JOURNAL OF VIROLOGY, Feb. 1996, p. 1288–1291 Vol. 70, No. 2
0022-538X/96/$04.00+0
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Immune $CD4^+$ T Cells Promote the Clearance of Influenza Virus from Major Histocompatibility Complex Class II −/− Respiratory Epithelium

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Received 8 September 1995/Accepted 15 November 1995

The experiments described establish that $CD4^+$ T-cell-dependent effector mechanisms can eliminate an H3N2 influenza A virus from lung cells that are unable to express class II major histocompatibility complex (MHC) glycoproteins. Radiation chimeras were made by using $CD4^+$ T cells and bone marrow from CD8-depleted, MHC class II +/+ mice and irradiated (950 rads) MHC class II −/− recipients. The influenza virus-specific $CD4^+$ T-cell responses in these +/+→−/− mice were not obviously different from those in the +/+→+/+ controls: the cytokine profiles, the spectra of plasma cells producing the various immunoglobulin isotypes, and the frequencies of virus-specific $CD4^+$ T cells were similar for the two groups. Expression of class II MHC glycoproteins on stimulator cells, B lymphocytes, and monocytes/macrophages is apparently sufficient for $CD4^+$ T cells to terminate influenza virus infection of MHC class II −/− respiratory epithelium. A possible explanation is that the local spread of this lytic virus in the lung is limited by cytokines and/or antibody.

Analysis with mice homozygous for disruption (−/−) of the β_2-microglobulin (β_2-m) gene has established that $CD4^+$ T cells, acting in the absence of the $CD8^+$ subset, can terminate respiratory infections induced by H1N1 and H3N2 influenza A viruses (3, 7). The inflammatory cells recovered by bronchoalveolar lavage (BAL) of these β_2-m −/− mice contain $CD4^+$ cytotoxic T lymphocytes (CTL) lytic for major histocompatibility complex (MHC) class I^+ II^+, but not MHC class I^+ II^-, virus-infected target cells. Similar findings have been made for a murine parainfluenza type 1 (Sendai) virus (16). These $CD4^+$ CTL (24) are never found in normal mice infected with these viruses. The functional competence of $CD4^+$ T cells has also been established by adoptive transfer experiments with helper T-cell (Th) lines, which show that Th1 cells, producing gamma interferon (IFN-γ), tumor necrosis factor, and interleukin 2 (IL-2), protect from lethal challenge with H1N1 and H2N2 influenza A viruses, while Th2 clones (producing IL-4 and IL-5) are ineffective (11, 29).

Virus clearance in wild-type β_2-m +/+ mice is normally mediated by $CD8^+$ effectors that (at least for Sendai virus) must target directly to MHC class I^+ virus-infected respiratory epithelium. This was established (15) with bone marrow (BM) chimeras, made by using lethally irradiated (950 rads) β_2-m −/− recipients and BM from normal β_2-m +/+ donors. When these β_2-m +/+→−/− chimeras were depleted of $CD4^+$ T cells and infected intranasally 10 to 12 weeks later, neither the reduced numbers of endogenous $CD8^+$ T cells that develop in such mice (4) nor adoptively transferred, $CD8^+$ memory T cells could clear Sendai virus from the radiation-resistant β_2-m −/− epithelial cells. Virus-specific $CD8^+$ CTL were, however, detected in the population recovered by BAL, reflecting stimulation by MHC class I^+ cells of BM origin, including inflammatory monocytes/macrophages.

The question asked in this study was whether clearance of the HKx31 (H3N2) influenza A virus by immune $CD4^+$ T cells also requires direct targeting to respiratory epithelium. This is the only site supporting substantial virus growth, reflecting the distribution of a trypsin-like enzyme required to cleave the viral hemagglutinin (12, 32). The experiments utilized radiation chimeras (5) made with MHC class II^- (H-2-IA^{b-}) C2DKO −/− recipients (12, 22) and normal +/+ donor BM and $CD4^+$ T cells. While the BM-derived elements in the lymphoid compartment and the inflammatory monocytes/macrophages in these +/+→−/− chimeric mice will express the class II MHC glycoproteins (Fig. 1) required to stimulate adoptively transferred $CD4^+$ effector T cells, production of infectious influenza virus should be limited to the radiation-resistant, class II MHC −/− lung epithelium.

The initial experiment (Table 1) was done with chimeric mice made with a cocktail of lymph node cells (1×10^7), spleen cells (5×10^7) and BM cells (2.5×10^7), injected intravenously 24 h after receiving 950 rads of irradiation, from +/+ C57BL/6 (B6) donors that had first been depleted in vivo by administering the 2.43.1 monoclonal antibody (MAb) to CD8 every 3 days beginning 9 days before transfer (15, 16), a treatment protocol that was continued for the −/− and control +/+ recipients throughout all the experiments (Tables 1 to 4). The mice were then infected 24 h after transfer. Flow cytometric analysis of the BAL and regional mediastinal lymph node (MLN) populations at time of sampling established that there were very few, if any, residual $CD8^+$ T cells in the chimeric mice (Table 1). The $CD4^+$ T-cell and $B220^+$ B-cell compartments were, however, substantially reconstituted (Fig. 1, Table 1). To check for the presence of virus, 10^{-1} and 10^{-2} of lung homogenates were injected into embryonated hen's eggs, and the presence of virus was confirmed by hemagglutination (2). The infectious process had been terminated in some of the chimeras from both groups by day 10 after intranasal challenge, but residual virus was still present in four of eight lung homogenates assayed on day 13 from the +/+→−/− set. The titers were low, with virus being detected only at the 10^{-1} dilution in two of the four positive mice, and there was no statistically significant difference (by chi-square analysis) in

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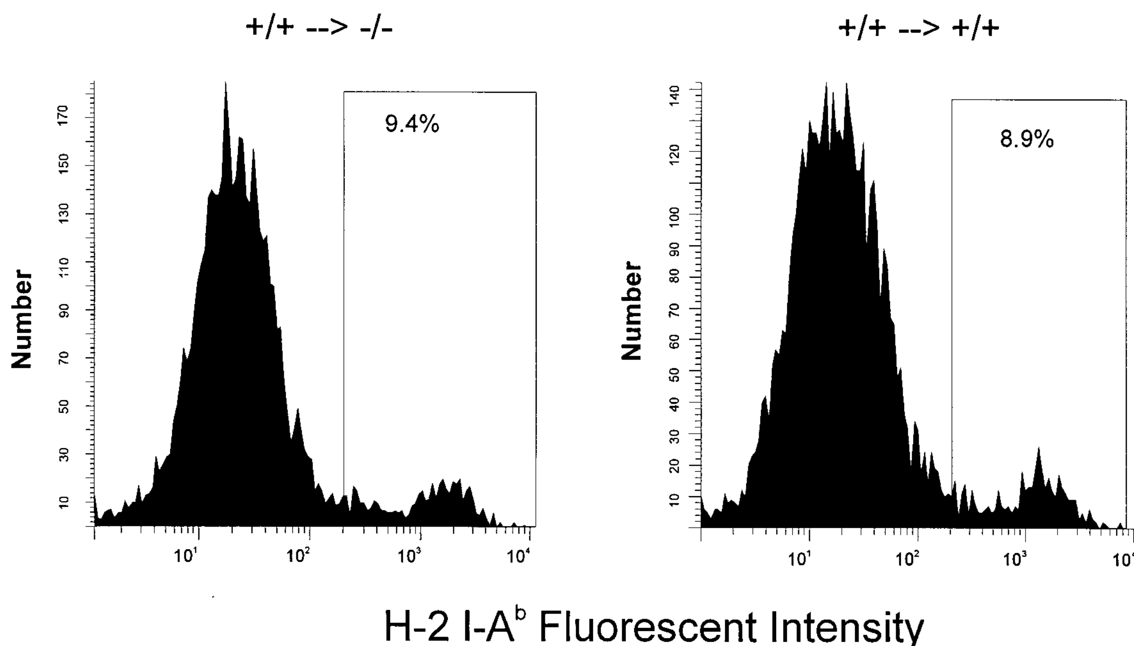

FIG. 1. Splenic nucleated cells were recovered on day 13 after infection, stained for the expression of H-2IA^b, and analyzed (6, 27) in a FACScan (Becton Dickinson). After the Fc receptors were blocked with 10% normal mouse serum, the cells were incubated with the rat TIB120 MAb to I-A^b and then exposed to a biotinylated anti-rat MAb (Pharmingen, San Diego, Calif.) followed by streptavidin 670 (Gibco BRL, Gaithersburg, Md.). The background staining in the absence of the TIB120 was <1.0% in each case. Other details of the experiment, including the construction of the chimeras, are described in the text.

virus clearance between the +/+→+/+ and +/+→−/− mice in this (Table 1) or subsequent (Tables 2 to 4) experiments.

Further experiments (Tables 2 to 4) utilized CD4$^+$ T cells enriched to >99% purity by well-established protocols (8, 14, 18, 31) that depend on magnetic depletion of cells which express MHC class II, immunoglobun (Ig), or CD8 followed by staining with anti-CD4 (Pharmingen) and sorting with a FACStar Plus (Becton Dickinson, Mountain View, Calif.) (8, 14). The in vivo CD8$^+$ T-cell depletion was continued throughout for donors and recipients in all experiments. Tables 2 to 4 deal variously with findings from cytokine (Table 2), B-cell (Table 3), and CD4$^+$ T-cell (Table 4) assays. Excluding the data presented in Table 1, the combined virus clearance results for the different experiments are that some virus was still detected in lung homogenates from: day 10, 5 of 8 +/+→−/− and 3 of 8 +/+→+/+; day 13, 2 of 10 +/+→−/− and 2 of 10 +/+→+/+.

TABLE 1. Clearance of virus from CD8-depleted chimeric mice

No. of days after infection[a]	Radiation chimera	Virus in lung[b]	Source of cells	% with phenotype		
				CD4$^+$	CD8$^+$	B220$^+$
10	+/+→+/+	3/8	BAL	64	<1	7
			MLN	33	<1	57
	+/+→−/−	5/8	BAL	75	<1	2
			MLN	27	<1	58
13	+/+→+/+	1/8	BAL	76	2	12
			MLN	24	1	65
	+/+→−/−	4/8	BAL	58	<1	21
			MLN	21	<1	68

[a] The chimeric mice were infected intranasally with 240 hemagglutination units of the HKx31 influenza A virus.

[b] Values are the numbers of mice with virus in their lungs per the total number of mice tested.

By day 19, no virus was detected in any of six +/+→−/− or six +/+→+/+ mice tested. Formal proof that the virus was not eliminated by a notional population of class I MHC-restricted CD8$^+$ T cells that persisted despite continued treatment with the 2.43.1 MAb, or an aberrant set of class I MHC-restricted CD4$^+$ effectors, was established by making chimeras with +/+ CD4$^+$ T cells and C2DKO −/− BM and recipients. While the class I MHC glycoproteins that are recognized by the CD8$^+$ subset should be present throughout the radiation-resistant (epithelium) and radiation-sensitive (BM) compartments, these chimeras cannot express class II MHC$^+$ cells on either stimulator (lymphoid tissue) or target (infected lung) cells. All six mice were dead within 13 days of infection, while the six C2DKO −/− recipients given +/+ CD4$^+$ T cells and +/+ BM concurrently survived and had cleared the virus from lungs by day 19 (Table 4).

Cytokine-producing cells were readily demonstrated (27, 28) by ELISPOT analysis (Table 2) of the BAL population from the +/+→−/− chimeras. Cytokine levels in the BAL fluid (concentrated fourfold) (23) and restimulated spleen cell supernatant (immune spleen cells were restimulated in vitro with irradiated, virus-infected splenocytes for 24 to 48 h) (28) were measured by enzyme-linked immunosorbent assay (ELISA) (23, 28). The broad spectrum of Th1 and Th2 cytokines (Table 2) was also found for the +/+ controls and is typical of the inflammatory process induced by this influenza virus (27). Furthermore, the levels of IFN-γ (Th1) made in the pneumonic lungs of the +/+→−/− mice were sufficient to allow detection in concentrated (23) BAL fluid (Table 2). The IL-6 that was also found in the BAL fluid can be made by a variety of cell types (1), including macrophages and B cells, in addition to CD4$^+$ T cells. At least some of this IL-6 is probably produced by CD4$^+$ T cells, as +/+→−/− spleen cell populations restimulated with virus in vitro also secreted substantial amounts of IL-6 and IFN-γ (Table 2).

TABLE 2. Cytokine profiles for BAL and spleen[a]

Source	Day	Mouse	IL-2	IL-4	IL-5	IL-6	IL-10	IFN-γ
BAL cells[b]	10	+/+	1.6	1.3	1.5	0.6	0.0	1.9
		+/+→−/−	1.9	1.7	2.1	0.9	0.3	1.0
	13[b]	+/+	2.1	2.2	1.0	0.2	0.2	1.0
		+/+→−/−	1.7	1.9	2.2	0.2	2.1	2.4
BAL fluid[c]	10	+/+	0.0	0.0	0.0	0.9	0.0	1.0
		+/+→−/−	0.0	0.0	0.0	4.0	0.0	12.3
	13	+/+	0.0	0.0	0.0	0.0	0.0	0.0
		+/+→−/−	0.0	0.0	0.0	3.4	0.0	7.2
	13[d]	++→−/−	0.0	0.0	1.4	8.0	0.0	2.1
Spleen[e]	10	+/+	0.0	0.0	0.0	8.0	0.0	1.6
		+/+→−/−	0.0	0.0	0.0	13.2	0.0	1.9
	13	+/+	0.0	0.0	0.0	9.3	0.0	8.2
		+/+→−/−	0.0	0.0	0.0	15.2	0.0	27.9
	13[d]	+/+→−/−	1.2	NT	NT	5.7	2.4	26.2

[a] The C2DKO −/− recipients were treated as described in the text except that the mice were given bone marrow plus $CD4^+$ T cells (1.4×10^6 per mouse) that were 99% $CD4^+$. Virus was detected in lung homogenates from two of two controls assayed on day 7 and zero of four from each group on day 13.

[b] Pooled BAL cells were assayed for cytokine producers by the single cell ELISPOT assay (9, 27, 28). The findings are presented as the percentage of total cells producing cytokines. The detection limit for each cytokine was 0.05%.

[c] Cytokine levels are expressed as units per milliliter or (for IL-10) nanograms per milliliter, before fourfold concentration. The detection limits were 0.1 ng/ml for IL-10, 0.4 U/ml for IFN-γ, and 0.2 U/ml for all other cytokines.

[d] A second experiment with the +/+→−/− chimeras showed that four of five +/+→−/− mice and four of five +/+→+/+ mice on day 10 and seven of eight from each group on day 13 had cleared the virus. The BAL cells from this repeat experiment were assayed for CTL activity by using virus-infected MHC I^+ II^+ target cells (17). No cytotoxicity was found for the lymphocytes obtained on day 10, while the values for day 13 (effector-to-target ratio, 40:1) were 15 and 16% for the respectivegroups. The lymphokine assays were only done on day 13.

[e] The detection limits were 0.4 ng/ml for IL-10, 1.6 U/ml for IFN-γ, and 0.8 U/ml for all other cytokines. Peak values are reported. NT, not tested.

To further demonstrate that the $CD4^+$ T-cell response in the +/+→−/− chimeras was essentially normal, 13 days after infection of chimeric mice (made as described for Table 2, except that each mouse received 4.0×10^6 FACS-enriched $CD4^+$T cells), the spectra of virus-specific plasma cells (20, 26) in cervical lymph nodes, MLN, and spleen were similar to the values found for the controls (Table 3). All the Ig isotypes that require "help" from Th1 or Th2 $CD4^+$ T cells were readily

TABLE 3. Prevalence of virus-specific-antibody-forming cells[a]

Antibody	No. of cells per group					
	+/+→+/+			+/+→−/−		
	CLN	MLN	Spleen	CLN	MLN	Spleen
IgM[b]	45	38	410	22	72	96
IgA	36	33	0	38	51	2
IgG1	38	123	13	9	183	3
IgG2a	103	55	5	33	185	2
IgG2b	93	68	8	27	244	4
IgG3	73	210	11	15	93	3

[a] The experiment followed the protocol described for Table 2, with the exception that each mouse was given 4.0×10^6 FACS-enriched (99% pure) $CD4^+$ T cells. Virus was recovered from lung homogenates of three of three mice from each group sampled on day 7 after infection, one of two on day 13, and zero of three on day 19.

[b] Specific-Ig-producing cells (antibody-forming cells) per 10^5 cells; values were obtained by single-cell ELISPOT assay of the cervical lymph nodes (CLN), MLN, and spleen (20, 26). Sera from two mice in each group were also analyzed for virus-specific Ig by ELISA end-point titration. The titers for all four sera were 1/27,000.

TABLE 4. Virus-specific $CD4^+$ T cells in +/+→−/− chimeras[a]

No. of days after infection	Source of cells	% with phenotype			Reciprocal Th frequency[b]	
		$CD4^+$	$CD8^+$	$B220^+$	NAPC	VAPC
13	CLN	22	<1	45	3,106	259
	MLN	34	<1	37	1,272	227
	Spleen	7	<1	17	1,962	254
19	CLN	11	1.1	67	8,251	106
	Spleen	7	<1	32	16,212	199

[a] The experiment was done as described for Table 2, with exception that the cells were enriched only by magnetic separation and were not sorted through the flow cytometer. Each mouse was given 9×10^5 $CD4^+$ T cells, and the infection was delayed until 5 days after cell transfer.

[b] Noninfected antigen-presenting cells (NAPC) or virus-infected antigen-presenting cells (VAPC). The only frequency values that were not significantly different (outside 95% confidence limits) were the day 13 results for the cervical lymph nodes (CLN). The high frequencies for the NAPC group on day 13 are typical when this assay is used during the acute phase of the infectious process (8) and may reflect "bystander activation" (31) of memory $CD4^+$ T cells specific for other antigens.

detected. Viral infection of unmanipulated C2DKO −/− mice leads only to an IgM response (19). We also performed limiting dilution analysis (LDA) of the $CD4^+$ Th precursors (Thp) by culturing FACS-enriched immune $CD4^+$ T cells under limiting dilution conditions (8) for 4 days with either uninfected or virus-infected, T-cell-depleted spleen cells as antigen-presenting cells. Supernatants from individual culture wells were then assayed for the presence of IL-2 (or IL-4) by [^{3}H]thymidine incorporation in a CTLL assay (8). Cultures with counts per minute that were greater than three times the standard deviation for CTLL cells cultured in medium alone were considered positive. The frequencies of virus-specific $CD4^+$ T cells for the +/+→−/− mice were clearly substantial and similar to values found previously in other experiments (Table 4) (8) (unpublished data).

Though the numbers of purified, immunologically naive $CD4^+$ T cells used to reconstitute the irradiated C2DKO −/− mice were as low as 9×10^5 (Table 4), the response generated when these T cells were primed in the context of MHC class II +/+ antigen-presenting cells was sufficient to clear influenza virus from the MHC class II −/− respiratory tract. Thus, unlike the situation for the $CD8^+$ set in β_2-m +/+→−/− chimeras given Sendai virus (15), the direct targeting of $CD4^+$ effectors to virus-infected respiratory epithelium expressing the appropriate MHC glycoprotein is not mandatory for virus clearance. Influenza virus can apparently be dealt with in the MHC class II +/+→−/− mice by cytokine- and/or antibody-mediated processes promoted by the $CD4^+$ Th populations. Furthermore, any cytokine effect does not (unlike the situation for $CD8^+$ effectors) require cognate interaction with the cells supporting virus growth. Given that influenza virus is cytolytic, the mechanism underlying $CD4^+$ T-cell effector function may reflect the interruption of virus dissemination (25) during the extracellular phase rather than T-cell-mediated elimination of the virus-producing cellular "factories." In addition to the chimera experiments (present data and reference 15), the fact that immune $CD4^+$ T cells can terminate the disease process caused by influenza virus (3, 7), Sendai virus (16), and vaccinia virus (30), but not the nonlytic lymphocytic choriomeningitis virus that is dealt with very adequately by the $CD8^+$ set (6, 10, 21), indicates that the fundamental basis of $CD4^+$ and $CD8^+$ T-cell-mediated effector function differs in virus infections. The present experiments do not, however, rule out the possi-

bility that $CD4^+$ CTL (24) can clear influenza virus, only that this aspect of the host response is not essential.

We thank Mehdi Mehrpooya and Anthony McMickle for technical assistance and Vicki Henderson for help with the manuscript.

This work was supported by grants CA 21765 and AI 29579 from the National Institutes of Health and by the American Lebanese Syrian Associated Charities.

REFERENCES

1. **Akira, S., T. Taga, and T. Kishimoto.** 1993. Interleukin-6 in biology and medicine. Adv. Immunol. **54:**1–78.
2. **Allan, W., Z. Tabi, A. Cleary, and P. C. Doherty.** 1990. Cellular events in the lymph node and lung of mice with influenza. Consequences of depleting CD4+ T cells. J. Immunol. **144:**3980–3986.
3. **Bender, B. S., T. Croghan, L. Zhang, and P. A. Small, Jr.** 1992. Transgenic mice lacking class I major histocompatibility complex-restricted T cells have delayed viral clearance and increased mortality after influenza virus challenge. J. Exp. Med. **175:**1143–1145.
4. **Bix, M., and D. Raulet.** 1992. Inefficient positive selection of T cells directed by haematopoietic cells. Nature (London) **359:**330–333.
5. **Doherty, P. C., R. Ceredig, and J. E. Allan.** 1988. Immunogenetic analysis of cellular interactions governing the recruitment of T lymphocytes and monocytes in lymphocytic choriomeningitis virus-induced immunopathology. Clin. Immunol. Immunopathol. **47:**19–26.
6. **Doherty, P. C., S. Hou, and P. J. Southern.** 1993. Lymphocytic choriomeningitis virus induces a chronic wasting disease in mice lacking class I major histocompatibility complex glycoproteins. J. Neuroimmunol. **46:**11–18.
7. **Eichelberger, M., W. Allan, M. Zijlstra, R. Jaenisch, and P. C. Doherty.** 1991. Clearance of influenza virus respiratory infection in mice lacking class I major histocompatibility complex-restricted $CD8^+$ T cells. J. Exp. Med. **174:**875–880.
8. **Ewing, C., D. J. Topham, and P. C. Doherty.** 1995. Prevalence and activation phenotype of Sendai virus-specific $CD4^+$ T cells. Virology **210:**179–185.
9. **Fujihashi, K., J. R. McGhee, K. W. Beagley, D. T. McPherson, S. A. McPherson, C.-M. Huang, and H. Kiyono.** 1993. Cytokine-specific ELISPOT assay. Single cell analysis of IL-2, IL-4 and IL-6 producing cells. J. Immunol. Methods **160:**181–189.
10. **Fung-Leung, W. P., T. M. Kundig, R. M. Zinkernagel, and T. W. Mak.** 1991. Immune response against lymphocytic choriomeningitis virus infection in mice without CD8 expression. J. Exp. Med. **174:**1425–1429.
11. **Graham, M. B., V. L. Braciale, and T. J. Braciale.** 1994. Influenza virus-specific $CD4^+$ T helper type 2 T lymphocytes do not promote recovery from experimental virus infection. J. Exp. Med. **180:**1273–1282.
12. **Grusby, M. J., R. S. Johnson, V. E. Papaioannou, and L. H. Glimcher.** 1991. Depletion of $CD4^+$ T cells in major histocompatibility complex class II-deficient mice. Science **253:**1417–1420.
13. **Horimoto, T., and Y. Kawaoka.** 1995. The hemagglutinin cleavability of a virulent avian influenza virus by subtilisin-like endoproteases is influenced by the amino acid immediately downstream of the cleavage site. Virology **210:** 466–470.
14. **Hou, S., and P. C. Doherty.** 1993. Partitioning of responder $CD8^+$ T cells in lymph node and lung of mice with Sendai virus pneumonia by LECAM-1 and CD45RB phenotype. J. Immunol. **150:**5494–5500.
15. **Hou, S., and P. C. Doherty.** 1995. Clearance of Sendai virus by $CD8^+$ T cells requires direct targeting to virus-infected epithelium. Eur. J. Immunol. **25:** 111–116.
16. **Hou, S., P. C. Doherty, M. Zijlstra, R. Jaenisch, and J. M. Katz.** 1992. Delayed clearance of Sendai virus in mice lacking class I MHC-restricted $CD8^+$ T cells. J. Immunol. **149:**1319–1325.
17. **Hou, S., M. Fishman, K. G. Murti, and P. C. Doherty.** 1993. Divergence between cytotoxic effector function and tumor necrosis factor alpha production for inflammatory $CD4^+$ T cells from mice with Sendai virus pneumonia. J. Virol. **67:**6299–6302.
18. **Hou, S., L. Hyland, K. W. Ryan, A. Portner, and P. C. Doherty.** 1994. Virus-specific $CD8^+$ T-cell memory determined by clonal burst size. Nature (London) **369:**652–654.
19. **Hou, S., X. Y. Mo, L. Hyland, and P. C. Doherty.** 1995. Host response to Sendai virus in mice lacking class II major histocompatibility complex glycoproteins. J. Virol. **69:**1429–1434.
20. **Hyland, L., M. Sangster, R. Sealy, and C. Coleclough.** 1994. Respiratory virus infection of mice provokes a permanent humoral immune response. J. Virol. **68:**6083–6086.
21. **Lehmann-Grube, F., J. Löhler, O. Utermöhlen, and C. Gegin.** 1993. Antiviral immune responses of lymphocytic choriomeningitis virus-infected mice lacking $CD8^+$ T lymphocytes because of disruption of the β_2-microglobulin gene. J. Virol. **67:**332–339.
22. **Markowitz, J. S., H. Auchincloss, Jr., M. J. Grusby, and L. H. Glimcher.** 1993. Class II-positive hematopoietic cells cannot mediate positive selection of $CD4^+$ T lymphocytes in class II-deficient mice. Proc. Natl. Acad. Sci. USA **90:**2779–2783.
23. **Mo, X. Y., S. R. Sarawar, and P. C. Doherty.** 1995. Induction of cytokines in mice with parainfluenza pneumonia. J. Virol. **69:**1288–1291.
24. **Muller, D., B. H. Koller, J. L. Whitton, K. E. LaPan, K. K. Brigman, and J. A. Frelinger.** 1992. LCMV-specific, class II-restricted cytotoxic T cells in β2-microglobulin-deficient mice. Science **255:**1576–1578.
25. **Palladino, G., K. Mozdzanowska, G. Washko, and W. Gerhard.** 1995. Virus-neutralizing antibodies of immunoglobulin G (IgG) but not of IgM or IgA isotypes can cure influenza virus pneumonia in SCID mice. J. Virol. **69:**2075–2081.
26. **Sangster, M., L. Hyland, R. Sealy, and C. Coleclough.** 1995. Distinctive kinetics of the antibody-forming cell response to Sendai virus infection of mice in different anatomical compartments. Virology **207:**287–291.
27. **Sarawar, S. R., S. R. Carding, W. Allan, A. McMickle, K. Fujihashi, H. Kiyono, J. R. McGhee, and P. C. Doherty.** 1993. Cytokine profiles of bronchoalveolar lavage cells from mice with influenza pneumonia: consequences of $CD4^+$ and $CD8^+$ T cell depletion. Reg. Immunol. **5:**142–150.
28. **Sarawar, S. R., and P. C. Doherty.** 1994. Concurrent production of interleukin-2, interleukin-10, and gamma interferon in the regional lymph nodes of mice with influenza pneumonia. J. Virol. **68:**3112–3119.
29. **Scherle, P. A., G. Palladino, and W. Gerhard.** 1992. Mice can recover from pulmonary influenza virus infection in the absence of class I-restricted cytotoxic T cells. J. Immunol. **148:**212–217.
30. **Spriggs, M. K., B. H. Koller, T. Sato, P. J. Morrissey, W. C. Fanslow, O. Smithies, R. F. Voice, M. B. Widmer, and C. R. Maliszewski.** 1992. β-Microglobulin, $CD8^+$ T-cell-deficient mice survive inoculation with high doses of vaccinia virus and exhibit altered IgG responses. Proc. Natl. Acad. Sci. USA **89:**6070–6074.
31. **Tripp, R. A., S. Hou, A. McMickle, J. Houston, and P. C. Doherty.** 1995. Recruitment and proliferation of $CD8^+$ T cells in respiratory virus infections. J. Immunol. **154:**6013–6021.
32. **Walker, J. A., T. Sakaguchi, Y. Matsuda, T. Yoshida, and Y. Kawaoka.** 1992. Location and character of the cellular enzyme that cleaves the hemagglutinin of a virulent avian influenza virus. Virology **190:**278–287.