INFECTION AND IMMUNITY, May 2003, p. 2938–2940 Vol. 71, No. 5
0019-9567/03/$08.00+0 DOI: 10.1128/IAI.71.5.2938–2940.2003

Humoral Immunity Reflects Altered T Helper Cell Bias in *Borrelia burgdorferi*-Infected γδ T-Cell-Deficient Mice

Linda K. Bockenstedt,[1]* Marie-Claude Shanafelt,[1] Alexia Belperron,[1] Jialing Mao,[1] and Stephen W. Barthold[2]

Department of Internal Medicine, Yale University School of Medicine, New Haven, Connecticut 06520,[1] and Center for Comparative Medicine, University of California, Davis, California 95616[2]

Received 26 November 2002/Returned for modification 30 December 2002/Accepted 14 January 2003

In murine Lyme borreliosis, the absence of γδ T lymphocytes augments the T helper cell 2 type humoral response but does not alter disease susceptibility. Arthropod transmission of *Borrelia burgdorferi* spirochetes results in similar antibody isotypes when γδ T cells are present, suggesting that vector effects can negate γδ T-cell functions in vivo.

Lyme borreliosis is a multisystem illness caused by infection with the arthropod-borne spirochete *Borrelia burgdorferi* (7, 27). In the mouse model of Lyme borreliosis, spirochetes introduced intradermally by syringe inoculation or by the tick vector first establish infection in the skin (2, 26), where they may encounter γδ T lymphocytes among the responding immune cells. γδ T cells have been shown to influence the T helper (Th) cell response and alter resistance to disease due to intracellular pathogens (10, 11, 13, 16–18), but their effects on the immune response to a vector-borne extracellular pathogen are unknown. We therefore used mice that had been rendered deficient in γδ T cells either by antibody (Ab) depletion (BALB/c mice) or by targeted disruption of the T-cell receptor (TCR) δ gene (B6.129 TCR $\delta^{-/-}$ mice [B6.129P2-*Tcrd*^{tm1Mom}; Jackson Laboratories, Bar Harbor, Maine] and BALB/c TCR $\delta^{-/-}$ mice [29], a gift of Mark Shlomchik, Yale University) to examine the consequences of γδ T-cell deficiency on the course of murine Lyme borreliosis. For Ab depletion, 150 μg of purified hamster anti-mouse γδ TCR monoclonal Ab UC7-13D5 or 150 μl of hamster serum was subcutaneously injected into 4- to 5-week-old BALB/c mice daily, beginning 5 days prior to experimental infection and continuing for a total of 9 days.

Mice were infected by either intradermal inoculation of 10^4 cloned N40 spirochetes in 100 μl of Barbour Stoenner Kelly medium (1) or by infestation with five N40-infected *Ixodes scapularis* nymphs (5). Fourteen days after infection, lymph node cells from γδ T-cell-deficient BALB/c mice produced less gamma interferon (IFN-γ) than controls, as measured by cytokine-specific enzyme-linked immunosorbent assay (ELISA) (Fig. 1). Interleukin 4 was detected in culture supernatants of stimulated lymph node cells, consistent with the known Th2 dominance of BALB/c mice (4, 19), and the level was modestly increased in the absence of γδ T cells (273 versus 408 pg/ml; $P < 0.07$). A more dramatic reduction in IFN-γ production was noted for B6.129 TCR $\delta^{-/-}$ mice (Fig. 1), but no interleukin 4 was detected. Thus, the absence of γδ T cells in *B. burgdorferi* infection reduces the Th1 cytokine response, consistent with previous studies showing that γδ T cells can direct polarization of αβ Th-cell subsets (11, 17, 18).

ELISA measurement of *B. burgdorferi*-specific immunoglobulin G (IgG) isotypes (25) reflected the reduction in the Th1 cytokine IFN-γ (Table 1). BALB/c TCR $\delta^{-/-}$ mice had a significant rise in endpoint titers of IgG1 ($P = 0.0317$, Mann-Whitney test) and tended to have lower IgG2a levels. In contrast, B6.129 TCR $\delta^{-/-}$ mice exhibited a marked reduction in IgG3 endpoint titers ($P = 0.0159$, Mann-Whitney test), and titers of IgG2b trended higher. One explanation for the different IgG isotypes induced in the two mouse strains is that γδ T cells serve to decrease the genetically dominant Th2 response in BALB/c mice, whereas they promote a Th1 response in B6.129 mice.

Saliva from *I. scapularis* nymphs, the vector for *B. burgdorferi*, contains factors that inhibit host defenses and retard the development of immunologic resistance to tick infestation (21, 22, 24). Although deposited locally in the skin during tick feeding, tick saliva appears to have systemic effects. The feeding of *I. scapularis* ticks on mice results in decreased IFN-γ production and downregulation of a Th1-type response by mitogen-stimulated splenocytes (23, 28). Interestingly, in our study, *B. burgdorferi*-specific IgG isotypes induced in control mice by vector-borne infection paralleled those seen in TCR $\delta^{-/-}$ mice infected with cultured spirochetes, supporting a vector-induced bias toward Th2 cytokine patterns. Tick-transmitted spirochetes elicited predominantly IgG1 in wild-type BALB/c mice, at levels comparable to those achieved in BALB/c TCR $\delta^{-/-}$ infected with cultured spirochetes (Table 1). The absence of γδ T cells did not lead to a further increase in this Ab subset after vector-borne infection.

Despite the altered cytokine and humoral immune responses in *B. burgdorferi*-infected γδ T-cell-deficient mice, we observed no difference in the severity or prevalence of disease as assessed by histopathology (3). When examined using TCR $\delta^{-/-}$ mice on two backgrounds or after depletion of γδ T cells by monoclonal Ab treatment, no significant differences were noted in arthritis or carditis (Table 2). The relative decrease in *B. burgdorferi*-specific Th1 responses in TCR $\delta^{-/-}$ mice did not lead to protracted episodes of carditis even though $CD4^+$ Th1

* Corresponding author. Mailing address: 609 LCI, Section of Rheumatology, Yale University School of Medicine, 333 Cedar St., New Haven, CT 06520-8031. Phone: (203) 785-7063. Fax: (203) 785-7053. E-mail: linda.bockenstedt@yale.edu.

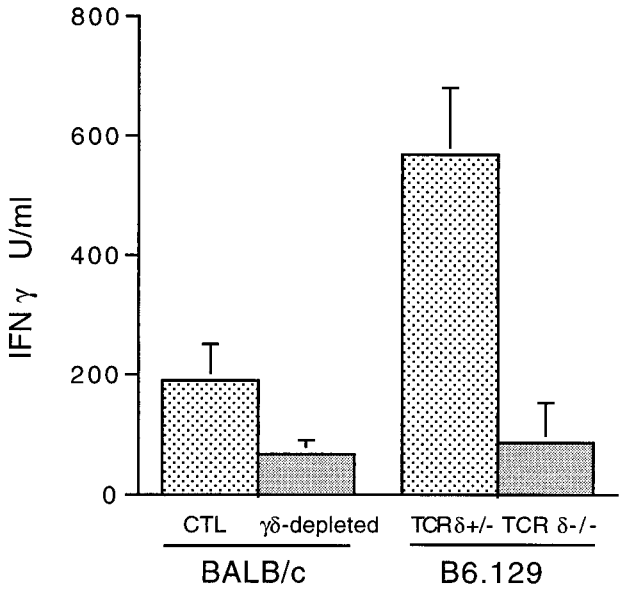

FIG. 1. Lymph node cells from mice deficient in γδ T cells produce less IFN-γ. Production of IFN-γ by popliteal lymph node cells isolated from individual mice was measured by ELISA. Each result is reported as the mean of values obtained for mice within each group ± the standard error of the mean.

TABLE 2. Absence of γδ T cells does not alter disease in *B. burgdorferi*-infected mice[a]

Expt or mouse strain + treatment	Day of infection	Results		
		Carditis	Arthritis	Arthritis severity
Expt 1				
BALB/c + UC7-13D5	14	0/10	11/20	1.1 ± 0.5
BALB/c + hamster serum	14	0/10	9/20	1.2 ± 0.5
Expt 2				
B6.129 (WT)	14	0/6	3/12	1.4 ± 0.6
B6.129 (TCR $\delta^{-/-}$)	14	0/6	5/12	1.1 ± 0.4
B6.129 (WT)	30	0/6	4/12	1.2 ± 0.3
B6.129 (TCR $\delta^{-/-}$)	30	0/6	5/12	1.3 ± 0.4
Expt 3				
BALB/c (WT)	14	5/5	12/13	1.0 ± 0.2
BALB/c (TCR $\delta^{-/-}$)	14	5/5	15/16	1.2 ± 0.3
BALB/c (WT)	42	1/5	3/17	0.6 ± 0.2
BALB/c (TCR $\delta^{-/-}$)	42	0/3	2/12	0.3 ± 0.3

[a] In experiment 1, BALB/c mice were treated with the indicated agents as described in Materials and Methods and then infected by inoculation of spirochetes into both hind feet. In experiments 2 and 3, mice were infected by inoculation of spirochetes into the skin of the back. Carditis results are reported as the number of mice with acute carditis over the total number of animals examined. Arthritis presence in tibiotarsal joints was assessed and expressed as the number of inflamed joints over the total number of joints examined. Arthritis severity was determined by averaging the individual scores for the most severely affected joint in each mouse ± the standard error of the mean.

cells have been shown to promote resolution of this disease manifestation (6). Two previous studies also failed to show an effect of absence of the Th1 cytokine IFN-γ on murine Lyme arthritis (8, 14). Mean pathogen burden among mouse groups was the same as assessed by quantitative PCR of the spirochete *osp*A gene in urinary bladders (data not shown).

B. burgdorferi TCR $\delta^{-/-}$ mice were able to develop protective Ab. Both wild-type and TCR $\delta^{-/-}$ mice that were passively immunized with 500 μl of 1:5 dilution of immune serum from 45-day-infected TCR $\delta^{-/-}$ mice 24 h prior to challenge infection with tick-borne spirochetes were completely protected as assessed by culture and histopathology (data not shown) (3). This was an expected outcome, because for *B. burgdorferi* infection, protective and arthritis-resolving antibodies arise in the absence of T-cell help (12, 20).

In summary, we used the murine model of Lyme borreliosis to investigate the effect of γδ T cells on the adaptive immune response to a vector-borne extracellular pathogen. While disease expression was not altered, our results show that γδ T cells influence the quality of the humoral immune response to *B. burgdorferi* introduced through the skin and suggest that tick transmission of spirochetes negates the γδ T-cell effect. γδ T-cell effector functions have been implicated in a variety of inflammatory and infectious processes (9, 15), yet the degree to which these cells play a part in the host immune response remains uncertain. In mammals, the location of these cells at sites of anatomic barriers to the environment suggests a primary role in the early immune response against agents, including arthropod vectors, which can penetrate the barrier. Given the similarity between IgG isotypes induced by vector-borne infection in wild-type mice to those of TCR $\delta^{-/-}$ mice, it is possible that the Th cell bias attributed to tick saliva may be

TABLE 1. Anti-*B. burgdorferi* IgG and IgG isotype reciprocal endopoint ELISA titers[a]

Mouse group or strain	Mean reciprocal endpoint ELISA titer				
	IgG	IgG1	IgG2a	IgG2b	IgG3
Syringe inoculated					
B6.129 (WT)	43,750 ± 6,250	4,800 ± 924	1,100 ± 300	14,400 ± 4,027	9,600 ± 1,848
B6.129 (TCR $\delta^{-/-}$)	42,560 ± 7,440	5,120 ± 784	1,840 ± 588	18,560 ± 4,571	1,280 ± 528[b]
BALB/c (WT)	17,920 ± 3,135	2,880 ± 320	12,800 ± 3,505	5,440 ± 2,147	1,100 ± 392
BALB/c (TCR $\delta^{-/-}$)	17,920 ± 3,135	10,880 ± 1920[c]	6,240 ± 4,843	5,440 ± 4,468	1,120 ± 196
Tick infected					
BALB/c (WT)	13,867 ± 2,569	10,133 ± 1,736	3,267 ± 1,950	8,000 ± 1,600	4,033 ± 2,673
BALB/c (TCR $\delta^{-/-}$)	13,867 ± 2,569	10,667 ± 1,349	5,067 ± 1,855	7,200 ± 1,927	4,000 ± 1,789

[a] Results from a minimum of five mice/group are reported as the mean of reciprocal endpoint ELISA titers ± standard error of the mean. WT, wild type.
[b] Significantly different ($P = 0.0159$) from value for wild-type mice as calculated by the Mann-Whitney test.
[c] Significantly different ($P = 0.0317$) from value for wild-type mice as calculated by the Mann-Whitney test.

due to its actions on dendritic epidermal γδ T cells. These cells may contribute to the local cytokine milieu for maturing dendritic cells in the skin and influence the priming of α/β T-cell responses in the lymph nodes. Although γδ T-cell effects do not alter the outcome from *B. burgdorferi* infection, our results have important implications for other vector-borne pathogens transmitted through the skin.

We thank Debbie Beck for excellent technical assistance and Ruth Montgomery for editorial review of the manuscript.

This work was supported by NIH AR42637, AI50604, and AR47058 and the Arthritis Foundation (L.K.B.) and NIH AI26815 (S.W.B.).

REFERENCES

1. **Barthold, S. W., M. S. de Souza, J. L. Janotka, A. L. Smith, and D. H. Persing.** 1993. Chronic Lyme borreliosis in the laboratory mouse. Am. J. Pathol. **143:**419–420.
2. **Barthold, S. W., D. H. Persing, A. L. Armstrong, and R. A. Peeples.** 1991. Kinetics of *Borrelia burgdorferi* dissemination and evolution of disease following intradermal inoculation of mice. Am. J. Pathol. **139:**263–273.
3. **Barthold, S. W., C. L. Sidman, and A. L. Smith.** 1992. Lyme borreliosis in genetically resistant and susceptible mice with severe combined immunodeficiency. Am. J. Trop. Med. Hyg. **47:**605–613.
4. **Bix, M., Z.-E. Wang, B. Thiel, N. J. Schork, and R. M. Locksley.** 1998. Genetic regulation of commitment to interleukin 4 production by a CD4+ T cell-intrinsic mechanism. J. Exp. Med. **188:**2289–2299.
5. **Bockenstedt, L. K., E. Hodzic, S. Feng, K. W. Bourrel, A. deSilva, R. R. Montgomery, E. Fikrig, J. D. Radolf, and S. W. Barthold.** 1997. *Borrelia burgdorferi* strain-specific Osp C-mediated immunity in mice. Infect. Immun. **65:**4661–4667.
6. **Bockenstedt, L. K., I. Kang, C. Chang, D. Persing, A. Hayday, and S. W. Barthold.** 2001. CD4+ T helper 1 cells facilitate regression of murine Lyme carditis. Infect. Immun. **69:**5264–5269.
7. **Bockenstedt, L. K., and S. E. Malawista.** 1995. Lyme disease, p. 1234–1249. *In* R. R. Rich (ed.), Clinical immunology. Mosby-Year Book, St. Louis, Mo.
8. **Brown, C. R., and S. L. Reiner.** 1999. Experimental Lyme arthritis in the absence of interleukin-4 or gamma interferon. Infect. Immun. **67:**3329–3333.
9. **Carding, S. R., and P. J. Egan.** 2002. γδ T cells: functional plasticity and heterogeneity. Nat. Rev. Immunol. **2:**336–345.
10. **Cardona, A. E., and J. M. Teale.** 2002. γ/δ T cell-deficient mice exhibit reduced disease severity and decreased inflammatory response in the brain in murine neurocysticercosis. J. Immunol. **169:**3163–3171.
11. **Ferrick, D. A., M. D. Schrenzel, T. Mulvania, B. Hsieh, W. G. Ferlin, and H. Lepper.** 1995. Differential production of interferon-gamma and interleukin-4 in response to Th1- and Th2-stimulating pathogens by gamma delta T cells in vivo. Nature **373:**255–257.
12. **Fikrig, E., S. W. Barthold, M. Chen, I. S. Grewal, J. Craft, and R. A. Flavell.** 1996. Protective antibodies in murine Lyme disease arise independently of CD40 ligand. J. Immunol. **157:**1–3.
13. **Fu, Y.-X., C. E. Roark, K. Kelly, D. Drevets, P. Campbell, R. O'Brien, and W. Born.** 1994. Immune protection and control of inflammatory tissue necrosis by γδ T cells. J. Immunol. **153:**3101–3115.
14. **Glickstein, L., M. Edelstein, and J. Z. Dong.** 2001. Gamma interferon is not required for arthritis resistance in the murine Lyme disease model. Infect. Immun. **69:**3737–3743.
15. **Hayday, A. C.** 2000. γδ cells: a right time and a right place for a conserved third way of protection. Annu. Rev. Immunol. **18:**975–1026.
16. **Hiromatsu, K., Y. Yoshikai, G. Matsuzaki, S. Ohga, K. Muramori, K. Matsumoto, J. A. Bluestone, and K. Nomoto.** 1992. A protective role of γ/δ T cells in primary infection with *Listeria monocytogenes* in mice. J. Exp. Med. **175:**49–56.
17. **Huber, S., C. Shi, and R. C. Budd.** 2002. Gamma delta T cells promote a Th1 response during coxsackievirus B3 infection in vivo: role of Fas and Fas ligand. J. Virol. **76:**6487–6494.
18. **Huber, S. A., D. Graveline, W. K. Born, and R. L. O'Brien.** 2001. Cytokine production by Vgamma(+)-T-cell subsets is an important factor determining CD4(+)-Th-cell phenotype and susceptibility of BALB/c mice to coxsackievirus B3-induced myocarditis. J. Virol. **75:**5860–5869.
19. **Kang, I., S. W. Barthold, D. H. Persing, and L. K. Bockenstedt.** 1997. T helper cell cytokines in the early evolution of murine Lyme arthritis. Infect. Immun. **65:**3107–3111.
20. **McKisic, M. D., and S. W. Barthold.** 2000. T-cell-independent responses to *Borrelia burgdorferi* are critical for protective immunity and resolution of Lyme disease. Infect. Immun. **68:**5190–5197.
21. **Nuttall, P. A., G. C. Paesen, C. H. Lawrie, and H. Wang.** 2000. Vector-host interactions in disease transmission. J. Mol. Microbiol. Biotechnol. **2:**381–386.
22. **Ribeiro, J. M. C., G. T. Makoul, J. Levine, D. R. Robinson, and A. R. Spielman.** 1985. Antihemostatic, antiinflammatory, and immunosuppressive properties of the saliva of a tick. Ixodes dammini. J. Exp. Med. **161:**332–344.
23. **Schoeler, G. B., S. A. Manweiler, and S. K. Wikel.** 1999. *Ixodes scapularis*: effects of repeated infestations with pathogen-free nymphs on macrophage and T lymphocyte cytokine responses of BALB/c and C3H/HeN mice. Exp. Pathol. **92:**239–248.
24. **Schoeler, G. B., and S. K. Wikel.** 2001. Modulation of host immunity by haematophagous arthropods. Ann. Trop. Med. Parasitol. **95:**755–771.
25. **Shanafelt, M.-C., I. Kang, S. W. Barthold, and L. K. Bockenstedt.** 1998. Modulation of murine Lyme borreliosis by interruption of the B7/CD28 T cell costimulatory pathway. Infect. Immun. **66:**266–271.
26. **Shih, C.-M., R. J. Pollack, S. R. Telford, III, and A. Spielman.** 1992. Delayed dissemination of Lyme disease spirochetes from the site of deposition in the skin of mice. J. Infect. Dis. **166:**827–831.
27. **Steere, A. C.** 2001. Lyme disease. N. Engl. J. Med. **345:**115–124.
28. **Zeidner, N., M. L. Mbow, M. Dolan, R. Massung, E. Baca, and J. Piesman.** 1997. Effects of *Ixodes scapularis* and *Borrelia burgdorferi* on modulation of the host immune response: induction of a Th2 cytokine response in Lyme disease-susceptible (C3H/HeJ) mice but not in disease-resistant (BALB/c) mice. Infect. Immun. **65:**3100–3106.
29. **Zuany-Amorim, C., C. Ruffie, S. Haile, B. B. Vargaftig, P. Pereira, and M. Pretolani.** 1998. Requirement for γδ T cells in allergic airway inflammation. Science **280:**1265–1267.

Editor: F. C. Fang