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Up-Regulation of NK Cytotoxic Activity Via IL-15 Induction by Different Viruses: A Comparative Study

Lama M. Fawaz, Ehsan Sharif-Askari, and José Menezes

IL-15 is a recently identified cytokine that belongs to the four α-helix bundle cytokine family and possesses biological activities similar to those of IL-2. Its ability to induce effectors of NK activity suggests its involvement in innate immunity. In this study, we analyzed the effect of different viruses (HSV, EBV, respiratory syncitial virus, vesicular stomatitis virus, influenza virus, reovirus, and Sendai virus) on the up-regulation of NK activity in vitro. Exposure of human PBMC to theses viruses resulted in an immediate up-regulation of NK activity of PBMC via IL-15 induction; this effect was abrogated in the presence of mAbs to IL-15. Results of experiments conducted in parallel using mAbs to IL-15, as well as to other cytokines (IL-2, IL-12, IFN-γ, and TNF-α), clearly indicated that IL-15 was specifically responsible for the observed effect. Furthermore, supernatants of virus-infected PBMC cultures significantly enhanced NK activity of uninfected PBMC in vitro. An increase of IL-15 protein levels 20 h postinfection was also confirmed in a bioassay using the IL-2-dependent cell line CTLL. Kinetic analysis of IL-15 mRNA expression using a semiquantitative RT-PCR revealed that the level of IL-15 messages peaked at different time points (up to 12 h) postinfection, depending on the nature of the virus. Taken together, these results suggest that the IL-15 response of the host to viral infection and the subsequent NK cell activation represent an important effector mechanism of the innate immune surveillance of the host against viral infections. The Journal of Immunology, 1999, 163: 4473–4480.

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Abbreviations used in this paper: ADCC, Ab-dependent cell-mediated cytotoxicity; HHV, human herpesvirus; VSV, vesicular stomatitis virus; RSV, respiratory syncitial virus; UTR, untranslated region; PI, postinfection.
whether different, unrelated viruses have the ability to enhance NK activity of human PBMC via IL-15 induction. Thus, we undertook a comparative study in which we investigated the following viruses belonging to different families, namely, influenza virus, HSV-1, EBV, reovirus, vesicular stomatitis virus (VSV), Sendai virus, and the respiratory syncytial virus (RSV), for their ability to up-regulate the NK cytotoxic activity of virus-infected PBMC in vitro. Here, we present data that suggest up-regulation of NK activity via IL-15 induction represents an early effector mechanism of the host’s innate immune response to viruses.

**Materials and Methods**

**Preparation and culture of PBMC**

Heparinized venous blood, freshly obtained from healthy donors, was centrifuged over a Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) gradient, and PBMC were collected as described (17). The separated PBMC were washed and cultured in RPMI 1640 culture medium (Life Technologies, Grand Island, NY) supplemented with 10% heat-inactivated FBS, 1,0% glutamine, 100 U/ml penicillin, 20 μg/ml streptomycin, and 1 μg/ml gentamicin.

**Cell treatment**

The PBMC (1 × 10^6 cells) were infected with optimal doses of viruses (as determined in preliminary experiments for maximal induction of NK cytotoxicity) and mock-infected culture supernatant for 2 h at 37°C, washed with Hank’s buffer (Life Technologies), and then resuspended in 1 ml RPMI 1640 medium supplemented with 10% heat-inactivated FBS for 20 h at 37°C with or without a mAb to IL-15. After 20 h, cell-free supernatants from mock- and virus-treated PBMC were collected and kept at −80°C until used, and cells were prepared for the NK cytotoxicity assay as described (17).

Cell-free supernatants were added to untreated resting PBMC (24 h postseparation) at 25% of the final volume, in the presence or absence of a mAb to IL-15, as well as mAbs to other cytokines such as IL-2, IL-12, IFN-γ, and TNF-α, before mixing with the NK cell targets, K562 cells. The NK activity of PBMC was tested in the presence of recombinant human IL-15 (50 ng/ml) and anti-IL-15 Abs (10 μg/ml). The choice of 50 ng/ml concentration of rIL-15 used in these experiments was based on the fact that, in our preliminary experiments, increasing concentrations of human rIL-15 from 10 to 50 ng/ml induced a linear increase of cytotoxic activity, whereas no significant differences were seen at concentrations between 50 and 100 ng/ml (data not shown). This is also in agreement with our previously published results (17).

**Cell lines and viruses**

The K562 cell line was purchased from the American Type Culture Collection (ATCC, Manassas, VA). The CTL assay cell line was obtained from Dr. R.-P. Sékaly’s laboratory (Clinical Research Institute of Montreal and Department of Microbiology, University of Montreal). K562 cells were cultured in RPMI 1640 supplemented with 10% FBS, and CTL-L2 cells were cultured in RPMI 1640 with 5% FBS supplemented with 5 × 10^-5 M 2-ME. Treatment of PBMC (1 × 10^6 cells) with each of the viruses used was as follows: 200 μl of EBV (B95-8 strain) preparation with a titer of 2 × 10^3 EBV nuclear Ag-inducing U/ml (19), 80 μl of HSV-1 at a multiplicity of infection of 50 PFU/cell (McIntyre strain) (19); 200 μl of human RSV, Long strain; ATCC) at a titer of 10^5 syncytia-forming units/ml (20); 200 μl of Sendai virus containing 750 hemaglutinating units in PBS (21); 200 μl of reovirus preparation (10^7 PFU/ml) (kindly provided by Dr. Guy Lemay, Department of Microbiology, University of Montreal); 50 μl of influenza virus A (H1N1) strain AI/FM/1/47 with a titer of 10^4.2 at a multiplicity of infection dose of 500 PFU/ml; 200 μl of VSV 10^5 dilution 10^5 median tissue culture infectious dose (TCID50) kindly supplied by Dr. Youssef Elazhary (Faculty of Veterinary Medicine, University of Montreal). Viral preparations were first titrated to determine the dose that up-regulate the NK cytotoxic activity of virus-infected PBMC in vitro, and were found to contain 10^5 PFU/ml of contaminating endotoxin.

**NK cell cytotoxicity assay**

All cytotoxicity assays were performed using either untreated resting PBMC or virus-infected PBMC (20 h postinfection: PI) as effectors. All assays were done using a standard 51Cr-release assay as described previously (17). Briefly, K562 target cells (1 × 10^6 cells/ml) were labeled by incubation with 100 μCi of ^51Cr (DuPont/NEN, Boston, MA) for 1 h at 37°C. The radiolabeled target cells were then washed four times with RPMI 1640, and resuspended in a concentration of 1 × 10^5 cells/ml in RPMI 1640 with 10% FBS. K562 cells were then added into V-bottom wells and mixed with 0.05 ml PBMC (4 × 10^5 cells/ml) from healthy donors at an E:T ratio of 20:1 and incubated for 16 h at 37°C. Radioactivity was then measured using a gamma-counter (Wallac model 1272; LKB, Turku, Finland). The percentage of cytolysis was calculated based on the following formula: [cpm experimental − cpm spontaneous]/(cpm maximum − cpm spontaneous) × 100. All these experiments were done in triplicate, and the results are presented as the mean ± SE of three independent determinations. The spontaneous cpm were determined by counting the radioactivity of the supernatant of the target cell suspensions, whereas the maximum cpm were determined from the radioactivity of the resuspended target cells with Triton-X (100 μl).

**Abs and cytokines**

Neutralizing mAbs to human IL-2, IL-12, and TNF-α were purchased from R&D Systems (Minneapolis, MN). Neutralizing mAbs to IFN-γ were purchased from Genzyme (Boston, MA). Human IL-15 and mAbs to IL-15 were a gift from Immunex (Seattle, WA). All mAbs were used at a concentration of 10 μg/ml, and IL-15 was used at a concentration of 50 ng/ml. The choice of this concentration (i.e., 10 μg/ml of mAbs in our experiments was established following preliminary experiments using HSV-1, HHV-6, and influenza virus) in which undiluted and diluted preparations of mAbs were tested for their ability to inhibit NK enhancement induced by different concentrations of supernatants from virus-infected PBMC cultures; only mAbs to IL-15 were found to significantly inhibit this enhancement, whereas Abs to other above-listed cytokines and to IFN-γ and IL-12 had no such effect (17). Neutralizing Abs to anti-IL-15 mAbs, concentrations of 5 to 10 μg/ml produced maximum inhibitory effect in all preliminary tests, including tests against 50 ng/ml of human rIL-15 (data not shown). An additional consideration for the use of the same concentration of mAbs in these experiments was also the fact that some of these mAbs represent the needed control (negative) Abs in relation to the inhibition that is observed upon neutralization of IL-15 by anti-IL-15 mAbs.

**CTL assay**

Supernatants from infected PBMC cultures (2 × 10^6 cells), obtained after 20 h incubation with each of the different viruses, were collected and tested for the presence of bioactive IL-15 by its ability to sustain the proliferation of the IL-2- and IL-15-responsive CTL cell line (1). Treatment of supernatants with a mAb to IL-2 ascertained that proliferation of CTL-L2 cells was not due to IL-2 (data not shown). Cells were washed twice and incubated in RPMI 1640 medium containing 5 × 10^-5 M 2-ME without FBS for 3 h at 37°C, in 5% CO2. CTL-L2 cells (5 × 10^5 cells/100 μl) were then dispensed into 96-well round-bottom plates containing 100 μl of supernatant. After 66 h of incubation at 37°C in 5% CO2, cells were pulsed with 1 μCi of H3/well (DuPont/NEN) for 6 h. Cells were then harvested on glass-fiber filter paper by an automated sample cell harvester (Tomtec, Orange, NJ) and dried. The incorporation of radioactivity was determined in a liquid scintillation counter. Results represent the mean of two replicate wells of two independent experiments and are expressed as cpm of [3H]thymidine incorporation.

**Preparation of IL-15 mRNA and RT-PCR**

PBMC (1 × 10^6 cells) were treated with different viruses for 2 h at 37°C, washed, and resuspended in 1 ml RPMI 1640 supplemented with 10% FBS. At various time intervals (2, 4, 8, 12, and 20 h) posttreatment, cells were counted and checked for mortality by trypan blue exclusion staining (<5%), and 5 × 10^5 cells were lysed and stored at −70°C until assayed for IL-15 mRNA expression.

Total mRNA was extracted from cells using a modified guanidium thiocyanate procedure as described previously (22). All reagents used in this test were purchased from Life Technologies unless indicated otherwise. The cDNA was extracted by final digestion with RNAse. RT-PCR was performed in a total volume of 40 μl diethylpyrocarbonate-treated ddH2O. The samples were then treated with 10 U DNase I for 30 min at 37°C. The isolated RNA (from infected and mock-treated PBMC) was subjected to RT-PCR to determine the level of expression of IL-15 mRNA.

For first-strand cDNA synthesis, 4 μl of total RNA was reverse transcribed to a total volume of 20 μl containing 100 U recombinant Moloney murine leukemia virus reverse transcriptase, 2 μl 5× first-strand buffer (250 mM Tris-HCl, 375 mM KCl, and 15 mM MgCl2, pH 8.32), 1 μl of 1 mg/ml random hexamer primer (pd N6), 0.01 M DTT, 0.5 μl of a 5 mM
mixture of all four dNTP, and 30 U RNase inhibitor (Pharmacia). Following
denaturation for 10 min at 65°C, RNA was reverse transcribed for 1 h at 42°C,
and then the RT enzyme was inactivated by incubation at 95°C for 5 min.

PCR was performed with an aliquot of 5 µl of synthesized cDNA product
in a reaction mixture containing 5 µl cDNA, 5 µl 10× PCR buffer (200
mM Tris-HCl, pH 8.4, and 500 mM KCl), 3 mM MgCl2, 50 pmol A and
B oligonucleotide primers, 1 µl 5 mM dNTPs, 2.5 U Taq polymerase, and
200 µl cDNA was detected using 32 P-labeled human actin cDNA excised by
EcoRI restriction enzyme from the actin containing the 1.1-kb plasmid
(BlueScript SK--; ATCC). After hybridization, the blots were washed (15
min/wash) with solutions comprising 0.1% SDS, twice at ambient tempera-
ture with 2× SSC and once with 0.1× SSC at 37°C, followed by 0.1×
SSC at 58°C. Intensity of the probe products was determined using a
PhosphorImager screen (Molecular Dynamics, Sunnyvale, CA). The
amount of IL-15 RNA was quantitated for each sample at each time point
relative to its own actin RNA level. The given ratio was compared with the
RNA transcripts from mock-infected cells infected as described previously
(23). All reactions were conducted in conditions in which amplification
was linear.

Southern analysis
Amplified DNA was blotted on a positively charged nylon membrane
(Boehringer Mannheim, Indianapolis, IN) overnight at ambient tempera-
ture in a 10× SSC (3 M NaCl and 300 mM sodium citrate, pH 7.0)
and immobilized by UV cross-linking. The membrane was then prehybridized
and hybridized at 58°C using rapid-hyb buffer (Amersham Life Sciences,
Arlington Heights, IL) for 4 h and overnight, respectively. The synthesized
PCR products were then probed with a 32P-labeled IL-15 probe comple-
tary to sequences recognized by the PCR amplification primers (5′-

ATGCTTTCTATTGAGCTTGTTTCAGTGCG-3′) (17). Amplified actin
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FIGURE 1. Induction of NK activity by different viruses and inhibition by a mAb to IL-15. Cytolytic activity was measured by mixing infected
PBMC with K562 cells at an E:T ratio of 20:1, and their cytotoxic activity
was assayed in a 16-h 51Cr-release assay as described in Materials and
Methods. Data, expressed as percentage of cytotoxicity, represent mean
± SE. The p values for each virus, and virus plus anti-IL-15 are, respectively,
as follows: 0.009 and 0.007 for influenza; 0.001 and 0.002 for HSV-1; 0.006 and 0.043 for EBV; 0.011 and 0.030 for reovirus; 0.003 and 0.013
for VSV; 0.004 and 0.003 for Sendai virus; and 0.036 and 0.011 for RSV.
These results were obtained from triplicate determinations for one donor.
When experiments were repeated with PBMC from four different donors,
similar results were obtained for each donor. The grey column above each
given viral preparation represents infected cells, whereas the black column
represents infected cells treated with anti-IL-15 mAbs.

Results
Enhancement of NK activity by different viruses and its inhibition by anti-IL-15 Ab
We first determined the NK cytotoxic activity of PBMC following
their infection with different viruses as described in Materials and
Methods. All viruses used (influenza virus, HSV-1, EBV, reovirus,
VSV, Sendai virus, and RSV) were able to significantly enhance
the NK activity of PBMC following 20 h of infection as compared
with the mock-infected PBMC (Fig. 1). Infection with the influ-
Enza virus induced a 38% increase in NK cytotoxicity as compared with 7% cytocidal activity of the mock-infected PBMC; these results correspond to a more than 5.5-fold increase in cytocidal activity. Also, VSV had an activity of 43%, the highest increase in NK activity among the different viruses. RSV (15%) and EBV (20%) induced a lower increase in NK cytotoxicity. To determine the role of IL-15 in the observed induction of NK cytotoxicity, a mAb to IL-15 was used. It was thus found that this Ab inhibited the

FIGURE 3. Specific role of IL-15 in NK induction by different viruses. PBMC (1 × 10⁶ cells) obtained from healthy donors were infected with optimal doses of the different viruses or mock-infected as described in Materials and Methods. Cells were then washed and resuspended in RPMI 1640 with 10% FBS for 20 h at 37°C in 5% CO₂. Culture supernatants were then collected and added to resting PBMC (4 × 10⁶ cells/ml) at 25% of final volume in the presence of mAbs to IL-15, IL-2, IL-12, TNF-α, and IFN-γ (all Abs were used at a concentration of 10 μg/ml). The lytic activity was measured in a standard 16-h ⁵¹Cr-release assay as described in Materials and Methods. A, Reovirus and RSV; B, HSV-1 and VSV; C, Sendai virus and influenza virus; D, EBV. Values obtained represent mean ± SE of triplicate experimental values. Results obtained from other experiments done using PBMC from three different donors were similar. Stars show that addition of anti-IL-15 significantly reduced NK activity induced with the viral supernatants with p values of 0.001 for PBMC with IL-15, 0.008 for PBMC with anti-IL-15; 0.018 for reovirus supernatant (SN) + anti-IL-15, 0.029 for RSV SN + anti-IL-15, 0.020 for HSV-1 SN + anti-IL-15, 0.003 for VSV SN + anti-IL-15, 0.081 for EBV SN + anti-IL-15, 0.025 for Sendai SN + anti-IL-15, and 0.016 for the influenza virus SN + anti-IL-15.
increase in NK activity following infection with the different viruses. This increase was statistically significant with a p value of ≤0.01 and was seen with all the viruses to a varying degree. Hence, the role of IL-15 in mediating the induction of NK activity following infection with influenza virus, HSV-1, EBV, reovirus, VSV, Sendai virus, and RSV was shown.

Specificity of NK induction by viruses via IL-15

The above observations pointed to a role for IL-15 in the up-regulation of NK cytotoxicity by the different viruses used. If this was true, the presence of bioactive IL-15 secreted in the supernatants of virus-infected cells should be detected. In fact, treatment of PBMC for 24 h with the supernatants of infected PBMC obtained 20 h PI showed that all supernatants were able to significantly enhance NK activity as measured by percentage of cytotoxicity in comparison with the mock-treated PBMC (Fig. 2). To document the specificity of NK induction by the supernatants of infected PBMC cultures via IL-15, a mAb to IL-15 was used. The results show that, when added to the supernatants, anti-IL-15 Ab inhibited the increase in NK activity. This abrogation of the increase of NK activity was statistically significant and observed with all viruses (p ≤ 0.01). A more drastic decrease was seen with the supernatants of EBV and RSV (i.e. from 18 to 2% and from 24 to 3%, respectively), suggesting that in the case of these two viruses NK cytotoxicity was solely induced by IL-15. These results provide strong evidence for the presence of IL-15 in the supernatants of virus-infected PBMC and its role in the induction of NK cytotoxicity. Moreover, when CD16-depleted PBMC (following two repeated treatments with anti-CD16 plus complement) were used in the presence of rIL-15, no up-regulation of NK cytotoxicity was observed (results not shown).

Furthermore, the specific role of IL-15 in the NK induction by the supernatants of infected PBMC cultures by the different viruses was supported by the results of experiments using mAbs with specificity to other relevant cytokines such as IL-12, IL-2, TNF-α, and IFN-γ (Fig. 3). These results clearly showed a significant decrease in NK activity following the use of anti-IL-15 Ab only, and not with mAbs to other cytokines. This was demonstrated in individual experiments with each virus (Fig. 3, A–C). Interestingly, previous studies with HIV-6 in which Abs to IFN-α were used also showed no decrease in NK cytotoxicity (17). Taken together, our results clearly indicate that IL-15 is the cytokine specifically responsible in our experimental system for the up-regulation of NK activity following infection of PBMC with different viruses.

Assessment of bioactivity of IL-15 in supernatants of infected PBMC

After ascertaining the presence of IL-15 in the supernatants of infected PBMC using a specific mAb, the next step was to test the bioactivity of the secreted protein. Attempts to detect IL-15 in the supernatants of infected PBMC by ELISA were unsuccessful (data not shown). This is because the detection limits of most commercially available ELISA kits are probably well above the actual bioactive protein concentrations present in the supernatants of infected PBMC. In fact, contrary to other cytokines, the concentration of IL-15 is not directly correlated with the cellular level of IL-15 mRNA expression (24, 25). However, we were able to detect bioactive IL-15 levels in the supernatants of infected PBMC 20 h PI using the CTLL-2 proliferation assay as described in Materials and Methods (Fig. 4). Indeed, all supernatants obtained from virus-infected PBMC cultures supported the growth of the CTLL cell line as illustrated by the significant increase in [3H]thymidine incorporation (cpm) (treatment of supernatants with a mAb to IL-2 ascertained that the proliferation of CTLL-2 was not due to IL-2; data not shown). Interestingly, supernatants from VSV-treated cells, for example, had a high increase correlating with their high induction of NK activity, whereas supernatants from Sendai virus-treated cultures showed lower values, which correlated with their lower NK-inducing activity (see Fig. 2 for comparison).

Kinetics of IL-15 mRNA expression

To determine the levels of IL-15 mRNA expression at different time intervals (i.e., 2, 4, 8, 12, and 20 h) PI of PBMC, cells were lysed and mRNA levels assessed by semiquantitative RT-PCR (Fig. 5). IL-15 mRNA levels for most viruses peaked at 8 h PI, with some variation among the different viruses: influenza virus, HSV-1, EBV, and Sendai virus peaked at 8 h PI, whereas reovirus and RSV had little increase between 8 and 12 h PI (Fig. 5, A and B), and VSV peaked at 4 h PI, probably accounting for the higher increase observed in NK induction (Fig. 1). However, there were differences with respect to the correlation between the increase in mRNA levels and protein expression levels. For instance, EBV mRNA expression peaked at 8 h PI, with a 40-fold increase in mRNA levels. In comparison, influenza IL-15 mRNA level also peaked at 8 h PI with a 28-fold increase. For both viruses, the bioactive protein levels (Fig. 4), as well as the NK activity (Fig. 1) did not correlate with the observed mRNA levels. Compared with EBV, influenza virus infection of PBMC was associated with a higher concentration of IL-15 protein and a much higher NK activity, thus pointing to the possibility of a differential regulation at the translational and posttranslational levels of IL-15 expression. This is also true if one compares other viruses such as HSV-1 and
influenza, which despite different expression levels of mRNA following infection (10- and 28-fold increase, respectively), induced NK activity of comparable strength and expressed similar levels of the bioactive protein (Figs. 1 and 4).

Discussion

NK cells represent a distinct lineage of lymphocytes that play an important role in the innate defense mechanisms of the host aimed at the elimination of a variety of target cells (tumor and transplant, as well as virus-infected cells) (10, 26, 27). The cytocidal activity of NK cells has been shown to be enhanced by cytokines such as α and β IFNs produced by infected cells that up-regulate killing mechanisms, and IL-12, which promotes IFN-γ secretion (28). The role of NK cells in early antiviral immune defense mechanisms seems to be crucial as evidenced by studies of several types of viral infections, particularly those due to herpesviruses (14, 29).

FIGURE 5. Kinetics of IL-15 mRNA levels in infected PBMC as determined by RT-PCR. PBMC (1 × 10^6 cells) were treated with different viruses for 2 h at 37°C, washed, and resuspended in 1 ml RPMI 1640 supplemented with 10% FBS. At various time intervals (2, 4, 8, 12, and 20 h) posttreatment, cells (5 × 10^5) were lysed and assayed for IL-15 mRNA levels. Total RNA was extracted, reverse-transcribed, and amplified using RT-PCR. Bands were detected following blotting on a nylon membrane as described in Materials and Methods. A. Panels represent bands obtained in mock- and virus-infected cells for IL-15 and β-actin at the different time intervals PI. B. Curves illustrating fold increase in IL-15 mRNA levels as expressed for different virus-infected cells.
Numerous studies have demonstrated that NK cells can selectively lyse virus-infected target cells while sparing uninfected cells (30, 31). The expression of viral Ags or other surface structures by infected cells appears to render them more sensitive to NK cytolysis (30–32). Mammalian cells infected by many different viruses such as herpes, vaccinia, measles, mumps, and influenza viruses can be lysed in vitro by NK effectors (30). Furthermore, several studies have shown that humans and mice with a relatively low NK activity are more susceptible to herpesvirus infections such as by HSV, CMV, and EBV (14, 30, 31, 33–35). Characterization of the virus-induced endogenous mechanisms regulating NK cell responses and functions has been limited to the IFN-α/β-mediated activation of NK cell cytotoxicity. However, Binder et al. (36), using a murine model of viral infection, showed that deletion of the IFN-α/β receptor gene did not affect NK cell expansion and activity, thus suggesting the existence of alternative pathways and/or cytokines that can mediate the enhancement of NK cytototoxicity. Recent work has shown that some, but not all, viral infections induce IL-12, the expression of which results in IFN-γ production by NK cells, which in turn contributes to an antiviral state (29). However, a newly identified cytokine, IL-15, with biologic activities similar to IL-2, including the ability to up-regulate NK cytolytic mechanisms (4), has been shown to be induced following infection with two related human lymphotropic herpesviruses, HHV-6 and HHV-7 (17, 18). A study by Carson et al. (37) showed that human monocytes produced IL-15 protein within 5 h of activation with LPS and that it was critical for optimal NK cell production of IFN-γ. Furthermore, Elloso et al. (38) reported that neutralization of endogenous IL-15 in PBMC from HIV-infected patients, in which dysfunction of both NK and ADCC activity is well documented (39, 40), resulted in a reduction in IFN-γ production in vitro. A previous report had shown that IL-15 and IL-12 had the ability to enhance cell-mediated immunity of HIV-infected PBMC (41), and recent work from our laboratory has shown that adding rIL-15 to PBMC culture from HIV-infected individuals could up-regulate NK and ADCC activity in vitro (42). Taken together, these observations suggested that IL-15 plays an important role in the up-regulation of NK effector function following viral infection.

To gain further insight into the role of IL-15 in the activation of the early nonspecific cellular immune response to viral infection, we studied the induction of IL-15 following exposure of human PBMC to several viruses belonging to different families (influenza virus, HSV-1, EBV, reovirus, VSV, Sendai virus, and RSV), and its role in the enhancement of the NK cytotoxic activity of virus-infected PBMC. Our data clearly show that all the viruses studied induced the expression of IL-15, both at the mRNA and protein levels, and that these viruses significantly enhanced the NK activity of PBMC as compared with the mock-infected cells. This increase in NK cytotoxicity was shown to be abrogated by the use of a mAb to IL-15. Furthermore, when the supernatants from virus-treated PBMC cultures were added to normal PBMC, the NK activity of the latter cells was up-regulated. These data thus show that NK activity is up-regulated following viral infection via IL-15 induction. Furthermore, the present results clearly show that IL-15 was specifically responsible for the NK induction of normal PBMC following treatment with supernatants of infected PBMC. Inhibition of NK activity was only seen with anti-IL-15 Abs and not with mAbs specific to other cytokines such as IL-12, IL-2, IFN-γ, or TNF-α. Based on these findings, it would appear that IL-15 is secreted earlier than the other cytokines following a viral infection. This finding is supported by the fact that monocytes are the primary source of IL-15 production in response to intracellular infection, as documented in several studies (17, 43–45). These findings emphasize the importance of studying the role of IL-15 early in infection as well as in the production of other cytokines.

It has been difficult to demonstrate IL-15 in the supernatants of the majority of the cells that express messages for this cytokine, despite its widespread expression in several human tissues (25). The results obtained in this study regarding the mRNA and the levels of IL-15 protein expression illustrate the differential regulation of this cytokine. In fact, IL-15 mRNA levels were not translated into equivalent proportions of bioactive protein as assessed by the CTLL-2 bioassay. This could be explained in the light of the translational and posttranslational levels of regulation of IL-15 protein synthesis. Unlike other cytokines that belong to the same family (such as IL-2, which is regulated at the level of message transcription and stabilization), synthesis and secretion of IL-15 appear to be controlled by the presence of upstream AUGs in the 5′ untranslated region (UTR) of the IL-15 messages (24, 25). It has been hypothesized that IL-15 is stored in a translationally inactive IL-15 mRNA form that can be readily translated in response to an infection through several mechanisms that are effective in the removal of the 5′ UTR blockade of transcription such as a splicing event or an internal initiation of translation (46–48). Interestingly, Bamford et al. (24) showed by analysis of the IL-15 message from the HuT-102 T cell line, that there was a 6- to 10-fold more protein expressed in HuT-102 cells compared with activated monocytes, correlating with the lack in HuT-102 cells of 8 of the 10 upstream AUGs normally present in the 5′ UTR of the IL-15 message. Furthermore, IL-15 secretion was found to be controlled by natural signal peptides that apparently regulate the efficiency of release of soluble IL-15 in biologically relevant amounts (49, 50). Meazza et al. (49) reported that substitution of the natural signal peptide encoded by IL-15 cDNA with another one from a secretory protein IgVx chain (VxL) increased significantly the secretion of biologically active IL-15. Hence, the results obtained in the present study are consistent with other findings, suggesting that IL-15 synthesis and secretion is controlled at multiple levels (translation and entry into the secretory pathway), in addition to transcription (24).

Innate immunity is an important first line of defense and plays a key role in immune surveillance, particularly against infections. Unraveling the role of cytokines involved in the regulation of the early immune response to infections should also lead to a better understanding of their role in host defense and pathogenesis of these infections. With respect to IL-15, the results presented confirm its role in the activation of NK cytotoxic activity. In this context, it is noteworthy that a recent study by Carson et al. (51) documented the ability of IL-15 to sustain NK cell survival with the NK cells expressing IL-15 mRNA. It was shown that picomolar amounts of IL-15 were sufficient, in the absence of serum or other growth factors, to sustain the survival of resting human NK cells for up to 8 days. It is thus likely that IL-15 plays a unique role in the activation and maintenance of the host’s innate cellular immune response to infection. Indeed, recent data from our laboratory showed that long-term incubation of IL-15 with EBV-infected PBMC in vitro resulted in the inhibition of EBV-transformed immortalized cells in these cultures (E. Sharif-Askari, L. M. Fawaz, and J. Menezes, in preparation), which further supports a role for IL-15 in antiviral immunity. Nevertheless, given that treatment with anti-IL-15 Abs did not block all the enhanced NK cell cytotoxicity observed with several of the viruses used in our assay system, it is likely that other factor(s) (e.g., other cytokines) is (are) contributing to the up-regulation of NK activity by these viruses. Whether the action of such factor(s) is synergistic with or complementary to that of IL-15 remains to be addressed in future studies.
In conclusion, the results presented demonstrate that exposure of PBMC to different, unrelated viruses leads to immediate activation of NK cytotoxic effector function via IL-15 induction. These events may indeed represent crucial steps in host’s innate immune response to, and immunosurveillance against, viral infections.

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References