Differential Antigen Burden Modulates the Gamma Interferon but Not the Immunoglobulin Response in Mice That Vary in Susceptibility to Sendai Virus Pneumonia

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Sendai virus, a paramyxovirus which causes murine pneumonia, grew to approximately 10-fold higher titers and was cleared less rapidly from the lungs of 129/J (129) than *H-2^b*-compatible C57BL/6J (B6) mice. The more susceptible 129 mice also made higher titers of gamma interferon (IFN- γ) and immunoglobulin G2a (IgG2a) virus-specific antibody. Analysis with acutely irradiated (950 rads) mice and immunologically reconstituted bone marrow (BM) radiation chimeras indicated that the enhanced virus growth was a function of the radiation-resistant respiratory epithelium. Prolonged exposure to more virus in turn influenced the magnitude of IFN- γ production, most of which was made by CD4⁺ T lymphocytes. Somewhat surprisingly, however, the 129 pattern of a higher virus-specific serum Ig response skewed towards IgG2a mapped to the reconstituting BM. Thus, the characteristics of the humoral response are at least partly dissociated from both the antigen load, resulting from viral replication, and the level of IFN- γ production. Further analysis of double chimeras (B6+129 BM→B6 recipients) confirmed that the divergent humoral immune response to Sendai virus in B6 and 129 mice is largely determined by the inherent characteristics of the lymphoid cells.

The outcome of any virus infection is a consequence of the nature of the pathogen and the characteristics of the host response (10). Productive infection with the murine parainfluenza type 1 virus, Sendai virus, is substantially confined to the superficial epithelium of the respiratory tract (5, 20). This pathogenesis reflects the distribution of a trypsin-like enzyme needed to cleave the viral fusion protein before progeny virus can initiate infection (50). The lack of both viremia and further virus growth cycles in lymphoid tissue leads to anatomical restriction of the primary T- and B-cell responses, with clonal expansion of specifically committed lymphocytes being detected first in the draining cervical and mediastinal lymph nodes (CLN and MLN) and then in the spleen (18, 43). The antigenic stimulus is presumably provided by virus and/or non-productively infected dendritic cells (29, 33) that have seeded from the lung via the lymph.

Inbred mice show diverse patterns of susceptibility to Sendai virus pneumonia (6, 38). Genetic resistance to Sendai virus infection is multifactorial and autosomal, being largely determined by loci that do not map to the major histocompatibility complex (4, 6). Otherwise, the mechanisms underlying inherited resistance are, as with many viruses, poorly understood. An exception is the *Mx* gene, which is activated via the alpha/beta interferon (IFN- α/β)-dependent pathway and mediates resistance to influenza virus infection in some mouse strains (39). However, expression of the *Mx* gene (38, 39) or differential IFN- α/β production has not correlated with resistance to Sendai virus (2). Little is known about the role of cytokines in parainfluenza virus infection though interleukin 2 (IL-2), IFN- γ , IL-6, and IL-10 are known to be induced during the course of the primary response to Sendai virus in regional lymph nodes (35).

Recent analysis of the humoral response in *H-2^b* mouse

strains that are relatively resistant (C57BL/6J [B6]) or susceptible (129/J [129]) to Sendai virus (38) indicates that dissection of the factors influencing the IFN- γ response could provide useful insights. IFN- γ is known to induce class switching to immunoglobulin G2a (IgG2a) (12, 49), an isotype that is prominent in many virus infections (15). Use of the ELISPOT assay for antibody-forming cells (AFCs) has shown that plasmablasts or plasma cells producing IgG2a predominate in the MLN and CLN of susceptible 129 mice, whereas the response in B6 mice is characterized by a more even distribution of IgG isotypes. This correlates with profiles of virus-specific Ig in serum (43).

The present analysis characterizes patterns of virus growth, IFN- γ production, and Ig response for B6 and 129 mice infected intranasally (i.n.) with Sendai virus (20). Bone marrow radiation chimeras (11) made between these two mouse strains are then used to dissect the interactions between events occurring in the radiation-resistant compartment (respiratory epithelium), which we show determines antigen load, and the radiation-sensitive hemopoietic cells that reconstitute the lymph nodes and spleen. Our results clearly indicate that the antigen load is the major determinant of IFN- γ levels, but the features of the humoral immune response are controlled primarily by the genotype of the hemopoietic or lymphoid cells.

MATERIALS AND METHODS

Mice and bone marrow chimeras. Female 129 and B6 mice were purchased from the Jackson Laboratory (Bar Harbor, Maine) and kept under pathogen-free conditions until infected with Sendai virus at 8 to 10 weeks of age. Bone marrow chimeras were made (11) with recipient mice that had been lethally irradiated (950 rads) 1 day prior to receiving 10⁷ donor bone marrow cells in an 0.5-ml volume given intravenously via the tail vein and then held for at least 10 weeks to allow full reconstitution prior to virus challenge. The bone marrow was depleted of donor T cells by first incubating it with a cocktail of the AT83 (anti-Thy1.2), J1j (anti-Thy1.2), and 31M (anti-Lyt2) monoclonal antibodies (MAbs) on ice for 30 min and then washed prior to further incubation with guinea pig complement for 45 min at 37°C. The double chimeras received 0.5 × 10⁷ cells from each of the donor mouse strains. The extent of chimerism in the

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hemopoietic compartment was analyzed by using the 30C7 MAb to Ly9.1 (27), the allele expressed on lymphoid cells in 129 but not B6 mice.

Virus infection and titration. Anesthetized mice were infected i.n. with 100 to 200 50% infectious doses for embryonated hen's eggs (EID₅₀) of the Enders strain of Sendai virus (20). Homogenates of mouse lung were titrated by inoculating 100- μ l aliquots of 10-fold dilutions into the allantoic cavity of 10-day-old embryonated hen's eggs. The eggs were then incubated at 35°C for 48 h, and virus titers were determined as $-\log_{10}$ EID₅₀ following analysis of allantoic fluid by the hemagglutination-inhibition assay.

Sampling the mice. The mice were anesthetized with Avertin (2,2,2-tribromoethanol) and eye bled to obtain serum, and bronchoalveolar lavage (BAL) was performed (1, 20) to obtain both inflammatory cell populations and (from the initial washing) BAL fluid (BALF). Pooled MLN and CLN were then collected and processed with tissue grinders to give single-cell suspensions which were depleted of stromal elements by passing through a nylon mesh.

Cytokine assays. The frequencies of freshly isolated BAL cells producing IL-2, IFN- γ , IL-4, IL-5, IL-6, and IL-10 were enumerated as spot-forming cells (SFCs) by the cytokine ELISPOT assay (13, 45, 46). The minimal BAL populations that can be recovered from naive mice contained <40 SFCs per 10,000 cells. Sandwich cytokine enzyme-linked immunosorbent assays (ELISAs) were used to analyze the levels of soluble cytokine in the BALF or the supernatant from MLN cells cultured in vitro with virus-infected, irradiated B6 stimulators (35, 46). The baseline for the assay is three times the standard deviation for eight medium blanks, which is >0.78 U/ml for each cytokine assay. Comparably stimulated MLN populations from naive 129 or B6 mice produced undetectable levels for all the cytokines assayed. Also, there was no difference in the capacity of stimulators from 129 or B6 mice to induce immune T cells to secrete cytokine in these cultures (data not shown).

Ig isotype analysis. The frequencies of Sendai virus-specific AFCs in pooled (from at least three mice) MLN and CLN populations were determined by single-cell ELISPOT assay (23, 24, 43) performed on plates coated with detergent-disrupted Sendai virus. Antibodies bound to viral antigen were detected with alkaline phosphatase-conjugated goat anti-mouse Ig with specificity for IgM, IgA, IgG1, IgG2a, IgG2b, and IgG3 and further developed with 5-bromo-4-chloro-3-indolylphosphate (Sigma, St. Louis, Mo.). Samples from naive mice were used as the negative controls. Results are expressed as the percentage of the total virus-specific AFCs that are producing Ig of a particular isotype. Virus-specific serum antibody titers were determined by sandwich ELISA (23). The serum titer is expressed as the reciprocal of the highest dilution giving an A_{405} value on a microplate reader more than twice that for simultaneously titrated samples from naive mice.

Lymphocyte phenotyping and separation. The BAL and MLN populations were analyzed on a FACScan or sorted (18) with a FACStar Plus (Becton Dickinson, Mountain View, Calif.). The MAbs used for staining were phycoerythrin (PE)-conjugated anti-T-cell receptor α/β (α/β -TCR) (H57.597) and anti-CD4 (RM4-5), or fluorescein isothiocyanate (FITC)-conjugated anti-CD8 α (53-6.7), anti-B220 (RA3-6B2), and anti-Ly9.1 (30C7). All MAbs were obtained from Pharmingen (San Diego, Calif.) and are described and referenced in the Pharmingen catalogue. For the T-cell populations that were analyzed for cytokine production, MLN cells were first depleted of B lymphocytes with magnetic conjugated goat anti-mouse IgG (PerSeptive Diagnostics, Cambridge, Mass.) and then stained with PE-anti-CD4 or FITC-anti-CD8 and sorted in two-color mode with the FACStar Plus.

Statistical analysis. Data were analyzed by Student's *t* test.

RESULTS

Virus titers and lymphocyte phenotypes. The *H-2^b* congenic B6 and 129 mice were infected i.n. with 200 EID₅₀ of Sendai virus and sampled at intervals. Both showed evidence of pathology in hematoxylin and eosin-stained paraffin sections, with lung consolidation and inflammation being much more apparent for the 129 mice (data not shown). This did not, however, translate into increased numbers of cells recovered by BAL, values (10^6) for the 129 and B6 mice being as follows: day 7, 1.6 ± 0.4 and 1.7 ± 0.4 ; and day 10, 0.5 ± 1.1 and 0.6 ± 0.3 , respectively. The more severe pneumonic changes correlated with enhanced virus titers, which were about 10-fold greater in the lungs of 129 mice at days 3 and 7 after infection and (contrary to the situation for the B6 animals) were still at high levels on day 8 (Table 1). Both mouse strains had cleared the virus by day 14.

The characteristics of the BAL population recovered from the 129 mice were generally similar to those described previously for the B6 strain (20). Contemporary comparison (Fig. 1A) showed the presence of few B220⁺ B lymphocytes, while the predominance of the CD8⁺ subset developed more quickly

TABLE 1. Virus titers in the lungs of B6 and 129 mice^a

Day postinfection	Virus titer ($-\log_{10}$ EID ₅₀ /100 μ l)	
	B6	129
3	5.4 ± 0.1	6.6 ± 0.1
7	5.0 ± 0.2	5.9 ± 0.1
8	1.7 ± 0.4	6.2 ± 0.2
10	0.4 ± 0.3	3.8 ± 0.1
14	0.00	0.00

^a The mice were infected i.n. with 200 EID₅₀ of Sendai virus. Diluted homogenates of virus-infected lung were injected (100 μ l) into embryonated hen's eggs and incubated for 48 h before testing for virus by hemagglutination. The values are means $\pm$ standard errors of the mean ($n = 5$ to 6). The differences between the two groups were statistically significant ($P < 0.05$) from days 7 to 10.

with the B6 mice. This correlated with the differential pattern of virus clearance (Table 1), presumably reflecting the earlier localization of the virus-specific cytotoxic T-lymphocyte effectors that normally terminate the infectious process (20, 21). The massive recruitment of all classes of lymphocytes to the MLN that follows infection with respiratory viruses (1, 20) was broadly comparable in magnitude for the two mouse strains, with the cell counts (10^7) for the 129 and B6 mice (Fig. 1B) being as follows: day 7, 0.6 ± 0.3 and 1.1 ± 0.3 ; and day 10, 0.5 ± 0.2 and 0.6 ± 0.1 , respectively. However, the T/B ratio was higher for the 129 mice at all time points, with the difference being statistically significant ($P < 0.01$) on day 10 for the three experiments summarized in Fig. 1B.

FIG. 1. The relative prevalence of different lymphocyte subsets is shown for BAL (A) and MLN (B) populations from B6 and 129 mice sampled at intervals after i.n. infection with 200 EID₅₀ of Sendai virus. The day 0 values are for mice sampled immediately prior to infection. The results are representative of two or three separate experiments.

FIG. 2. Cytokine profiles were determined for BAL populations recovered from B6 and 129 mice at 7 and 10 days after infection. (A) The frequencies of cytokine-producing cells detected by ELISPOT analysis are shown for pooled (five or six mice) BAL cells, the results being expressed as means $\pm$ standard errors of the mean (SEM) ($n = 3$ or 4). No IL-5-producing cells were found. (B) The cytokine levels in the BALF pooled from 6 to 12 mice were measured by ELISA after concentrating 6- to 18-fold (35) and are presented as units per milliliter prior to concentration (mean $\pm$ SEM, $n = 3$). Levels of IL-4 and IL-5 in the BALF were below the limit of detection (<0.78 U/ml).

Cytokine profiles in the BAL and MLN. Analysis at the time points (days 7 and 10) found to be critical for termination of the infectious process (Table 1) confirmed earlier experiments with B6 mice (35) showing that cells producing IL-2, IFN- γ , IL-4, IL-6, and IL-10 were present in the BAL population (Fig. 2A). The maximal frequencies for these cytokine-producing cells were broadly comparable (particularly for IL-10), with the peak values for SFCs producing IL-2 and IL-4 being found for the B6 mice on day 7 and the 129 mice on day 10 (Fig. 2A). A high-level IFN- γ response was also sustained longer in the 129 mice, again reflecting the pattern of virus clearance (Table 1). At least a proportion of the IL-10-producing SFCs could be monocyte/macrophages (36), which comprise 30 to 70% of the inflammatory exudate (18, 20). Analysis of extracellular cytokine levels in the fluid phase of the lung lavage (BALF) showed the presence of IL-2 (minimal amounts), IFN- γ , IL-6, and IL-10 (but no IL-4), with the maximal levels of IFN- γ , IL-6, and IL-10 being greater in the BALF from the 129 mice at both time points. This could be thought to reflect the extent of stimulation, resulting from the differences in level and duration of virus growth and thus antigen load.

The theme that greater virus growth in vivo (Table 1) promotes the development of higher IFN- γ levels in the 129 mice (Fig. 2B) was replicated for both total MLN populations (Fig. 3) and for fluorescence-activated cell sorter (FACS)-separated T cells (Fig. 4). Culture with irradiated, virus-infected B6 stim-

FIG. 3. Pooled MLN cells (4×10^6 /ml) from groups of five mice were restimulated with irradiated (3,900 rads), syngeneic Sendai virus-infected splenocytes (4×10^6 /ml) for 24, 48, or 72 h. Maximal levels of cytokine production detected by ELISA of culture supernatants are expressed as means $\pm$ SEM ($n = 3$). No IL-4 or IL-5 was found.

ulators also induced the production of IL-2 and IL-10 and minimal amounts of IL-6, but the differences in activity for the supernatants derived from B6 and 129 responders were clearly greatest for IFN- γ (Fig. 3). Most, though not all, of this virus-induced IFN- γ production was associated with the CD4 $^+$ subset (Fig. 4). The absence of IL-4-secreting cells from the MLN (Fig. 3), but not from the BAL (Fig. 2A), has been shown previously for B6 mice infected with both Sendai virus and an influenza A virus (35, 45, 46).

Viral replication in reciprocal bone marrow radiation chimeras. The pattern of greater virus growth in the 129 mice (Table 1) could reflect either some inherent property of the target respiratory tract or that both the early nonspecific response and later immune response are much less effective than in the *H-2^b*-compatible B6 mice. Infection of lethally irradiated (950 rads) mice that were not given bone marrow indicates that differential susceptibility of the lung cells is a major factor, as the virus titers in the 129 mice were $>10\times$ higher 3 days after i.n. infection (first two rows, Table 2).

However, it is also possible that nonspecific effectors of bone

FIG. 4. Comparison of IFN- γ production for CD4 $^+$ and CD8 $^+$ T cells. Pooled MLN cells from 10 to 12 B6 or 129 mice were magnetically depleted of Ig $^+$ cells and then stained with anti-CD8-FITC and anti-CD4-PE in two-color mode and sorted with a FACStar Plus (18). The lymphocytes were then restimulated in vitro, and culture supernatants were assayed as described in the legend to Fig. 3. Data are represented as means $\pm$ SEM ($n = 3$).

TABLE 2. Virus titers in the lungs of irradiated mice and bone marrow radiation chimeras^a

Mice	Day	Virus titer ($-\log_{10}$ EID ₅₀ /100 μ l)
Irradiated B6	3	4.1 $\pm$ 0.2
Irradiated 129	3	5.4 $\pm$ 0.3 ^b
B6 $\rightarrow$ 129	7	4.9 $\pm$ 0.2
	9	4.9 $\pm$ 0.4
	10	0.3 $\pm$ 0.2
B6 $\rightarrow$ B6	7	4.3 $\pm$ 0.2 ^c
	9	0.6 $\pm$ 0.3 ^{c,d}
	10	0.00
129 $\rightarrow$ B6	7	4.0 $\pm$ 0.2 ^c
	9	0.1 $\pm$ 0.1 ^{c,d}
	10	0.00
129 $\rightarrow$ 129	7	6.0 $\pm$ 0.2 ^d
	9	4.6 $\pm$ 0.5
	10	2.8 $\pm$ 0.2 ^d

^a The lethally irradiated (950 rads) B6 or 129 mice were infected i.n. with 100 EID₅₀ of Sendai virus on the following day, while the bone marrow chimeras were left for at least 10 weeks after irradiation and reconstitution and then infected i.n. with 100 EID₅₀ of Sendai virus. FACS analysis of both MLN and spleen cells with an allelic MAb to Ly9.1 antigen showed that >98% of the T cells and B cells were of donor origin (data not shown). Virus titers are shown as means $\pm$ standard errors of the mean ($n = 4$ to 9).

^b Significantly greater ($P < 0.01$) than the values for the irradiated B6 mice.

^c Significantly different ($P < 0.01$) from the values shown for the 129 $\rightarrow$ 129 chimeras.

^d Significantly different ($P < 0.01$) from the values for the same day for the B6 $\rightarrow$ 129 chimeras.

marrow origin play a role: there was a 10-fold difference in virus titers on day 7 for fully reconstituted B6 $\rightarrow$ 129 chimeras and 129 $\rightarrow$ 129 controls (Table 2). However, the profiles of virus growth on day 7 and clearance by day 9 were identical for the 129 $\rightarrow$ B6 and B6 $\rightarrow$ B6 mice. Furthermore, effectors of both B6 and 129 origin showed about the same capacity to reduce virus titers in the 129 respiratory tract on day 9 (B6 $\rightarrow$ 129 and 129 $\rightarrow$ 129, Table 2). Thus, though the effect is not absolute, by far the greater determinant of virus growth and persistence is the characteristics of the radiation-resistant host component. Given the findings with the recently irradiated mice, that the two mouse strains are *H-2^b* compatible, and that the chimeric mice had 10 weeks to reconstitute their stimulator compartment with donor cells, the difference probably reflects the nature of the respiratory epithelium (9).

Cytokine production in the chimeric mice. The most dramatic divergence in the cytokine response for Sendai virus-infected B6 and 129 mice was for IFN- γ production by (predominantly) CD4⁺ T cells (Fig. 2 to 4). This was again true for the chimeric mice (Table 3), with the highest IFN- γ titers in culture supernatants from restimulated MLN populations being found for lymphoid cells that had been primed in vivo in the 129 radiation-resistant environment (B6 $\rightarrow$ 129 and 129 $\rightarrow$ 129, Table 3). However, there was also a lesser effect from the hemopoietic compartment, as the level of IFN- γ production was consistently lower for the B6 and 129 mice that had been reconstituted with B6 (rather than 129) bone marrow (Table 3). Again (Table 1 and Fig. 2 and 3), the correlation of virus titers (Table 2) and IFN- γ profiles (Table 3) suggests that the main determinant for IFN- γ is the antigen load, and thus the extent of CD4⁺ T-cell stimulation (Fig. 4), occurring during the primary response in vivo (Table 3).

The antibody response in the chimeric mice. Previous studies have shown that the Ig response in 129 mice is much more skewed (compared with the B6 response) to IgG2a (43). The primary inducer of class switching to IgG2a is IFN- γ (12, 49),

TABLE 3. Cytokine levels in supernatants of restimulated MLN populations from chimeric mice^a

Day postinfection	Mice	Expt	Cytokine level (U/ml)			
			IL-2	IFN- γ	IL-10	IL-6
7	B6 $\rightarrow$ 129	1	6.9	113.9	5.2	3.1
		2	3.6	95.8	4.5	3.2
	129 $\rightarrow$ B6	1	5.5	71.7	14.0	2.9
		2	4.8	52.7	17.3	2.3
	B6 $\rightarrow$ B6	1	2.6	24.9	2.9	3.7
		2	2.1	38.7	3.1	2.8
	129 $\rightarrow$ 129	1	7.7	140.3	9.7	5.4
		2	4.7	125.9	10.5	4.2
	B6 $\rightarrow$ 129	1	1.6	81.1	0.8	6.1
		1	1.0	62.5	1.4	3.9
10	B6 $\rightarrow$ B6	1	1.4	42.7	0.00	3.3
		1	1.0	151.7	1.0	7.4

^a The mice were infected i.n. with 100 EID₅₀ of Sendai virus, the MLN population was restimulated in vitro, and culture supernatants were assayed as described in the legend to Fig. 3.

which is made in much greater amounts in 129 mice (Fig. 2 to 4) and in B6 $\rightarrow$ 129 and 129 $\rightarrow$ 129 reconstituted mice (Table 3). Analysis of freshly isolated MLN and CLN populations by the single-cell ELISPOT assay showed that IgG2a-producing AFCs clearly predominated in the 129 $\rightarrow$ B6 and the 129 $\rightarrow$ 129 chimeras, while this was not the case with the B6 $\rightarrow$ 129 or the B6 $\rightarrow$ B6 mice (Fig. 5). Furthermore, both the total virus-specific Ig and IgG2a serum antibody levels (experiment 1, Table 4) were at least 10-fold higher for the chimeras reconstituted with the 129 bone marrow, the level of response being only minimally influenced by the recipient host environment. Thus, both the character and the magnitude of the virus-specific antibody response are apparently determined within the hemopoietic-lymphoid compartment (Fig. 5; experiment 1, Table 4) and are independent of the differential in antigen load (virus growth) and IFN- γ production that is more associated with the radiation-resistant host genotype (Tables 1 to 3 and Fig. 2 to 4).

The question then became whether the greater virus-specific Ig and IgG2a production associated with the 129 lymphoid compartment was a function of the 129 B lymphocytes themselves or reflected the absence of some negative regulatory mechanism (31) modulating the emergence of B6 plasma cells and consequent serum antibody levels. We thus made double chimeras with B6 and 129 bone marrow and irradiated B6 recipients, allowed the mice to reconstitute fully, and then determined the relative prevalence of AFCs in the MLN and CLN (Fig. 6) and measured the serum antibody response (experiment 2, Table 4). Phenotyping with the allelic Ly9.1 MAb (27) established that 129 (Ly9.1⁺) and B6 (Ly9.1⁻) lymphocytes were almost equally represented in peripheral blood of naive double chimeras (legend to Fig. 6). Analysis of FACS-separated Ly9.1⁺ versus Ly9.1⁻ cells in CLN and MLN populations showed that the relative frequencies of Ly9.1⁺ IgG2a AFCs in the 129+B6 $\rightarrow$ B6 chimeras were less polarized than those for the 129 $\rightarrow$ 129 and 129 $\rightarrow$ B6 controls (Fig. 6), with the values for the Ly9.1⁺ cells from the double chimeras being significantly lower ($P < 0.05$) than that for 129 $\rightarrow$ B6 controls, implying the existence of a negative regulatory effect from B6 lymphoid cells. Nevertheless, for both CLN (two experiments) and MLN (three experiments) populations collected from the double chimeras, IgG2a AFCs always formed the largest proportion of the Ly9.1⁺ cells producing the various isotypes. This was never the case for the corresponding Ly9.1⁻ cells.

FIG. 5. The spectrum of Sendai virus-specific plasmablasts/plasma cells producing virus-specific Ig of different isotypes is shown for the MLN (A) and CLN (B) of bone marrow chimeras sampled at 10 days after i.n. infection. The percentage of AFCs is generally shown as means $\pm$ SEM ($n = 4$ to 6), except that only two experiments were done with the MLN from the B6 $\rightarrow$ 129 mouse chimeras. The mean total numbers of AFCs in the MLN and CLN of these mice were as follows: 129 $\rightarrow$ B6, 1,145 and 1,187; B6 $\rightarrow$ 129, 526 and 1,430; 129 $\rightarrow$ 129, 1,125 and 2,345; and B6 $\rightarrow$ B6, 603 and 919, respectively.

The high virus-specific Ig and IgG2a serum antibody profiles characteristic of the 129 mice (43) were clearly dominant in the double chimeras (experiment 2, Table 4), and the levels were not significantly different from the titers for the 129 $\rightarrow$ B6 single

TABLE 4. Virus-specific serum Ig levels in the chimeras^a

Expt and mice	Ig titer (1/dilution [10^3])			
	Total Ig	IgG1	IgG2a	IgG2b
Expt 1				
129 $\rightarrow$ B6	34.2 $\pm$ 12.2	1.3 $\pm$ 0.8	55.8 $\pm$ 15.7	18.6 $\pm$ 5.2
B6 $\rightarrow$ 129	3.0 $\pm$ 0.0 ^b	1.5 $\pm$ 0.6 ^c	3.0 $\pm$ 0.0 ^b	3.0 $\pm$ 0.0 ^b
129 $\rightarrow$ 129	27.0 $\pm$ 0.0 ^{b,c}	1.5 $\pm$ 0.6 ^c	45.0 $\pm$ 18.0 ^{b,c}	27.0 $\pm$ 0.0 ^{b,c}
B6 $\rightarrow$ B6	5.0 $\pm$ 2.0 ^b	1.3 $\pm$ 0.7 ^c	2.3 $\pm$ 0.7 ^b	7.0 $\pm$ 2.0 ^b
Expt 2				
129+B6 $\rightarrow$ B6	19.3 $\pm$ 3.6	0.7 $\pm$ 0.1	21.9 $\pm$ 3.3	9.0 $\pm$ 0.0
B6 $\rightarrow$ B6	3.0 $\pm$ 0.0 ^b	0.3 $\pm$ 0.0 ^c	3.0 $\pm$ 0.0 ^b	3.0 $\pm$ 0.0 ^b
129 $\rightarrow$ B6	17.0 $\pm$ 0.0 ^{b,c}	0.8 $\pm$ 0.2 ^c	27.0 $\pm$ 0.0 ^{b,c}	9.0 $\pm$ 0.0 ^{b,c}

^a ELISA titers (mean $\pm$ standard error of the mean) were determined for three to five individual mice. Serial threefold dilutions of sera were assayed on plates coated with detergent-disrupted Sendai virus. The sera tested were obtained from mice analyzed for Fig. 5 (experiment 1) and Fig. 6 (experiment 2) by the ELISPOT technique.

^b Significantly different ($P < 0.05$) from the group on the line above.

^c Not significantly different from the group shown on the first line of the experiment.

FIG. 6. The prevalence of AFCs producing virus-specific Ig of different isotypes from double chimeras (129+B6 $\rightarrow$ B6) and from 129 $\rightarrow$ B6 and B6 $\rightarrow$ 129 single chimeras analyzed concurrently as controls is shown for pooled lymphocytes (five to six mice) from the MLN (A) and CLN (B). The lymph node cells from 129+B6 $\rightarrow$ B6 mice shown on the left-hand histograms were stained with a cocktail of anti- α/β -TCR-PE, anti-B220-PE, and anti-Ly9.1-FITC MABs: 95 to 99% of cells were α/β -TCR⁺ B220⁺. They were then sorted into α/β -TCR⁺ B220⁺ Ly9.1⁺ or α/β -TCR⁺ B220⁺ Ly9.1⁻ populations with the FACStar Plus. The sorting process did not influence the percentage of cells secreting particular Ig isotypes (data not shown). Data for the MLN are presented as means $\pm$ SEM ($n = 3$), and data for the CLN are presented as the average of two experiments. Prior to infection, peripheral blood lymphocytes from the double chimeras were typed as α/β -TCR⁺ B220⁺ Ly9.1⁺ or α/β -TCR⁺ B220⁺ Ly9.1⁻. The mean $\pm$ SEM for the ratio between these two sets of lymphocytes was 1.07 ± 0.05 , the range being 0.67 to 1.52 ($n = 20$). After infection, the average ratios of α/β -TCR⁺ B220⁺ Ly9.1⁺ α/β -TCR⁺ B220⁺ Ly9.1⁻ cells were 1.89 and 2.40 for the MLN and CLN populations, respectively.

chimeras. Although this suggests that any regulatory influence by B6 lymphoid cells is minor, it should be noted that Ly9.1⁺ cells were approximately twice as prevalent as Ly9.1⁻ cells in the MLN and CLN of the double chimeras when samples were collected 10 days after infection (legend to Fig. 6). Even when the difference in lymphocyte numbers is taken into account, however, any negative regulatory effect from the Ly9.1⁻ B6 cells in the double chimeras would seem to have less determining effect on the humoral response than the genotype of the Ly9.1⁺ 129 plasma cells.

DISCUSSION

The divergence in virus growth characteristics and clearance for susceptible 129 and resistant B6 mice (38) has allowed a somewhat different analysis of cytokine and Ig production pro-

files in an infectious process. So far as we are aware, this is the first time that bone marrow chimeras have been used to dissect the interaction between host and viral genotypes in the regulation of a humoral immune response. Previous studies with chimeras made between *H-2^s*-compatible mouse strains with varying susceptibility to experimental allergic encephalomyelitis mapped the induction of this CD4⁺ T-cell-mediated autoimmune disease to the radiation-resistant host phenotype (25, 28). Other experiments addressed the nature of CD8⁺ T-cell targeting and effector function in chimeric mice infected with lymphocytic choriomeningitis virus and Sendai virus (9, 19).

The analysis of Sendai virus pneumonia in both wild-type mice and radiation chimeras indicates that antigen load, assessed here by determining titers of infectious virus, drives the IFN- γ response. The more severe lung pathology seen in the 129 animals may reflect both direct virus-induced cytopathology and immunopathology related to IFN- γ , which can promote tissue damage via a tumor necrosis factor-nitric oxide pathway (37, 44, 48). Greater numbers of IFN- γ -producing cells were present longer in the 129 BAL populations. This resulted in the presence of more IFN- γ , which is presumably excess to consumption, in the BALF. Apart from the quantitative effect, there was no obvious difference in the quality of the cytokine response profiles for either the pneumonic lung or the regional lymph nodes of the B6 and 129 mice.

The more persistent, and higher-level, infection in the 129 mice probably causes prolonged clonal expansion of virus-specific CD4⁺ T cells. Though we have not attempted to quantify the precursor frequencies (7, 34) for the B6 and 129 T helper cells in these experiments, the divergence in cytokine production following *in vitro* stimulation of MLN populations is consistent with differences in virus-specific T-cell numbers in MLN. Viruses are well known to be prominent inducers of IFN- γ (16, 35, 47, 52), and it is hardly surprising that the effect is quantitative. Apart from being *H-2^b* compatible, and thus restricted to *H-2IA^b*, the chimera experiments suggest that genotype of the responding T cells is much less important.

The dissociation between levels of IFN- γ production and class switching to IgG2a indicates that, though some IFN- γ may be needed (12, 41), the amount is not limiting. The chimera experiments show that the primary control of total virus-specific antibody production, including IgG2a, seems to rest in the genotype of the B lymphocyte-plasma cells. The Ig heavy chain (Igh) allotypes are known to differ for the two mouse strains studied, with the 129 being Igh^a and the B6 mice being Igh^b (3, 26). In the present experiments, high and low IgG2a responses were associated with the Igh^a and Igh^b allotypes, respectively, an association that has been described in studies of the antibody response to nonviral antigens (26). The genotype at the Igh locus may thus be one of the genetic factors controlling the magnitude of the IgG2a response.

Any association of disease severity with allotype-associated subclass regulation is of interest, as patterns of restricted usage have been found for autoantibodies that are thought to be important in human pathology (17, 30). Given the possibility that at least some autoimmune diseases are triggered by virus infections (8, 14), the correlation between allotype dominance and level of response in viral immunity may be worth pursuing further. There was a hint that a measure of allotype-related suppression might be operating to regulate Sendai virus-specific IgG2a production in the current analysis of double chimeric mice, but any such effect was minimal and not reflected in serum Ig profiles. Such suppression has been shown (for other antigens) to be mediated by CD8⁺ T cells (31, 32), but CD8-depleted mice have not been found to make an enhanced IgG2a or IgG2b response to Sendai virus (22).

The present experiments do not directly address the effects of host genotype on the CD8⁺ T-cell response that, at least in B6 mice, is responsible for terminating the acute infectious process (20, 21). However, comparing the kinetics of virus clearance in, for example, the B6 $\rightarrow$ 129 and B6 $\rightarrow$ B6 chimeras indicates that the elimination of more virus-infected cells, represented here by prolongation of virus titers, takes longer. The present findings also confirm that the magnitude of the virus-specific Ig response is not a major factor in the resolution of this primary infection. Though antibody is very important for promoting resistance to reinfection (10, 42) and antibodies of both IgG1 and IgG2a isotypes neutralize Sendai virus effectively (40), cell-mediated immunity is the major mechanism operating in the normal primary response. It is likely that the enhanced susceptibility of 129 mice to primary Sendai virus infection is essentially a function of the greater virus growth in the respiratory tract. Whether this reflects the characteristics (51) of viral replication in the epithelial cells themselves or some other physiological difference related to (for instance) mucociliary transport (4) in the lung has yet to be determined.

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