Brief Definitive Report

Interleukin 13 Inhibits Human Immunodeficiency Virus Type 1 Production in Primary Blood-derived Human Macrophages In Vitro

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Summary

The mechanisms by which cellular immunity maintains the asymptomatic state after human immunodeficiency virus type 1 (HIV-1) infection are poorly understood. $CD4^+$ T lymphocytes play a complex role in regulating anti-HIV effector pathways, including activation of macrophages, which are themselves implicated in clinical latency and pathogenesis of symptomatic acquired immune deficiency syndrome. We have found that a newly identified T helper type 2 lymphokine, interleukin 13 (IL-13), inhibits HIV-1_{ADA} and $_{Ba-L}$ replication in primary tissue culture–derived macrophages but not in peripheral blood lymphocytes. Viral production in cells was measured by viral protein (p24) and reverse transcriptase levels, while entry was assessed by proviral DNA analysis at timed intervals after infection. Inhibition by IL-13 was dose and time dependent and not mediated through altered viral entry, reverse transcription, or viral release. IL-13 is therefore a candidate cytokine for the suppression of HIV infection within monocytes and macrophages in vivo.

Macrophages as well as T lymphocytes play a central role during the pathogenesis of AIDS (1–5). During asymptomatic AIDS infected circulating cells of both types have been shown to harbor unexpressed HIV in vivo (6), while virus is continually being produced within lymph nodes (7, 8). The mechanisms that regulate HIV expression during the different stages of disease are not well understood. Cytokines, while regulating immune responses, may influence viral expression in vivo, since studies in vitro have shown clear effects upon HIV replication (9).

IL-13, a recently characterized human lymphokine produced by activated T cells, displays immunomodulatory effects on B cells and macrophages. This new cytokine is a member of the IL-4 superfamily and shares Th-2 type activities on macrophages with IL-4 and IL-10, even though its described synergy with IL-2 in IFN-γ release by NK cells suggests its activity is not restricted to a generalized shutdown of Th-1 responses (10). Therefore, the present study of IL-13 and its effect on HIV replication in T cells and macrophages is of interest in understanding the possible mechanisms that regulate HIV replication during the different stages of AIDS pathogenesis. We show that human (h)rIL-13 has an inhibitory effect on HIV replication in tissue culture–differentiated macrophages (TCDM), and not in T cells.

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Materials and Methods

Isolation and Culture of Peripheral Monocytes and Lymphocytes. Total human PBMC were isolated from healthy donors as described (11); in short, Ficoll-Hypaque-isolated mononuclear cells were incubated for 1 h in 2% gelatin-coated plates. Adherent cells (TCDM), >94% $CD14^+$ by FACS® (Becton Dickinson & Co., Mountain View, CA), were cultivated in 5% of pooled human serum for 48 h before transfer to either a 96-well plate at a density of 10^5 cells/well (200 μl total volume), or to eight-chamber culture slides (177402; Nunc Inc., Naperville, IL) at a density of 2×10^5 (400 μl total volume). Nonadherent lymphoid cells (PBL) were stimulated with 1 μg/ml PHA-P (Wellcome Laboratories, Beckenham, Kent) for 72 h in 20% FCS and subsequently maintained with 10% FCS and 80 ng/ml IL-2 (Pharmacia, Uppsala, Sweden) in a 96-well plate at a density of 10^5 cells/well (200 μl total volume).

HIV Strains. Macrophage-tropic viral stocks HIV-1_{ADA} (3) and HIV-1_{Ba-L} (2) were grown and titrated in TCDM as described (11) to 1.9×10^5 tissue culture infectious dose ($TCID^{50}$) and 2.7×10^4 $TCID^{50}$ respectively. T cell–tropic viral stock HIV-1_{IIIB} (12) was grown in C8166 cells and titrated in PBL to 4.4×10^4 $TCID^{50}$.

Cytokine Treatment and HIV Infection of Target Cells. IL-13 was provided by Sanofi-Elf as supernatant from COS-transfected cells (COS IL-13) and as purified recombinant protein from CHO cells (hrIL-13).

hrIL-13 titration studies were performed in triplicate cultures of TCDM and PBL from the same donor with decreasing concentrations of hrIL-13 (<30 pg endotoxin/μg of protein) starting from 250 ng/ml. The cytokine was added to cultures for 72 h, leaving an equal portion of untreated cells for postinfection treatment and

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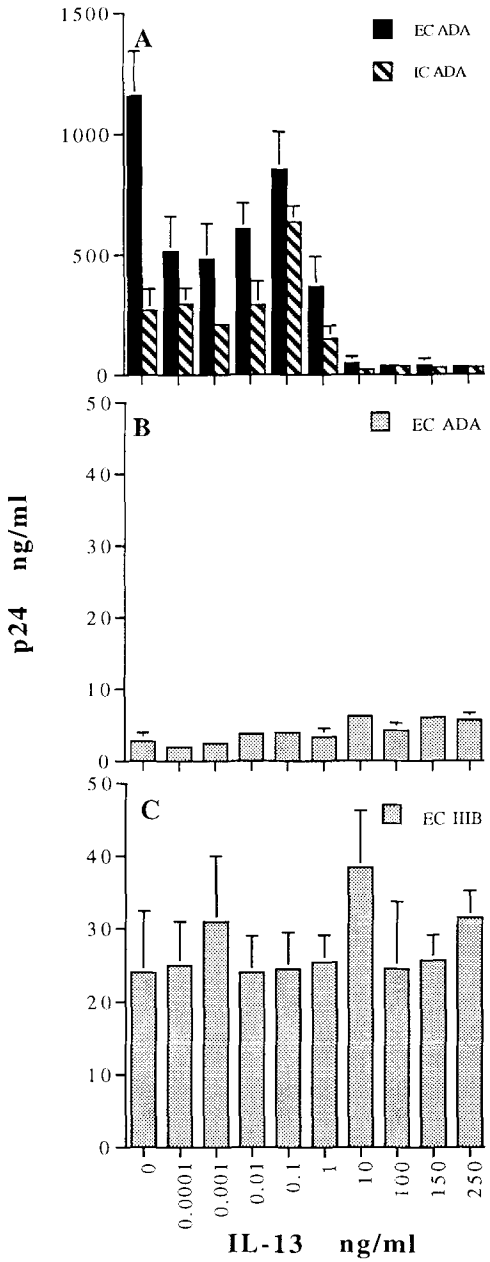

Figure 1. hrIL-13 inhibits HIV-1 replication in TCDM but not PBL. (*A*) Coulter™ ELISA: p24 antigen levels (intracellular [IC] and extracellular [EC]) at 11 d postinfection in TCDM treated with hrIL-13 for 72 h before viral challenge (HIV-1_{ADA} [m.o.i., 0.12]). TCDM were plated at 10^5 cells per well; results are presented in nanograms per milliliter as the mean (± SD) of triplicate assays. (*B*) In-house ELISA: p24 antigen EC levels (note scale) in corresponding PBL at 7 d postinfection under identical conditions of infection (HIV-1_{ADA} [m.o.i., 0.12]) and treatment as for TCDM. (*C*) In-house ELISA: p24 antigen EC levels in PBL 7 d after infection with HIV-1_{IIIB} (m.o.i. 0.03) under identical conditions to those shown in *B*.

HIV-infected controls. HIV-1_{ADA} (multiplicity of infection [m.o.i], 0.12) was added to TCDM, PBL, and respective control triplicate cultures, while HIV-1_{IIIB} (m.o.i., 0.03) was only added to PBL and its control triplicates. Medium and hrIL-13 were replenished every 3 d until 7 d in PBL and 11 d in TCDM. Culture supernatants and cell lysates prepared in 100 μl 1% Empigen lysis buffer for 1 h at 60°C were stored at −70°C before assay of extra- and intracellular p24 antigen.

Growth curves of HIV-1_{ADA} (m.o.i., 0.04) and HIV-1_{Ba-L} (m.o.i., 0.02) in TCDM were performed in eight-chamber slides. hrIL-13 (10 ng/ml) was added in duplicate 72 h before challenge with HIV-1 or 18 h postinfection; the remaining chambers in each slide served as controls (hrIL-13, infected, uninfected). Medium and hrIL-13 were replenished every 3 d. Supernatants were collected every 2 d and kept at −70°C until tested.

Proliferation Assays. After cultivation of TCDM in microtiter plates for 24, 48, and 72 h, in the presence or absence of hrIL-13, [^{3}H]thymidine was added (0.25 μCi/well) and incubated for a further 18 h. Triton X-100 was then added to a concentration of 0.25% (vol/vol), released nuclei were harvested onto glass fiber filters using an automated harvester, and incorporated [^{3}H]thymidine was measured by liquid scintillation spectrometry (LS5000 CE; Beckman Instrs., Inc., Fullerton, CA).

Virus Entry Assay by PCR. TCDM were cultured in 96-well plates (10^5 cells/well) and treated for 72 h with hrIL-13 (10 ng/ml) before infection with HIV-1_{ADA} (m.o.i., 0.12). Cells were lysed at 0, 2, 4, 8, 12, 16, and 20 h postinfection in a buffer containing 100 mM KCl, 20 mM Tris, pH 8.4, 500 μg/ml proteinase K, and 0.2% (vol/vol) NP-40. PCR was performed on cell lysates as described (11), using a Programmable Thermal Controller (M.J. Research Inc., Watertown, MA). The PCR primer sequences for LTR, *gag*, and human β-globin gene (DNA control) were as follows. HIV-1 LTR primers: U3(sense) CACACAAGGCTACTTCCCTGA; U5(antisense)GATCTCTAGTTACCAGAGTCAC; PCR product, 540 bp. HIV-1 *gag* primers: *gag* (sense) GGTACATCAGGCCATATCACC; *gag* (antisense) GGTACATCAGGCCATATCACC; PCR product, 627 bp. Human β-globin control primers; glb (sense) CCTTTGTTCCCTAAGTCCAA; glb (antisense) CCTCACCTTCTTTCATGGAG; PCR product, 238 bp.

Detection of CD4 in TCDM. TCDM were treated with hrIL-13 (10 ng/ml) and assayed for CD4 expression by FACS®. ADP 318/C4120 mouse IgG1 mAb (MRC AIDS Directed Programme, London, UK) was used to detect CD4 on TCDM. MOPC-21 (Sigma, Inc., Poole, UK) mouse IgG1 was used as an isotype-matched Ab control and 15.2 mouse IgG1 against CD54 (provided by Dr. Nancy Hogg, Imperial Cancer Research Fund Labs., London, UK) as a positive control for antigen expression. FITC-conjugated rabbit anti-mouse Ig (RAM-FITC) (Serotec Ltd., Oxford, UK) was used to detect bound Ab.

Reverse Transcriptase Activity Assay. 5 μl of culture supernatants was added to 50 μl of a mixture containing poly(A), oligo(dT) (Pharmacia Fine Chemicals, Piscataway, NJ), $MgCl_2$, and ^{32}P-labeled deoxythymidine 5′-triphosphate (dTTP) (Amersham Corp., Amersham, Buckinghamshire, UK), and incubated for 1.5 h at 37°C. 3 μl of the mixture was spotted in duplicate onto DE81 paper, air dried, and washed four times in 2× SSC buffer and two additional times in 95% ethanol. The paper was then dried and either cut and assayed by scintillation counting or analyzed by phosphoimaging (Molecular Dynamics, Kent, UK).

HIV ELISA. Supernatants were initially tested for p24 antigen by an in-house–adapted ELISA (13) as described (14). Macrophage-tropic viral supernatants were further tested with a Coulter™ HIV-1p24 kit (Luton, Bedfordshire, UK). The sensitivity for macrophage-tropic strains between the two ELISAs differed by a factor of 100.

Results and Discussion

We have defined a model system in which TCDM and corresponding PBL from normal individual donors are challenged

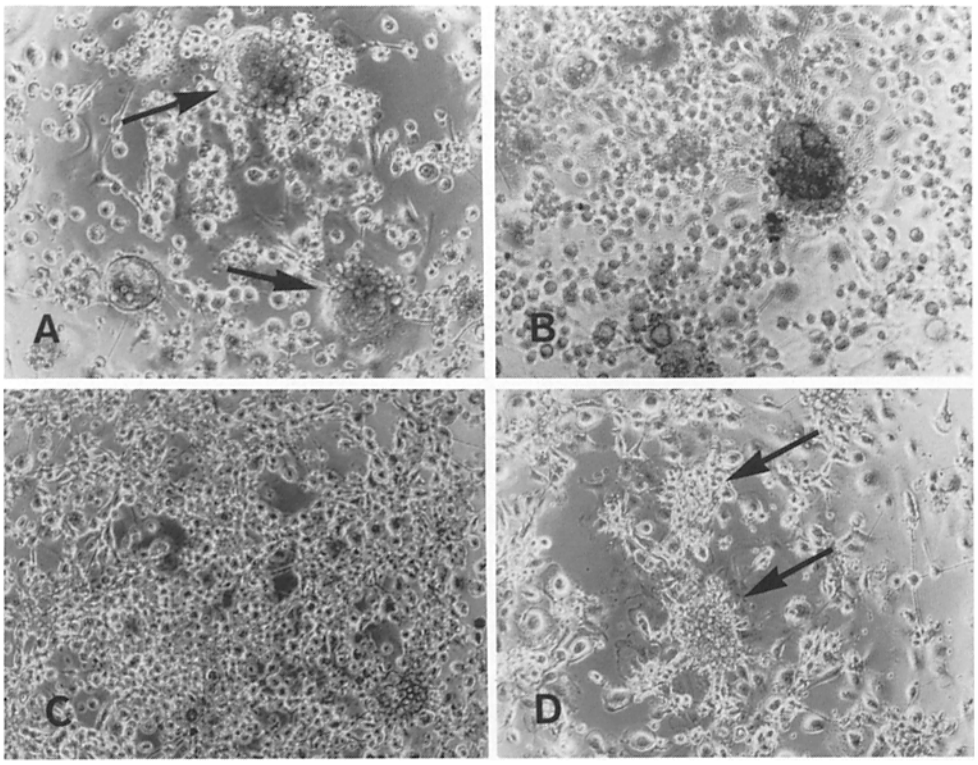

Figure 2. Phase-contrast micrographs to illustrate the morphologic effects of hrIL-13 in TCDM (9 d postinfection with HIV-1_{ADA} [m.o.i. 0.12]) in 96-well plates. (*A*) No cytokine, (*B*) 1 ng/ml, (*C*) 10 ng/ml, and (*D*) 150 ng/ml. *A* (*arrows*) shows examples of HIV-induced syncytium formation and cytolysis in TCDM. *C* shows hrIL-13-dependent aggregation of viable macrophages (more pronounced in *D*, *arrows*) and HIV c.p.e. protection, which are lost at 1 ng/ml IL-13 (*B*). These pictures correspond to the experiment from which Fig. 1 *A* was derived (×20).

with a defined infectious challenge of different HIV-1 strains (ADA, Ba-L, and III_B) that express different tropism for primary macrophages and lymphocytes. Viral replication during the 2 wk after infection was measured by several independent assays: intra- and extracellular p24 antigen, reverse transcriptase, cytopathic effect (c.p.e.), and PCR analysis for proviral DNA.

The effect of IL-13 on HIV-1 replication was determined using a range of lymphokine concentrations added before, at the time of, or after challenging with virus at various m.o.i. In a series of eight initial independent experiments COS IL-13 inhibited viral replication of both HIV-1_{ADA} and $_{Ba-L}$-infected TCDM compared with COS control supernatants, while not affecting PBL infected with HIV-1_{IIIB}. These studies with COS IL-13 showed characteristic aggregation of TCDM when viewed by phase contrast microscopy, which correlated with the anti-HIV effect and was absent from control supernatants (data not shown).

To confirm that the anti-HIV and morphological effects were due to IL-13, purified recombinant protein was used to titrate both effects. 1–10 ng/ml hrIL-13 inhibited, up to 30-fold, intra- and extracellular p24 production in TCDM that had been pretreated with cytokine for 72 h before challenge with HIV-1_{ADA} and then maintained in its presence (Fig. 1 *A*). The morphological effects of hrIL-13 (Fig. 2) treatment in the same HIV-1_{ADA}-infected cultures resembled those observed with COS IL-13. These micrographs illustrate dose-dependent aggregation of TCDM treated with hrIL-13 and inhibition of virus-induced c.p.e., characterized by giant cell formation followed by membrane disruption and cytolysis. Control experiments confirmed that both activities were destroyed by heat inactivation (not shown). Uninfected TCDM remained fully viable at all hrIL-13 concentrations tested, and a similar concentration-dependent cell aggregation was observed. Indeed, at the same doses of hrIL-13, levels of mannose receptor are increased on these cells (our unpublished observations), confirming a lack of cytotoxicity. Inhibition of p24 antigen production by hrIL-13 treatment 18 h postinfection and maintained in its presence thereafter was comparable to inhibition observed after hrIL-13 pretreatment in 10 of 12 experiments. Two experiments showed no inhibi-

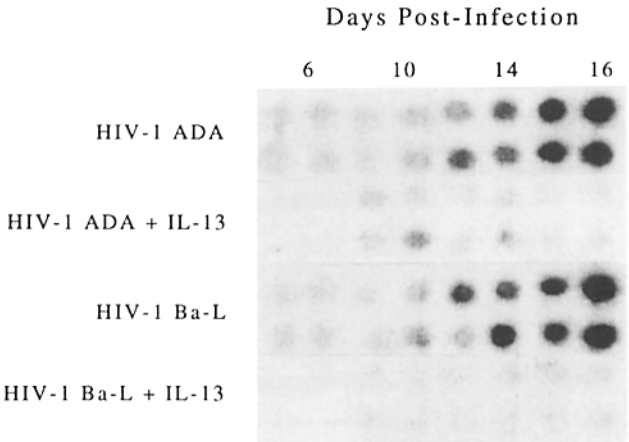

Figure 3. hrIL-13 inhibits HIV-1 reverse transcriptase production in TCDM. Shown is a photophosphoimager exposure of DE81 paper spotted with 3 μl of extracellular supernatants derived from duplicate 16-d growth curves of HIV-1_{ADA} and HIV-1_{Ba-L} in control and 10 ng/ml IL-13-treated (72 h pre-HIV infection) TCDM.

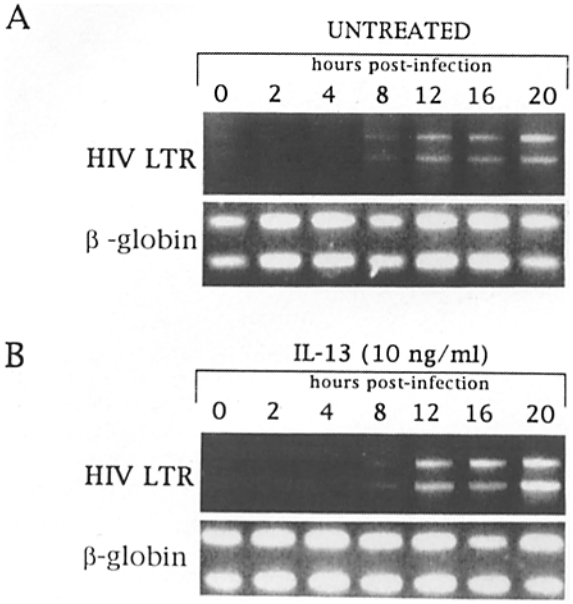

Figure 4. hrIL-13 does not inhibit viral entry into TCDM. Shown is a time sequence >20 h postinfection by HIV-1_{ADA}(m.o.i. 0.12). (*A*) Two double-loaded 2% agarose gels with PCR products for HIV LTR (540 bp) and human β-globin control (238 bp) after HIV infection in untreated TCDM. (*B*) Similar format for TCDM pretreated with 10 ng/ml hrIL-13 72 h before HIV infection.

tion when hrIL-13 treatment was delayed until after infection. hrIL-13 HIV-1 inhibition in TCDM at 10 ng/ml was further confirmed with additional viral growth curves measuring supernatant reverse transcriptase (Fig. 3) and p24 antigen levels after infection with either HIV-1_{ADA} or $_{Ba-L}$.

Analysis of cellular morphology of TCDM in the absence of virus showed that hrIL-13 at 10 ng/ml induced aggregation and time-dependent formation of multinucleated giant cells, compared with background polykaryon levels with 5% human serum alone. IL-4 has also been reported to induce giant cell formation (15), corresponding to the apparent biological similarities described between IL-4 and IL-13 (8). Further experiments are needed to establish the mechanism and efficacy of TCDM giant cell formation by hrIL-13, and its relationship to HIV-induced cell fusion and cytolysis.

hrIL-13 did not inhibit HIV-1 replication in PBL under comparable conditions, with either the identical strain and m.o.i. as used in TCDM (HIV-1_{ADA}[m.o.i., 0.12]), or a more infectious lymphotropic strain (HIV-1_{IIIB}[m.o.i., 0.03]) (Fig. 1, *B* and *C*, respectively). There was no inhibition of p24 antigen produced in either instance and no cytotoxicity resulting from hrIL-13 itself at all concentrations tested. These results were confirmed in two additional experiments.

The possible mechanism by which hrIL-13 (10 ng/ml) inhibited HIV-1 replication in TCDM was investigated. HrIL-13 did not affect cell surface $CD4^+$ or thymidine incorporation in uninfected cells (not shown). Viral entry and reverse transcription as measured by PCR for viral LTR (Fig. 4) and *gag* primers (not shown) were similar in treated and untreated controls. The HIV LTR signal appeared simultaneously at 8 h in both hrIL-13-treated and untreated TCDM. Proviral DNA remained detectable throughout the period of culture, and recovery experiments (no replenishment of IL-13) showed that HIV had not been eradicated by IL-13 (not shown). Experiments such as those described above (Fig. 1 *A*) established that IL-13 did not inhibit release of virus p24 antigen by TCDM into the culture supernatants. Further experiments are needed to establish the intracellular process between reverse transcription and p24 translation by which IL-13 inhibits HIV-1 production in TCDM.

Only IFN-α and IFN-β have been consistently reported to inhibit HIV-1 replication in macrophages or monocytoid cell lines, while other cytokines, including IFN-γ, IL-4, and TGF-β, are categorized "bifunctional" since they have been reported to enhance and/or inhibit HIV-1 (9, 16–18). The factors responsible for the variable effects in studies concerning this latter group of cytokines have not been defined, other than possible differences in monocyte culture conditions, timing of cytokine addition, cytokine doses used, and donor variation. In the present study, variation in the efficiency of replication of HIV-1_{ADA} and $_{Ba-L}$ in TCDM from different apparently healthy donors may partly account for the difference in inhibition observed when hrIL-13 treatment was delayed until after infection.

IL-13 provides a potential pathway by which activated T lymphocytes can suppress HIV replication in macrophages in vivo and inhibit dissemination of monocytotropic variants (19). IL-13 may also contribute to the maintenance of postintegration HIV latency (8) by its inhibition of monocyte IL-6 and TNF-α production (9). IL-13 treatment in HIV^+ patients could have a beneficial virostatic action to reduce the systemic viral load and be of use in combination with other forms of antiviral therapy. Further studies are needed to study its mechanism of action in vitro and the contribution of host IL-13 during different stages of the disease in vivo.

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