively transported into nasal secretions and to protect against challenge with the homologous virus (15). This protection could be abrogated by intranasal administration of anti-IgA antiserum. This latter observation has made it possible to now determine whether S-IgA does mediate local immunity against influenza virus. Our approach has been to selectively inhibit nasal S-IgA antibody in immune mice by giving anti-IgA antibody by nose drops and thereby to show that the previously immune mice are made susceptible to influenza virus infection.

The experimental design is summarized in Fig. 1. Nonimmune control mice and convalescent mice, i.e., mice that had recovered from an influenza virus infection 4 to 6 weeks earlier and would therefore be expected to be immune, were treated intranasally with saline, normal rabbit serum (NRS), or antiserum to α, γ, or μ chain during a 24-h period. Ten minutes after the initial treatment, the mice were challenged intranasally while awake with 200 50% mouse infective doses of influenza virus mixed with saline, NRS, or antiserum. One day later they were killed, and their nasal washes were assayed for virus shedding. Nasal tissue was not assayed for virus because of the fear that serum and extracellular tissue antibodies released during tissue homogenation would neutralize virus and obscure infection.

Figure 2 summarizes the results of five separate experiments. The normal range of virus shedding in nasal washes of nonimmune mice is shown in the left column of Fig. 2. In

experiment 1, convalescent mice pretreated with 10 μl of undiluted anti-IgA and then challenged with influenza virus in anti-IgA antiserum shed amounts of virus comparable to the amount of virus shed in the nasal secretions of control nonimmune anti-IgA treated mice (*P* was not significant), and both groups shed more virus than saline-treated convalescent controls (*P* < 0.01). The anti-α-chain antiserum did not affect influenza virus growth in eggs but did inhibit monoclonal IgA anti-influenza virus neutralization in vitro (data not shown). In experiment 2, convalescent mice were pretreated with 20 μl of undiluted anti-IgA antiserum or saline and then challenged as previously described. Five of six anti-IgA antiserum-treated convalescent mice in this experiment shed virus in their nasal secretions, and the titer was comparable (*P* was not significant) to that of nonimmune anti-IgA-treated mice. Saline-treated convalescent mice again shed less virus than either of the anti-IgA-treated groups (versus anti-IgA-treated convalescent mice, *P* < 0.05; versus anti-IgA-treated nonimmune mice, *P* < 0.001).

To demonstrate that the suppression of convalescent immunity was due to the rabbit anti-IgA antibodies and not to nonspecific factors in rabbit serum, convalescent mice were pretreated with 20 μl of undiluted NRS and then challenged with influenza virus in a 1:5 dilution of NRS (experiment 3). None of the six NRS-treated convalescent animals shed virus, while all five nonimmune mice treated with NRS shed virus (*P* = 0.002, one-tail Fisher exact test [20]).

To determine whether IgG or IgM made detectable contributions to nasal anti-influenza virus immunity, convalescent mice were pretreated intranasally with anti-IgG antiserum (experiment 4) or a mixture of anti-IgG and anti-IgM antisera (experiment 5). Anti-IgG-treated and anti-IgG plus anti-IgM-treated convalescent mice remained immune. Both experimental groups shed little or no virus, comparable to saline-treated convalescent mice but significantly less (*P* < 0.001) than nonimmune mice treated with anti-γ chain or the mixture of anti-γ chain and anti-μ chain. The anti-γ-chain antiserum did not affect influenza virus growth in eggs but did inhibit monoclonal IgG anti-influenza virus neutralization in vitro (data not shown). The titer of the anti-IgG antibody was similar to the titer of the anti-IgA antibody discussed above. The anti-μ-chain antiserum was shown by Ouchterlony double diffusion to be specific for the μ chain (data not shown), but because we did not have an IgM anti-influenza virus monoclonal antibody, its ability to inhibit neutralization was not measured.

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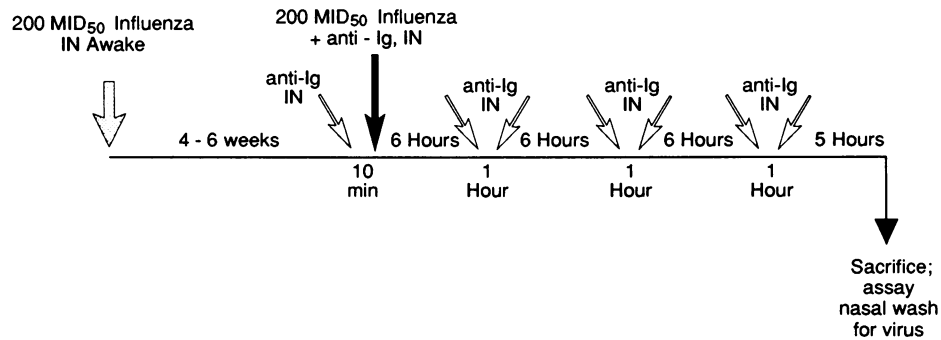
FIG. 1. Convalescent immunity abrogation protocol. Female BALB/c mice were infected intranasally (IN) while awake (stippled arrow) with 200 50% mouse infective doses ($MID_{50}s$) of A/PR8-Mt. Sinai (H1N1) influenza virus (the gift of Walter Gerhard, Wistar Institute, Philadelphia, Pa.). This produces an initial nasal infection (21) and induces prolonged immunity (10). Four to six weeks after infection, mice were pretreated intranasally (first open arrow) with undiluted rabbit anti-mouse IgA (rabbit anti-mouse α chain antiserum purchased from ICN) or sterile saline, which was followed 10 min later by 200 50% mouse infective doses of influenza virus in 20 μl of either a 1:5 dilution of rabbit anti-mouse IgA or saline (black arrow). Mice received further IN treatments with 20 μl of a 1:5 dilution of anti-IgA or saline at 6, 7, 13, 14, 20, and 21 h postinfection (open arrows). Control (nonimmune) mice were treated similarly with virus and anti-IgA. In some experiments NRS or anti-IgG and/or anti-IgM (affinity-purified goat anti-mouse γ chain and goat anti-mouse μ chain antibodies purchased from Sigma) was substituted for the anti-IgA. Twenty-five to 27 h postinfection, mice were anesthetized intravenously with pentobarbital and exsanguinated. The nasopharynxes were lavaged (9) with saline containing 1:100 anti-IgA (to prevent post facto in vitro neutralization of virus by IgA antibody), and the nasal wash fluid was assayed for virus by using embryonated hen's eggs as previously described (15). 50% egg infective dose viral titers were calculated by the method of Reed and Muench (14). The immune statuses of both the convalescent and nonimmune animals were confirmed by measuring serum IgG anti-influenza virus antibody. Convalescent mice had enzyme-linked immunosorbent assay titers ranging from 0.1 to 0.8; noninfected mice had serum IgG anti-influenza virus antibody titers ranging from 0.3 × 10^{-3} to 0.5 × 10^{-3}.

Analysis of the pooled data from the 5 individual experiments revealed that, following influenza virus challenge, all 40 nonimmune mice shed virus in their nasal secretions (mean titer $\log_{10}$ 50% egg infective dose, 2.26 ± 0.92), regardless of whether the virus was administered in the presence of saline, NRS, or any of the antisera. Of the 27 convalescent mice challenged with influenza virus in saline or NRS, 17 failed to shed virus in their nasal secretions, while the remaining 10 shed virus at a reduced level (mean titer $\log_{10}$ 50% egg infective dose, 0.56 ± 0.44) relative to the nonimmune mice ($P < 0.001$). Thus, all convalescent mice treated with saline or NRS showed complete or partial protection against influenza virus challenge. In contrast, 11 of 12 convalescent mice pretreated with anti-IgA antiserum and challenged with influenza virus shed virus (mean titer $\log_{10}$ 50% egg infective dose, 1.08 ± 0.6), and the amount shed was significantly greater ($P < 0.001$) than that shed by the 27 convalescent control mice treated with saline or NRS.

The results presented here, in which convalescent immunity was abrogated by anti-IgA but not by anti-IgG or anti-IgM, demonstrate that IgA is the major, if not the sole, mediator of mucosal immunity to influenza virus in the murine nose. The degree of abrogation of nasal immunity by anti-IgA treatment seen in convalescent animals was comparable to the degree of anti-IgA abrogation seen in polymeric IgA passively protected mice, in which the only mucosal immunity present is due to IgA (15). It is possible that IgG or IgM plays a role in convalescent mucosal immunity, especially in IgA deficiency. However, since neither anti-IgG nor anti-IgM antiserum had any effect upon the protection against influenza virus challenge in convalescent mice, it appears that these immunoglobulins make negligible contributions to nasal immunity in normal mice.

The major role IgA plays in mucosal immunity to influenza virus has implications for vaccine development strategies. Influenza virus is an important pandemic respiratory pathogen of humans which results in tremendous economic losses
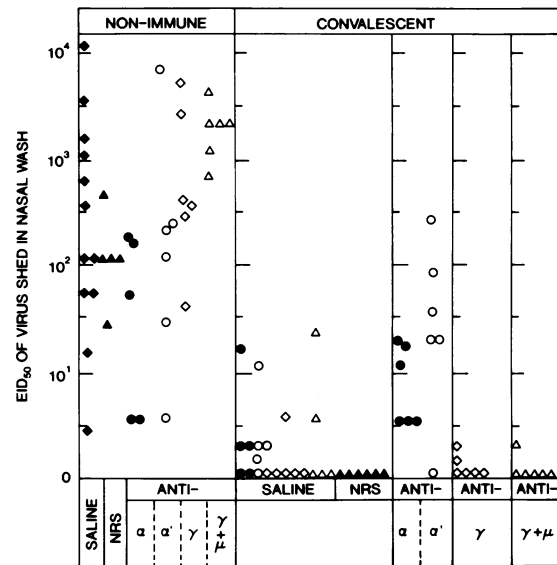

FIG. 2. Effect of intranasal installation of anti-IgA, anti-IgG, anti-IgM, or NRS on nasal immunity of convalescent mice. Virus shedding from 12 nonimmune saline-treated mice (◆) is shown in the left column. Experiment 1 (●): nonimmune and convalescent mice were treated intranasally with 10 μl of anti-IgA (anti-α) and challenged with influenza virus in anti-IgA, as described in the legend to Fig. 1. Convalescent control mice were similarly treated, except saline was substituted for anti-IgA. Experiment 2 (○): nonimmune and convalescent mice were treated with 20 μl of anti-IgA (anti-α'). Convalescent control mice were treated similarly with saline. Experiment 3 (▲): nonimmune and convalescent mice were treated with 20 μl of NRS. Experiment 4 (◇): nonimmune and convalescent mice were treated with 20 μl of anti-IgG (anti-γ). Convalescent control mice were treated with saline. Experiment 5 (△): nonimmune and convalescent mice were treated with 20 μl of anti-IgG and anti-IgM (anti-γ + μ). Convalescent control mice were treated with saline. EID_{50}, 50% egg infective dose.

(6, 12, 16) as well as deaths in the very young and the very old (5, 6). If IgA is primarily responsible for defense against influenza virus infection in humans as it is in mice, influenza virus vaccination protocols should exploit routes of immunization which activate the common mucosal immune system and lead to the production of S-IgA.

The development of the abrogation technique has made it possible to determine the contribution IgA makes to local immunity in the upper respiratory tract. The model may be applicable to the investigation of immunity at other mucosal surfaces and should lead to a better understanding of specific host defenses against a variety of mucosal pathogens.

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