

## Immunoglobulin A Monoclonal Antibodies Protect against Sendai Virus

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**Immunoglobulin A anti-Sendai virus HN protein monoclonal antibodies, generated via a mucosal immunization protocol, were shown to neutralize virus in vitro and, when passively administered to the mouse respiratory tract, to protect against Sendai virus in vivo. Thus, immunoglobulin A antibodies by themselves can protect against respiratory virus infection.**

The secretory immune system with its predominant antibody, immunoglobulin A (IgA), poses the initial immunological barrier to viruses at mucosal surfaces, the portal of entry for many viruses, including respiratory viruses. Moreover, resistance to pulmonary virus infections correlates with the presence of specific IgA antibodies in respiratory secretions (10, 11, 16). Whether IgA alone can afford antiviral protection without the participation of other classes of antibody such as IgG and IgM or antiviral T cells is not known. This question could be approached by using monoclonal antibodies (MAB). However, conventional immunization protocols tend not to result in IgA hybridomas. For example, among more than 150 Sendai virus-specific MAB described in the literature, none have been reported to be of the IgA isotype (5, 12, 15, 18).

Using instead a mucosal immunization procedure similar to published methods for generating IgA hybridomas (2, 3, 8) but employing cholera toxin as a mucosal adjuvant (4), we were able to produce a large number of IgA MAB against Sendai virus, a member of the parainfluenza group and a natural respiratory pathogen of mice. The availability of such IgA MAB has enabled us to inquire whether IgA antibodies by themselves are capable of offering protection against a respiratory pathogen.

**Immunization procedure.** For immunization, Sendai virus (ATCC VR-105) was grown in eggs and concentrated and purified by differential centrifugation (6). Virus was quantified in a plaque assay on LLCMK<sub>2</sub> monkey kidney cells and, when desired, inactivated with 0.05%  $\beta$ -propiolactone plus 6 min of UV irradiation 20 cm from a germicidal lamp.

Sendai virus-free BALB/c mice obtained from the Jackson Laboratory (Bar Harbor, Maine) and subsequently housed in isolated rooms were immunized intragastrically four times over a 6-week period, the first three times with  $10^{10}$  PFU of live Sendai virus plus 10  $\mu$ g of cholera toxin (Sigma Chemical Co., St. Louis, Mo.). For the last immunization, cholera toxin was omitted, inactivated virus was used, and the mice also received an intravenous boost with  $10^9$  PFU equivalents of inactivated virus. Three days later, the mice were sacrificed, and their spleen cells were hybridized to SP2/0 myeloma cells (7). Clones, obtained by limiting dilution, were screened for secretion of anti-Sendai virus IgA antibody by an enzyme-linked immunosorbent assay (ELISA). After multiple subclonings, stable IgA secretors were injected intraperitoneally into pristane-primed BALB/c mice, and the ascitic fluid was harvested and clarified.

**ELISA and other assays.** The anti-Sendai virus ELISA was modified slightly from that of Parker et al. (14), with endpoints determined by serial dilution and isotype detected with individual alkaline phosphatase-conjugated antisera specific for mouse IgA, IgG, and IgM (Zymed, South San Francisco, Calif.). The specificity of these conjugates was confirmed with purified MOPC-315 IgA myeloma protein and other IgG and IgM myeloma and hybridoma proteins as standards. Immunoblotting (Western blotting) was performed by the method of Towbin et al. (17) with clarified hybridoma ascites fluid to determine the antigenic specificity of the individual MAB.

Virus neutralization was measured in a plaque reduction assay; the titer was scored as the MAB dilution which yielded a 50% reduction in the number of plaques compared with that in hybridoma ascites of irrelevant specificity. MAB were tested for hemagglutination inhibition, a measure of reactivity to the hemagglutinin (H) portion of the Sendai virus hemagglutinin-neuraminidase (HN) molecule, and hemolysis inhibition, a measure of antibody interference with either the HN or the fusion (F) protein, in accordance with the methods of Orvell et al. (12, 13) and Aymard-Henry et al. (1).

**MAB production and characterization.** Of approximately 800 hybridoma colonies, 10% were IgA anti-Sendai virus antibody secretors as determined by ELISA. Ouchterlony immunoprecipitation was used to confirm the IgA isotype. After multiple subclonings, 50 hybridomas remained stable IgA secretors. Of these, 25 were characterized further.

Table 1 summarizes the characteristics of nine of the most reactive MAB. All nine have anti-Sendai virus titers by ELISA, and viral antigen reactivity can be definitely assigned by Western blot analysis in four, all of which react with the HN protein. Eight of the nine hybridomas produce antibody with both hemagglutinin and hemolysis inhibition reactivities. Inasmuch as all MAB with hemolysis inhibition activity also possess hemagglutinin inhibition activity, both tests likely reflect HN protein rather than F protein reactivity.

The ability of MAB to neutralize virus in tissue culture was ascertained by plaque reduction assay. Seven of the nine MAB gave a neutralization titer  $\geq 10^3$ .

**Passive immunization studies.** Ascites containing IgA MAB (50  $\mu$ l) was administered intranasally to lightly etherized Sendai-free (C57BL/6  $\times$  DBA/2)F<sub>1</sub> (B6D2) mice. The mice were subsequently challenged with  $10^2$  or  $10^4$  PFU of Sendai virus intranasally, again while under light ether anesthesia. After challenge, IgA MAB was again administered intrana-

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TABLE 1. Characterization of IgA anti-Sendai virus MAb<sup>a</sup>

Clone no.	Anti-Sendai virus titer (ELISA) <sup>a</sup>	Response in <sup>b</sup> :		Western blot binding	Neutralization titer <sup>c</sup>
		HI (HN)	HLI (HN, F)		
9	>2 × 10 <sup>5</sup>	+	+	HN	>10 <sup>3</sup>
37	>2 × 10 <sup>5</sup>	+	+	HN	>10 <sup>3</sup>
77	2.5 × 10 <sup>4</sup>	+	+		10 <sup>3</sup>
170	>2 × 10 <sup>5</sup>	+	+	HN	>10 <sup>3</sup>
380	2 × 10 <sup>5</sup>	+	+	HN	>10 <sup>3</sup>
390	1 × 10 <sup>5</sup>	+	+		>10 <sup>3</sup>
474	3.2 × 10 <sup>3</sup>	-	-		10 <sup>1</sup>
561	2 × 10 <sup>5</sup>	+	+		10 <sup>3</sup>
568	>2 × 10 <sup>5</sup>	+	+		<10 <sup>1</sup>

<sup>a</sup> Of nine clones, eight had anti-Sendai virus titers (ELISA) ≥2.5 × 10<sup>4</sup>.

<sup>b</sup> HI, Hemagglutination inhibition assay; HLI, hemolysis inhibition assay.

<sup>c</sup> Of nine clones, eight had neutralization titers ≥10<sup>1</sup>, and seven had neutralization titers ≥10<sup>3</sup>.

sally. Three days later, virus titers of lung homogenates were determined. Based on the in vitro characterizations (Table 1), we used a 1:1 mixture of IgA MAb from clones 9 and 37 for the in vivo experiments. Control animals received either saline or an irrelevant IgA MAb (MOPC-315).

Figure 1 shows the results of four passive-immunization experiments involving 14 animals which received antiviral IgA MAb and 14 control animals. All control animals but one were productively infected, with lung virus titers between 10<sup>5</sup> and 10<sup>8</sup> PFU 3 days after virus challenge. In contrast, all animals which received virus-specific IgA MAb exhibited

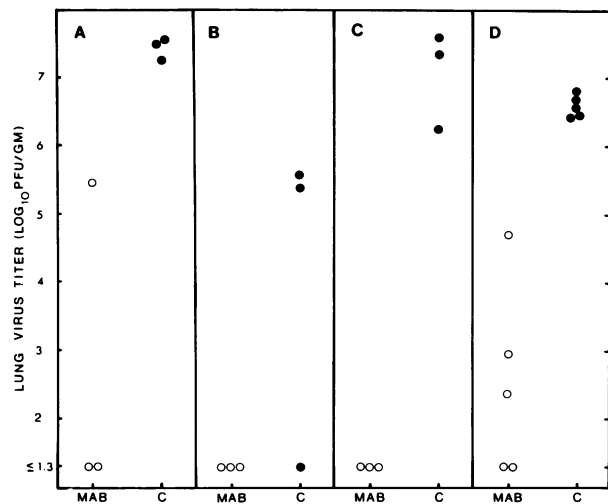


FIG. 1. Results of four experiments in which mice were passively immunized intranasally with anti-Sendai virus IgA MAb before and after intranasal challenge with live Sendai virus. Three days after challenge, lung virus titers were determined by plaque assay. Each symbol represents a single animal. (A) Mice received MAb 1 h before and 24 and 48 h after intranasal challenge with 10<sup>2</sup> PFU of Sendai virus. Virus titers were determined for animals (MAB) which received anti-viral MAb (○) and for control animals (C) which received saline (●). (B) Antiviral MAb (○) or MOPC-315 (●), an irrelevant IgA myeloma protein, was administered 4 h before, 30 min before, and 4 h after intranasal challenge with 10<sup>2</sup> PFU of Sendai virus. (C) Mice received either antiviral MAb (○) or MOPC-315 (●) 30 min before and 4 h after challenge with 10<sup>4</sup> PFU of Sendai virus. (D) Mice received either anti-Sendai virus MAb (○) or MOPC-315 (●) 4 h before, 30 min before, and 4 h after a 10<sup>4</sup>-PFU challenge dose of Sendai virus.

reduced virus titers when compared with the controls. Of the 14 experimental animals, 10 were completely protected, 2 had virus titers more than 1,000-fold lower than those of controls, and 2 had virus titers 80-fold lower than those of controls. When the log<sub>10</sub> PFU data from all 14 control animals are pooled and compared by the Fischer unpaired *t* test with the pooled log<sub>10</sub> PFU data from the 14 animals which received MAb, the difference is highly significant (*P* < 0.0005).

Viral infections of the respiratory tract account for a significant proportion of the morbidity and mortality seen in medical practice today. The mucosal immune system, with its primary effector, secretory IgA, is thought to play a pivotal role in defense of the airways against viral agents. While many studies have correlated the presence of IgA antibodies in respiratory secretions with resistance to viral infection, to our knowledge this is the first report directly demonstrating that IgA antibodies by themselves can mediate resistance to respiratory virus challenge. The results thus strengthen the rationale for developing mucosal immunization regimens designed to lead to the production of mucosal IgA antibodies. If effective, IgA antibodies are especially desirable since they are much less phlogistic and less likely to participate in immunopathologic sequelae than antibodies of the IgG or IgM class (9).

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