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Fusogenic selectivity of the envelope glycoprotein is a major determinant of human immunodeficiency virus type 1 tropism for CD4+ T-cell lines vs. primary macrophages

(cell fusion/β-galactosidase reporter gene/syncytia formation/virus entry/recombinant vaccinia viruses)

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ABSTRACT **We investigated the relationship between the fusion selectivity of the envelope glycoprotein (env) and the tropism of different human immunodeficiency virus type 1 (HIV-1) isolates for CD4+ human T-cell lines vs. primary macrophages. Recombinant vaccinia viruses were prepared encoding the envs from several well-characterized HIV-1 isolates with distinct cytotropisms. Cells expressing the recombinant envs were mixed with various CD4+ partner cell types; cell fusion was monitored by a quantitative reporter gene assay and by syncytia formation. With CD4+ continuous cell lines as partners (T-cell lines, HeLa cells expressing recombinant CD4), efficient fusion occurred with the envs from T-cell line-tropic isolates (IIIB, LAV, SF2, and RF) but not with the envs from macrophage-tropic isolates (JR-FL, SF162, ADA, and Ba-L). The opposite selectivity pattern was observed with primary macrophages as cell partners; stronger fusion occurred with the envs from the macrophage-tropic than from the T-cell line-tropic isolates. All the envs showed fusion activity with peripheral blood mononuclear cells as partners, consistent with the ability of this cell population to support replication of all the corresponding HIV-1 isolates. These fusion selectivities were maintained irrespective of the cell type used to express env, thereby excluding a role for differential host cell modification. We conclude that the intrinsic fusion selectivity of env plays a major role in the tropism of a HIV-1 isolate for infection of CD4+ T-cell lines vs. primary macrophages, presumably by determining the selectivity of virus entry and cell fusion.**

Genotypic and phenotypic variation is a hallmark of infection by human immunodeficiency virus type 1 (HIV-1). Of particular note is the marked difference in cytotropism of individual isolates *in vitro*; some productively infect continuous CD4+ T-cell lines but not primary macrophages, whereas others show the opposite preference (1–3). This biological diversity has critical implications for pathogenesis. Isolates obtained during the asymptomatic phase generally display the macrophage-tropic phenotype; indeed, macrophages probably serve as major viral reservoirs *in vivo*. T-cell line-tropic isolates usually appear during progression to the symptomatic phase, and their emergence often foreshadows CD4+ T-cell decline and demise of the immune system. HIV-1 variation is also important for transmission between individuals; whereas a donor typically harbors a diverse virus population, early isolates from the recipient are relatively homogenous and generally display the macrophage-tropic phenotype (4). Variation must also be considered during *in vitro* manipulation of HIV-1, since culture in continuous T-cell lines enriches for isolates with high replication capacity in these cells but not in macrophages. The selection pressures and molecular mechanisms underlying HIV-1 biological diversity thus have profound implications for the infection and disease processes.

Analyses of the viral determinants responsible for T-cell line vs. macrophage tropism have highlighted the central role of the envelope glycoprotein (env), specifically the external subunit gp120 (5–11). Particular importance has been assigned to the V3 loop, although sequences elsewhere within gp120 have significant influences. Since the primary function of env is to promote the membrane fusion events associated with virion entry and syncytia formation, a reasonable hypothesis is that tropism results from a corresponding selectivity of virus entry. Attempts to test this proposal have yielded conflicting results. Several groups investigating T-cell line vs. macrophage tropism have reported that HIV-1 DNA production fails to occur upon infection of the nonpermissive cell type; in some cases, it was shown that the block could be overcome by introducing the viral genome via transfection or pseudotype formation (6, 10, 12–15). These findings suggest that the infectivity restriction for a discordant virus–cell combination is associated with a block in the early phase of the viral replication cycle prior to reverse transcription and presumably involving entry. However, other reports have contradicted this view. Direct measurement of virion–cell fusion by fluorescence dequenching methods indicated that a T-cell line-tropic isolate fuses efficiently with primary macrophages, suggesting the replication block is at a postentry step (16). A subsequent study assessing production of circular viral DNA led to the conclusion that the defect is attributable not to early events in the virus life cycle (including entry) but rather to later events, perhaps involving migration of the viral DNA into the nucleus (17). The role of selective virus entry was also questioned by an *in situ* analysis, which indicated that nearly all cells in a primary macrophage culture contained retrotranscribed HIV-1 DNA after infection with either T-cell line-tropic or macrophage-tropic isolates (18). Finally, similar questions have arisen for the related simian immunodeficiency virus (SIV). As for HIV-1, env is the principle determinant of SIV infectivity for macrophages, and the V3 analog contributes strongly to target cell tropism (19–21). Yet SIV isolates with env-dependent differences in macrophage replication showed comparable levels of viral DNA synthesis upon macrophage infection, arguing against virus entry as the determining step (19). By contrast, analysis of SIV V3 site-directed mutants indicated that selective impairment of infectivity for a CD4+ cell line vs. peripheral blood mononuclear cells (PBMCs) or macrophages correlated with selective reduction of DNA synthesis in the cell line, suggesting restriction at the entry level (20). The mechanisms by which the

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Abbreviations: HIV-1, human immunodeficiency virus type 1; SIV, simian immunodeficiency virus; β-gal, β-galactosidase; PBMC, peripheral blood mononuclear cell; unc, uncleavable.

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HIV and SIV envs determine tropism for T-cell lines vs. primary macrophages are thus controversial.

In the present report, we directly analyze the ability of recombinant envs from different HIV-1 isolates to promote fusion with different CD4$^+$ cell types in the absence of other HIV-1 components. We use a versatile quantitative reporter gene assay as well as syncytia analysis to measure fusion between cells expressing vaccinia-encoded envs and different types of CD4$^+$ partner cells. Our results highlight the intrinsic fusion selectivity of env as a critical determinant of HIV-1 tropism for CD4$^+$ T-cell lines vs. primary macrophages.

MATERIALS AND METHODS

Cells. HeLa, BS-C-1, and Jurkat cells were obtained from the American Type Culture Collection, the HeLa-CD4 transformant 1022 (22) was obtained from B. Chesebro (National Institute of Allergy and Infectious Diseases, Hamilton, MT). Cultures were maintained in the following media: HeLa and 1022 monolayers, DMEM-10 [Dulbecco's modified Eagle's medium (Quality Biologicals, Gaithersburg, MD) supplemented with 10% fetal bovine serum (FBS), 2 mM L-glutamine, and antibiotics]; BS-C-1 monolayers, MEM-10 [Eagle's medium (Quality Biologicals) supplemented with 10% FBS, 2 mM L-glutamine, and antibiotics]; Jurkat cell suspensions, RPMI-10 [RPMI 1640 medium (Quality Biologicals) supplemented with 10% FBS, 2 mM L-glutamine, 10 mM Hepes, and antibiotics]. PBMCs were prepared from healthy donors and stimulated with phytohemagglutinin (M form) for 3–4 days in RPMI-10 (23). Monocyte-derived macrophages were prepared by countercurrent centrifugation elutriation of PBMCs and differentiation (24) and were plated in flat-bottom 96-well microtiter plates as described (23).

Plasmids and Recombinant Vaccinia Viruses. Purified vaccinia stocks were used (derived from the WR strain); the multiplicity of infection was 10 plaque-forming units per cell for each indicated virus. For env expression, we used a battery of vaccinia recombinants containing the *env* genes from several T-cell line-tropic and macrophage-tropic HIV-1 isolates. All *env*-containing viruses were produced by us except for vSC60 (S. Chakrabarti and B. Moss, National Institute of Allergy and Infectious Diseases, Bethesda, MD, personal communication). The final vector was plasmid pSC59, which contains a synthetic strong early/strong late vaccinia promoter (S. Chakrabarti and B. Moss, personal communication). Recombinant vaccinia viruses were isolated by thymidine kinase-negative selection and Western blot screening for env expression (25). The nomenclature of the vaccinia recombinants containing the indicated *env* genes (and the donors of the corresponding env DNAs) is as follows: vSC60, Lai, HTLV-IIIB BH8 *env* (26) (R. Gallo, National Cancer Institute, Bethesda, MD; modified as described in ref. 27); vCB41, Lai, LAV *env* (28) (M. Martin, National Institute of Allergy and Infectious Diseases, Bethesda, MD); vCB-36, RF *env* (29) (R. Gallo); vCB-34, SF2 *env* (30) and vCB-32, SF162 *env* (5) (C. Cheng-Mayer and J. Levy, University of California, San Francisco); vCB-28, JR-FL *env* (6) (I. Chen, University of California, Los Angeles); vCB-39, ADA *env* (9) (L. Ratner, Washington University, St. Louis); vCB-32, Ba-L *env* (8) (B. Cullen, Duke University Medical Center, Durham, NC). As a negative control, vCB-16 contains a mutated *env* gene (IIIB BH8) with a deletion of the gp120/gp41 cleavage site (31); the resulting uncleavable (unc) env is nonfusogenic. For recombinant CD4, we used vCB-3 (32) containing the same promoter as the env viruses. For bacteriophage T7 RNA polymerase, we generally used vP11gene1 (33) with the polymerase gene linked to the P11 late promoter; cell fusion assays using this virus were conducted in the presence of cytosine arabinoside (40 μg/ml) (34). Alternatively, we used vTF7-3 (35), which employs the P7.5 early/late promoter; this vector enables polymerase production in primary macrophages, which are nonpermissive for vaccinia late gene expression (23). Plasmid pG1NT7β-gal (R. A. Morgan, National Center for Human Genome Research, National Institutes of Health, personal communication) contains the *Escherichia coli LacZ* gene linked to the T7 promoter and the 5′ untranslated region of encephalomyocarditis virus. Transfection of monolayers was performed with DOTAP (Boehringer Mannheim).

Cell Fusion Assays. Fusion between env-expressing and CD4-expressing cells was measured by a reporter gene assay wherein the cytoplasm of one cell population contains vaccinia-encoded T7 RNA polymerase and the cytoplasm of the other contains the transfected plasmid with the *LacZ* gene linked to the T7 promoter; β-galactosidase (β-gal) is synthesized in fused cells (34). Vaccinia-encoded proteins were produced by incubating infected cells at 31°C overnight. Fusion reactions were conducted with the indicated cell mixtures in 96-well plates (0.2 ml per well) at 37°C. For quantitative analyses, Nonidet P-40 was added (0.5% final concentration) and aliquots of the lysates were assayed for β-gal at ambient temperature with the substrate chlorophenol red β-D-galactopyranoside; A_{570} was measured. Alternatively, β-gal was detected by *in situ* staining. In some cases, instead of assessing β-gal, syncytia were monitored by fixing the cultures with formaldehyde and staining with crystal violet.

RESULTS

Expression of Recombinant envs. Production of the recombinant vaccinia-encoded HIV-1 envs is analyzed in Fig. 1. The envs from four T-cell line-tropic isolates (IIIB, SF2, RF, and LAV) and four macrophage-tropic isolates (JR-FL, SF162, ADA, and Ba-L) were examined; as a control, we used a nonfusogenic unc env. Flow cytometry (Fig. 1*A*) indicated that all envs were expressed at comparable levels at the cell surface. To examine processing of the gp160 precursor, we performed radioimmunoprecipitation (Fig. 1*B*). For each wild-type env, significant levels of gp120 and gp41 were observed (although the presence of residual uncleaved gp160 indicated that processing was incomplete, as is typically observed with the vaccinia system). The mutant unc env yielded only the expected unc gp160.

Fusion Selectivities of Each env for Different CD4$^+$ Cell Types. The fusogenic selectivities of the recombinant envs were examined by the reporter gene assay in which β-gal is produced selectively in fused cells. The design of each fusion experiment involved expressing the panel of envs on a particular cell type and comparing their fusogenic activities when mixed with the designated CD4$^+$ partner cell type. This approach avoids ambiguities introduced by comparing cell types with different properties (e.g., differences in permissiveness for vaccinia expression, variations in env or CD4 levels, differences in membrane properties that might affect fusion, etc.).

Fig. 2 shows results using the *in situ* β-gal assay in which HeLa cells were used to express a representative env from each type of isolate: IIIB (T-cell line tropic), JR-FL (macrophage tropic), and unc (nonfusogenic control). When the partner population was the HeLa cell line producing vaccinia-encoded CD4 (Fig. 2 *Upper*), large blue syncytia were observed with the IIIB env; by contrast, fusion did not occur with the JR-FL env, as indicated by the presence of only occasional blue single cells (similar to the background with the unc env). The opposite result was obtained with macrophages as partners (Fig. 2 *Lower*); only occasional individual blue cells were observed with the IIIB env (similar to unc), whereas giant blue syncytia formed with the JR-FL env.

In Fig. 3, the analysis was extended to include more envs from each class (all expressed on HeLa cells) as well as a greater variety of CD4$^+$ partner cell types; fusion was assessed by quantitative assay of β-gal activity in cell lysates. With

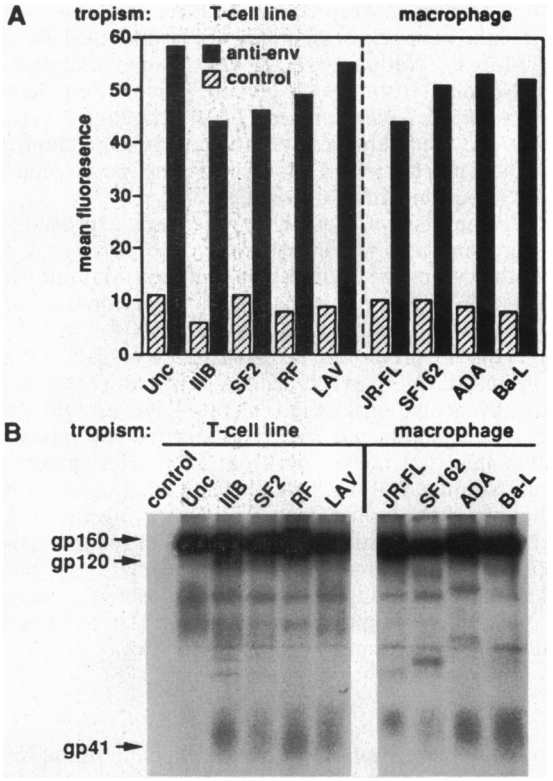

FIG. 1. Expression of recombinant HIV-1 envs. (*A*) Flow cytometry. HeLa cells were infected with vaccinia recombinants encoding the indicated envs and incubated overnight at 31°C. Cells were stained with polyclonal rabbit antiserum raised by us against purified baculovirus-encoded gp120 (IIIB; American Biotechnologies, Columbia, MD) followed by fluorescein isothiocyanate-labeled swine anti-rabbit IgG (Dako); for control cells, normal rabbit serum was used in the first step. Samples were fixed with 0.2% paraformaldehyde and analyzed on a FACScan flow cytometer (Becton Dickinson). (*B*) Radioprecipitation. BS-C-1 monolayers were infected with vaccinia recombinants encoding the indicated envs at 37°C. Beginning 4 hr postinfection, the cells were labeled overnight with L-[^{35}S]methionine (>800 Ci/mmol; 1 Ci = 37 GBq; Amersham). Lysates were prepared in buffer containing Triton X-100 and clarified by centrifugation. Immunoprecipitation was performed with rabbit polyclonal antiserum against a recombinant soluble construct representing the ectodomain of unc env (IIIB) followed by protein A-Sepharose. The radiolabeled proteins were resolved by SDS/PAGE on 10% reducing gels and were detected by fluorography. BS-C-1 cells were chosen for this experiment since, compared to other cell types, they are relatively efficient at processing recombinant vaccinia-encoded envs (although significant amounts of unprocessed gp160 are typically observed even in BS-C-1 cells, particularly when strong promoters are used).

CD4-expressing continuous cell lines as partners (Fig. 3 *A–C*), potent fusion occurred with envs from the T-cell line-tropic but not the macrophage-tropic isolates. This pattern was observed with various continuous cell line partners including the Jurkat T-cell line (Fig. 3*A*; similar results not shown for Hut-78 and MT-4) or the 1022 HeLa-CD4 transformant (Fig. 3*B*) expressing endogenous CD4, or HeLa cells producing the highly elevated levels of vaccinia-encoded CD4 (32) (Fig. 3*C*). Essentially the opposite selectivity pattern was observed when macrophages were used as the partner cell population (Fig. 3 *D* and *E*). Potent fusion occurred with the envs from the macrophage-tropic isolates, whereas consistently less fusion was observed with envs from the T-cell line-tropic isolates. When the level of CD4 on the macrophages was greatly augmented by using a vaccinia vector, the fusion efficiency increased dramatically (higher β-gal levels), but the selectivity pattern was unchanged. Finally, with PBMCs as partners (Fig. 3*F*), all the envs were active, consistent with the ability of both

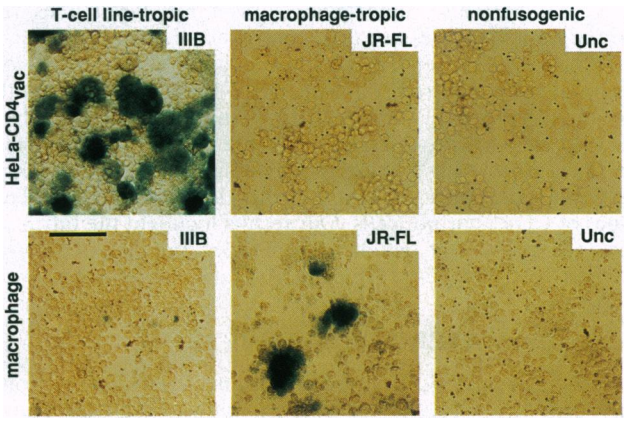

FIG. 2. Fusion specificities of envs for different CD4$^+$ cell types analyzed by *in situ* staining. HeLa cells were transfected with the *LacZ* plasmid and infected with vaccinia recombinants encoding the indicated envs and mixed with the indicated partner cell types. (*Upper*) HeLa cells expressing CD4 encoded by vCB-3 and T7 RNA polymerase encoded by vP11gene1. (*Lower*) Macrophage monolayers expressing endogenous CD4 and T7 RNA polymerase encoded by vTF7-3. Mixtures were prepared in 96-well microtiter plates (1×10^5 cells per well of each indicated cell type). After 3 h at 37°C, cells were fixed and stained for β-gal *in situ*. (Bar = 100 μm.)

T-cell line-tropic and macrophage-tropic HIV-1 isolates to productively infect PBMCs (3). In all cases, fusion was potently inhibited by the anti-CD4 monoclonal antibody OKT4A (data not shown). This control, coupled with the results using the unc env, verify that fusion observed with the vaccinia system resulted from the env–CD4 interaction.

The quantitative analyses in Fig. 3 reveal that previously reported departures from "all or none" infection cytotropism of various HIV-1 isolates correlated with the fusion specificities of the corresponding envs. Thus, with macrophage partners, we observed lower but significant fusion activity with the envs from the T-cell line-tropic compared to the macrophage-tropic isolates (Fig. 3 *D* and *E*). This finding is consistent with reports that primary macrophages can be infected at low efficiency with most T-cell line-tropic isolates (3), including IIIB (36), NL4-3 which contains the LAV env (10), and SF2 (6). With the reciprocal combinations, we detected little or no fusion when envs from the macrophage-tropic isolates were tested against CD4-expressing continuous cell lines (Fig. 3 *A–C*), consistent with the inability of the corresponding HIV-1 strains to replicate in these cell types. Also of note is that envs from isolates of a given class were not equally efficient at supporting fusion with a permissive partner cell type. The SF2 env was considered weaker than the other T-cell line-tropic envs at mediating fusion with the HeLa-CD4 1022 cell line or PBMCs (Fig. 3 *B* and *F*), consistent with the relatively low syncytia-forming capacity reported for SF2 in certain CD4$^+$ cell lines and PBMCs (37).

Fusion Selectivities Are Independent of the env-Producing Cell Type. Cytotropism is typically assessed by measuring production of HIV components as the infection spreads throughout the culture. Such protocols raise the possibility that the observed cytotropism results from cell type-specific modifications of env that adapt its fusion selectivity for the cell type used. The vaccinia system circumvents this issue, since the recombinant envs can be produced in alternative cell types and directly compared for their fusion selectivities against different CD4$^+$ partner cells. In Fig. 4. the envs were expressed in macrophages and then tested for fusion with different CD4$^+$ partners. The reporter gene assay proved unsuitable when macrophages were used as both partners in the fusion assay (data not shown), presumably because of restricted vaccinia gene expression in these cells (23); therefore, we used the

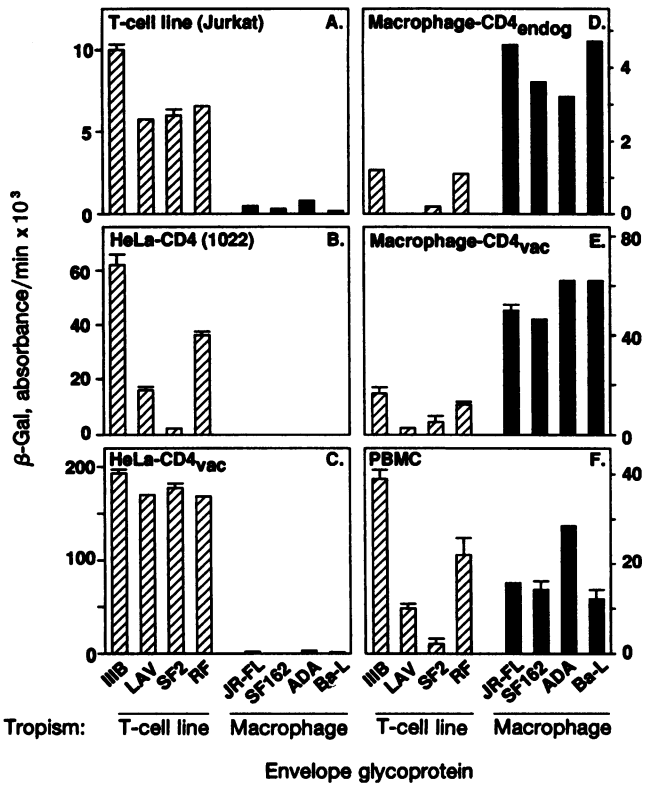

FIG. 3. Quantitation of fusion activities of envs against different CD4+ cell types. HeLa cells were transfected with the *LacZ* plasmid and infected with vaccinia recombinants encoding the indicated envs. Each designated partner cell type was infected with a vaccinia recombinant encoding T7 RNA polymerase. (*A–C* and *F*) vP11gene1 was used to infect cells in suspension. (*D* and *E*) Macrophage monolayers in 96-well plates were coinfected with vTF7-3 and either WR (*D*) or vCB-3 (*E*). The env-expressing cells (1×10^5 cells) were mixed with each CD4-expressing cell type (1×10^5 cells in *A–E*; 5×10^5 cells in *F*) in duplicate wells of 96-well microtiter plates. After 3.5 h at 37°C, Nonidet P-40 was added and β-gal activity was quantitated. Data shown are β-gal values obtained with each env minus the background values obtained with unc env for each partner cell type; bars represent sample standard deviations. The CD4+ partner cell types and the corresponding values obtained with unc env were as follows: Jurkat cells, 0.35 (*A*); HeLa-CD4 1022 cells, 1.1 (*B*); HeLa cells expressing CD4 encoded by vCB-3, 0.72 (*C*); macrophages, 1.2 (*D*); macrophages expressing CD4 encoded by vCB-3, 7.7 (*E*); PBMCs, 0.75 (*F*).

syncytia assay for all combinations. The results demonstrate that the fusion selectivity of each env produced in macrophages was similar to the selectivity described above when produced in HeLa cells. Thus, with the HeLa-CD4 1022 cells as partners (Fig. 4 *Upper*), large syncytia formed with the T-cell line-tropic IIIB env but not the macrophage-tropic JR-FL env. Conversely, with macrophages as partners (Fig. 4 *Lower*), large syncytia formed with the JR-FL env but not the IIIB env (where only aggregates were observed, similar to the unc env control). Similar results were obtained with the other envs of each class (data not shown). Thus, fusion selectivity for different CD4+ cell types is an intrinsic property of each env and does not arise from modifications occurring in different cell types.

DISCUSSION

The results presented here demonstrate a marked parallel between the infection tropism of a HIV-1 isolate for CD4+ T-cell lines vs. primary macrophages and the intrinsic cell-type fusion selectivity of the corresponding env. These analyses provide direct evidence that the restriction to replication with

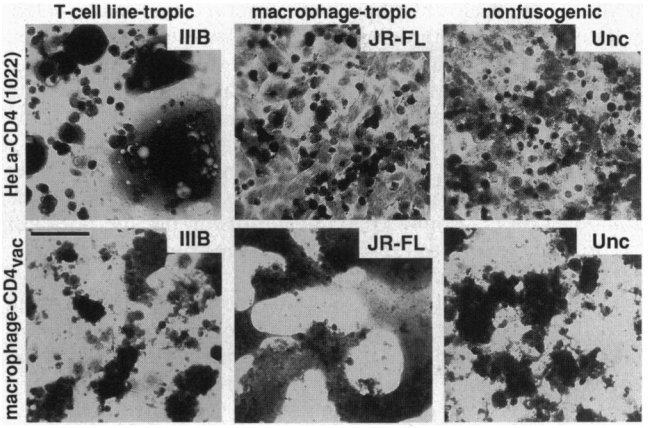

FIG. 4. Fusion specificities of envs expressed on macrophages. (*Upper*) Macrophage monolayers in 96-well plates (1×10^5 cells per well) expressed the designated vaccinia-encoded envs. HeLa-CD4 1022 cells previously infected in suspension for 2 h with WR were added to each well (1×10^5 cells per well). After 12 h at 37°C, the cultures were fixed, stained with crystal violet, and photographed. (*Lower*) Macrophages expressing the designated vaccinia-encoded envs were dislodged with a rubber policeman, dispersed by gentle pipetting, washed, and resuspended. Aliquots (1×10^5 cells) were added to individual wells of a 96-well microtiter plate containing adherent macrophages infected with vCB-3 (1×10^5 cells per well). After 6 h at 37°C, the cultures were fixed and stained with crystal violet. (Bar = 100 μm.)

a discordant virus–cell combination occurs at the membrane fusion level. Several points can be considered regarding the conflicting view that the restriction occurs at a later stage. One argument was based on virus–cell fusion measurements by fluorescence dequenching (16). However, recent studies of virus glycoproteins have indicated that fluorescence dequenching, as well as fluorescent membrane probe redistribution analyses, may detect lipid mixing under conditions where fusion pore formation fails to occur (38–40). Lipid mixing therefore does not necessarily indicate complete membrane fusion necessary for productive infection. Moreover, potential artifacts associated with fluorescence dequenching have been noted (41). Another challenge to the role of selective virus entry was based on *in situ* analysis showing that most cells in a macrophage culture contained retrotranscribed HIV-1 DNA after infection with T-cell line-tropic isolates (18). However, since most T-cell line-tropic isolates have at least some capacity to replicate in macrophages, these results do not exclude low entry efficiency as the major factor limiting infection. Indeed quantitative measurements indicated much higher viral RNA and DNA levels with a macrophage-tropic strain, which were attributed to either more efficient entry or postentry steps (18).

When CD4+ T cells are used as target for HIV-1 infection, macrophage-tropic isolates typically display a non-syncytium-inducing (NSI) phenotype, whereas T-cell line-tropic isolates are syncytium inducing (SI) (3). In our system, envs from the macrophage-tropic (NSI) isolates induced large syncytia with macrophage partners (Figs. 2 and 4). This is not a discrepancy, since the capacity of an isolate to form syncytia is highly target cell dependent. Indeed, with macrophage targets, syncytia formation has been reported for NSI isolates, including SF162 (42) and Ba-L (11). It is thus inappropriate to draw conclusions about the inherent fusogenic activity of a particular env by simply extrapolating from the NSI vs. SI nomenclature.

Our finding that fusion selectivity of env is independent of the cell type in which it is expressed is consistent with reports that the cytotropism of a HIV-1 isolate is maintained after short-term cultivation in diverse cell types (e.g., ref. 18). Tropism, therefore, does not result from differential host cell

modification of env. Moreover, we observed that fusion selectivity is maintained under conditions of widely varying fusion efficiencies. For example, when CD4-expressing continuous cell lines were examined, the fusion was much greater with partner cells expressing extremely high CD4 levels attained with a vaccinia vector compared to cells expressing endogenous CD4; however, the selectivities were similar in all cases (Fig. 3 *A–C*). Likewise, vaccinia-mediated elevation of the CD4 level on macrophages enhanced fusion but did not alter the preference for envs from macrophage-tropic isolates (Fig. 3 *D* and *E*). This point also applied to variations in the level of env. Because of the restrictions to vaccinia gene expression (23), the amount of vaccinia-encoded env in macrophage is much lower than in HeLa cells; yet this difference had no influence on fusion specificity for different $CD4^+$ cell types. Thus, selectivity is preserved under conditions of highly varying gp120/CD4-mediated binding between the fusing cells, thereby arguing against the notion that tropism is influenced by the varied affinity of env on different isolates for CD4 (1). An attractive alternative model derives from findings regarding another form of HIV-1 tropism—namely, the requirement that CD4 be expressed on a human cell type. Experiments based on hybrid cell formation (32, 43, 44) and biochemical membrane transfer (45) indicated that, for envs of the T-cell line-tropic class, an accessory membrane component(s) of the $CD4^+$ cell is required for fusion. It is tempting to propose (see ref. 1) that different accessory components are required for envs from the T-cell line-tropic vs. macrophage-tropic isolates; cytotropism would result from the varied expression of these components on different $CD4^+$ cell types. An accessory component could serve as a "coreceptor" directly interacting with env (perhaps via the V3 loop); alternatively, it could create a permissive environment wherein CD4 interaction with a compatible env leads to fusion. These concepts provide a framework for future studies.

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