11. R. A. Fournelle and J. B. Clark, *Metall. Trans.* **3**, 2757 (1972).
12. J. P. Poirier, J. Peyronneau, M. Madon, F. Guyot, A. Revcolevschi, *Nature* **321**, 603 (1986).
13. J. E. Burke, in *Grain Control in Industrial Metallurgy*, (American Society for Metals, Cleveland, 1949), pp. 1–73; R. J. Brook, in *Treatise on Materials Science and Technology*, vol. 9, *Ceramic Fabrication Process*, F. F. Wang, Ed. (Academic Press, New York, 1976), pp. 331–364; M. Hillert, *Acta Metall.* **13**, 227 (1965); A. M. Glaeser, Yogyo Kyokaishi, *J. Cer. Soc. Japan* **92**, 538 (1984).
14. J. B. Baldo and R. C. Bradt, *J. Am. Ceram. Soc.* **71**, 720 (1988); T. Senda and R. C. Bradt, *ibid.* **73**, 106 (1990); A. Hoshikuma, H. Sato, E. Ito, *J. Seismol. Soc. Japan* **48**, 159 (1995).
15. G. C. Nicholson, *J. Am. Ceram. Soc.* **49**, 47 (1966); T. M. Harkulich, J. Magder, M. S. Vukasovich, R. J. Lockhart, *ibid.*, p. 295; T. K. Gupta, *ibid.* **54**, 413 (1971); J. B. Baldo and R. C. Bradt, *ibid.* **71**, 720 (1988); I. Chen and L. A. Xue, *ibid.* **73**, 2585 (1990).
16. F. A. Nichols, *J. Appl. Phys.* **37**, 4599 (1966).
17. E. Hanitzch and M. Kalweit, *Z. Phys. Chem.* **57**, 145 (1968).
18. Y. Oishi and W. D. Kingery, *J. Chem. Phys.* **33**, 905 (1960).
19. We thank K. Onuma for the use of the high-pressure apparatus, H. Horiuchi for the use of the micro-area x-ray diffractometer, I. Shimuzu for discussion, S. Karato for useful comments of an early version of the manuscript and two anonymous referees for valuable comments.

3 June 1996; accepted 30 October 1996

Late Complications of Immune Deviation Therapy in a Nonhuman Primate

Claude P. Genain,* Kristina Abel, Nicole Belmar, François Villinger, Daniel P. Rosenberg, Christopher Linington, Cedric S. Raine, Stephen L. Hauser

The administration of antigens in soluble form can induce antigen-specific immune tolerance and suppress experimental autoimmune diseases. In a marmoset model of multiple sclerosis induced by myelin oligodendrocyte glycoprotein (MOG), marmosets tolerized to MOG were protected against acute disease, but after tolerization treatment a lethal demyelinating disorder emerged. In these animals, MOG-specific T cell proliferative responses were transiently suppressed, cytokine production was shifted from a T helper type 1 (T_H1) to a T_H2 pattern, and titers of autoantibodies to MOG were enhanced. Thus, immune deviation can increase concentrations of pathogenic autoantibodies and in some circumstances exacerbate autoimmune disease.

Experimental allergic encephalomyelitis (EAE) is an autoimmune disease of the central nervous system (CNS) that serves as a laboratory model for the human demyelinating disease multiple sclerosis (MS) (*1*, *2*). In rodents, EAE is mediated by effector T cells that respond to myelin antigens and secrete proinflammatory (T_H1) cytokines, primarily interleukin-2 (IL-2), tumor necrosis factor–α (TNF-α), and interferon-γ (IFN-γ) (*3*). The therapeutic administration of myelin antigens in nonimmunogenic form can suppress disease-inducing T cells and protect against EAE (*4*). This protection may result from enhanced production of anti-inflammatory (T_H2) cytokines, notably IL-4, IL-6, and IL-10 (*5*), an effect known as immune deviation (*6*). Protection can be conferred by adoptive transfer of antigen-specific T_H2 cells or administration of T_H2 cytokines, and is abrogated by antibodies to IL-4 (*7*).

We have developed a primary demyelinating form of EAE in the common marmoset *Callithrix jacchus* that has a clinical and pathological similarities to human MS (*8*, *9*). In this species, synergistic T cell and B cell responses result in the MS-like lesion. Diverse populations of T_H1-cells recognizing myelin basic protein (MBP) or myelin oligodendrocyte glycoprotein (MOG) mediate the inflammatory component of the lesion, whereas antibodies to MOG mediate demyelination (*9*). Thus, MOG, a minor constituent of myelin comprising less than 0.05% of myelin proteins by weight, is a major encephalitogenic antigen capable of inducing pathogenic T cell and B cell responses in *C. jacchus*. Here, we characterize the effects of immune deviation in this model.

Seven *C. jacchus* marmosets were immunized with a recombinant protein corresponding to the extracellular domain of rat MOG (rMOG) in adjuvant (*10*). From day 7 to day 18 after immunization, animals received either soluble rMOG (rMOG-tolerized) or placebo (control-tolerized). Consistent with previous observations in rMOG-immunized marmosets (*9*), clinical signs of EAE developed in four control-tolerized animals between 9 and 16 days after immunization. In contrast, in the three animals treated with soluble rMOG, signs of EAE were suppressed, indicating that tolerization was successful. However, after cessation of treatment a rapidly progressive, lethal form of hyperacute EAE developed that was clinically more severe than in controls (Fig. 1) or than in any of more than 40 previously studied marmosets with acute EAE.

Neuropathologic findings confirmed the presence of widespread and histologically severe lesions. Lesions in the cerebral hemispheres and spinal cord of rMOG-tolerized animals were visible to the naked eye and were microscopically centered on blood vessels around which there was residual inflammation rich in plasma cells, demyelination, and macrophage activity. In the spinal cord and optic nerve (Fig. 2), lesions consisted of broad bands of subpial and perivascular white matter involvement. In contrast to control-tolerized animals in which lesion activity was limited to perivascular and subpial areas, in rMOG-tolerized animals there was a prominent zone of myelin pallor, sometimes up to 2 mm in width, surrounding a narrow band of demyelination and extending into the adjacent white matter. Within this zone of myelin pathology, cellular infiltration was absent and affected nerve fibers displayed dilated myelin sheaths with the axon either lying to one side of a large myelin vacuole or within a web of

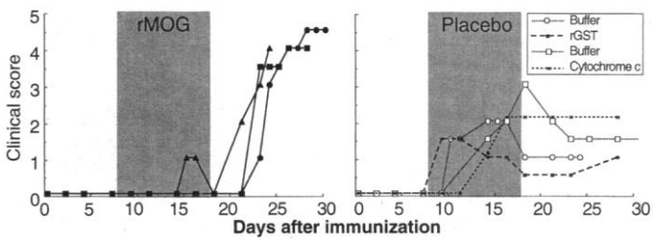

Fig. 1. Clinical course of EAE in placebo-treated and rMOG-treated *C. jacchus* marmosets. Animals were treated from day 7 until day 18 (shaded area) after immunization and received either 300 μg of rMOG dissolved in 0.025M Na-acetate buffer, pH 3.0 (left panel) or placebo [right panel: buffer alone, recombinant glutathione-S-transferase (rGST), or cytochrome c] by intraperitoneal injection every other day for a total of six injections. Clinical signs were graded by blinded observers according to a scale of 1 to 5 as described (*19*).

C. P. Genain, K. Abel, N. Belmar, D. P. Rosenberg, S. L. Hauser, Department of Neurology, University of California, San Francisco, CA 94143, USA.
F. Villinger, Department of Pathology and Laboratory Medicine, Emory University School of Medicine, Atlanta, GA 30322, USA.
C. Linington, Department of Neuroimmunology, Max Planck Institut für Psychiatrie, Martinsried, Germany.
C. S. Raine, Department of Pathology, Albert Einstein College of Medicine, Bronx, NY 10461, USA.
*To whom correspondence should be addressed.

dissociated membranes. These changes, confirmed at the ultrastructural level, suggested that the CNS demyelination was humorally mediated (*11*). Within this zone of myelin vacuolation, except for a small number of oligodendrocytes that displayed evidence of cytolysis but not apoptosis, oligodendrocyte degeneration was not seen. The histopathologic and ultrastructural characteristics of these lesions were similar to those of typical acute MS (*12*).

Consistent with previous experience (*9*), control-tolerized marmosets developed T cell proliferative responses to rMOG in circulating peripheral blood mononuclear cells (PBMCs) and in lymph node cells (LNCs). In contrast, no response could be detected in any rMOG-tolerized marmoset during the period of antigen administration (Fig. 3), but after treatment proliferative responses appeared concurrent with the development of lethal EAE. Circulating antibodies to rMOG were present in all animals in both groups; however, by day 21 after immunization, titers were higher in rMOG-tolerized animals despite the fact that they remained asymptomatic (Fig. 4, A and B). Epitope mapping studies indicated that rMOG-tolerized and control marmosets developed antibodies to rMOG with similar fine specificities (Fig. 4C). At the time of lethal EAE, rMOG-tolerized marmosets had identical proliferative responses to rMOG as did controls, but autoantibodies were detected in serum at dilutions that were four- to eightfold higher (Figs. 3 and 4). Prior studies indicated that polyclonal marmoset antibodies to MOG can transfer severe demyelination in marmosets, but only in the presence of disease-inducing T cells capable of disrupting the blood brain barrier (*9*). In hyperacute EAE, it is likely that antibody-mediated demyelination was similarly facilitated by disease-inducing T cells that were activated after cessation of tolerance treatment.

Cytokine gene expression was measured in LNC and PBMC by reverse transcriptase–polymerase chain reaction (RT-PCR) after stimulation in vitro with either rMOG or MBP. Compared to control-tolerized animals, rMOG-tolerized marmosets had increased synthesis of IL-10 and IL-6 mRNA, and decreased IFN-γ and TNF-α mRNA, in response to stimulation with rMOG (Fig. 5). No significant stimulation of any cytokine mRNA was observed after stimulation with MBP. The shift from a T_H1-like to a T_H2-like pattern of cytokine production in response to rMOG was present by day 14 after immunization, and persisted until the end of the study. The inability to detect a proliferative response to rMOG in tolerized animals may have resulted from antiproliferative effects of T_H2 cytokines (*13*), from reversible T cell anergy or apoptotic T cell death (*14*). The appearance of proliferative responses after cessation of treatment suggested that the state of unresponsiveness was reversible.

Our data show that induction of a T_H2 response may exacerbate autoimmunity by enhancing production of pathogenic autoantibodies. This effect is likely mediated by the known functions of T_H2 cytokines on induction of B cell differentiation, switch of immunoglobulin production from low-affinity immunoglobulin M (IgM) to high-affinity IgG, and synthesis of IgG1 (*6*, *15*). In human MS, T cell mediation is one likely pathogenic mechanism (*2*, *16*), but myelin-specific autoantibodies are also present (*17*) and antibody- and complement-mediated tissue damage may occur (*18*). This raises the possibility that in MS, as in *C. jacchus* EAE, induction of a T_H2 response to myelin antigens might promote humoral autoimmunity. Strategies that induce long-term tolerance both for T cells and B cells may be required for successful immunotherapy of complex autoimmune disorders.

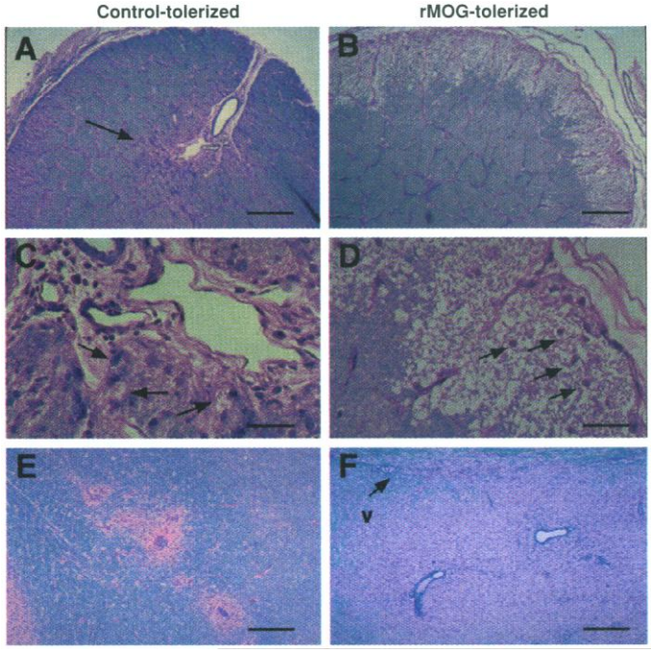

Fig. 2. Neuropathologic findings. Representative control-tolerized (left) and rMOG-tolerized (right) animals. Low (A, B, E, F) and high (C, D) power views stained with luxol fast blue/periodic acid Schiff. Bars = 200 μm and 40 μm, respectively. (A) and (B) represent transverse sections of the optic nerves, showing a small perivascular infiltrate with concentric demyelination in the control (**A**, arrow), and a broad band of extensive subpial demyelination in the rMOG-tolerized marmoset (**B**). (C) and (D) illustrate lesion detail; the cellular infiltrate in the control animal is comprised of mononuclear cells and macrophages (arrows) with astrogliosis (**C**), whereas there is sparse mononuclear cell infiltration (arrows) but extensive demyelination in the rMOG-tolerized animal (**D**). (E) and (F) represent comparison of deep periventricular (v) white matter lesions in typical control (**E**) and hyperacute (**F**) EAE; in hyperacute EAE there is extensive confluence of infiltrates, and demyelination is widespread.

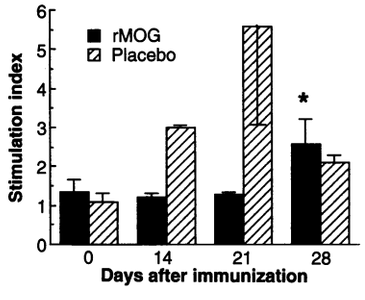

Fig. 3. T cell responses in PBMC of control-tolerized and rMOG-tolerized marmosets. T cell proliferative responses were measured by a standard proliferation assay (*9*). Briefly, 10^5 cells were plated in triplicate in 96 well plates at a cell density of 10^6 cells/ml in AIM-V medium (Gibco-BRL). The following antigens were added to the wells: none (control), rMOG (10 μg/ml), or phytohemagglutinin (2.5 μg/ml). After 48 hours, 0.5 μCi per well of [^{3}H]thymidine was added and cells harvested 18 hours later. Stimulation indices (SI) were calculated as the ratio of cpm in stimulated/unstimulated (control) wells. Data are presented as mean ± SD. *, $P < 0.05$ when compared to days 0, 14, and 21 (ANOVA). Identical proliferative responses were observed from LNC obtained at biopsy.

REFERENCES AND NOTES

1. C. S. Raine, in *Multiple Sclerosis. Pathology, Diagnostic and Management*, J. F. Hallpike, G. W. M. Adams, W. W. Tourtelotte, Eds. (William and Wilkins, Baltimore, MD, 1983), pp. 413–460.
2. R. Martin, H. F. McFarland, D. E. McFarlin, *Annu. Rev. Immunol.* **10**, 153 (1992).
3. C. B. Pettinelli and D. E. McFarlin, *J. Immunol.* **127**, 1420 (1981); M. B. Powell *et al.*, *Int. Immunol.* **2**, 539 (1990); V. K. Kuchroo *et al.*, *J. Immunol.* **151**, 4371 (1993); J. L. Baron, J. A. Mardi, N. H. Ruddle, G. Hashim, C. A. Janeway, *J. Exp. Med.* **177**, 57 (1993).
4. A. Miller, Z. J. Zhang, R. A. Sobel, A. Al-Sabbagh, H. L. Weiner, *J. Neuroimmunol.* **46**, 73 (1993); J. M. Critchfield *et al.*, *Science* **263**, 1139 (1994); S. D. Miller and W. J. Karpus, *Immunol. Today* **15**, 356 (1994).
5. S. Khoury, W. W. Hancock, H. L. Weiner, *J. Exp.*

Med. **176**, 1355 (1992); Y. Chen, V. K. Kuchroo, J.-I. Inobe, D. A. Hafler, H. L. Weiner, *Science* **265**, 1237 (1994).
6. M. Rocken, M. Racke, E. M. Shevach, *Immunol. Today* **17**, 225 (1996).
7. V. K. Kuchroo *et al.*, *Cell* **80**, 707 (1995); M. K. Racke *et al.*, *J. Exp. Med.* **180**, 1961 (1994); O. Rott, B. Fleischer, E. Cash, *Eur. J. Immunol.* **24**, 1434 (1994); A. H. Cross *et al.*, *J. Clin. Invest.* **95**, 2783 (1995).
8. L. Massacesi *et al.*, *Ann. Neurol.* **37**, 519 (1995).
9. C. P. Genain *et al.*, *J. Clin. Invest.* **96**, 2966 (1995).
10. *C. jacchus* marmosets used for these studies were from the New England Primate Research Center (Southborough, MA). Animals were cared for in accordance with the guidelines of the Institutional Committee on Care and Use of Laboratory Animals. A maximum of 2.5 ml of blood every other week was taken from each animal. All procedures were done under brief anesthesia administered intramuscularly [ketamine (10 to 20 mg/kg) and midazolam (0.1 mg/kg)]. Fifty micrograms of rMOG were dissolved in 100 μl of phosphate-buffered saline and emulsified with an equal volume of complete Freund's adjuvant (Gibco-BRL) containing killed *Mycobacterium tuberculosis* (3 mg/ml, H37Ra, Difco), and administered intradermally in four injection sites on the scapular and hip regions. On the day of immunization, and again 2 days later, 10^{10} inactivated *Bordetella pertussis* organisms diluted in 5 ml of isotonic saline were administered intravenously. At the termination of the experiments animals were euthanized under pentobarbital anesthesia by intracardiac perfusion with 2.5% buffered glutaraldehyde solution. rMOG is a non-glycosylated recombinant fusion protein containing the extracellular domain (residues 1 to 124) of rat MOG (*20*). Although the sequence of marmoset MOG is not known, this domain of the protein is highly conserved between species (*21*), and rMOG is encephalitogenic in *C. jacchus* marmosets (*9*).
11. C. S. Raine and M. B. Bornstein, *J. Neuropathol. Exp. Neurol.* **29**, 177 (1970); M. C. Dal Canto *et al.*, *J. Neurosci. Res.* **24**, 313 (1975); K. W. Selmaj and C. S. Raine, *Ann. Neurol.* **23**, 339 (1988).
12. C. F. Brosnan and C. S. Raine, *Brain Pathol.* **6**, 243 (1996).
13. K. Taga and G. Tosato, *J. Immunol.* **148**, 1143 (1992); P. A. Sieling *et al.*, *ibid.* **150**, 5501 (1993); H. Groux, M. Bigler, J. E. de-Vries, M.-G. Roncarolo, *J. Exp. Med.* **183**, 19 (1996).
14. R. H. Schwartz, *J. Exp. Med.* **184**, 1 (1996); A. Mondino, A. Khoryts, M. K. Jenkins, *Proc. Natl. Acad. Sci. U.S.A.* **93**, 2245 (1996); L. Van Parijs, A. Ibraghimov, A. K. Abbas, *Immunity* **4**, 321 (1996).
15. T. R. Mosmann and R. L. Coffman, *Annu. Rev. Immunol.* **7**, 145 (1989); W. E. Paul and R. A. Seder, *Cell* **76**, 241 (1994); R. S. Liblau, S. M. Singer, H. O. McDevitt, *Immunol. Today* **16**, 34 (1995).
16. J. R. Oksenberg *et al.*, *Nature* **362**, 68 (1993); N. Kerlero de Rosbo *et al.*, *J. Clin. Invest.* **92**, 2602 (1993).
17. H. S. Panitch, C. J. Hooper, K. P. Johnson, *Arch. Neurol.* **37**, 206 (1980); K. G. Warren, I. Catz, E. Johnson, B. Mielke, *Ann. Neurol.* **35**, 280 (1994); B. G. Xiao, C. Linington, H. Link, *J. Neuroimmunol.* **31**, 91 (1991).
18. D. A. S. Compston *et al.*, *Neuropathol. Appl. Neurobiol.* **15**, 307 (1989); N. J. Scolding *et al.*, *Nature* **339**, 620 (1989).
19. C. P. Genain *et al.*, *J. Clin. Invest.* **94**, 1339 (1994).
20. M. V. Gardinier, P. Amiguet, C. Linington, J. M. Matthieu, *J. Neurosci. Res.* **33**, 177 (1992); S. Amor *et al.*, *J. Immunol.* **153**, 4349 (1994).
21. D. Pham-Dinh *et al.*, *J. Neurochem.* **63**, 2353 (1994); A. A. Hilton, A. J. Slavin, D. J. Hilton, C. C. A. Bernard, *ibid.* **65**, 309 (1995).
22. The sequences of the primers were as follows: 5′-CATGTGGGCCATGAGGTCCACCAC-3′ (3′-G3PDH); 5′-TGAAGGTCGGAGTCAACGGATTTGGT-3′ (5′-G3PDH); 5′-GCAATGATCCCAAAGTAGACCTGCC-CAGACT-3′ (3′-TNF-α); 5′-GAGTGACAAGCCTG-TAGCCCATGTTGTAGCA-3′ (5′-TNF-α); 5′-GCAT-CGTTTTGGGTTCTCTTGGCTGTTACTGC-3′ (3′-IFN-γ); 5′-CTCCTTTTTCGCTTCCCTGTTTTAGCT-GCTGG-3′ (5′-IFN-γ); 5′-TCTCAAGGGGCTGGGTC-AGCTATCCCA-3′ (3′-IL-10); 5′-ATGCCCCAAGCT-GAGAACCAAGACCCAGAC-3′ (5′-IL-10); 5′-GAA-GAGCCCTCAGGCTGGACTG-3′ (3′-IL-6); and 5′-ATGAACTCCTTCTCCACAAGCGC-3′ (5′-IL-6). PCR amplification was carried out for 25 cycles (TNF-α and IFN-γ) or 35 cycles (IL-10 and IL-6).
23. Supported by the Nancy Davis Center Without Walls; the National Institutes of Health NS 30727 (S.L.H.) and NS 08952 and NS 11920 (C.S.R.); the National Multiple Sclerosis Society NMSS Reg 1001-I-9 (C.S.R.); the Deutsche Forschungsgemeinschaft SFB217 (C.L.); and the National Multiple Sclerosis

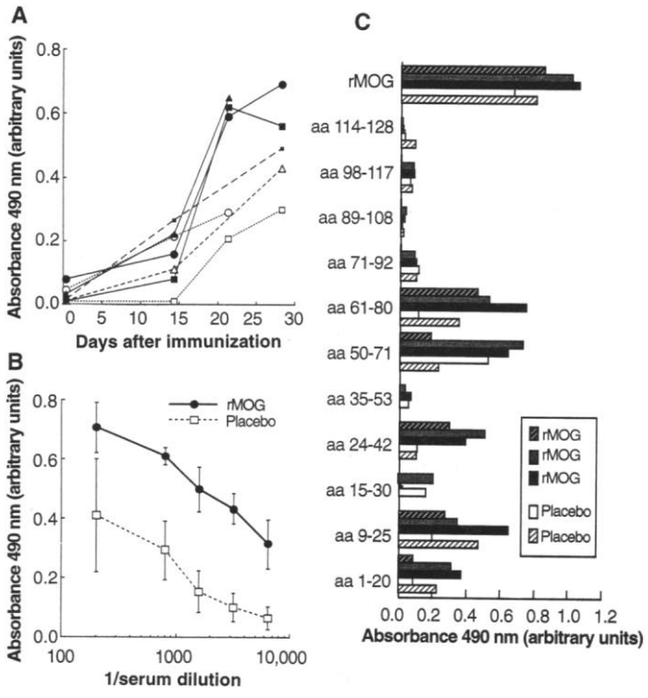

Fig. 4. Antibody responses in control-tolerized and rMOG-tolerized marmosets. (**A**) Time course of the appearance of antibodies to rMOG in individual control (open symbols) and rMOG-tolerized (closed symbols) animals. Serum antibody titers were serially measured by ELISA. ELISA plates (Pierce) were coated overnight with rMOG (1 μg/ml) in 0.25 M carbonate buffer, pH 8.6, washed with phosphate-buffered saline containing 0.05% Tween 20 and blocked with 1% bovine serum albumin in the same buffer. After washing, 100 μl of a 1:800 dilution of immune sera were incubated in the wells for 2 hours at 37°C, and immunoperoxidase-conjugated anti-monkey IgG (Sigma, 1:6000) was applied for 1 hour at 37°C. Plates were developed with *o*-phenylenediamine dihydrochloride in 0.05 M phosphate-citrate buffer, pH 5.0 (Sigma) for 30 min and read at 490 nm in a Vmax ELISA reader (Molecular Devices). (**B**) Serum titers at day 21 after immunization (mean ± SD). Control-tolerized, open symbols; rMOG-tolerized, closed symbols. Titers were measured by ELISA using serial dilutions of immune sera (1:200 to 1:6400). (**C**) Fine specificity of anti-MOG antibodies was studied in five animals with the same ELISA system with 20 residue synthetic overlapping peptides corresponding to the published sequence of rat MOG (*20*) (1 μg/well), and 1:200 dilutions of immune sera. (**Inset**) The legend indicates individual animals with their respective treatments. Antibodies from placebo- and rMOG-tolerized marmosets recognized similar regions of the rMOG molecule, located between aa 1 to 42 and aa 50 to 80.

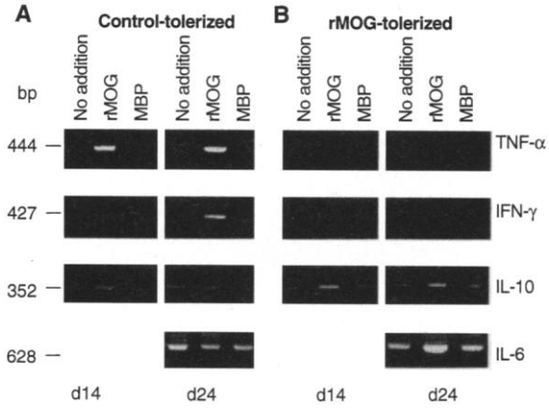

Fig. 5. Cytokine gene expression in control-tolerized and rMOG-tolerized marmosets. Sequential analysis of TNF-α, IFN-γ, IL-10, and IL-6 mRNA in LNCs obtained at biopsy or autopsy in representative control-tolerized (**A**) and rMOG-tolerized (**B**) animals at day 14 and day 24 after immunization, respectively. For measurement of cytokine mRNA levels, 10^6 LNC were cultured in 1 ml AIM-V in the presence of: no antigen (control); rMOG (10 μg/ml) or MBP (50 μg/ml). After 4 hours, cells were harvested and total RNA extracted using TriZol (Gibco-BRL). A first strand cDNA was synthesized using 2.5 μg of total RNA in a 100 μl reaction containing PCR buffer (Promega), 1.5 mM $MgCl_2$, 1 unit of Moloney murine leukemia virus reverse transcriptase (Gibco-BRL), 1 mM dNTP, 25 nM random hexamers and ribonuclease inhibitor (Pharmacia). Ten microliters of cDNA was used for PCR amplification in a 25-μl reaction in the presence of PCR buffer, 0.5 mM $MgCl_2$, 1 unit of Taq polymerase (Gibco), and 0.5 μM of each of G3PDH upstream and downstream primers (as internal control for the RT-PCR reaction) and primers specific for TNF-α, IFN-γ, IL-10, and IL-6 (*22*).

Society (C.P.G.). rGST was supplied by R. H. Edwards and D. Krantz, and *Bordetella pertussis* from Wyeth Lederle Vaccine and Pediatrics, Pearl River, NY. We thank J. R. Oksenberg for helpful discussions and K. Lovett and L. Brovarney for their dedicated work with the animals.

18 July 1996; accepted 18 November 1996

Loss of Heterozygosity in Normal Tissue Adjacent to Breast Carcinomas

Guoren Deng, You Lu, Galina Zlotnikov, Ann D. Thor, Helene S. Smith*

Loss of heterozygosity (LOH) was detected in morphologically normal lobules adjacent to breast cancers. The most frequent aberration was at chromosome 3p22-25; of ten cases with this LOH in the carcinoma, six displayed the same LOH in adjacent normal lobules. This suggests that in a subset of sporadic breast cancers, a tumor suppresser gene at 3p22-25 may be important in initiation or early progression of tumorigenesis. Among sixteen breast cancers with LOH at 17p13.1 and five breast cancers with LOH at 11p15.5, one case each displayed the same LOH in adjacent normal lobules. Thus the molecular heterogeneity that characterizes invasive breast cancers may occur at the earliest detectable stages of progression.

The mature breast contains lobules, clusters of closed glandular spaces that produce milk during lactation. These lobules are connected to the nipple–areolar complex by a system of branching ducts that are surrounded by varying amounts of fat and connective tissue. Breast cancer is thought to develop within a terminal ductal-lobular unit (TDLU), which includes the lobule and its most proximal ducts (*1*).

Breast cancer evolves by clonal selection of cells that acquire multiple molecular changes. One model suggests that breast cancer, like colon cancer (*2*), develops through a defined progression of morphologically distinguishable stages beginning with benign hyperplasia, which progresses to atypical hyperplasia, then to in situ carcinoma, and finally to invasive cancer (*1*). This sequential progression may not be the only way that breast cancers develop, however. Many small invasive cancers do not have atypical components, which suggests that they may have developed directly from morphologically normal epithelium. If this were true, one might expect to find evidence of a "field effect" in which at least some of the genetic aberrations found in invasive cancers are also present in the morphologically normal epithelium.

To test this hypothesis, we carefully microdissected hematoxylin-eosin–stained sections of breast cancers so as to isolate morphologically discrete regions (Fig. 1A). DNA was prepared from malignant areas of the section and from adjacent normal TDLUs. As a control for each case, DNA was also prepared from normal breast skin (usually from a separate section) that had been similarly microdissected.

We studied LOH at chromosome 3p24, 11p15.5, 13q13, and 17p13.1 because these loci show LOH in a high percentage (~30 to 60%) of invasive ductal breast cancers (*3*, *4*). For the carcinomatous regions, the frequency of LOH at 3p24 (48%) and 11p15.5 (29%) was similar to that previously reported (*4*). The frequency of LOH in the invasive components was higher than the literature values for 13q13 (64% here versus ~40%) and for 17p13.1 (80% here versus ~60%). These discrepancies may be due to random variation because our sample size was small.

In 8 of 30 cases we detected LOH in the adjacent morphologically normal TDLUs (Table 1). In all eight cases, the same allele was missing in the adjacent carcinoma (Fig. 2, A and B). LOH in normal TDLUs was seen in 6 of the 10 cases where LOH at 3p24 was found in the carcinoma. LOH at 11p15.5 in the normal TDLUs was seen in one of five cases with this LOH in the carcinoma; LOH at 17p13.1 in the normal TDLUs was seen in 1 of 16 cases with this LOH in the carcinoma. None of 10 cases with LOH at 13q13 in the carcinoma had this lesion in the normal TDLUs. Among tumors with and without LOH in adjacent normal tissues, there was no significant difference in grade, hormone receptor status,

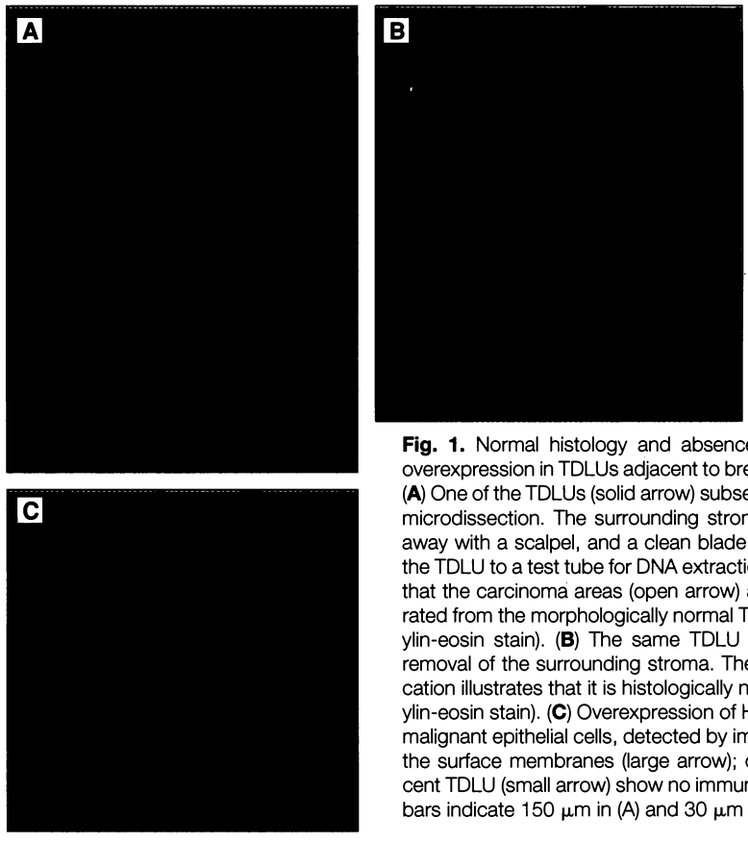

Fig. 1. Normal histology and absence of HER-2/neu overexpression in TDLUs adjacent to breast carcinomas. (**A**) One of the TDLUs (solid arrow) subsequently used for microdissection. The surrounding stroma was scraped away with a scalpel, and a clean blade used to remove the TDLU to a test tube for DNA extraction (*11*, *14*). Note that the carcinoma areas (open arrow) are clearly separated from the morphologically normal TDLUs (hematoxylin-eosin stain). (**B**) The same TDLU as in (A) before removal of the surrounding stroma. The higher magnification illustrates that it is histologically normal (hematoxylin-eosin stain). (**C**) Overexpression of HER-2/neu in the malignant epithelial cells, detected by immunostaining of the surface membranes (large arrow); cells in the adjacent TDLU (small arrow) show no immunostaining. Scale bars indicate 150 μm in (A) and 30 μm in (B) and (C).

G. Deng, Y. Lu, G. Zlotnikov, Geraldine Brush Cancer Research Institute, California Pacific Medical Center, 2330 Clay Street, San Francisco, CA 94619, USA.
A. D. Thor, Department of Pathology, Northwestern University School of Medicine, Evanston Hospital, 2650 Ridge Avenue, Evanston, IL 60201, USA.
H. S. Smith, Geraldine Brush Cancer Research Institute, California Pacific Medical Center, 2330 Clay Street, San Francisco, CA 94619, and Department of Medicine, University of California, San Francisco, CA 94143, USA.
*To whom correspondence should be addressed.