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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 NFκB 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.

Introduction

Interleukin-11 (IL-11), a 19 kDa secreted protein, is a plieotrophic 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 trails in chemotherapy-induced thrombocytopenia, mucositis, inflammatory bowl 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, NFκB 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

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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/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-1β) 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/Agilant, 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 NFκB inhibitors on IL-1β 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 NFκB signal transduction pathway or other inhibitors dissolved in DMSO. Then cells were incubated for 24 h with a mixture of TNF-α and IL-1β 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-β1 or TGF-β2 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 RNAeasy mini kit (Quiagen 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 NFκB, 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-β1. No other interleukin or chemokine gene expression was altered by TGF-β1 (data not shown). Microarray analysis of HRPE cells treated with a mixture of inflammatory cytokines (TNF-α + IL-1β + 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-1α 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-1β + 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 HCHF 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-1β + 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-β1 and TGF-β2 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).

NFκB pathway is involved in IL-1β and TNF-α induced IL-11 secretion

We used selective inhibitors to evaluate the role of NFκB signal transduction pathway in IL-1 and TNF-α induced IL-11 secretion in HRPE cells. Ro106-9920 and NFκB activation inhibitor at 1 μM concentration significantly ($p < 0.01$) inhibited IL-1β + 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-1β + TNF-α induced IL-11 secretion (Fig. 2A). HRPE cells were treated with IL-1β + TNF-α in the absence or presence of NFκB inhibitors and cytoplasmic and nuclear extracts were prepared for NFκB, p65 analysis. Results from one representative experiment are shown in Fig. 2B and C.

NFκB p65, one of the dissociated and activated form of NFκB heterodimer complex, levels were increased by about 4-fold in both cytoplasmic and nuclear fractions of HRPE cells treated with