and whole cells were found to be reactive with 54.2.8, it is possible that a surface component that extends into the streptococcal membrane is involved in the cross-reactivity. Alternately, it is possible that the membranes contained fragments of cell wall components that reacted with 54.2.8. Adsorption of the reactivity of antibody 54.2.8 with several M-protein types of *S. pyogenes* suggests that the cross-reactivity was not type-specific. Type-specific cross-reactivity of antibodies to *S. pyogenes* type 5 M protein with other heart components has been reported (*10*).

The role that cross-reactions might have in the pathogenesis of streptococcal sequelae is not known. However, several reports of the immunopathology of heart lesions subsequent to rheumatic fever have described the degeneration of the muscle cell (*20*). Whether or not the lesions are a direct result of streptococcal infection is not known. Our discovery that myosin may share immunodeterminants with a component of *S. pyogenes* is apparently the first report of a cross-reactive tissue component. The stimulation of immune clones against myosin by the streptococcus may be an attractive hypothesis for the mechanism of the production of heart-reactive antibodies in streptococcal diseases and the development of sequelae of uncomplicated infections.

KAREN KRISHER
MADELEINE W. CUNNINGHAM
Department of Microbiology and Immunology, University of Oklahoma Health Sciences Center, Oklahoma City 73190

References and Notes

1. M. H. Kaplan, R. Bolande, L. Rakita, J. Blair, *N. Engl. J. Med.* **271**, 637 (1964).
2. J. B. Zabriskie, K. C. Hsu, B. C. Seegal, *Clin. Exp. Immunol.* **7**, 147 (1970).
3. J. B. Zabriskie and E. H. Freimer, *J. Exp. Med.* **124**, 661 (1966).
4. I. van de Rijn, A. S. Bleiweis, J. B. Zabriskie, *J. Dent. Res.* **55**, 59 (1976).
5. J. J. Ferretti, C. Shea, M. W. Humphrey, *Infect. Immun.* **30**, 69 (1980).
6. E. H. Beachey *et al.*, *J. Exp. Med.* **150**, 862 (1979); J. R. McGhee and S. M. Michalek, *Annu. Rev. Microbiol.* **35**, 595 (1981).
7. I. van de Rijn, J. B. Zabriskie, M. McCarty, *J. Exp. Med.* **146**, 579 (1977).
8. I. M. Lyampert, N. A. Borodiyuk, G. A. Ygryumova, *Immunology* **15**, 845 (1968).
9. I. M. Lyampert and T. A. Danilova, *Progr. Allergy* **18**, 423 (1975).
10. J. B. Dale and E. H. Beachey, *J. Exp. Med.* **156**, 1165 (1982).
11. I. Goldstein, P. Reybeyotte, J. Parlebas, B. Halpern, *Nature (London)* **219**, 866 (1968).
12. V. A. Fischetti and B. N. Manjula, *Seminars in Infectious Disease* (Thieme-Stratton, New York, 1982), vol. 4, pp. 411–418.
13. M. W. Cunningham, K. Krisher, D. C. Graves, *Infect. Immun.* **46**, 34 (1984).
14. W. A. Clark, Jr., R. A. Chizzonite, A. W. Everett, M. Rabinowitz, R. Zak, *J. Biol. Chem.* **257**, 5449 (1982).
15. W. A. Clark, B. R. Eisenberg, W. W. Tambone, R. Zak, abstracts presented at *23rd Annu. Meet. Am. Soc. Cell. Biol.* **97**, 262 (1983).
16. I. Arndt and F. A. Pepe, *J. Histochem. Cytochem.* **23**, 159 (1975).
17. N. K. Hall and M. W. Cunningham, in preparation.
18. G. C. Nicholson, R. L. Dawkins, B. L. McDonald, J. D. Wetherall, *Clin. Immunol. Immunopathol.* **7**, 349 (1976).
19. A. J. Fairfax and U. Gröschel-Stewart, *Clin. Exp. Immunol.* **28**, 27 (1977).
20. C. G. Becker and G. E. Murphy, *Streptococcal Diseases and the Immune Response* (Academic Press, New York, 1980), pp. 23–40.
21. M. W. Cunningham and S. M. Russell, *Infect. Immun.* **42**, 531 (1983).
22. I. van de Rijn and R. E. Kessler, *ibid.* **27**, 444 (1980).

23. We thank J. Ferretti, N. Hall, D. Graves, M. Lerner, S. Tobin, J. Tang, and W. Clark, Jr., for discussions; H. Whyte and M. Patterson for technical assistance; V. Fischetti for a gift of a heart obtained at autopsy from a patient with acute rheumatic fever; and W. Clark for a gift of monoclonal antibody CCM-52. Supported by a Faculty Senate Award from the University of Oklahoma Health Sciences Center. M.W.C. is the recipient of American Heart Association grant 841337 funded in part by the American Heart Association, Oklahoma Affiliate.

10 September 1984; accepted 16 October 1984

Reversal of Experimental Allergic Encephalomyelitis with Monoclonal Antibody to a T-Cell Subset Marker

Abstract. *Administration of a monoclonal antibody (GK1.5) that recognizes the L3T4 marker present on helper T cells prevented the development of experimental allergic encephalomyelitis (EAE) in mice. Furthermore, treatment with GK1.5 reversed EAE when the antibody was given to paralyzed animals. In vivo injection of GK1.5 selectively reduced the number of L3T4$^+$ cells in the spleen and the lymph nodes. These results suggest that manipulation of the human equivalent of the murine L3T4$^+$ T-cell subset with monoclonal antibodies may provide effective therapy for certain autoimmune diseases.*

Experimental allergic encephalomyelitis (EAE), an autoimmune inflammatory disease of the central nervous system, is a model for the human disease multiple sclerosis (MS) (*1*). Results of several studies suggest that particular T-lymphocyte subsets are implicated in the pathogenesis of both EAE and MS (*2, 3*). In the case of murine EAE, T cells capable of provoking EAE belong to the helper (inducer) subset of T cells that carries the L3T4 surface marker (*4, 5*). Cells belonging to a similar human T-cell subset are associated with the progression of lesions in the brains of MS patients (*3*). Administration of monoclonal antibodies directed against T-cell subset markers might constitute an effective strategy for the treatment of MS and other human disorders, particularly when a specific T-cell subset or an imbalance between regulatory T-cell subsets (*6*) is involved in disease pathogenesis.

To induce EAE, susceptible SJL/J mice were given pertussis vaccine intravenously and immunized with mouse spinal cord homogenate (MSCH) emulsi-

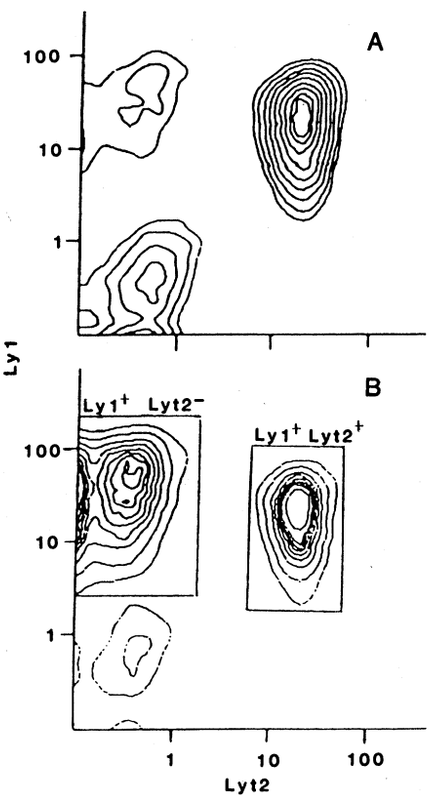


Fig. 1. Monoclonal antibody GK1.5 reduces the number of Ly1$^+$Lyt2$^-$ (L3T4$^+$) lymph node cells. (A) GK1.5-treated; (B) control. Preparation of cells, staining procedures, and data collection were as described (*13*). Lymph node cells were stained with fluorescein-conjugated antibody to Lyt2 (*12*) plus biotin-conjugated antibody to Ly1 (*12*) followed by staining with Texas red avidin and analysis on a dual laser FACS equipped with logarithmic amplifiers (*14*). Axes represent the relative amounts of the respective antigens as revealed by fluorescence intensity. Two-color staining data are presented as "contour plots" that are representations of three-dimensional surfaces in which the levels of green and red fluorescence per cell define locations on a two-dimensional surface, and the frequency of cells with that value of fluorescence defines the elevation at that location. After this surface was smoothed, contour lines were drawn to divide the sample into equal fractions. The integration boundaries used to determine the frequency of Ly1$^+$Lyt2$^-$ and Ly1$^+$Lyt2$^+$ cells are shown on Fig 1B. Treated mice received 100 μg of GK1.5 on each of the 2 days preceding analysis.

Table 1. Prevention of experimental allergic encephalomyelitis with GK1.5. On day 0, all mice were immunized in the hind foot pads with 5 mg of mouse spinal cord homogenate (MSCH) in 0.1 ml of an emulsion of equal volumes of complete Freund's adjuvant and phosphate-buffered saline (PBS) containing *Mycobacterium tuberculosis* (H37Ra) at a concentration of 4 mg/ml. *Bordetella pertussis* (30×10^9 organisms in 0.5 ml PBS) was injected into the tail vein before immunization with MSCH on day 0 and again on day 2.

Monoclonal antibody treatment*		Clinical disease			Perivascular cuffs
Monoclonal antibody	Injection (days)	Incidence†	P‡	Mean onset§ (day)	Frequency‖
GK1.5	9, 10, 11, 12 13, 14, 16, 18 20, 22	0/10	.001	—	1/6
GK1.5	9, 10, 11, 12	8/18	.02	19	1/8
GK1.5	−2, −1, 1	4/15	.002	27	0/9
53-6.7	−2, −1, 1	8/9	N.S.	12	5/5
53-6.7	9, 10, 11, 12	17/19	N.S.	14	11/12
None (PBS)	−2, −1, 1	26/30	—	14	13/13

*Monoclonal antibodies to L3T4 (GK1.5) and to Lyt2 (53-6.7) were purified from culture supernatants of hybridomas GK1.5 (*5*) and 53-6.7 (*12*) grown in serum-free medium HB101. Antibody (100 μg) was given intraperitoneally on each day, shown as days after immunization with MSCH (day 0). †The ratio of number of sick mice to the total number of mice. Mice were examined at least through day 32. Mice were scored as sick if they exhibited any signs of illness. ‡P values were computed by comparing the treated groups to the PBS group utilizing the continuity correction and taking account of multiple comparisons; N.S., not significant. §The standard deviation for all values is ±2 days. ‖The ratio of number of mice with perivascular cuffs to the total number of mice examined histologically. Six standard sections of brain and spinal cord were examined for each mouse (*9*). Slides were coded and read by an observer who was not informed of the treatment protocol.

fied in Freund's complete adjuvant. The disease induced in this way had a characteristic clinical course. (i) The first signs of illness (loss of weight, disheveled coat, and tail weakness) occurred 12 to 15 days after immunization; (ii) hind-limb paralysis and sometimes complete paralysis developed during the next 5 to 7 days; and (iii) by day 21, mice either died or recovered and became essentially asymptomatic. We examined the effects of injection of a monoclonal antibody (GK1.5) that recognizes the L3T4 surface molecule (*5*) on the development of EAE.

All clinical signs of EAE were prevented when GK1.5 was administered before the onset of EAE and were reversed when the antibody was administered shortly after the first symptoms of EAE appeared (Table 1 and 2). Injection of GK1.5 prevented the clinical and histologic manifestations of EAE when the antibody was administered after the appearance of autoimmune T cells capable of transferring EAE (Table 1). Thus, 9 days after immunization with MSCH, mice have already produced a T-cell population that can transfer EAE to untreated recipients (*7*). Such MSCH-immunized mice failed to develop EAE when injected repeatedly with GK1.5 beginning on day 9. When mice were injected with GK1.5 on the 2 days preceding and the day after immunization with MSCH, no disease symptoms were observed 2 weeks later, a time when nearly 90 percent of saline-injected controls were paralyzed (Table 1). Similar treatments with a monoclonal antibody to Lyt2, which does not bind to $L3T4^+$ peripheral T cells but does bind to suppressor-cytotoxic T cells, did not significantly influence the incidence of EAE (Table 1).

Treatment with GK1.5 was effective even when mice were injected with the antibody after the first symptoms of EAE appeared (on days 12 to 14). In this protocol, mice were observed daily and were selected at random to receive GK1.5 or phosphate-buffered saline (PBS) injection as soon as tail weakness, paraparesis, and weight loss were observed. Unlike the control mice, the GK1.5-treated mice did not develop hind-limb paralysis or quadriplegia and they did not die. By 72 hours after injection of GK1.5, 90 percent of the treated mice showed clinical improvement with no residual neurologic impairment (Table 2). Treatment of quadriplegic or moribund mice with GK1.5 did not ameliorate paralysis or prevent death.

We used multiparameter fluorescent-activated cell sorter (FACS) analyses to investigate the changes in the relative numbers of T cells belonging to different T-cell subsets after treatment with GK1.5. To estimate the frequency of $L3T4^+$ cells in animals treated with GK1.5 we used the Ly1 and Lyt2 surface markers. T helper (inducer) cells are $Ly1^+Lyt2^-$, while T suppressor–cytotoxic cells are $Ly1^+Lyt2^+$. With dual immunofluorescence analyses these surface markers provide a measure of $L3T4^+$ T cell frequency, since $L3T4^+$ cells are $Ly1^+Lyt2^-$ and $L3T4^-$ cells are $Ly1^+Lyt2^+$ (*5*).

Treatment with antibody to the L3T4 marker selectively reduced the number of $L3T4^+$ T cells. Two injections of GK1.5 at 24-hour intervals were sufficient to deplete about half of the $L3T4^+$ cells from the spleen and nearly all of this T-cell subset from the lymph nodes (Fig. 1 and Table 3). Similar depletions of the $L3T4^+$ subset occurred in mice that had already been immunized with MSCH in order to induce EAE and treated with GK1.5 on days 9 to 12 (*8*). The T-cell depletion was specific for the $L3T4^+$ subset since the numbers of $Ly1^+Lyt2^+$ ($L3T4^-$ T cells) were not altered by the

Table 2. Reversal of EAE with GK1.5.

Treatment*	Mice (No.) with clinical symptoms 72 hours after treatment†			Deaths‡
	None	Mild	Severe	
GK1.5	14	1	1	1
None (PBS)	1	2	13	6

*Treatment was begun when mice exhibited mild EAE. At this time, mice received 300 μg of GK1.5 in saline intraperitoneally. An injection of 100 μg of GK1.5 was made on each of the 2 days after initiation of treatment. A total of 16 mice received GK1.5 and 16 mice received PBS. †The clinical status of the mice was graded according to the following scale: none, no neurologic symptoms or residual tail weakness with weight gain; mild, a flaccid tail and paraparesis with weight loss and poor coat texture; severe, quadriplegia with hind-limb scissoring. The clinical conditions were graded by an observer who was not informed of the treatment protocol. ‡Seven days after initiation of treatment.

Table 3. Selective depletion of the $L3T4^+$ T cell subset with GK1.5. The integration boundaries shown in Fig. 1 were used to determine the frequencies of $Ly1^+Lyt2^-$ and $Ly1^+Lyt2^+$ cells. The number of cells with the indicated phenotype was estimated by multiplying the cell frequency × the cell count for the organ in question. Pooled popliteal, inguinal, axillary, and brachial lymph nodes were used for analyses. Preparation of cells, staining procedures, and data collection procedures were as described (*13*).

T-cell subset	Spleen* ($\times 10^6$ cells)		Lymph nodes* ($\times 10^6$ cells)	
	Untreated	Treated†	Untreated	Treated†
$Ly1^+Lyt2^-$ ($L3T4^+$)	28	16	25	3.1
$Ly1^+Lyt2^+$ ($L3T4^-$)	8.3	8.8	14	16

*The standard error for all values is less than 15 percent. Each group had a minimum of four mice. †Treated SJL/J mice were injected intraperitoneally with 100 μg of GK1.5 on each of the 2 days prior to FACS analysis.

GK1.5 treatment (Table 3). Although nearly all thymocytes express L3T4 (*5*), the percentage of L3T4$^+$ thymocytes was not significantly changed in treated mice (*8*).

The mechanism of the therapeutic actions of GK1.5 administration is still unknown. The observed decrease in the numbers of peripheral L3T4$^+$ helper (inducer) or effector T cells may be sufficient to account for the amelioration of the disease. Alternatively, the ability of the antibody to bind L3T4 and block the functional activities of this molecule could also play a role. In vivo administration of monoclonal antibodies to the I-A subregion of the major histocompatibility complex can prevent and reverse EAE (*9*). It is possible that antibody to L3T4 and antibodies to I-A effect EAE because both antibodies block T-cell activation, either at the site of the T-cell receptor for I-A [in the case of treatment with antibody to L3T4 (*5, 10*)] or at the site of I-A on antigen-presenting cells (in the case of treatment with antibody to I-A). In addition, if there is a reduction in the number of L3T4$^+$ cells or if their activation is blocked (or both), there may be a shift in the balance between regulatory helper and suppressor T cells to an alternate stable state (*11*) in which suppression becomes the dominant response to the encephalitogenic components of MSCH.

Regardless of the mechanism of action of GK1.5 treatment, we have shown that in vivo administration of GK1.5 has a dramatic effect on the course of EAE. The successful treatment of murine EAE with GK1.5 suggests that therapy with monoclonal antibodies to the human equivalent of the murine L3T4$^+$ subset might prove effective in the treatment of MS and possibly in other diseases where this T-cell subset plays a central role.

MATTHEW K. WALDOR*
Departments of Genetics and *Neurology, Stanford University, Stanford, California 94305*
SUBRAMANIAN SRIRAM
Department of Neurology, Stanford University
RICHARD HARDY
LEONORE A. HERZENBERG
LEONARD A. HERZENBERG
Department of Genetics, Stanford University
LEWIS LANIER
Becton Dickinson Monoclonal Antibody Center, Mountain View, California 94043
MAE LIM
LAWRENCE STEINMAN
Departments of Neurology and *Pediatrics, Stanford University*

References and Notes

1. E. C. Alvord, M. W. Kies, A. J. Suckling, *Experimental Allergic Encephalomyelitis, A Useful Model for Multiple Sclerosis* (Liss, New York, 1984); P. Y. Paterson, in *Autoimmunity*, N. Talal, Ed. (Academic Press, New York, 1977), pp. 644–692.
2. B. G. Arnason *et al.*, *J. Exp. Med.* **116**, 177 (1962); N. K. Gonatas and J. C. Howard, *Science* **186**, 839 (1974); L. Ortiz-Ortiz and W. O. Weigle, *J. Exp. Med.* **144**, 604 (1976).
3. U. Traugott, E. L. Reinherz, C. S. Raine, *Science* **219**, 308 (1982).
4. C. B. Pettinelli and D. E. McFarlin, *J. Immunol.* **127**, 1420 (1981); Z. Lando and A. Ben-Nun, *Clin. Immunol. Immunopathol.* **30**, 290 (1984); J. Trotter and L. Steinman, *J. Immunol.*, in press.
5. D. P. Dialynas *et al.*, *Immunol. Rev.* **74**, 29 (1983).
6. E. L. Reinherz and S. F. Schlossman, *N. Engl. J. Med.* **303**, 370 (1980); E. L. Reinherz *et al.*, *ibid.*, p. 125.
7. C. Bernard and I. R. MacKay, *J. Neuroimmunol.* **4**, 61 (1983).
8. M. K. Waldor *et al.*, unpublished data.
9. L. Steinman *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **78**, 7111 (1981); S. Sriram and L. Steinman, *J. Exp. Med.* **158**, 1362 (1983).
10. P. Marrack *et al.*, *J. Exp. Med.* **158**, 1077 (1983).
11. L. A. Herzenberg, S. J. Black, L. A. Herzenberg, *Eur. J. Immunol.* **10**, 1 (1980).
12. J. A. Ledbetter and L. A. Herzenberg, *Immunol. Rev.* **47**, 63 (1979).
13. K. Hayakawa, R. R. Hardy, D. R. Parks, L. A. Herzenberg, *J. Exp. Med.* **157**, 202 (1983).
14. D. R. Parks, R. R. Hardy, L. A. Herzenberg, *Immunol. Today* **4**, 145 (1983).
15. Supported by NIH grants NS-571, NS-18235, GM-17367, HD-01287, and CA-04681; a grant from the Multiple Sclerosis Society 1440-A-1; and a grant from the Kroc Foundation.
* To whom correspondence should be addressed at Department of Neurology, C-338, Stanford University, Stanford, Calif. 94305.

27 August 1984; accepted 5 November 1984

Hernandulcin: An Intensely Sweet Compound Discovered by Review of Ancient Literature

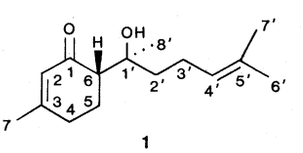
Abstract. *Ancient Mexican botanical literature was systematically searched for new plant sources of intensely sweet substances.* Lippia dulcis *Trev., a sweet plant, emerged as a candidate for fractionation studies, and hernandulcin, a sesquiterpene, was isolated and judged by a human taste panel as more than 1000 times sweeter than sucrose. The structure of the sesquiterpene was determined spectroscopically and confirmed by chemical synthesis. Hernandulcin was nontoxic when administered orally to mice, and it did not induce bacterial mutation.*

Because of such factors as the caloric and cariogenic potential of sucrose, alternative means are being sought to satisfy the human habit of consuming sweets. Synthetic substances such as saccharin, cyclamate, and aspartame have been or are being used in the United States for this purpose, while naturally occurring substances such as phyllodulcin, stevioside and glycyrrhizin are used in Japan (*1, 2*). None of these substances is ideal, however, either because of taste characteristics that are generally perceived as unpleasant, questionable safety, chemical instability, or because of the relatively high cost of production (*1, 3*).

The discovery of practically every intensely sweet, prototype molecule has been fortuitous; there is still insufficient knowledge to design sweet compounds that are structurally unrelated to existing sweeteners (*3*). In addition, large-scale screening of potential sweeteners is impractical because of the lack of convenient bioassay systems (*4*). Thus, as part of a program to develop new sweetening agents and to provide compounds that could aid in understanding the relation between moleclular structure and sweetness, the Mexican ethnobotanical literature was searched with the express purpose of uncovering records of intensely sweet-tasting plants.

While examining a monograph entitled *Natural History of New Spain*, written between 1570 and 1576 by the Spanish physician Francisco Hernández (*5*), our attention was drawn to a remarkably sweet plant known to the Aztec people by the Nahuatl name *Tzonpelic xihuitl*. The literal translation of these words is "sweet herb." The accurate description and illustration of the plant provided by Hernández combined with another mention (*6*), enabled the assignment of *Tzonpelic xihuitl* as *Lippia dulcis* Trev. (Verbenaceae). The constituents of this plant do not appear to have been studied in recent years, but investigators have suggested that the sweet principle is volatile (*7*) and distinguishable from the sweet glycoside glycyrrhizin (*8*).

The principal sweet component of *L. dulcis* (*9*), which was present mainly in the leaves and flowers, was isolated in pure form after solvent partition and chromatographic fractionation. This compound, **1**, which has been named



1

hernandulcin in honor of Francisco Hernández, was obtained as a colorless oil $[\alpha]_D^{25}$ +109° (c, 0.11 g/100ml in ethanol). Its molecular formula is $C_{15}H_{24}O_2$, based on a high-resolution mass spectral measurement of the molecular ion, 236.18005 amu. Analysis of the ^{1}H- and ^{13}C-NMR