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IL-11 expression in retinal and corneal cells is regulated by interferon- γ

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Abstract

Interleukin-11 (IL-11) is an anti-apoptotic, anti-inflammatory cytokine with hematopoietic potential. The expression and protective actions of IL-11 have not been explored in the eye. The expression of IL-11 in primary cultures of human retinal pigment epithelial (HRPE) and human corneal fibroblast (HCRF) cells were evaluated in these studies. Constitutive secretion of IL-11 was not observed in either HRPE or HCRF. TNF- α + IL-1 induced IL-11 secretion and this production was inhibited by NF κ B pathway inhibitors. IFN- γ significantly inhibited TNF- α and IL-1 induced IL-11 secretion and inhibitors of JAK-STAT pathway reversed this inhibition. TGF- β induced IL-11 secretion that was blocked by TGF- β receptor 1 inhibitor but not by IFN- γ . RT-PCR analysis confirmed the effects of IL-1, TNF- α , IFN- γ and TGF- β on IL-11 secretion at mRNA levels. Our results demonstrate that IL-11 is dramatically up regulated in retina and cornea cells and that IFN- γ is a physiological inhibitor of IL-11 expression.

Keywords

IL-11; IFN- γ ; TNF- α ; IL-1; TGF- β ; Retina; Cornea

Introduction

Interleukin-11 (IL-11), a 19 kDa secreted protein, is a pleiotrophic cytokine with anti-inflammatory, cytoprotective and hematopoietic actions [1–3]. Circulating levels of IL-11 are almost undetectable in normal individuals while increased serum levels are observed in certain disease conditions like rheumatoid arthritis, thrombocytopenia and psoriasis [3]. Human IL-11, highly helical thermostable protein with no cysteine residues, acts through a specific IL-11 receptor α that acts in concert with a common gp130 receptor of IL-6 ligand family [1–3]. Recently, IL-11 has been shown to (1) act as anti-inflammatory agent by down regulating proinflammatory cytokine production [4], (2) prevent acute graft-versus-host disease by T cell polarization and inhibition of inflammatory cytokine production [5], (3) up regulate expression of cytoprotective protein survivin in keratinocytes and endothelial cells [6], (4) promote oligodendrocyte survival, maturation and myelin formation [7], (5) reduce the ischemic/reperfusion injury in the hearts by protecting myocardial cells [8], and (6)

protect intestinal epithelial cells from radiation-induced injury [3]. Because of its multifunctional potential, IL-11 has been approved for clinical trials in chemotherapy-induced thrombocytopenia, mucositis, inflammatory bowel disease and psoriasis [3,9,10]. However, the expression of IL-11, its association with pathological states and its potential use as therapeutic agent in the eye have not been investigated.

The expression of IL-11 in the normal physiology in the ocular tissues has not been reported. Furthermore, its role in ocular diseases has not been described. While studying gene expression profiles of human retinal pigment epithelial (HRPE) cells, we found elevated expression of IL-11 in HRPE treated with TGF- β but not in cells treated with an inflammatory cytokine mix (IL- β + TNF- α + IFN- γ). Since previous studies in various cells have shown induction of IL-11 by TNF- α and IL-1 [11–14], we hypothesized and demonstrated that IL-11 is induced in the retina and cornea as a key immunosuppressive cytokine and that IFN- γ inhibits this expression.

Materials and methods

Materials

Fetal bovine serum and cell culture media were obtained from Invitrogen, Carlsbad, CA. Human recombinant IFN- γ and TNF- α were purchased from Roche Applied Science, Indianapolis, IN. All other human recombinant cytokines, growth factors and interleukin-11 ELISA kits were purchased from R&D Systems, Minneapolis, MN. JAK inhibitor 1, TGF- β R1 kinase inhibitors, NF κ B activation inhibitor and Ro106-9920 were obtained from Calbiochem, San Diego, CA. RNA PCR kits and PCR supplies were obtained from Applied Biosystems, Foster City, CA. Affymetrix GeneChips (HG U133 plus 2.0) were purchased from Affymetrix Inc., Santa Clara, CA.

Cell cultures

Human retinal pigment epithelial (HRPE) cell cultures and human choroidal fibroblast (HCHF) cultures were prepared from donor eyes as described before [15,16]. Human corneal fibroblast (HCRF) cells were prepared from corneal buttons or from cornea obtained from donor eyes [17]. Cells were grown in MEM supplemented with 10% FBS, non-essential amino acids and antibiotic–antimycotic mixture. Serum free medium (SFM) is similar to the above medium without FBS. Primary cell lines of HRPE, HCHF and HCRF derived from 2 to 5 donor eyes were used at passages 6–10.

Cell cultures were treated with cytokines at the following concentrations: IFN- γ (100 μ g/ml), IL-1 (10 ng/ml), TNF- α (10 ng/ml) and TGF- β (10 ng/ml) in serum free medium.

Microarray analysis of gene expression in HRPE cells

Confluent cultures of HRPE cells were treated with TGF- β or inflammatory cytokine mix (IFN- γ , TNF- α and IL-1 β) for 8 h in SFM. Total RNA prepared from these cultures was used for global gene expression profiling using Affymetrix GeneChips following manufacturer's protocol. Affymetrix GeneChip operating software and GeneSpring software (Silicon Genetics/Agilent, CA) was used for absolute expression and normalization [18].

Analysis of IL-11 secretion by HRPE, HCRF and HCHF

Cells maintained in medium containing 10% FBS were grown to confluence in 24 well plates. Cultures were washed with SFM and incubated in SFM overnight. Then cells were incubated for 24 h in SFM containing various cytokines or growth factors. Culture supernatants were collected and used for the determination of IL-11 levels by ELISA. This

assay recognizes both natural and recombinant human IL-11 with a mean minimum detectable dose of 8 pg/ml.

Effect of NF κ B inhibitors on IL-1 β and TNF- α induced IL-11 secretion

Confluent cultures of HRPE and HCRF were grown in 24 well plates as described above and appropriate wells were pretreated for 30 min with NF κ B signal transduction pathway or other inhibitors dissolved in DMSO. Then cells were incubated for 24 h with a mixture of TNF- α and IL-1 β in the absence or presence of inhibitors. Supernatant fluids were collected and IL-11 levels determined by ELISA.

Effects of JACK-STAT pathway inhibitor on IL-11 secretion

Confluent cultures of HRPE and HCRF were grown in 24 well plates as described above and appropriate wells were pretreated for 30 min with JAK inhibitor 1. Then cultures were treated with various cytokine preparations in the absence or presence of JAK inhibitor. After 24 h incubation, supernatants were collected and the levels of secreted IL-11 were measured by ELISA.

Effects of TGF- β on IL-11 secretion

Confluent cultures of HRPE and HCRF were grown in 24 well plates as described above and appropriate wells were pretreated for 30 min with TGF- β R1 kinase inhibitors dissolved in DMSO. Then TGF- β 1 or TGF- β 2 were added to the cultures. After 24 h incubation, culture supernatants were collected and IL-11 levels determined by ELISA.

RT-PCR analysis of IL-11 mRNA expression

The following primers were used for PCR [14,19]. The numbers in the parenthesis indicate the size of PCR products.

IL-11-F: 5'-CTG AGC CTG TGG CCA GAT ACA-3'

IL-11-R: 5'-CTC CAG GGT CTT CAG GGA AGA-3' (336 bp)

GAPDH-F: 5'-CCA CCC ATG GCA AAT TCC ATG GCA-3'

GAPDH-R: 5'-TCT AGA CGG CAG GTC AGG TCC ACC-3' (600 bp).

HCRF cultures were grown to confluence in 60 mm dishes and treated with various cytokines and/or inhibitors in SFM for 8 h. Total cellular RNA was prepared from the cells using RNeasy mini kit (Qiagen Sciences, MD) or RNA STAT-60 (Tel-Test, Friendswood, TX). RNA PCR kit (Applied Biosystems, Foster City, CA) was used for reverse transcription and polymerase chain reaction according to the protocols provided by the manufacturer and procedure described by us earlier [17,19]. We used GAPDH gene as a control gene, the expression of which was unchanged in all our control and treatment samples.

Transcription factor assays

HRPE cultures grown to confluence in 100 mm dishes were treated with cytokines for 2 or 6 h in SFM. Nuclear and cytoplasmic extracts were prepared by using nuclear extract kit (Active Motif, Carlsbad, CA) as described earlier [19]. Nuclear or cytoplasmic fractions were used for NF κ B, p65 and pSTAT-1 transcription factor assays by using ELISA based kits (Active Motif, Carlsbad, CA or Invitrogen, Carlsbad, CA) as described earlier [19].

Results

Microarray analysis of HRPE gene expression

Microarray analysis revealed 25 and 10-fold increase in IL-11 expression in two primary HRPE cell lines treated with TGF- β 1. No other interleukin or chemokine gene expression was altered by TGF- β 1 (data not shown). Microarray analysis of HRPE cells treated with a mixture of inflammatory cytokines (TNF- α + IL-1 β + IFN- γ) showed several fold increased levels of expression of IL-6, IL-8, CCL-2, 5, 7, 20 and CXCL-2, 3, 6, 9, 10, 11 but not IL-11 (data not shown). Previous studies have demonstrated that IL-11 expression was enhanced by TGF- β , IL-1 α and TNF- α in a number of cell lines [9–12]. Lack of elevated expression of IL-11 in the presence of IFN- γ in (TNF- α + IL-1 β + IFN- γ mix) suggested inhibitory effect of IFN- γ on IL-11 expression, since TNF- α and IL-1 are known to induce IL-11.

IFN- γ inhibits IL-1 and TNF- α induced IL-11 secretion by HRPE and HCRF

We used HRPE and HCRF cells for these studies. Comparisons were always made between control and treated cells under similar conditions. HRPE and HCRF cells did not secrete IL-11 constitutively even after 24 h of incubation (Fig. 1A and C). Incubation of cells in the presence of IL-1 or TNF- α induced secretion of IL-11, but the presence of both IL-1 and TNF- α significantly enhanced IL-11 secretion (Fig. 1A and C). Addition of IFN- γ inhibited IL-1 + TNF- α induced IL-11 secretion significantly ($p < 0.001$). Dose dependent effects of these cytokines were observed at 10 to 100-fold dilutions (data not shown). In HCRF cultures also, IFN- γ inhibition of IL-1 and TNF- α induced IL-11 secretion was observed (data not shown). Interleukin-2, -4, -6, -8, -10 and -12 had no effect on constitutive or IL-1 β + TNF- α induced IL-11 secretion by HRPE (data not shown).

IFN- γ does not inhibit TGF- β induced IL-11 secretion by HRPE and HCRF

TGF- β 1 and TGF- β 2 induced IL-11 secretion in both HRPE and HCRF (Fig. 1B and D). IFN- γ had no inhibitory effects on IL-11 secretion induced by TGF- β . In the same batch of cultures, IFN- γ inhibited IL-1 and TNF- α induced IL-11 secretion by HRPE and HCRF (Fig. 1B and D). Other growth factors, EGF, bFGF, PDGF, TGF- α , IGF-1, BMP-4, activin-A and inhibin-A had no effect on IL-11 secretion by HRPE (data not shown).

NF κ B pathway is involved in IL-1 β and TNF- α induced IL-11 secretion

We used selective inhibitors to evaluate the role of NF κ B signal transduction pathway in IL-1 and TNF- α induced IL-11 secretion in HRPE cells. Ro106-9920 and NF κ B activation inhibitor at 1 μ M concentration significantly ($p < 0.01$) inhibited IL-1 β + TNF- α induced IL-11 secretion (Fig. 2A). Under similar conditions, negative control of Ro106-9920 and Ly294002 (PI3k inhibitor) had no effects on IL-1 β + TNF- α induced IL-11 secretion (Fig. 2A). HRPE cells were treated with IL-1 β + TNF- α in the absence or presence of NF κ B inhibitors and cytoplasmic and nuclear extracts were prepared for NF κ B, p65 analysis. Results from one representative experiment are shown in Fig. 2B and C.

NF κ B p65, one of the dissociated and activated form of NF κ B heterodimer complex, levels were increased by about 4-fold in both cytoplasmic and nuclear fractions of HRPE cells treated with IL-1 β + TNF- α treatment. NF κ B pathway inhibitors, Ro106-9920 and NF κ B activation inhibitor abolished this increase in p65 protein in both cytoplasmic and nuclear fractions (Fig. 2B and C). Negative control of Ro106-9920 had no effect on cytoplasmic or nuclear p65 levels. These data suggest that NF κ B pathway contributes to IL-11 secretion induced by IL-1 β and TNF- α .

JAK-STAT inhibitor reverses inhibition of IL-11 secretion by IFN- γ

In both HRPE and HCRF cells, IFN- γ inhibited significantly ($p < 0.001$) TNF- α + IL-1 induced IL-11 secretion (Fig. 3A and B) as seen Fig. 1. Incubation of cultures in the presence of JAK-1 inhibitor, inhibitor of JAK-STAT pathway, reversed the inhibition caused by IFN- γ significantly ($p < 0.001$). Nuclear translocation of pSTAT-1 in HRPE cells treated with IFN- γ increased by 7–12-fold in comparison to control cultures, and JAK-1 inhibitor abolished this pSTAT-1 nuclear translocation (data not shown) [19]. These data confirm the inhibitory actions of IFN- γ on IL-11 secretion.

TGF- β receptor 1 inhibitor abolishes TGF- β induced IL-11 secretion

Two TGF- β receptor 1 inhibitors were used to ascertain TGF- β induction of IL-11 in HRPE and HCRF cells. There was a dose dependent effect of TGF- β receptor inhibitors on TGF- β induced IL-11 secretion and TGF- β effects are abolished at 100 nM concentration (Fig. 3C and D). NF κ B and JAK-1 inhibitors, that were shown to influence TNF- α , IL-1 β and IFN- γ effects on IL-11 secretion, had no effects on TGF- β 1 induced IL-11 secretion (Fig. 3C and D). Similar results were observed with TGF- β 2 induction of IL-11 secretion. This and previous data (Fig. 1) clearly demonstrate that TGF- β actions are independent of IL-1, TNF- α and IFN- γ .

Regulation of IL-11 mRNA expression by TGF- β , IL-1, TNF- α and IFN- γ

RT-PCR analysis was performed to examine the mRNA levels of IL-11 under different treatment conditions. House keeping gene GAPDH and IL-11 were amplified for 25 and 30 cycles, respectively. In both HRPE and HCRF cells TNF- α + IL-1 β enhanced IL-11 mRNA and IFN- γ down regulated this increase (Fig. 4A and C). In the presence of JAK-1 inhibitor, IFN- γ inhibition of IL-11 expression was reversed (Fig. 4A and C). TGF- β 1 and TGF- β 2 enhanced IL-11 mRNA expression in both HRPE and HCRF cells and TGF- β receptor 1 inhibitor abolished TGF- β induced IL-11 expression (Fig. 4B and D). IFN- γ did not have any inhibitory effect on TGF- β induced IL-11 mRNA levels in HCRF cells (Fig. 4D) and in HRPE cells (data not shown). Thus, RT-PCR results demonstrate that mRNA levels of IL-11 are regulated by IL-1, TNF- α , TGF- β and IFN- γ (Fig. 4), which in turn influence IL-11 secretion by HRPE and HCRF cells (Figs. 1–3).

Discussion

In this study we investigated the production of IL-11 by ocular cells. Our data demonstrate that human retinal and corneal cells do not constitutively produce IL-11. However, treatment with TGF- β , IL-1 and TNF- α results in the upregulation of IL-11 gene expression and protein production. Moreover, this is the first report to show significant inhibition of IL-11 production by IFN- γ .

IL-1, TNF- α , TGF- β , LPS, RSV and Rhinoviral infections are known to induce IL-11 expression in cells derived from various tissues [11–14]. To date, no physiological molecules have been identified as negative regulators of IL-11 expression. Herein, we have shown here that IFN- γ , but none of the cytokines tested, significantly inhibited IL-11 expression in primary HRPE and HCRF cells. Interestingly, IFN- γ had no effect on TGF- β induced IL-11 expression in these cells.

The molecular mechanisms by which IFN- γ [20] is inhibiting TNF- α and IL-1 induced IL-11 secretion by HRPE and HCRF cells may be complex. Neither JAK inhibitor and IFN- γ affected NF κ B p65 levels induced by TNF- α + IL-1 nor IFN- γ induced pSTAT-1 was affected by TNF- α + IL-1 [19]. Therefore, the effects of IFN- γ on IL-11 secretion most likely are mediated through some mechanisms interconnected to the primary signaling pathways.

The presence of IFN- γ together with TNF- α + IL-1 augments secretion of several cytokines, chemokines and growth factors in HRPE cells [15,16,18,19]. IFN- γ can also act as a double edge sword by inhibiting IL-1 + TNF- α or TGF- β induced VEGF-A secretion and by enhancing IL-1 + TNF- α or TGF- β induced sVEGF-r1 secretion in HCRF cells [17]. In HRPE cells, stimulatory and inhibitory effects of IFN- γ were observed on TGF- β 1 and TGF- β 2 expression, respectively [19]. These studies indicate that IFN- γ can act differentially under certain conditions.

Retinal pigment epithelium (RPE) is a single layer of pigmented epithelial cells interspersed between vascular rich choroid on the posterior side and neuro-sensory retina on the anterior side behind vitreous in the eye [21,22]. Normal structure and functioning of RPE is crucial for retaining visual acuity by maintaining blood retinal barrier, retinal structure, fluid transport, ingestion of shed photoreceptors and protection from light damage [21,22]. Our previous studies have demonstrated that TNF- α and IL-1 alone or together stimulate HRPE cells to secrete a number of cytokines, chemokines and growth factors [15,16,18,19]. The secretion of IL-11 by HRPE cells in the presence of inflammatory cytokines TNF- α and IL-1, and TGF- β may be a mechanism for protection of HRPE cells as well as neighboring retinal and choroidal cells. It is important to note that RPE cells rarely divide after birth and any damage or degeneration of these cells leads to permanent defect in the RPE cell layer and ultimately to retinal damage and loss of vision [21,22].

Cornea, transparent tissue on the external part of the eye critical for transmission of light, consists of 4–5 layers of epithelial cells on the external surface, stromal connective tissue in the middle and a single layer of endothelial cells facing the aqueous humor [23,24]. Stroma with its dispersed fibroblast cells make up to 90% of the cornea. Viral infections, chemical or mechanical injury and/or chronic inflammation may lead to disorganization of stromal connective tissue resulting in loss of transparency [23,24]. In HCRF cells derived from corneal stroma, TNF- α and IL-1, products of infiltrating and activated leukocytes, induce secretion of IL-11. HCRF cells produce large quantities of IL-11 in the presence of TGF- β that is not inhibited by IFN- γ (Fig. 1D). Since HCRF cells produce TGF- β 1 under inflammatory conditions (unpublished observations), IL-11 production under these conditions would impart protection to corneal tissue that is exposed to a variety of environmental insults such as intense light, heat, chemicals and infectious organisms [23,24]. Corneal transplantation, using donor corneal buttons, is only option for patients with damaged corneas to restore vision. IL-11 may be useful in preventing graft protection by T cell polarization and immunomodulation as reported in bone marrow transplantation models [5].

The eye is an immune privileged organ and the retinal pigment epithelium and cornea are key barrier sites. We have previously shown that the HRPE cell is a potent source of immunosuppressive cytokines, TGF- β and IFN- β (18,19). Herein, we demonstrate that IL-11, an immunosuppressive and cytoprotective cytokine, is produced by HRPE and HCRF. Since inflammation and RPE cell alterations are now considered basic components of age related macular degeneration, it is possible that IL-11 regulation may play a role in this pathogenic process. The potential application of IL-11 as a therapeutic agent in retinal degeneration disorders and in corneal diseases needs to be explored.

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Fig. 1.

Effect of inflammatory cytokines on IL-11 secretion by HRPE (A) and HCRF (C) cells. Cultures were incubated in the presence of various indicated agents in serum free medium (SFM) for 24 h. Culture supernatants were used for the determination of secreted IL-11 by ELISA. IFN- γ inhibits TNF- α + IL-1 induced IL-11 secretion in both HRPE and HCRF. IFN- γ does not inhibit TGF- β induced IL-11 secretion by HRPE (B) and HCRF (D). Cultures were incubated with TNF- α + IL-1 or TGF- β 1 or TGF- β 2 alone or in the presence of IFN- γ for 24 h in SFM. IL-11 levels in the culture supernatants were determined by ELISA. Results are means $\pm$ SEM of 4–5 experiments each performed with at least duplicate samples. * $p < 0.001$.

Fig. 2.

NF κ B signaling pathway is involved in TNF- α + IL-1 β induced IL-11 secretion by HRPE cells. (A) Inhibition of IL-1 β + TNF- α induced IL-11 secretion by NF κ B inhibitors. Cultures were pre-incubated with the indicated inhibitors for 30 min before treating with IL-1 β + TNF- α . After 24 h incubation in SFM, culture supernatants were collected and used for the determination of IL-11 levels by ELISA. Results are means $\pm$ SE for 4 experiments each performed with duplicate samples. * p < 0.01. NF κ B inhibitors abolish NF κ B, p65 levels elevated by IL-1 β + TNF- α in the cytoplasmic (B) and nuclear (C) fractions of HRPE cells. Experimental details are described in the methods section. Results are from one representative experiment with triplicate samples.

Fig. 3.

JAK-STAT inhibitor reverses IFN- γ inhibition of TNF- α + IL-1 β induced IL-11 secretion by HRPE (A) and HCRF (B) cells. Cultures were pre-incubated with JAK inhibitor 1 for 30 min before treating with TNF- α + IL-1 β for 24 h in SFM. The levels of IL-11 in culture supernatants were determined by ELISA. * $p < 0.001$ for inhibition by IFN- γ . @ $p < 0.01$ for reversal of IFN- γ inhibition by JAK-1 inhibitor. TGF- β 1 receptor inhibitor abolishes TGF- β 1 induced IL-11 secretion by HRPE (C) and HCRF (D). The cultures were pre-incubated with the indicated inhibitors for 30 min before treating with TGF- β 1 for 24 h in SFM. Levels of IL-11 in the culture supernatants were determined by ELISA. * $p < 0.001$ for inhibition of TGF- β 1 effects by TGF- β receptor inhibitors. The results are means $\pm$ SEM of 4 experiments each performed with duplicate samples.

Fig. 4.

RT-PCR analysis of IL-11 mRNA in HRPE and HCRF cells. Cultures were incubated in serum free medium for 8 h in the presence of various combinations IFN- γ (100 μ /ml), TNF- α (10 ng/ml), IL-1 β (10 ng/ml) and JAK-1 inhibitor (1 μ M), TGF- β (10 ng/ml), TGF- β receptor 1 inhibitor (1 μ M) as indicated in the figures. Total RNA prepared was used for RT-PCR as described in the methods section. PCR reactions for IL-11 and GAPDH were performed for 30 and 25 cycles, respectively. Effects of TNF- α , IL-1 β , IFN- γ and JAK-1 inhibitor on IL-11 expression in HRPE (A) and HCRF (C) cells. Effects of TGF- β , IFN- γ and TGF- β R1 inhibitor on IL-11 expression in HRPE (B) and HCRF (D) cells.