The development of variations of envelope with the resulting subtypes of human immunodeficiency virus type 1 (HIV-1) has created new challenges for the development of both therapeutic and preventive AIDS vaccines. We examined T-helper proliferative responses to HIV-1 clade A, B, C, G, and E whole-killed virus and to HIV-1 clade G and B core (p24) antigens in HIV-1-infected subjects taking potent antiviral drugs who received HIV immunogen (Remune) therapeutic vaccination. Subjects who were immunized mounted strong proliferative responses to both whole virus and core antigens of the different clades. These results suggest that a whole-killed immunogen may have broad applications as a therapeutic as well as a preventive vaccine in the current multiclade HIV-1 pandemic.
B) were obtained from Advanced Biotechnologies Incorporated. rp24 (clade B) was obtained from Protein Science (Meriden, Conn.).

Native p24 was preferentially lysed from purified inactivated HIV-1 (HZ321) with 1% Triton X-100 and then purified using Pharmacia Sepharose Fast Flow S resin. Chromatography was carried out at pH 5.0, and p24 was eluted using a linear salt gradient. The purity of the final product was estimated by both sodium dodecyl sulfate-polyacrylamide gel electrophoresis and reverse-phase high-pressure liquid chromatography to be >99%.

For the lymphocyte proliferation assays, fresh PBMCs from HIV-1-seropositive subjects were cultured in RPMI medium with 10% human AB serum at a concentration of 2 × 10^6 cells per well with medium alone or with inactivated HIV antigens, including whole gp120-depleted HZ321 (clade A) (5 μg/ml), native p24 (clade G) (5 μg/ml), whole Bal (clade B) (1 μg/ml), whole clade E (5 μg/ml), clade C (5 μg/ml), IIIB (5 μg/ml), IIIB p24 (5 μg/ml), and rp24 (5 μg/ml). PBMCs were seeded in a round-bottom 96-well plate (Falcon) at 2 × 10^5 cells/well in RPMI (Gibco) containing 10% heat-inactivated (56°C for 30 min) human AB serum (Gemini) and 1% antibiotics (100 U of penicillin per ml and 100 μg of streptomycin per ml) (Gibco). All assays were done in triplicate. After 6 days of incubation, supernatants were harvested from each well (100 μl), and the cells were labeled with 1 μCi of [3H]thymidine in complete RPMI. On day 7 before the harvest, 20 μl of BPL (1:400 final concentration) was added to each well to neutralize any virus produced during the incubation period. Cells were harvested after a 2-h incubation in BPL at 37°C, and incorporated label was determined by scintillation counting in a beta counter. Geometric mean counts per minute were calculated from the triplicate wells with and without antigen. Results were calculated as a lymphocyte stimulation index (LSI), which is the geometric mean counts per minute for the cells without antigen divided by the geometric mean counts per minute for the cells without antigen (cells incubated in medium alone). Spearman rank correlation was performed to examine relationships between lymphocyte proliferative responses to different antigens. The Mann-Whitney nonparametric U test was utilized to compare lymphocyte proliferation responses before and after immunization. All P values are two-tailed.

RESULTS

The baseline characteristics of the HIV-1-infected subjects on potent antiviral drug therapy who also received the HIV-1 immunogen and of unimmunized controls are listed in Table 1. As reported previously, subjects treated with the HIV-1 immunogen responded with strong lymphocyte proliferative responses to whole HIV antigens, including the gp120-depleted immunizing antigen (HZ321) (clade A) (P = 0.0008) and whole virus Bal (clade B) (P = 0.003), as shown in Fig. 1. We also demonstrated that these same subjects developed strong lymphocyte proliferative immune responses to HIV-1 IIIB (clade B) (P = 0.002). Overall, unimmunized controls showed weaker proliferative responses to HZ321 (n = 4; mean LSI ± standard error [SE] = 9.1 ± 2.0), Bal (n = 4; mean LSI ± SE = 3.1 ± 1.8), and IIIB (n = 4; mean LSI ± SE = 5.0 ± 3.1) whole HIV-1 antigens.

We also examined the response to core proteins of different clades. As shown in Fig. 2, subjects responded to np24 (clade G) (P = 0.0001), rp24 (clade B) (P = 0.01), and IIIB p24 (clade B) (P = 0.001). These core protein immune responses correlated with whole-protein responses (e.g., np24 correlated with HIV-1 [r = 0.88; P < 0.0001]). Overall, unimmunized subjects (n = 4) displayed weaker proliferative responses to np24 (mean LSI ± SE = 4.2 ± 1.1), rp24 (mean LSI ± SE = 15.4 ± 6.8), and IIIB p24 (mean LSI ± SE = 8.3 ± 2.9).

Finally, we examined responses to both clade E and clade C whole virus in these subjects. Strong lymphocyte proliferative responses to both HIV-1 type E (mean LSI ± SE = 26.0 ± 12.6) and HIV-1 type C (mean LSI ± SE = 34.5 ± 12.7) were observed, as shown in Fig. 3. HIV-1 type C T-helper immune responses correlated with type E (r = 0.87; P = 0.0009), Bal (r = 0.8; P = 0.005), IIIB (r = 0.8; P = 0.006), np24 IIIB (r = 0.9; P = 0.002), and rp24 (r = 0.9; P = 0.0007) compared to other whole-virus antigens tested. Unimmunized subjects (n = 4) displayed weaker proliferative responses to HIV-1 clade E (mean LSI ± SE = 0.8 ± 0.1) and HIV-1 clade C (mean LSI ± SE = 3.4 ± 1.8).

**DISCUSSION**

In this study we tested T-helper immune responses to a number of HIV-1 whole and core antigens from different clades of HIV-1. Subjects were on potent antiviral drug therapy and concomitantly received therapeutic HIV-1 immunogen. In unimmunized subjects and at baseline prior to immunization, subjects expressed low proliferative responses to HIV-1 antigens. This is consistent with work by others suggesting that the partial immune reconstitution with potent antiviral therapy does not include the full repertoire of HIV-specific clones (4, 14). Furthermore, recent work suggests that the frequency of both CD4 and CD8 HIV-specific T cells may decrease in subjects on potent antiviral drug therapy (R. Koup, M. Betts, J. Casazza, D. Douek, L. Picker, Abstr. 2000 Palm Springs Symposium on HIV/AIDS, p. 30, 2000). In this study we utilized HIV-1 protein antigens which most likely stimulate
the class II major histocompatibility complex pathway to activate CD8 T-helper cells (17). Studies using HIV-1 peptides which may activate the class I major histocompatibility complex pathway in order to better examine the CD8 T-cell response to this immunogen are ongoing.

This study further suggests that proliferative responses to clade B, C, and E whole-virus antigens can be stimulated in HIV-1-infected subjects on antiviral drug therapy who receive the HIV-1 immunogen. This observation expands our previous findings and suggests that treatment with an envelope-depleted clade A envelope and clade G Gag can stimulate T-helper responses to a number of clades of HIV-1. While the exact mechanism is unknown, this is probably due to the cellular response to the more conserved proteins of the virus. The response demonstrated here is most likely not due solely to alloantigen stimulation or nonspecific stimulation, as these immune responses to whole-virus antigens correlated with the highly purified core proteins.

Recently, strong core protein T-helper immune responses have been observed both in subjects with primary HIV-1 infection on potent antiviral drug therapy and in subjects with nonprogressive HIV disease receiving no therapy (5, 25). Studies to determine whether the induction of such responses with this immunogen can delay viral load rebound in patients on potent antiviral drug therapy or during structured treatment interruption are ongoing. Additionally, such an approach may offer a logical prototype for a preventive vaccine, particularly if it can elicit antiviral immune responses against different clades in seronegative subjects.

In summary, HIV-1-infected subjects on potent antiviral drug therapy were able to mount strong proliferative responses to different whole-killed HIV-1 and core proteins from different clades after treatment with HIV-1 immunogen (Remune). Such an immunogen may have broad applications as a therapeutic vaccine as well as a preventive vaccine in the current multiclade HIV-1 epidemic (16).

REFERENCES


