Respiratory Virus Infection of Mice Provokes a Permanent Humoral Immune Response

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We have observed that respiratory virus infection of mice provokes an extremely persistent humoral immune reaction, due to a long-sustained population of antibody-secreting cells in the bone marrow. Theories of humoral immunity that strongly distinguish primary and secondary reactions thus may not adequately describe the immune response to respiratory viruses.

Intending to compare the characteristics of primary and secondary exposure of mice to infectious Sendai virus, a natural pathogen of mice, we inoculated Avertin-anesthetized 129/Sv or C57BL/6 mice intranasally, using a 50% egg-infectious dose of 500 for each C57BL/6 mouse and a dose of 30 per mouse for the much more-susceptible 129/Sv strain. The serum antibody response is shown in Fig. 1. The titer was determined by plotting the extent of substrate conversion in enzyme-linked immunosorbent assays (ELISAs) (7) against the logarithm of the reciprocal of the serum dilution and taking the midpoint of the linear section of the sigmoid curve produced. This yields a titer which is about 10-fold lower than that determined from the highest dilution giving a significant reaction. The humoral response was dominated from the outset by immunoglobulin G (IgG) in both mouse strains but with a marked strain dependence of IgG isotype distribution. IgG2a was hugely predominant in 129/Sv mice (Fig. 1A), while IgG2b, IgG1, and IgG2a shared in the response of C57BL/6 mice (Fig. 1B). These strain-specific patterns of isotype expression had already been established 10 days after infection and were maintained thereafter. Coutelier et al. (3, 4) reported that while IgG1 typified the response to protein antigens, IgG2a predominated in the antibody response of mice to a range of viruses but observed, in contrast to the data presented in Fig. 1, that this was equally marked in 129/Sv and C57BL/6 mice exposed to Sendai virus. Conceivably, some genetic drift between ostensibly similar mouse colonies may account for the difference. More surprising was the longevity of the response, which showed little diminution throughout a period of 3 months after infection. As the bone marrow has been reported to be an important source of serum antibody in multiply immunized animals (1), we checked bone marrow from C57BL/6 mice exposed to Sendai virus for the presence of virus-specific antibody-forming cells (AFC), using the enzyme-linked immunospot ELISPOT technique (5, 13) for the detection of individual AFC. Indeed, a single round of infection with Sendai virus did elicit long-sustained populations of bone marrow AFC (Fig. 1C).

Because Sendai virus can spread notoriously rapidly throughout mouse colonies and establish an endemic infection, we were concerned that a long-sustained response might simply reflect continual reexposure to exogenous virus. Therefore, although routine monitoring had not shown any evidence of virus spread, we tested serum obtained from sentinel

C57BL/6 mice that were housed (in cages without filter tops) in the same negative pressure cubicle on a shelf below the experimental animals (held in cages with filter tops) throughout the course of the experiments for which the results are shown in Fig. 1. In ELISAs run in parallel with the titration of sera from experimentally infected mice (Fig. 1), no serum antibody capable of binding Sendai virus could be detected in any of six sentinel mice.

In order to gain further information about the longevity and generality of humoral responses in mice exposed to respiratory viruses, we examined groups of C57BL/6 mice that had been infected more than 1 year previously with Sendai virus or with the mouse-adapted HKx31 (H3N2) influenza A virus (11). Sera from such animals generally had antiviral-binding titers (data not shown) comparable to those of the C57BL/6 group described above, sampled earlier in their response. Frequencies of virus-specific AFC detected in the spleen, mediastinal lymph node, and bone marrow are listed in Table 1 for four groups of mice that had been infected 15 months previously with influenza HKx31 and 14 or 15 months previously with Sendai virus or that were uninfected. Mice that had been exposed to virus had small but significant populations of virus-specific AFC in the spleen and in the mediastinal lymph node (which drains the lungs), but they exhibited much larger populations in the bone marrow. Mice that had been exposed to Sendai virus, a natural mouse pathogen, tended to show somewhat higher and more-consistent frequencies of overall virus-specific AFC in the bone marrow than did those infected with influenza virus but displayed a very marked variation from animal to animal in the incidence of isotype-specific AFC, as indicated by large standard deviations. This effect is more readily appreciated by inspection of Fig. 2, photographs of the ELISPOTs developed from bone marrow samples from the group of mice sampled 15 months after Sendai virus infection (Table 1). ELISPOTs of Sendai virus-specific AFC in samples of 10^5 bone marrow cells are shown, in duplicate, for three mice that had been infected at the same time and housed together; IgG2a dominated in mouse 1, IgG2b dominated in mouse 2, and IgG1 dominated in mouse 3. Evidently, not every detail of the long-term isotype pattern of antiviral antibody secretion is determined by circumstances at the time of infection; some stochastic features must play a role. The overall AFC frequency indicates the persistence of approximately 10^5 IgG-secreting plasma cells in the bone marrow of individual mice once exposed to Sendai virus, sufficient to support the secretion of roughly 0.25 μ g of IgG per day.

Serum antiviral IgM develops a significant titer shortly after infection, which dwindles rapidly to negligible levels. Yet,

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FIG. 1. Response of mice to Sendai virus infection. (A) Serum antibody response of 129/Sv mice. Only IgG1, IgG2a, and IgG2b are shown; the titers of other isotypes were generally negligible. The titer represents the midpoint of the linear section of the curve relating to the logarithmic reciprocal of the serum dilution to the ELISA reading. Each point represents the mean for three mice, analyzed individually, with one standard deviation above and below the mean indicated by a narrow line. Mice were sampled 10, 16, 27, and 90 days after infection; points on the graph are slightly offset to avoid crowding. (B) Serum antibody response of C57BL/6 mice. Groups of three mice were sampled, as described for panel A, at 10, 18, 23, 41, 61, and 90 days after infection. The star indicates the mean antiviral titer for IgG isotypes (background) of six uninfected control mice housed near the experimental animals to act as sentinels for virus spread. (C) Bone marrow AFC of C57BL/6 mice. Points represent the means of frequency estimates of Sendai virus-specific AFC producing isotypes as shown for three mice.

Table 1 shows antiviral IgM AFC persisting in the bone marrow of mice infected long before, at a frequency comparable to that of AFC secreting the major serum IgG components, and in the spleen at a frequency well above that of any other isotype. In compiling these data, all spots that appeared to have the appropriate morphology when viewed under low-power magnification were included. However, Fig. 2 reveals that spot intensity varies greatly both between isotypes and within a specific isotype; in particular, IgM spots are generally much weaker than IgG spots. When a general intensity difference is obvious between populations of spots of different isotypes, we think that this most likely reflects a genuine discrepancy in the secretion rate and/or binding affinity of the Igs produced by the different classes of AFC. It seems likely that the intense IgG spots result from terminally differentiated plasma cells, secreting antibody at a maximal rate, while many of the less-intense IgM spots might be due to less completely differentiated immunoblasts, which are much less efficient in antibody secretion. Nevertheless, IgM AFC

frequencies in the spleens of infected mice are severalfold above background, suggesting a continued activation of virus-specific IgM-expressing B cells, remarkable in animals more than 1 year after the clearance of virus.

It can be concluded from these observations that a single round of virus infection of the respiratory tract of mice prompts an active secretion of antiviral antibodies that persists for more than 1 year. Consistent with this conclusion are the observations of Jones and Ada (9, 10), who reported the persistence of lung-associated antiviral AFC many months after challenge with influenza virus, and Liang et al. (12), who noted elevated serum hemagglutination inhibition titers long after influenza infection.

Ecological study of mice in a natural habitat has indicated that their average life expectancy at birth is only about 100 days (2), though it can be considerably longer in commensal populations. The period of active humoral immunity following virus infection is therefore essentially lifelong for mice in habitats that may equate to those typically experienced throughout rodent evolution; this humoral response can thus be considered permanent. This is in marked contrast to the consensus view of the humoral response which predicts that while a single primary exposure to antigen may elicit only a weak and transitory secreted antibody response, it effectively primes the system so that subsequent secondary challenge will provoke a much stronger and more-sustained response. Possibly the departure of the humoral response to respiratory virus infection from this pattern reflects the fact that the consensus view

TABLE 1. Virus-specific AFC in various tissues from infected and uninfected C57BL/6 mice

Sample source and Ig isotype	AFC/10 ⁵ cells ^a			
	Influenza virus	Sendai virus		Uninfected
		15 mo	14 mo	
Spleen				
IgA	2 ± 1	1 ± 1	ND	0
IgG1	1 ± 1	1 ± 1	ND	0
IgG2a	5 ± 2	2 ± 1	ND	0
IgG2b	1 ± 1	1 ± 0	ND	0
IgG3	0	1 ± 1	ND	0
IgM	37 ± 9	51 ± 17	ND	6 ± 3
MLN^b				
IgA	1	2	ND	ND
IgG1	3	1	ND	ND
IgG2a	5	1	ND	ND
IgG2b	1	2	ND	ND
IgG3	1	1	ND	ND
IgM	10	17	ND	ND
Bone marrow				
IgA	7 ± 2	4 ± 2	9 ± 3	0
IgG1	7 ± 3	18 ± 17	19 ± 14	0
IgG2a	16 ± 9	41 ± 37	44 ± 30	1 ± 1
IgG2b	14 ± 4	63 ± 91	44 ± 26	0
IgG3	4 ± 1	3 ± 2	4 ± 2	0
IgM	19 ± 3	33 ± 3	36 ± 22	1 ± 0

^a Virus-specific AFC per 10⁵ nucleated cells were determined in spleen, mediastinal lymph node, and bone marrow samples from C57BL/6 mice infected more than 1 year before sampling or uninfected mice specified by the heavy-chain isotype. Antiviral ELISPOTs were determined for spleen, mediastinal lymph node, and bone marrow samples from four groups of three mice each, infected either with HKx31 influenza virus 15 months earlier or with Sendai virus 15 or 14 months earlier or uninfected, as indicated. Spleen and bone marrow samples were treated individually, and the results are the means and standard deviations for each group of AFC detected per 10⁵ cells, rounded to the nearest integer. Mediastinal lymph nodes were pooled for each group of three mice before sampling for AFC, and the results are the AFC detected per 10⁵ cells of the pooled nodes. Spleen and bone marrow cells from uninfected mice were screened for Sendai virus-specific AFC. ND, not determined.

^b MLN, mediastinal lymph nodes.

depends heavily upon studies with inert antigens. Why should viruses provoke a permanent active response? As mice are communal animals, a type of virus encountered once would likely be encountered again, and arguably, members of such a short-lived species might ill-afford the reduction in overall reproductive fitness implied by even transient debility following a second round of infection by such a virus.

Infectious Sendai virus particles, having declined several millionfold from their abundance 7 days after infection, could not be detected in the lungs and associated tissues of C57BL/6 mice 10 days following intranasal inoculation (8). Infectious particles of mouse-adapted influenza viruses were eliminated, with similar kinetics, and viral RNA sequences were undetectable by PCR in the lungs and surrounding tissues 14 days after exposure (6). Viruses that are normally lytic can set up a persistent infection, frequently in neuronal cells that lack major histocompatibility complex expression. The persistence of Sindbis virus that thus follows the resolution of acute encephalitis induced by intrathecal inoculation is accompanied by a long-sustained incidence of antiviral AFC within the brains of infected animals (14). However, it seems unlikely that the permanent humoral immune responses described here, elicited by respiratory virus infections with no known neuronal involvement, are due to viral persistence. Rather, we suggest

FIG. 2. Long-term bone marrow AFC response to Sendai virus infection of C57BL/6 mice. Photographs of ELISPOTs generated from duplicate samples of 10⁵ bone marrow cells from each member, labelled 1, 2, and 3, at the heads of the relevant columns, of a group of three mice infected with Sendai virus 15 months before sampling are shown. Each spot represents a single virus-specific AFC producing the Ig isotype indicated at the left of each row. These data were used to generate the numbers of bone marrow AFC that appear in column 2 of Table 1.

that they reflect an evolutionarily selected program of the mouse immune system set in motion by some immunogenic feature possessed by viruses but lacking in inert antigens. A better understanding of this mechanism would provide a partial basis for the tenet of the conventional wisdom of immunology that live vaccines are much more efficacious than inert vaccines and might, moreover, be of considerable practical significance: effective protection from certain important human pathogens, such as malaria and human immunodeficiency virus, demands that the invading pathogen be neutralized before it can infect a host cell. It would therefore seem desirable that vaccination against such agents should provoke a permanent, active humoral immune response, similar to that elicited here in mice by respiratory virus infection, rather than simply prime for a vigorous secondary response.

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