A CCR5-dependent novel mechanism for type 1 HIV gp120 induced loss of macrophage cell surface CD4

Citation for published version:

Link:
Link to publication record in Edinburgh Research Explorer

Document Version:
Publisher's PDF, also known as Version of record

Published In:
Journal of Immunology

Publisher Rights Statement:
© 2001 by The American Association of Immunologists

General rights
Copyright for the publications made accessible via the Edinburgh Research Explorer is retained by the author(s) and / or other copyright owners and it is a condition of accessing these publications that users recognise and abide by the legal requirements associated with these rights.

Take down policy
The University of Edinburgh has made every reasonable effort to ensure that Edinburgh Research Explorer content complies with UK legislation. If you believe that the public display of this file breaches copyright please contact openaccess@ed.ac.uk providing details, and we will remove access to the work immediately and investigate your claim.
A CCR5-Dependent Novel Mechanism for Type 1 HIV gp120 Induced Loss of Macrophage Cell Surface CD4

This information is current as of January 17, 2014.

Tim J. Hewson, James J. Logie, Peter Simmonds and Sarah E. M. Howie

*J Immunol* 2001; 166:4835-4842; ;
http://www.jimmunol.org/content/166/8/4835

**References**

This article cites 52 articles, 25 of which you can access for free at:
http://www.jimmunol.org/content/166/8/4835.full#ref-list-1

**Subscriptions**

Information about subscribing to *The Journal of Immunology* is online at:
http://jimmunol.org/subscriptions

**Permissions**

Submit copyright permission requests at:
http://www.aai.org/ji/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at:
http://jimmunol.org/cgi/alerts/etoc
A CCR5-Dependent Novel Mechanism for Type 1 HIV gp120 Induced Loss of Macrophage Cell Surface CD4

Tim J. Hewson, James J. Logie, Peter Simmonds, and Sarah E. M. Howie

Type 1 HIV gp120 is especially effective in disrupting immune cell function because it is able to cause dysregulation of both infected and uninfected cells. We report a novel CCR5-dependent mechanism of gp120-induced CD4 loss from macrophages. An M-tropic gp120, using CCR5, is able to induce 70% loss of cell surface CD4 from macrophages within an hour. This cell surface CD4 loss is more substantial and rapid than the 20% loss observed with T-tropic gp120IIIB by 3 h. The rapid and substantial CD4 loss induced by M-tropic gp120 is not observed on macrophages homozygous for the ccr5Δ32 mutation, which fail to express cell surface CCR5. We have used confocal imaging to show that gp120 and CD4 are internalized together by a process resembling receptor-mediated endocytosis, and that both proteins enter HLA-DR containing compartments of the macrophage. We have also shown by semiquantitative RT-PCR that, in response to CD4 loss from the cell surface, mRNA for CD4 is up-regulated and the intracellular pool of CD4 increases. CCR5 mRNA levels are also increased. It is proposed that internalization of self and viral protein and increased pools of intracellular CD4 could modulate Ag presentation efficiencies and have implications for the induction and maintenance of both productive immune responses and self-tolerance. The Journal of Immunology, 2001, 166: 4835–4842.

Copyright © 2001 by The American Association of Immunologists 0022-1767/01/$02.00

Received for publication June 28, 2000. Accepted for publication January 16, 2001.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

*Immunobiology Group, Centre for Inflammation Research and †Laboratory for Clinical and Molecular Virology, University of Edinburgh, and ‡Department of Pathology, University of Edinburgh Medical School, Edinburgh, United Kingdom

Unless otherwise stated, all reagents and Abs were obtained from Sigma (Poole, Dorset, U.K.).

Materials and Methods

Reagents and Abs
Plate (Life Technologies). In separate experiments 5
Facility for AIDS Reagents) or human rIL-16 (R&D Systems) were added
incubated with a mixture of mAbs and loaded onto the depletion column.
1 –18 h (overnight) before preparation
Monocytes
Technologies) and allowed to adhere to the flask for1h in 5% CO 2 37°C
washing and fresh medium was added (as before with the addition of 5%
Gp120 was conjugated to FITC, using a published protocol (48) and Slide-
FITC conjugation of protein
Gp120 was conjugated to FITC, using a published protocol (48) and Slide-
T cell isolation and culture
Mononuclear cells (PBMC) were isolated by centrifugation
Macrophage isolation and culture
Briefly, 50 μg gp120 was diluted to 500 μl with distilled water and dia-
DMSO was incubated with dialyzed gp120 for2h in the dark to allow
All Ab labeling steps were conducted on ice and all solutions were made
To demonstrate that neither R5-tropic gp120 nor gp120muc competes for
Detection of surface-bound gp120
Six-day-old cc5 wild-type macropores were harvested from culture
Staining of macrophages for fluorescence microscopy
Monocyte-derived human macrophages were grown from buffy coats as
After fixation, the coverslips were washed three times in PBS.
After fixation, the coverslips were washed three times in PBS.
All Ab labeling steps were conducted on ice and all solutions were made
plates (Life Technologies) at 1 × 10^5 cells/well and washed by centrifu-
gellation. The supernatant was removed and 10 μl/900ng of R-PE-conjugated
Dose response of CD4 loss
Under similar conditions to those described above, R5-tropic gp120 was incubated at a range of doubling concentrations from 1/16– 4 μg/ml with
Demonstration of competitive and noncompetitive binding
To demonstrate that neither R5-tropic gp120 nor gp120muc competes for
Detection of CD4 by flow cytometry
Detection of CD4 by flow cytometry)
Covered slips were removed, dried, and mounted onto glass slides (BDH) using Vector-shield antifade reagent (Polysciences, Warrington, Pa.) and stained against
FITC conjugated against human CD4 was used as the isotype control between samples.
Furthermore, to avoid triggering apoptosis, cells were resuspended in 400 μl
and retained identical binding for both short plasmids. A determination of the
To demonstrate that either R5-tropic gp120 or gp120muc competes for
Detection of CD4 levels assayed as described above.
After incubation, the cells were harvested and replated into 96-well microtiter U-bottom
Laser-scanning confocal microscopy and image analysis

Stained macrophages were visualized on a DMRE laser scanning confocal microscope with a TCS NT image capture computer system (Leica Microsystems, Heidelberg, Germany). Images were saved as tagged image files and analyzed by the TCS NT system and Scion Image (Scion, Frederick, MD).

Macrophage ccr5 Genotyping by RT-PCR

Total RNA was extracted from freshly isolated PBMC using the Qiagen RNeasy spin column kit as per the manufacturer's instructions (Qiagen, Crawley, U.K.). RNA content was measured using a GeneQuant photometer (Pharmacia Biotech, St. Albans, U.K.).

A total of 0.1 μg RNA was used for cDNA synthesis using the Expand reverse transcriptase kit (Boehringer Mannheim, Roche Diagnostic Systems, Lewes, U.K.) and oligot(dT) (Oswel, Southampton, U.K.) and primers (Oswel) spanning the Δ32 deletion site in the ccr5 gene or a portion of the housekeeping gene β-actin. Primer sequences were: ccr5: antisense, CCT GTG CCT CTT CTT CTC ATT TCG; sense, CAA AAA GAA GGT CTT CAT TAC ACC; β-actin: antisense, CTA GAA GCA TTT GCG GTG GAC GAT GGA GGG; sense, TGA CGG GGT CAC CCA CAC TGT GCC CAT CTA.

The thermal cycler program consisted of 5 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min; 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min; 35 cycles of 94°C for 0.5 min, 55°C for 0.5 min, and 72°C for 1.5 min; 35 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s; and 72°C for 10 min.

PCR products were run on a 3% agarose gel with appropriate m.w. markers (pGEM; Promega, Southampton, U.K.). Bands were visualized by ethidium bromide staining and UV trans-illumination. Donors were ccr5 genotyped based on PCR fragment size (see Fig. 1 for example).

Semiquantitative RT-PCR

RNA was extracted and assayed, and 0.1 μg was reverse transcribed from samples as described above. A variable cycle number PCR was conducted to determine the optimum number of cycles required for a near-linear relationship between the RNA level and resultant DNA band intensity. All cDNAs were then amplified by PCR for this number of cycles and the band intensities following ethidium bromide staining and trans-illumination was determined by the Enhanced Analysis System (EASY, version 4.19; Scotlab, Coatbridge, Lanarkshire, U.K.). To allow for errors in the initial equalization of RNA mass between samples, samples were compared as ratios of the intensity of a band amplified from the gene of interest to the intensity of a housekeeping gene band from the same cDNA amplified by the same thermal cycler program (47). CD4 and ccr5 message levels were compared thus using the following programs and primers: CD4: sense, GCA GTG CGG AGC TGT GGT; antisense, GGG TCC CCA CAC CTC ACA GG; and ccr5 and β-actin primers as above. The CD4 thermal cycler program consisted of 32 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min.

Results

CCR5-dependent loss of cell surface CD4 is induced by R5 tropic gp120

All buffy coat donors were screened for the ccr5Δ32 mutation (Fig. 1), of 67 samples 52 were found to be homozygous wild type, 10 donor samples were heterozygous and 5 donors were homozygous for the Δ32 mutation. Donors were ccr5 genotyped based on PCR fragment size (see Fig. 1 for example).

CD4 and ccr5 message levels were compared thus using the following programs and primers: CD4: sense, GCA GTG CGG AGC TGT GGT; antisense, GGG TCC CCA CAC CTC ACA GG; and ccr5 and β-actin primers as above. The CD4 thermal cycler program consisted of 32 cycles of 94°C for 1 min, 58°C for 2 min, and 72°C for 2 min.

The Ccr5 thermal cycler program consisted of 5 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min; then 29 cycles of 94°C for 30 s, 60°C for 30 s, and 72°C for 45 s.

FIGURE 1. Genotyping gel. RNA extracted from buffy coats 1, 2, and 3 was subjected to RT-PCR for ccr5. The products show a length polymorphism with wild-type message amplifying to a 190-bp product and the Δ32 mutant message amplifying to a 158-bp product. This allows sample 1 to be detected as homozygous for the Δ32 mutation. Sample 2 is heterozygous, and sample 3 is homozygous wild type.

FIGURE 2. The effect of gp120s on the cell surface CD4 level of ccr5Δ32 wild-type macrophages. Macrophages were incubated with R5-tropic gp120 or gp120IIIB for the time intervals indicated. The normalized MnIX values are calculated from the mean fluorescence intensity of the anti-CD4 labeled cells. The MnIX value above the background fluorescence obtained with the isotype control is then rescaled to be expressed as a percentage decrease of the initial (untreated) staining intensity. The graph (a) shows the mean data values obtained from six different donors. The bars show SEM and the p values were calculated using an unpaired Mann Whitney U test. Raw data showing CD4 (b) and CD18 (c) levels before (black line) and after (solid gray) 3 h of incubation with R5-tropic gp120 in wild-type macrophages showing a typical response is also given.
were heterozygous, and 5 were homozygous mutant. Using wild-type donors’ macrophages, 1 μg/ml gp120 IIIB induces a maximal 12–25% CD4 loss at 3 h, which does not reach statistical significance. A total of 1 μg/ml R5-tropic gp120 induced substantial and significant (65–75%) loss of cell surface CD4 at both 1 and 3 h. After 18 h, the loss was still significant, while CD4 levels had started to recover (Fig. 2). Levels of a non-gp120-binding cell surface marker, CD18, remained unchanged (Fig. 2). Although R5-tropic gp120 caused a substantial loss of surface CD4, the CD4 ligand, IL-16, and the CCR5 ligand, MIP-1α, had no effect (Table I).

To demonstrate that both gp120s were able to bind to macrophage cell surfaces, gp120 was allowed to bind to Ccr5 wild-type macrophages and was then detected by an anti-gp120 Ab (Fig. 3).

To study the dose dependency of surface CD4 loss, both gp120s were incubated separately at a range of concentrations for 3 h with Ccr5 wild-type macrophages. Table II shows that changes in CD4 level are not greatly influenced by gp120 dose (as opposed to coreceptor usage) over the concentrations examined. To demonstrate that apparent cell surface CD4 loss was not simply due to R5-tropic gp120 competing with anti-CD4 (clone MT310) for CD4 binding, both gp120s were separately incubated with Ccr5 wild-type macrophages from CCR5Δ32 mutant donors and neither gp120IIIB or R5-tropic gp120 was able to induce a significant cell surface CD4 loss (Table IV).

To confirm that this effect was dependent on intact CCR5 receptor, the same experiment was repeated on macrophages from CCR5Δ32 mutant donors and neither gp120IIIB or R5-tropic gp120 was able to induce a significant cell surface CD4 loss (Table IV).

Loss of surface CD4 is accompanied by increased levels of CD4 in an internal HLA-DR-positive pool

Ccr5 wild-type macrophages were permeabilized and stained for CD4 after various periods of incubation with R5-tropic gp120. Cell surface CD4 declines after incubation with R5-tropic gp120. Intracellular pools of CD4, indicated by cytoplasmic staining (Fig. 4) increase after gp120 incubation.

Fig. 4 shows a coincidence of intracellular staining for CD4 and HLA-DR in the cytoplasm of macrophages that have been incubated with R5-tropic gp120. This suggests that the intracellular CD4 staining seen in Fig. 6 is localized to the endoplasmic reticulum.

![FIGURE 3](http://www.jimmunol.org/)

**FIGURE 3.** Detection of macrophage surface-bound gp120. Macrophages were incubated with R5-tropic gp120 (a) or gp120Δhom (b). The surface-bound gp120s were then detected by flow cytometry using an anti-gp120 polyclonal Ab, which was linked to FITC-conjugated strepavidin. Incubation of cells with gp120 resulted in a higher fluorescence signal than cells stained with anti-gp120 in the absence of gp120.

**Table I.** R5-tropic, but not X4-tropic, gp120 induces macrophage CD4 loss

<table>
<thead>
<tr>
<th>Length of Treatment (h)</th>
<th>MnIX Relative to 100% Starting Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R5-gp120</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
</tr>
<tr>
<td>3</td>
<td>32</td>
</tr>
<tr>
<td>18</td>
<td>34</td>
</tr>
</tbody>
</table>

**Table II.** R5-tropic gp120 induces macrophage CD4 loss at low concentration

<table>
<thead>
<tr>
<th>Concentration (μg/ml)</th>
<th>Donor 1</th>
<th>Donor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R5-gp120</td>
<td>gp120Δhom</td>
</tr>
<tr>
<td>Nil</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>0.0625</td>
<td>40</td>
<td>92</td>
</tr>
<tr>
<td>0.125</td>
<td>41</td>
<td>104</td>
</tr>
<tr>
<td>0.25</td>
<td>38</td>
<td>92</td>
</tr>
<tr>
<td>0.5</td>
<td>37</td>
<td>98</td>
</tr>
<tr>
<td>1</td>
<td>35</td>
<td>84</td>
</tr>
<tr>
<td>2</td>
<td>30</td>
<td>84</td>
</tr>
<tr>
<td>4</td>
<td>31</td>
<td>83</td>
</tr>
</tbody>
</table>

**Table III.** gp120 binding does not compete with MT310 anti-CD4 Ab

<table>
<thead>
<tr>
<th>Anti-CD4 Clone Used for Detection</th>
<th>MnIX Relative to 100% Value Obtained Without Preincubation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor 1</td>
</tr>
<tr>
<td>R5-gp120 (1 μg/ml)</td>
<td>100</td>
</tr>
<tr>
<td>gp120 IIIB (1 μg/ml)</td>
<td>102</td>
</tr>
<tr>
<td>Nil</td>
<td>100</td>
</tr>
<tr>
<td>gp120 IIIB (1 μg/ml)</td>
<td>92</td>
</tr>
<tr>
<td>gp120 IIIB (1 μg/ml)</td>
<td>104</td>
</tr>
<tr>
<td>QS4120</td>
<td>98</td>
</tr>
</tbody>
</table>

**Table IV.** R5-tropic gp120-induced CD4 loss is dependent on the presence of CCR5

<table>
<thead>
<tr>
<th>Length of Treatment (h)</th>
<th>MnIX Relative to 100% Starting Value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Donor 1</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
</tr>
<tr>
<td>18</td>
<td>101</td>
</tr>
</tbody>
</table>
R5-tropic gp120 is endocytosed by ccr5-wild-type macrophages in a way distinct from other proteins

Macrophages were stained red for HLA-DR at various time-points after being exposed to FITC-conjugated (green) R5-tropic gp120 or BSA (Data for gp120IIIB is not shown but is similar to that for BSA). After 20–60 min (Fig. 5c) BSA enters the macrophages as numerous, small endocytic vesicles, these persist for many hours but by 2 h there is evidence of breakdown of the BSA-FITC conjugate in the form of a more diffuse green stain. In contrast, R5-tropic gp120 enters as one or two larger and much more brightly staining vesicles from 20 min (Fig. 5b), smaller vesicles only become visible from ~1–2 h (visible at 18 h, Fig. 5d). There is evidence of gp120-FITC conjugate breakdown from 2–3 h. Both proteins are detected in HLA-DR containing compartments (at 3–4 h for BSA and at 2 h for R5-tropic gp120).

R5-tropic gp120 enters cells along with CD4, gp120IIIB and BSA does not

A few large vesicles of R5-tropic gp120 appear in macrophages after 20 min of incubation and these also contain CD4 (Fig. 6a).

FIGURE 4. Confocal micrographs of a macrophage that has been incubated with R5-tropic gp120 for 3 h and then fixed, permeabilized, and stained red for MHC class II (a) and green for CD4 (b). CD4 and MHC are colocalized into the same intracellular compartments (these will include the endoplasmic reticulum and the Golgi apparatus) as indicated by the coincidence of the red and green stains to give a yellow stain (c).

FIGURE 5. Confocal micrographs showing macrophages that have been incubated with FITC-conjugated R5-tropic gp120 or a control FITC-conjugated protein (BSA) for the times indicated then fixed, permeabilized, and stained red for MHC class II. Internalized protein is visible in green.

The vesicles containing endocytosed X4-tropic gp120IIIB (Fig. 6b) or BSA (Fig. 6c) do not contain CD4 at any time-point studied and are smaller, less brightly stained, and more numerous. Such characteristics resemble the appearance of pinocytic vesicles. Cell surface CD4 staining is less diminished and more visible in Fig. 5.

FIGURE 6. Confocal micrographs showing macrophages that have been incubated with FITC-conjugated R5-tropic gp120 (a), gp120IIIB (b) or a control FITC-conjugated protein (BSA, c) for 20 min then fixed, permeabilized, and stained red for CD4. Internalized protein is visible in green. Coincidence of the red and green stains gives a yellow stain. The coincidence of the two stains in a, but not in b or c, is confirmed by software analysis of the image and shown by the resultant intensity plot, which shows the intensity of red and green staining long the line drawn through the image of the cell as indicated.
after incubation with gp120

R5 tropic gp120 induces an up-regulation of mRNA for CCR5 and CD4

Figs. 7 and 8 show that R5-tropic gp120 but not X4-tropic gp120 induces a concomitant up-regulation in CD4 and CCR5 transcript. The increased mRNA is visible from 1 h and persists until ~18 h (overnight) when levels start to fall again.

Surface CD4 loss does not occur on T cells

Incubating with either M- or T-tropic gp120 does not cause a loss of surface CD4 from CD4+ T cells (Table V).

Discussion

It has been reported that HIV-1 gp120 is able to disrupt the function of immune cells (1, 11, 50) and that one of its actions is to cause a loss of cell surface CD4 and the functions associated with this protein (18, 37, 38, 40). Our evidence identifies a novel, CCR5-dependent mechanism of CD4 decline.

We observed a relatively small (~25%) surface CD4 decline induced on macrophages by gp120 (Fig. 2) This X4-tropic gp120-induced response may be similar to the CD4 loss reported by Wahl et al. (38) and Karsten et al. (18). Although CXCR4 is expressed on human macrophages and can, in some circumstances, be used for HIV-1 entry, it appears to be unusable and possibly inaccessible to many T cell line adapted X4-tropic gp120s including gp120, which was used in this study as a prototypic X4-tropic gp120 (51).

When R5-tropic gp120 was used, we observed a cell surface CD4 loss that was more substantial than both previous reports and our observations with X4-tropic gp120. Approximately 75% of the surface CD4 was lost by 3 h after the addition of the gp120. This observation requires that a novel mechanism of CD4 loss be proposed. Further evidence implicates CCR5 binding, in addition...
Table V. gp120 binding does not down-regulate CD4 on T cells

<table>
<thead>
<tr>
<th>Length of Treatment (h)</th>
<th>Donor 1</th>
<th>Donor 2</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>R5-gp120</td>
<td>gp120mum</td>
</tr>
<tr>
<td>0</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>1</td>
<td>96</td>
<td>108</td>
</tr>
<tr>
<td>3</td>
<td>100</td>
<td>86</td>
</tr>
<tr>
<td>18</td>
<td>90</td>
<td>83</td>
</tr>
</tbody>
</table>

to CD4 binding, as a requirement for the operation of this novel mechanism.

The requirement for gp120 to bind to CCR5 to obtain a substantial CD4 loss is suggested by the strain specificity of the effect, with substantial loss only observed when R5-tropic gp120 is used. Further evidence for a CCR5 binding requirement comes from the observation that CD4 loss is not observed in mutant macrophages, which do not express CCR5 (Table II). Binding to CD4 but not CCR5 (by gp120mum, IL-16, or on ccr5-null cells) or binding to CCR5 only (by MIP-1α) does not induce substantial CD4 loss (Table I).

Therefore, substantial CD4 loss by this newly described mechanism requires gp120 to bind to both CD4 and CCR5. We propose that the CD4 loss observed be due to cross-linking of CD4 and CCR5 on the macrophage cell surface followed by endocytosis of the tri-protein complex. Single ligation of CD4 or CCR5 does not produce sufficient cross-linking to allow endocytosis and CD4 loss by this mechanism. An assumption of this model is that it is the same molecule of gp120 that binds to both cell surface receptors. CXCR4 on the macrophage appears to be inaccessible to X4-tropic gp120mum binding in this way: whereas, CCR5 is present on the macrophage cell surface in a form that allows R5-tropic gp120 to bind to both it and CD4. It may be that CD4 and CCR5 are pre-associated in some way on the macrophage cell surface (52, 53) and that this allows molecules of R5-tropic gp120 to bind to these surface molecules and produce the CD4 loss demonstrated. Such a preassociation could explain the apparent selection by HIV-1 of CCR5 for almost exclusive use as a coreceptor for macrophage infection. Alternatively CCR5 and CD4 ligation may be required to cause macrophage specific intracellular signaling events required for the induction of endocytosis. If this were the case and receptor cross-linking was not also a requirement for substantial CD4 loss, one would expect to see macrophage surface CD4 loss induced by coinubation with gp120mum and MIP-1α. Preliminary experiments (data not shown) fail to show this.

The analogous in vivo situation may be more complicated. Gp120 has been found at high levels in the serum of AIDS and AIDS-related complex patients (54). Serum Abs to gp120 may increase the extent of macrophage surface receptor cross-linking, bring the Fc receptor into play, and reduce the CCR5-binding requirement. It has been observed that Abs to gp120 can enhance cell infection (55) by increasing virion-to-cell binding.

Our model of cross-linking-induced endocytosis is strengthened by the confocal images obtained. Figs. 4 and 5 show that R5-tropic gp120 enters macrophages in a form that is different, in terms of size and number of vesicle and localization with CD4, from pino-cytosis of BSA, a protein with no specific cell surface receptor, and endocytosis of gp120mum, a protein that is unable to use both a cell surface receptor and a coreceptor. R5-tropic gp120 enters cells as a few large vesicles, possibly by a process of capping. Endocytosed gp120mum and BSA appears in macrophages in a form that suggests that it entered by a process more akin to pinocytosis. Loss of surface CD4 from CD4+ T cells could not be induced by either gp120. One might have expected X4-tropic gp120mum to cause a loss of T cell surface CD4 by cross-linking CD4 and CXCR4. It may be that despite gp120mum producing TCR cross-linking, T cells lack the cellular machinery for phagocytosis that is present in macrophages.

If our proposed model of cross-linking-induced endocytosis operates in vivo, there are implications for processing and presentation of large amounts of both viral and self-protein entering an APC. The induction of protective and autoimmune responses could be influenced if the endocytosed proteins were finding their way into Ag-presentation pathways and being presented with unusually high efficiency.

Figs. 4, 7, and 8 show that after R5-tropic gp120-induced cell surface loss of CD4 (and presumably CCR5 also), the cellular production of these proteins is stepped up. This is manifested as an up-regulation of mRNA transcript (Figs. 6 and 7) and, certainly in the case of CD4 (Fig. 3) at the level of increased translation of mRNA into intracellular pools of protein. Increased levels of intracellular self-protein must have in vivo implications for the possible breaking of tolerance and the induction of autoimmune responses to these proteins.

Acknowledgments
We thank Marilyn Moore and Donald Innes who initiated the macrophage isolation and culture methods for their assistance in setting up this project; and the Edinburgh center of the Scottish National Blood Transfusion Service for generous provision of buoyf coats and human serum. Invaluable technical assistance in the capture of confocal images was provided by Linda Sharp (Department of Biomedical Sciences, University of Edinburgh).

References


