

1 **The Engagement of Activating Fcγ Receptors Inhibits Primate Lentivirus**

2 **Replication in Human Macrophages**

3 Annie David*, Asier Sáez-Ciri3n*, Pierre Versmisse*, Odile Malbec^{†¶}, Bruno Iannascoli^{†¶},
4 Florence Herschke*, Marianne Lucas[‡], Françoise Barré-Sinoussi*, Jean-François Mouscadet[§],
5 Marc Daéron^{†¶} and Gianfranco Pancino*

6 * Institut Pasteur, Unité de Régulations des Infections Rétrovirales, Paris, France ; [†] Institut

7 Pasteur, Unité d'Allergologie Moléculaire et Cellulaire, Paris, France ; [¶] INSERM U760,

8 Paris, France ; [‡] Institut Pasteur, Unité Postulante Interactions Moléculaires Flavivirus-Hôtes,

9 Paris, France ; [§]LBPA- CNRS UMR 8113, Ecole Normale Supérieure de Cachan, Cachan,

10 France.

11
12 Running title: Lentivirus inhibition in FcγR-activated macrophages

13
14 Key words: HIV-1, macrophages, Fc Receptors, Cell Activation, Lentiviruses

15
16 Corresponding author:

17 Gianfranco Pancino, e-mail : gpancino@pasteur.fr

18
19
20 **Footnote:** This study was supported by the Agence Nationale de Recherches sur le SIDA
21 (ANRS), France

22

1 Abstract

2 We previously reported that the stimulation of monocyte-derived macrophages
3 (MDM) by plate-bound intravenous immune globulins (IVIg) inhibits HIV-1 replication
4 (Perez-Bercoff et al, J Virol, 2003, 77:4081). Here, we show that IgG immune complexes also
5 suppress HIV-1 replication in MDMs and that activating receptors for the Fc portion of IgG -
6 Fc γ RI, Fc γ RIIA and Fc γ RIII are responsible for the inhibition. MDM stimulation through
7 Fc γ Rs induces activation signals and the secretion of HIV-1 modulatory cytokines, such as
8 M-CSF, TNF- α and MDC. However, none of these cytokines contribute to HIV-1
9 suppression. HIV-1 entry and post-integration steps of viral replication are not affected,
10 whereas reduced levels of reverse transcription products and of integrated proviruses, as
11 determined by real time PCR analysis, account for the suppression of HIV-1 gene expression
12 in Fc γ R-activated MDMs. We found that Fc γ R-dependent activation of MDMs also inhibits
13 the replication of HIV-2, SIVmac and SIVagm, suggesting a common control mechanism for
14 primate immunodeficiency lentiviruses in activated macrophages.

15

1 **Introduction**

2
3 Unlike other retroviruses, lentiviruses can integrate their DNA into the genome of
4 non-dividing cells and can therefore replicate in monocytes and macrophages. HIV and SIV
5 infection of monocytes and differentiated tissue-resident macrophages may play a major role
6 in viral transmission, dissemination and persistence (1-4). The capacity of monocytes and
7 macrophages to migrate in tissues makes them potential conveyors of HIV and SIV
8 infections. Monocytes are thought to carry the virus to the central nervous system, and the
9 expansion of subsets of activated monocytes has been associated with neurological diseases in
10 AIDS (5, 6). Virions generated in infected macrophages are more efficient in establishing
11 lymphocyte infection than cell-free virions (7). Macrophages may also favor cell-to-cell
12 transmission to CD4 T cells by producing chemotactic cytokines and by interacting with cells
13 during antigen presentation (8). In addition, HIV-1-infected macrophages can also induce the
14 apoptosis of uninfected bystander T cells and neuronal cells (9). Finally, infected monocytes
15 and macrophages may act as viral reservoirs for HIV and SIV and be the main source of virus
16 production during the late stages of disease in pathogenic infections when the numbers of
17 CD4 T cells are substantially reduced (10-14).

18 Macrophages play a major role in mounting innate and adaptive immune responses to
19 pathogens. Macrophages react to HIV-1 infection by secreting cytokines, chemokines and
20 other molecules having anti-viral activity or can directly control HIV-1 replication (15).
21 However, HIV-1 infection may affect essential macrophage functions, such as antigen
22 presentation, intracellular killing and phagocytosis (16). Therefore, the regulation of HIV-1
23 and related lentivirus replication in monocytes and macrophages might affect the host
24 susceptibility to infection and could help to control viral dissemination and pathogenesis in
25 infected individuals. In a SCID mouse model, virus spread and pathology was abolished by
26 suppressing macrophage infection with anti-nerve growth factor antibodies (17).

1 We previously showed that the incubation of macrophages with IVIg bound on culture
2 plates potently inhibit HIV-1 replication independently of viral tropism (18). Inhibition was
3 not observed when macrophages were incubated with IVIg-F(ab')₂ fragments suggesting that
4 it was mediated by receptor(s) for the Fc portion of IgG (FcγR) (18). FcγRs are a group of
5 integral membrane proteins that bind to the Fc portion of IgG (19), and which can either
6 activate or inhibit cell activation when engaged by IgG immune complexes. Activating FcγRs
7 include the high-affinity receptor FcγRI (CD64), which can bind monomeric IgG, and the
8 low-affinity receptors FcγRIIA/C (CD32) and FcγRIIIA (CD16), which do not bind
9 monomeric IgG but bind IgG aggregates and antigen-antibody immune complexes (ICs) with
10 a high avidity. Activating FcγRs possess immunoreceptor tyrosine-based activation motifs
11 (ITAMs) that become phosphorylated upon FcγR clustering. ITAM phosphorylation promotes
12 the recruitment of cytosolic protein tyrosine kinases. These kinases phosphorylate other
13 proteins involved in signaling pathways, leading to the activation of PI-3K and MAP kinases
14 (19). Inhibitory FcγRs consist of FcγRIIB, which contains an immunoreceptor tyrosine-based
15 inhibition motif (ITIM). This motif enables FcγRIIB to negatively regulate cell activation
16 triggered by ITAM-containing receptors when co-engaged with them.

17 In this study we aimed at identifying which FcγR(s) is(are) involved in viral inhibition.
18 We quantitatively analyzed FcγR-mediated inhibition of HIV-1 replication using real time
19 PCR. We also determined whether FcγR-mediated inhibition was limited to HIV-1 or was a
20 general anti-retroviral mechanism by studying the effect of FcγR cross-linking on the
21 replication of other primate lentiviruses in human macrophages.

22

23 **Materials and Methods**

24 *Monocyte derived macrophages (MDM)*

1 Human monocytes were isolated from buffy coats of healthy seronegative donors (Centre de
2 Transfusion Sanguine Ile-de-France, Rungis and Hôpital de la Pitié-Salpêtrière, Paris, France)
3 using lymphocyte separation medium (PAA laboratories GmbH, Haidmannweg) density
4 gradient centrifugation and plastic adherence as previously described (18). Monocytes were
5 then differentiated into macrophages by seven to 11 days culture in MDM medium (RPMI
6 1640 medium supplemented with 200 mM L-glutamine, 100 U penicillin, 100 µg
7 streptomycin, 10 mM HEPES, 10 mM sodium pyruvate, 50 µM β-mercaptoethanol, 1%
8 minimum essential medium vitamins, and 1% nonessential amino acids) supplemented with
9 15% of human AB serum in hydrophobic Teflon dishes (LumoxTM. D Dutcher, Brumath,
10 France) as previously described (18). Monocyte derived macrophages (MDM) were then
11 harvested, washed and resuspended in MDM medium containing 10% heat-inactivated fetal
12 calf serum (FCS) for experiments. The purity of CD14⁺ macrophages was usually more than
13 95% as assessed by immunofluorescent staining and flow cytometry analysis. FcγRI (CD64),
14 FcγRII (CD32) and FcγRIII (CD16) were all expressed on the MDM surface but the
15 proportion of cells expressing each receptor varied with different donors and MDM
16 preparations.

17 LPS content in the media and all the reagents used for culture and stimulation of MDM was
18 below the limit of detection of the QCL1000 Limulus amoebocyte lysate test (LAL)
19 (BioWhittaker, France).

20 *Antibodies*

21 Monoclonal Abs (MAbs) against FcγRs (CD64, clone 32.2; CD32, clones IV.3 and AT.10;
22 CD16, clone 3G8) were purified from hybridoma supernatants. F(ab')₂ from MAbs was
23 generated by pepsin digestion (ImmunoPure F(ab')₂ Preparation kit from PIERCE). Anti
24 CD64 F(ab')₂ 10.1 was from Ancell (COGER, France). Isotype-matched uncoupled or FITC-
25 or PE-coupled irrelevant control MAbs or F(ab')₂ were from SIGMA (St Quentin Fallavier,

1 France). All F(ab')₂ preparations used for stimulating MDM were passed through a polymixin
2 B-column (Detoxi-Gel Endotoxin Removing Gel, PIERCE, Perbio Science France, Brebières,
3 France) to eliminate potential endotoxin contamination and were then verified as LPS-free by
4 the LAL test.

5 Human FcγRIIA-specific and FcγRIIB-specific polyclonal antibodies were generated in
6 rabbits immunized with GST fusion proteins containing the intracytoplasmic domain of either
7 human FcγRIIA (ICIIA) or FcγRIIB2 (ICIIB). Briefly, cDNAs encoding the intracytoplasmic
8 domains of human FcγRIIA and FcγRIIB2 were amplified by PCR using the following
9 primers: FcγRIIA, *forward*: CGCGGATCCGCGAATTCCACTGATCCTGTGAAG;
10 *reverse*: CGGAATTCGTTAGTTATTACTGTTGACATGGTC;
11 FcγRIIB2, *forward*: GCGGATCCGCGAATCCACTAATCCTGATGAG;
12 *reverse*: CGGAATTCCTAAATACGGTTCTGGTCATC.

13 Purified amplicons were ligated into the PGEX4T1 vector (Pharmacia Biotech 27-4580-01)
14 and expressed in *E. coli* DH5α cells. GST-ICIIA and GST-ICIIB were purified on glutathion-
15 agarose gel and were then used to immunize rabbits (one 200 μg injection in complete
16 Freund's adjuvant followed three weeks later by three 200 μg injections in incomplete
17 Freund's adjuvant every two weeks). Serum IgG were purified by affinity chromatography on
18 Protein A-sepharose (Pharmacia). IgG from rabbits immunized with GST-ICIIA were
19 absorbed by two passages through GST-ICIIB-coated sepharose 4B beads (Pharmacia),
20 whereas IgG from rabbits immunized with GST-ICIIB were absorbed by passage through
21 GST-ICIIA-coated sepharose 4B beads, to remove anti-GST and any possibly cross-reacting
22 antibodies. Anti-FcγRIIA Abs recognized FcγRIIA but not FcγRIIB in rat basophilic
23 leukemia (RBL) transfectants, whereas anti-FcγRIIB Abs recognized FcγRIIB but not
24 FcγRIIA in the same transfectants, as assessed by western blotting analysis and intracellular
25 immunofluorescence.

1 The above described FcγRIIA and FcγRIIB2 specific primers were used to analyze the
2 receptor transcripts in RNA preparations from MDM by RT-PCR. RNA was extracted using
3 RNeasy Kit (Qiagen, France). cDNA was synthesized from 0.5 μg total cell RNA using
4 Taqman Reverse Transcription Reagents Kit (Applied Biosystem). 1/5, 1/50 or 1/500 of the
5 volume of the reaction mixture were used for PCR amplification (30 cycles), using Taq
6 (Invitrogen,) in a GeneAmp PCR9700 (Applied Biosystems). The PCR products were
7 analyzed by gel electrophoresis on 2% agarose gel.

8 Anti-TNF-α rabbit IgG (gift of J-M. Cavillon, Institut Pasteur), anti-M-CSF goat IgG or
9 anti-MDC chicken IgYs (both from R&D systems, Minneapolis, MN) were used for TNF-α,
10 M-CSF or MDC neutralization respectively. Isotypic controls were rabbit, goat or chicken
11 irrelevant Abs. Commercial Abs were detoxified by passage through polymixin B columns
12 before utilization. TNF-α, M-CSF and MDC levels in culture supernatants were measured by
13 using quantikine ELISAs (R&D systems).

14 The following Abs were also used: unconjugated Mouse anti-phosphotyrosine mAb 4G10:
15 purified from hybridoma supernatant on Protein G-sepharose; Rabbit anti-phospho-PLC-
16 gamma1(tyrosine-783) Abs: Santa Cruz (Santa Cruz, CA); Rabbit anti-erk1/2 and rabbit anti-
17 phospho-erk1/2 (Thr202/tyr204) Abs: Cell Signaling (Beverly, MA); fluorochrome-
18 conjugated CD11b-PE (clone Bear1) and CD4-PE (clone 13B8.2) (both from Beckman
19 Coulter), CD14-FITC (clone Leu M3) and CD3-FITC (clone Leu3) (both from Becton
20 Dickinson, San Jose, Calif.).

21 *Flow cytometry analysis*

22 Cells were stained either with FITC-conjugated or PE-conjugated mAbs or with unconjugatd
23 mAbs or F(ab')₂ fragments followed by secondary FITC-goat anti-mouse IgG F(ab')₂ or
24 FITC-goat anti-mouse Fab F(ab')₂ (Immunotech) and analyzed using a FACSCalibur flow
25 cytometer (Beckman Coulter).

1 *Immunoblotting*

2 For tyrosine phosphorylation analysis, cells were lysed by three cycles of incubation for 1 min
3 in liquid nitrogen followed by 1 min at 37°C in lysis buffer at pH 8.0 (50 mM Tris, pH8, 150
4 mM NaCl, 1% Tx100, 1 mM Na₃VO₄, 5 mM NaF, 10 µg/ml aprotinin, 10 µg/ml leupeptin, 10
5 µg/ml pepstatin and 1mM PMSF). For FcγRII expression analysis, cells were lysed by boiling
6 5 min in 10 mM Tris pH 7.4, 1% SDS. Proteins were quantified using a Biorad protein assay
7 (Hercules, CA). 40 µg proteins for tyrosine phosphorylation analysis or 10 µg proteins for
8 FcγRII analysis were boiled in sample buffer, fractionated by SDS-PAGE and then
9 transferred onto Immobilon-P membranes (Millipore, Bedford, MA). The membranes were
10 saturated with either 5% BSA (Sigma Chemical Co) or 5% skimmed milk (Régilait, Saint-
11 Martin-Belle-Roche, France) diluted in western buffer (150 mM NaCl, 10 mM Tris and 0.5%
12 Tween 20 (Merk, Schuchardt, Germany) pH 7.4) and incubated with the indicated antibodies
13 and then incubated with HRP-conjugated goat anti-rabbit or goat anti-mouse Ig antibodies.
14 Labeled antibodies were detected using an ECL kit (Amersham Pharmacia biotech, Little
15 Chalfont, Buckinghamshire). Blots for FcγRII analysis were first probed with the anti-
16 FcγRIIB antibody, then stripped with stripping buffer (reblot+, Chemicon, Temecula,CA) as
17 indicated by the manufacturer, and re-probed with the anti-FcγRIIA antibody.

18

19 *MDM stimulation*

20 MDM were stimulated using three different methods.

21 (1) Immobilized IVIg stimulation was carried out with hIgG for therapeutic use (IVIg)
22 (Endobuline, BAXTER, Maurepas, France) (0.1 mg/ml in PBS) as previously described (18).

23 (2) Stimulation with preformed immune complexes (ICs) was as follows: dinitrophenyl
24 (DNP) groups were conjugated to LPS-free bovine serum albumin (BSA) (SIGMA) using
25 dinitrobenzene sulfonate (DNBS, Eastman Kodak) in alkaline medium and then dialyzed

1 against PBS. Culture plates were coated with 0.1 mg/ml DNP-BSA antigen by incubation for
2 2 hours at 37°C, washed with PBS, saturated by incubation with 1 mg/ml BSA in PBS for 30
3 minutes at 37°C and then incubated with 30 µg/ml rabbit anti-DNP antibodies (SIGMA) for 1
4 hour at 37°C to form ICs. MDMs were stimulated by plating on IC coated wells.

5 In some experiments 3 µm polystyrene beads (Polysciences Inc., France) opsonized with
6 DNP-anti-DNP ICs were used for MDM stimulation. Polystyrene beads were absorbed with
7 400 µg/ml DNP-BSA in PBS according to manufacturer instructions, washed, and incubated
8 with 100 µg/ml rabbit anti-DNP antibodies in PBS-BSA (IC-beads) or with PBS-BSA alone
9 (Ag-beads). MDM were plated on 96-well plates and incubated with 100 µl medium
10 containing IC-beads or Ag-beads at a bead:cell ratio of 10:1 and 30:1 and immediately
11 infected.

12 (3) Each FcγR was separately cross-linked on an MDM surface by incubating MDMs with
13 the appropriate specific F(ab')₂ (5 µg F(ab')₂ per 10⁶ MDM) for 30 min at 4°C. The MDMs
14 were then washed with PBS and seeded on plates previously coated with 0.2 mg/ml goat anti-
15 mouse Fab F(ab')₂ (SIGMA) and saturated with 1 mg/ml BSA. MDMs were incubated in
16 parallel with irrelevant mouse F(ab')₂ as controls.

17 Cell viability was not affected in IgG or IC-stimulated MDM cultures, as evaluated by a
18 WST-1-based colorimetric assay (not shown).

19 *Viruses and MDM infection*

20 HIV-1 infections: The following viral strains were used for productive infections: HIV-1_{Bal},
21 HIV-2_{SBL}, SIVmac251, SIVagmGril. Strains were propagated in PHA-activated human
22 PBMCs (except SIVagm, propagated on SupT1 cells) and the culture supernatants were
23 collected at times of peak p24 (HIV-1) or p27 (HIV-2, SIV) production. p24 and p27 were
24 measured with commercial ELISA kits (Beckman Coulter, Paris, France). Viral stocks were

1 titrated on PHA-activated human PBMCs except SIV_{agm}, which was titrated on SupT1 cells.
2 Multiplicities of infection (m.o.i.) used in this study were between 10^{-2} and 2×10^{-2} .
3 For single-round infections, HIV-1 particles containing the *luc* reporter gene and pseudotyped
4 with the VSV-G envelope protein (HIV-1_{VSV-G}) that allows HIV-receptor independent entry
5 into cells were used. HIV-1_{VSV-G} virions were produced by transiently co-transfecting
6 (SuperFect, Qiagen GmbH, Hilden, Germany) 293T cells with the proviral pNL-Luc-E_R⁺ (20)
7 vector and the VSV-G expression vector pCMV-G, as previously described (18).
8 Supernatants were harvested 72 h after transfection, and p24_{gag} levels were measured using a
9 commercial ELISA kit (Beckman Coulter, Paris, France) with MDMs. Between 3×10^{-1} and 3
10 $\times 10^{-2}$ m.o.i. were used for MDM infection. Mock infections with equivalent amounts of p24
11 from supernatants from 293T cells transfected with pNL-Luc-E_R⁺ only were carried out in
12 parallel as controls.

13 MDMs (0.8×10^5 - 1×10^5 cells/well in 96 well plates or 10^6 cells/well in 12 well plates) were
14 infected either with viral strains or with pseudotyped particles by incubating cell with viral
15 inoculum 1h at 37°C, or by a spinoculation protocol (1h centrifugation at room temperature at
16 1200 x g followed by 1h incubation at 37°C), to increase the efficiency of infection (21).
17 MDMs were then washed with PBS and cultured in MDM medium.

18 In the experiments for detecting HIV DNA by PCR, HIV-1_{VSV-G} preparations were previously
19 treated with DNase I (Roche Diagnostics GmbH, Mannheim, Germany).

20 In cytokine/chemokine neutralization experiments, immobilized-IgG-stimulated or
21 unstimulated MDMs were infected in triplicate with HIV-1_{Bal}, and then cultured in 96 well-
22 plates in the presence of neutralizing concentrations of the each specific Ab (anti-TNF- α ,
23 1:150 dilution; anti-M-CSF, 7,5 μ g/ml, anti-MDC, 10 μ g/ml) or equal concentrations of the
24 appropriate control Ab. Half culture supernatant was changed with fresh medium containing
25 the appropriate concentrations of each Ab each 2 days.

1 WN virus infection: Production of WN virus strain IS-98-ST1 (GenBank accession number
2 AF 481864) from mosquito *Aedes pseudoscutellaris* AP61 cell monolayers and virus titration
3 on AP61 cells by focus immunodetection assay (FAI) were performed as previously
4 described (22). Infectivity titres were expressed as focus forming units (FFU). RPMI medium
5 supplemented with 2% FCS was used for washing, infection and culture. MDM were washed
6 three times and infected with 1 m.o.i of WN virus for 1 h at 37°C. MDM were then washed
7 twice and incubated at 37°C for 72 hours, then cell culture supernatants were harvested and
8 processed for viral titration. As a control for inhibition of WN virus replication, MDM were
9 exposed to 10 IU/ml human recombinant IFN- α A/D (Biosource, France), during and after
10 infection.

11 *Measure of luciferase activity in cell lysates*

12 At various times after infection with pseudotyped HIV-1 virions, each well of MDMs was
13 lysed with 100 μ l of luciferase cell culture lysis reagent (Promega France, Charbonnières,
14 France). The luciferase activity was quantified in 20 μ l of each lysate using the Promega
15 Luciferase reporter 1000 Assay System and an LUMAT LB9501 luminometer (Berthold
16 Technologies).

17 *Real time PCR quantification of HIV-1 cDNA forms*

18 At different times after infection, MDMs were washed in PBS and total DNA was extracted
19 using the DNeasy Tissue Kit (Qiagen). The HIV-1 DNA forms R-U5, U5-Gag and 2-LTR
20 were quantified using real time PCR with an ABI PRISM 7000 instrument (Applied
21 Biosystems Applera France, Courtaboeuf, France). For all the real-time PCR, we used 100 ng
22 of template DNA per reaction, corresponding to about 2×10^4 MDMs. DNA loading was
23 controlled by concurrently amplifying the albumin gene by real-time PCR and quantifying
24 with reference to a control human genomic DNA (Roche). The reaction mixture contained 1X
25 Taqman Universal PCR master mix, 300 nM of each primer (except R-U5 primers, 200 nM)

1 and 200 nM of the appropriate fluorogenic probe, in a final volume of 30 μ l. PCR cycle
2 conditions were: 50°C for 2 min, 95°C for 10 min and 40 cycles of 95°C for 15 sec and 60°C
3 for 1 min. Copy numbers of R-U5 and U5-Gag were determined with reference to a standard
4 curve prepared by concurrent amplification of serial dilutions of 8E5 cells containing one
5 integrated copy of HIV-1 per cell (23). The copy number of 2-LTR was determined with
6 reference to standard curves generated by serial dilutions of CEM cells infected with HIV-
7 1_{NL4-3}. The number of 2-LTR copies/per CEM cell was previously quantified against a
8 standard curve generated by dilution of cloned DNA with matching sequences (pSLL-IIIb,
9 gift of Audrey Brussel, Institut Pasteur, Paris, France) (24). R-U5 primers are described
10 elsewhere (25), the probe was (FAM)-AGACGGGCACACACTA-(MGB). Primers and
11 probes for U5-Gag (26), 2-LTR (27) and albumin (28) have been reported.

12 Integrated HIV-1 DNA was quantified by real time *Alu*-Gag nested PCR using primers and
13 probes supplied by Norio Yamamoto, Tokyo Medical and Dental University, Tokyo, Japan.
14 The first round of amplification was conducted on a Gene Amp PCR system 9700 (Applied
15 Biosystems). Integrated HIV-1 sequences were amplified with the expand high fidelity kit
16 (Roche) using an *Alu* primer (NY1F) and a Gag primer extended with an artificial tag
17 sequence at the 5' end of the oligonucleotide (NY1R). The reaction mixture contained 100 ng
18 of DNA, 0.2 mM dNTP, 300 nM primers, 1X buffer with 1.5 mM MgCl₂, and 1.05 U of
19 polymerase. Reaction conditions were as follows: 95°C for 2 minutes, 15 cycles of 95°C for
20 15 seconds, 57°C for 30 seconds and 72°C for 3 minutes, and 72°C for 2 minutes. Real time
21 nested PCR was carried out on the ABI PRISM 7000 system (Applied Biosystems) using 10
22 μ l of 1/20 dilution of the first-round PCR product as a template with 300nM of LTR primer
23 (NY2F), 300nM tag sequence primer (NY2R) and 200 nM *Alu*-LTR probe (NY2ALU) and
24 with 1X Taqman Universal PCR master mix. The integrated HIV-1 DNA copy number was
25 determined with reference to a standard curve generated by concurrent amplifications of a

1 standard HeLa R7 Neo cell DNA. The HeLa R7 Neo cell line was generated as described
2 (29). Briefly, Hela cells were infected with a VSV-G pseudotyped HIV-1 R7 Neo virus (gift
3 from Audrey Brussel) containing a neomycin resistance gene and cultured for five weeks in
4 the presence of G418. For each sample, a whole nested PCR procedure omitting the *Alu*
5 primer in the first round PCR was carried out in parallel, showing a very low background. The
6 number of integrated HIV-1 DNA copies was then adjusted by subtracting the copy number
7 measured in the absence of the *Alu* primer in the first-round PCR from the copy number
8 measured in the presence of *Alu* primer. Primers and probes for the *Alu*-Gag nested PCR are
9 as follows:

10 NY1F: GGCTGAGGCAGGAGAATGG

11 NY1R: CAATATCATACGCCGAGAGTGCGCGCTTCAGCAAG

12 NY2F : AATAAAGCTTGCCTTGAGTGCTC

13 NY2R: CAATATCATACGCCGAGAGTGC

14 NY2ALU: (FAM)-AGTGTGTGCCCGTCTGTTGTGTGACTC-(TAMRA).

15

1 **Results**

2

3 *Immune complexes inhibit HIV-1 replication in macrophages*

4 We reported previously that HIV-1 replication is inhibited in macrophages stimulated with
5 immobilized IVIg, but not by F(ab')₂ fragments of the same IVIg preparations (18). This
6 observation suggested that Ag-Ab complexes might regulate viral infection by interacting
7 with macrophage FcγRs. To confirm this interpretation, MDMs were plated onto immobilized
8 immune complexes (ICs) made of DNP-BSA and IgG anti-DNP, and infected with HIV-1_{VSV-G}
9 pseudotype. We used HIV-1_{VSV-G} pseudotyped viruses to assess the impact of IC-
10 stimulation of MDM on a single cycle of viral replication. HIV-1 replication was dose-
11 dependently inhibited in MDMs exposed to ICs, compared to unstimulated MDMs, but not in
12 MDMs exposed to antigen or antibody alone (Fig 1A). In some experiments, MDM were
13 incubated with IC-opsonized polystyrene beads, which are readily phagocytosed by
14 macrophages and may mimic antibody-opsonized bacteria or cell debris. IC-coated
15 polystyrene beads, but not beads coated with antigen only, inhibited HIV-1_{VSV-G} replication in
16 a dose-dependent manner (Fig 1B). However, inhibition induced by immobilized ICs was
17 more reproducible (data not shown) and as efficient as when induced by immobilized IVIg
18 (Fig 1C). The levels of IC- or IVIg-induced viral inhibition vary in parallel in MDM
19 preparations from different donors (Fig 1C). Replication competent viruses, including HIV-1
20 Bal, were also inhibited by immobilized ICs (data not shown).

21 When equivalent concentrations of anti-DNP F(ab')₂ fragments were used to form DNP-
22 anti-DNP ICs, no inhibition of HIV-1 replication was observed (Fig. 1D), indicating that the
23 Fc portion of IgG is required for inhibition. Since complement is not present in incubation
24 medium, this result implies that HIV-1 inhibition induced by ICs is mediated by FcγR.

25

1 *Inhibition of HIV-1 replication is mediated by activating FcγRs*

2 Human macrophages express several FcγRs. To identify which FcγR(s) account(s) for
3 IC-induced inhibition of HIV-1 replication, we investigated first the effect of engaging
4 separately the three activating receptors known to be expressed on macrophages, and for
5 which specific antibodies are available *i.e.* FcγRI, FcγRIIA and FcγRIIIA. MDMs were
6 incubated with F(ab')₂ fragments of mAbs specific for each receptor or with irrelevant mouse
7 F(ab')₂, and seeded onto wells coated with F(ab')₂ fragments of goat anti-mouse Fab Abs.
8 MDMs were then infected with HIV-1 BaL, and viral replication was evaluated by measuring
9 p24 production. The incubation of MDMs with each of the FcγR-specific F(ab')₂, but not with
10 control F(ab')₂, decreased p24 production, compared with unstimulated MDMs. Anti-FcγRIIA
11 IV.3 F(ab')₂ induced the strongest inhibition (Fig 2). These results indicated that cross-linking
12 activating receptors can induce an inhibition of HIV-1 replication. The level of viral
13 suppression induced by F(ab')₂ fragments of antibodies against activating FcγRs was however
14 generally lower than that induced by either IVIg or ICs ((Fig. 2 and data not shown).

15 Human monocytes having been reported to express the inhibitory FcγRIIB, we
16 investigated next the expression and the potential role of this receptor in HIV-1 inhibition.
17 Because there are no available antibodies (Abs) that recognize specifically the extracellular
18 domain of FcγRIIB, we generated FcγRIIB-specific and, as controls, FcγRIIA-specific
19 polyclonal Abs by immunizing rabbits with peptides corresponding to the intracytoplasmic
20 domain of each receptor. By Western blotting, anti-FcγRIIA Abs recognized proteins of the
21 expected MW in lysates of cells stably transfected with cDNA encoding FcγRIIA but not in
22 lysates of cells stably transfected with cDNA encoding FcγRIIB (Fig. 3A, left). Conversely,
23 anti-FcγRIIB Abs recognized proteins of the expected MW in lysates of cells stably
24 transfected with cDNA encoding FcγRIIB, but not in lysates of cells transfected with cDNA

1 encoding Fc γ RIIA (Fig. 3A, left). We used these Abs to evaluate the expression of Fc γ RIIA
2 and Fc γ RIIB in monocytes and macrophages. As a control, we also examined B lymphocytes
3 which express Fc γ RIIB. As expected, Fc γ RIIB was readily detected in B lymphocytes by the
4 anti-Fc γ RIIB Ab (Fig. 3A, right). In contrast, Fc γ RIIB was undetectable in monocyte or
5 macrophage lysates at concentrations that showed very strong Fc γ RIIA signals (Fig. 3A,
6 right). The same results were found with MDMs from four different donors. Thus, Fc γ RIIB
7 was not detectably expressed in MDMs under our experimental conditions. When analyzed by
8 RT-PCR with Fc γ RIIB or Fc γ RIIA specific primers, Fc γ RIIB transcripts were detected in
9 MDM RNA, but in lower amount than Fc γ RIIA transcripts (Fig 3B). Indeed, when analyzing
10 three ten-fold dilutions of MDM cDNA, Fc γ RIIB transcripts were clearly detected in the first
11 dilution and barely detected in the second dilution, whereas Fc γ RIIA transcripts were detected
12 in all three dilutions (Fig 3B). The same results were found with MDMs from two different
13 donors. Altogether, WB and RT-PCR results indicate that Fc γ RIIA is the predominant Fc γ RII
14 in MDMs. To assess whether, although undetected by Western blotting, Fc γ RIIB could
15 modulate Fc γ RIIA-mediated inhibition of HIV-1 replication, we compared the effect of IV.3
16 F(ab')₂, which recognize the extracellular domain of Fc γ RIIA but not that of Fc γ RIIB, and the
17 effect of AT10 F(ab')₂, which recognize the extracellular domains of Fc γ RIIA, Fc γ RIIB and
18 Fc γ RIIC. Similar percentages of positive cells and similar mean fluorescence intensities were
19 found when MDM were stained with either AT10 or IV.3 F(ab')₂ (Fig. 3C). AT10 and IV.3
20 F(ab')₂ induced comparable HIV-1 inhibition in infected MDM (Fig 3D).

21 Both expression and functional analyses altogether suggest that activating, rather than
22 inhibitory Fc γ Rs account for the IC-induced inhibition of HIV-1 replication in MDM.

23

24 *MDM stimulation through Fc γ R induces activation signals and cytokine secretion*

1 We then investigated the signaling events induced by Fc γ R_s in MDMs and their consequences
2 on infection by HIV-1_{VSV-G}. As expected, tyrosine phosphorylation of a number of
3 intracellular proteins, including PLC- γ and Erk1/2, increased in IVIg-stimulated MDMs (Fig.
4 4A). PLC- γ phosphorylation was transient whereas Erk1/2 phosphorylation was sustained in
5 non infected MDMs (Fig. 4A,C). HIV-1_{VSV-G} infection did not detectably modify the
6 phosphorylation patterns or kinetics (Fig. 4B), even when examined over an extended period
7 of time (Fig. 4C).

8 In order to assess the effect of blocking activating Fc γ R-mediated signaling on HIV-1
9 inhibition in IC-stimulated MDMs, we used piceatannol, reported as an inhibitor of the
10 tyrosine kinase Syk that is recruited by phosphorylated ITAMs in Fc γ R aggregates (30).
11 Piceatannol concentrations of 20-40 μ M partially but significantly ($p=0.001$) removed viral
12 suppression in IC-stimulated MDM infected with HIV-1_{VSV-G} (data not shown). However,
13 piceatannol treatment also caused a dose-dependent inhibition of HIV-1 replication in
14 unstimulated MDM, which was almost complete at concentrations higher than 50 μ M (data
15 not shown), possibly because of the inhibition of phosphorylation pathways involved in HIV-
16 1 replication. Indeed piceatannol can inhibit not only Syk but also numerous tyrosine and
17 serine-threonine kinases (31-33).

18

19 MDM stimulation by either IVIg or ICs induced chemokine and cytokine secretion,
20 including M-CSF, MDC and TNF- α (Fig. S1 A and (18)). Cross-linking of activating Fc γ R
21 with anti-Fc γ R F(ab')₂ on MDMs also induced the secretion of M-CSF (Fig. 4D) and other
22 cytokines (not shown). Whether using IVIg or ICs or anti-Fc γ R F(ab')₂, in all cases the
23 amounts of cytokines secreted by Fc γ R-activated MDMs, and particularly M-CSF, correlated
24 with the magnitude of inhibition of HIV-1 replication (Figs. 2 & 4A, (18) and data not
25 shown). However, we previously reported that HIV-1 suppression could not be induced by

1 exposing MDMs to cytokine-containing supernatants from IVIG-stimulated MDMs and that
2 MDC neutralization in IVIG-stimulated MDM cultures did not restore HIV-1 replication (18).
3 These results indicate that neither MDC nor other secreted factors are responsible for Fc γ R-
4 mediated HIV-1 inhibition. Supporting this conclusion, anti-M-CSF or anti-TNF- α
5 neutralizing Abs reduced, rather than enhanced HIV-1 infection in unstimulated MDMs, and
6 increased IVIG-induced inhibition (Fig. S1 B).

7 *HIV-1 cDNA and integrated proviruses are decreased in Fc γ R-activated macrophages*

8 To identify the steps in the HIV-1 replicative cycle that are inhibited in Fc γ R-activated
9 MDMs, we measured the intermediate products of HIV-1 replication using real time PCR
10 (rtPCR) in single-round infections with HIV-1_{VSV-G} from entry to integration. We used
11 primers and probes amplifying early (R-U5) and late (U5-Gag) products of reverse
12 transcription (RT), 2-LTR circles (2-LTR), and integrated proviruses (*Alu*-LTR). We found
13 similar HIV-1 replication inhibition profiles in MDMs activated either by IVIg or by ICs in
14 MDMs from three different donors. A representative experiment is shown in Fig 5. Luciferase
15 activity in cell lysates was much lower in IC-activated MDMs (90% inhibition at 96h) than in
16 unstimulated MDMs (Fig. 5A). Similar levels of R-U5 products were found by rtPCR in
17 unstimulated and in IC-stimulated MDMs at 4 and 24 h post infection (p.i.) (Fig. 5B). At
18 these early times, R-U5 products essentially reflect the input virus entered into the cells and
19 the initial synthesis of the first products of retrotranscription. However, at later times p.i., the
20 levels of both early and late RT products were decreased in IC-activated MDMs compared to
21 unstimulated MDMs (Fig. 5B,C). Among the nuclear forms of HIV-1 cDNA, 2-LTR circles
22 were only slightly less abundant in IC-activated MDMs than in unstimulated MDMs (Fig.
23 5D), whereas the number of integrated copies, determined from *Alu*-LTR levels, progressively
24 decreased over time in IC-activated MDMs (64 and 76% inhibition at 48 and 96 hours p.i.
25 respectively) (Fig. 5E). Accordingly, the ratio between 2-LTR circles and integrated forms

1 was higher in IC-activated MDMs than in control MDMs and increased over time (2-LTR
2 copies were at 6% and 2,6% of *Alu*-LTR copies at 48 h p.i. and at 38% and 15% at 96h p.i. in
3 IC-activated and in unstimulated MDMs respectively) (Fig. 5F). This result suggests that
4 unintegrated viral forms accumulate in FcγR-activated MDM while integrated forms decrease.
5

6 *Early post-integration steps are not inhibited in FcγR-activated macrophages*

7 We then determined whether FcγR-mediated activation of macrophages could affect
8 HIV-1 post-integration steps, including transcription. MDMs were infected with HIV-1_{VSV-G}
9 and cells were kept in suspension for 72 or 96 hours before they were plated onto IVIg-coated
10 or uncoated wells. Under these conditions, MDMs activation was triggered after most of the
11 viral DNA should be integrated (Fig. 5 and results not shown). Forty eight hours after
12 activation, similar levels of luciferase activity were found in lysates from unstimulated and
13 from IVIg-stimulated MDMs (Fig. 6). By contrast, the same MDM preparation activated at
14 the same time as infection showed a luciferase activity 82% lower in IVIg-stimulated MDMs
15 than in unstimulated cells (Fig. 6). These results indicate that HIV-1 transcription and protein
16 synthesis are not affected by FcγR-mediated activation in macrophages.

17

18 *FcγR-mediated activation of macrophages inhibits the replication of primate lentiviruses*

19 All lentiviruses can complete integration in non-dividing cells and can thus replicate in
20 macrophages. We therefore wondered whether other primate lentiviruses would also be
21 susceptible to FcγR-mediated inhibition in human macrophages. MDMs were infected with
22 HIV-2sbl, SIVmac or SIVagm, plated onto wells coated with ICs, and viral p27 levels were
23 measured in culture supernatants every three days for 23 days (Fig. 7A-C). All three
24 lentiviruses replicated efficiently in control MDMs, with p27 production becoming greater
25 than 1 μg/ml. p27 levels were markedly reduced in the cultures of MDMs infected with each

1 of the three viruses and stimulated with ICs (Fig. 7A-C). Fc γ R-mediated inhibition of viral
2 infection is therefore not limited to HIV-1 but affects other primate lentiviruses.

3 Fc γ R-mediated inhibition affects HIV-1 reverse transcription and integration that are
4 peculiar features of retroviral replication. We therefore investigated whether Fc γ R-mediated
5 activation of MDMs could affect other viruses. For this experiment, we used the West Nile
6 (WN) Virus, an unrelated macrophagotropic *Flaviviridae* virus. WN virus replication in
7 unstimulated MDMs was compared with that in Fc γ R-stimulated MDMs (Fig. 7D). As a
8 control for inhibition, MDMs were treated with IFN- α (34-36). MDMs were infected with
9 WN virus IS-98-ST1 strain at 1 m.o.i. No cytopathic effect was observed in any condition
10 (not shown). After 72 hours, titres of virus produced in cell culture supernatants were
11 determined. No difference was observed in the virus titre between unstimulated and
12 stimulated MDMs (Fig. 7D). However, as expected, virus titre was strongly decreased in IFN-
13 α treated MDMs. These results suggest therefore that Fc γ R-mediated inhibition selectively
14 affects lentiviruses.

15

16 **Discussion**

17 In the present study, we show 1) that the inhibition of HIV-1 replication that we
18 previously reported in macrophages plated onto immobilized IVIg (18), can also be induced
19 by IgG immune complexes; 2) that activating Fc γ Rs account for HIV-1 inhibition, but that
20 Fc γ R-induced cytokines are not responsible for HIV-1 inhibition; 3) that inhibition affects
21 neither viral entry nor post integration steps, but causes a reduction of viral cDNA and blocks
22 viral integration; 4) that Fc γ R-mediated suppression is not limited to HIV-1 but also affects
23 other primate lentiviruses.

24 We showed that the three known ITAM-bearing Fc γ Rs can mediate HIV-1 replication
25 inhibition. Although the levels of HIV-1 inhibition varied depending on the MDM

1 preparation, it was consistently higher upon FcγRIIA cross-linking than upon FcγRI or
2 FcγRIIIA cross-linking (Fig. 2). Whether this difference is due to a higher expression of
3 FcγRIIA on MDMs, to specific properties of this receptor, or to a higher affinity of the anti-
4 FcγRIIA mAb used is not known. Inhibition of HIV-1 correlated with MDM activation, as
5 judged by M-CSF and MDC secretion. Accordingly, the partial recovery of HIV-1 replication
6 by piceatannol in IC-stimulated MDM may suggest that blocking signalling pathways
7 downstream activating FcγRs can remove HIV-1 inhibition. However, the significance of this
8 result was obscured by a direct inhibitory effect of piceatannol on HIV-1 replication. Both
9 inhibition of HIV-1 and MDM activation were generally lower when induced by anti-FcγR
10 F(ab')₂ than when induced by IVIg or ICs. This is consistent with previous studies showing
11 that FcγR cross-linking with immobilized IgG induces TNF secretion by human monocytes,
12 whereas cross-linking with anti-FcγR antibodies does not (37). It also indicates that ICs or
13 IVIg are more efficient at aggregating FcγRs than anti-FcγR antibodies.

14 We found no evidence that FcγRIIB contributes to IC-induced HIV-1 inhibition in
15 MDMs. One possible reason is the low expression of FcγRIIB in these cells. We did not detect
16 FcγRIIB proteins by Western blotting, but we found relatively low levels of FcγRIIB
17 transcripts by RT-PCR, in MDMs. These results are consistent with previous reports where
18 FcγRIIB detection by Western blotting required very high amounts of monocytes (38, 39).
19 FcγRIIB is highly regulated by culture conditions and the presence of cytokines, including IL-
20 4 (38-40). Culture conditions used for macrophages differentiation, *i.e.* culture medium
21 supplemented with human serum but with no added cytokines, may not be optimal for
22 FcγRIIB expression. Our data, however, do not exclude that, if expressed at sufficient levels,
23 FcγRIIB could negatively regulate HIV-1 replication inhibition by activated FcγRs. The

1 coengagement of Fc γ RIIA/C and Fc γ RIIB by AT10 F(ab')₂, however, had the same effect on
2 HIV-1 replication as the engagement of Fc γ RIIA alone by IV.3 F(ab')₂.

3 Fc γ Rs have been involved in either enhancement or inhibition of HIV-1 infection
4 when engaged by anti-HIV-1 antibodies. Fc γ RI has been suggested to contribute to the control
5 of infection in HIV-1-infected patients by favoring the internalization of HIV-1-IgG
6 complexes and the degradation of the virus in macrophages (41). Likewise, bispecific Abs
7 that could target HIV-1 to macrophage activating Fc γ Rs inhibited HIV-1 infection (42). On
8 the contrary, Fc γ RI or Fc γ RIII have been suggested to enhance the entry of Ab-opsonized
9 HIV-1 virions into macrophages (43-45). Whatever the effects of activating Fc γ Rs when
10 engaged by HIV-anti-HIV immune complexes, we consistently found that activating Fc γ Rs
11 inhibited HIV-1 infection when engaged by irrelevant IgG immune complexes. It was
12 recently reported that Fc γ RIIA/IIIA polymorphisms which confer higher avidity binding to
13 ICs are associated with protection against HIV infection, but, on the contrary, these same
14 polymorphisms are associated with the likelihood of infection in HIV gp120-vaccinated
15 individuals (46). Based on these data, one may speculate that in the presence of preexistent
16 HIV-gp120 specific antibodies induced by vaccination, higher avidity for ICs may be
17 deleterious favoring Ab-dependent enhancement of HIV infection. In contrast, in the absence
18 of preexistent HIV-1 antibodies, higher avidity of Fc γ Rs for circulating ICs may favor
19 protection against incoming infection by limiting viral replication in IC-activated
20 macrophages.

21 Macrophage activation by Fc γ Rs affects the mechanisms that eventually lead to
22 proviral integration. Previous qualitative PCR analysis of IVIg-stimulated MDMs infected
23 with a replication competent virus detected a reduction in integrated proviral DNA but not in
24 reverse transcription products (18). Using one-round infections, which avoid overlapping
25 replication cycles, and quantitative PCR, we now show that, whereas reverse transcription

1 was not affected at the earliest times p.i., the levels of both early and late cDNA products
2 were eventually reduced in activated MDMs (Fig. 5). Levels of integrated proviruses were
3 further inhibited in either IVIg or IC-stimulated MDMs. By contrast, the ratio of circular 2-
4 LTR forms to integrated forms was higher in activated macrophages than in controls (Fig.
5 5F). 2-LTR circles are unintegrated nuclear forms of HIV-1 DNA (47-49) and thus reflect the
6 translocation of viral transcripts into the nucleus of infected cells. Our results suggest that
7 unintegrated HIV-1 DNA accumulates because of an inhibition of integration, as observed
8 with anti-integrase drugs (50). The level of HIV-1 integrated forms in Fc γ R-activated MDMs
9 decreased to levels similar as viral replication inhibition levels, as shown by reduced
10 luciferase activity (Fig. 5 and data not shown), suggesting that the postintegration steps of
11 replication are unaffected. We confirmed this hypothesis by showing that Fc γ R-stimulation
12 did not alter viral gene expression, once integration was achieved (Fig. 6).

13 The activation of PI3K or of the MAPK pathways has been shown to be essential for
14 an efficient replication of HIV-1 (51-53). Therefore, one expects early signaling events
15 triggered by activating Fc γ R cross-linking to favor HIV-1 replication rather than to exert an
16 anti-viral effect. We thus suggest that Fc γ R-induced late signaling events, which need to be
17 identified in future work, are involved in HIV-1 inhibition. These might include the
18 mobilization, the neosynthesis or the suppression of molecules that are critical for reverse
19 transcription and/or integration. Fc γ R-activated MDMs secrete several cytokines, such as M-
20 CSF, TNF- α and MDC, which either up- or down-regulate HIV-1 infection in macrophages
21 (54-56). Neutralization of these cytokines in MDM cultures showed that the inhibitory
22 mechanisms induced by Fc γ R-aggregation overcome the enhancing effects of M-CSF and
23 TNF- α on HIV-1 replication and are not linked to MDC (18) and data not shown). The pre-
24 integration inhibition induced by Fc γ R stimulation is reminiscent of the inhibitory effects of
25 IFN- α et β (57). IFN- α/β was, however, not detected in Fc γ R-activated MDM supernatant

1 (18). In addition, West Nile virus infection, which is inhibited by IFN- α and β (Fig. 7B and
2 (34, 35)), was not affected in Fc γ R-activated MDM. The participation of type I IFNs in HIV-
3 1 inhibition in Fc γ R-activated MDM is therefore unlikely.

4 Two distinct inhibition mechanisms may be operating: one affecting the
5 retrotranscription process, and another inhibiting viral integration after nuclear translocation
6 of HIV-1 cDNA. A single mechanism may however inhibit both steps of the viral cycle. An
7 increased degradation of reverse transcripts by endonucleases, as suggested for APOBEC3G
8 (58), or of incoming viral proteins by the proteasome (59, 60), would reduce both the levels of
9 reverse transcripts and the reverse transcription products available for integration.
10 Alternatively, mechanisms that hinder HIV-1 integrase activity and/or affect the
11 preintegration complex (PIC) stability would have a negative impact on both integration and
12 reverse transcription. Indeed, although reverse transcription and integration occur in distinct
13 cellular compartments, they take place in the same molecular environment formed by the PIC
14 (61). Moreover, HIV-1 integrase was involved in different steps of the HIV-1 life cycle,
15 including reverse transcription (62, 63).

16 Remarkably, Fc γ R-mediated anti-viral activity is not limited to HIV-1 as it also affects
17 other primate lentiviruses. By contrast, unrelated macrophage-tropic viruses such as the West
18 Nile Virus were not affected, suggesting that Fc γ R-mediated anti-viral activity is not a general
19 anti-viral defense mechanism. If it targets highly conserved lentiviral proteins and/or their
20 functions, such as reverse transcription and integration, one would expect inhibition to affect
21 other HIV-1-related lentiviruses. It would be interesting to study the effect of Fc γ R-mediated
22 activation of macrophages on the replication of lentiviruses in their natural hosts in more
23 distant animal systems. This would be especially relevant for diseases caused by lentiviruses
24 having a restricted tropism for macrophages, such as the caprine arthritis and encephalitis
25 virus (CAEV) or the Maedi-Visna virus.

1

2 Acknowledgements

3 We are grateful to Audrey Brussel and Norio Yamamoto for reagents and methods for rtPCR,
4 Emmanuelle Lenôtre (Applied Biosystems) for help in designing the R-U5 probe. We thank
5 Hugues Sudry for his contribution to cytokine analysis and Philippe Despres for expert
6 assistance in experiments of infection with the West Nile Virus. We thank Roger Legrand and
7 Michael Ploquin for providing HIV-2, SIVmac and SIVagm strains.

8

1 References:

- 2 1. Ignatius, R., K. Tenner-Racz, D. Messmer, A. Gettie, J. Blanchard, A. Luckay, C.
3 Russo, S. Smith, P. A. Marx, R. M. Steinman, P. Racz, and M. Pope. 2002. Increased
4 macrophage infection upon subcutaneous inoculation of rhesus macaques with simian
5 immunodeficiency virus-loaded dendritic cells or T cells but not with cell-free virus. *J.*
6 *Viol.* 76:9787-9797.
- 7 2. Gendelman, H. E., J. M. Orenstein, L. M. Baca, B. Weiser, H. Burger, D. C. Kalter,
8 and M. S. Meltzer. 1989. The macrophage in the persistence and pathogenesis of HIV
9 infection. *Aids* 3:475-495.
- 10 3. Hirsch, V. M., M. E. Sharkey, C. R. Brown, B. Brichacek, S. Goldstein, J. Wakefield,
11 R. Byrum, W. R. Elkins, B. H. Hahn, J. D. Lifson, and M. Stevenson. 1998. Vpx is
12 required for dissemination and pathogenesis of SIV(SM) PBJ: evidence of
13 macrophage-dependent viral amplification. *Nat. Med.* 4:1401-1408.
- 14 4. Sharova, N., C. Swingler, M. Sharkey, and M. Stevenson. 2005. Macrophages archive
15 HIV-1 virions for dissemination in trans. *Embo J.* 24:2481-2489.
- 16 5. Williams, K., S. Westmoreland, J. Greco, E. Ratai, M. Lentz, W.-K. Kim, R. A.
17 Fuller, J. P. Kim, P. Autissier, P. K. Sehgal, R. F. Schinazi, N. Bischofberger, M.
18 Piatak, Jr., J. D. Lifson, E. Masliah, and R. G. Gonzalez. 2005. Magnetic resonance
19 spectroscopy reveals that activated monocytes contribute to neuronal injury in SIV
20 neuroAIDS. *J. Clin. Invest.* 115:2534-2545.
- 21 6. Thieblemont, N., L. Weiss, H. M. Sadeghi, C. Estcourt, and N. Haeffner-Cavaillon.
22 1995. CD14^{low}CD16^{high}: a cytokine-producing monocyte subset which expands
23 during human immunodeficiency virus infection. *Eur. J. Immunol.* 25:3418-3424.

- 1 7. Carr, J. M., H. Hocking, P. Li, and C. J. Burrell. 1999. Rapid and efficient cell-to-cell
2 transmission of human immunodeficiency virus infection from monocyte-derived
3 macrophages to peripheral blood lymphocytes. *Virology* 265:319-329.
- 4 8. Swingler, S., B. Brichacek, J. M. Jacque, C. Ulich, J. Zhou, and M. Stevenson. 2003.
5 HIV-1 Nef intersects the macrophage CD40L signalling pathway to promote resting-
6 cell infection. *Nature* 424:213-219.
- 7 9. Mahlknecht, U., and G. Herbein. 2001. Macrophages and T-cell apoptosis in HIV
8 infection: a leading role for accessory cells? *Trends Immunol.* 22:256-260.
- 9 10. Gartner, S., D. Markovits, D. M. Markovitz, M. H. Kaplan, R. C. Gallo, and M.
10 Popovic. 1986. The role of mononuclear phagocytes in HTV-III/LAV infection.
11 *Science* 233:215.
- 12 11. Williams, K. C., S. Corey, S. V. Westmoreland, D. Pauley, H. Knight, C. deBakker,
13 X. Alvarez, and A. A. Lackner. 2001. Perivascular macrophages are the primary cell
14 type productively infected by simian immunodeficiency virus in the brains of
15 macaques: implications for the neuropathogenesis of AIDS. *J. Exp. Med.* 193:905-
16 915.
- 17 12. Zhu, T., D. Muthui, S. Holte, D. Nickle, F. Feng, S. Brodie, Y. Hwangbo, J. I.
18 Mullins, and L. Corey. 2002. Evidence for human immunodeficiency virus type 1
19 replication in vivo in CD14(+) monocytes and its potential role as a source of virus in
20 patients on highly active antiretroviral therapy. *J. Virol.* 76:707-716.
- 21 13. Orenstein, J. M., C. Fox, and S. M. Wahl. 1997. Macrophages as a source of HIV
22 during opportunistic infections. *Science* 276:1857-1861.
- 23 14. Igarashi, T., C. R. Brown, Y. Endo, A. Buckler-White, R. Plishka, N. Bischofberger,
24 V. Hirsch, and M. A. Martin. 2001. Macrophage are the principal reservoir and sustain
25 high virus loads in rhesus macaques after the depletion of CD4+ T cells by a highly

- 1 pathogenic simian immunodeficiency virus/HIV type 1 chimera (SHIV): Implications
2 for HIV-1 infections of humans. *Proc. Natl. Acad. Sci. U S A* 98:658-663.
- 3 15. Verani, A., G. Gras, and G. Pancino. 2005. Macrophages and HIV-1: dangerous
4 liaisons. *Mol. Immunol.* 42:195-212.
- 5 16. Kedzierska, K., R. Azzam, P. Ellery, J. Mak, A. Jaworowski, and S. M. Crowe. 2003.
6 Defective phagocytosis by human monocyte/macrophages following HIV-1 infection:
7 underlying mechanisms and modulation by adjunctive cytokine therapy. *J. Clin. Virol.*
8 26:247-263.
- 9 17. Garaci, E., S. Aquaro, C. Lapenta, A. Amendola, M. Spada, S. Covaceuszach, C. F.
10 Perno, and F. Belardelli. 2003. Anti-nerve growth factor Ab abrogates macrophage-
11 mediated HIV-1 infection and depletion of CD4+ T lymphocytes in hu-SCID mice.
12 *Proc. Natl. Acad. Sci. U S A* 100:8927-8932.
- 13 18. Perez-Bercoff, D., A. David, H. Sudry, F. Barre Sinoussi, and G. Pancino. 2003. Fcγ-
14 mediated suppression of human immunodeficiency virus type 1 replication in primary
15 human macrophages. *J. Virol.* 77:4081-4094.
- 16 19. Daeron, M. 1997. Fc receptor biology. *Annu. Rev. Immunol.* 15:203-234.
- 17 20. Connor, R. I., K. C. Benjamin, S. Choe, and N. R. Landau. 1995. Vpr Is Required for
18 Efficient Replication of Human Immunodeficiency Virus Type-1 in Mononuclear
19 Phagocytes. *Virology* 206:935-944.
- 20 21. O'Doherty, U., W. J. Swiggard, and M. H. Malim. 2000. Human immunodeficiency
21 virus type 1 spinoculation enhances infection through virus binding. *J. Virol.*
22 74:10074-10080.
- 23 22. Despres, P., M. P. Frenkiel, and V. Deubel. 1993. Differences between cell membrane
24 fusion activities of two dengue type-1 isolates reflect modifications of viral structure.
25 *Virology* 196:209-219.

- 1 23. Folks, T., D. Powell, M. Lightfoote, S. Koenig, A. Fauci, S. Benn, A. Rabson, D.
2 Daugherty, H. Gendelman, and M. Hoggan. 1986. Biological and biochemical
3 characterization of a cloned Leu-3- cell surviving infection with the acquired immune
4 deficiency syndrome retrovirus. *J. Exp. Med.* 164:280-290.
- 5 24. Brussel, A., D. Mathez, S. Broche-Pierre, R. Lancar, T. Calvez, P. Sonigo, and J.
6 Leibowitch. 2003. Longitudinal monitoring of 2-long terminal repeat circles in
7 peripheral blood mononuclear cells from patients with chronic HIV-1 infection. *Aids*
8 17:645-652.
- 9 25. Rouet, F., D. K. Ekouevi, M.-L. Chaix, M. Burgard, A. Inwoley, T. D. A. Tony, C.
10 Danel, X. Anglaret, V. Leroy, P. Msellati, F. Dabis, and C. Rouzioux. 2005. Transfer
11 and Evaluation of an Automated, Low-Cost Real-Time Reverse Transcription-PCR
12 Test for Diagnosis and Monitoring of Human Immunodeficiency Virus Type 1
13 Infection in a West African Resource-Limited Setting. *J. Clin. Microbiol.* 43:2709-
14 2717.
- 15 26. Chiu, Y. L., V. B. Soros, J. F. Kreisberg, K. Stopak, W. Yonemoto, and W. C. Greene.
16 2005. Cellular APOBEC3G restricts HIV-1 infection in resting CD4+ T cells. *Nature*
17 435:108-114.
- 18 27. Suzuki, Y., N. Misawa, C. Sato, H. Ebina, T. Masuda, N. Yamamoto, and Y.
19 Koyanagi. 2003. Quantitative analysis of human immunodeficiency virus type 1 DNA
20 dynamics by real-time PCR: integration efficiency in stimulated and unstimulated
21 peripheral blood mononuclear cells. *Virus Genes* 27:177-188.
- 22 28. Douek, D. C., J. M. Brenchley, M. R. Betts, D. R. Ambrozak, B. J. Hill, Y. Okamoto,
23 J. P. Casazza, J. Kuruppu, K. Kunstman, S. Wolinsky, Z. Grossman, M. Dybul, A.
24 Oxenius, D. A. Price, M. Connors, and R. A. Koup. 2002. HIV preferentially infects
25 HIV-specific CD4+ T cells. *Nature* 417:95-98.

- 1 29. Brussel, A., and P. Sonigo. 2003. Analysis of early human immunodeficiency virus
2 type 1 DNA synthesis by use of a new sensitive assay for quantifying integrated
3 provirus. *J. Virol.* 77:10119-10124.
- 4 30. Oliver, J. M., D. L. Burg, B. S. Wilson, J. L. McLaughlin, and R. L. Geahlen. 1994.
5 Inhibition of mast cell Fc epsilon R1-mediated signaling and effector function by the
6 Syk-selective inhibitor, piceatannol. *J. Biol. Chem.* 269:29697-29703.
- 7 31. Geahlen, R. L., and J. L. McLaughlin. 1989. Piceatannol (3,4,3',5'-tetrahydroxy-trans-
8 stilbene) is a naturally occurring protein-tyrosine kinase inhibitor. *Biochem. Biophys.*
9 *Res. Commun.* 165:241-245.
- 10 32. Wang, B. H., Z. X. Lu, and G. M. Polya. 1998. Inhibition of eukaryote
11 serine/threonine-specific protein kinases by piceatannol. *Planta Med.* 64:195-199.
- 12 33. Ashikawa, K., S. Majumdar, S. Banerjee, A. C. Bharti, S. Shishodia, and B. B.
13 Aggarwal. 2002. Piceatannol inhibits TNF-induced NF-kappaB activation and NF-
14 kappaB-mediated gene expression through suppression of IkappaBalpha kinase and
15 p65 phosphorylation. *J. Immunol.* 169:6490-6497.
- 16 34. Lucas, M., T. Mashimo, M. P. Frenkiel, D. Simon-Chazottes, X. Montagutelli, P. E.
17 Ceccaldi, J. L. Guenet, and P. Despres. 2003. Infection of mouse neurones by West
18 Nile virus is modulated by the interferon-inducible 2'-5' oligoadenylate synthetase 1b
19 protein. *Immunol. Cell. Biol.* 81:230-236.
- 20 35. Anderson, J. F., and J. J. Rahal. 2002. Efficacy of interferon alpha-2b and ribavirin
21 against West Nile virus in vitro. *Emerg. Infect. Dis.* 8:107-108.
- 22 36. Samuel, M. A., and M. S. Diamond. 2005. Alpha/beta interferon protects against lethal
23 West Nile virus infection by restricting cellular tropism and enhancing neuronal
24 survival. *J. Virol.* 79:13350-13361.

- 1 37. Debets, J., J. Van de Winkel, J. Ceuppens, I. Dieteren, and W. Buurman. 1990. Cross-
2 linking of both Fc gamma RI and Fc gamma RII induces secretion of tumor necrosis
3 factor by human monocytes, requiring high affinity Fc-Fc gamma R interactions.
4 Functional activation of Fc gamma RII by treatment with proteases or neuraminidase.
5 *J. Immunol.* 144:1304-1310.
- 6 38. Pricop, L., P. Redecha, J. L. Teillaud, J. Frey, W. H. Fridman, C. Sautes-Fridman, and
7 J. E. Salmon. 2001. Differential modulation of stimulatory and inhibitory Fc gamma
8 receptors on human monocytes by Th1 and Th2 cytokines. *J. Immunol.* 166:531-537.
- 9 39. Tridandapani, S., K. Siefker, J. L. Teillaud, J. E. Carter, M. D. Wewers, and C. L.
10 Anderson. 2002. Regulated expression and inhibitory function of Fc gamma RIIb in
11 human monocytic cells. *J. Biol. Chem.* 277:5082-5089.
- 12 40. Liu, Y., E. Masuda, M. C. Blank, K. A. Kirou, X. Gao, M. S. Park, and L. Pricop.
13 2005. Cytokine-mediated regulation of activating and inhibitory Fc gamma receptors
14 in human monocytes. *J. Leukoc. Biol.* 77:767-776.
- 15 41. Holl, V., S. Hemmerter, R. Burrer, S. Schmidt, A. Bohbot, A. M. Aubertin, and C.
16 Moog. 2004. Involvement of Fc gamma RI (CD64) in the mechanism of HIV-1
17 inhibition by polyclonal IgG purified from infected patients in cultured monocyte-
18 derived macrophages. *J. Immunol.* 173:6274-6283.
- 19 42. Connor, R. I., N. B. Dinces, A. L. Howell, J. L. Romet-Lemonne, J. L. Pasquali, and
20 M. W. Fanger. 1991. Fc receptors for IgG (Fc gamma Rs) on human monocytes and
21 macrophages are not infectivity receptors for human immunodeficiency virus type 1
22 (HIV-1): studies using bispecific antibodies to target HIV-1 to various myeloid cell
23 surface molecules, including the Fc gamma R. *Proc. Natl. Acad. Sci. USA* 88:9593-
24 9597.

- 1 43. Takeda, A., and F. A. Ennis. 1990. FcR-mediated enhancement of HIV-1 infection by
2 antibody. *AIDS Res. Hum. Retroviruses* 6:999-1004.
- 3 44. Homsy, J., Meyer, M., Rateno, M., Clarkson, S., Levy, J. A. 1989. The Fc and not
4 CD4 receptor mediated antibody enhancement of HIV infection in human cells.
5 *Science* 244:1357-1360.
- 6 45. Trischmann, H., D. Davis, and P. J. Lachmann. 1995. Lymphocytotropic strains of
7 HIV type 1 when complexed with enhancing antibodies can infect macrophages via Fc
8 gamma RIII, independently of CD4. *AIDS Res. Hum. Retroviruses* 11:343-352.
- 9 46. Forthal, D., G. Landucci, T. Phan, H.-T. R, and G. P. 2006. Fcg Receptor IIa and IIIa
10 polymorphisms are associated with the risk of HIV infection. In *13th Conference on*
11 *Retroviruses and Opportunistic Infections*, Denver, USA.
- 12 47. Kim, S. Y., R. Byrn, J. Groopman, and D. Baltimore. 1989. Temporal aspects of DNA
13 and RNA synthesis during human immunodeficiency virus infection: evidence for
14 differential gene expression. *J. Virol.* 63:3708-3713.
- 15 48. Butler, S. L., E. P. Johnson, and F. D. Bushman. 2002. Human immunodeficiency
16 virus cDNA metabolism: notable stability of two-long terminal repeat circles. *J. Virol.*
17 76:3739-3747.
- 18 49. Pierson, T. C., T. L. Kieffer, C. T. Ruff, C. Buck, S. J. Gange, and R. F. Siliciano.
19 2002. Intrinsic stability of episomal circles formed during human immunodeficiency
20 virus type 1 replication. *J. Virol.* 76:4138-4144.
- 21 50. Hazuda, D. J., P. Felock, M. Witmer, A. Wolfe, K. Stillmock, J. A. Grobler, A.
22 Espeseth, L. Gabryelski, W. Schleif, C. Blau, and M. D. Miller. 2000. Inhibitors of
23 strand transfer that prevent integration and inhibit HIV-1 replication in cells. *Science*
24 287:646-650.

- 1 51. Yang, X., and D. Gabuzda. 1999. Regulation of Human Immunodeficiency Virus
2 Type 1 Infectivity by the ERK Mitogen-Activated Protein Kinase Signaling Pathway.
3 *J. Virol.* 73:3460-3466.
- 4 52. Francois, F., and M. E. Klotman. 2003. Phosphatidylinositol 3-kinase regulates human
5 immunodeficiency virus type 1 replication following viral entry in primary CD4+ T
6 lymphocytes and macrophages. *J. Virol.* 77:2539-2549.
- 7 53. Lee, C., B. Tomkowicz, B. D. Freedman, and R. G. Collman. 2005. HIV-1 gp120-
8 induced TNF- α production by primary human macrophages is mediated by
9 phosphatidylinositol-3 (PI-3) kinase and mitogen-activated protein (MAP) kinase
10 pathways. *J. Leukoc. Biol.* 78:1016-1023.
- 11 54. Kutza, J., L. Crim, S. Feldman, M. P. Hayes, M. Gruber, J. Beeler, and K. A. Clouse.
12 2000. Macrophage colony-stimulating factor antagonists inhibit replication of HIV-1
13 in human macrophages. *J. Immunol.* 164:4955-4960.
- 14 55. Herbein, G., Montaner, L. J., and Gordon, S. 1996. Tumor necrosis factor alpha
15 inhibits entry of human immunodeficiency virus ype 1 into primary human
16 macrophages: a selective role for a 75-kilodalton receptor (published erratum appears
17 in *J. Virol.* 1997 Mar; 71(3):1581). *J. Virol.* 70:7388-7397.
- 18 56. Cota, M., M. Mengozzi, E. Vicenzi, P. Panina-Bordignon, F. Sinigaglia, P. Transidico,
19 S. Sozzani, A. Mantovani, and G. Poli. 2000. Selective inhibition of HIV replication in
20 primary macrophages but not T lymphocytes by macrophage-derived chemokine.
21 *Proc. Natl. Acad. Sci. U S A* 97:9162-9167.
- 22 57. Meylan, P. R., J. C. Guatelli, J. R. Munis, D. D. Richman, and R. S. Kornbluth. 1993.
23 Mechanisms for the inhibition of HIV replication by interferons-alpha, -beta, and -
24 gamma in primary human macrophages. *Virology* 193:138-148.

- 1 58. Mariani, R., D. Chen, B. Schrofelbauer, F. Navarro, R. Konig, B. Bollman, C. Munk,
2 H. Nymark-McMahon, and N. R. Landau. 2003. Species-specific exclusion of
3 APOBEC3G from HIV-1 virions by Vif. *Cell* 114:21-31.
- 4 59. Schwartz, O., V. Marechal, B. Friguet, F. Arenzana-Seisdedos, and J. M. Heard. 1998.
5 Antiviral activity of the proteasome on incoming human immunodeficiency virus type
6 1. *J. Virol.* 72:3845-3850.
- 7 60. Wei, B. L., P. W. Denton, E. O'Neill, T. Luo, J. L. Foster, and J. V. Garcia. 2005.
8 Inhibition of lysosome and proteasome function enhances human immunodeficiency
9 virus type 1 infection. *J. Virol.* 79:5705-5712.
- 10 61. Bukrinsky, M., N. Sharova, T. McDonald, T. Pushkarskaya, W. Tarpley, and M.
11 Stevenson. 1993. Association of Integrase, Matrix, and Reverse Transcriptase
12 Antigens of Human Immunodeficiency Virus Type 1 with Viral Nucleic Acids
13 Following Acute Infection. *Proc. Natl. Acad. Sci. U S A* 90:6125-6129.
- 14 62. Parrill, A. L. 2003. HIV-1 integrase inhibition: binding sites, structure activity
15 relationships and future perspectives. *Curr. Med. Chem.* 10:1811-1824.
- 16 63. Zhu, K., C. Dobard, and S. A. Chow. 2004. Requirement for integrase during reverse
17 transcription of human immunodeficiency virus type 1 and the effect of cysteine
18 mutations of integrase on its interactions with reverse transcriptase. *J. Virol.* 78:5045-
19 5055.
- 20
21

1 **Figure legends**

2 **Figure 1.** IgG-immune complexes inhibit HIV-1 replication in MDMs. (A) MDMs infected
3 with HIV-1_{VSV-G} were plated on wells treated with BSA only (unstimulated, US) or coated
4 with 30 µg/ml anti-DNP Abs (a-DNP) or with decreasing concentrations of DNP-BSA (10, 1,
5 0.1 µg/well) followed or not by 30 µg/ml anti-DNP Abs; (B) MDMs were plated and
6 incubated with medium (US) or with 3µm polystyrene beads coated with DNP-BSA-anti
7 DNP ICs (IC) or with DNP-BSA only (Ag) at a bead:cell ratio of 10:1 and 30:1 (10, 30) and
8 infected with HIV-1_{VSV-G}; (C) HIV-1_{VSV-G} infected MDMs from three different donors were
9 stimulated with either immobilized IVIg or ICs. Results are expressed as percentage of
10 inhibition of luciferase activity (means and SD of three independent wells) found in
11 unstimulated MDMs plated on BSA or DNP-BSA. (D) HIV-1_{VSV-G} infected MDMs were
12 plated on wells coated with 10 µg/well DNP-BSA or with complexes formed by DNP-BSA
13 with 30 µg/ml of either anti-DNP IgG or anti-DNP F(ab')₂ and infected with HIV-1_{VSV-G}. In
14 all the experiments luciferase activity was measured in cell lysates 72 hours p.i. Results are
15 expressed as means and SD of three independent wells.

16 **Figure 2.** Cross-linking of activating FcγRs inhibit HIV-1 replication. MDMs were incubated
17 with medium (unstimulated, US), with irrelevant F(ab')₂ (C) or with F(ab')₂ fragments of
18 mAbs specific for FcγRI (32.2), FcγRIIA (IV.3) or FcγRIII (3G8), and plated on anti-mouse-
19 Fab F(ab')₂-coated wells. As a control of HIV-1 inhibition, MDMs were stimulated in parallel
20 with IVIg. MDMs were then infected with HIV-1 Bal. Results are expressed as means and SD
21 of the percentage of infection in three independent wells (evaluated by p24 levels in
22 supernatants) on day 8 p.i. with respect to unstimulated MDMs (p24 = 202 ng/ml).
23 Comparisons among data sets were performed by independent sample t-test. A representative
24 experiment of three performed with MDM from different donors is shown.

1 Figure 3. Fc γ RIIB is undetectable by Western blotting in MDM and does not apparently
2 regulate activating Fc γ R-mediated HIV-1 inhibition. (A) Left: RBL cells transfected with
3 cDNA encoding Fc γ RIIA or anti-Fc γ RIIB were western blotted with polyclonal rabbit anti-
4 Fc γ RIIA or anti-Fc γ RIIB. Right: B-lymphocytes, monocytes or MDM lysates (10 μ g) were
5 western blotted with polyclonal rabbit anti-Fc γ RIIA or anti-Fc γ RIIB. (B) RNA from MDMs
6 was analyzed by RT-PCR for expression of Fc γ RIIA and Fc γ RIIB transcripts. 0.5 μ g of RNA
7 were reverse transcribed. PCR amplification with primers specific to each receptor was
8 performed on sequential ten-fold dilutions of the cDNA mixture. (C) FACS analysis of
9 MDMs stained with F(ab')₂ of mAbs directed against Fc γ RIIA (IV.3) (solid line) or against
10 the Fc γ Rs IIA, IIB and IIC (AT.10) (dashed line). (D) MDMs were incubated with medium
11 (unstimulated, US), with ICs (IC), with 5 μ g/10⁶ MDM of irrelevant F(ab') (C) or with F(ab')₂
12 derived from IV.3 or AT10 mAbs at decreasing concentrations (5, 1, 0.2, 0.04 μ g/10⁶ MDM)
13 and plated on anti-mouse-Fab F(ab')₂ coated wells to cross-link bound F(ab')₂. MDMs were
14 infected with HIV-1 Bal. Results are expressed as percentage of infection (evaluated by p24
15 levels in supernatants) on day 6 p.i. with respect to unstimulated MDM (p24 = 658 ng/ml).
16 Values are means and SD of three independent wells. Similar results were obtained in
17 experiments performed with MDM from three different donors.

18 **Figure 4.** Fc γ R aggregation induces activation signals in MDMs and cytokine secretion (A-C)
19 Non-infected or HIV-1_{VSV-G} infected MDMs were stimulated by being plated onto IgG-coated
20 (S) or non-coated wells (US) for the indicated times before being lysed. Proteins (40 μ g) were
21 electrophoresed and western blotted with anti-phosphotyrosine (anti-pY), anti-phospho-PLC-
22 γ 1, or anti-phospho-Erk Abs. Anti-Erk Abs were used as loading controls. (D) M-CSF
23 secretion after cross-linking of activating Fc γ Rs. M-CSF secreted in the supernatants of
24 unstimulated MDM (US), MDM incubated with irrelevant F(ab')₂ (C) or with F(ab')₂ specific

1 for each activating Fc γ R and plated on anti-mouse-Fab F(ab')₂-coated wells was measured
2 48h after cell plating by ELISA (means of duplicates).

3 **Figure 5.** Fc γ R-mediated activation causes a reduction of HIV-1 retrotranscripts and of
4 integrated proviruses in MDMs. MDMs were infected with DNase treated HIV-1_{VSV-G} and
5 plated on DNP-BSA coated plates (unstimulated, US) or stimulated with ICs (S). Luciferase
6 activity was monitored at 24, 48 and 96 h p.i. in MDM lysates (A). Early and late
7 retrotranscription products and 2-LTR circles were analyzed by rtPCR using the appropriate
8 primers (R-U5, U5-Gag, 2-LTR) and probes (B-D). Integrated copies were evaluated by *Alu*-
9 LTR nested rtPCR. Values are means of duplicate measures at the indicated times p.i. (E).
10 Ratios between 2-LTR circles and integrated nuclear forms of HIV-1 at 48 and 96 h p.i. as
11 measured by rtPCR in unstimulated or IC-activated MDMs. Results from a representative
12 experiment of three performed with MDM from different donors are shown.

13 **Figure 6.** Fc γ R-mediated activation of MDMs after viral integration does not affect HIV-1
14 gene expression. MDMs were infected with HIV-1_{VSV-G} and immediately stimulated (0 h)
15 with immobilized IgG or kept in suspension for 72 h or 96 h before stimulation. Luciferase
16 activity in cell lysates was measured 72 h p.i. for MDM stimulated at time 0 and 48 h after
17 activation for MDM stimulated 72 or 96 h p.i. Results are expressed as percentage of the
18 luciferase activity (means and SD of 3 independent wells) found in unstimulated MDM. The
19 experiment shown is representative of experiments on MDM from three different donors.

20 **Figure 7.** Fc γ R-mediated activation inhibits HIV-2, SIVmac and SIVagm replication, but not
21 WN virus replication in MDMs. (A-C) Unstimulated (dotted line) or IC-stimulated (solid line)
22 MDMs were infected with HIV-2_{SBL}, SIVmac251 or SIVagmGril and infection was
23 monitored between seven and 23 days by p27 levels in culture supernatants. The results
24 shown (means and SD of 3 independent wells) are representative of experiments on MDMs
25 from three different donors. (D) MDMs were infected with WN virus. MDMs, unstimulated

1 (US), stimulated with immobilized IgG (S), or treated by IFN- α were infected with IS-98-ST1
2 strain. Supernatants were harvested at 72 hours p.i. and virus infectivity was titrated by focus
3 immunodetection assay (FIA). Data are expressed as means and SD of 3 independent wells.