IL-15 Induces the Expression of Chemokines and Their Receptors in T Lymphocytes

Liyanage P. Perera, Carolyn K. Goldman and Thomas A. Waldmann

*J Immunol* 1999; 162:2606-2612; http://www.jimmunol.org/content/162/5/2606

**References**

This article *cites 61 articles*, 26 of which you can access for free at: http://www.jimmunol.org/content/162/5/2606.full#ref-list-1

**Why *The JI***? Submit online.

- **Rapid Reviews! 30 days** from submission to initial decision
- **No Triage!** Every submission reviewed by practicing scientists
- **Fast Publication!** 4 weeks from acceptance to publication

*average

**Subscription**

Information about subscribing to *The Journal of Immunology* is online at: http://jimmunol.org/subscription

**Permissions**

Submit copyright permission requests at: http://www.aai.org/About/Publications/JI/copyright.html

**Email Alerts**

Receive free email-alerts when new articles cite this article. Sign up at: http://jimmunol.org/alerts
IL-15 Induces the Expression of Chemokines and Their Receptors in T Lymphocytes

Liyanage P. Perera,1 Carolyn K. Goldman, and Thomas A. Waldmann

IL-15 is a T cell growth factor that shares many biological activities with IL-2 and uses the same βγ polypeptides of the IL-2R complex for signal transduction. Accumulating evidence implicates an important role for this cytokine in the inflammatory response of the host. Consistent with such a role, IL-15 has been shown to be a chemoattractant for T lymphocytes, NK cells, and neutrophils. Extending these observations, we now show that IL-15 is a potent inducer of CC-, CXC-, and C-type chemokines in T lymphocytes. In addition, we demonstrate that IL-15 induces CC chemokine receptors, but not CXC chemokine receptors, in a dose-dependent manner. Thus, our findings suggest that the proinflammatory effects of IL-15 at least in part may be due to the induction of chemokines and their receptors in T cells. Furthermore, we demonstrate that IL-15 promotes entry and replication of macrophage-tropic HIV in T lymphocytes and suggest a plausible mechanism by which IL-15, a cytokine that is elevated in HIV-infected individuals, may promote the transition of HIV displaying the M-tropic phenotype primarily associated with the initial transmission into the T cell-tropic phenotype that predominates as the disease progresses. The Journal of Immunology, 1999, 162: 2606–2612.

Interleukin-15 is a 15-kDa polypeptide that was discovered by its ability to promote the growth of T lymphocytes. It belongs to the four α-helix bundle family of cytokines (1–3). IL-15 uses the β- and γ-components of the IL-2R (reviewed in Ref. 4) and, as anticipated from the receptor subunit sharing it, exhibits a spectrum of immune functions that largely overlaps with that of the T cell growth factor IL-2. However, IL-15 and IL-2 use different α-chain receptor components, i.e., IL-15Rα and IL-2Rα, respectively (5, 6). IL-15Rα binds IL-15 with high affinity (Kd = ~10^{-11} M) and is expressed in a wide variety of tissues, unlike its counterpart IL-2Rα, which binds IL-2 less avidly (Kd = ~10^{-8} M) and is expressed largely in lymphocytes upon activation (7).

Unlike IL-2, which is secreted by activated T lymphocytes, IL-15 mRNA is expressed by macrophages, dendritic cells, endothelial cells, keratinocytes, and other cell types as well in response to environmental/stress stimuli and infectious agents (3, 8–14). There is increasing evidence to suggest that IL-15 may play an important role in protective immune responses, allograft rejection (15), and the pathogenesis of autoimmune diseases (16–19) where mononuclear cell infiltration is a hallmark feature. Recruitment of immune cells, especially lymphocytes, NK cells, and neutrophils, to sites of inflammation appears to be greatly influenced by IL-15 (20–22). More recently, it has been suggested that the effects of IL-15 on T cell motility are more similar to chemokinesis than chemotaxis due to its ability to stimulate motility in the absence of environmental cues (19,20).

Addressing this issue, we demonstrate that IL-15 is a potent inducer of chemokines and their receptors in peripheral blood-derived T lymphocytes, thus establishing an important link between IL-15 expression and the induction of chemokines leading to a prolific inflammatory response. Furthermore, considering the importance of chemokines and their receptor expression in the pathogenesis of HIV infection (reviewed in Refs. 27–29), we suggest a plausible mechanism by which IL-15 may promote the spread into T lymphocytes of monocytophoric, nonsyncytial-inducing strains of HIV that are primarily responsible for the initial transmission (30–32).

Materials and Methods

Cell culture

Peripheral blood-derived lymphocytes were obtained from normal volunteers by a two-step procedure: initiating with an automated leukopheresis and cold flow elutriation. Following elutriation, the lymphocytic fraction was collected, and any residual contaminating monocytes were further removed by incubation of the cells with carbonyl iron (100 mg/10^6 cells) to facilitate engulfment by monocytes and their subsequent removal by exposure to a magnetic field. NK cells and B lymphocytes were removed by using CD56 and CD19 microbeads with MACS separation columns from Miltenyi Biotech (Auburn, CA). The resultant T lymphocyte-enriched cell population was >96% CD3-positive as assessed by FACS analysis.

Cytokine reagents

Human recombinant IL-15 and IL-2 were purchased from PeproTech (Rocky Hill, NJ). Rabbit polyclonal Abs to human IL-1β and human TNF-α were purchased from Genzyme (Cambridge, MA). The mAb to IL-2Rβ (Mikβ-1) was a gift from Matsuru Tsudo (Kyoto, Japan). MIP-1α, MIP-1β, and RANTES ELISA kits were purchased from R&D Systems (Minneapolis, MN) and were used to measure the levels of these chemokines in the cell culture supernatants.

Received for publication July 17, 1998. Accepted for publication November 16, 1998.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Address correspondence and reprint requests to Dr. L. P. Perera, Building 10, Room 4B40, Metabolism Branch, Division of Clinical Sciences, National Cancer Institute, Bethesda, MD 20892.

2 Abbreviations used in this paper: MIP, macrophage inflammatory protein; RPA, ribonuclease protection assay; GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Copyright © 1999 by The American Association of Immunologists 0022-1767/99/$02.00
Ribonuclease protection assay (RPA)

Total cellular RNA was isolated from cytokine-treated T lymphocytes using TRIZol (Life Science Technologies, Gaithersburg, MD) according to the manufacturer’s instructions. The expression of various chemokines and their receptors was measured by multiprobe RPAs (33). Template sets for the multiprobe RPA were purchased from PharMingen (San Diego, CA), and the assays were performed according to the manufacturer’s instructions. Briefly, 50 ng of DNA from each multiprobe set was used to generate 32P-labeled riboprobes of defined length with T7 RNA polymerase in the presence of 150 μCi of [32P]UTP. Template DNA was then eliminated by digestion with DNase free of RNase, followed by precipitation of labeled RNA. Fifteen micrograms of total cellular RNA was then mixed with 6 × 10^6 cpm of 32P-labeled riboprobe mixture in a hybridization buffer consisting of 40 mM PIPES, 1 mM EDTA, and 0.4 M NaCl in 80% formamide and incubated at 90°C for 5 min followed by 56°C for 12 h. The hybridized RNA duplexes were then treated with an RNase mixture consisting of RNase A and RNase T1 followed by proteinase K digestion. RNase-resistant duplex RNA was extracted with phenol once and precipitated by the addition of an equal volume of 4 M ammonium acetate and 2 vol of ethyl alcohol. The RNA pellet was then solubilized and resolved on a 6% sequencing gel, dried, and subjected to autoradiography or phosphorimage analysis.

Results and Discussion

IL-15 induces the expression of receptors for C-C chemokines but not for CXC chemokines

To evaluate the effect of IL-15 on chemokine receptor expression in peripheral blood-derived T lymphocytes, cells were cultured in the presence of recombinant human IL-15 (10 ng/ml). In parallel, T lymphocytes were treated with IL-2 (10 ng/ml)-supplemented medium for comparison of these functionally related cytokines. Cells were harvested 12 h later, and total cellular RNA was extracted and subjected to a RPA to evaluate the modulation of chemokine receptor expression. As shown in Fig. 1A, the mRNAs for CXCR1 and CXCR2 receptors that bind IL-8 and for the CXCR3 receptor that binds IP10/Mig were not detectable in resting T cells or in T cells cultured in the presence of IL-15 or IL-2. However, there was abundant expression of the CXCR4 receptor that binds stromal-derived growth factor-1 (SDF-1) and functions as a coreceptor for syncytial-inducing, T cell-tropic HIV in resting T lymphocytes (34–36). The levels of CXCR4 expression remained unaltered regardless of whether the cells were cultured in the presence of IL-2 or IL-15. In addition, there was abundant expression of BLR-2 (CCR7) receptor (37) but not BLR-1 (CXCR5) in resting T lymphocytes, although no modulation of its expression was evident in the presence of either of these cytokines. We also noted a detectable signal for the orphan receptor V28 (38), which appears to function as an entry molecule for certain strains of HIV-1 and HIV-2 (39), but again no modulation of its expression was evident in the presence of IL-15 or IL-2. Thus, resting T lymphocytes express a subset of CXC chemokine receptors, and the expressions of these chemokine receptors remain unchanged by the presence of T cell growth factors IL-15 or IL-2.

In contrast to the CXC chemokine receptors that were evaluated previously and remained unmodulated in the presence of IL-15 or IL-2, the receptors for C-C chemokines examined in Fig. 1B displayed differential responses to IL-15 and IL-2. The receptors CCR1, CCR4, CCR5, CCR2a, and CCR2b were expressed, although in low abundance, in resting T lymphocytes and were detected by the exceedingly sensitive RPA. Transcripts for CCR3 and TER-1 were, however, not detected. More importantly, all the transcripts that were constitutively expressed in resting T cells were induced when cultured in the presence of IL-15, and the magnitude of the induction was fivefold or more (compare lane 2 vs lane 4), whereas in the presence of IL-2, no discernible induction was apparent (compare lane 2 vs lane 3).

Although both IL-15 and IL-2 signaling occurs through the engagement of IL-2/15Rβ and IL-2/15Rγ (γc) components of the receptor complex, IL-2 requires the presence of IL-2Rα for high affinity interactions and subsequent signal transduction (reviewed in Refs. 4 and 7). However, IL-2Rα, which is induced by the engagement of the TCR/CD3 complex, is minimally expressed in resting T lymphocytes, unlike the IL-2/15Rβ and γc polypeptides (4, 7). Thus, it is conceivable that in resting T cells, IL-15 efficiently engages IL-2/15Rβ and IL-2/15Rγ in the presence of IL-15Rα for signal transduction, leading to significant up-regulation of receptors for C-C chemokines relatively rapidly, while IL-2 is unable to do so. Nonetheless, it is important to note that the inability of IL-2 to up-regulate these receptors in T cells does not appear to be absolute. Recently, Loetscher et al. (40) reported that when T lymphocytes were cultured in the presence of IL-2, in addition to displaying chemotaxis to both MCP-1 (monocyte chemoattractant protein-1, and RANTES, their cognate receptor expression was also up-regulated coincidentally with that of the IL-2Rα expression. However, this induction required prolonged culture of cells (>4–10 days) in the presence of IL-2.
IL-15 and IL-2 induce expression of chemokines in resting T lymphocytes

The expression of chemokines in response to IL-15 and IL-2 was next examined. When resting T lymphocytes were cultured in the presence of either recombinant human IL-2 or IL-15 at a concentration of 10 ng/ml for 12 h, total cellular RNA was extracted, and ribonuclease protection assays were performed. The expression levels of ribosomal L32 and cellular GAPDH serve as internal controls. In lane 1, RPA was performed with yeast transfer RNA; in lane 2, RPA was performed with RNA derived from cells cultured in medium alone; in lane 3, RPA was performed with RNA derived from cells cultured in medium supplemented with IL-2; in lane 4, RPA was performed with RNA derived from cells cultured in medium supplemented with IL-15. Similar results were obtained from cells derived from two other donors.

Having demonstrated that the expression of both chemokines and their receptors are induced in response to IL-15 treatment in T cells, we next examined the kinetics of this induction by culturing cells in the presence of IL-15 at a concentration of 10 ng/ml and evaluating the mRNA profiles of chemokines and their receptors at various post-treatment time points up to 24 h by RPA. As shown in Fig. 3, by 12 h following IL-15 treatment an increase in the mRNA levels of all responsive chemokines was apparent, although highest induction levels were seen in the samples harvested 18 h after IL-15 treatment. It should be noted that the gradual decline in the mRNA levels seen in samples collected after 24 h suggests that the induction in response to IL-15 is probably transitory. Moreover, when cells were exposed to increasing amounts of IL-15, there was a concordant increase in the magnitude of the induction.
of mRNA expression for all the responsive chemokines examined (Fig. 3).

The inductive kinetics of the C-C chemokine receptor expression paralleled those of chemokines themselves as shown in Fig. 4, with peak expression occurring 18 h post-treatment. In addition, as demonstrated above for the expression of chemokine gene expression, exposure of cells to increasing amounts of IL-15 resulted in an enhancement of receptor gene expression in a dose-dependent manner up to 500 ng/ml, although at 1 μg/ml some diminution in the level of induction was apparent.

To determine whether the increase in the steady state mRNA levels of chemokines seen above in the presence of IL-2 or IL-15 actually results in augmented secretion of these chemokines, we measured the chemokine levels in the culture supernatants of treated cells using an ELISA. The basal levels of chemokines assessed varied from donor to donor, but as shown in Fig. 5, a dose-dependent increase in the secreted levels of MIP-1α, MIP-1β, and RANTES was observed. This enhanced secretion was not affected by the presence of Abs to TNF-α or IL-1β, thus excluding the possibility that the chemokine secretion observed was secondary to induction of other proinflammatory cytokines, such as IL-1β or TNF-α, by IL-15 (data not shown). More importantly, the observed dose dependency extending to high concentrations of IL-15, exemplified by cultures treated with 500 or 1000 ng/ml, was somewhat surprising considering the fact that T lymphocytes express only about 2 × 10^3 IL-2/15Rβ and γc receptors on the cell surface, and saturation of these receptors should have occurred at the lower amounts of IL-15 used (42). Since IL-15 binds to the IL-2/15Rβ,γc heterodimer in the absence of IL-15Rα with low (10^-8–10^-9 M) affinity, one possible explanation for this could be that peripheral blood-derived T lymphocytes use a receptor system that does not involve IL-15Rα. Alternatively, IL-15 might use a receptor that does not involve any of the IL-2/15R elements in freshly isolated peripheral blood T lymphocytes. To explore this possibility, a mAb, Mikβ-1, that binds to IL2Rβ and prevents IL-15 signaling via the IL-2R complex subunits (43) was added (20 μg/ml) to cells 15 min before the addition of various amounts of IL-15. As shown in Fig. 6, Mikβ-1, which has an IC_{50} of 5 nM for the induction of IL-15-mediated proliferation of the Kit225/K6 T lymphocytic cell line via the IL-2/15R complex (44), inhibited 75% of the MIP-1α secretion induced by low concentrations of IL-15 in peripheral blood-derived T lymphocytes, in accord with the use of IL-2/15Rβ,γc with or without IL-15Rα. More importantly, when T cells were treated with higher doses of IL-15, the Ab was less effective (<25% inhibition) in blocking IL-15-mediated MIP-1α secretion. From the results presented in Fig. 6, it is conceivable that the response elicited with lower amounts of IL-15, which is inhibitable by Mikβ-1, represents a more efficient signaling pathway, whereas when IL-15 is present in excess, an alternate receptor system other than the IL-2/15Rβ,γc with attenuated ligand affinity becomes operational. In this regard it is noteworthy that an alternate receptor system for IL-15 has been described in mast cells, although the actual components of this system have yet to be identified (45). However, if the receptor densities of IL-2/15Rβ and γc are markedly different in peripheral blood-derived T lymphocytes compared with those in the well-studied T lymphocytic cell line
In a parallel set of experiments, mAb Mikβ-1, which binds to the IL-2/15Rβ polypeptide, was added (20 μg/ml) to the culture medium 15 min before the addition of IL-15. MIP-1α levels in the culture supernatants were measured after 24 h by an ELISA, and the reduction in MIP-1α levels in the presence of Mikβ-1 Ab is expressed as a percentage and represents the mean ± SEM of samples performed in triplicate.

Kit225/k6, then the failure of Mikβ-1 Ab to block high dose IL-15 effects may simply be due to a limitation of the Ab.

The importance of chemokine receptors for HIV entry and AIDS pathogenesis has recently become increasingly apparent (27–29). The C-C chemokine receptor CCR5 and, to a lesser extent, CCR3 and CCR2b mediate entry of M-tropic, nonsyncytial-inducing strains of HIV that are primarily responsible for the transmission of HIV (30–32). In quiescent T cells these C-C chemokine receptors are minimally expressed (46); as a result, T cells are refractory to HIV infection. Our observation of an enhanced expression of these receptors in the presence of IL-15 in T lymphocytes taken together with the fact that unlike IL-2 production, which declines as the disease progresses, the elevated levels of serum IL-15 often persist in AIDS patients (47) suggest a potential role for this cytokine in the pathogenesis of HIV infection. It is possible that IL-15 could facilitate the spread into the T cell population of M-tropic virus that is usually confined to monocytes/macrophages. IL-15 is ideally poised for this function because of its inherent ability to induce the expression of CCR5 receptor as well as CD3-independent proliferation of T lymphocytes, two pre-requisites for efficient replication of HIV. To examine this possibility, in the presence of IL-15, freshly isolated, quiescent T lymphocytes were infected with a primary M-tropic, nonsyncytial-inducing HIV isolate (HIVUS-1) that had previously been shown to use the CCR-5 receptor exclusively for entry (48). Viral replication was monitored by measuring the secreted p24 levels in infected culture supernatants. It is important to note that unlike previous studies (49–51) that used either cell lines or artificially activated T cells with mitogens or CD3 cross-linking, our studies were performed using unactivated cells and thus are more likely to represent the natural state of T cells that would encounter the virus in the body. As can be seen in Fig. 7, there was efficient replication of HIV in T cells as assessed by the detection of p24 as early as day 3 postinfection in cultures treated with IL-15 but not in untreated control cultures. The addition of IL-15 and virus simultaneously to cells or pretreatment of cells with IL-15 before the addition of virus did not significantly affect the virus replication profile (data not shown). It is established that CCR-5-mediated entry can be competitively inhibited by the presence of its ligands, MIP-1α, MIP-1β, and RANTES, in T lymphocytes (52). The fact that IL-15 induced these chemokines efficiently with kinetics similar to those of the CCR-5 gene and yet was able to promote HIV entry suggests that the receptor induction is more pronounced than that of its ligands, thus quantitatively favoring virus entry. The levels of IL-15-induced MIP-1α, MIP-1β, and RANTES in peripheral blood-derived T lymphocytes (<6 ng/ml) may not sufficiently saturate the induced cell surface CCR-5 coreceptors. This is in contrast to what is seen in NK cells, in which, either in combination with IL-12 or following CD16 cross-linking, IL-15 leads to copious secretion of these chemokines (53, 54) and, in fact, is able to efficiently suppress M-tropic HIV entry and replication in T lymphocytes (54). Thus, the overall impact of IL-15 on HIV replication and pathogenesis may be dependent upon the balance of the HIV-suppressive and HIV-inductive activities of this cytokine.

Additionally, the association of IL-15 with aberrant inflammatory responses such as rheumatoid arthritis (55, 56), ulcerative colitis (57, 58), pulmonary sarcoidosis (19), and multiple sclerosis (59) strongly suggests an etiologic role for this cytokine in the perpetuation of inflammation. Although physiological inflammation is pivotal in host defense, a breakdown in this finely tuned response may result in an exaggerated response that is detrimental to the host, leading to chronic inflammatory, autoimmune, or allergic diseases. Parallel evidence from animal models of such de-ranged inflammatory processes have clearly defined a critical role for chemokines (60). For example, lung reperfusion injury, and urate-crystal-induced arthritis in rabbits showed regression after treatment with an anti-IL-8 Ab (61, 62). Adjuvant-induced arthritis in Lewis rats, which mimics rheumatoid arthritis, responded to anti-RANTES therapy (63). Similarly, administration of Abs to
MIP-2 and monocyte chemoattractant protein-1 led to rapid resolution of glomerulonephritis and cutaneous delayed hypersensitivity in rats, respectively (64, 65). In addition, Abs against MIP-1α and RANTES reversed allergic airway inflammation in mice (66). In this context, the ability of IL-15 to directly and rapidly augment the synthesis of both C-C as well as CXC chemokines by T lymphocytes and their receptors in the induction of inflammatory chemokines and their receptors.

Acknowledgments

We thank Drs. Keizo Furuke, Hiyoriyo Moriuchi, Masako Moriuchi, Joe Mosca, and Pin-Yu Perera for their assistance.

References


