Proc. Natl. Acad. Sci. USA
Vol. 89, pp. 5582–5585, June 1992
Medical Sciences

Viricidal effect of stimulated human mononuclear phagocytes on human immunodeficiency virus type 1

(monocyte/macrophage/myeloperoxidase/hydrogen peroxide)

MARLEEN J. CHASE AND SEYMOUR J. KLEBANOFF*

Department of Medicine, SJ-10, University of Washington, Seattle, WA 98195

Contributed by Seymour J. Klebanoff, March 23, 1992

ABSTRACT **Human monocytes stimulated with phorbol 12-myristate 13-acetate or opsonized zymosan *in vitro* were viricidal to human immunodeficiency virus type 1 (HIV-1) as measured by the inability of the virus to replicate in CEM cells. Monocytes, when stimulated, release myeloperoxidase (MPO) and produce H_2O_2; MPO reacts with H_2O_2 and chloride to form hypochlorous acid, a known microbicidal agent. The viricidal activity of stimulated monocytes was inhibited by the peroxidase inhibitor azide, implicating MPO, and by catalase but not heated catalase or superoxide dismutase, implicating H_2O_2. Stimulated monocytes from patients with chronic granulomatous disease (CGD) or hereditary MPO deficiency were not viricidal to HIV-1 unless they were supplemented with the H_2O_2-generating enzyme glucose oxidase or MPO, respectively. The viricidal activity of stimulated, glucose oxidase-supplemented CGD monocytes and MPO-supplemented MPO-deficient monocytes, like that of normal stimulated monocytes, was inhibited by azide and catalase. Monocytes maintained in culture differentiate into macrophages with loss of MPO and decreased H_2O_2 production. The viricidal activity of 3- to 9-day monocyte-derived macrophages was decreased unless MPO was added, whereas the loss of viricidal activity by 12-day-old monocyte-derived macrophages was not reversed by MPO unless the cells were pretreated with γ-interferon. These findings suggest that stimulated monocytes can be viricidal to HIV-1 through the release of the MPO/H_2O_2/chloride system and that the decreased viricidal activity on differentiation to macrophages results initially from the loss of MPO and, with more prolonged culture, also from a decreased respiratory burst that can be overcome by γ-interferon.**

Mononuclear phagocytes are major targets for the establishment, dissemination, and persistence of human immunodeficiency virus type 1 (HIV-1) in the infected human host (1). Since mononuclear phagocytes permit HIV-1 replication, are capable of wide-ranging migration, and are relatively resistant to the cytopathic consequences of viral infection, they play a major role in the pathogenesis of HIV-1-induced disease. Although there has been considerable research on the cellular uptake and replication of HIV-1 in mononuclear phagocytes and on the functional consequences to mononuclear phagocytes of HIV-1 infection (1), little attention has been paid to the possible toxic effect of mononuclear phagocytes on HIV-1.

Blood monocytes respond to stimulation with a respiratory burst that converts oxygen to the superoxide anion, hydrogen peroxide, and, in the presence of added iron, hydroxyl radicals (2). Degranulation occurs with the release of a number of granule constituents with microbicidal potential. Among these is myeloperoxidase (MPO), which reacts with H_2O_2 and a halide (usually chloride) to form hypochlorous acid and chloramines. These and other products of the respiratory burst combine with granule components effective in the absence of oxygen to be toxic to ingested organisms or extracellular targets.

Monocytes cultured *in vitro* differentiate into cells expressing many of the biochemical and morphologic characteristics of mature tissue macrophages. This differentiation is associated with a reduction in antimicrobial activity due in part to a decrease in oxygen-dependent mechanisms of toxicity. Thus, the granule MPO is lost, with a 41% decrease on day 1 and a 95% decrease on day 6 in culture in one study (3). The respiratory burst also falls sharply, often following an initial increase at 3 days, to reach very low levels after 6 days in culture (3). Activation of monocyte-derived macrophages by certain cytokines, in particular γ-interferon (IFN-γ) (4, 5), results in heightened microbicidal activity associated with an increase in the respiratory burst.

The MPO/H_2O_2/chloride system either in cell-free form (6) or following release by stimulated neutrophils (7) is viricidal to HIV-1. We report here that stimulated human monocytes can be viricidal to HIV-1 through the action of the MPO system. This effect is decreased when monocytes differentiate into macrophages in culture. The addition of MPO restores viricidal activity following short-term culture; with longer-term culture, MPO is effective only when the monocyte-derived macrophages are pretreated with IFN-γ.†

MATERIALS AND METHODS

Special Reagents. MPO was isolated from human neutrophils as described (8) and was assayed by guaiacol oxidation (9). Phorbol 12-myristate 13-acetate (PMA) and glucose oxidase (*Aspergillus niger*, type V-S) were obtained from Sigma and superoxide dismutase (bovine erythrocytes, 5000 units/mg) was obtained from Boehringer Mannheim. Catalase (bovine liver, 50,514 units/mg), obtained from Worthington, was heated at 100°C for 20 min where indicated. Zymosan was obtained from ICN and was opsonized as described (10).

Isolation and Culture of Mononuclear Phagocytes. Peripheral blood was obtained after informed consent from healthy volunteers, a patient with chronic granulomatous disease (CGD), and a patient with hereditary MPO deficiency. All normal volunteers (including those providing AB serum) and patients tested negative for HIV-1 antibody. Whole blood was anticoagulated with 0.2% K_2EDTA. Twenty-milliliter batches were diluted with 15 ml of sterile phosphate-buffered

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: CGD, chronic granulomatous disease; HIV-1, human immunodeficiency virus type 1; IFN-γ, γ-interferon; MPO, myeloperoxidase; PMA, phorbol 12-myristate 13-acetate; NS, not significant.

*To whom reprint requests should be addressed.

†This work was presented in part at the Seventh International Conference on AIDS, June 16–21, 1991, Florence, Italy (Vol. 2, p. 41, abstr. WA 74), and at the regional meeting of the American Federation for Clinical Research, February 5–8, 1992, Carmel, CA (27).

saline without calcium and magnesium (PD) supplemented with 0.3 mM Na_2EDTA (PD/EDTA), underlaid with 13 ml of lymphocyte separation medium (Organon Teknika–Cappel), and centrifuged at $1000 \times g$ for 20 min. Peripheral blood mononuclear cells were removed and washed twice in PD/EDTA at $350 \times g$ for 10 min and resuspended in RPMI 1640 with 25 mM Hepes buffer (Whittaker Bioproducts). An aliquot of the mononuclear cell suspension containing 1×10^6 monocytes was added to each well of six-well tissue culture plates (Primaria, Falcon 3846 Becton Dickinson) containing 2 ml of RPMI 1640 with 25 mM Hepes buffer supplemented with 20% AB serum, 2 mM glutamine, penicillin (50 units/ml), and streptomycin sulfate (50 μg/ml) (monocyte medium). The tissue culture plates were incubated for 2 hr at 37°C in a humidified atmosphere of 5% CO_2/95% air. The nonadherent cells were removed by five washes with warm phosphate-buffered saline containing calcium and magnesium (PBS). More than 98% of the adherent cells had typical monocyte morphology when examined by light microscopy using Wright's stain and were peroxidase-positive. No neutrophils were detected.

Monocyte-derived macrophages were prepared by adding 2 ml of monocyte medium to each well containing adherent cells and returning the tissue culture plates to the incubator for the periods indicated. The cultures received fresh medium every 3 days. Where indicated, IFN-γ (kindly provided by Genentech) was added (100 units/ml) to the cultures daily for 3 days. Less than 5% of the monocyte-derived macrophages detached when cultured for periods of up to 2 weeks.

Measurement of HIV Viability. The mononuclear phagocytes were overlaid with 1.0 ml of a standard salt solution (5 mM sodium phosphate buffer, pH 7.4/0.128 M NaCl/12 mM KCl/1 mM $CaCl_2$/2 mM $MgCl_2$/2 mM glucose) containing the LAV strain of HIV-1 [500 median tissue culture infective doses ($TCID_{50}$)/ml; prepared as described (6)]. The following supplements were added where indicated: 100 ng of PMA, 1 mg of opsonized zymosan, 0.1 mM sodium azide, 9.8 μg of catalase or heat-inactivated catalase, 10 μg of superoxide dismutase, 0.226 unit of human MPO, and 0.190 unit of glucose oxidase. The tissue culture plates were incubated at 37°C in 5% CO_2/95% air for 2 hr, following which >95% of the cells remained viable as shown by trypan blue exclusion. Twenty microliters of the supernatant fluid was added to 2×10^5 CEM cells (11) (CCL 119, American Type Culture Collection) suspended in 1 ml of RPMI 1640 with 25 mM Hepes buffer, 20% fetal bovine serum, 2 mM glutamine, penicillin (50 units/ml), and streptomycin sulfate (50 μg/ml) in 48-well plates (Costar 3548), and the plates were incubated at 37°C in 5% CO_2/95% air. On the sixth day of incubation, 200 μl of the supernatant fluid was removed for measurement of HIV p24 antigen accumulation by an enzyme immunoassay (Abbott). If the absorbance was >2.0, the supernatant fluid was diluted and remeasured. The data are reported as median HIV-1 p24 antigen (pg/ml) and as the number of samples with HIV-1 growth/total number of samples. Growth of HIV-1 was defined arbitrarily as the production of >50 pg of p24 antigen per ml following a 6-day incubation with CEM cells. The background level of HIV-1 p24 antigen was determined to be 9 pg/ml by incubating HIV-1 in the standard salt solution for 2 hr in wells without monocytes and transferring 20 μl of the reaction mixture to 1 ml of CEM medium without CEM cells, which was then incubated for 6 days. Although the number of CEM cells, the volume of the reaction mixture added to the CEM cells, and the period of replication in the CEM cells were kept constant in all experiments, some variation in maximum replication was observed, which appeared to be related to the number of passages of the CEM cells.

Statistical Analyses. The Mann–Whitney U rank-sum test was used to analyze differences for significance [$P > 0.05$, not significant (NS)].

RESULTS

Normal Monocytes. When HIV-1 was incubated with freshly isolated monocytes for 2 hr and an aliquot of the supernatant fluid was added to CEM cells for 6 days, viral replication occurred (p24, 115,350 pg/ml) that was not significantly different from that observed when HIV-1 was incubated in the standard salt solution alone (148,700 pg/ml) (Table 1). Viral replication was also observed in all 33 samples in which HIV-1 was incubated with PMA alone, although the total amount of p24 antigen generated (26,080 pg/ml) was reduced ($P < 0.05$ versus HIV-1 in standard salt solution alone). When HIV-1 was exposed to monocytes stimulated with PMA, intact virus could not be detected in the supernatant fluid in 46 of 48 samples as measured by the lack of replication in CEM cells ($P < 0.001$ versus PMA alone, monocytes alone, or no supplements). The viricidal effect of PMA-stimulated monocytes was lost when chloride was replaced by sulfate and was inhibited by the peroxidase inhibitor azide, implicating MPO, and by catalase but not heated catalase or superoxide dismutase, implicating H_2O_2. Similar results were obtained when monocytes were incubated with a particulate stimulus, opsonized zymosan.

CGD Monocytes. Monocytes of patients with CGD do not respond to stimulation with a respiratory burst, due to the absence or malfunction of one of the components of the phagocyte NADPH oxidase system. CGD monocytes alone did not lower HIV-1 levels (171,685 versus 133,200 pg/ml, NS), nor was the effect of PMA-stimulated CGD monocytes different from that of PMA alone (41,575 versus 16,370 pg/ml, NS) (Table 2) under conditions in which PMA-stimulated normal monocytes were highly toxic (Table 1). Glucose oxidase, in the presence of its substrate glucose, generates H_2O_2. The addition of glucose oxidase to the PMA-stimulated CGD monocytes in the standard salt solution (which contains glucose) resulted in a return of viricidal activity, which was inhibited by azide and catalase but not by heated catalase or superoxide dismutase. At the concentrations employed, glucose and glucose oxidase alone were not toxic to HIV-1 (47,950 versus 133,200 pg/ml, NS).

MPO-Deficient Monocytes. The monocytes of patients with hereditary MPO deficiency lack MPO in their cytoplasmic granules, whereas the respiratory burst, in contrast to CGD monocytes, is not depressed; indeed, it may be greater than normal (12). MPO-deficient monocytes stimulated with PMA or opsonized zymosan did not decrease HIV-1 levels below

Table 1. Viricidal effect of stimulated monocytes on HIV-1

Supplement(s)	HIV-1 p24 antigen		No. of samples with growth/total
	pg/ml	P	
None	148,700	<0.001*	32/33
Monocytes	115,350	<0.001*	30/30
PMA	26,080	<0.001*	33/33
Monocytes + PMA	11		2/48
Chloride deleted	23,700	<0.001*	9/9
Azide added	31,500	<0.001*	24/24
Catalase added	66,690	<0.001*	21/21
Heated catalase added	9	NS*	0/18
Superoxide dismutase added	9	NS*	0/21
Opsonized zymosan	616,250	<0.001†	6/6
Monocytes + opsonized zymosan	2		0/12
Azide added	437,700	<0.001†	12/12
Catalase added	306,350	<0.001†	12/12
Heated catalase added	4	NS†	0/12
Superoxide dismutase added	13	NS†	1/3

*Significance of the difference from Monocytes + PMA.
†Significance of the difference from Monocytes + opsonized zymosan.

Table 2. Viricidal effect of stimulated CGD monocytes on HIV-1

Supplement(s)	HIV-1 p24 antigen pg/ml	P*	No. of samples with growth/total
None	133,200	NS	5/6
CGD monocytes	171,685	<0.05	8/8
PMA	16,370	NS	6/6
CGD monocytes + PMA	41,575		8/8
CGD monocytes + PMA + GO	8	<0.001	0/8
Azide added	10,585	NS	7/8
Catalase added	14,985	NS	8/8
Heated catalase added	3	<0.001	0/8
Superoxide dismutase added	10	<0.005	0/5

GO, glucose oxidase.
*Significance of the difference from CGD monocytes + PMA.

that observed with MPO-deficient monocytes, PMA, or opsonized zymosan alone, with viral replication occurring in essentially all samples (Table 3). However, when MPO was added to MPO-deficient monocytes that had been stimulated with PMA or opsonized zymosan, complete loss of viral replication was observed. This viricidal activity was inhibited by azide and catalase but not by heated catalase or superoxide dismutase.

Monocyte-Derived Macrophages. Table 4 demonstrates the effect of the differentiation of monocytes to macrophages in culture on toxicity to HIV-1. In contrast to freshly isolated monocytes, monocytes maintained in culture for 3 days were not completely viricidal to HIV-1 when stimulated by PMA. Replication of virus was observed in all 21 samples, although the HIV-1 p24 antigen level was depressed (90,000 versus 3800 pg/ml, $P < 0.001$). The addition of glucose oxidase to PMA-stimulated 3-day-old monocyte-derived macrophages did not increase viricidal activity, whereas the addition of MPO did (3800 versus 13 pg/ml, $P < 0.001$). Similar results were observed with 6-day-old monocyte-derived macrophages. By day 9, the viricidal activity of MPO-supplemented, PMA-stimulated, monocyte-derived macrophages began to subside (74 pg/ml), and by day 12, the viricidal effect was lost (6680 pg/ml) unless the cells were exposed to IFN-γ for 3 days prior to study (without IFN-γ 6680 pg/ml; with IFN-γ 13 pg/ml; $P < 0.001$).

Table 3. Viricidal effect of stimulated MPO-deficient monocytes on HIV-1

Supplement(s)	HIV-1 p24 antigen pg/ml	P	No. of samples with growth/total
None	25,255	NS*	6/6
MPO-def monocytes	28,660	NS*	8/9
PMA	13,340	NS*	6/6
MPO-def monocytes + PMA	18,990		9/9
MPO-def monocytes + PMA + MPO	4	<0.001*	0/12
Azide added	2,780	NS*	10/10
Catalase added	18,537	NS*	9/9
Heated catalase added	7	<0.001*	0/12
Superoxide dismutase added	6	<0.001*	0/9
Ops zym	67,290	NS†	12/12
MPO-def monocytes + Ops zym	35,565		12/12
MPO-def monocytes + Ops zym + MPO	6	<0.001†	0/9
Azide added	57,260	NS†	9/9
Catalase added	28,390	NS†	9/9
Heated catalase added	8	<0.001†	1/9
Superoxide dismutase added	8	<0.001†	0/9

Ops zym, opsonized zymosan.
*Significance of the difference from MPO-deficient monocytes + PMA.
†Significance of the difference from MPO-deficient monocytes + Ops zym.

DISCUSSION

Human monocytes, stimulated *in vitro*, are viricidal to HIV-1 through the release of components of the MPO/H_2O_2/halide system. Thus, infectious extracellular HIV-1 was decreased to undetectable levels on exposure to monocytes stimulated with either the soluble stimulus PMA or the particulate stimulus opsonized zymosan. Both stimuli induce a respiratory burst in monocytes with the formation of reactive oxygen species, including H_2O_2, and cause degranulation with the release of granule constituents, including MPO. That the loss of extracellular infectious HIV-1 was due to the release of components of the MPO/H_2O_2/halide system was suggested by the inhibitory effect of azide, which is a potent inhibitor of peroxidase, and by the inhibitory effect of catalase but not heated catalase, implicating H_2O_2. Superoxide dismutase was without effect, suggesting that superoxide was not required for toxicity.

Additional support for the involvement of the MPO system in the viricidal effect of stimulated monocytes on HIV-1 came from studies with cells from patients with an inborn error in phagocyte function. When stimulated normal monocytes were replaced by stimulated CGD or MPO-deficient monocytes, toxicity to HIV-1 was absent unless the H_2O_2-generating enzyme glucose oxidase was added to the CGD monocytes and MPO was added to the MPO-deficient monocytes. Azide and catalase abolished the viricidal effect of the appropriately supplemented and stimulated patient monocytes, again implicating the MPO/H_2O_2/halide system in the viricidal effect.

When the monocytes were maintained in culture for 3–12 days, toxicity to HIV-1 by the PMA-stimulated monocyte-derived macrophages was decreased. Differentiation of monocytes to macrophages in culture is associated with a loss of granule peroxidase and the attenuation of the respiratory burst (3). That MPO is the limiting factor in viricidal activity during the early stages of differentiation is suggested by the return of toxicity on the addition of MPO, but not the H_2O_2-generating enzyme glucose oxidase, to the monocyte-derived macrophages. H_2O_2 production thus appears to be adequate in these cells. However, following 12 days in culture, viricidal activity was depressed, even when MPO was added. IFN-γ can activate macrophages *in vitro* with increased respiratory burst activity and H_2O_2 production (4, 5), and when the monocyte-derived macrophages were treated with IFN-γ, toxicity to HIV-1 by MPO-supplemented cells was again observed.

In the MPO system, MPO catalyzes the oxidation of chloride by H_2O_2 to form hypochlorous acid (HOCl) (2), which, with hypochlorite (OCl^-) (pK_a 7.3), is a potent oxidant (Clorox, household bleach) with recognized disinfectant activity against HIV-1 (13). Our findings suggest that freshly isolated, normal monocytes, when appropriately stimulated *in vitro*, can form HOCl/OCl^- in amounts sufficient to inactivate HIV-1. It is probable that viricidal activity is largely or totally extracellular under our experimental conditions. Thus, the incubation of HIV-1 with monocytes alone for 2 hr did not lead to a significant decrease in extracellular infectious virus, suggesting that only a small proportion, if any, of the extracellular HIV-1 was bound to or taken up by the monocytes. We used the LAV strain of HIV-1, which originally was a lymph node isolate (14). Although our strain is strongly lymphotropic (15–17), it has also been reported to infect human monocytes (18). The inhibition of viricidal activity by catalase, a large molecule

Table 4. Viricidal effect of monocyte-derived macrophages

Days in culture	IFN-γ	No supplement		PMA		PMA + GO		PMA + MPO	
		p24 antigen, pg/ml	No. of samples with growth/total	p24 antigen, pg/ml	No. of samples with growth/total	p24 antigen, pg/ml	No. of samples with growth/total	p24 antigen, pg/ml	No. of samples with growth/total
3	−	90,000	21/21	3,800	21/21	29,075	6/6	13	7/22
6	−	53,915	24/24	1,062	23/24	5,350	6/6	14	8/24
9	−	37,115	10/10	5,107	10/10	9,480	3/3	74	6/11
9	+	118,850	2/2	9,260	3/3	4,340	3/3	8	0/3
12	−	215,650	10/10	20,900	11/11	55,580	6/6	6,680	9/11
12	+	114,900	5/5	24,592	6/6	14,490	6/6	13	0/6

GO, glucose oxidase.

that would not pass readily into cells, would also argue for an extracellular effect of the MPO system on HIV-1 in our studies. It should be emphasized that the microenvironment of inflammatory sites is complex and not necessarily represented by our *in vitro* conditions. Substances such as protein, low molecular weight reducing agents, and catalase are potential inhibitors of the MPO system in some tissue fluids. Furthermore, we have employed a cell-free HIV-1 preparation in this study; toxicity to lymphocyte- or macrophage-associated virus was not addressed.

HIV-1, particularly macrophage-tropic strains, can infect monocytes/macrophages, with a considerably higher proportion of tissue macrophages than blood monocytes being HIV-1-positive in the human host (1). Blood monocytes and monocyte-derived macrophages can be infected with HIV-1 *in vitro* (18–23); however, studies comparing the susceptibility of freshly isolated monocytes and monocyte-derived macrophages to infection with HIV-1 have yielded conflicting results. Freshly isolated monocytes have been reported to be more susceptible (24), to be equally susceptible (25), to show different kinetics and efficiency of infection (26), and to be less susceptible (23) to infection with HIV-1, when compared with monocyte-derived macrophages. These findings may reflect the use of different HIV-1 strains or experimental conditions. Further studies are needed to determine whether HIV-1 taken up by mononuclear phagocytes is subject to attack intracellularly by endogenous toxins.

We are grateful to Kristie Schlechte and Ann Waltersdorph for excellent technical assistance and to Sandi Larsen for manuscript preparation. This work was supported by National Institutes of Health Grants AI07763, AI17758, and AI27757 and by the Howard Hughes Medical Institute through a Medical Student Research Training Fellowship to M.J.C.

1. Meltzer, M. S., Skillman, D. R., Gomatos, P. J., Kalter, D. C. & Gendelman, H. E. (1990) *Annu. Rev. Immunol.* **8**, 169–194.
2. Klebanoff, S. J. (1992) in *Inflammation: Basic Principles and Clinical Correlates*, eds. Gallin, J. I., Goldstein, I. M. & Snyderman, R. (Raven, New York), 2nd Ed., pp. 541–588.
3. Nakagawara, A., Nathan, C. F. & Cohn, Z. A. (1981) *J. Clin. Invest.* **68**, 1243–1252.
4. Nathan, C. F., Murray, H. W., Wiebe, M. E. & Rubin, B. Y. (1983) *J. Exp. Med.* **158**, 670–689.
5. Murray, H. W., Rubin, B. Y. & Rothermel, C. D. (1983) *J. Clin. Invest.* **72**, 1506–1510.
6. Klebanoff, S. J. & Coombs, R. W. (1991) *J. Exp. Med.* **174**, 289–292.
7. Klebanoff, S. J. & Coombs, R. W. (1992) *J. Clin. Invest.* **89**, 2014–2017.
8. Rakita, R. M., Michel, B. R. & Rosen, H. (1990) *Biochemistry* **29**, 1075–1080.
9. Klebanoff, S. J., Waltersdorph, A. M. & Rosen, H. (1984) *Methods Enzymol.* **105**, 399–403.
10. Klebanoff, S. J., Vadas, M. A., Harlan, J. M., Sparks, L. H., Gamble, J. R., Agosti, J. M. & Waltersdorph, A. M. (1986) *J. Immunol.* **136**, 4220–4225.
11. Foley, G. E., Lazarus, H., Farber, S., Uzman, B. G., Boone, B. A. & McCarthy, R. E. (1965) *Cancer* **18**, 522–529.
12. Locksley, R. M., Wilson, C. B. & Klebanoff, S. J. (1983) *Blood* **62**, 902–909.
13. Martin, L. S., McDougal, J. S. & Loskoski, S. L. (1985) *J. Infect. Dis.* **152**, 400–403.
14. Barre-Sinoussi, F., Chermann, J. C., Rey, F., Nugeyre, M. T., Chamaret, S., Gruest, J., Dauguet, C., Axler-Blin, C., Vezinet-Brun, F., Rouzioux, C., Rozenbaum, W. & Montagnier, L. (1983) *Science* **220**, 868–871.
15. Klatzmann, D., Barre-Sinoussi, F., Nugeyre, M. T., Dauguet, C., Vilmer, E., Griscelli, C., Brun-Vezinet, F., Rouzioux, C., Gluckman, J. C., Chermann, J.-C. & Montagnier, L. (1984) *Science* **225**, 59–63.
16. Montagnier, L., Gruest, J., Chameret, S., Dauguet, C., Axler, C., Guetard, D., Nugeyre, M. T., Barre-Sinoussi, F., Chermann, J.-C., Brunet, J. B., Klatzmann, D. & Gluckman, J. C. (1984) *Science* **225**, 63–66.
17. McDougal, J. S., Mawle, A., Cort, S. P., Nicholson, J. K. A., Cross, G. D., Scheppler-Campbell, J. A., Hicks, D. & Sligh, J. (1985) *J. Immunol.* **135**, 3151–3162.
18. Nicholson, J. K. A., Cross, G. D., Callaway, C. S. & McDougal, J. S. (1986) *J. Immunol.* **137**, 323–329.
19. Gartner, S., Markovits, P., Markovitz, D. M., Kaplan, M. H., Gallo, R. C. & Popovic, M. (1986) *Science* **233**, 215–219.
20. Ho, D. D., Rota, T. R. & Hirsch, M. S. (1986) *J. Clin. Invest.* **77**, 1712–1715.
21. Crowe, S., Mills, J. & McGrath, M. (1987) *Arch. AIDS Res.* **1**, 145–146.
22. Collman, R., Hassan, N. F., Walker, R., Godfrey, B., Cutilli, J., Hastings, J. C., Friedman, H., Douglas, S. D. & Nathanson, N. (1989) *J. Exp. Med.* **170**, 1149–1163.
23. Rich, E. A., Chen, I. S. Y., Zack, J. A., Leonard, M. L. & O'Brien, W. A. (1992) *J. Clin. Invest.* **89**, 176–183.
24. Kazazi, F., Mathijs, J.-M., Foley, P. & Cunningham, A. L. (1989) *J. Gen. Virol.* **70**, 2661–2672.
25. Olafsson, K., Smith, M. S., Marshburn, P., Carter, S. G. & Haskill, S. (1991) *J. Acquired Immune Defic. Syndr.* **4**, 154–164.
26. Valentin, A., Von Gegerfelt, A., Matsuda, S., Nilsson, K. & Asjo, B. (1991) *J. Acquired Immune Defic. Syndr.* **4**, 751–759.
27. Chase, M. J. & Klebanoff, S. J. (1992) *Clin. Res.* **40**, 52 (abstr.).