-0.6 ($P < 0.05$). The Mini PSE, however, rates only reported positive symptoms. Many of our patients were noted to be suspicious and guarded, probably resulting in an artificially low symptom score. Many patients had more severe symptoms on following examinations.

29. In a preliminary study of two patients with bipolar depression (44 and 52 years of age and one patient with unipolar depression (39 years of age), who were severely depressed at the time of PET scanning, the B_{max} values were 5, 15, and 12.5 pmoles/g respectively.

30. Supported by U.S. Public Health Service grants NS15080 and NIMH 1RO1 53146 and Scottish Rite Foundation, N.M.J., U.S.A. We thank F. Gilbart, S. Herda, S. Bosley, D. Clough, M. Stumpf, C. Steele, M. Haden, F. Schaerf, C. Ross, M. Murrell, K. Prendergast, D. Starkey, D. Goldberg, H. Rothenberg, H. Drew, B. Scheinin, C. Trauma, J. Schmidt, C. Schultz, K. Kofsky, A. Rosenbaum, K. H. Douglass, A. Bice, B. Zeeberg, E. London, A. Weissman, B. Kuyatt, C. Cidis, R. Gungon, P. D. Wilson, R. Parker, L. Widerman, L. Wilkins, P. Hartig, and V. Villemagne for assistance and discussions.

14 October 1986; accepted 21 November 1986

CD8$^+$ Lymphocytes Can Control HIV Infection in Vitro by Suppressing Virus Replication

CHRISTOPHER M. WALKER, DEWEY J. MOODY, DANIEL P. STITES, JAY A. LEVY

Lymphocytes bearing the CD8 marker were shown to suppress replication of human immunodeficiency virus (HIV) in peripheral blood mononuclear cells. The effect was dose-dependent and most apparent with autologous lymphocytes; it did not appear to be mediated by a cytotoxic response. This suppression of HIV replication could be demonstrated by the addition of CD8$^+$ cells at the initiation of virus production as well as after several weeks of virus replication by cultured cells. The observations suggest a potential approach to therapy in which autologous CD8 lymphocytes could be administered to individuals to inhibit HIV replication and perhaps progression of disease.

THE ACQUIRED IMMUNE DEFICIENCY syndrome (AIDS) is caused by a newly recognized human retrovirus that is now termed human immunodeficiency virus (HIV) (*1*). This virus can be recovered from cultured peripheral blood mononuclear cells (PMC) of individuals with AIDS, AIDS-related conditions (ARC), and many asymptomatic individuals in the known risk groups (*2*). Studies in our laboratory have indicated that cultured PMC from 50% of seropositive healthy individuals do not yield infectious virus (*3*). Moreover, we have studied some individuals whose PMC in cultures have initially released virus and then ceased to yield any infectious virus for more than 1 year (*4*). These individuals remain clinically healthy and show an improvement in their immune status. Their clinical state suggests a control of the virus infection.

Cellular immune responses provide a major mechanism for reducing the growth of virus-infected cells as well as tumors (*5*). We therefore examined whether the lack of production of infectious HIV by the PMC of some individuals was due to selected cellular immune responses. We found that the CD8 (OKT8/Leu-2) subset of T lymphocytes (*6*) suppresses HIV replication in PMC.

For these studies, we removed the CD8$^+$ cells from the PMC of HIV antibody–positive individuals by the panning method of Wysocki and Sato (*7*) (see legend to Table 1). The cells remaining in the CD8-depleted fraction were then cultured in the presence of phytohemagglutinin (PHA) and interleukin-2 (IL-2) (*2, 3*). The removed CD8$^+$ cells were cultured in a similar manner. The supernatants of all cultures were assayed at 3- to 4-day intervals for the presence of HIV (*2, 3*).

In several repeated experiments, cultured unseparated PMC obtained from three healthy, HIV antibody–positive homosexual men (subjects 1, 2, and 3) did not yield infectious HIV (*8*). In contrast, when cultures of PMC from these individuals were depleted of CD8$^+$ cells, substantial levels of reverse transcriptase (RT) activity were detected in the supernatants (Table 1). That these supernatants contained infectious virus was demonstrated by their ability to infect cultured PMC from virus-negative donors, in which they induced RT activity and HIV antigen production (*3*). PMC from subjects 2 and 3 did not release virus after depletion of cells expressing CD16 (Leu-11), a marker associated with natural killer (NK) cells (*9*). However, HIV was detected in the CD16-depleted fraction of PMC from subject 1, a seropositive Asian male. Like other Asian individuals (*10*), he may have a large proportion of CD16$^+$ lymphocytes that co-express the CD8 marker.

High levels of virus-associated RT activity were also detected in CD8-depleted PMC from subject 4, an asymptomatic individual who has had Kaposi's sarcoma for over 4 years. However, low but detectable levels of HIV were also consistently detected in cultures of his unseparated PMC (Table 1). This finding suggests that his CD8$^+$ cells have a reduced capacity to control HIV replication. Virus was not recovered from the cultured CD8$^+$ cells of any of these four individuals; this observation confirms the lack of replication of HIV in this subset of lymphocytes (*11*).

In examining further the role of CD8$^+$ cells in suppressing HIV replication, we performed additional studies on subject 3, who agreed to be tested on several occasions. First, we added his separated CD8$^+$ cells to his autologous CD8-depleted PMC prior to the initiation of culture (Fig. 1). No RT activity was detected in the fluid of the reconstituted culture. We found a clear dose-response relation between the number

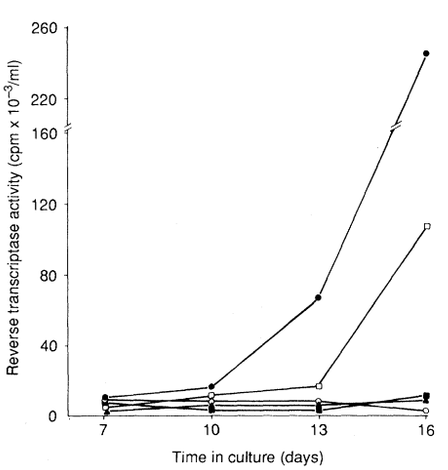

Fig. 1. Reconstitution of CD8-depleted PMC cultures with autologous CD8$^+$ lymphocytes prevents HIV replication. A representative experiment is shown. PMC from subject 3 were separated into CD8$^-$ and CD8$^+$ fractions by the panning method and cultures were established as described in the legend to Table 1; 4×10^6 CD8$^-$ PMC were cultured alone (●) or with 0.375×10^6 (□), 0.75×10^6 (■), or 1.5×10^6 (▲) autologous CD8$^+$ lymphocytes that were added prior to the initiation of culture. A control culture of 4×10^6 CD8$^+$ positive cells was also established (○). All culture supernatants were monitored for HIV-associated RT activity at 3- to 4-day intervals, and the presence of HIV antigen in cultured cells was confirmed by an indirect immunofluorescence assay (see legend to Table 1).

C. M. Walker and J. A. Levy, Cancer Research Institute, Department of Medicine, University of California, School of Medicine, San Francisco, CA 94143.
D. J. Moody and D. P. Stites, Department of Laboratory Medicine, University of California, School of Medicine, San Francisco, CA 94143.

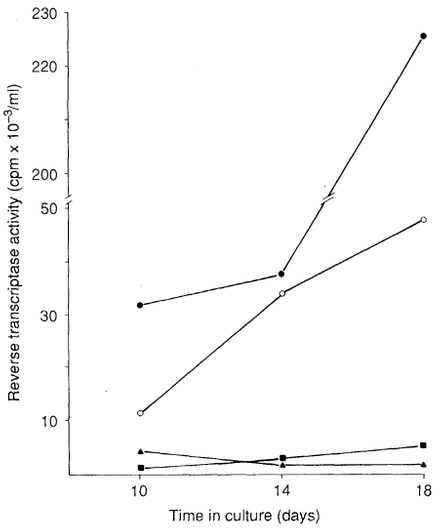

Fig. 2. Autologous but not allogeneic $CD8^+$ lymphocytes prevent virus replication in CD8-depleted PMC cultures. A population of CD8-depleted PMC from subject 3 was prepared (see legend to Table 1); then 5×10^6 cells were cultured alone (●) or with 10^6 $CD8^+$ lymphocytes recovered from the PMC of subject 3 (■) or subject 1 (○). Control cultures were established with 5×10^6 unseparated PMC from subject 3 (▲). At 4-day intervals the cultures were monitored for HIV-associated RT activity and for the presence of HIV antigen by an indirect immunofluorescence assay.

of $CD8^+$ cells added to the CD8-depleted PMC cultures and the degree of virus suppression. As few as 0.75×10^6 $CD8^+$ cells (less than the number found in the unseparated PMC culture), when added to 4×10^6 CD8-depleted PMC prior to the initiation of culture, prevented virus replication. However, after reconstitution with only 0.375×10^6 $CD8^+$ cells, HIV could be recovered as early as 10 days after the initiation of the culture (Fig. 1).

To control for the possibility that the reconstituted $CD8^+$ lymphocytes suppressed virus replication by a nonspecific "overcrowding" effect, we added an equal number of $CD8^+$ cells from subject 1 to the CD8-depleted cultures from subject 3. The amount added (10^6 cells) approximated the number of $CD8^+$ cells initially found in this amount of unseparated PMC (see legend to Table 1). As shown in Fig. 2, only the $CD8^+$ lymphocytes recovered from subject 3, and not those from subject 1, inhibited HIV replication in the CD8-depleted PMC cultures. However, large numbers of $CD8^+$ cells (for example, 3×10^6) from either subject inhibited virus replication when added to the CD8-depleted PMC cultures. In an independent experiment in which $CD8^+$ cells from subject 3 or 4 were added to CD8-depleted PMC from subject 4, similar results were obtained; only the $CD8^+$ cells from subject 4 were able to reduce the replication of HIV.

These first experiments dealt with prevention of virus replication at initiation of culture. We next examined the ability of $CD8^+$ cells to abrogate ongoing virus replication in CD8-depleted PMC (Table 2). The CD8-depleted PMC from subject 3, when cultured for 3 weeks, produced high levels of RT activity. This culture was then split into three sublines and reconstituted with 10^6 $CD8^+$ cells from subjects 3 or 5 that had been grown in the presence of IL-2 for 14 days. By day 7 the amount of RT activity in the culture that received 10^6 $CD8^+$ cells from subject 3 was clearly reduced. In contrast, virus replication in the unreconstituted PMC culture or that receiving $CD8^+$ cells from subject 5 was increased. By day 10, high levels of RT activity were only present in the CD8-depleted PMC and these cells reconstituted with $CD8^+$ cells from subject 5.

The mechanism for this control of HIV replication by $CD8^+$ cells could involve soluble mediators, such as interferon, that inhibit virus production (*12*), or directly kill virus-infected cells (*13*). In examining these possibilities, we added $CD8^+$ cells to autologous CD8-depleted PMC prior to the initiation of culture (see Fig. 3A), and then removed them again 3 weeks later using the panning technique (Fig. 3B). Culture supernatants were then assayed at regular intervals for HIV-associated RT activity. Initially, as noted above, HIV replication was not detected in unseparated PMC nor in CD8-depleted cells mixed prior to culture with the separated autologous $CD8^+$ cells. However, as previously shown, the CD8-depleted cells readily released HIV in culture (Fig. 3A). Subsequently, when the $CD8^+$ cells were removed at day 23, the resulting CD8-depleted fraction (containing 75% $CD4^+$ cells and 2% $CD8^+$ cells) started to produce HIV within 9 days (Fig. 3B), and high levels of RT activity were continuously detected in the culture supernatants until the adherent cell fraction obtained by panning on day 23 contained 99% $CD8^+$ positive cells and failed to produce virus in culture. It

Table 1. Isolation of HIV from cultures of PMC depleted of $CD8^+$ lymphocytes. PMC from four subjects (*8*) were separated on Ficoll-Hypaque gradients, washed, and cultured at approximately 10^6 cells per milliliter in RPMI 1640 medium containing 10% fetal bovine serum (FBS), 1% antibiotics, 10% human IL-2, and Polybrene (1 μg/ml) (*2, 3*). Phytohemagglutinin (PHA) (Wellcome) (3 μg/ml) was added at the initiation of a 5-ml cell culture in a 25 cm^2 flask. Culture supernatants were assayed for Mg^{2+}-dependent RT activity at 3- to 5-day intervals as described (*3*). Additional PMC were not added to the cultures at any time. The presence of HIV antigens in cultured cells was confirmed by an indirect immunofluorescence assay with the use of serum from AIDS patients containing antibodies to HIV proteins (*2, 3*). Cultures were also established from PMC depleted of $CD8^+$ or of $CD16^+$ cells (*7*). Briefly, plastic-adherent cells were removed and then 20×10^6 to 30×10^6 of the nonadherent PMC were incubated in 2 ml of phosphate-buffered saline (PBS) containing 10 μg/ml of monoclonal antibodies to either Leu-2b or Leu-11b (Becton-Dickinson) for 20 minutes at room temperature. These antibodies recognize epitopes on the CD8 and CD16 antigens, respectively. The cells were then washed twice, resuspended in 4 ml of PBS containing 1% fetal bovine serum (FBS), and incubated on a plastic petri dish coated with antibody for 2 hours at 4°C. These capture plates were prepared with bacteriologic grade petri plates coated with the F(ab)'2 portion of goat antibody to mouse immunoglobulin G (Tago, Burlingame, CA). The plates were coated with the capture antibody (10 μg/ml, 0.05*M* tris, *p*H 9.5) for 40 minutes at room temperature, and excess antibody was washed off the plate with PBS. To prevent nonspecific attachment of PMC, plates were coated with PBS containing 1% FBS prior to use. After incubation, nonadherent cells (that is, the $CD8^-$ or $CD16^-$ fraction) were washed off the plate with cold PBS, and adherent cells ($CD8^+$ or $CD16^+$) were removed from the plate with a forceful jet of PBS. Adherent and nonadherent PMC fractions were then cultured for the presence of HIV as described above. The effectiveness of the procedure was assessed by flow cytometry as described (*22*). ND, not done.

Time in culture (days)	Reverse transcriptase activity in culture supernatants* (cpm × 10^{-3}/ml)		
	Unseparated	CD8-depleted	CD16-depleted
	Subject 1		
8	1.7	1.2	1.4
11	2.5	9.5	11.2
15	3.6	9.5	4.9
20	1.2	32.5	1.0
25	3.4	63.5	22.5
	Subject 2		
10	2.3	11.7	1.0
14	5.9	22.2	2.2
18	5.9	725.1	1.3
	Subject 3		
10	2.6	26.3	2.1
14	0.8	490.9	0.8
	Subject 4		
10	11.6	50.5	ND
14	5.9	106.5	ND
18	6.0	44.7	ND

*Analysis of the results from 11 independent experiments on the subjects revealed that before removal of monocytes, unseparated PMC cultures contained 24% $CD4^+$ cells (SD = 4.1) and 36% $CD8^+$ cells (SD = 11.6). The remainder of the cells were B cells (Leu-12–positive), NK cells (Leu-11–positive), and monocytes (Leu-M3–positive) in approximately equal numbers. Incubation of PMC on plastic dishes reduced the monocyte population to less than 2%. After planning, 93% of the adherent cells were Leu-2b–positive (SD = 3.8) and 7% were Leu-3–positive (SD = 5.0). Nonadherent cells were 64% Leu-3–positive (SD = 9.1) and 7% Leu-2b–positive (SD = 2.5). The remainder of the cells in this fraction were B cells (approximately 10%) and NK cells (approximately 15%).

is noteworthy that after 23 days in culture, the number of cells in the unseparated PMC increased tenfold (4×10^6 cells grew to 4×10^7 cells), but the ratio of $CD4^+$ to $CD8^+$ cells remained constant (1:1). All these findings strongly suggest that $CD8^+$ cells do not inhibit virus replication by suppression of $CD4^+$ cell proliferation or by killing virus-containing cells. This latter conclusion is supported by the recent observation that peripheral blood T lymphocytes activated with mitogens and lymphokines do not lyse HIV-infected target cells (*14*).

We favor the hypothesis that $CD8^+$ cells produce a soluble factor that interferes with HIV replication. In preliminary studies we observed that the supernatant from cultured $CD8^+$ cells reduced virus production by CD8-depleted PMC. It is possible that γ-interferon, which is made by $CD8^+$ lymphocytes (*15*), is responsible for this antiviral effect. However, we could not induce HIV expression in unseparated PMC from subject 3 by adding antibodies to γ-interferon to the cultures. Moreover, γ-interferon has no effect on HIV replication (*16*).

Allogeneic $CD8^+$ cells from healthy HIV-seropositive donors were not as effective as autologous $CD8^+$ cells in suppressing HIV replication by PMC (Fig. 2 and Table 2). This finding could not result from differences among individuals in $CD8^+$ positive cells with antiviral activity since this result was observed with three different subjects. It is more likely that, as has been observed with other T-suppressor/cytotoxic cell systems (*17*), virus-specific $CD8^+$ lymphocytes recognize viral antigens in association with class I HLA glycoproteins expressed on the surface of infected cells. This recognition linked to histocompatibility antigens could lead to an enhanced production of the putative antiviral lymphokine discussed above, and thus explain the preferential suppression of virus production by autologous $CD8^+$ cells. Further experiments are needed with HLA matched and mismatched HIV-seropositive individuals to confirm this conclusion. The inhibition of HIV replication, however, does appear to be dependent on the relative number of $CD8^+$ cells present (Fig. 1). Thus, HIV production in infected individuals might correlate with low percentages of $CD8^+$ cells.

To date, after depletion of $CD8^+$ lymphocytes, we have successfully isolated large quantities of HIV from the cultured PMC of four out of seven asymptomatic, seropositive homosexual men who were initially virus-negative or, in the case of subject 4, released very low levels of HIV. The result does not reflect enrichment of the $CD4^+$ cells in culture after panning, since addition of even relatively low quantities of autologous $CD8^+$ cells abrogated virus replication (Fig. 1). We do not know why virus could not be recovered from the other three subjects. Conceivably, the virus had been cleared from their PMC, or is harbored in other nonlymphoid tissues such as those of the nervous or reticuloendothelial systems (*4, 18*). Alternatively, HIV may have established a latent infection in the PMC of these subjects (*4, 19*). However, addition of iododeoxyuridine, an inducer of latent viruses (*4, 19*), to the CD8-depleted PMC of one of these individuals (subject 5) did not result in HIV production.

It is not yet clear if $CD8^+$ cells play a role in vivo in preventing HIV replication and disease progression. Virus was not detected in the serum of the three healthy subjects who were able to suppress virus replication in vitro (subjects 1, 2, and 3). Thus, this control mechanism could be active in the host.

Patients with AIDS have a reduced capacity to generate cell-mediated immune responses against a variety of viruses, but in many instances these responses can be restored in vitro by adding IL-2 to the cultures (*20*). This finding suggests that in HIV-infected, symptomatic individuals a defect in the generation of adequate suppressor T-cell control results from the lack of T-helper (that is, $CD4^+$) cells. Thus, if PMC from individuals with AIDS or related conditions show virus replication, it may be reasonable to treat their $CD8^+$ cells in vitro with IL-2 and then adoptively transfer them back. This selective treatment would avoid the known effect of IL-2 in increasing T-helper cells that could become additional target cells for HIV replication. A similar procedure for growing T-suppressor/cytotoxic cells has been used in treating certain tumors of mice and humans (*21*). However, in that procedure, the cells generated in vitro are cytotoxic and thus differ from the $CD8^+$ cells mediating the suppression of HIV. Our results encourage further basic studies that might eventually lead to treat-

Table 2. Effect of $CD8^+$ lymphocytes on HIV replication by CD8-depleted PMC cultures. An HIV-producing CD8-depleted culture established from the PMC of subject 3 was split into three sublines (each containing 5×10^6 cells) after 3 weeks in culture. Two of these cultures were reconstituted with 1.5×10^6 $CD8^+$ lymphocytes from subject 3 or 5 (*8*). These CD8-enriched lymphocytes were originally recovered from unseparated PMC and maintained in IL-2–containing medium for 14 days prior to being added to the HIV-producing cultures. Culture supernatants were assayed for RT activity and the results are expressed in counts per minute $\times 10^{-3}$ per milliliter as described previously (*3*).

Time after subculture (days)	CD8-depleted PMC from subject 3	Source of $CD8^+$ cells added to CD8-depleted PMC from subject 3	
		Subject 3	Subject 5
4	64.7	59.8	64.9
7	69.3	23.2	101.4
10	50.4	9.5	75.2

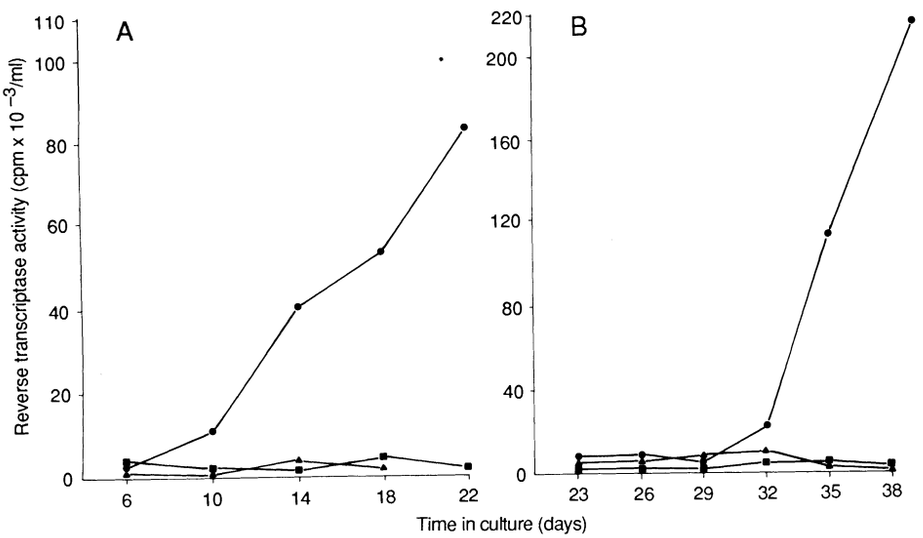

Fig. 3. Lymphocytes with the CD8 marker suppress HIV replication in vitro without eliminating virus-infected cells. (A) Cultures of PMC were established from subject 3 (see legend to Table 1). These cultures included 5×10^6 unseparated PMC (▲), and 5×10^6 CD8-depleted PMC cultured with (■) or without (●) the addition of 3×10^6 $CD8^+$ lymphocytes prior to the initiation of culture. (B) On day 23 of culture, the flask originally seeded with 5×10^6 CD8-depleted cells and cultured with 3×10^6 $CD8^+$ PMC was subcultured; a portion containing 5×10^6 cells was immediately reseeded into a 25-cm^2 tissue culture flask (■), and the remaining cells were again separated by the panning method into cultures of a similar number of $CD8^+$ positive (▲) and CD8-depleted (●) cells (see legend to Table 1). Supernatants were assayed for HIV-associated RT activity at 3- to 4-day intervals.

ment of AIDS virus infections with cultured $CD8^+$ cells to prevent or delay progression of disease.

REFERENCES AND NOTES

1. J. Coffin *et al.*, *Science* **232**, 697 (1986).
2. F. Barré-Sinoussi *et al.*, *ibid.* **220**, 868 (1983); R. C. Gallo *et al.*, *ibid.* **224**, 500 (1984); J. A. Levy *et al.*, *ibid.* **225**, 840 (1984).
3. J. Shimabukuro and J. A. Levy, *J. Infect. Dis.* **152**, 734 (1985); A. D. Hoffman *et al.*, *Virology* **147**, 326 (1985).
4. J. A. Levy *et al.*, *Ann. Int. Med.* **103**, 694 (1985); J. A. Levy *et al.*, *Ann. Inst. Pasteur (Paris)*, in press.
5. P. C. Doherty *et al.*, *Adv. Cancer Res.* **42**, 1 (1985); R. M. Zinkernagel and P. C. Doherty, *Adv. Immunol.* **27**, 51 (1979).
6. E. L. Reinherz *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **76**, 4061 (1979); E. G. Engelman *et al.*, *J. Exp. Med.* **154**, 193 (1981); L. L. Lanier *et al.*, *Immunol. Rev.* **74**, 143 (1983).
7. L. J. Wysocki and V. L. Sato, *Proc. Natl. Acad. Sci. U.S.A.* **75**, 2844 (1978).
8. Subjects 1, 2, and 3 are asymptomatic homosexual men; subject 4, of a homosexual man, is at present asymptomatic, with Kaposi's sarcoma in a quiescent state; subject 5 is a healthy homosexual man whose unseparated as well as CD8-depleted PMC do not release HIV. All the subjects have antibodies to HIV.
9. L. L. Lanier *et al.*, *J. Immunol.* **131**, 1789 (1983); B. Perussia *et al.*, *ibid.* **130**, 2142 (1983); H. Rumpold *et al.*, *ibid.* **129**, 1458 (1982).
10. H. E. Prince *et al.*, *Diagn. Immunol.* **3**, 33 (1985).
11. D. Klatzmann *et al.*, *Science* **225**, 59 (1984).
12. R. M. Friedman and S. N. Vogel, *Adv. Immunol.* **34**, 97 (1983).
13. A. J. McMichael and B. A. Askonas, *Eur. J. Immunol.* **8**, 705 (1978); G. V. Quinnan *et al.*, *N. Engl. J. Med.* **307**, 7 (1982); H. M. Mitsuya *et al.*, *J. Exp. Med.* **158**, 994 (1983); I. S. Misko *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **77**, 4247 (1980); see review by R. M. Zinkernagel and P. C. Doherty, *Adv. Immunol.* **27**, 51 (1979).
14. F. W. Ruscetti *et al.*, *J. Immunol.* **136**, 3619 (1986).
15. J. J. Hooks *et al.*, *Blood* **59**, 198 (1982); T. Kasahara *et al.*, *J. Immunol.* **130**, 1784 (1983).
16. J. K. Yamamoto *et al.*, *J. Interferon Res.* **6**, 143 (1986).
17. S. C. Meuer *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* **79**, 4395 (1982); H. Spits *et al.*, *J. Immunol.* **129**, 1563 (1982).
18. G. Shaw *et al.*, *J. Immunol.* **227**, 117 (1985); J. A. Levy *et al.*, *Lancet* **1985-II**, 586 (1985); D. Ho *et al.*, *N. Engl. J. Med.* **313**, 1493 (1985).
19. T. Folks *et al.*, *Science* **231**, 600 (1986).
20. A. H. Rook *et al.*, *J. Clin. Invest.* **72**, 398 (1983); G. M. Shearer *et al.*, *ibid.* **76**, 1699 (1985); H. W. Murray *et al.*, *ibid.*, p. 1959; J. F. Sheridan *et al.*, *J. Clin. Immunol.* **4**, 304 (1984).
21. S. A. Rosenberg, *J. Natl. Cancer Inst.* **75**, 595 (1985); S. A. Rosenberg *et al.*, *N. Engl. J. Med.* **313**, 1485 (1985).
22. J. A. Levy *et al.*, *Clin. Immunol. Immunopathol.* **35**, 328 (1984).

23. These studies were supported by a grant from the California State Universitywide Task Force on AIDS. C.M.W. is the recipient of a fellowship from the Medical Research Council of Canada. Monoclonal antibodies were a gift from N. Warner of Becton-Dickinson Corporation. We thank T. McHugh, M. Whalen, and H. Legg for technical assistance.

9 October 1986; accepted 12 November 1986

Catalytic Antibodies

ALFONSO TRAMONTANO,* KIM D. JANDA, RICHARD A. LERNER

Monoclonal antibodies elicited to haptens that are analogs of the transition state for hydrolysis of carboxylic esters behaved as enzymic catalysts with the appropriate substrates. These substrates are distinguished by the structural congruence of both hydrolysis products with haptenic fragments. The haptens were potent inhibitors of this esterolytic activity, in agreement with their classification as transition state analogs. Mechanisms are proposed to account for the different chemical behavior of these antibodies with two types of ester substrates. The generation of an artificial enzyme through transition state stabilization by antibodies was thus demonstrated. These studies indicate a potentially general approach to catalyst design.

A BASIC PRINCIPLE OF ENZYME CATALYSIS states that strong binding interactions are required by enzymes to reduce the energy barriers along the chemical reaction pathway (*1*). One way in which this may be done is by helping to stabilize the transition state. This insight, attributed to Pauling, foretold that substances that mimic the structure of a transition state in a particular enzymic reaction would be bound tightly to enzymes involved in the reaction (*2*). In fact, the transition state analog concept is now firmly established as a viable approach to the design of enzyme inhibitors (*3*). The converse proposition, that a receptor designed to optimally bind a suitable analog of a transition state would achieve the catalytic function of an enzyme, continues to intrigue experimentalists. The demonstration of this would reinforce the correlation of enzyme function with the principle of active site–transition state complementarity and indicate a powerful approach to devising artificial enzymes.

The immunological reservoir provides the variety of receptor sites with the required specificities for such a study. The combining sites of antibodies have been considered as useful templates for simulating the environment of an enzyme active site (*4*). Previous constructs (*5, 6*), which achieved only limited success, were not based on the Pauling mechanism of binding energy utilization (*7*). Our studies have focused on the use of transition state analogs as the haptens to elicit the desired antibodies (*8*). The hapten would then behave as an inhibitor in the catalytic system. Accordingly, we have demonstrated that such antibodies can exhibit some of the chemical attributes of enzymes (*9*). We now describe how these antibodies are capable of true enzyme catalysis when their proper substrates are identified. These findings portend the emergence of a class of proteins, having the antibody-enzyme dichotomy, for which the term "abzyme" is suggested.

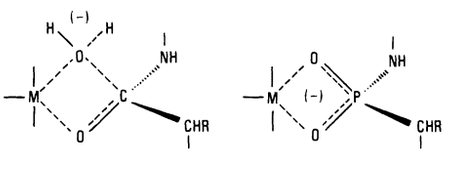

Fig. 1. (A) A possible structure of the transition state in metallopeptidases. The bidentate coordination of the partially hydrated amide to the metal ion is one model for a stabilizing interaction that has been proposed to occur in the mechanism of peptide cleavage by a zinc peptidase (*17*). (B) The interactions of a phosphonamidate analog with the metalloenzyme that allow it to simulate the transition state configuration according to the model shown.

We chose initially to examine a protocol for the hydrolysis of carboxylic esters as an example of a transacylation reaction. Enzymes that catalyze such reactions are expected to bind well those analogs of the substrate having a tetrahedral configuration, thus resembling an intermediate or transition state for nucleophilic addition to the acyl group (*10*). This is true for serine proteases, where a covalent bond between the ligand and the enzyme is formed temporarily (*11, 12*), as well as for metallopeptidases. Metallopeptidases are inhibited by substrate analogs having a tetrahedral phosphoryl or phosphonyl group in place of the cleaved amide unit (*13–15*). A proposed mechanism of peptide bond hydrolysis in metalloenzymes employs the metal ion to either polarize the amide carbonyl by coordination, or deliver a coordinated hydroxide to that group (*16, 17*) (Fig. 1A). Recent structural studies have shown that complexes of enzymes with transition state analogs

Department of Molecular Biology, The Research Institute of Scripps Clinic, La Jolla, CA 92037.

*To whom communications should be addressed.