pronounced selectivity of reconstituted PLM channels for taurine and the increased taurine fluxes in oocytes expressing PLM demonstrate that PLM is involved in membrane ion transport.

FIG. 3a, PLM molecules induce similar currents in Xenopus oocytes. Currents across an excised patch of an oocyte expressing PLM immediately after excision (trace 1) and 2 min later (trace 2). Trace 3 is current through the broken pipette, near 0 mV. PLM expression increases taurine fluxes in Xenopus oocytes. After incubation in $^3$H]taurine for 4 h (short) or 15–18 h (long incubation), oocytes previously injected with PLM RNA or KCl were incubated in a taurine-free hypotonic solution. Radioactivity in the solution and the dried oocytes was then determined. After short incubation, there were significant increases in taurine influx and efflux in oocytes expressing PLM ($P < 0.001$, Mann–Whitney rank sum test). After long incubation, oocytes expressing PLM had significantly higher taurine efflux ($P < 0.001$) but about the same taurine influx (not significant). The simplest interpretation is that PLM expression increased the rates of taurine influx and efflux but did not increase the maximum taurine capacity of the oocytes.

METHODS. Oocytes from Xenopus laevis were collected, injected through the follicular layer with 25–70 nl 150 mM KCl, or 150 mM KCl and 2–40 ng of PLM RNA using methods described previously, and incubated for 24–72 h (refs 4, 25). Patch-clamp experiments were performed by conventional techniques. The bath contained (in mM) KCl 200, EGTA 10, HEPES 10, the electrode containing KCl 50, HEPES 10, CaCl$_2$ 3, MgCl$_2$ 2.0 (pH 7.4), KOH. For taurine flux measurements, groups of 5–17 defolliculated oocytes were placed into a physiological saline solution containing 150 mM NaCl, osmolality 280, and 20 μl ml$^{-1}$ $^3$H]taurine (New England Nuclear). After 4 h (short) or 15–18 h (long incubation), the solution was aspirated, and the oocytes were washed 6 times with 5 ml portions of saline solution without taurine. The oocytes were then placed in modified Barth’s solution (MBS), osmolality 220, for 1 h. The MBS was then collected, and the oocytes washed in 3.5 ml fresh MBS, which was also collected. The MBS and the dried oocytes were placed in separate scintillation vials with 5 ml scintillation cocktail and counts were determined. The counts in the MBS were taken as a measure of taurine efflux, and the sum of the counts in the MBS and in the oocytes was taken as a measure of the taurine influx. Long-incubation experiments were performed with 57 control and 62 PLM-expressing oocytes from 6 frogs; short-incubation experiments were performed with 144 and 99 oocytes from 8 frogs. Data are displayed as the mean of the normalized c.p.m. per oocyte; error bars are 95% confidence intervals.

Follicular dendritic cells and human immunodeficiency virus infectivity

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LARGE amounts of human immunodeficiency virus (HIV) localize on follicular dendritic cells (FDC) in the follicles of secondary lymphoid tissues following viral infection[1,2]. During clinical latency, active viral infection occurs primarily at these sites[3-5]. As HIV on FDC is in the form of immune complexes[6], some of which may be formed with neutralizing antibody, we investigated whether HIV on FDC is infectious. We report here that HIV on FDC is highly infectious. Furthermore, FDC can convert neutralized HIV into an infectious form even in the presence of a vast excess of neutralizing antibody. Thus FDC may provide a mechanism whereby HIV infection can continue in the presence of neutralizing antibody.

We used an in vitro model of the germinal centre, designed to mimic events during clinical latency, to determine whether or not FDC-retained HIV could cause infection. We obtained FDC from the tonsils of non-HIV-infected individuals and incubated...
FIG. 1 Infection of T cells by FDC-trapped HIV-1. CD4⁺ T cells and autologous FDC-bearing HIV immune complexes trapped in vitro were cultured and infection monitored using PCR. Proviral HIV-1 gag (115 bp) DNA was detected where FDC-bearing HIV-1 were used as the only source of virus for infection of the T cells. Lyssates of decreasing numbers of ACH-2 cells, a chronically infected T-cell line containing one copy of proviral DNA per cell, were run in parallel for comparison. The intensity of the signal from T cells infected by FDC-bearing HIV immune complexes appeared to vary between the intensity obtained from non-infected and 2 x 10⁶ ACH-2 cells. As a control, β-globin DNA was amplified simultaneously (bottom). T + SEE, T cells activated with staphylococcal enterotoxin E; FDC - HIV(II); FDC incubated with HIV-linm immune complexes; FDC, control FDC (same donor) not incubated with HIV.

METHODS. Human FDC were obtained from tonsils of non-HIV-infected individuals using a modification of the procedure used for murine FDC⁶,¹³. The modification was to increase the incubation times and enzyme volumes to accommodate the larger mass of tissue present in the tonsils. The resulting FDC show the typical dendritic morphology, structure and function. FDC were further enriched using fluorescence-activated cell sorting (FACS) as described in the Methods. FDC were stained using two (murine IgM, anti-human FDC) monoclonal antibodies: H2 (from M. Nahm¹¹,¹⁵) and DRC-1 (Dako). The FDC preparation was γ-irradiated (3,000 R) before incubation with HIV immune complexes to block proliferation and minimize the ability of the cells to support viral infection. FDC (6 x 10⁴) were incubated overnight (4°C) with HIV immune complexes, formed by incubating (for 2 h at 37°C) 100-µl fresh frozen serum from an HIV-infected individual (as a source of specific antibody and complement) with 100 µl HIV Env cell-free supernatant (5,000 TCID₅₀/µl). This dose of HIV provided sufficient virus for immune complex formation and subsequent trapping by FDC. HIV immune complexes bound to FDC were removed by washing. FDC incubated with HIV immune complexes or control FDC were co-cultured in triplicate in 48-well plates, with FACS-purified, positively selected, autologous CD4⁺ T cells that had been activated overnight with SEE (100 µg/ml; from Toxin Technology). After 4 days of culture, DNA was isolated as described in the Methods and stored at −20°C until tested. PCR analysis for HIV proviral gag and β-globin sequences was performed in the same reaction vessel using primer pairs SK38/39⁶ and GH20/PC04¹⁸, respectively. Control cultures of ACH-2 cells were incubated, diluted as indicated with uninfected H9 cells (these reagents were obtained through the AIDS Research and Reference Reagent Program, Division of AIDS, NIAID, NIH (ARRRP)); ACH-2 was from T. Folkes¹⁸,²⁰ H9 cells were from R. C. Gallo¹¹,²² to provide a constant amount of cellular DNA before isolation for PCR analysis. PCR amplification was performed for 35 cycles (94°C for 2 min for first cycle, 92°C for 1 min all other cycles; then 55°C for 1.5 min; 72°C for 2 min; after cycle 35, an additional 7-min incubation at 72°C was performed). Amplified products (10 µl) were analysed by electrophoresis (2% agarose), stained with ethidium bromide for β-globin DNA detection, and blotted onto Nytran. Blots were probed using 3²⁹-labelled SK19¹⁷. Autoradiography was for 3 d at −80°C. Autoradiograms and Polaroid prints of ethidium bromide-stained gels were scanned at 400 dots per inch (d.p.i.), aligned using Microsoft Power Point, transferred to film and printed on Kodak F5 resin-coated paper.

FIG. 2 T-cell infection by HIV-1 trapped on murine FDC in vivo. Xeno- geneic (murine) FDC bearing HIV immune complexes trapped in vivo were cultured with human CD4⁺ T cells and infection monitored using PCR. Infection of human T cells was clearly evident in cultures where FDC bearing in vivo trapped HIV were used as the sole source of virus (T + SEE + FDC – HIV (trapped in vivo)). Infection was also detected when control human FDC (no trapped HIV) were incubated with human T cells in the presence of exogenous HIV (5,000 TCID₅₀) and this signal appeared slightly more intense than that obtained from the FDC with in vivo trapped HIV. In contrast, no proviral DNA signal was obtained when the same FDC bearing virus were cultured with resting human T cells (T + FDC – HIV (trapped in vivo)), indicating the necessity for T-cell activation to support infection by the trapped virus. No proviral DNA signal was observed in control cultures of either the human T cells alone or the murine FDC-bearing HIV trapped in vivo. ACH-2 (5 x 10⁴) were included as a positive control. The ability of HIV on xenogeneic FDC to infect human T cells was not unexpected as there is a priori reason why gp120 on FDC-trapped virus should not interact with CD4 present on human T cells. Furthermore, murine dendritic cells (DC, not to be confused with FDC) have also successfully infected human T cells in vitro⁶,¹³.

METHODS. HIV immune complexes were formed and trapped on FDC in vivo by injection (sc) of 1.2 mg per mouse, murine anti-gp120 mAb followed 2 h later by exposure to 600 R γ-irradiation to eliminate radiation-sensitive lymphocytes and thus enrich for radiation-resistant FDC⁶,¹³. One day later, mice received injections of 5,000 TCID₅₀ HIVIII in the feet and behind the neck to distribute the virus to FDC in several lymph nodes. This dose of HIV resulted in consistent binding of HIV on FDC in draining lymph nodes. Five days later, when antigens including virus are confined to murine FDC⁶, the mice were killed and the FDC isolated as described in the Methods. These preparations contain 25-45% FDC, with the remaining cells comprising equal numbers of T and B lymphocytes. Human peripheral blood CD4⁺ T cells were obtained from normal donors and enriched on lymphocyte-separation medium (Organon Technika), followed by sorting on the magnetic-activated cell sorter (MACS) using positive selection with anti-CD3 and anti-CD4 magnetic beads (Micro-
FIG. 3  FDC mediate HIV infection of T cells in the presence of neutralizing antibody. PCR amplification of HIV proviral DNA (top and control β-globin DNA (bottom) from cultures of SEE-activated CD4⁺ T cells (T+SEE) and HIV complexes(HIV-IC) formed with various doses of neutralizing antibody in the absence (a) or presence of human FDC (b and c). Infection was confirmed by electron microscopy (d). a. Infection of SEE-activated CD4⁺ T cells by HIV-1mib immune complexes formed with 1 μg of neutralizing anti-gp120 (H902) was evident, whereas infection was blocked when viral complexes were formed using 1 ng, 1 μg and 1 mg antibody. Signal intensity is compared with 50,000 ACH-2 cells. b and c (left). Addition of FDC to cultures reverses the effect of neutralizing antibody even in vast excess of the 1 ng needed to block infection when FDC were not present. c. right panel. Tonsilar macrophages (MO) and tonsilar cells depleted of FDC (FDC dep) could not substitute for FDC in promoting infection by HIV-IC formed with 1 ng of neutralizing antibody. Addition of anti-CD4 blocked infection even when FDC were present in cultures (+aCD4). d. Electron micrograph of a small portion of an infected cell from an FDC-T-cell culture containing HIV and 1 ng H902. Note the numerous HIV virions budding from the cell surface (magnification, ×54,000).

**METHODS.** All cells were isolated from human tonsils as described previously. SEE-activated T cells (5 × 10⁴) were incubated at 37 °C for 1 h with HIV immune complexes formed using the indicated dose of neutralizing murine anti-HIV-1mib gp120 (the reagent, hybridoma 902 (anti-gp120) from B. Chesebro was obtained through the ARRRP) and 5,000 TOID₅₀ HIV₄₅. This dose of HIV was used to provide a high level of virus-anti-virus immune complexes, comparable to that which might exist on FDC in vivo. All cultures were performed in triplicate and PCR analysis was as for Fig. 1. MACS was used to prepare tonsilar macrophages (positively selected using anti-CD14 Microbeads) and to remove FDC from tonsilar cells (using HJ2 followed by biotin-conjugated, rat anti-murine IgM and streptavidin Microbeads). Anti-CD4 (Leu 3a + 3b; Becton Dickinson) was added to cultures at a dose of 1 μg. Cells for electron microscope evaluation were prepared using tannic acid during fixation.
these with HIV immune complexes in vitro. Viral immune complexes not bound to FDC were removed by washing and FDC were cultured with activated, autologous CD4+ T cells. Infection was monitored using the polymerase chain reaction (PCR) to analyse DNA from the cultures. Infection was demonstrated by isolation of proviral HIV-1 DNA from cultures of T cells and FDC-bearing HIV immune complexes, but not from cultures containing control FDC (Fig. 1). Syncytium formation was also observed in cultures of T cells and FDC-bearing HIV immune complexes. HIV binding to FDC was confirmed by electron microscopy, which also revealed the presence of infected cells budding virus (Fig. 3d). Portions of FDC dendritic processes bore 5–10 virus particles extracellularly, which is consistent with in vitro observations. The distribution of HIV virions on FDC processes suggests that a T cell can interact with several virus particles on a single portion of an FDC process.

Trapping of HIV immune complexes on FDC in vitro could differ from trapping in vivo, so we examined the infectivity of HIV trapped on FDC in vitro using a murine model. Previous work has shown that FDC can act as accessory cells to lymphocytes across species (G.F.B., unpublished) and we reasoned that the HIV-1 glycoprotein gp160 on FDC-trapped virus could interact with human CD4 on T cells regardless of FDC species. Antigens, including virus, can be localized on murine FDC in vitro by passive immunization followed by antigen challenge. FDC trapping of HIV was accomplished by passively immunizing mice with HIV-1-specific anti-gp120, and one day later injecting 5,000 TCID50 (half-maximal tissue culture infectious dose) HIV1167 in several sites to allow trapping of HIV immune complexes on FDC in multiple lymph nodes. Five days later, draining lymph nodes were obtained and the FDC isolated. The murine FDC bearing HIV trapped in vitro were co-cultured with activated human T cells obtained from the blood of a normal donor. Infection of human T cells was detected when HIV immune complexes on murine FDC were used as the only source of virus. Transfer of infection by murine FDC also indicated that HIV infection of FDC was not needed because the mouse is a non-permissive host. This model also excluded the possibility that human CD4+ cells in the FDC preparation were needed for transfer of infection.

The monoclonal antibody (H902) used to trap HIV on the murine FDC has been used to neutralize HIV-1167 (ref. 10). The HIV complexes trapped on FDC in vitro were infectious: we therefore reasoned that FDC may be able to reverse the effect of neutralizing antibody. To test this, neutralized HIV-1167 immune complexes were formed in vitro using increasing doses of neutralizing antibody and cultured with activated T cells in the presence or absence of FDC (Fig. 3). One nanogram of antibody consistently neutralized 5,000 TCID50 HIV-1167 (Fig. 3c). Infection occurred when FDC were present at doses of neutralizing antibody ranging from one thousand- to one million-fold above the one nanogram needed to prevent infection in the absence of FDC (Fig. 3b, c). Furthermore, infection was confirmed by electron microscopy which showed infected cells budding virus (Fig. 3d). Tonsilar macrophages and tonsilar cells specifically depleted of FDC failed to promote infection by neutralized HIV-1 immune complexes (Fig. 3c). Anti-CD4 in cultures of HIV immune complexes, T cells and FDC blocked infection, confirming the importance of the surface marker CD4. Increasing amounts of neutralizing antibody reduced the amount of proviral HIV-1 gag DNA detected, indicating that even though antibody did not block HIV on FDC, it did appear to decrease infection (Fig. 3b, c).

Immune complexes were formed with laboratory isolates HIV1167, HIV-1MN, and HIV-1392w, the primary isolate 301714, and human neutralizing monoclonal antibodies (F105 or IgG1b12), in order to determine the general nature of infection by neutralized HIV on FDC (Fig. 4). In the absence of FDC, HIV-1 immune complexes were unable to infect T cells regardless of the strain of HIV-1 used. But, when FDC were present, neutralized virus was able to infect the T cells, indicating that this FDC-mediated process was not restricted to a single antibody or HIV-1 strain. It is noteworthy that the laboratory isolate SF2, which is easily neutralized by antibody, is rendered infectious when FDC are present. Thus under conditions where antibody is particularly efficient at neutralizing virus, FDC can still negate its effect—suggesting that this FDC activity is very potent. The ability of FDC to facilitate HIV infection in the presence of neutralizing antibody may help to explain why some individuals with a high titre of neutralizing antibody have ongoing infection. It may also help to explain why much of the viral replication occurring during the clinically latent stage of HIV infection is confined to lymphoid follicles where virus-laden FDC reside in intimate contact with germinal centre T and B.

**FIG. 4** FDC-mediated infection by neutralized HIV-1 immune complexes is not restricted to viral strain or antibody. PCR analysis of HIV-1 gag proviral DNA (115 bp) and β-globin in cultures containing various strains of neutralized HIV-1 immune complexes (HIV-1C) and SEE-activated T cells (T + SEE) (2.6 × 10^4) or SEE-activated T cells and human FDC (5 × 10^4). Infection by HIV-1 immune complexes formed with human, neutralizing anti-gp120 (1 μg F105 or 20 μg IgG1 b12) was blocked in the absence of FDC with HIV-1 strains IIIB, MN and SF2 as reported (ARRRP data sheet) and with the primary strain 301714. In contrast, infection was apparent in cultures when FDC were present, indicating that the FDCs ability to reverse the effect of neutralizing antibody was not restricted to a single strain of virus or monoclonal antibody.

**METHODS.** Cultures of SEE-activated T cells and MACS-sorted FDC were incubated with HIV-1 immune complexes assessed for infection as described for Fig. 3, with the exception that the HIV immune complexes were formed using HIV-1 strains (IIIB, MN and SF2) (obtained through the ARRRP: strains IIIB and MN from R. Gallo25, 26, 27, strain SF2 from J. Levy28) mixed with 1 μg human anti-HIV-1 gp120 (obtained through the ARRRP: HIV-1 gp120 monoclonal antibody (F105) from M. Posner27, 28) Immune complexes were also prepared using primary isolate 301714 (obtained from the ARRRP) and monoclonal antibody b12 (ref. 29; from D. R. Burton and P. Parren).
cells. Although the mechanism for converting neutralized virus into an infectious form by FDC is unclear, there are similarities to events that occur in anamnestic responses, in which antigens are trapped by FDC in the form of immune complexes. Even though antibody should 'mask' epitopes and prevent recognition by the immunoglobulin receptors of B cells, experiments have shown that antigen-specific B cells are able to recognize the immune-complexed antigen on FDC or icosomes even in vast antibody excesses.\(^1,2\)

We envisage a similar process in the case of FDC-trapped HIV. The V3 loop of HIV-1 gp160 would potentially be 'masked' by neutralizing antibody, but the FDC can display the virus in such a manner that gp120 can bind CD4 on adjacent T cells and thus cause infection. These possible mechanisms for negating the effect of neutralizing antibody are being actively pursued. Nevertheless, the finding that FDC can convert neutralized HIV immune complexes into an infectious form may have important implications for the design of therapeutic and vaccine strategies, regardless of the mechanism(s) involved.

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**Behavioural and cardiovascular effects of disrupting the angiotensin II type-2 receptor gene in mice**

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ANGIOTENSIN II, a potent regulator of blood pressure and of water and electrolyte balance, binds to two different G-protein-coupled receptors. The type-1 receptor (AT\(_1\)) mediates the vasoactive and aldosterone-secreting effects of angiotensin II, but the function of the type-2 receptor (AT\(_2\); refs 1, 2) is unknown, although it is expressed in both adult and embryonic life. To address this question, we have generated mice lacking the gene encoding the AT\(_2\) receptor. Mutant mice develop normally, but have an impaired drinking response to water deprivation as well as a reduction in spontaneous movements. Their baseline blood pressure is normal, but they show an increased vasopressor response to injection of angiotensin II. Thus, although the AT\(_2\) receptor is not required for embryonic development, it plays a role in the central nervous system and cardiovascular functions that are mediated by the renin–angiotensin system.

The angiotensin II AT\(_2\) receptor gene (Agr2) on the X chromosome\(^2\) was disrupted in embryonic stem (ES) cells to generate mice deficient in this receptor subtype (Fig. 1). Crossing heterozygous female mice with a male germ-line-transmitting chimera gave 24/94 non-mutant (+/Y), 21/94 heterozygous (−/ +), 28/94 homozygous (−/−) and 21/94 hemizygous (−/ Y) mutant mice. This distribution does not deviate significantly from mendelian prediction (1/4:1/4:1/4:1/4; \(\chi^2 = 1.405, P > 0.05\)), indicating that there was no increase in embryonic or perinatal mortality in mice of the Agr2−/− Agr2−/Y genotype. Mice carrying the mutated Agr2 allele apparently developed normally. Histological sections of heart, lung, kidney, adrenal, spleen, brain, aorta, ovary, uterus, pancreas, eye, skeletal muscle and blood did not reveal any differences in morphology between non-mutant and hemizygous or homozygous mutant mice (data not shown). The skeletal system was differentially stained with alizarin red S for bone and alcin blue for cartilage in 1-day-old Agr2−/− mutant mice and showed no sign of malformation or retarded ossification (data not shown). Furthermore, homozygous mutant females and hemizygous mutant males (age 8–16 weeks) produced healthy litters of normal size (average size of litter: 4.9 ± 0.5 (Agr2−/− × Agr2−/− or Agr2−/− × Agr2−/Y); n = 7) versus 6.0 ± 0.5 (Agr2−/− × Agr2−/− Y; n = 15); \(P < 0.05\).

To demonstrate that functional AT\(_2\) receptors were missing in Agr2−/− mutant mice, their RNA was analysed and radioligand binding experiments were done on membranes prepared from whole mouse fetuses (minus placenta) on day 18.5 of embryonic development (E18.5). In normal rat embryos, the level of AT\(_2\) receptor expression is maximal a few days before birth.\(^3\) We detected no intact AT\(_2\) messenger RNA in hemizygous or homozygous Agr2−/− mutant mice (Fig. 1c). Binding studies confirmed the expression of AT\(_2\) receptors in non-mutant E18.5 mice. The amount of bound ligand (275 ± 21 fmol mg\(^{-1}\) (n = 6) and females 703 ± 29 fmol mg\(^{-1}\) (Fig. 1d). In heterozygous Agr2−/− female embryos, AT\(_2\) receptor expression was 395 ± 38 fmol mg\(^{-1}\) (n = 8), approximately half that in non-mutant mice; in homozygous female or hemizygous male fetuses, none was detected (Fig. 1d). Expression of AT\(_2\) receptors did not differ between non-mutant mice and mice carrying the mutated Agr2 allele (data not shown).

Agr2−/− mice influence several central nervous system functions, including dopaminergic, activation of the sympathetic nervous system, modulation of hormone release from the pituitary, interference with learning and motor activity, and regulation of body temperature and angio genesis. Although AT\(_1\) and AT\(_2\) receptors may both participate in the dopaminergic response to angiotensin II (refs 9–12), the function of AT\(_1\) is unclear.\(^10,11\)

We therefore examined the drinking behaviour of knockout mice and found no difference in daily water intake compared with