Evidence That HIV Budding in Primary Macrophages Occurs through the Exosome Release Pathway*

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Lipid rafts are specialized regions of cell membranes enriched in cholesterol and sphingolipids that are involved in immune activation and signaling. Studies in T-cells indicate that these membrane domains serve as sites for release of human immunodeficiency virus (HIV). By budding through lipid rafts in T-cells, HIV selectively incorporates raft markers and excludes nonraft proteins. This process has been well studied in T-cells, but it is unknown whether lipid rafts serve as budding sites for HIV in macrophages. Recently, we proposed a new model of retroviral biogenesis called the Trojan exosome hypothesis (Gould, S. J., Booth, A., and Hildreth, J. E. K. (2003) Proc. Natl. Acad. Sci. U. S. A. 100, 10592–10597). This model proposes that retroviruses cannot escape the exosomal compartment until they reach the plasma membrane. Using macrophages, we examined the relative abundance of several host proteins on the cell surface, in lipid rafts, and on both HIV particles and exosomes derived from these cells. Our results show that some host proteins on the cell surface and in HIV do not correspond to the abundance of proteins in lipid rafts and in HIV. Moreover, our data demonstrate discordance in the abundance of some proteins in liposomes and in HIV. Finally, our data reveal a strong concordance between the host cell protein profile of exosomes and that of HIV. These results strongly support the Trojan exosome hypothesis and its prediction that retroviral budding represents the host cell vesicle trafficking.

Retroviruses are enveloped (+)-strand RNA viruses that replicate through a DNA intermediate inserted in the host genome. Although retroviral gene products drive the replication cycle, recent studies have made it clear that host cell proteins and lipids play critical roles in many phases of this cycle. Lipid rafts in the membranes of infected T-cells have been shown to be essential for budding and entry of retroviruses such as human immunodeficiency virus (HIV) and human T-cell lymphotropic virus type 1 (1−6). Lipid rafts are highly specialized regions of the plasma membrane characterized by a high content of cholesterol, sphingolipids, and glycosylphosphatidylinositol-anchored proteins (7). These membrane domains have been implicated in a multitude of processes in cells of the immune system (8). Several other viruses also require the integrity of lipid rafts on target cells for infection, including filoviruses (9) and measles virus (10). Recent studies show that lipid rafts are present on HIV particles and that depletion of viral membrane cholesterol blocks HIV infection further highlighting the importance of lipid rafts in the biology of this virus (11−14).

Most of the evidence supporting the HIV raft budding model was generated in T-cells, and there are few extensive mechanistic studies on HIV budding in infected primary macrophages. Unlike T-cells, monocyte-derived macrophages (MDMs) do not appear to release HIV in significant amounts from the plasma membrane. Early electron microscopy studies revealed that HIV accumulates in large membrane-limited vacuoles in infected primary macrophages (15). Recent studies have confirmed these earlier experiments and extended them to show that, in macrophages, HIV is present in major histocompatibility complex (MHC) class II-loading compartments or vesicles that have features of late endosomes (16, 17). Published data indicate that HIV released from primary macrophages incorporates lipid raft-associated proteins such as the heavily palmitoylated protein CD36 (18). Thus, although HIV does not appear to be released primarily at the plasma membrane in macrophages, such viruses nonetheless bear proteins associated with rafts.

The budding of HIV in macrophages appears to confound the lipid raft model of budding for this virus. We have recently proposed an alternative model for retroviral budding summarized as the Trojan exosome hypothesis (19). This model proposes that retroviruses actually exploit the pre-existing pathway of exosomal exchange for the formation of retroviral particles and for a low efficiency mechanism of infection (19). Published data on HIV-infected macrophages support this model. The HIV-containing vacuoles in macrophages are reminiscent of multivesicular endosomes (MVEs), also called MHC class II-containing compartments (20). These endosomal organelles contain vesicles resulting from membrane budding into the vacuolar lumen, creating intraluminal vesicles containing cytoplasmic proteins. These intraluminal vesicles can be released from the cell by fusion of the MVE with the plasma membrane, at which time they are called exosomes (for review, see Ref. 21). Exosomes contain membrane proteins normally found in late endosomes, such as Lamp-2 and CD63, as well as MHC molecules, both classes I and II. They also contain co-stimulatory molecules such as CD86, supporting findings that these vesicles can serve as miniature antigen-presenting cells capable of activating T-cells (22−24). Much like lipid rafts, the MVEs that...
esosomes are derived from and the resulting esosomes themselves are rich in cholesterol and sphingolipids (25). A link between the formation of esosomes and HIV budding in macrophages is supported by recent evidence showing viral budding into intracellular compartments containing MHC II and CD63 and by the presence of these two MVE-associated proteins at high levels on the resulting virion particles within the MVE (16). Previous studies have also shown a requirement for proteins critical to the formation of esosomes in MVEs, such as Tsg-101 (26) and ubiquitin (27), for HIV budding. To date, esosomes from T-cells, Epstein-Barr virus-transformed B-cells, erythrocytes, and dendritic cells have been analyzed (21), but esosomes from primary macrophages have not been studied. Thus, it is not possible to ascertain whether the host membrane protein phenotype of HIV released by primary macrophages is similar to that of macrophage-derived esosomes, as the Trojan esosome hypothesis predicts.

Here, we used primary macrophages to perform the first test designed to differentiate between the lipid raft hypothesis of retroviral biogenesis and the Trojan esosome hypothesis. Using human MDMs, we examined the relative abundance of several host cell proteins on the cell surface, in lipid rafts, and on both HIV particles and esosomes derived from these cells. Our results show that there are significant differences in the abundance of host cell proteins on the cell surface and in HIV particles. Furthermore, our data demonstrate that some lipid raft-associated proteins in macrophages are not incorporated by the virus. Finally, our data reveal a strong concordance between the host protein profile of macrophage-derived esosomes and that of HIV particles. Taken together, these results strongly support the Trojan esosome hypothesis and its prediction that retroviral budding is the exploitation of a pre-existing cellular pathway of intercellular vesicle trafficking.

MATERIALS AND METHODS

Antibodies — The following murine monoclonal antibodies (mAbs) were used in flow cytometry, virus capture, and dot-blot assays: anti-MHC I (HHF.5, anti-CD68 (H35), and anti-MHC II (HM36 and H53) (28); anti-CD63 and biotinylated anti-CD63 (H5C6) (29); anti-CD14 (63D3) (30); anti-Lamp-1 (HA43) and anti-Lamp-2 (HB43) (31); anti-CD36 (produced as described previously) (32); anti-CD55, anti-macrophage mannose receptor (MMR), and anti-CD81 (PharMingen); and fluorescein isothiocyanate (FITC)-conjugated anti-CD36, fluorescein isothiocyanate-conjugated anti-CD45, and mouse IgM control mAb (Jackson Immunoresearch Laboratories, Inc., West Grove, PA). For flow cytometry and virus capture, antibodies were used at 10 μg/ml except for anti-CD36 mAb, which was a hybridoma supernatant used at a dilution of 1:2. For dot blots, antibodies were used at 1 μg/ml except for mAb HHF.5 and anti-CD61 mAb (0.5 μg/ml and anti-CD36 mAb hybridoma supernatant diluted 1:20).

Cells and Virus — MDMs were derived from the adherent fraction of human peripheral blood mononuclear cells. Peripheral blood mononuclear cells (5 × 10⁷/7T-75 flask) were cultured for 1 h in RPMI 1640 medium supplemented with 2.5% normal human AB serum (Sigma) (complete RPMI 1640 medium). The non-adherent fraction was removed, and fresh complete RPMI 1640 medium containing 600 μg/ml penicillin/streptomycin and 5 μg/ml amphotericin B was added. Cells were cultured for 18 h in RPMI 1640 medium supplemented with 10% FCS until the cell density reached 10⁶ cells/mL. The culture supernatant was then removed, and the adherent cells were used for virus infection.

To obtain MDM-derived HIV strain BaL, day 4 MDM cultures were exposed to HIV BaL (600 ng of p24, Advanced Biotechnologies Inc., Cockeysville, MD) over a period of 48 h. Supernatants were subjected to a series of centrifugation steps: 200 × g for 10 min (Pellet 1 cells and large cell debris), two cycles at 500 × g for 10 min (Pellet 2), two cycles at 2000 × g for 15 min (Pellet 3), 10,000 × g for 30 min (Pellet 4), and 70,000 × g for 60 min (Pellet 5 = exosomal pellet). Each resuspended pelleted fraction was assayed using the BCA assay, and then a series of dilutions were made in PBS. Equal volumes of each dilution (100 μl) were added to the wells of a Bio-Dot apparatus and allowed to flow through the nitrocellulose membrane by gravity for 1 h. Any remaining sample was then gently suc-
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RESULTS

Lipid Raft Localization of MDM Surface Proteins—Previous work in this laboratory has shown that HIV preferentially buds from lipid raft regions of the plasma membrane in infected T-cells, thus selectively incorporating plasma membrane lipid raft proteins (3). To address whether this holds true for viruses produced in macrophages, we first phenotyped HIV-infected and uninfected MDMs by flow cytometry and then isolated lipid rafts from the cells to determine the raft localization of the surface proteins detected by flow cytometry. Immunoblot analysis of the fractions from the sucrose gradient raft isolations revealed that, as in T-cells and monocytes, MDM lipid rafts were enriched in glycosylphosphatidylinositol-anchored proteins such as CD55 and CD14 (Fig. 1). CD63, another known raft marker containing four palmitoylation sites, also localized primarily to raft regions, as has been shown previously (36). Other membrane proteins tested, including Lamp-1, CD63, MHC I, and MHC II, had at least partial lipid raft localization. Interestingly, CD45 also localized to low density fractions, with very strong lipid raft localization in HIV BaL-infected MDMs. Confocal immunofluorescence studies on uninfected MDMs confirmed that a substantial fraction of CD45 on the cell surface co-localized with the raft marker CD36 (data not shown). This is in contrast to HIV-infected and control T-cells, in which CD45 is excluded from lipid rafts (3, 37). It is unlikely that the presence of CD45 in raft fractions was due to aggregation of proteins at the plasma membrane or some other nonspecific mechanism because little CD45 was found in Pellet 5 (70,000 × g; as described below) despite the abundance of this membrane protein.

Incorporation of MDM Surface Proteins into HIV Virions—After establishing a panel of proteins that localize to lipid rafts on macrophages, we carried out virus capture assays (3, 38) to determine whether HIV particles released from macrophages incorporate these proteins. HIV virions incorporated the raft markers CD55 and CD36 as well as MHC I, MHC II, and MMR (Fig. 2). Surprisingly, the virus failed to incorporate CD45 or CD14 even though both localize to lipid rafts in MDMs. This is in contrast to HIV produced in T-cells, where all lipid raft proteins investigated so far have been found in virions (2). Macrophage-derived HIV also efficiently incorporated CD63 and CD81, members of the tetraspannin family of proteins that are highly enriched in exosomes (21), and a significant amount of the lysosomal protein Lamp-1, which is also present in exosomes. The virus capture assay was used for this analysis instead of immunoblotting of purified virus because HIV purified by methods other than antibody pull-down assays appears to always contain contaminating cell membranes or vesicles.

FIG. 1. Protein profile of MDM lipid rafts. Day 4 differentiated adherent MDMs (A) or 2-week HIV-infected adherent MDMs (B) were lysed in 1% Triton X-100 for 1 h on ice. After pelleting large cell debris, lysates were brought to 40% sucrose, overlaid with a discontinuous sucrose gradient, and subjected to equilibrium centrifugation. Eleven 1-ml fractions were collected and subjected to immunoblotting with mAbs as described under “Materials and Methods.” Fraction 1 (bottom) corresponds to soluble proteins. Lipid raft fractions (indicated at the bottom of each panel) were determined by the location of raft markers CD55 and CD36. Gag, HIV core antigen.

FIG. 2. HIV derived from MDMs excludes CD45 and CD14. HIV BaL from primary macrophages was subjected to host protein phenotyping by virus capture with mAbs as described under “Materials and Methods.” The background level of capture was determined using mouse IgG1 as a negative control, and this background (<3% of input) was subtracted from all other values. The results are expressed as the percentage of input virus captured by mAb after background subtraction.

A. Uninfected MDM

| Lipid Rafts |
|------------|
| CD14 |
| CD45 |
| CD55 |
| CD63 |
| MHC I |
| MHC II |
| Lamp-1 |
| CD36 |
| CD81 |

B. HIV-BaL Infected MDM

| Lipid Rafts |
|------------|
| CD14 |
| CD45 |
| CD55 |
| CD63 |
| MHC I |
| MHC II |
| Lamp-1 |
| CD36 |
| CD81 |

The background level of capture was determined using mouse IgG1 as a negative control, and this background (<3% of input) was subtracted from all other values. The results are expressed as the percentage of input virus captured by mAb after background subtraction.
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Flow cytometry analysis of surface markers on cultured MDMs

Cells were harvested, blocked, and stained for the proteins of interest using mAbs, followed by FITC-conjugated secondary antibodies. Samples were then analyzed using a Coulter EPICS-XL flow cytometer. MTF, mean total fluorescence.

|                     | Day 4 MDMs | 2-Week uninfected MDMs | 2-Week HIV BaL-infected MDMs |
|---------------------|------------|------------------------|-----------------------------|
|                     | Positive   | MTF        | Positive | MTF           | Positive | MTF          |
|                     | %          | %          | %        | %             | %        | %            |
| MHC I               | 98.93      | 33.8       | 79.8     | 55.2          | 83.5     | 67.4         |
| CD36                | 79.37      | 5.5        | 38.1     | 8.3           | 48.0     | 9.8          |
| CD63                | 98.2       | 9.4        | 87.5     | 92.3          | 86.39    | 84.5         |
| CD45                | 98.63      | 40.3       | 69.9     | 17.3          | 72.7     | 21.1         |
| CD55                | 53.23      | 2.2        | 27.8     | 6.3           | 18.1     | 5.4          |
| CD14                | 95.27      | 10.3       | 72.3     | 12.7          | 48.0     | 9.4          |
| MHC II              | 95.83      | 54.9       | 61.0     | 27.1          | 54.9     | 46.9         |
| Lamp-1              | 95.73      | 7.2        | 83.0     | 66.0          | 89.1     | 78.6         |
| Lamp-2              | 12.3       | 1.0        | 39.9     | 17.1          | 24.5     | 11.1         |

Table II

Ratio of mean percent input virus captured to mean total fluorescence of surface proteins on HIV-infected MDMs

The mean percentage of input virus captured by mAbs against proteins of interest was calculated from the data shown in Fig. 2. Mean total fluorescence (MTF) levels of each protein (from Table I) were then expressed as a percentage of the mean total fluorescence of CD63 (percent relative expression). CD63 was chosen for determining relative expression because of its high expression and the quantitative or near-quantitative precipitation of virus always observed with the mAb against this protein. The ratio of the mean percent input virus captured to percent relative expression was determined (approaches 1 if the incorporation of the protein by HIV correlates with the cell-surface expression levels).

|               | Mean input virus captured | Relative expression (% of CD63 MTF) | Ratio of mean percent input virus captured to relative expression |
|---------------|---------------------------|------------------------------------|---------------------------------------------------------------|
|               | %                         | %                                  | %                                                             |
| CD63          | 85.94                     | 100                                | 0.86                                                          |
| Lamp-1        | 33.2                      | 93.02                              | 0.36                                                          |
| MHC I         | 41.84                     | 79.76                              | 0.52                                                          |
| MHC II        | 42.2                      | 55.5                               | 0.76                                                          |
| CD45          | 5.53                      | 24.97                              | 0.22                                                          |
| Lamp-2        | 1.4                       | 13.14                              | 0.11                                                          |
| CD96          | 120.31                    | 11.6                               | 10.37                                                         |
| MMR           | 18.38                     | 11.24                              | 1.64                                                          |
| CD14          | 0.65                      | 11.12                              | 0.06                                                          |
| CD55          | 21.48                     | 6.39                               | 3.36                                                          |

TABLE I

FMH, mean total fluorescence.

FIG. 3. Limiting dilution immunoblot analysis of MDM exosomes shows enrichment of MHC II. Exosomes from MDM were isolated using differential centrifugation as described under “Materials and Methods.” The resulting pellets from each of the five centrifugation steps (with Pellet 5 representing the exosomal pellet) were lysed in 1% Triton X-100, serially diluted in PBS, and subjected to immunoblot assays as described under “Materials and Methods.” Blots were developed using ECL reagent and Hyperfilm ECL. The resulting films were scanned, and densitometry was performed using Kodak 1D 3.6.1 software. Mean dot intensities are plotted against dilution for each pellet. Shown are the limiting dilution dot blot (A) and a plot of mean dot intensities (B) for MHC II after MDM exosomal isolation. Data are representative of three independent MDM exosomal isolations from three separate donors.

lysosomal protein, Lamp-2, remained very low on all cells tested.

A comparison between the mean fluorescence intensity of the proteins on HIV-infected MDMs and the percentage of input virus captured was performed to determine whether virion incorporation of proteins directly correlates with MDM surface expression. We calculated the relative expression of each protein as a percentage of the mean total fluorescence signal of CD63, whose signal was consistently highest on MDMs (Table II). CD63 was chosen also because antibodies against this molecule consistently yield quantitative or near-quantitative precipitation of MDM-derived HIV. The expression of each protein was then compared with the amount of virus captured with antibodies against that protein (as a ratio; see Table II). Proteins with both high relative surface expression and high virion incorporation (CD63) and those with both low relative expression and low virion incorporation (MMR) had ratios close to 1, indicating that virion incorporation could be solely a function of surface expression levels for these proteins. However, although some proteins such as MHC II and CD63 showed viral incorporation levels that correlated with expression ratios ap-

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proaching 1), others appeared to be preferentially incorporated (CD36 and CD55; ratios of 10.37 and 3.36, respectively) or excluded (CD45, CD14, and Lamp-2; ratios of 0.22, 0.06, and 0.11, respectively). Assuming that a threshold level of expression is required for incorporation by HIV and that a threshold density on the viral particles exists for precipitation of virus by mAb, one might expect that any protein expressed above a certain level should mediate HIV precipitation. However, our comparison of viral precipitation and flow cytometry data presented here is not consistent with this model, suggesting that another mechanism is responsible for incorporation of host membrane proteins from primary macrophages.

The Host Protein Phenotype of MDM-derived HIV Matches That of MDM Exosomes—In light of the similarity between the protein phenotype of exosomes from several cell types (21) and that demonstrated for macrophage-derived HIV BaL (CD63, MHC I, MHC II, and Lamp-1), we examined whether exosomes from MDMs show a protein phenotype similar to that of HIV BaL. Exosomes were isolated from MDM culture supernatants by a series of centrifugation steps as previously reported (39). Each pellet was lysed and subjected to immunoblotting with mAbs. By performing densitometry analysis and plotting the mean signal intensity versus the dilution factor for each fraction, we determined whether proteins recognized by the mAbs were enriched in the exosomes. The immunoblot for the consensus exosomal marker MHC II and the resultant densitometry graph are shown in Fig. 3. Consistent with data from other cell types, MHC II was highly enriched in exosomes from macrophages, as evidenced by the persistence of signal at higher dilutions of Pellet 5 (exosomes) compared with other fractions (Fig. 3B). Similar graphs for the other known exosomal markers, CD81 and CD63, confirmed their enrichment in macrophage exosomes (Fig. 4). In fact, the data show that CD81 was enriched by >10-fold in the exosomal pellet compared with other pellets. In contrast, CD14 and CD45, which were not incorporated by HIV, were present at very low levels in exosomes, with the exosomal (Pellet 5) signal decreasing to near-background levels beyond a 5-fold dilution (Fig. 4). Unfortunately, we were not able to assess the presence of CD36 in the five fractions because none of the available mAbs against CD36 worked in the dot-blot assay. Other approaches such as capture enzyme-linked immunosorbent assays also failed to provide consistent data.

For comparison, exosomal immunoblotting curves corrected for background at each dilution were generated for each protein. When plotted together, the curves show a striking difference between the exosome-enriched proteins (CD81, CD63, and MHC II) and CD14 and CD45, confirming that the latter proteins are poorly represented in the exosomal pellet (Fig. 5A). The highest dilution at which the Pellet 5 (exosome) signal for each of the panel of proteins reached 50% of the maximum was determined for exosomes derived from MDMs from three different normal donors (Table III). For all three donors, those proteins enriched in exosomes (CD81, CD63, and MHC II) showed a half-maximum signal at a dilution of 1:1280, whereas CD14 and CD45 had half-maximum titers of 1:20, despite the fact that MHC II, CD14, and CD45 showed similar cell-surface expression (Table I). The ratio of the exosomal signal for each

![Examination of exosomal sorting of MDM proteins. MDM exosomes were isolated as described under “Materials and Methods” and analyzed for the proteins of interest by limiting dilution immunoblotting as described in the legend to Fig. 3. Plots of mean dot intensity versus dilution for each fraction are shown for CD81, CD63, CD14, and CD45. The pattern observed for MHC II in Fig. 3 was also seen for CD81 and CD63, two tetraspannin proteins, with high signal over a large dilution range for the exosomal pellet (Pellet 5) compared with the other pellets. In contrast, the signals for Pellet 5 for CD14 and CD45 decreased sharply with increasing dilution, much like the signal in the other pellets, showing their lack of enrichment in exosomes.]{fig4}

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The highest dilution at which the mean dot intensity signal in Pellet 5 retained 50% of the maximum signal was determined for the five proteins after subtracting the background. The process was carried out using exosomes isolated from MDMs from three different donors (Donors 1–3).

Table III
Half-maximal signal titers in exosomes for five proteins of interest

| Protein | Donor 1 | Donor 2 | Donor 3 |
|---------|---------|---------|---------|
| CD81    | 1:1280  | 1:1280  | 1:1280  |
| CD63    | 1:1280  | 1:1280  | 1:1280  |
| MHC II  | 1:1280  | 1:1280  | 1:1280  |
| CD14    | 1:20    | 1:20    | 1:20    |
| CD45    | 1:20    | 1:20    | 1:20    |

Together, these observations strongly suggest that exosomes and HIV particles produced by infected macrophages have very similar (if not identical) physical and biochemical properties, i.e. HIV-1 is a “viral” exosome.

DISCUSSION

There is substantial evidence that HIV preferentially buds from lipid rafts, including the enrichment of cholesterol and sphingolipids in the viral envelope and the selective incorporation of raft markers into virions (2, 3). Although phenotypic analysis of HIV from macrophages has shown incorporation of some raft markers such as CD55 into the virus, most of the evidence supporting the raft budding model was generated in T-cells. To determine the validity of the lipid raft budding model in HIV-infected macrophages, we first isolated lipid rafts from primary MDMs and probed for various raft and non-raft proteins. As expected, the glycosylphosphatidylinositol-anchored proteins CD55 and CD14 and the palmitoylated scavenger receptor CD36 localized to rafts. Interestingly, a marker of non-raft regions in T-cells, the tyrosine phosphatase CD45, had raft localization in macrophages, indicating that rafts on macrophages may be functionally distinct from those on T-cells. Phenotyping of HIV produced in MDMs showed high levels of incorporation of the raft marker CD36, as had been shown previously (18). Virions also incorporated CD55, MHC I, MHC II, CD63, Lamp-1, and CD81. As has also been shown by Esser et al. (2), CD45 was mostly excluded from macrophage-derived HIV BaL. It is possible that CD45 might be excluded from the virion particle despite its raft localization due to its large cytoplasmic phosphatase domains or its association with other proteins.

Even more surprising, CD14 was also absent in MDM-derived viral particles, thus becoming the first glycosylphosphatidylinositol-anchored raft protein studied that is not incorporated into budding HIV particles (40, 41). This observation is consistent with data recently published by Pelchen-Matthews et al. (17). Combined with our data on exclusion of raft-localized CD45, lack of CD14 incorporation suggests that HIV budding in macrophages occurs by a mechanism distinct from the plasma membrane raft model. Early clues as to the possible site of HIV budding in macrophages came from electron microscopy studies on infected macrophages, where numerous progeny virions in intracellular vacuoles were observed with little or no budding seen at the plasma membrane (15, 42). These compartments are reminiscent of MVEs (also called MHC II-containing compartments), the sites of exosomal biogenesis (20). A link between formation of exosomes and HIV budding in macrophages is supported by recent evidence showing viral budding into intracellular compartments containing MHC II and CD63 and by the presence of these two MVE residents at high levels on the resulting virion particles within the MVE (16, 17). Our data are consistent with the results of a recent study showing that HIV produced by MDMs carries proteins known to be present on late endosomal membranes (17). In our previous studies in T-cells, we used confocal immunofluorescence to show co-localization of HIV-1 proteins and cellular proteins (3). Such experiments were not carried out in the present study because elegant and definitive immunoelectron microscopy experiments have been carried out on HIV-infected MDMs by others. These studies very clearly show co-localization of viral proteins and host cell proteins in late endosomal compartments in these cells (16, 17).

The profile of host molecule incorporation we observed for MDM-derived HIV particles and that found in other recent studies match completely that of exosomes described for other cell types. If HIV is budding into MVEs as a viral exosome, the protein phenotype of the exosomes released by primary macro-
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The presence of MHC proteins, co-stimulatory molecules, and adhesion molecules on exosomes suggests that they may represent cell-independent activation of immune responses in vivo. Exosomes purified from dendritic cells pulsed with tumor antigens have been shown to stimulate antigen-specific responses both in vitro and in vivo, leading to destruction of established murine tumors (53). Human and mouse B-cell-derived exosomes have also been shown to elicit specific MHC II- and MHC I-mediated responses in vitro (24, 39). We have demonstrated that macrophages release exosomes with a protein phenotype similar to that of exosomes released by other cell types. It is highly likely that the MHC molecules on HIV (viral exosomes) from macrophages carry peptides derived from viral proteins, allowing HIV to activate T-cells specific for itself. The result could be preferential infection and elimination of HIV-specific T-cells. Indeed, a recent study provides evidence strongly supporting this idea (54). Should this hold true, it suggests that vaccine strategies that elicit robust HIV-specific T-cell responses could actually facilitate HIV transmission rather than prevent it. These and other implications (19) underscore the importance of further study of the Trojan exosome model of HIV biology.

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