Immunology 1993 **78** 611–615

Cell-mediated immunity to HIV-1 in Walter Reed stages 1–6 individuals: correlation with virus burden

R. J. TRAUGER, W. K. GIERMAKOWSKA, F. FERRE, P. C. DUFFY, M. R. WALLACE,* D. E. LEWIS,* H. J. BEECHAM,* K. G. BURNETT, F. C. JENSEN & D. J. CARLO *The Immune Response Corporation, Carlsbad, California and * US Navy Hospital, San Diego, California, U.S.A.*

Accepted for publication 20 October 1992

SUMMARY

Cell-mediated immunity (CMI) to human immunodeficiency virus-1 (HIV-1) was assessed in a blinded fashion for a patient group ($n = 79$) representing Walter Reed (WR) stages 1–6. At the same time, viral load was quantitatively measured by two different methods, specifically, virus isolation and HIV viral DNA copy number as measured by the polymerase chain reaction (PCR). After unblinding it was determined that the ability to generate a lymphoproliferative response to an inactivated gp120-depleted HIV (HIV-ag) and tetanus toxoid diminished with advancing WR staging, with complete anergy to HIV-ag and tetanus at stage 6. As a group, individuals whose peripheral blood mononuclear cells (PBMC) proliferated to HIV-ag were either virus isolation negative or produced low levels of virus as measured by p24 antigen (< 250 pg p24) on day 7. Similarly, HIV DNA copy number in the HIV-ag responders was low (< 200 copies/4×10^5 PBMC). In contrast, antigen proliferation to tetanus toxoid did not correlate with virus load. Thus, clinical progression as defined by the WR staging system appears to coincide with a loss of CMI to HIV. More importantly, the low viral load measured in HIV-ag responders suggests a link between viral burden and CMI to HIV which might be exploited in the design of immunotherapies for HIV-infected individuals.

INTRODUCTION

The clinical progression to acquired immune deficiency syndrome (AIDS) in the human immunodeficiency virus (HIV)-infected individual is most notably characterized by a steady decline in CD4 lymphocytes.[1] Efforts to categorize the stages of progression to AIDS have been made to provide insight into the sequence of events which leads to the onset of life-threatening opportunistic infections in the HIV-infected individual.[2,3] Coincident with this clinical decline is an array of immunological abnormalities such as hypergammaglobulinaemia, delayed cutaneous hypersensitivity (DCH) anergy, and a gradual loss of cell-mediated immunity (CMI) to HIV and subsequently other antigens.[4–7]

Serum p24 levels in newly infected individuals would suggest that virus replication in the acute phase of infection is quite high.[8] These levels then fall dramatically and maintain a steady level typically referred to as an asymptomatic state until a rise in serum p24 viral antigen occurs, heralding the onset of opportunistic infection. While no clear-cut association regarding virus burden and clinical progression has been demonstrated, recent evidence would suggest that an increase in virus burden as estimated by measurements of HIV-1 DNA by polymerase chain reaction (PCR) is correlated with progression to clinical disease.[9,10]

CMI responses to HIV-1 and other antigens have been extensively employed to study the decline of $CD4^+$ T-cell function in HIV-infected individuals.[11–13] Measurements of the lymphoproliferative response to specific antigens suggest that the HIV-specific response is lost very early in the course of the infection.[14] To gain insight into the relationship between viral burden and immune function, particularly to HIV, we have analysed the cellular responses to HIV-1 in a cohort of HIV-infected individuals representing Walter Reed (WR) stages 1–6. These data were then compared to two estimates of viral burden: (1) virus isolation through co-cultivation with seronegative target peripheral blood mononuclear cells (PBMC) and (2) viral DNA as estimated by a quantitative PCR assay. We report here a correlation of cellular immunity to HIV and viral burden in individuals comprising WR stages 1–6.

MATERIALS AND METHODS

Patient selection

Patients were selected to represent all stages of HIV-related clinical disease as assessed by WR staging system.[3] Forty millilitres of whole blood from each of 79 HIV-seropositive and

Correspondence: Dr R. J. Trauger, The Immune Response Corporation, 5935 Darwin Court, Carlsbad, CA 92008, U.S.A.

eight HIV-seronegative Navy personnel were drawn into heparinized vacutainer tubes. Samples were given a blinding code and delivered within 2 hr from phlebotomy to The Immune Response Corporation Laboratories. Cells were separated over Ficoll–Paque (Pharmacia, Alameda, CA) and assayed immediately for viral burden and CMI. The patient breakdown was as follows: 27 WR1, 10 WR2, 16 WR3, eight WR4, 10 WR5, and eight WR6. It should be noted that these individuals were recently boosted with tetanus toxoid in accordance with military procedures. Seven additional seronegative PBMC samples were also used in this study (one for each assay) as negative controls, thus the total number of seronegative samples studied was 15.

T-cell proliferation assay

PBMC from either HIV-seropositive or HIV-seronegative individuals were seeded in round-bottom 96-well plates (Costar, Pleasanton, CA) at 2×10^5 cells/well (140 μl) of RPMI (ICN-Flow, Costa Mesa, CA) containing 10% human AB^- serum and 1% antibiotics (complete media). The response to HIV-1 was assayed using a density gradient purified HZ_{321} virus.[15] This virus preparation was inactivated by β-propiolactone (BPL; 1:4000 dilution in complete media at 37° for 5 hr) and ^{60}Co-irradiation (4·5 Mrads of frozen pelleted virus). The gp120 envelope glycoprotein was depleted as a consequence of the purification process, i.e. freeze thawing and multiple sucrose gradients. The gp120 content in the resulting highly purified virus stock was measured by a quantitative gp120 capture ELISA kit (American Biotechnologies Inc, Cambridge, MA). Total gp120 remaining in the virus stock was determined to be <1 ng gp120/ml. Results from previous experiments demonstrated that the optimum concentration of HIV antigen for lymphoproliferation was 5 μg/ml (1 μg/2×10^5 cells), so this concentration was employed throughout this study. Tetanus toxoid (Massachusetts Department of Public Health) was freshly prepared as a 50 μl stock solution. The final concentration of tetanus was 0·250 μg/2×10^5 cells. All assays were performed in triplicate. After 6 days incubation the cells were labelled with 30 μl of [^{3}H]thymidine (Amersham Radiochemicals, Arlington Heights, IL) at 0·5 μCi/ml, specific activity 45·5 Cu/mmol, in complete RPMI. On day 7 prior to harvest 20 μl of BPL (1:4000 final concentration) was added to the well to neutralize any virus produced during the incubation period. Cells were harvested (Ph.D. Cell Harvester, Cambridge Technology, Watertown, MA) after 2 hr incubation in BPL and incorporated label was determined by scintillation counting. Results are expressed as a 'stimulation index' (SI) which is the geometric mean c.p.m. of the cells plus antigen divided by the geometric mean c.p.m. of the cells without antigen. The mean SI for the 15 seronegative samples was 1·01 [$\pm$0·06 (standard error)] with a range of 0·83–1·29. The 95% confidence interval was 0·88–1·14. An SI equal to or greater than 2 was considered a positive response to the HIV-ag based on analysis of the response of 15 seronegative individuals ($P < 0{\cdot}0001$). Tetanus was also considered positive if a SI of $\geqslant 2$ was measured.

Determination of viral burden

The methodology for the quantitative PCR assay for HIV and the virus isolation assay has been previously described.[16] Briefly, PBMC were isolated by Ficoll–Hypaque and washed in sterile saline. A standard curve of HIV DNA was generated by serial dilution of the 85-14-F2 cell line (gift of S. Z. Salahuddin, University of Southern California, CA), which contains one truncated HIV genome/cell. The standard curve was generated by analysis of 1000 cells to 10 cells in a background of 4×10^5 seronegative PBMC. Cell lysates of either the standard curve samples or patient PBMC were treated with proteinase K for 1 hr and boiled for 1 hr. Oligonucleotide pairs from the gag region of HIV (SK38, SK39) were used as primers. Direct detection of the PCR product was obtained by end-labelling one of the primers (SK39) with γ [^{32}P]ATP (6000 Ci/mmol). PCR was performed on 4×10^5 cells in a total volume of 50 μl in PCR buffer (10 mM Tris, pH 8·3, 50 mM KCl, 2·5 mM $MgCl_2$, 0·22 mM dNTP, 33 pM of SK38, 5 pM 5 end-labelled SK39, 1 U Taq polymerase from Cetus/Perkin-Elmer, Emeryville, CA). After 30 years (95°, 1 min; 65°, 2 min) the samples were separated by PAGE. For quantitative analysis, the 115 base pair (bp) HIV PCR product was cut from the gel and incorporated c.p.m. determined by scintillation counting. A set of standard curve samples was run with each set of patient PBMC. A statistical program (STATPACK) was employed to generate the regression coefficients used to calculate HIV copy number.

Table 1. Proliferative response to HIV-ag and tetanus toxoid in WR 1–6 individuals

WR stage	(*n*)*	% HIV$^+$ (%)	% TET$^+$ (%)
SN†	15	0	86
1	27	22	70
2	10	30	70
3	16	12·5	56
4	8	12·5	50
5	10	10	30
6	8	0	0

* No. of individuals/group.

† SN, both the seronegative blinded and control PBMC samples.

Virus isolation

PBMC were purified by Ficoll–Hypaque gradient centrifugation. Cultures were initiated by adding 1×10^7 patient PBMC to 5×10^6 PBMC from an HIV-1-seronegative individual which had been stimulated for 24 hr with phytohaemagglutinin (PHA). Cultures were fed weekly with a complete media change and 3×10^6 PHA-stimulated PBMC from the same donor were added to the cultures. PBMC from HIV-1-seronegative individuals were obtained weekly from blood bank donors and were stored at $-70°$ after PHA stimulation for use as feeders. All seronegative PBMC were also infected with a standard stock to HIV-1 to assure similar susceptibilities to HIV-1 infection between blood bank donor cells. Co-cultivations were tested for p24 antigen by the Coulter p24 antigen capture assay (Coulter Immunology) on a weekly basis.

RESULTS

Lymphoproliferative response

To determine the extent of the CMI response to HIV and tetanus, PBMC isolated from patients were tested for their ability to respond to HIV-ag and tetanus toxoid by cell proliferation. Each assay also included an HIV-seronegative

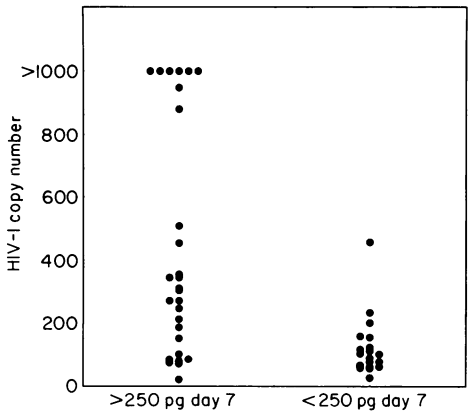

Figure 1. Correlation of antigen levels detected on day 7 of virus isolation with HIV-1 DNA copy number: viral p24 antigen levels at day 7 of co-cultivation of patient PBMC with seronegative feeder PBMC were segregated into either high (> 250 pg/ml) or low (< 250 pg/ml) p24 antigen producers. Viral DNA as assessed by quantitative PCR.

PBMC sample. Blinded samples contained at least one HIV-seronegative bleed with every set of samples. Through the course of this study none of the seronegative samples responded to HIV-ag in any of these assays. Control (no antigen) c.p.m. ranged from 419 to 4106 for the seronegative (SN) group and 292 to 3650 for the HIV-seropositive (SP) group. The ranges of c.p.m. in response to antigen and mitogen were as follows: HIV-ag c.p.m. ranged from 415 to 4079 for the SN group and 309 to 6962 for the SP group; tetanus c.p.m. ranged from 1336 to 23,727 (SN) and 377 to 46,192 (SP).

After the analysis was completed the clinical data for the patient group were unblinded and the lymphoproliferative data were segregated according to WR stage. We determined that a stimulation index of $\geqslant 2$ represented a significant positive response to the HIV-ag based on the response to HIV-ag measured in the seronegative control samples. As can be seen in Table 1, the proliferative responses to tetanus and HIV generally declined with advancing WR stage. By far the bulk of the responders (52%) were found in WR stages 1 and 2. These stages are comprised of HIV-1-seropositive individuals with PGL who have greater than 400 CD4 cells/mm^3.[3] It is interesting to note that the response to HZ_{321} for healthy seropositive individuals is comparable to that obtained with HIV_{IIIb}.[12] Thus, immunity to this isolate accurately reflects immunity to HIV-1 in the patient population studied. All HIV-ag responders demonstrated an intact tetanus response suggesting, as has been described earlier,[14] that the ability to respond to HIV is compromised before other antigen responses in HIV-infected individuals.

A proliferative response to HIV was seen in 16% (13/79) of the individuals studied. Responsiveness to tetanus also declined with advanced disease stage paralleling the decline in reactivity to HIV, with complete anergy to HIV-ag and tetanus observed in all WR6 individuals. A profound drop in reactivity to tetanus was seen in WR5 individuals, with only 30% of patients from this stage responding to tetanus *in vitro* compared to 70% in WR1 or 2. These data are in agreement with the WR staging criteria of partial to complete anergy to skin test antigen in WR5 individuals.[3] These patients were also skin tested for responsiveness to tetanus as part of the CMI multitest panel (Merieux Pasteur) on the day blood was drawn, providing an *in vivo* measure of the CMI response to tetanus. A comparison of the *in vivo* and *in vitro* measurements of CMI to tetanus demonstrated a 74% correlation of the cell proliferation data with the skin test results from these individuals (data not shown).

Determination of virus burden

Patients' viral burden was independently determined by two methods, virus isolation through co-cultivation of seropositive PBMC with seronegative feeder cells and quantitative PCR for HIV DNA. Both estimates of viral burden demonstrated an increase in virus as clinical staging increased.[16] Viral burden for values in this group of patients as measured by virus isolation could be segregated into high (> 250 pg/ml) and low (< 250 pg/ml) p24 antigen producers on day 7 of co-cultivation. A comparison of the amount of virus produced on day 7 of co-cultivation and HIV DNA copy number revealed a significant difference ($P < 0{\cdot}005$) in the amount of viral DNA between the high (> 250 pg/ml) and low (< 250 pg/ml) p24 antigen producers on day 7 (Fig. 1). Thus, these assays demonstrated comparable values for viral burden and provided two independent methods of determining the viral burden in infected individuals.

To assess the relationship between virus isolation and CMI, we compared the antigen levels from the day 7 co-cultivation with the ability to respond to either the HIV-ag or tetanus toxoid. As shown in Fig. 2, there is a striking correlation of CMI to HIV-ag with low viral load as determined by virus isolation at day 7 ($P < 0{\cdot}05$). Of the 13 individuals who were stimulation positive ($SI \geqslant 2$), 12 produced low or no HIV p24 on day 7. Similarly, all 13 SI-positive individuals had low (< 200 copies/4×10^5 PBMC) HIV DNA copy numbers ($P < 0{\cdot}001$). Tetanus responders showed no such correlation for either measurement of viral burden ($P > 0{\cdot}05$), with 40% of the tetanus responders producing > 250 pg of p24 in the virus isolation assay by day 7. Thus, it appears that specific immunity to HIV is strongly associated with low viral burden at all stages of infection, since this relationship is observed in WR1–6 individuals.

DISCUSSION

We have analysed the CMI response to HIV and tetanus toxoid in a cohort of HIV-seropositive individuals representing WR stages 1–6. The goals of this study were twofold. First, we wished to establish in a blinded study if our *in vitro* measurements of CMI to HIV-ag and tetanus correlated with a clinical segregation of HIV-infected individuals, specifically the WR clinical staging system. Second, we wanted to determine if a correlation existed between the CMI response to HIV-ag and viral burden. With regard to the CMI analysis presented here, it is well documented that the CMI response to HIV and other antigens decline as a consequence of HIV infection.[17–19] Thus, it was important to confirm this observation in order to validate correlations with viral burden, which was the ultimate goal of this study.

Through the course of this analysis we observed that the CMI to HIV-1, as measured by the ability to respond to a gp120-depleted inactivated HIV-1 antigen, steadily declined with each progressive WR stage. This observation is in agreement with the current view of immune dysfunction in HIV-infected indi-

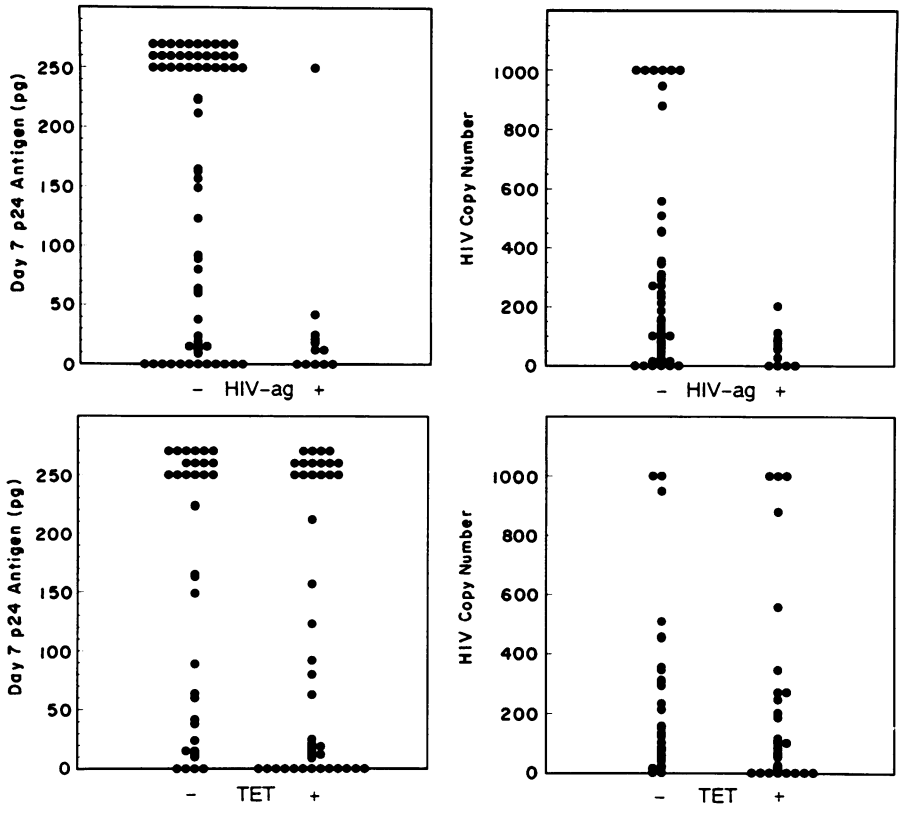

Figure 2. Correlation of the CMI response to HIV-ag and tetanus with viral burden. The level of virus in the supernatant is represented as the amount of p24 antigen detected on day 7 of co-cultivation of patient PBMC with PHA-stimulated seronegative PBMC. If no p24 antigen was detectable by day 7, the level of viral antigen was scored as 0. The amount of viral DNA as determined by PCR was determined according to Materials and Methods. The CMI response to HIV-ag and tetanus was scored positive if the stimulation index was equal to or greater than 2.

viduals. The fact that no response could be detected in any of the WR6 individuals, while striking, is not surprising. Explanations that have been proposed to account for the drop in the CMI response to antigens as a consequence of infection include a specific loss of memory cells,[20] anergy to interleukin-2 (IL-2),[21] elicitation of suppressor factors from infected macrophages,[22] suppressor cell activity,[23] decreased accessory cell function,[24,25] as well as direct suppression via HIV-encoded gene products such as TAT.[26]

Recent reports have focused on the increase in viral load in HIV-infected individuals as a potential indicator of clinical progression.[9,10] When the proliferation data were compared with two independent measurements of viral burden to determine whether specific immunity to HIV correlated with the viral load, we observed a much lower viral load in individuals responding to the HIV-ag *in vitro*. Our results extend the hypothesis that the cell-mediated compartment of immunity to HIV-1 may be the critical component in the control of viral burden in HIV-infected individuals. Given the central role that $CD4^+$ cells play in initiating and amplifying the immune response, a protective effect as a consequence of HIV-1 antigen-specific cells would be expected. For example, this response could translate into antiviral activity through the production of soluble immunopotentiating factors such as IL-2 and/or interferon-γ (IFN-γ), which have the effect of expanding both cytotoxic T cells and natural killer (NK) cells.[27] Cytotoxic activity of $CD4^+$ cells has also been described[28] which could contribute directly to the control of virus-infected cells.

The observation that immunity to HIV as measured by the cell-mediated response to HIV-ag and low viral burden appear to correlate, while intuitively obvious, is clearly demonstrated here. However, the dynamics between the immunity to HIV and viral burden are far from being understood. We have demonstrated, as have others, that functional antigen-specific $CD4^+$ cells can be found at their highest levels in the early stages of infection. However, two scenarios may be proposed to explain the correlation between low viral burden and HIV-specific CMI. On the one had, it is possible that HIV CMI is detectable in individuals with low viral load simply because a limited amount of virus is present to cause cytopathic effects in CD4 cells, resulting in the eventual decay of CMI to HIV. Alternatively, these results could suggest that the correlation of HIV CMI to low viral burden is a consequence of the HIV-specific CMI and hence immunity to HIV. The observation that immunity to HIV-1 but not tetanus correlates with low viral burden would seem to support the latter hypothesis since immunity to tetanus, which was even more robust than the HIV-ag response in these individuals, did not appear to be associated with low viral burden. The fact that the CD4 cell dysfunction, i.e. the loss of CMI to HIV-1 as well as other antigens, generally precedes the drop in CD4 cell number in clinically declining patients would also be in agreement with this hypothesis.

In conclusion, our results suggest that the immunological control of virus does contribute directly to the maintenance of a low viral burden in the initial stages of infection and throughout the asymptomatic phase. The strong association of HIV antigen-specific cells with low viral burden suggests that therapies directed at qualitatively improving the HIV antigen-specific $CD4^+$ cell compartment may provide a measure of immunity capable of stemming the progression to disease.

ACKNOWLEDGMENTS

The authors wish to thank D. Ripley and R. Griffin for expert technical help, J. Ande for review of the manuscript, and D. Rollins and S. Liu for providing database assistance.

REFERENCES

1. Gottlieb M.S., Schroff R., Schanker M.H., Weisman J.D., Fan P.T., Wolf R.A. & Saxon A. (1981) Pneumocystis carinii pneumonia and mucosal candidiasis in previously healthy homosexual men: evidence of a new acquired cellular immunodeficiency. *New Engl. J. Med.* **305,** 1425.
2. Centers for Disease Control (1986) *Classification System for Human T-lymphotropic Virus Type III/Lymphadenopathy-Associated Virus Infection. Morbid Mortal Weekly Rep.* **35,** 334.
3. Redfield R.R., Wright D.C. & Tramont E.C. (1986) The Walter Reed staging classification for HTLV-III/LAV infection. *New Engl. J. Med.* **314,** 131.
4. Giorgi J.V., Fahey J.L., Smith D.C., Hultin L.E., Cheng H.L., Mitsuyasu R.T. & Detels R. (1987) Early effects of HIV on CD4 lymphocytes *in vivo*. *J. Immunol.* **138,** 3725.
5. Lane H.C. & Fauci A.S. (1985) Immunologic abnormalities in the acquired immunodeficiency syndrome. *Ann. Rev. Immunol.* **3,** 477.
6. Nicholson J.K.A., McDougal J.S., Jaffe H.W., Spira T.J., Kennedy M.S., Jones B.M., Darrow W.W., Morgan M. & Hubbard M. (1985) Exposure to human T-lymphotropic virus type III/lymphadenopathy-associated virus and immunologic abnormalities in asymptomatic homosexual men. *Ann. Int. Med.* **103,** 37.
7. Ballet J.J., Couderc L.J., Rabian-Herzog C., Duval-Roy C., Janier M., Danon F., Clauvel J.P. & Seligmann M. (1988) Impaired T-lymphocyte-dependent immune responses to microbial antigens in patients with HIV-1-associated persistent generalized lymphadenopathy. *AIDS*, **2,** 291.
8. Lange J.M.A., Paul D.A., Huisman H.G., de Wolf F., van den Berg H., Coutinho R.A., Danner S.A., van der Noordaa J. & Goudsmit J. (1986) Persistent HIV antigenaemia and decline of HIV core antibodies associated with transition to AIDS. *Br. med. J.* **293,** 1459.
9. Schnittman S.M., Greenhouse J.J., Psallidopoulos M.C., Baseler M., Salzman N.P., Fauci A.S. & Lane H.C. (1990) Increasing viral burden in $CD4^+$ T cells from patients with human immunodeficiency virus (HIV) infection reflects rapidly progressive immunosuppression and clinical disease. *Ann. Int. Med.* **113,** 438.
10. Michael N.L., Vahey M., Burke D.S. & Redfield R.R. (1992) Viral DNA and mRNA expression correlate with the stage of human immunodeficiency virus (HIV) type 1 infection in humans: evidence for viral replication in all stages of HIV disease. *J. Virol.* **66,** 310.
11. Ahearne P.M., matthews T.J., Lyerly H.K., White G.C., Bolognesi D.P. & Weinhold K.J. (1988) Cellular immune response to viral peptides in patients exposed to HIV. *AIDS Res. Hum. Retroviruses*, **4,** 259.
12. Wahren B., Morfeldt-Mansson L., Biberfeld L., Moberg A., Sönnerborg A., Ljungman P., Werner A., Kurth R., Gallo R. & Bolognesi D. (1987) Characteristics of the specific cell-mediated immune response in human immunodeficiency virus infection. *J. Virol.* **61,** 2017.
13. Schrier R.D., Gnann J.W., Jr, Landes R., Lockshin C., Richman D., McCutchan A., Kennedy C., Oldstone M.B.A. & Nelson J.A. (1989) T cell recognition of HIV synthetic peptides in a natural infection. *J. Immunol.* **142,** 1166.
14. Wahren B., Morfeldt-Månsson L., Biberfeld G., Moberg L., Ljungman P., Nordlund S., Bredberg-Rådén U., Werner A., Löwer J. & Kurth R. (1986) Impaired specific cellular response to HTLV-III before other immune defects in patients with HTLV-III infection. *New Engl. J. Med.* **315,** 393.
15. Getchell J.P., Hicks D.R., Svinivasan A., Heath J.L., York D.A., Malonga M., Forthal D.N., Mann J.M. & McCormick J.B. (1987) Human immunodeficiency virus isolated from a serum sample collected in 1976 in Central Africa. *J. infect. Dis.* **156,** 833.
16. Ferre F., Marchese A., Duffy P.C., Lewis D.E., Wallace M.R., Beecham H.J., Burnett K.G., Jensen F.C. & Carlo D.J. (1992) Quantitation of HIV viral burden by PCR in HIV seropositive Navy personnel representing Walter Reed stages 1 to 6. *AIDS Res. Hum. Retroviruses*, **8,** 269.
17. Reddy M.N. & Grieco M.H. (1989) Cell-mediated immunity to recombinant human immunodeficiency virus (HIV) antigens in HIV-infected populations. *J. infect. Dis.* **159,** 120.
18. Krowka J.F., Stites D.P., Jain S., Steimer K.S., George-Nascimento C., Gyenes A. *et al.* (1989) Lymphocyte proliferative responses to human immunodeficiency virus antigens *in vitro*. *J. clin. Invest.* **83,** 1198.
19. Laurence J., Friedman S.M., Chartash E.K., Crow M.K. & Posnett D.N. (1989) Human immunodeficiency virus infection of helper T cell clones: early proliferative defects despite intact antigen-specific recognition and interleukin 4 secretion. *J. clin. Invest.* **83,** 1843.
20. Schnittman S.M., Lane H.C., Greenhouse J., Justement J.S., Baseler M. & Fauci A.S. (1990) Preferential infection of $CD4^+$ memory T cells by human immunodeficiency virus type 1: evidence for a role in the selective T-cell functional defects observed in infected individual. *Proc. natl. Acad. Sci. U.S.A.* **87,** 6058.
21. Clerici M., Stocks N.I., Zajac R.A., Boswell R.N., Bernstein D.C., Mann D.L., Shearer G.M. & Berzofsky J.A. (1989) Interleukin-2 production used to detect antigenic peptide recognition by T-helper lymphocytes from asymptomatic HIV-seropositive individuals. *Nature*, **339,** 383.
22. Fernández-Cruz E., Gelpi E., Longo N., González B., de la Morena M.T., Garcia Montes M. *et al.* (1988) Increased synthesis and production of prostaglandin E_2 by monocytes from drug addicts with AIDS. *AIDS*, **3,** 91.
23. Mullin G. & Mayer L. (1991) Correlation of spontaneous suppressor cell activity with progression of human immunodeficiency virus infection. *AIDS Res. Hum. Retroviruses*, **7,** 387.
24. Macatonia S.E., Patterson S. & Knight S.C. (2989) Suppression of immune responses by dendritic cells infected with HIV. *Immunology*, **67,** 285.
25. Melendez-Guerrero L.M., Nicholson K.A. & McDougal J.S. (1991) Infection of human monocytes with $HIV\text{-}1_{Ba\text{-}L}$. Effect on accessory cell function for T-cell proliferation *in vitro*. *AIDS Res. Hum. Retroviruses*, **7,** 465.
26. Viscidi R.P., Mayur K., Lederman H.M. & Frankel A.D. (1989) Inhibition of antigen-induced lymphocyte proliferation by tat protein from HIV-1. *Science*, **246,** 1606.
27. Swain S.L., Weinberg A.D. & English M. (1990) Lymphokine secretion of memory cells and of effector cells that develop from precursors *in vitro*. *J. Immunol.* **144,** 1788.
28. Powrie F. & Mason D. (1988) Phenotypic and functional heterogeneity of $CD4^+$ T cells. *Immunol. Today*, **9,** 274.