meters as the dose of antigen (*30*), the type of adjuvant (*31*), and the type of APC (Figs. 2 to 4). They are also compatible with the view that tolerance or activation to a peripheral antigen is not determined by the self or nonself origin of the antigen but rather by the conditions under which it is introduced.

REFERENCES AND NOTES

1. F. M. Burnet, *The Clonal Selection Theory of Acquired Immunity* (Vanderbilt Univ. Press, Nashville, TN, 1959).
2. _____ and F. Fenner, *The Production of Antibodies* (Macmillan, Melbourne/London, 1949).
3. J. Lederberg, *Science* 129, 1649 (1959).
4. G. J. V. Nossal, *Aust. J. Exp. Biol.* **35**, 549 (1957).
5. F. M. Burnet, J. D. Stone, M. Edney, *ibid.* **28**, 291 (1950).
6. R. E. Billingham, L. Brent, P. B. Medawar, *Nature* **172**, 603 (1953); R. E. Billingham, *Proc. R. Soc. London Ser. B* **239**, 44 (1956).
7. P. J. Morrissey, D. Bradley, S. O. Sharrow, A. Singer, *J. Exp. Med.* **158**, 365 (1983); M. Inaba *et al.*, *ibid.* **173**, 549 (1991).
8. A. Bandeira, A. Coutinho, C. Carnaund, F. Jacquemart, L. Forni, *Proc. Natl. Acad. Sci. U.S.A.* **86**, 272 (1989); B. J. Roser, *Immunol. Rev.* **107**, 179 (1989); D. Bellgrau, D. Smilek, D. B. Wilson, *J. Exp. Med.* **153**, 1660 (1981); R. K. Gershon and K. Kondo, *Immunology* **21**, 903 (1971); C. Y. Lu, E. G. Calamai, E. R. Unanue, *Nature* **282**, 327 (1979).
9. S. Schurmans *et al.*, *J. Immunol.* **145**, 2465 (1990); T. J. Powell and J. W. Streilein, *ibid.* **144**, 854 (1990); N. Chen and E. H. Field, *Transplantation* **59**, 933 (1995).
10. A. Miller, L. Ofer, A. Oded, H. Weiner, *Eur. J. Immunol.* **24**, 1026 (1994); S. Strobel and A. Ferguson, *Pediatric Res.* **18**, 588 (1984).
11. J. Chiak and F. Lehmann-Grube, *Immunology* **34**, 265 (1978); P. M. Hoffman, D. S. Robbins, H. C. Morse, *J. Virol.* **52**, 734 (1984).
12. P. Matzinger, *Annu. Rev. Immunol.* **12**, 991 (1994).
13. C. A. Janeway, *Immunol. Today* **13**, 11 (1992)
14. P. Bretscher and M. Cohn, *Science* **169**, 1042 (1970); K. J. Lafferty and A. Cunningham, *Aust. J. Exp. Biol. Med. Sci.* **53**, 27 (1975).
15. F. Finkleman, A. Lees, R. Birnbaun, W. C. Gause, S. C. Morris, in preparation.
16. J. R. Lamb *et al.*, *J. Exp. Med.* **157**, 1434 (1983); M. K. Jenkins and R. H. Schwartz, *ibid.* **165**, 302 (1987).
17. R. I. Lechler and J. R. Batchelor, *ibid.* **156**, 1835 (1982); N. N. Shehadeh, R. G. Gill, K. J. Lafferty, *Springer Sem. Immunopathol.* **14**, 203 (1993); B. Arnold and G. J. Hammerling, *Annu. Rev. Immunol.* **9**, 297 (1991); J. Markmann *et al.*, *Nature* **336**, 476 (1988).
18. V. Bal *et al.*, *Eur. J. Immunol.* **20**, 1893 (1990).
19. O. Lassila, O. Vainio, P. Matzinger, *Nature* **334**, 253 (1988).
20. E. J. Fuchs and P. Matzinger, *Science* **258**, 1156 (1992).
21. E. E. Eynon and D. C. Parker, *Transplant. Proc.* **23**, 729 (1991); *J. Exp. Med.* **175**, 131 (1992).
22. W. Lauchart, B. J. Alkins, D. A. Davies, *Transplantation* **29**, 259 (1980).
23. R. E. Billingham and W. K. Silvers, *J. Immunol.* **85**, 14 (1960).
24. S. T. Ishizaka, C. Carnaud, O. Stutman, *ibid.* **137**, 2093 (1986).
25. S. W. Umlauf *et al.*, *Immunol. Rev.* **133**, 177 (1993); S. E. Macatonia, P. M. Taylor, S. C. Knight, B. A. Askonas, *J. Exp. Med.* **169**, 1255 (1989); H. G. Rammensee, P. J. Fink, M. J. Bevan, *J. Immunol.* **133**, 2390 (1984).
26. Maintaining tolerance is, of course, different from inducing it. Here we agree with the passive model described earlier. Once the neonatal or adult mouse has become tolerant, stem cells in the donor inoculum can set up residence and establish microchimerism to maintain both central and peripheral tolerance. By quantitative polymerase chain reaction, we find that tolerant animals maintain low levels of cells bearing the Y chromosome and that they lose tolerance when the microchimerism wanes (E. A. Bonney, J. P. Ridge, O. Lantz, P. Matzinger, in preparation).
27. K. J. Gollob and E. Palmer, *J. Immunol.* **150**, 3705 (1993); J. A. Donohoe *et al.*, *Transplantation* **35**, 62 (1983); D. J. Lenschow *et al.*, *Science* **257**, 789 (1992); P. S. Linsley *et al.*, *ibid.*, p. 792; S. Guerder and P. Matzinger, *J. Exp. Med.* **176**, 553 (1992); S. Qin *et al.*, *Science* **259**, 974 (1993); M. A. Rees, A. S. Rosenberg, T. I. Munitz, A. Singer, *Proc. Natl. Acad. Sci. U.S.A.* **87**, 2765 (1990); S. Guerder and P. Matzinger, *Cold Spring Harbor Symp. Quant. Biol.* **54**, 799 (1989); S. P. Cobbold, G. Martin, H. Waldmann, *Eur. J. Immunol.* **20**, 2747 (1990); K. J. Lafferty, S. K. Babcock, R. G. Gill, *Prog. Clin. Biol. Res.* **224**, 87 (1986).
28. L. L. Johnson, *Transplantation* **46,170**, 167 (1988); A. E. Busker, S. D. Miller, R. W. Melvold, *Cell Immunol.* **125**, 225 (1990).
29. Y. Chen, V. K. Kuchroo, J.-i. Inobe, D. A. Hafler, H. L. Weiner, *Science* **265**, 1237 (1994)
30. M. Sarzotti, D. S. Robbins, P. M. Hoffman, *Science* **271**, 1726 (1996).
31. T. Forsthuber and P. V. Lehmann, *ibid.*, p. 1728.
32. P. Matzinger, *J. Immunol. Methods* **145**, 185 (1991).
33. J. P. Ridge, E. J. Fuchs, P. Matzinger, data not shown.
34. We obtained the enriched dendritic cell preparations by incubating B6 male spleen cells in Iscove's modified Dulbecco's medium plus 10% fetal bovine serum in Falcon tissue culture dishes (number 3025) for 2 hours at 37°C, removing nonadherent cells, and incubating the remaining adherent cells overnight at 37°C in medium containing mouse recombinant granulocyte-macrophage colony-stimulating factor (5 ng/ml) (Pharmingen, San Diego, CA). Nonadherent cells were then harvested and further purified over a 50% Percoll density gradient. By flow cytometery, the cell populations were typically bright for MHC class II staining and contained <2% T cells, <5% B cells, and >80 to 90% dendritic cells, as seen by staining for the dendritic cell markers 33D1 and N418.

35. We thank L. Yuan I for suggesting the day 7 tolerance experiment; A. Bendelac for other useful suggestions and encouragement; and A. Bendelac, L. Chiodetti, L. D'Adamio, M. Epstein, R. Germain, R. Schwartz, and A. Sher for reading the manuscript. We especially thank B. Tineo and C. Fenton for caring for the 351 baby mice and their mothers reported here, as well as several hundred mice that did not make it into these pages.

28 November 1995; accepted 28 February 1996

Induction of Protective CTL Responses in Newborn Mice by a Murine Retrovirus

Marcella Sarzotti,* Deanna S. Robbins, Paul M. Hoffman

The susceptibility of neonates to virus-induced disease is thought to reflect, in part, the immaturity of their immune systems. However, inoculation of newborn mice with low doses of Cas-Br-M murine leukemia virus induced a protective cytotoxic T lymphocyte (CTL) response. The inability of neonates to develop a CTL response to high doses of virus was not the result of immunological immaturity but correlated with the induction of a nonprotective type 2 cytokine response. Thus, the initial viral dose is critical in the development of protective immunity in newborns.

In neonates, B cell and T cell responses to antigen are impoverished compared to those in adults (*1*). In part, these reduced responses are the result of deficient accessory cell numbers or function (*2*). However, T cells from neonates express receptors for cytokines and costimulatory molecules in amounts similar to those expressed by adult T cells (*3*), and in vitro CTL responses to alloantigen can be detected by 4 to 6 days postpartum, gradually increasing to adult amounts by 11 to 20 days postpartum (*4*).

Infection of neonatal NFS/N mice ($Fv\text{-}1^{nn}$, $H\text{-}2^{sq4}$) with Cas-Br-M murine leukemia virus (Cas) [1000 plaque-forming units (PFU) per mouse] (*5*) results in rapid virus replication, detectable (*6*) in the spleen (10^4 to 10^6 PFU/g) and brain (10^2 to 10^4 PFU/g) within 2 weeks of infection (*7*). This perinatal infection does not elicit protective CTL and interferon γ (IFN-γ) responses and results in virus-induced neurologic disease (*8*, *9*). However, these mice do not exhibit a generalized suppression of T cell function and remain fully competent to generate allogeneic CTL responses (*8*). In contrast, Cas infection (1000 PFU) in 21-day-old mice leads to a protective $CD8^+$ CTL response and no neurologic disease (*8*, *9*). Thus, as in other viral systems, the ability to develop a CTL response influences the outcome of viral disease (*10*).

Because the number of T cells per spleen is 3 to 3.5 log units lower in neonates than in adult mice (*11*), we tested whether inoculation of newborn mice with a dose of Cas proportional to their splenic T cell number

M. Sarzotti, Research Service, Veterans Affairs Medical Center, 10 North Greene Street, Baltimore, MD 21201, USA, and Department of Microbiology and Immunology, University of Maryland School of Medicine, Baltimore, MD 21201, USA.
D. S. Robbins, Research Service, Veterans Affairs Medical Center, 10 North Greene Street, Baltimore, MD 21201, USA, and Department of Pathology, University of Maryland School of Medicine, Baltimore, MD 21201, USA.
P. M. Hoffman, Research Service, Veterans Affairs Medical Center, 10 North Greene Street, Baltimore, MD 21201, USA, and Department of Neurology, University of Maryland School of Medicine, Baltimore, MD 21201, USA.

*To whom correspondence should be addressed. E-mail: msarzott@umabnet.ab.umd.edu

would induce a CTL response comparable to that seen in 21-day-old mice, or whether newborn mice were intrinsically incapable of developing protective immunity. Decreasing doses of infectious Cas were used to inoculate groups of neonatal NFS/N mice. These mice were tested at 4 to 6 weeks of age for cytotoxicity to a Cas-infected, B-lineage lymphoma line (NS467) (*8*) after in vitro restimulation with Cas-infected cells.

Inoculation of neonatal mice with Cas resulted in the induction of CTL responses that were inversely correlated to viral dose (Fig. 1). The highest frequency of CTL responders was seen in cohorts inoculated with 0.3 PFU (70.4%; 19/27) and 1 PFU (61.5%; 8/13) of Cas, whereas no responders were found at the higher dose of 1000 PFU (0/42). The CTLs were antigen-specific, as determined by tests with syngeneic NFS/N target cells infected with the Friend murine leukemia virus (TP-3) (*12*), allogeneic target cells (EL-4, H-2^b), and natural killer–sensitive target cells (YAC-1) (*13*). These results indicated that a CTL response could be reliably induced in newborn mice, provided that the dose of Cas was ≤1 PFU per mouse. The dose of 0.3 PFU of Cas was used for further characterization of the CTL response.

The CTL response of newborn mice to low-dose infection, like that of mice infected at 21 days of age (*8, 14*), was mediated by $CD8^+$ T cells. The phenotype of the cytotoxic cells was determined by flow cytometric sorting (*15*). The cytotoxic response of splenocytes from neonatally inoculated mice was first detectable 10 to 15 days after infection and persisted for at least 28 weeks (*16*), which demonstrated long-lasting CTL memory. Induction of CTL responses to Cas in adult mice also requires 10 to 15 days (*8*). This suggests that CTL responses detected 2 weeks after neonatal inoculation resulted from the priming of newborns rather than from delayed activation by lingering virus in more mature mice.

The ability of splenocytes from mice inoculated at birth with Cas to protect neonatal NFS/N mice from Cas-induced disease was shown in vivo in adoptive transfer experiments (*8, 17*). Newborn mice received 5×10^6 lymphocytes from donor mice that had been inoculated at birth with 0.3 PFU of Cas. After 24 hours, these recipients were injected with a neuropathogenic dose of Cas (1000 PFU) (Fig. 2). Of the mice that received neonatally primed lymphocytes, 94% (15/16) were protected from Cas-induced disease, a result similar to that for mice that received adult primed lymphocytes, whereas neurologic disease developed in 100% (6/6) of mice that were given unprimed lymphocytes.

High doses of antigen preferentially stimulate type 2 cytokine responses [eliciting interleukin-4 (IL-4), IL-5, and IL-10 and promoting humoral immunity], whereas low doses of antigen induce type 1 cytokine responses [eliciting IL-2, IFN-γ, and tumor necrosis factor–α (TNF-α) and promoting cell-mediated immunity] (*18, 19*). In addition, enhanced type 2 and diminished type 1 responses are associated with neonatal tolerance to alloantigens and prolonged skin graft survival (*20*). We therefore investigated whether inoculation of newborn mice with a high dose of Cas (1000 PFU) resulted in a type 2 cytokine response. Production of IL-4 was used as an indicator of a type 2 response, and IFN-γ production was used as an indicator of a type 1 response (*19–21*). Splenocytes from adult mice infected at birth with a high dose of Cas produced substantial amounts of IL-4 but not IFN-γ when cultured in vitro (Fig. 3) (*22*). Conversely, inoculation of neonatal mice with a low dose of Cas resulted in the production of IFN-γ but not IL-4 in culture; this response was similar to that of mice infected as adults with 1000 PFU of Cas (Fig. 3).

Our results show that inoculation of newborn mice with a high dose of Cas does not result in immunological nonresponsiveness but leads to the induction of a nonprotective type 2 response (*21*). This response is likely to have a negative effect on the recruitment of $CD8^+$ CTL precursors (pCTL) into mature effector cells in vivo (*23*), but it does not result in CTL

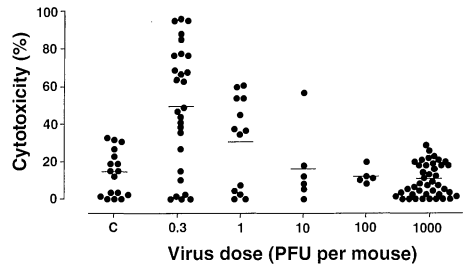

Fig. 1. Inoculation of newborn mice with low-dose Cas induces a CTL response. Splenocytes from NFS/N mice (*Fv1*^{nn}, *H-2*^{sq4}) injected intraperitoneally at 2 days of age with a single dose of Cas (0.3, 1, 10, 100, or 1000 PFU per mouse) or with medium (C, control) were taken 4 to 6 weeks after infection, restimulated in vitro (*28*), and tested for cytotoxicity against ^{51}Cr-labeled NS467 cells in a 4-hour ^{51}Cr release assay at an E/T ratio of 20:1. Each dot represents the percent cytotoxicity of an individual mouse. The horizontal lines represent the mean percent cytotoxicity for each group. CTL responders are mice with percent cytotoxicity 2 SDs above the mean percent cytotoxicity of sham-inoculated mice.

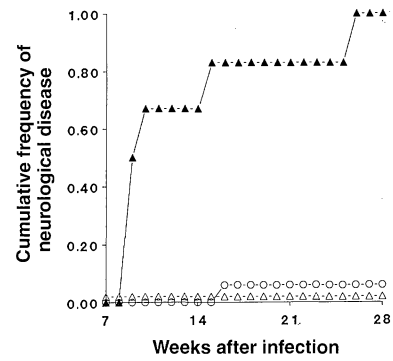

Fig. 2. Immune splenocytes from mice inoculated with low-dose Cas are protective. Splenocytes taken from adult mice injected at birth with 0.3 PFU of Cas (○; n = 16) or at 21 days of age with 1000 PFU of Cas (△; n = 7), cocultured in vitro with irradiated NS467 cells, were injected intraperitoneally into 1-day-old mice, as were splenocytes from control mice cultured with medium (▲; n = 6). Recipient mice were then injected with Cas (1000 PFU) 24 hours later on the opposite side of the abdomen. Beginning 4 weeks after infection, mice were monitored weekly for evidence of tremor, weakness, and hind limb paralysis (*7, 8*). Parallel results were obtained with donor splenocytes from the same groups of mice, without in vitro restimulation.

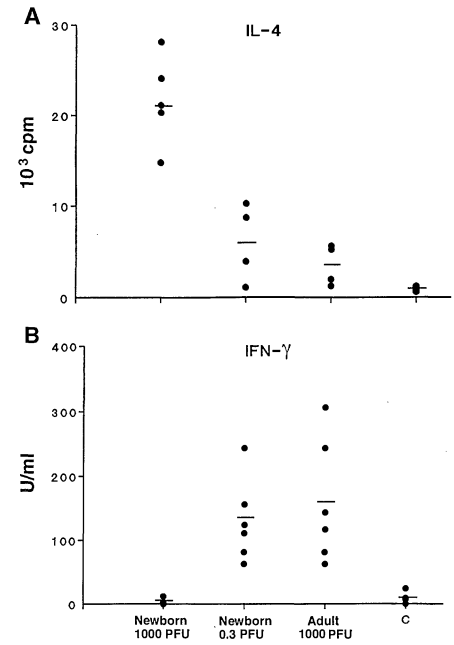

Fig. 3. IL-4 is produced by mice infected with a high dose of Cas at birth. Splenocytes from adult mice inoculated at 2 days of age [newborn, 1000 PFU, 0.3 PFU, or medium (C)] or at 21 days of age (adult, 1000 PFU) were cultured with irradiated NS467 cells. In (**A**), culture supernatants (day 3) were tested for IL-4 activity with the CT.4S proliferation assay (*29*); in (**B**), they were tested for IFN-γ activity with a standard plaque reduction assay (*9*). The IFN samples were fully neutralized by IFN-γ monoclonal antibody treatment (*9*). IL-4 or IFN-γ activity was not detected in the supernatants of the irradiated stimulator cells cultured alone. Dots represent individual mice; horizontal lines represent mean values for each group.

deletion (*24*), because clonal deletion of pCTL was not observed in mice infected at birth with a high dose of Cas (*25*). Our data indicate that the dose of virus encountered by the immune system of a newborn mouse determines the development of type 1 or type 2 responses and influences the generation of protective immunity. Thus, T cells in newborn mice, like those in adults, may be activated to either type of response by the appropriate antigen-presenting cells (*26*), costimulatory signals (*27*), and dose of antigen (Figs. 1 and 3) (*18*).

REFERENCES AND NOTES

1. P. G. Spear and G. Edelman, *J. Exp. Med.* **139**, 249 (1974); D. E. Mosier and B. M. Johnson, *ibid.* **141**, 216 (1975).
2. C. Y. Lu, D. I. Beller, E. Unanue, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 1597 (1980).
3. B. Adkins, A. Ghanai, K. Hamilton, *J. Immunol.* **153**, 3378 (1994).
4. L. M. Pilarski, *J. Exp. Med.* **146**, 887 (1977).
5. M. B. Gardner *et al., J. Natl. Cancer Inst.* **51**, 1243 (1973).
6. W. P. Rowe, W. E. Pugh, J. W. Hartley, *Virology* **42**, 1136 (1970).
7. P. M. Hoffman, S. K. Ruscetti, H. C. Morse III, *J. Neuroimmunol.* **1**, 275 (1981).
8. P. M. Hoffman, D. S. Robbins, H. C. Morse III, *J. Virol.* **52**, 734 (1984); D. S. Robbins and P. M. Hoffman, *J. Neuroimmunol.* **31**, 9 (1991); P. M. Hoffman, E. F. Cimino, D. S. Robbins, *ibid.* **33**, 157 (1991).
9. M. Sarzotti, D. S. Robbins, P. M. Hoffman, *Viral Immunol.* **6**, 207 (1993).
10. J. A. Byrne and M. B. A. Oldstone, *J. Virol.* **51**, 682 (1984); A. E. Lukacher, V. L. Braciale, T. J. Braciale, *J. Exp. Med.* **160**, 814 (1984); B. D. Jamieson and R. Ahmed, *Proc. Natl. Acad. Sci. U.S.A.* **85**, 2265 (1988); D. Moskophidis, F. Lechner, H. Pircher, R. M. Zinkernagel, *Nature* **362**, 758 (1993).
11. P.-F. Piguet, C. Irle, E. Kollatte, P. Vassalli, *J. Exp. Med.* **154**, 581 (1981).
12. A. Oliff, S. Ruscetti, E. C. Douglass, E. Scolnick, *Blood* **58**, 244 (1981).
13. The cytotoxic response of mice primed with 0.3 PFU of Cas was high when tested against Cas-infected NS467 cells [85 ± 2% cytotoxicity at an effector/target (E/T) ratio of 20:1], but remained at background amounts when tested against the other targets (8 ± 2 against TP-3; 15 ± 1 against EL-4; 26 ± 4 against YAC-1; E/T ratio, 20:1) (mean ± SE of three to seven individually tested mice). Cytotoxic activity in splenocytes from sham-inoculated mice that were cultured in vitro with Cas-infected cells was uniformly low against all targets (14 ± 4 against NS467; 6 ± 2 against TP-3; 11 ± 3 against EL-4; 23 ± 2 against YAC-1). The specificity and efficacy of the CTL response from newborn mice were the same as in adult mice (*8, 17*).
14. D. S. Robbins, M. P. Remington, M. Sarzotti, D. St. Louis, P. M. Hoffman, *J. Virol.* **69**, 6847 (1995).
15. Splenocytes from adult mice infected at birth with 0.3 PFU of Cas were restimulated in vitro, enriched for T cells with the use of affinity columns (*17*), labeled with phycoerythrin-conjugated monoclonal antibodies to CD4 and fluorescein isothiocyanate–conjugated monoclonal antibodies to CD8, and positively sorted. Virtually the entire CTL response was recovered in the $CD8^+$ T cell population (>95% of the cytotoxic activity of unsorted cells), with no appreciable amount of cytotoxicity detected in the $CD4^+$ population.
16. M. Sarzotti, D. S. Robbins, P. M. Hoffman, unpublished results.
17. M. Sarzotti, T. A. Dean, M. Remington, P. M. Hoffman, *AIDS Res. Hum. Retroviruses* **10**, 1695 (1994).
18. C. R. Parish, *Transplant. Rev.* **13**, 35 (1972); P. A. Bretscher, G. Wei, J. N. Menon, H. Bielefeldt-Ohmann, *Science* **257**, 539 (1992); C. Pfeiffer, J. Murray, J. Madri, K. Bottomly, *Immunol. Rev.* **123**, 65 (1991); N. A. Hosken *et al., J. Exp. Med.* **182**, 1579 (1995).
19. T. R. Mosmann and R. L. Coffman, *Annu. Rev. Immunol.* **7**, 145 (1989).
20. T. J. Powell Jr. and J. W. Streilein, *J. Immunol.* **144**, 854 (1990); N. Chen and E. H. Field, *Transplantation* **59**, 933 (1995).
21. F. P. Heinzel, M. D. Sadick, B. J. Holaday, R. L. Coffman, R. M. Locksley, *J. Exp. Med.* **169**, 59 (1989); A. Sher *et al., Immunol. Rev.* **127**, 183 (1992); S. Schurmans *et al., J. Immunol.* **145**, 2465 (1990).
22. The IL-4 response of splenocytes to neonatal infection with a high dose of Cas was mediated by $CD4^+$ T cell populations (unfractionated splenocytes, 10,311 ± 1220 cpm; CD4 T cells, 15,368 ± 1247 cpm; $CD8^+$ T cells, 419 ± 98 cpm, as measured with the CT.4S proliferation assay).
23. J. K. Actor *et al., Proc. Natl. Acad. Sci. U.S.A.* **90**, 948 (1993).
24. R. Zamoyska, H. Waldmann, P. Matzinger, *Eur. J. Immunol.* **19**, 111 (1989).
25. M. Sarzotti and D. S. Robbins, in *Modern Approaches to New Vaccines Including Prevention of AIDS*, R. M. Chanock, H. S. Ginsberg, F. Brown, R. A. Lerner, Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 1991), pp. 355–358.
26. E. J. Fuchs and P. Matzinger, *Science* **258**, 1156 (1992); P. Matzinger, *Annu. Rev. Immunol.* **12**, 991 (1994); J. P. Ridge, E. J. Fuchs, P. Matzinger, *Science* **271**, 1723 (1996).
27. K. J. Lafferty and A. Cunningham, *Aust. J. Exp. Biol. Med. Sci.* **53**, 27 (1975); G. R. Otten and R. N. Germain, *Science* **251**, 1228 (1991); K. J. Lafferty, S. K. Babcock, R. G. Gill, *Prog. Clin. Biol. Res.* **224**, 87 (1986); M. K. Jenkins and R. H. Schwartz, *J. Exp. Med.* **165**, 302 (1987); V. K. Kuchroo *et al., Cell* **80**, 707 (1995).
28. Responder splenocytes (3×10^6) and irradiated, Cas-infected splenocytes (4×10^6) or irradiated, Cas-infected NS467 lymphoma cells (5×10^5) were cocultured for 5 days in each well of 24-well tissue culture plates in Eagle's minimal essential medium, 10% fetal bovine serum, antibiotics, 2-mercaptoethanol, and L-glutamine (*8, 9, 14, 17*). The cytotoxicity of the responder cells against uninfected syngeneic concanavalin A blasts was <15% and was omitted for clarity.

29. J. Hu-Li, J. Ohara, C. Watyson, W. Tsang, W. E. Paul, *J. Immunol.* **142**, 800 (1989).
30. We thank T. A. Dean and R. Germain for excellent assistance; M. Sztein for cell sorting; C. Via for the CT.4S cell line; P. Matzinger and A. O'Garra for sharing their unpublished data; and G. Kelsoe for critically reading the manuscript. Supported by grants from the Medical Research Service, Department of Veterans Affairs, and by the Bressler Research Fund, University of Maryland at Baltimore (to M.S.).

28 November 1995; accepted 27 February 1996

Induction of T_H1 and T_H2 Immunity in Neonatal Mice

Thomas Forsthuber, Hualin C. Yip, Paul V. Lehmann*

The neonatal period has been thought of as a window in ontogeny, during which the developing immune system is particularly susceptible to tolerization. In the present study, the classic system for induction of neonatal tolerance to protein antigens was reexamined in mice. The presumably tolerogenic protocol was found to trigger a vigorous T helper cell type 2 (T_H2) immune response. Thus, neonatal "tolerization" induces immune deviation, not tolerance in the immunological sense. Neonates are not immune privileged but generate T_H2 or T_H1 responses, depending on the mode of immunization.

Dizygotic twin cattle, which share a placental blood supply during gestation, have erythrocytes of both their own and their twin's genotype, that is, these cattle do not reject the allogenic cells to which they were exposed early in life (*1*). On the basis of this observation, reported in 1945, the neonatal period was postulated to represent a critical window in ontogeny, during which the developing immune system learns to discriminate self from nonself by developing a tolerance to antigens it encounters (*2*). This hypothesis has defined immunological thinking ever since and has been supported by subsequent experiments (*3–5*).

The mechanism underlying neonatal tolerance has remained controversial. Proposed mechanisms include suppressor cell development (6) and the clonal deletion of antigen-reactive T cells (*7*). Because these two models conflict, with suppression representing an active tolerance mechanism and clonal deletion a passive one, we revisited the neonatal tolerance paradigm.

We followed the classic protocol for inducing neonatal tolerance to protein antigens, injecting the protein into mice within 24 hours of birth in incomplete Freund's adjuvant (IFA) intraperitoneally (i.p.) (*5*), a regimen that is considered to be tolerogenic in adults as well (*8, 9*). When they reached adult age, the mice were reinjected with the antigen in complete Freund's adjuvant (CFA) subcutaneously (s.c.). Mice injected neonatally with hen egg lysozyme (HEL) displayed an impaired response in the lymph nodes (LN) that has been considered a hallmark of tolerization (*10*) (Fig. 1A). However, the spleen cells of these mice proliferated vigorously in response to HEL, even when HEL was not reinjected at the adult age (Fig. 1B). Because of technical limitations, earlier studies were confined to LN responses; splenic recall responses to protein antigens

Department of Pathology, Biomedical Research Building, Case Western Reserve University, Cleveland OH, 44106–4943, USA.

* To whom correspondence should be addressed.