Role of IgE Immune Complexes in the Regulation of HIV-1 Replication and Increased Cell Death of Infected U1 Monocytes: Involvement of CD23/FcεRII–Mediated Nitric Oxide and Cyclic AMP Pathways

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ABSTRACT

Background: IgE/anti-IgE immune complexes (IgE-IC) induce the release of multiple mediators from monocytes/macrophages and the monocytic cell line U937 following the ligation of the low-affinity Fcε receptors (FcεRII/CD23). These effects are mediated through an accumulation of cAMP and the generation of L-arginine–dependent nitric oxide (NO). Since high IgE levels predict more rapid progression to acquired immunodeficiency syndrome, we attempted to define the effects of IgE-IC on human immunodeficiency virus (HIV) production in monocytes.

Materials and Methods: Two variants of HIV-1 chronically infected monocytic U1 cells were stimulated with IgE-IC and virus replication was quantified. NO and cAMP involvement was tested through the use of agonistic and antagonistic chemicals of these two pathways.

Results: IgE-IC induced p24 production by U1 cells with low-level constitutive expression of HIV-1 mRNAs and extracellular HIV capsid protein p24 levels ($U1^{low}$), upon their pretreatment with interleukin 4 (IL-4) or IL-13. This effect was due to the crosslinking of CD23, as it was reversed by blocking the IgE binding site on CD23. The IgE-IC effect could also be mimicked by crosslinking of CD23 by a specific monoclonal antibody. p24 induction by IgE-IC was then shown to be due to CD23-mediated stimulation of cAMP, NO, and tumor necrosis factor α (TNFα) generation. In another variant of U1 cells with >1 log higher constitutive production of p24 levels ($U1^{high}$), IgE-IC addition dramatically decreased all cell functions tested and accelerated cell death. This phenomenon was reversed by blocking the nitric oxide generation.

Conclusions: These data point out a regulatory role of IgE-IC on HIV-1 production in monocytic cells, through CD23-mediated stimulation of cAMP and NO pathways. IgE-IC can also stimulate increased cell death in high HIV producing cells through the NO pathway.

INTRODUCTION

It is now well established that in addition to T cells, monocytes/macrophages play a central role in the pathogenesis of acquired immunodeficiency syndrome (AIDS) (1–3). The recently described ability of both cell types to harbor microbiologically latent human immunodeficiency virus (HIV) (4–7), (where viral expression can be induced to produce infectious virus) as well as to maintain restricted chronic low level viral expression (8,9), suggests a mechanism for viral persistence during the long asymptomatic period

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seen in AIDS. Even in the lymph nodes, where virus is continually produced (7,10), most T cells and macrophages containing provirus do not have detectable viral mRNA (7). It is clear that the macrophage serves as a viral reservoir, since HIV has been detected in tissue macrophages of many organs.

It is interesting that Con A–activated T cells can reactivate expression of latent virus in monocytes obtained from AIDS patients (6), suggesting that immune activation can play a role in HIV pathogenesis. In various human immune responses, IgE levels increase and correlate with an enhancement of *in situ* infiltration by hemopoietic effector cells and the release of multiple mediators from these cells (11–17). Allergy and IgE levels are not dramatically elevated in HIV patients, but high IgE levels predict more rapid progression to AIDS (18–20).

IgE and antigen directly activate the secretion of various cytokines from mast cells following cross-linking of the high-affinity receptor, FcεRI (12,13). IgE/antigen also induce the synthesis and the release of tumor necrosis factor α (TNFα) and other mediators from the human mononuclear phagocytic cell lineage (14–17). These cells are potent effectors of inflammation through their capacity to secrete preformed and newly synthesized monokines, respiratory burst products, and arachidonate metabolites (21).

Although monocytes/macrophages are a main reservoir of HIV-1, the role of IgE on HIV-infected cells and AIDS pathogenesis remains unclear. Monocytes/macrophages have a restricted FcεRI expression (22), but can easily bind IgE following expression of a specific isoform of the low-affinity receptor CD23b/FcεRII, undetected in mice (17,23,24). Recently, we have shown that CD23b is a functional IgE receptor on normal human monocytes and U937 cells (17,25). Ligation of CD23 by IgE/anti-IgE or specific monoclonal antibody (mAb) induces various monocyte functions through two second messengers: cAMP and nitric oxide (NO) (17,25,26). In the present study, we attempted to define the role of IgE-anti-IgE immune complexes (IgE-IC) on HIV production in monocytes using two variants of the HIV-1 chronically infected monocytic U1 cells (a clone of U937). Our data point to a regulatory role of IgE-IC on HIV production in monocytic cells, through CD23-mediated stimulation of cAMP and NO pathways. In addition, we suggest that IgE-IC can stimulate increased cell death in high HIV producing cells through the NO pathway.

MATERIALS AND METHODS

Reagents

Cells were grown in RPMI medium supplemented with L-glutamine, penicillin, streptomycin, and 10% fetal calf serum (FCS) (all from Gibco Laboratories, Grand Island, NY, U.S.A.). Culture medium, chemicals, and FCS were tested for the absence of direct effects on U1 cells (CD23 expression, TNFα, and p24 expression as activation markers). Cultures were also supplemented with: TNFα and neutralizing anti-TNFα mAb (Genzyme, Paris, France); human IgE (Stallergene, Paris, France); goat anti-human-IgE (Nordic Immunology, Tilburg, The Netherlands); N^G-monomethyl-L-arginine (L-NMMA), D-NMMA, L-arginine, D-arginine, superoxide dismutase (SOD), and catalase (all from Sigma, St. Louis, MO, U.S.A.); S-nitroso-acetyl-penicillamine (SNAP; Alexis Corporation, Läufelfingen, Switzerland); 6-morpholino-sydnonimine (SIN-1; Glaxo, Paris, France); FITC-conjugated CD14-mAb, CD71-mAb, and CD19-mAb (clone BC3, IgG1κ) (all from Immunotech, Marseille Lumigny, France). FITC-conjugated CD23-mAb (clone 25) was used for cell labeling and CD23-mAb (clone 135: IgG1κ, $F(ab')^2$ or Fab) for biologic investigation (27). The mAb 135 have better specificity than mAb 25 in recognizing the IgE-binding site of CD23 (27) and, in its Fab form, to prevent CD23 cross-linking with no cell activation (17,28).

Cells

U1 cells with low p24 production levels (<250 pg/10^5 cells/ml in 3-day supernatants) designed later as U1^{low} were a gift of T. Folks (CDC, Atlanta, GA, U.S.A.). They do not express CD23 (<4%). Following 48 hr of incubation at 37°C with 10 ng/ml of interleukin 4 (IL-4) or IL-13, the cells were collected, washed and analyzed for their reactivity with FITC-conjugated CD23-mAb. U1^{low} cells became >63% CD23$^+$. In addition to U1^{low} cells, we have also used a variant of U1 cells which raised spontaneously from long-term cultures of U1^{low} cells, and stably express higher (>1 log) surface density of CD23/FcεRII and CD14 compared with U1^{low} cells. These cells express nonspecific esterases, and have 1 log higher extracellular p24 levels (>2.5 ng/ml/10^5 cells per 3 days) than U1^{low} (see results). These cells are designated in this work as U1^{high} cells.

Cell Cultures

$U1^{low}$ or $U1^{high}$ cells were treated with IgE (1–10 μg/ml) for 1 hr, washed and incubated with anti-IgE (1–30 μg/ml) plus other factors, as indicated, for 48 hr. Other treatments were CD23-mAb (clone 135) (27); an isotype-matched control (CD19-mAb, 20 μg/ml); and anti-FcεRI-mAb (Clone 15-1, a gift from Dr. J. P. Kinet, National Institutes of Health, Bethesda, MD, U.S.A.) (29). In control experiments, this anti-FcεRI antibody induced the release of 23% histamine from human basophils. Some cultures were also supplemented with anti-human TNFα mAb (15 μg/ml); SOD (120 U/ml) and catalase (240 U/ml); and chemical NO donors SIN-1 and SNAP. SOD and catalase used in this work inhibited >80% superoxide levels induced by lipopolysaccharide (LPS) in human macrophages (data not shown). To some infected cultures, CD23-Fab (20 μg/ml), L-NMMA, and D-NMMA (1–1000 μM) were added for 1 hr before IgE treatment, washed; and afterwards incubated with the required above factors plus L-NMMA in D-NMMA for 48 hr. To reverse L-NMMA effect, the cultures were supplemented with L-arginine, compared with D-arginine. Preliminary analysis using various concentrations of these reagents (not shown) permitted definition of optimal doses used in the present work. Following 1–3 day of incubation, cell supernatants were collected and the levels of p24, nitrites, and TNFα were determined. Viable cells were counted through trypan blue exclusion.

Factor Assays

To assay virus replication levels, we have quantified the HIV capsid protein p24 by enzyme-linked immunosorbent assay (ELISA; Institut Pasteur, Paris; or Dupont de Nemours, les Ulis, France) in cell supernatants, as recommended by the manufacturer. This test was yet reported to correlate with cell infection levels, in U1 cells in particular (6,30). TNFα measurement was performed by ELISA (Genzyme, Cambridge, MA, U.S.A.). Stable end product of NO, NO_2^-, was assayed using Greiss reaction modified as detailed elsewhere (31). Cell surface phenotype was assayed using immunofluorescence with FITC-conjugated mAb from Immunotech as previously described (25). Nonspecific butyrate esterase and its inhibition by sodium fluoride were performed as detailed elsewhere (25). Results were analyzed and compared using the Student *t* test for paired data.

HIV-1 mRNA Detection

The experiments were performed as follows: U1 cells (3×10^5/ml) were treated with 10 ng/ml IL-4 or IL-13 for 48 hr, washed and treated with IgE (10 μg/ml) 1-hr incubation followed by anti-IgE (30 μg/ml) addition. After 48 hr, RNA was isolated using RNA-STAT (Tel-Test Inc., Freindswood, TX, U.S.A.). For reverse transcriptase–PCR (RT-PCR), amplification reactions of 1 μg of RNA (32,33) contained 2 mM $MgCl_2$, 10 mM Tris-HCl, pH 8.3, 50 mM KCl, 1 mM of each four deoxynucleotides (Perkin Elmer Cetus, Emerville, CA, U.S.A.). 100 units of M-MLV Reverse Transcriptase (BRL, Bethesda, MD, U.S.A.), 2.5 units AmpliTaq DNA polymerase, 1 unit RNase inhibitor (Promega), 0.01 M DTT, and 1 μM of each primer. Primer pairs used were: SK38/39 HIV gag (1543–1570, 1630–1657) (34); and GAPHD splice product (371–388, 546–565) (35). Thirty amplification cycles were carried out using a thermocycler (Perkin Elmer Cetus), denaturing at 94°C for 1 min, annealing at 55°C for 1 min, and extending at 72°C for 2 min, followed by liquid hybridization as described (32). Probes used were SK19 HIV gag (1587–1627) and GAPDH (514–532). Analysis of hybridized products was visualized on a 8% nondenaturing acrylamide/1X TAE gel and exposure to Kodak XAR-5 film at −50°C for 1–2 hr (with intensifying screens). Amplification products were quantified on a densitometer (Molecular Dynamics, Sunnyvale, CA, U.S.A.) using the GPDH as a control.

RESULTS

IgE-IC Enhances HIV Replication in IL-4– or IL-13–Treated U1 Cells

The effect of IgE-IC on p24 production by $U1^{low}$ cells was assayed. $U1^{low}$ cells were also pretreated with IL-4 or IL-13 prior to incubation with IgE-IC. In fact, we have recently shown (17) that these cytokines are necessary to induce IgE responses in the monocyte/macrophage lineage, probably through the induction of FcεRII (see below). As shown in Fig. 1, sequential treatment of U1 cells with IL-4 or IL-13 for 48 hr, then IgE-IC resulted in a significant induction of p24 levels ($p < 0.003$). Treatment with IL-4 or IgE-IC alone had no effect on p24 levels over three days of incubation, while IL-13 caused a suppression in p24 levels (Fig. 1B). To determine the role of IgE-IC on HIV-1 transcription, we

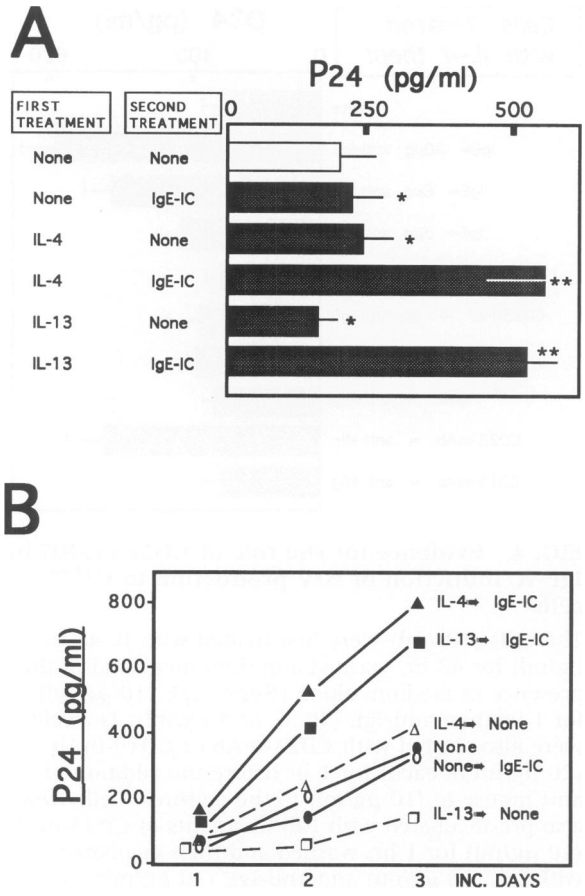

FIG. 1. Induction of p24 production in U1^{low} cells by IgE-IC

The cells were incubated (10^5/ml) for 48 hr in medium alone (None), or in the presence of IL-4 or IL-13 (10 ng/ml). After washing, they were incubated for 1 hr with IgE (10 μg/ml), washed, and reincubated for 48 hr (A) or for 24, 48, or 72 hr (B) with anti-IgE (30 μg/ml). Cell supernatants were then collected and their p24 quantified. Values are mean (B) or mean ± SD from three distinct assays (A) done on three different U1 cell preparations.

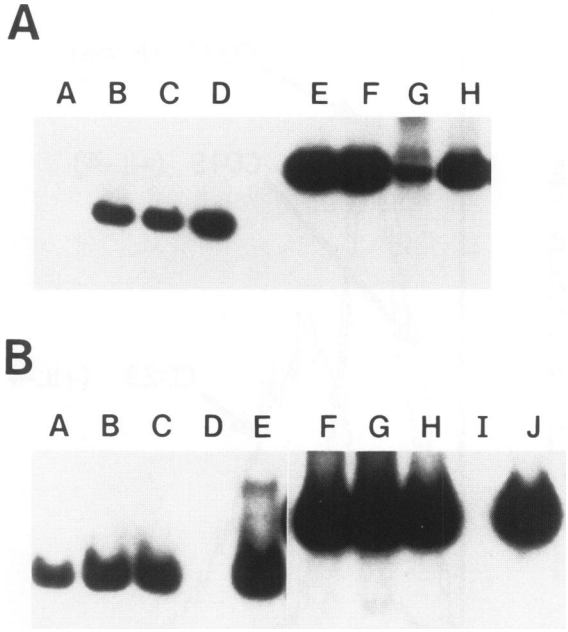

FIG. 2. Effect of IgE-IC on HIV mRNA accumulation in U1^{low} cells

RNA from U1 cultured for 48 hr was isolated and 1 μg of RNA was amplified as described in Materials and Methods. The oligomer hybridization autoradiography demonstrates the presence of amplified HIV-1 gag (Panel A, A–D; Panel B, A–E) and GAPDH-RNA (Panel A, E–H; Panel B, F–J). (A) Lane A, no RNA; Lane B, U1; Lane C, U1 + IL-4; Lane D, U1 + IL-4/IgE-IC; Lane E, U1; Lane F, U1 + IL-4; Lane G, U1 + IL-4/IgE-IC (1 μg anti-IgE); Lane H, U1 + IL-4/IgE-IC (2 μg anti-IgE). (B) Lane A, U1 + IL-13; Lane B, U1 + IL-13/IgE-IC; Lane C, U1 + IL-13 + IL-4/IgE-IC; Lane D, same as Lane C with no RT; Lane E, THP-1 productively infected with HIV; Lane F, U1 + IL-13; Lane G, U1 + IL-13/IgE-IC; Lane H, U1 + IL-13 + IL-4/IgE-IC; Lane I, same as Lane H with no RT; Lane J, THP-1 productively infected with HIV.

have assayed the effect of IgE-IC on HIV-gag mRNA accumulation in U1^{low} cells (Fig. 2). As shown, while IL-4 and IL-13 had no effect on basal gag levels, treatment with IgE-IC enhanced gag mRNA levels in U1^{low} cells (2- to 4-fold amplification, as quantified by a densitometer). These results indicate that IgE-IC can augment HIV production in U1 cells.

Requirement for FcεRII/CD23 for IgE-Mediated Activation of U1 Cells

Normal human monocytes express various potential ligands for IgE including FcεRI, FcεRII/CD23, Mac-2, FcγRII, and FcγRIII (22,36–38). Having recently shown that IgE-IC trigger normal monocytes and U937 cell activation through the ligation of FcεRII/CD23 (17,25,26,39), we asked whether CD23 mediates IgE-induced p24 production by U1^{low} cells, HIV-infected counterpart of U937 cells. The need for IL-4 or IL-13 for the induction of IgE-IC response is indicative of CD23 involvement as these cytokines are potent inducers of CD23 transcription and surface expression (36,40) (Fig. 3). U1^{low} cells, preincubated with IL-4, were thus treated with blocking Fab fragments of anti-CD23 mAb (CD23-Fab) 1 hr before the addition of IgE-IC (Fig. 4). Pre-

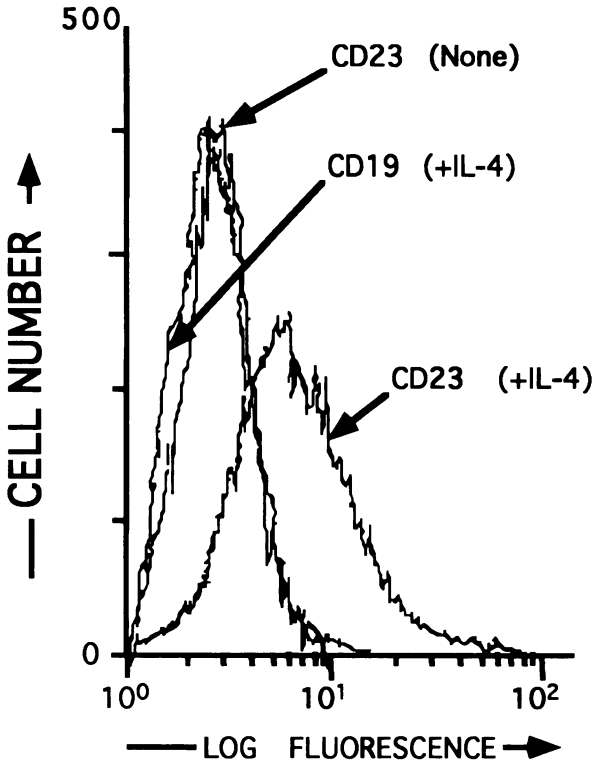

FIG. 3. Induction of CD23 expression on U1^{low} cells by IL-4

Cells were incubated (10^5/ml) for 48 hr in the presence of 10 ng/ml IL-4. They were then washed and assayed for their reactivity with FITC-CD23-mAb and isotype-matched control (FITC-CD19-mAb).

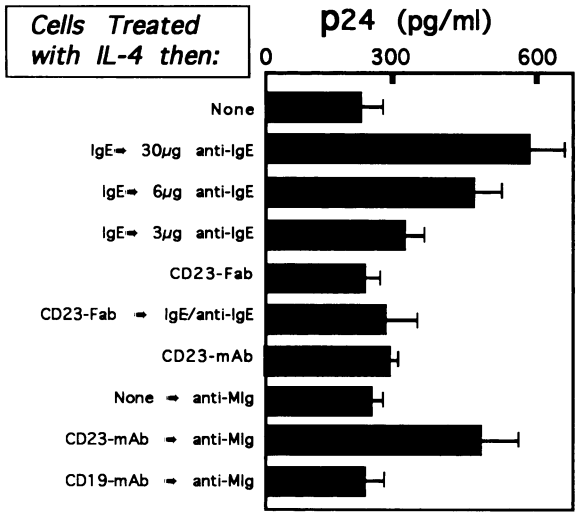

FIG. 4. Evidence for the role of CD23/FcεRII in IgE-IC induction of HIV production in U1^{low} cells

The cells (10^5/ml) were first treated with IL-4 (10 ng/ml) for 48 hr, washed and then incubated in the presence of medium alone (None), IgE (10 μg/ml) for 1 hr then anti-IgE (30, 6, or 3 μg/ml). The cells were also treated with CD23-mAb or CD19-mAb (20 μg/ml of each) for 1 hr before the addition of anti-mouse Ig (10 μg/ml) to the cultures. Cells were also pre-incubated with Fab fragments of CD23-mAb (10 μg/ml) for 1 hr, washed and then incubated with IgE (10 μg/ml) and anti-IgE (30 μg/ml). Cell supernatants were harvested following 48 hr post IL-4 and the p24 quantified. Shown are mean ± SD values of two distinct U1 cell preparations, each done in duplicates.

treatment of U1 cells with CD23-Fab, and not an isotype matched mAb (CD19-mAb), inhibits the effects of IgE-IC on p24 production by these cells. Furthermore, treatment of IL-4 induced U1^{low} cells with CD23-mAb + anti-mouse Ig (MIg) induces significant increases in p24 levels ($p < 0.02$) (Fig. 4). Anti-MIg was used in order to induce the crosslinkage of surface CD23, necessary to obtain optimal monocyte activation. No such effect was observed with anti-MIg alone or with an isotype-matched control mAb (CD19) (Fig. 4). In addition, we failed to detect FcεR1 on U1 cells, even after their activation by IL-4 or IL-13, and anti-FcεRI mAb failed to inhibit IgE-IC effects (data not shown). Finally, these culture conditions did not induce significant cell death and cell recovery was >70% of control cultures pretreated with IL-4 alone. Together, these data indicate the involvement of FcεRII/CD23 in IgE-IC mediated induction of U1^{low} cell activation and p24 production, while no role could be found for FcεRI in the phenomenon.

Involvement of CD23 Transduction Signals in IgE-IC–Mediated U1^{low} Cell Activation

Ligation of CD23b by IgE-IC induces two major intracellular signals in monocytic lineage, cAMP and NO (17,25,26,28). The respective role of each of these intracellular events in IgE-dependent enhancement of p24 production in U1^{low} cells was then assayed. Biochemical modulators of these factors were added to the cells prior to the treatment with IgE-IC. The inhibition of cAMP pathway by Rp-cAMP significantly reversed IgE-IC–dependent enhancement of p24 ($p < 0.004$) (Fig. 5A). Furthermore, addition of cAMP agonistic analogue (Dibut-cAMP) greatly enhanced the extracellular p24 levels in both untreated and IgE-IC–treated cells in a dose-dependent manner (Fig. 5B). Addition of the NO synthase inhibitor, L-NMMA also decreased IgE-IC induction of p24, but to a lesser extent than Rp-cAMP ($p < 0.03$). No such effects were

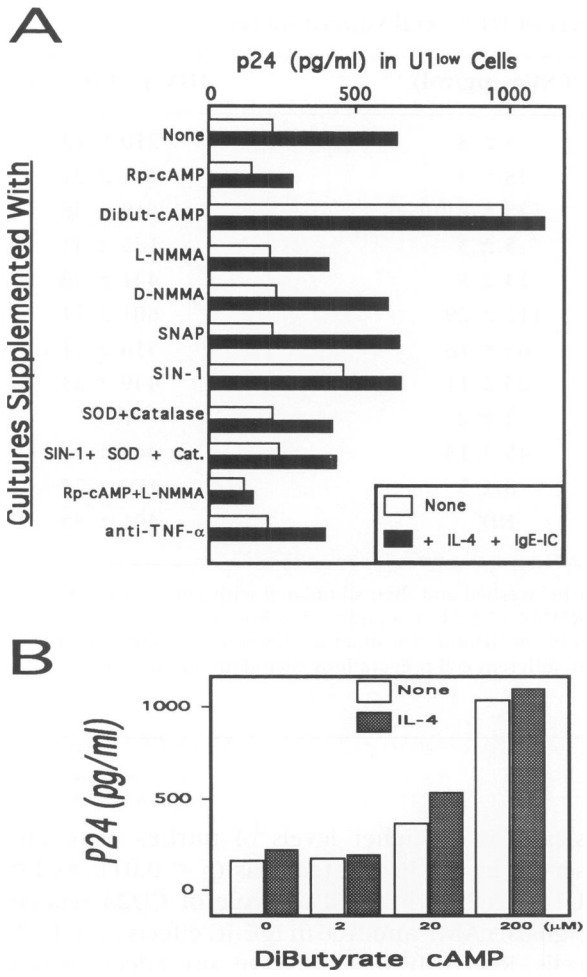

FIG. 5. Involvement of CD23-stimulated intracellular signaling in IgE-IC–mediated induction of HIV production in U1^{low} cells

(A) IL-4–treated (as in Fig. 1) cells were supplemented (10^5/ml) with the following: 200 μM Rp-cAMP, 100 μM Dibut-cAMP, 1 mM L-NMMA, 1 mM D-NMMA, 1 mM SNAP, 200 μM SIN-1, SOD (120 U/ml) + catalase (240 U/ml), or 10 μg/ml anti-TNFα mAb. Following 2 hr of incubation, the cells were washed and treated with IgE for 1 hr then by anti-IgE and above factors as in Fig. 4. Cell supernatants were then assayed for their p24 content. Shown are mean values (SD < 17%) from three different U1 cell preparations. (B) U1^{low} cells were or were not treated with IL-4 for 48 hr, washed and then incubated for 48 hr with decreasing concentrations of Dibut-cAMP. A dose-dependent induction of p24 is observed. Shown are mean values from two different experiments (SD < 13%).

observed with D-NMMA. The addition of the NO donor SNAP had little or no effect on p24 production by untreated U1^{low} cells, while another NO donor compound, SIN-1, significantly increased p24 production by untreated U1^{low} cells. Neither compound had any effect on IgE-IC–stimulated cells. In contrast to SNAP, SIN-1 also gives rise to peroxynitrites as well as to NO (41). Peroxynitrites are potent oxidants (42–44) and can be neutralized by anti-oxidant enzymes. We thus asked whether these oxidants play a role in IgE-IC effect on U1^{low} cells. Cell treatment with SOD + catalase partially reversed IgE-IC mediated induction of p24 ($p < 0.02$), at levels close to those observed with L-NMMA (Fig. 5A). SOD + catalase also inhibits p24 enhancement by SIN-1 (Fig. 5A). The addition of both Rp-cAMP and L-NMMA completely reversed IgE-IC induced p24 production in U1^{low} cells (Fig. 5A). In addition to p24 induction and as in U937 cells, U1^{low} cell treatment with IgE-IC induces nitrites levels in the same cell supernatants (from 0.5 ± 0.1 μM in medium alone to 3.8 ± 0.7 μM with IgE-IC), completely reversed by L-NMMA treatment (data not shown). Altogether, these data suggest that cAMP accumulation and the formation of peroxynitrites (inhibited by both L-NMMA and anti-oxidants) play a role in p24 induction by IgE-IC.

Involvement of TNFα in IgE-IC–Mediated Activation of HIV Production in U1^{low} Cells

CD23 has previously been shown to induce the production of TNFα in human monocytes (17,39). We then asked the role of this cytokine in IgE-IC–mediated regulation of HIV expression. Anti-TNFα antibody decreased IgE-IC mediated p24 induction (Fig. 5A), which suggests a role of TNFα in this phenomenon. The presence of TNFα is confirmed through the ability of IgE-IC to induce the production of TNFα from IL-4–treated U1^{low} cells (Table 1). TNFα increase by IgE-IC is correlated with p24 levels detected in the same supernatants (Table 1). TNFα production is inhibited by L-NMMA, and to lesser extent by Rp-cAMP, while D-NMMA addition had no effect on this phenomenon. L-NMMA effect was partially reversed through the addition of L-arginine but not D-arginine to IgE-IC–stimulated cultures. Addition of both Rp-cAMP and L-NMMA completely abolished TNFα induction by IgE-IC (Table 1). Together, these data demonstrate the involvement of CD23-related TNFα in IgE-IC activation of U1^{low} cells.

TABLE 1. IgE-mediated induction of TNFα and p24 levels in U1^{low} cell supernatants

U1^{low} Cells Cultured with:	TNFα (pg/ml)	HIV-p24 (pg/ml)
None[a]	5 ± 3	210 ± 42
IgE (2 μg)-Anti-IgE (10 μg)	38 ± 4	321 ± 21
IgE (10 μg)-Anti-IgE (10 μg) = (IgE-IC)	125 ± 21	619 ± 36
L-NMMA	5 ± 3	183 ± 41
L-NMMA + IgE-IC	14 ± 5	441 ± 38
D-NMMA + IgE-IC	112 ± 29	601 ± 11
L-NMMA + L-arginine + IgE-IC	65 ± 18	516 ± 71
L-NMMA + D-arginine + IgE-IC	25 ± 11	449 ± 33
Rp-cAMP	2 ± 2	ND[a]
Rp-cAMP + IgE-IC	45 ± 13	349 ± 10
Rp-cAMP + L-NMMA + IgE-IC	8 ± 5	170 ± 32
IgE-IC + anti-TNFα mAb	ND[a]	401 ± 35

IL-4–treated cells (as in Fig. 1) were incubated (10^5/ml) with IgE for 1 hr, washed and then stimulated with anti-IgE. Some cells were supplemented with anti-TNFα (10 μg/ml), L-NMMA (1 mM), D-NMMA (1 mM), L-arginine (5 mM), D-arginine (5 mM), or/and Rp-cAMP (100 μM) 1 hr before IgE-IC treatment. Following 48 hr incubation, cell supernatants were collected and assayed for their TNFα and p24 content. Values are mean ± SD from two different cell preparations each done in duplicates.
[a]ND = not determined.

Effects of IgE-IC on U1^{high} Cells

IgE-IC effects on U1^{low} cells led us to assay their effect on another variant of U1 cells with high HIV production levels. As observed in activated normal human monocytes/macrophages (17) or differentiated U937 cells (25), these cells are stably CD23$^+$, CD14^{low} (Table 2). They produce higher p24 (>2.5 ng/ml) and TNFα (>235 pg/ml) levels after IgE-IC incubation (Table 2) compared to U1^{low} cells (Fig. 1 and Table 1). These cells were designated as U1^{high} cells in this study. U1^{high} cells expressed CD23 and did not require pretreatment with IL-4 to respond to IgE-IC or CD23-mAb + anti-MIg (Fig. 6). In contrast to U1^{low} cells, 48-hr U1^{high} cell incubation with IgE-IC resulted in a significant decrease of p24 levels, correlating with decreases of the viable cell numbers recovered from these culture (Fig. 6).

The Role of Nitric Oxide in IgE-IC–Mediated Cytostasis of U1^{high} Cells

Following treatment with IgE-IC, U1^{high} cell death is preceded by a marked cytostasis as the cells displayed lower TNFα production and a dramatic decreases of surface CD23, CD14, and CD71 expression levels (Table 2). In contrast, significantly higher levels of nitrites were observed in IgE-IC–treated cells ($p < 0.01$). As for U1^{low} cells, we asked the role of CD23-related signals, cAMP and NO, in IgE-IC effects on U1^{high} cells. Rp-cAMP did not have any effect on cell death and growth arrest induced by IgE-IC and

TABLE 2. Cytostatic effect of IgE-IC on U1^{high} cells

	None	+IgE-IC[a]
Cell number/ml	10×10^5	2×10^5
HIV-p24 (ng/ml)	3.1 ± 0.3	0.6 ± 0.2
TNF-α (pg/ml)	300 ± 21	62 ± 13
Nitrites (μM)	1.2 ± 0.2	4.3 ± 0.7
	Mean fluorescence intensity	
CD23$^+$	230	82
CD14$^+$	112	41
CD71$^+$	182	93

[a]Cells (2×10^5/ml) treated with IgE (10 μg) for 1 hr then 48 hr with anti-IgE (10 μg). Cells were then collected, viable cells counted, and their surface CD23, CD14, and CD71 expression levels quantified by direct immunofluorescence. Cell supernatants were collected and assayed for their p24, TNFα, and nitrite content. Values are mean or mean ± SD from two different U1^{high} cell preparations.

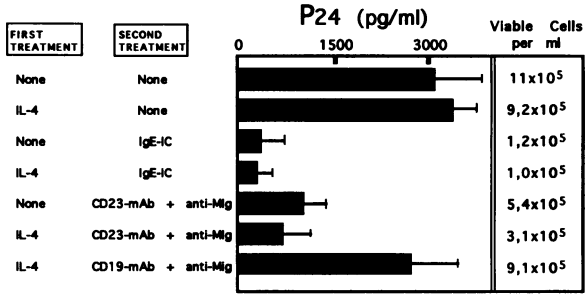

FIG. 6. Effects of IgE-IC and CD23-ligation on p24 levels and cell numbers in U1^{high} cells

The cells were preincubated for 48 hr in medium alone or with IL-4 (10 ng/ml), then washed and postincubated (2×10^5/ml) with IgE (10 μg/ml) for 1 hr before the addition of anti-IgE (10 μg/ml). Some IL-4–treated cells were also incubated with CD23-mAb or CD19-mAb (20 μg/ml) for 1 hr before the addition of anti-MIg (10 μg/ml). Following 48 hr, p24 levels and the number of cells were evaluated. Shown are mean ± SD values from three distinct U1^{high} cell experiments.

further addition of Dibut-cAMP did not modify cell numbers (Fig. 7) but increased p24 levels (from 1.2 ± 0.3 to 2.4 ± 0.5 ng/ml). By contrast, the addition of L-NMMA or SOD + catalase nearly abolished the accelerated cell death stimulated by IgE-IC (Fig. 7). L-NMMA effect was reversed through the addition of L-arginine but not D-arginine to the cultures (data not shown),

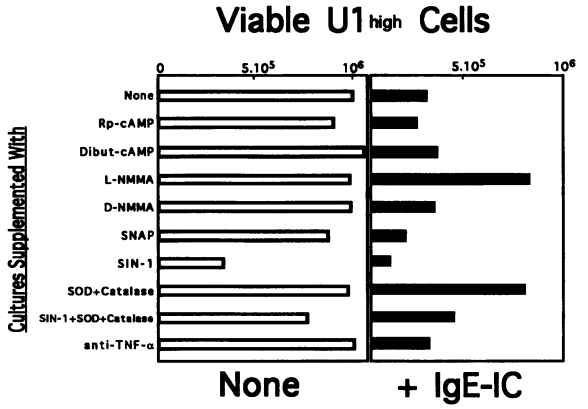

FIG. 7. Effects of cAMP and NO agonistic and antagonistic compounds on U1^{high} cell proliferation

Cells were incubated in medium alone (None) or with IgE-IC and various compounds as in Fig. 5A. Mean from two different U1^{high} cell cultures, each done in duplicates (SD < 18%). Data suggest the role of peroxynitrites in IgE-mediated inhibition of U1^{high} cell growth.

and IgE-IC effect was not affected by D-NMMA. Furthermore, addition of SIN-1 dramatically decreased cell numbers in untreated U1^{high} cells, while little or no effect was observed with SNAP. SIN-1 effect can partially be reversed by SOD + catalase (Fig. 7). Finally, the addition of neutralizing anti-TNFα had no effect on IgE-IC function (Fig. 7). Together, these data suggest a role of peroxynitrites in anti-proliferative and/or cell death induced by IgE-IC on U1^{high} cells.

DISCUSSION

The present work demonstrated biphasic regulatory effects of IgE-IC on HIV replication and the cell growth and function of monocytic, chronically HIV-infected U1 cells. These IgE effects depended upon the activation state of these cells. In U1^{low} cells with no CD23 or CD14 on the cell surface and low production of TNFα and HIV (Fig. 1 and Table 1), IgE-IC increased the production of HIV and the production of p24. This effect was dose dependent, and required the expression of CD23 on the surface of U1^{low} cells (Fig. 4). CD23 expression could easily be obtained following cell treatment with various physiologic factors: IL-4, IL-13, GM-CSF, or IFN-γ (25,36,40) (Fig. 3). We also assayed the effect of IgE-IC on a variant of U1 cells with high HIV transcription and p24 levels (U1^{high}). These cells expressed CD23 without a need for IL-4, and their treatment with IgE-IC dramatically inhibited their functions, including proliferation; TNFα, HIV, and p24 production; and the expression of CD14, CD71, and CD23 (Table 2). Preliminary analysis of the mechanism of accelerated U1^{high} cell death by IgE-IC shows apoptosis, necrosis, or both. The reason for such differences is now under investigation. High doses of oxidants induce necrosis consistent with the suggestion of Duvall and Wyllia that the severity of the insult determines the form of cell death (45). NO has previously been implicated as an inducer of apoptosis in the monocyte/macrophage lineage (46).

Therefore, IgE-IC displays a potent proviral effect on infected monocytic U1 cells. It up-regulates viral production in U1^{low} cells and stimulates the death of highly infected cells. However, similar studies on in vivo infected macrophages will permit further definition of IgE role on HIV pathogenesis. Our data, however, may explain the rapid progression to AIDS or bad prognosis of AIDS patients with high serum IgE levels (17–

20). IgE could mediate induction of viral expression of latent HIV in infected macrophages (6), increasing patient viremia. Cell death and the liberation of HIV particles from activated macrophages may also contribute to the viral infection of adjacent cells.

Similar to normal human monocytes (17), ligation of CD23 seems to be required for IgE-mediated U1 cell stimulation. This is evidenced through the ability of CD23-Fab fragments to block IgE-IC effects. In addition, cell stimulation was also observed following the ligation of CD23 by specific mAb. It is unlikely that FcεRI accounted for IgE effects, as we failed to detect this receptor on these cells, and treatment with neutralizing anti-FcεRI mAb was unable to reverse IgE mediated effects (data not shown). The use of isotype-matched control to CD23-mAb further supports that this phenomenon was not due to nonspecific FcγR.

By contrast to most Fc receptors, CD23 does not belong to Ig super family, but is a member of animal c-type lectins, type II transmembrane glycoproteins (23). In addition to IgE binding site, CD23 was shown to display other functional domains, including a cytokine-like site, a lectin region, and a binding site for CD21 antigen (23,27,47–49). Ligation of b isoform of CD23 by IgE-IC or by appropriate mAb rapidly induces the accumulation of cAMP and the generation of nitric oxide in human monocytes (17), myelomonocytic precursors (25), eosinophils (50), and keratinocytes (28). Of interest, two other hematopoietic cell surface molecules, CD69 (51) and NKP-R1 (52), which belong to type II protein, c-type lectin family, were recently shown to mediate activation of nitric oxide pathway in human monocytes and rat natural killer cells respectively.

The role of CD23-linked transduction signals in the dichotomy of IgE-IC effects on U1 cells was then analyzed. On $U1^{low}$ cells, CD23-mediated HIV induction is due to both cAMP and NO pathways. The addition of cAMP antagonist (Rp-cAMP) reversed 62–79% of p24 levels induced by IgE-IC in $U1^{low}$ cells, while Dibut-cAMP produced a dose-dependent increase of p24 in U1 cells, regardless of their activation state ($U1^{low}$ or $U1^{high}$). These data correlate with many reports which showed the pro-viral effects of cAMP and its induction in HIV pathogenesis (53–55).

The present work also points out the contribution of CD23-mediated NO pathway in p24 enhancement. The NO synthase inhibitor, L-NMMA, decreased by 20–39% the IgE-IC–mediated increases of p24 expression. This is likely due to peroxynitrites rather than NO itself, as chemical NO donor, SNAP, had little or no effect on $U1^{low}$ cells, while SIN-1, which donates both NO and peroxinitrites (41), had the capacity to increase by 34–52% p24 levels produced by these cells. In contrast to $U1^{low}$ cells, our data indicate that NO is the major factor involved in the cytostatic effect of IgE-IC in $U1^{high}$ cells. This IgE-IC effect is completely abolished through cell treatment by L-NMMA or SOD + catalase, while the addition of SIN-1 further accelerated cell death. The effects of NO on $U1^{high}$ cells are thus likely related to the generation of peroxinitrites. Comparative levels of nitrites detected in $U1^{low}$ and $U1^{high}$ cell supernatants suggested that $U1^{high}$ cells became sensitive to apoptotic effect of peroxynitrites, compared with $U1^{low}$ cells. This may be due to the failure of these cells to generate enough antioxidants, due to their high degree of infection or to their differentiation fate. In this phenomenon, no effect of cAMP modulators was observed, and the addition of cAMP to $U1^{high}$ cells even increased p24 levels in a dose-dependent manner without affecting their survival.

Therefore, while NO has an anti-viral action on other viruses (56–58), it seems to be a stimulatory pathway for HIV infection and thus may contribute to HIV pathogenesis. HIV products may be induced by peroxynitrites directly (as potent oxygen radicals) (59) or indirectly through their ability to induce TNFα production by these cells (Fig. 5). The similarity between p24 inhibition by L-NMMA and by anti-TNFα is in favor of the role of this cytokine in this IgE-IC effect. Nitrogen radicals and TNFα are potent inducers of the transcription factor NFκB in various cells (60,61) including U937 cells (25). Of interest, anti-viral effects of NO were found on viruses (56–58) which, by contrast to HIV, do not contain κB sites. This hypothesis corroborates with Chowdhury et al. work (53), which showed the synergy between cAMP and TNFα in stimulating HIV-1 from chronic low-level HIV production in cells of monocyte/macrophage lineage.

Induction of nitric oxide synthase was observed in asthma (62), which corroborates with the role of NO in proviral effect of IgE-IC. NO generation by human monocytes was also observed following their activation with gp120 (63). Bukrinsky et al. recently showed that HIV infection promoted the generation of NO in human monocytes (64), and that NO mediates neu-

rotoxicity in HIV-infected patients (63,64). While the precise mechanism(s) of NO effects remain(s) to be clarified, above data make likely the involvement of NO in HIV pathogenesis.

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