Proc. Natl. Acad. Sci. USA
Vol. 90, pp. 1411–1415, February 1993
Medical Sciences

Combined use of an immunotoxin and cyclosporine to prevent both activated and quiescent peripheral blood T cells from producing type 1 human immunodeficiency virus

KENNETH D. BELL*†, OCTAVIO RAMILO*‡, AND ELLEN S. VITETTA*§

*Department of Microbiology, †Molecular Microbiology Graduate Program, ‡Department of Pediatrics, and the §Cancer Immunobiology Center, University of Texas Southwestern Medical Center, 5323 Harry Hines Boulevard, Dallas, TX 75235-9048

Communicated by Jonathan W. Uhr, November 12, 1992

ABSTRACT **Two different populations of infected T cells are present in human immunodeficiency virus (HIV)-infected individuals: activated cells that produce virions and quiescent cells that harbor the viral genome but are unable to produce virus unless they are activated. Using an *in vitro* model of acute HIV infection, we have evaluated the effect of depleting activated T cells with an immunotoxin and subsequently inhibiting activation of quiescent T cells with an immunosuppressive agent. CD25 (Tac, p55), the α chain of the interleukin 2 receptor, is expressed on activated, but not quiescent, T cells. An anti-CD25–ricin A chain immunotoxin eliminated activated, CD25⁺ HIV-infected cells and, thereby, inhibited viral production by these cells. Subsequent addition of cyclosporine to the residual CD25⁻ cells prevented their activation and thereby suppressed their ability to produce virus and to propagate the infection to uninfected T cells.**

Treatment of human immunodeficiency virus (HIV) infection has involved three different approaches: anti-viral drugs that inhibit viral transcription or assembly (1–3), vaccines that enhance an anti-HIV immune response (4–7), and molecules targeted to HIV or HIV-infected cells. The latter include soluble cell surface receptors (e.g., CD4) (8–12), anti-HIV antibodies (13–15), and cell-reactive toxin conjugates (16–21). With regard to toxin-based therapy, there are two different populations of HIV-infected cells in seropositive individuals to be considered: activated cells that produce virus and spread the infection and quiescent cells that do not produce virus but harbor the viral genome (22). *In vitro* experiments have demonstrated that quiescent cells are unable to produce virus unless they are activated, at which time the virus is produced and transmitted to other cells (23, 24). Consistent with these findings, HIV titers in plasma increase after vaccination or infection with influenza viruses (25). Hence, a strategy aimed at suppressing viral production should include both the elimination of activated, infected cells and the prevention of activation of quiescent, infected cells.

Using an *in vitro* model of acute HIV infection, we have evaluated two pharmaceuticals to test the above strategy. The elimination of activated, CD25⁺ cells was accomplished with an anti-CD25–ricin A chain immunotoxin, RFT5-dgA. CD25 (Tac, p55), the α chain of the interleukin 2 (IL-2) receptor, is a cell surface glycoprotein expressed on activated, but not quiescent, T cells (26, 27). It, therefore, represents an excellent marker to distinguish activated (CD25⁺) from quiescent (CD25⁻) T cells that are infected with HIV. RFT5-dgA selectively kills activated, but not quiescent, T cells and hence eliminates T cells actively producing virus (28). An immunosuppressive drug, cyclosporine (CsA), was then used to prevent the remaining, CD25⁻, HIV-infected, quiescent cells from becoming activated. CsA is an inhibitor of T-cell activation (29), which is used clinically, and, therefore, is a candidate for evaluating the hypothesis that viral production from quiescent, CD25⁻, latently HIV-infected cells can be suppressed by inhibiting their activation. CsA cannot, however, induce activated, CD25⁺ cells to become quiescent.

Here we report that each pharmaceutical alone failed to prevent production of HIV in cultures containing both activated and quiescent infected cells that were later activated. However, when infected cells were first treated with immunotoxin and subsequently stimulated in the presence of CsA, no detectable HIV was produced.

MATERIALS AND METHODS

Immunotoxin. RFT5 is a murine anti-CD25 monoclonal antibody (IgG1) selected from a group of 25 antibodies based on its ability to form a potent immunotoxin–dgA (see below) and to stain only activated T cells in a panel of 28 normal human tissues (30). RFT5-dgA was prepared by using the hindered heterobifunctional crosslinker *N*-succinimidyloxycarbonyl-α-methyl-(2-pyridyldithio)toluene (31) and chemically deglycosylated ricin A chain (dgA) according to published reports (32).

Virus. A clinical isolate of HIV-1 (BAG) obtained from a patient in Alabama was kindly provided by Thomas Folks (33). Cell-free supernatants were utilized to infect phytohemagglutinin (PHA)-activated peripheral blood mononuclear cells (PBMNs). After 6–7 days in culture, infected PBMNs were harvested, and supernatants were divided into aliquots and stored at −70°C.

Elimination of CD25⁺ PBMNs Prior to Infection with HIV. PBMNs were obtained from a HIV-1 seronegative donor by centrifugation over Ficoll/Hypaque. Cells were divided into two culture flasks. One contained complete medium [RPMI-1640/15% fetal calf serum/10% IL-2 (Applied Biotechnologies, Columbia, Maryland)/glutamine/antibiotics]. IL-2 was used to support the viability of the T cells without inducing their entry into the cell cycle. The other flask contained complete medium with 10 nM RFT5-dgA to eliminate all CD25⁺ cells. Cells were then incubated for 3 days. On the fourth day, the cells were washed and then exposed to viral supernatants for 4–5 h, washed again, and cultured in 24-well plates at 10^6 cells per ml. In another group of experiments, PBMNs were incubated in complete medium containing 10 nM RFT5-dgA for 3 days, infected with HIV, and then cultured in 24-well plates previously coated with an anti-CD3 monoclonal antibody (64.1) (34). Cells were incubated at 10^6 cells per ml in complete medium or in complete medium with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: HIV, human immunodeficiency virus; CsA, cyclosporine; PHA, phytohemagglutinin; PBMN, peripheral blood mononuclear cell; IL-2, interleukin 2; dgA, deglycosylated ricin A chain.

CsA. Viral production was determined by measuring p24 concentrations in cell supernatants 6 days after infection.

Treatment of HIV-Infected PBMNs. A coculture system was used to test the effect of treatment with RFT5-dgA and, subsequently, with CsA on production of HIV. On day 1, PBMNs were exposed to viral supernatants for 4–5 h, washed, and incubated overnight. On day 2, HIV-infected PBMNs were incubated in 24-well plates at 10^6 cells per ml with complete medium or complete medium containing 10 nM RFT5-dgA. Cells were passaged on day 5, and the media were replaced with either complete medium or complete medium containing 10 nM RFT5-dgA. On day 8 (after 6 days of treatment), the immunotoxin (or medium in untreated controls) was removed, and cells were resuspended in complete medium. Three days later (day 11) 10^6 PHA-activated PBMNs (obtained from the same donor) were added to each well of cells treated with RFT5-dgA or complete medium. In addition, half the wells in both groups also received CsA at 1 μg/ml. Media were replaced every 3 days, and cell-free supernatants were assayed for p24 concentrations. To assess the effect of RFT5-dgA and CsA on the initially infected PBMNs, we included cultures of HIV-infected PBMNs treated with either RFT5-dgA or complete medium that were not cocultured with PHA blasts; on day 11, half of the wells in each treatment group also received CsA at 1 μg/ml.

p24 Assay. Concentrations of p24 antigen in the cell supernatants were measured by using a commercially available immunoassay (Coulter) (35). The limit of detection of this assay is 25 pg/ml.

RESULTS

The Effect of Culturing PBMNs with Anti-CD25–dgA Prior to Infection. To test the effect of eliminating $CD25^+$ cells prior to infection with HIV, freshly-obtained PBMNs were incubated with 10 nM RFT5-dgA in complete medium to eliminate the small (3–5%) population of $CD25^+$ cells normally present in fresh PBMNs. Treatment with RFT5-dgA prior to infection with HIV resulted in a >99% decrease in the levels of p24 secreted 6 days after infection (Fig. 1*A*). When the $CD25^-$ cells were infected with HIV and subsequently stimulated with a solid-phase anti-CD3 (64.1) monoclonal antibody, p24 production (determined 6 days after infection) was comparable to that of control cells from which the $CD25^+$ cells had not been eliminated (Fig. 1*B*). This result indicates that cell activation is not a prerequisite for infection. It further demonstrates that $CD25^-$ cells do not produce virus after infection unless they are activated. These results confirm and extend those of Zack *et al.* (23, 36), Bukrinsky *et al.* (22), and Stevenson *et al.* (24), who showed that HIV can efficiently enter fresh, unstimulated PBMNs. However, their studies did not address the issue of the role of the small (3–5%) population of activated cells normally present in fresh PBMNs.

The Effect of CsA on HIV-Infected $CD25^-$ PBMNs. We next asked whether CsA could prevent viral production by HIV-infected, $CD25^-$ cells when they were stimulated. To this end, a population of $CD25^-$, HIV-infected cells was generated as described. PBMNs were treated with RFT5-dgA to eliminate the $CD25^+$ cells, and the remaining cells were then exposed to HIV. These cells were then cultured in the presence of the potent T-cell activator, solid-phase anti-CD3, either with or without CsA. Fig. 1*B* shows that CsA prevented these cells from producing virus, <25 pg/ml in CsA treated cells vs. 12 ng/ml in cells that did not receive CsA.

In these studies, a high dose of CsA (4 μg/ml) was used, because anti-CD3 is a highly potent stimulus. To determine the minimum effective concentration of CsA necessary to suppress virus production, experiments were performed in which various concentrations of CsA were used (Fig. 2); 0.5 μg of CsA per ml reduced p24 production by >95%, and 1 μg of CsA per ml was sufficient to completely suppress detectable viral production. Therefore, 1 μg/ml was used in all subsequent experiments.

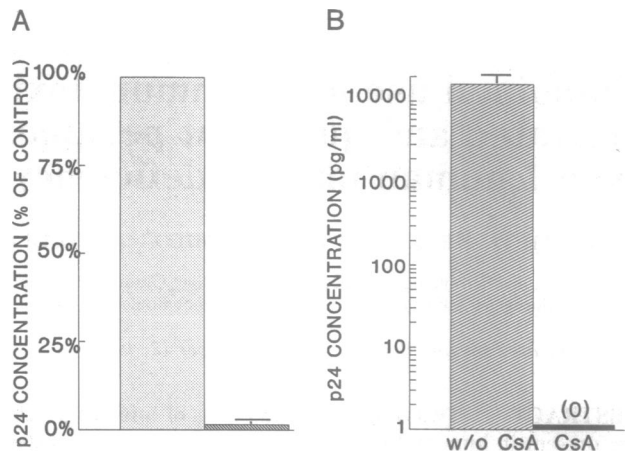

FIG. 1. Effect of eliminating the $CD25^+$ PBMNs prior to infection with HIV. (*A*) PBMNs were divided into separate culture flasks, one containing complete medium (stippled bar) and the other containing complete medium with 10 nM RFT5-dgA (hatched bar). Cells were incubated for 3 days and then washed before exposure to viral supernatants. Cells were again washed and then plated at 10^6 cells per ml. Medium was replaced every 3 days, and supernatants were analyzed for p24. The values represent the mean ± SE of two independent experiments, each run in duplicate, and are expressed as a percentage of the p24 levels of untreated cultures. The mean p24 level in the untreated control was 270 ng/ml. (*B*) Effect of CsA on $CD25^-$ HIV-infected PBMNs when stimulated with anti-CD3. PBMNs were incubated in complete medium containing 10 nM RFT5-dgA for 3 days. Cells were then washed, infected, washed again, and cultured in wells previously coated with an anti-CD3 monoclonal antibody (64.1). Cells were incubated at 10^6 cells per ml in complete medium (hatched bar) or complete medium with 4 μg of CsA per ml (black bar). p24 levels were determined 6 days after infection. The values represent the mean ± SE of two independent experiments, each run in duplicate.

The Effect of CsA on HIV-Infected $CD25^+$ PBMNs. Fresh PBMNs were infected with HIV and then treated with RFT5-dgA for 6 days. Viral production by these cells was suppressed >99% as compared to the peak production in untreated cells (Table 1). An identical group of cells, not treated with RFT5-dgA, was cultured with CsA continuously from day 11. These cells produced p24 in quantities almost identical to those produced by cells that did not receive CsA (Table 1), demonstrating that at 1 μg/ml CsA alone lacked both anti-viral activity and a cytotoxic effect. In contrast, cultures that received RFT5-dgA followed by CsA produced little or no p24. These results demonstrate that CsA inhibits $CD25^-$ cells from producing virus but has no effect on $CD25^+$ cells. Further, viral production in this experimental system can be attributed to the $CD25^+$ cells.

The Effect of RFT5-dgA and CsA on Transmission of HIV from Infected to Uninfected Cells. HIV production can also be induced in $CD25^-$, quiescent HIV-infected cells by coculturing these cells with activated PBMNs from the same donor. This assay is highly sensitive for the detection of latently infected cells since it activates these cells and amplifies virus production because of infection of the added, activated PBMNs. Hence, such an experiment is a more stringent test of the effectiveness of using the two above pharmaceuticals to suppress viral production.

Fresh PBMNs were infected and then treated with RFT5-dgA for 6 days (days 2–8) (Fig. 3). On the eighth day, RFT5-dgA was removed, and the cells were resuspended in complete medium. Three days later, PHA blasts and CsA were added to the HIV-infected cultures. The cultures were

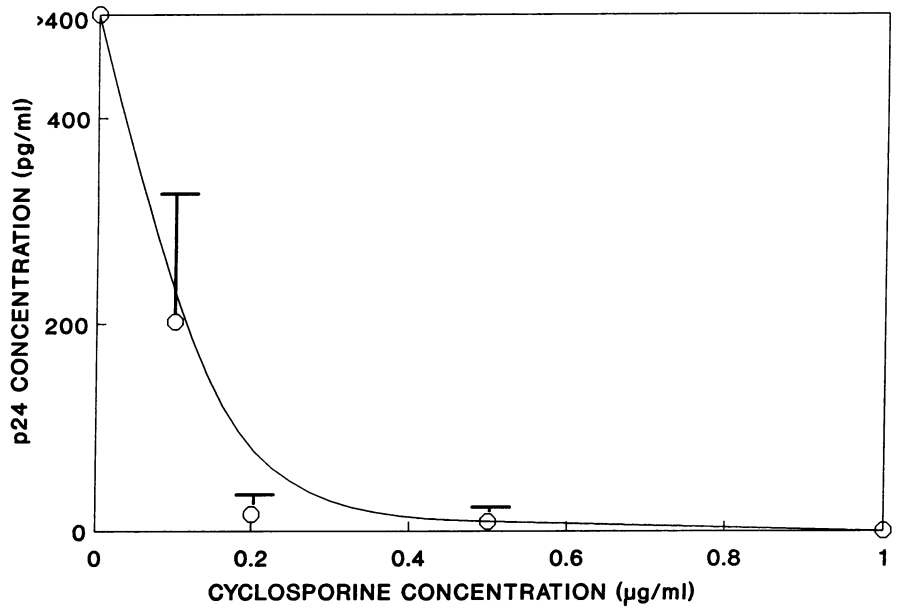

FIG. 2. Dose–response curve for inhibition of p24 production by CsA. PBMNs were cultivated in complete medium containing RFT5-dgA for 3 days and then washed. Cells were then exposed to viral supernatants, washed, and cultured at 10^6/ml in anti-CD3-coated 24-well plates. Cells were incubated in medium containing various concentrations of CsA. p24 was determined in samples collected 6 days after infection. The values represent the mean ± SE of two independent experiments, each run in triplicate.

then followed for 6 more days and then analyzed for the production of p24. At this time (days 14–17), cultures that did not receive additional cells were producing 3% of the p24 they were producing on day 11 (Table 1). This is due to the decline in cell number and viability generally observed in these HIV-infected, PBMN cultures (Table 1). Hence, secretion of p24 on days 14–17 can be attributed to PHA blasts that became infected during coculture. The results (Fig. 4) indicate the following: (*i*) HIV production can be completely suppressed by RFT5-dgA until day 11. Coculture at this time results in slow recovery of virus production, which eventually reaches control levels. This probably reflects the production capacity of the PHA blasts rather than the size of the viral reservoir in the original infected cells. The results demonstrate that the viral reservoir in the latently HIV-infected cells can be detected by coculture. (*ii*) CsA has little effect on p24 production in cocultures of HIV-infected cells that have not been treated with RFT5-dgA, supporting the concept that the $CD25^+$, productively infected cells are capable of spreading the infection. (*iii*) CsA does not affect the ability of infected, $CD25^+$ cells to transmit the virus, the ability of the PHA blasts to become infected, or the ability of PHA blasts to secrete p24. (*iv*) The combination of RFT5-dgA followed by CsA completely suppresses the spread of infection to PHA blasts and subsequent viral production. We speculate that CsA is suppressing the activation of the $CD25^-$ HIV-infected cells by cytokines secreted by the PHA-stimulated blasts.

Table 1. Effect of RFT5-dgA and CsA on viral production by HIV-infected PBMNs

Treatment	p24, pg/ml			
	Day 8	Day 11	Day 14	Day 17
None	12,996	85,278	7,574	2,848
RFT5-dgA	77	171	27	<25
CsA	9,806	53,977	2,795	660
RFT5-dgA + CsA	<25	<25	<25	<25

PBMNs were infected on day 1 and then treated with RFT5-dgA or medium for 6 days (days 2–8). On the eighth day, the immunotoxin (or medium in untreated controls) was removed, and cells were resuspended in complete medium. Three days later (day 11), half the wells in each treatment group received 1 μg of CsA per ml. After day 11, the number of viable PBMNs in culture began to decrease. Data represent p24 concentrations (in pg/ml) from a representative experiment.

DISCUSSION

The rationale of these studies was to eliminate HIV-infected T cells that produce virus by means of an immunotoxin specific to an activation marker on these cells and, subsequently, to prevent stimulation of HIV-infected, quiescent T cells with the immunosuppressive agent CsA. Although neither pharmaceutical by itself was effective in eliminating virus production in an acute *in vitro* infection after the quiescent T cells were activated, the use of these two pharmaceuticals in sequence resulted in virtually complete elimination of detectable viral production (i.e., p24 values were reduced by >99%). In our model, quiescent cells were activated by solid-phase anti-CD3, which stimulates virtually all quiescent T cells (34). This is likely to be a more vigorous activation signal than a physiological one and was employed to test the effectiveness of CsA in preventing activation of T cells and resultant HIV production. In addition, the ability of the above regimen to prevent spread of infection to cocultured PHA-stimulated, uninfected T-cell blasts was demonstrated.

The above strategy was based on prior results obtained in other experimental systems and clinical observations in HIV^+ individuals. Thus, IL-2–diphtheria toxin conjugates (20) and anti-CD25–dgA (28) are highly effective in decreasing viral production *in vitro* in HIV-infected T cells. In addition, anti-gp120, anti-gp41, and CD4-based immunotoxins (17–19, 21, 37) are effective in eliminating cells that are actively producing HIV *in vitro*. However, none of these

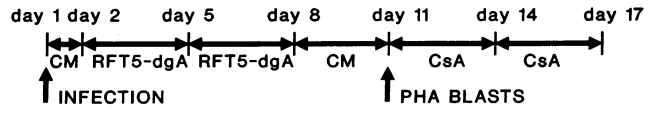

FIG. 3. Protocol of the coculture experiment. CM, complete medium.

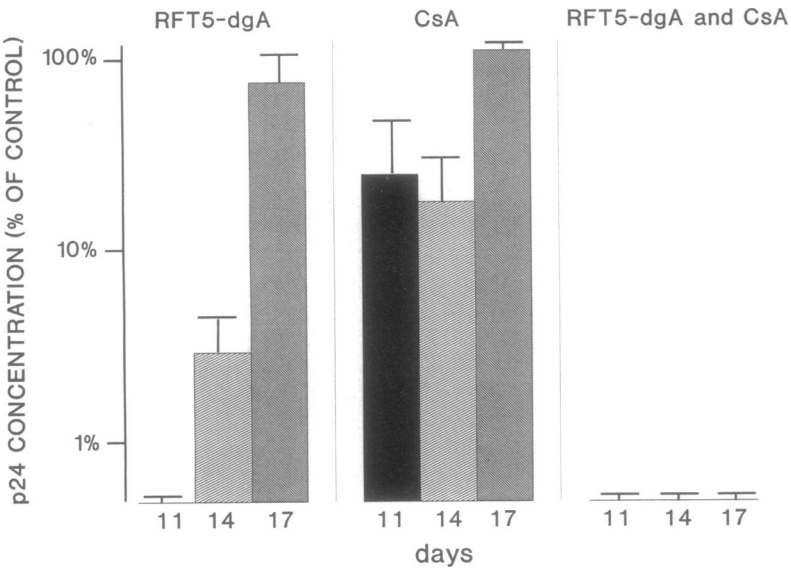

FIG. 4. The combination of RFT5-dgA and CsA can markedly inhibit p24 production and viral transmission to uninfected cells. Fresh PBMNs were infected on day 1 and then treated with RFT5-dgA for 6 days (days 2–8) as illustrated in Fig. 3. On the eighth day, RFT5-dgA was removed, and the cells were resuspended in complete medium. Three days later, PHA blasts and CsA were added to the HIV-infected cultures. The cultures were then followed for 6 more days and then analyzed for the production of p24. Data are shown as percentages of p24 concentrations, for each day, in control cultures, which did not receive RFT5-dgA or CsA. The values represent the mean ± SE of two independent experiments, each run in duplicate. The mean p24 level in untreated controls was 60 ng/ml on day 11, 104 ng/ml on day 14, and 94 ng/ml on day 17.

studies has addressed the issue of quiescent T cells that harbor the HIV genome and, upon subsequent activation, produce virus. To this end, CsA was used in our studies to prevent these cells from producing virus after a potent activating signal.

Previous studies have shown that CsA can inhibit viral production *in vitro* by infected T-cell lines (38, 39). In the present studies, CsA alone had little effect on viral production by HIV-infected PBMNs (Fig. 4). CsA has also been reported to inhibit the formation of the NκB complex, which is critical to both the production of IL-2 and the generation of HIV (40). CsA has also been used in AIDS patients, but little or no efficacy has been reported (41, 42). However, the treated patients were in late stages of their disease where viral burdens are large, and prevention of activation of quiescent cells would be predicted to have little effect on the course of disease. In this regard, when CsA was used in patients with less advanced HIV infection, CsA induced a temporary increase in $CD4^+$ cells counts and appeared to slow the progression of disease in a proportion of patients (42). Additionally, the course of HIV infection was no more severe or acute in transplant patients receiving CsA than in other HIV^+ individuals (43). Thus, CsA may prevent HIV production by initially quiescent infected cells in patients, and it has not yet shown adverse effects on the progression of disease in patients. It should be noted that in transplant patients, blood concentrations of 800 ng/ml to 1 μg/ml of CsA were used until quite recently (43).

There are major concerns in considering the use of a CD25-reactive immunotoxin and CsA in HIV-infected individuals. Thus, $CD8^+$ cells, which also express CD25 and probably must be activated to become cytotoxic, may be critical in suppressing HIV production. Their elimination by immunotoxin and suppression of their activation by CsA could be detrimental. HIV^+ individuals are generally immunosuppressed even during early stages of their disease, and it is not known whether further suppression with CsA might predispose them prematurely to progressive disease, opportunistic infections, and/or neoplasms. Finally, our studies do not address the problem of other HIV-infected cells, such as macrophages, dendritic cells, and neural cells. More information concerning these and other issues will have to be obtained if the above strategy is to be considered for clinical use.

There is an interesting parallel between the present studies and our previously employed strategy to prevent development of a murine T-cell lymphoma (44). Thus, radiation leukemia virus-induced premalignant disease is also characterized by cells actively producing virus and a premalignant (latent) population that is subsequently transformed. Replication of the latter is required for the development of a malignant phenotype and requires an IL-4-dependent, CsA-sensitive autocrine growth loop (44, 45). Immunotoxin directed against viral p70 alone or CsA alone (which inhibits IL-4 production) delayed the development of lymphoma but did not prevent the development of lymphoma. However, development of lymphoma was prevented in a large proportion of mice by a combination of the immunotoxin and CsA (44).

We thank Dr. Victor Ghetie for preparing the RFT5-dgA, Ms. Robin Reiber and Ms. Sue Chadwick for excellent secretarial assistance, and Dr. Jonathan Uhr for many helpful comments and discussions. The work was supported by National Institutes of Health Grants AI-28149 and AI-27336.

1. Skowron, G. (1992) *Hosp. Pract.* **27**, Suppl. 2, 5–13.
2. Scharpe, S., DeMeester, I., Hendriks, D., Vanhoof, G., Van-Sande, M. & Vriend, G. (1992) *Biochimie* **73**, 121–126.
3. Fischl, M. A., Richman, D. D. & Grieco, M. H. (1987) *N. Engl. J. Med.* **317**, 185–191.
4. Birx, D. & Redfield, R. R. (1991) *Int. J. Immunopharmacol.* **13**, Suppl. 1, 129–132.
5. Hersh, E. M. (1991) *Int. J. Immunopharmacol.* **13**, Suppl. 1, 9–18.
6. Taylor, R. (1992) *J. Natl. Inst. Health Res.* **4**, 89–93.
7. Redfield, R. R., Birx, D. L., Ketter, N., Tramont, E., Polonis, V., Davis, C., Brundage, J. F., Smith, G., Johnson, S., Fowler, A., Wierzba, T., Shafferman, A., Volvovitz, F., Oster, C. & Burke, D. S., Military Medical Consortium for Applied Retroviral Research (1991) *N. Engl. J. Med.* **324**, 1677–1684.
8. Smith, D. H., Byrn, R. A., Marsters, S. A., Gregory, T., Groopman, J. E. & Capon, D. J. (1987) *Science* **238**, 1704–1707.
9. Traunecker, A., Luke, W. & Karjalainen, K. (1988) *Nature (London)* **331**, 84–86.
10. Deen, K. C., McDougal, J. S., Inacker, R., Folena-Wasserman, G., Arthos, J., Rosenberg, J., Maddon, P. J., Axel, R. & Sweet, R. W. (1988) *Nature (London)* **331**, 82–84.
11. Hussey, R. E., Richardson, N. E., Kowalski, M., Brown, N. R., Chang, H.-C., Siliciano, R. F., Dorfman, T., Walker, B., Sodroski, J. & Reinherz, E. L. (1988) *Nature (London)* **331**, 78–81.
12. Fisher, R. A., Bertonis, J. M., Meier, W., Johnson, V. A., Costopoulos, D. S., Liu, T., Tizard, R., Walker, B. D., Hirsch, M. S., Schooley, R. T. & Flavell, R. A. (1988) *Nature (London)* **331**, 76–78.
13. Jackson, G. G., Rubenis, M., Knigge, M., Perkins, J. T., Paul, D. A., Despotes, J. C. & Spencer, P. (1988) *Lancet* **9**, 647–652.

14. Prince, A. M., Horowitz, B., Baker, L., Shulman, R. W., Ralph, H., Valinsky, J., Cundell, A., Brotman, B., Boehle, W., Rey, F., Piet, M., Reesnik, H., Lelie, N., Tersmette, M., Miedema, F., Barbosa, L., Nemo, G., Nastala, C. L., Allan, J. S., Lee, D. R. & Eichberg, J. W. (1988) *Proc. Natl. Acad. Sci. USA* **85,** 6944–6948.
15. Marasco, W. A., Bagley, J., Zani, C., Posner, M., Cavacini, L., Haseltine, W. A. & Sodroski, J. (1992) *J. Clin. Invest.* **90,** 1467–1478.
16. Chaudhary, V. K., Mizukami, T., Fuerst, T. R., FitzGerald, D. J., Moss, B., Pastan, I. & Berger, E. A. (1988) *Nature (London)* **335,** 369–372.
17. Ghetie, V., Slaughter, C., Wheeler, H. T., Uhr, J. W. & Vitetta, E. S. (1991) *Proc. Natl. Acad. Sci. USA* **88,** 5690–5693.
18. Till, M. A., Ghetie, V., Gregory, T., Patzer, E. J., Porter, J. P., Uhr, J. W., Capon, D. J. & Vitetta, E. S. (1988) *Science* **242,** 1166–1168.
19. Pincus, S. H., Wehrly, K. & Chesebro, B. (1989) *J. Immunol.* **142,** 3070–3075.
20. Finberg, R. W., Wahl, S. M., Allen, J. B., Soman, G., Strom, T. B., Murphy, J. R. & Nichols, J. C. (1991) *Science* **252,** 1703–1705.
21. Zarling, J. M., Moran, P. A., Haffar, O., Sias, J., Richman, D. D., Spina, C. A., Myers, D. E., Kueblbeck, V., Ledbetter, J. A. & Uckun, F. M. (1990) *Nature (London)* **347,** 92–95.
22. Bukrinsky, M. I., Stanwick, T. L., Dempsey, M. P. & Stevenson, M. (1991) *Science* **254,** 423–427.
23. Zack, J. A., Arrigo, S. J., Weitsman, S. R., Go, A. S., Haislip, A. & Chen, I. S. Y. (1990) *Cell* **61,** 213–222.
24. Stevenson, M., Stanwick, T. L., Dempsey, M. P. & Lamonica, C. A. (1990) *EMBO J.* **9,** 1551–1560.
25. Ho, D. D. (1992) *Lancet* **339,** 1549.
26. Waldmann, T. A., Pastan, I. H., Gansow, O. A. & Junghans, R. P. (1992) *Ann. Int. Med.* **116,** 148–160.
27. Schwarting, A. & Stein, H. (1991) in *Leukocyte Typing IV*, ed. Knapp, W. (Oxford Univ. Press, Oxford), p. 339.
28. Ramilo, O., Bell, K. D., Uhr, J. W. & Vitetta, E. S. (1992) *Pediatr. Res.* **31,** 176a (abstr.).
29. Schreiber, S. L. & Crabtree, G. R. (1992) *Immunol. Today* **13** (4), 136–141.
30. Engert, A., Martin, G., Amlot, P., Wijdenes, J., Diehl, V. & Thorpe, P. (1991) *Int. J. Cancer* **49,** 450–456.
31. Thorpe, P. E., Wallace, P. M., Knowles, P. P., Relf, M. G., Brown, A. N. F., Watson, G. J., Knyba, R. E., Wawrzynczak, E. J. & Blakey, D. C. (1987) *Cancer Res.* **47,** 5924–5931.
32. Ghetie, V., Thorpe, P. E., Ghetie, M.-A., Knowles, P., Uhr, J. W. & Vitetta, E. S. (1991) *J. Immunol. Methods* **142,** 223–230.
33. Benn, S., Rutledge, R., Folks, T., Gold, J., Baker, L., McCormick, J., Feorino, P., Piot, P., Quinn, T. & Martin, M. (1985) *Science* **230,** 949–951.
34. Geppert, T. D. & Lipsky, P. E. (1988) *J. Clin. Invest.* **81,** 1497–1505.
35. Allain, J. P., Laurian, Y. & Paul, D. A. (1987) *N. Engl. J. Med.* **317,** 1114–1121.
36. Zack, J. A., Haislip, A. M., Krogstad, P. & Chen, I. Y. S. (1992) *J. Virol.* **66,** 1717–1725.
37. Till, M. A., Zolla-Pazner, S., Gorny, M. K., Patton, J. S., Uhr, J. W. & Vitetta, E. S. (1989) *Proc. Natl. Acad. Sci. USA* **86,** 1987–1991.
38. Karpas, A., Lowdell, M., Jacobson, S. K. & Hill, F. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 8351–8355.
39. Wainberg, M. A., Dascal, A., Blain, N., Fitz-Gibbon, L., Boulerice, F., Numazaki, K. & Tremblay, M. (1988) *Blood* **72,** 1904–1910.
40. Schmidt, A., Hennighausen, L. & Siebenlist, U. (1990) *J. Virol.* **64,** 4037–4041.
41. Phillips, A., Wainberg, M. A., Coates, R., Klein, M., Rachlis, A., Read, S., Sheperd, F., Vellend, H., Walmsley, S., Halloran, P. & Fanning, M. (1989) *Can. Med. Assoc. J.* **140,** 1456–1460.
42. Andrieu, J.-M., Even, P., Venet, A., Tourani, J.-M., Stern, M., Lowenstein, W., Audroin, C., Eme, D., Masson, D., Sors, H., Israel-Biet, D. & Beldjord, K. (1988) *Clin. Immunol. Immunopathol.* **47,** 181–198.
43. Dummer, J. S., Erb, S., Breinig, M. K., Ho, M., Rinaldo, C. R., Jr., Gupta, P., Ragni, M. V., Tzakis, A., Makowa, L., VanThiel, D. & Starzl, T. E. (1992) *Transplantation* **47,** 134–139.
44. Yefenof, E., Abboud, G., Epszteyn, S. & Vitetta, E. S. (1992) *Proc. Natl. Acad. Sci. USA* **89,** 728–732.
45. Yefenof, E., Epszteyn, S. & Kotler, M. (1991) *Cancer Res.* **51,** 2179–2184.