Modification of the Sendai Virus-Specific Antibody and CD8⁺ T-Cell Responses in Mice Homozygous for Disruption of the Interleukin-4 Gene

X. YANIE MO, MARK Y. SANGSTER, RALPH A. TRIPP, AND PETER C. DOHERTY*

Department of Immunology, St. Jude Children's Research Hospital, Memphis, Tennessee 38105

Received 21 October 1996/Accepted 13 December 1996

Homozygous disruption (–/–) of the interleukin-4 (IL-4) gene did not obviously modify the severity of Sendai virus infection in the highly susceptible 129/J mouse strain. The virus was cleared from the respiratory tract, and potent cytotoxic T lymphocyte (CTL) effectors were present in the cell population recovered by bronchoalveolar lavage. However, the prevalence of virus-specific CTL precursors (p) was consistently diminished in the spleen and regional lymph nodes of the IL-4 –/– mice at day 7 after infection. Also, virus-specific serum immunoglobulin G1 (IgG1) levels were greatly reduced and few IgG1-producing cells were detected in the lymphoid tissue. The effect on IgG1 class switching was to be expected, but the decrease in CTLp numbers has not been observed previously for a virus-specific immune response.

Current thinking about the role of cytokines in disease processes emphasizes the divergent functional consequences of skewing the response towards a T_H1-like or T_H2-like profile (1, 15, 20). The T_H1 cytokine interferon gamma (IFN-γ) is prominent in most viral infections, while the prototypic T_H2 cytokine interleukin-4 (IL-4) is usually much less apparent (7, 18, 24, 25). Part of the reason for this may be that IL-4 is rapidly consumed and is thus difficult to detect in culture supernatants following *in vitro* restimulation protocols. Enhanced production of the T_H2 cytokines (IL-4 and IL-5) is generally considered to promote the emergence of a more “allergic” type of response, a syndrome that has been described for BALB/c mice infected with respiratory syncytial virus (RSV). Treating RSV-infected mice with a neutralizing monoclonal antibody (MAb) to IL-4 decreases the severity of the lung pathology (4, 8, 29). There is also evidence that excessive IL-4 production may compromise the development of virus-specific cell-mediated immunity. The magnitude of the vaccinia-specific CD8⁺ CTL response was greatly diminished following infection with a recombinant virus incorporating the murine IL-4 gene (28).

Intranasal (i.n.) infection of *H-2^b* congenic C57BL/6J (B6) and 129/J mice with the parainfluenza type 1 virus Sendai virus (SV) leads to the development of pneumonia, with both the rate of virus replication in the lung and the likelihood of fatal consequences being much greater in the 129 mice (17, 22). The 129 lymph node cells produced more IFN-γ following *in vitro* restimulation with SV, and the amounts of IFN-γ detected in the supernatant fluid obtained by bronchoalveolar lavage (BAL) were higher (17). No IL-4 was detected in either preparation from any of the mice. The BAL cell populations from both mouse strains, however, contained substantial numbers of both IL-4- and IFN-γ-producing cells. In addition, though the prominent IFN-γ-associated IgG2a response associated much more strongly with the 129 lymphoid compartment, the lower level IL-4-related production of SV-specific IgG1 was generally comparable in magnitude for the 129 and B6 mice.

The biological role of IFN-γ in SV infection has been analyzed by using IFN-γ –/– and +/+ BALB/c mice (5, 19). The

absence of IFN-γ had minimal consequences for the pathogenesis of this viral pneumonia. The amount of virus-specific IgG2a was slightly decreased and the rate of development of virus-specific CTL precursors (p) was reduced, with the latter effect being observed only when the IFN-γ –/– mice were concurrently depleted of the CD4⁺ T-cell subset. The overall conclusion was that the CD4⁺ T cells are making some other cytokine which substitutes for IFN-γ to promote the CD8⁺ T-cell response. This is in accord with evidence from a range of virus models that the lack of IFN-γ does not greatly compromise immunity, though it may tend to promote more of a T_H2-like response (9, 21).

The part played by the T_H2 cytokines in respiratory virus infections has largely been addressed for the RSV model (4, 8, 29). However, it is also clear from single-cell ELISPOT analysis of BAL populations that there are substantial numbers of IL-4-producing cells in the pneumonia resulting from infection with either SV or influenza virus (18, 24) and that (at least for the influenza model) cells expressing IL-4 mRNA are present at significant frequency in the lymphoid tissue (3). Treating β2-microglobulin –/– mice with a neutralizing MAb to IL-4 throughout the course of an influenza A virus infection neither altered the prevalence of the IL-4 producing cells in the BAL nor compromised virus clearance by CD4⁺ T-cell-mediated processes (26). The pathogenesis of influenza virus infection was not obviously modified in IFN-γ –/– mice, though it was possible to clone CD4⁺ T_H2 cells that secreted IL-4 from the spleens of convalescent animals (10). These T_H2 cells were unable to clear influenza virus on adoptive transfer (9). Otherwise, the role of IL-4 in respiratory virus infections has not been addressed in a direct way. Thus, we took advantage of the opportunity to analyze the host response to SV in IL-4 –/– (cell line 129) mice (14). Such IL-4 –/– mice have been shown to respond normally to both lymphocytic choriomeningitis virus and to vesicular stomatitis virus (2).

No external disease signs developed at any time in IL-4 –/– and +/+ mice infected i.n. with a SV dose of 100 EID₅₀ (equivalent to 0.5 50% lethal doses in +/+ mice). Three mice in each group were observed for 20 days, and then their lungs were tested and found to be free of infectious virus. The lung titers (mean ± standard deviation log₁₀ EID₅₀/ml of lung homogenate, *n* = 3) for mice sampled at day 11 after infection

* Corresponding author. Mailing address: Department of Immunology, St. Jude Children's Research Hospital, 332 N. Lauderdale, Memphis, TN 38105. Phone: (901) 495-3470. Fax: (901) 495-3107.

FIG. 1. The distribution of the various lymphocyte subsets in lymphoid tissue of IL-4 $-/-$ and $+/+$ mice 11 days after infection was determined by two-color flow cytometric analysis (30) in a FACScan (Becton Dickinson, Mountain View, Calif.). Single-cell suspensions were stained with fluorescein isothiocyanate-conjugated Abs specific for CD8 (53.6.72), and the B-cell marker B220 (RA3.6B2), or phycoerythrin-conjugated MAbs to CD4 (RM4-5) and the T-cell receptor chain (H57-697), all of which were purchased from Pharmingen (San Diego, Calif.). Repeat experiments were done with pooled samples from three individual mice. Shown is the mean $\pm$ standard error of the mean for three separate experiments.

were 3.4 ± 1.1 ($-/-$) and 3.8 ± 0.1 ($+/+$), which is at least 100-fold lower than the peak values (day 7) recorded previously for this mouse strain (17). The distribution profiles for the various lymphocyte subsets (30) in the regional cervical and mediastinal lymph nodes (CLN and MLN) and spleen were also comparable for the two groups (Fig. 1). As expected, the levels of virus-specific IgG1 in serum (23) were minimal for the IL-4 $-/-$, though the response profiles for the other Ig isotypes were essentially normal (Table 1). This profile was reflected in the frequencies (Fig. 2) of antibody-forming cells (AFCs) detected by ELISPOT analysis of the CLN (23).

Virus-specific CTL activity in the BAL (12) was equivalent for the IL-4 $-/-$ and $+/+$ mice at day 11 after infection (Fig. 3). However, the frequencies (data not shown) and the available numbers of virus-specific CTLps (11) were lower at day 7 in the spleen, the CLN, and the MLN (Table 2). The effect was less apparent on day 11, but the values for the spleen were still consistently different between $-/-$ and $+/+$ mice (Table 2). Thus, it seems that IL-4 is playing a part in promoting clonal expansion of the CTLp population (Table 2), though the single

experiment that was done at day 11 after infection suggests that this cytokine is not essential for the transition to CTL effector function (Fig. 3) that occurs in the virus-infected lung (6, 12). Previous, *in vitro* studies have also indicated that IL-4 can act as a growth factor for CD8⁺ T cells (13, 16, 27, 33), though this is (to our knowledge) the first report of such an effect operating in a whole animal system. The possibility exists that the effect of an IL-4 deficiency on the development of CTL responses may be in part compensated by enhanced production of IL-12 and T_H1 cytokines in IL-4 $-/-$ mice.

A lower prevalence of CTLp in the lymphoid tissue of ge-

TABLE 1. Consequences of IL-4 disruption for virus-specific serum antibody response

Mouse type	Titer (10^3) ^a			
	IgG1	IgG2a	IgG2b	IgG3
+/+	9.4 ± 5.2	113.4 ± 36.2	24.1 ± 12.1	2.7 ± 1.4
-/-	$<0.5 \pm 0.2$	189.0 ± 37.3	22.0 ± 9.8	8.1 ± 0.0

^a Titers (mean $\pm$ SEM) were determined by enzyme-linked immunosorbent assay for 5 or 6 individual mice infected i.n. with 100 EID₅₀ of SV 11 days after infection. Serial threefold dilutions of serum were assayed on plates coated with detergent-disrupted SV virus. Virus titers were expressed as the reciprocal of the highest dilutions giving an absorbance value greater than twice that for corresponding normal uninfected serum (23).

FIG. 2. Prevalence of SV-specific AFCs in the CLN of IL-4 $-/-$ and $+/+$ mice infected i.n. with 100 EID₅₀ of SV 11 days previously. Single-cell suspensions of the CLN were assayed by a well-established ELISPOT procedure (23) that uses detergent-disrupted SV coated onto the plates as an immunoabsorbant and antibodies to the various Ig isotypes to develop the "spots" characteristic of a single B cell/plasma cell. The data are the average values for two experiments for the $+/+$ mice and mean $\pm$ standard error of the mean for six IL-4 $-/-$ mice.

FIG. 3. The level of virus-specific CTL activity (12) was analyzed for the inflammatory population recovered by BAL at 11 days after i.n. infection of the IL-4 ^{+/+} and ^{-/-} mice with 100 EID₅₀ of SV. Pooled BAL cells recovered from 6 mice in each group were used in the analysis. The BAL cells were first adsorbed on plastic to remove most of the macrophages. The levels of specific ⁵¹Cr release are shown for a 6-h assay with SV-infected major histocompatibility complex class I⁺ II⁻ MC57G fibroblasts. The maximal levels of ⁵¹Cr release against uninfected MC57G targets were less than 5% for both ^{+/+} and ^{-/-} mice (data not shown).

netically disrupted mice that still mount an effective CD8⁺ T-cell response has been seen previously for major histocompatibility complex (MHC) class II ^{-/-} mice infected with an influenza A virus (32), and in the response to SV in CD4⁺ T-cell-depleted IFN- γ ^{-/-} mice (19). Comparison with the appropriate controls gives the impression that a smaller CTLp pool is utilized more completely in the various ^{-/-} mice to give an adequate CTL response. The numbers of responding CTLp generated in the acute response to virus infections of the lung may, at least in young adult mice, normally be greatly in excess of those required to terminate the infectious process (31).

TABLE 2. Virus-specific CTLp in IL-4 ^{-/-} and ^{+/+} mice^a

Days after infection and expt no.	CTLp Numbers					
	Spleen		CLN		MLN	
	^{-/-}	^{+/+}	^{-/-}	^{+/+}	^{-/-}	^{+/+}
7						
1	160*	8,650	369*	1,779	28*	274
2	1,619*	5,988	270*	1,247	<5*	251
11						
1	1,671*	3,652	320*	10,884	268	299
2	1,644*	6,521	1,036*	2,500	2,500	2,222
3	1,677*	5,294	604*	958	500	638

^a The mice were infected i.n. with 100 EID₅₀ of Sendai virus and sampled 7 or 11 days later. Graded dilutions of spleen, CLN, and MLN cells were cultured (replicates of 24) for 7 days in 96-well plates with irradiated (3,000 rads) virus-infected splenocytes + rIL-2 (10 U/ml). Virus-infected, ⁵¹Cr-labeled, MHC class I⁺ II⁻ MC57G targets were added to each well, and the level of specific ⁵¹Cr release was determined after a further 6-h incubation. Virus-specific CTLp frequencies (data not shown) were determined as described previously (11, 12), and CTLp numbers were calculated from the frequencies and the nucleated cell counts for each organ. The day 7 analysis was done on two separate occasions (expts. 1 and 2) with individual mice. The day 11 analysis was done on three separate occasions (expts. 1, 2, and 3) with pooled cells from three mice. Asterisks indicate that the CTLp frequencies (data not shown) that were used to calculate the CTLp numbers for the IL-4 ^{-/-} and ^{+/+} mice were not within 95% confidence limits and are thus significantly different.

The absence of any IgG1 response in these IL-4 ^{-/-} mice was expected from the known properties of this cytokine (1, 20). The decrease in CTLp frequencies is in accord with findings from in vitro systems that IL-4 can promote the CD8⁺ T-cell response, perhaps by a synergistic effect with IL-2 (13, 16, 27, 33). This is, however, the first time (to our knowledge) that the IL-4 that is always produced during the course of these virus infections (3, 18, 19, 24, 25) has been shown to have some beneficial effect. Analyzing how cytokines influence the virus-specific host response can lead to very different conclusions (28) depending on the mode of delivery and the effective dose in the lymphoid tissue and in sites of inflammatory pathology.

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REFERENCES

- Abbas, A. K., K. M. Murphy, and A. Sher. 1996. Functional diversity of helper T lymphocytes. *Nature* **383**:787-793.
- Bachmann, M. F., H. Schorle, R. Kühn, W. Müller, H. Hengartner, R. M. Zinkernagel, and I. Horak. 1995. Antiviral immune responses in mice deficient for both interleukin-2 and interleukin-4. *J. Virol.* **69**:4842-4846.
- Carding, S. R., W. Allan, A. McMickle, and P. C. Doherty. 1993. Activation of cytokine genes in T cells during primary and secondary murine influenza pneumonia. *J. Exp. Med.* **177**:475-482.
- Connors, M., N. A. Giese, A. B. Kulkarni, C. Y. Firestone, H. C. Morse, III, and B. R. Murphy. 1994. Enhanced pulmonary histopathology induced by respiratory syncytial virus (RSV) challenge of formalin-inactivated RSV-immunized BALB/c mice is abrogated by depletion of interleukin-4 (IL-4) and IL-10. *J. Virol.* **68**:5321-5325.
- Dalton, D. K., S. Pitts-Meek, S. Keshav, I. S. Figari, A. Bradley, and T. A. Stewart. 1993. Multiple defects of immune cell function in mice with disrupted interferon-gamma genes. *Science* **259**:1739-1742.
- Doherty, P. C. 1995. Anatomical environment as a determinant in viral immunity. *J. Immunol.* **155**:1023-1027.
- Frei, K., T. P. Leist, A. Meager, P. Gallo, D. Leppert, R. M. Zinkernagel, and A. Fontana. 1988. Production of B cell stimulatory factor-2 and interferon gamma in the central nervous system during viral meningitis and encephalitis. Evaluation in a murine model infection and in patients. *J. Exp. Med.* **168**:449-453.
- Graham, B. S., G. S. Henderson, Y. W. Tang, X. Lu, K. M. Neuzil, and G. Colley. 1993. Priming immunization determines T helper cytokine mRNA expression patterns in lungs of mice challenged with respiratory syncytial virus. *J. Immunol.* **151**:2032-2040.
- 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.
- Graham, M. B., D. K. Dalton, D. Giltinan, V. L. Braciale, T. A. Stewart, and T. J. Braciale. 1993. Response to influenza infection in mice with a targeted disruption in the interferon gamma gene. *J. Exp. Med.* **178**:1725-1732.
- 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.
- 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.
- Hu-Li, J., E. M. Shevach, J. Mizuguchi, J. Mosmann, J. Ohara, T. Mosmann, W. E. Paul. 1987. B cell stimulatory factor 1 (interleukin 4) is a potent co-stimulant for normal resting T lymphocytes. *J. Exp. Med.* **165**:157-72.
- Kuhn, R., K. Rajewsky, and W. Muller. 1991. Generation and analysis of interleukin-4 deficient mice. *Science* **254**:707-710.
- Locksley, R. M., and P. Scott. 1991. Helper T-cell subsets in mouse leishmaniasis: induction, expansion and effector function. *Immunol. Today* **12**: A58-A61.
- Miller, C. L., J. W. Hooton, S. Gillis, and V. Paetkau. 1990. IL-4 potentiates the IL-2-dependent proliferation of mouse cytotoxic T cells. *J. Immunol.* **144**:1331-1337.
- Mo, X. Y., M. Sangster, S. Sarawar, C. Coleclough, and P. C. Doherty. 1995. Differential antigen burden modulates the gamma interferon but not the immunoglobulin response in mice that vary in susceptibility to Sendai virus pneumonia. *J. Virol.* **69**:5592-5599.

18. Mo, X. Y., S. R. Sarawar, and P. C. Doherty. 1995. Induction of cytokines in mice with parainfluenza pneumonia. *J. Virol.* **69**:1288–1291.
19. Mo, X. Y., R. A. Tripp, M. Y. Sangster, and P. C. Doherty. 1997. The cytotoxic T-lymphocyte response to Sendai virus is unimpaired in the absence of gamma interferon. *J. Virol.* **71**:1906–1910.
20. Mosmann, T. R., and R. L. Coffman. 1989. T_H1 and T_H2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu. Rev. Immunol.* **7**:145–173.
21. Muller, U., U. Steinhoff, L. F. Reis, S. Hemmi, J. Pavlovic, R. M. Zinkernagel, and M. Aguet. 1994. Functional role of type I and type II interferons in antiviral defense. *Science* **264**:1918–1921.
22. Parker, J. C., M. D. Whiteman, and C. B. Richter. 1978. Susceptibility of inbred and outbred mouse strains to Sendai virus and prevalence of infection in laboratory rodents. *Infect. Immun.* **19**:123–130.
23. 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.
24. 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.
25. 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.
26. Sarawar, S. R., M. Sangster, R. L. Coffman, and P. C. Doherty. 1994. Administration of anti-IFN-gamma antibody to β_2 -microglobulin-deficient mice delays influenza virus clearance but does not switch the response to a T helper cell 2 phenotype. *J. Immunol.* **153**:1246–1253.
27. Selvan, R. S., P. S. Nagarkatti, and M. Nagarkatti. 1990. Role of IL-2, IL-4 and IL-6 in the growth and differentiation of tumor-specific CD4⁺ T helper and CD8⁺ T cytotoxic cells. *Int. J. Cancer* **45**:1096–1104.
28. Sharma, D. P., A. J. Ramsay, D. J. Maguire, M. S. Rolph, and I. A. Ramshaw. Interleukin-4 mediates down regulation of antiviral cytokine expression and cytotoxic T-lymphocyte responses and exacerbates vaccinia virus infection in vivo. 1996. *J. Virol.* **70**:7103–7107.
29. Tang, Y. W., and B. S. Graham. 1994. Anti-IL-4 treatment at immunization modulates cytokine expression, reduces illness, and increases cytotoxic T lymphocyte activity in mice challenged with respiratory syncytial virus. *J. Clin. Invest.* **94**:1953–1958.
30. 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.
31. Tripp, R. A., J. M. Lahti, and P. C. Doherty. 1995. Laser light suicide of proliferating virus-specific CD8⁺ T cells in an in vivo response. *J. Immunol.* **155**:3719–3721.
32. Tripp, R. A., S. R. Sarawar, and P. C. Doherty. 1995. Characteristics of the influenza virus-specific CD8⁺ T cell response in mice homozygous for disruption of the H-21A^b gene. *J. Immunol.* **155**:2955–2959.
33. Widmer, W. B., R. B. Acres, H. M. Sassenfeld, and K. H. Grabstein. 1987. Regulation of cytolytic cell populations from human peripheral blood by B cell stimulatory factor 1 (interleukin 4). *J. Exp. Med.* **166**:1447–1455.