T-Helper-Cell Proliferative Responses to Whole-Killed Human Immunodeficiency Virus Type 1 (HIV-1) and p24 Antigens of Different Clades in HIV-1-Infected Subjects Vaccinated with HIV-1 Immunogen (Remune)

RONALD B. MOSS,1,4 WIESLAWA GIERMAKOWSKA,1 MARK R. WALLACE,2 JAY SAVARY,1 FRED JENSEN,1 AND DENNIS J. CARLO1

The Immune Response Corporation, Carlsbad, California 92008,1 and Division of Infectious Diseases, U.S. Naval Hospital, San Diego, California 921342

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The discovery of multiple subtypes of human immunodeficiency virus type 1 (HIV-1) worldwide 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.

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 worldwide (27). For example, HIV-1 clade C, which is endemic in Africa and parts of Asia, may now account for one-half of the infections with HIV-1 worldwide (6, 12, 19, 26). Subtype-specific HIV preventive vaccines are being tested, but an alternative, more global, approach might utilize a whole-killed vaccine, which might be capable of inducing cross-clade CD4 and CD8 antiviral immune responses.

The lack of CD4 T-helper cell activity in response to HIV-1 antigens is characteristic of very early HIV infection and is a defect not restored in chronic HIV infection with antiviral drug treatment (1, 2, 8, 10, 15, 21, 22, 24). The rare exceptions to this are individuals with nonprogressive HIV-1 disease, and they may represent the best model of control of HIV-1 by the immune system. HIV-1-seropositive individuals with nonprogressive disease typically have low but measurable HIV-1 viral loads but do not progress clinically or develop profound CD4 depletion for at least 10 years (20, 28). One explanation of such sustained control of viral replication is that cell-mediated immunity is able to suppress viral replication below a threshold that results in clinical disease. A low level of viral replication may be an important source of antigenic stimulation necessary for the immune system to maintain host immunosurveillance.

We hypothesized that an inactivated, gp120-depleted HIV immunogen (clade A/G) (Remune) might be capable of inducing T-helper immune responses to multiple HIV-1 clades. Previously we had characterized and quantitated viral antigens currently receiving antiviral drug therapy. Previously we reported that some cross-clade CD4 T-helper cell responses could be elicited to whole virus types B and E in HIV-1 immunogen-treated patients (13). Here we extend those observations and show that subjects were able to mount cross-clade lymphocyte proliferative immune responses to different whole and p24 antigens, including HIV-1 type C. These results suggest that a gp120-depleted, whole-killed virus is capable of inducing T-helper immune responses and may have important implications for HIV-1 therapy and prevention.

MATERIALS AND METHODS

The HIV-1 Immunogen (Remune) is composed of an HIV-1 isolate (HZ321) from serum collected from a patient in Zaire in 1976. This virus has been sequenced and classified as clade A and clade G Gag and grown in the Hut 78 T-cell line (3). Eleven HIV-1-seropositive subjects were enrolled in an open-label research study as part of an expanded access program. Institutional review boards approved all research. Subjects received one intramuscular injection of the HIV-1 immunogen on day 1 and every 12 weeks, which consisted of 10 U of p24 (100 μg of total protein inactivated) gp120-depleted HIV-1 (HZ321) in incomplete Freund’s adjuvant. Lymphocyte proliferative assays were performed on 11 HIV-1-seropositive subjects, using strain HZ321 (clade A), strain BaL (clade B), strain TH022E (clade E), strain 96BW01 (clade C), strain BNL (clade C), strain TH022E (clade E), strain 96BW01 (clade C), and native p24 (p24) (clade G), recombinant p24 (p24) (clade B), and IIB p24 (clade B) antigens for comparison.

The HIV-1 HZ321 immunogen is obtained by concentration and purification from the supernatant fluid of HZ321-infected Hut-78 T cells (7). In the preparation of the immunogen, envelope gp120 is depleted during freezing and thawing during the purification process. HIV-1 BaL antigen (Advanced Biotechnologies Incorporated, Columbia, Md.) was propagated in primary human macrophages. HZ321 virus was collected from a 72-h harvest obtained from a 14-day-infected culture exhibiting extensive syncytium formation, maximum p24 antigen production, and reverse transcriptase activity. The supernatant was clarified at 250 × g for 15 min, aliquoted, and frozen at −70°C. The suspending buffer consisted of Dulbecco modified Eagle medium (high glucose), 20% fetal bovine serum, and 50 mg of gentamicin per ml. HIV-1 clade E (strain TH022E; Advanced Biotechnologies Incorporated) and clade C (18) (kindly provided by the Harvard AIDS Institute) were propagated in primary adult human phytohemagglutinin-stimulated peripheral blood mononuclear cells (PBMCs). Infectious fluids were clarified by differential pelleting, and virus was purified through a 20% sucrose cushion. The suspending buffer consisted of 10 mM Tris, 150 mM NaCl, 1 mM EDTA (pH 7.5). Clade E, clade C, and HZ321 antigen preparations were inactivated through a sequential application of beta-propiolactone (BPL) (11) and 106Co irradiation (9). Whole IIB (clade B) and p24 IIB (clade

* Corresponding author. Mailing address: The Immune Response Corporation, 5935 Darwin Court, Carlsbad, CA 92008. Phone: (760) 431-7080. Fax: (760) 431-8636. E-mail: shotdoc@imnr.com.
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 controls showed mean LSI ± SE = 426 < 400 Zidovudine (40) Lamivudine (35) Indinavir (19)

2 607 < 400 Lamivudine (32) Stavudine (31) Nelfinavir (16)

3 1,969 < 400 Lamivudine (21) Stavudine (21) Nelfinavir (21)

4 1,270 < 400 Zidovudine (30) Lamivudine (30) Indinavir (30)

5 657 < 400 Zidovudine (88) Zalcitabine (49) Indinavir (60)

6 954 < 400 Efavirenz (3) Stavudine (15) Lamivudine (15)

7 597 < 400 Zidovudine (29) Lamivudine (29) Efavirenz (2)

8 834 < 400 Zidovudine (37) Lamivudine (37) Indinavir (32)

9 568 < 400 Zidovudine (17) Lamivudine (17) Nelfinavir (17)

10 630 < 400 Stavudine (10) Lamivudine (10) Nevirapine (10)

11 520 < 400 Stavudine (38) Lamivudine (43) Sustiva (7)

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 drug 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 CD4 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


