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C/T conversion alters interleukin 1A promoter function in a human astrocyte cell line

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Abstract

Recently, association of an interleukin-1A promoter polymorphism (–889, thymine/thymine (T/T)) with Alzheimer's disease was reported, suggesting that this cytokine may play an important role in disease development. To understand the mechanism underlying the interleukin-1A promoter's role in Alzheimer's disease, a study comparing promoter function of an interleukin-1A polymorphism was performed in the SVG astroglia cell line. The effects of thymine and cytosine on transcriptional activity of the interleukin-1A promoter were analyzed by testing luciferase reporter activity in transfected SVG cells. Our results demonstrate that cytosine/thymine conversion increases activity of the interleukin-1A promoter in SVG cells. Both sodium salicylate and lovastatin are able to block induced promoter activities in astroglial cells. Induced promoter activity by the polymorphism (T/T) may result in the upregulation of interleukin-1 α protein and "cytokine cycle" amplification, which may promote disease development.

Keywords

Alzheimer's disease; interleukin-1 α ; neuroinflammation; neurodegeneration; promoter polymorphism; A-beta; LPS

Introduction

Although different hypotheses on the etiology of Alzheimer's disease (AD) have been advanced, the cause of the progressive cell death and the underlying mechanisms remains unknown. Recently, an increasing number of studies have suggested that inflammatory processes take part in the pathogenic cascade of events that leads to AD brain pathology (Koistinaho and Koistinaho, 2005; McGeer et al, 2006). It has been reported that activated glial cells, including astrocytes and microglia, are intimately associated with extracellular beta-amyloid (A β) (Dickson et al., 1993; McGreer et al., 1993), and the use of anti-inflammatory agents, in particular, non-steroid anti-inflammatory drugs, are associated with delayed onset or slower progression of disease (Hull et al., 1996). Activated glial cells producing

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proinflammatory cytokines, such as interleukin-1 α (IL-1 α) (Griffin et al., 1995), interleukin-1 β (Stanley and Griffin, 1990), and interleukin-6 (Bauer et al., 1991), have been found in areas surrounding extracellular amyloid plaques.

Furthermore, others and we have found that the risk for developing AD is considerably altered in carriers with a polymorphism of the IL-1A (-889) (Nicoll et al., 2000; Grimaldi et al., 2000; Du et al., 2000; Rebeck, 2000). Most recently, it has been reported that IL-1 A(-889, T/T) slightly upregulates IL-1A promoter function in a human pancreatic tumor cell line (Dominici et al., 2002). However, it remains unclear whether IL-1A(-889, T/T) is functional in glia and how it is involved in AD pathogenesis. In the present study, we investigate the promoter function of IL-1A polymorphisms (-889, T/T and C/C, (cytosine/cytosine)) in a human astroglial cell line (SVG) treated with or without A β and lipopolysaccharide (LPS) by transiently transfecting plasmids carrying the T/T or C/C genotypes on IL-1A promoters (linked to a reporter gene, luciferase) into SVG cells.

Materials and Methods

SVG cell line culture

The SVG cell line is a human astroglial cell line that has been previously used in our APOE promoter study (Du et al., 2005). In the present study, we maintained SVG cells in DMEM (Invitrogen) containing 10% fetal bovine serum (Invitrogen), 100 U/ml penicillin (Sigma) and 100 U/ml streptomycin (Sigma). The cells were fed three times a week with fresh growth medium.

PCR

The IL-1A primers, 5'-GCCTCGAGTGTCTACCACCTGAACTAGGC-3' and 5'-GGAAGCTTGTGAGGACAATACCTTTGCTG-3' containing XhoI and HindIII sites, was used for the amplification of a 5' flanking fragment (-968 to +64) from genomic DNA isolated from individuals homozygous for allele 1 or allele 2. In addition to buffer and nucleotide components, each amplification reaction contained approximately 100 ng of genomic DNA, 100 pmol of each primer and 2 U of Taq polymerase (Invitrogen) in a final volume of 100 μ l. The reaction mixture was initially denatured at 95°C for 5 min, followed by 35 cycles of PCR (94°C for 60 s, 55°C for 60 s, and 72°C for 120 s) and incubated at 72°C for 5 min. The amplification product was then purified by using a Qiagen PCR kit according to the manufacturer's recommendations and the promoter regions were confirmed by sequence analyses. After digested by XhoI and HindIII for 1 h at 37°C, the PCR products were size fractionated by electrophoresis on a 1% or 2% agarose gel with 1 μ g/ml ethidium bromide (Sigma) for 20 min at 200 V and directly detected under UV light. A Qiagen kit was then used for purification of the resulting fragments to be utilized for subsequent ligation into a luciferase-reporter construct for activity studies.

Construction of plasmid

A chimeric promoter/luciferase plasmid pGL2-IL-1A was made by ligation of segments (1.1 kb) containing the promoter regions of IL-1A (-889, T/T or C/C) into the promoterless luciferase plasmid, pGL2-basic (Promega). For this purpose, the pGL2-basic vector was cut within the polylinker region using XhoI and HindIII. Orientation of the insert was verified by restriction mapping and sequencing. Following confirmation of plasmid constructs, the constructs were transformed and amplified in XL-1 blue cells (Stratagene).

Transfection and luciferase assays

The IL-1A -Luciferase vectors were transiently transfected into the SVG cell line that was seeded at a density of 3×10^5 cells/24-well culture plate by using lipofectamine™ 2000 (Invitrogen). After 24-h of incubation, transfected cells were washed with growth media and treated with or without 10 μ M lovastatin or 3 mM salicylate for 24 h. The treated or untreated cells were exposed to 10 ng/ml LPS (Sigma, Dodel et al., 1999) or 50 μ M fibrillar A β_{25-35} (QCB Biosource International, Koenigsnecht-Talboo and Landreth, 2006; Meme et al., 2006) and A β_{1-40} (QCB Biosource International,) for an additional 24 h, then washed twice with PBS and lysed with the lysis buffer provided by Roche as a part of the luciferase assay system. In order to make fibrillar A β , synthetic A β peptides were first dissolved in the dH₂O and then added into PBS buffer overnight at 37°C (Du et al., 2003). A 50- μ l aliquot of cell extract was mixed with 100 μ l of reagent solution containing luciferin and ATP and the signal was detected at 562 nm. The transfection efficacy was controlled by co-transfection with β -gal plasmids. The data represent the average of three different wells within the same experiment.

Results

In this study, IL-1A promoters (–889, T/T and –889, C/C) were transiently transfected into astroglial cells (SVG) for 24 h and the luciferase activity from cell extracts was analyzed. The activity of IL-1A promoter, as shown in Fig. 1, was increased by 15% with a conversion from C to T at the –889 site, indicating that the nucleotide at this site affects IL-1A promoter activity in astrocytes.

We then investigated whether and how inflammatory stimuli (LPS and A β_{25-35}) stimulate the activity of IL-1A promoters. The SVG cells transfected with IL-1A(T/T) and IL-1A(C/C) constructs were treated with LPS (10 ng/ml) or A β_{25-35} (50 μ M) and A β_{1-40} (50 μ M). As shown in Fig. 2a and 2b, both LPS, A β_{25-35} , and A β_{1-40} significantly stimulated the activity of both IL-1A promoters (T/T and C/C). The promoter activity was greater with the –889T/T (7.4-fold with LPS, 2.3-fold with A β_{25-35} , and 4.1-fold with A β_{1-40}) than with the –889C/C promoter (2.3-fold with LPS, 1.1-fold with A β_{25-35} , and 1.3-fold with A β_{1-40}). These results indicated that both basal and stimulated activity of the –889T/T IL-1A promoter was higher than the –889C/C constructs in astrocytes.

Next, we performed a more in-depth study by treating transfected cells with salicylate and lovastatin, both of which were selected since anti-inflammatory agents and statins may play important roles in the treatment of Alzheimer's disease. We pretreated SVG cells transfected with IL-1A promoters with salicylate (3 mM) and lovastatin (10 μ M) for 24 h and the cells were then stimulated by A β or LPS for another 24 h. As shown in Fig. 3 and 4, salicylate and lovastatin markedly inhibited the luciferase activities of transfected SVG cells stimulated by A β or LPS, indicating that salicylate significantly blocks A β -induced IL-1 α expression and lovastatin inhibits LPS-induced IL-1 α production. Here, we did not observe that treatments of salicylate (3 mM) and lovastatin (10 μ M) affect cell viability in our experiments.

Discussion

IL-1 overexpression in the brain of patients with Alzheimer's disease relates directly to the development and progression of the disease (Griffin and Mrak, 2002). A polymorphism in the 5'-flanking regulatory region at –889 of the IL-1A promoter (a C-toT transition, designated as IL-1A[–889] allele 2) was reported to be associated with an increased risk of AD (Nicoll et al., 2000; Grimaldi et al., 2000; Du et al., 2000; Rebeck, 2000) and age of onset and correlated with a 4-fold increase in IL-1 α levels in vivo (Shirodaria et al., 2000). Although the promoter activity of IL-1A has been investigated in human pancreatic tumor cells, no information has

been provided as to whether this single nucleotide change modifies promoter function in the brain. In the present study, we transiently transfected constructs carrying human IL-1A promoters with either T or C at the -889 site into SVG cells and have found that both types of promoters are functional in astroglial cells. The C/T conversion significantly increases the activity of the IL-1A promoter, suggesting that the IL-1A T/T alleles may cause an overexpression of IL-1 α in the brain.

Additionally, previous studies demonstrate that AD is accompanied by an inflammatory reaction that is considered to be a response to A β deposition in the brain. In this study, we provide evidence that inflammatory stimuli LPS, A β ₂₅₋₃₅ and A β ₁₋₄₀ enhance both IL-1A promoters' activities and stimulate higher activity with the -889T/T IL-1A promoter than with the -889C/C IL-1A promoter. High activity of IL-1A promoter stimulated by A β or LPS may result in an upregulation of the IL-1 α protein, which initiates an amplification of the "cytokine cycle" by stimulating IL-1 β production as well as other cytokines. An increased inflammatory reaction may ultimately contribute to neuronal dysfunction and death. It should be noted that in this study, we focus on the effect of fibrillar A β , not soluble A β peptides on IL-1A promoters. Additionally, A β ₁₋₄₀ fibrils showed stronger stimulatory activity on IL-1A promoters than A β ₂₅₋₃₅ possibly resulting from its amino-terminal fragment or from the quality of peptides. Recently, it has also found that interleukin 1 α expression is regulated by the allele-specific expression and the promoters of this study may be involved in this regulation, although the data presented by those investigators does not support this hypothesis indirectly (Bayley et al., 2003). A further study on this hypothesis by using our promoters is necessary.

Furthermore, we have shown that A β can trigger an inflammatory response, and sodium salicylate is able to block such an induction possibly via a reduction of NF- κ B levels in astroglial cells (Dodel et al., 1999). Consistent with a previous study, here we have shown that salicylate is able to block A β -induced -889T IL-1A promoter activity. Moreover, it has also been shown that lovastatin inhibits LPS-induced NF- κ B and IL-1 β expression, which may be associated with delayed onset and/or slower progression of the disease (Pahan et al., 1997). Here, our new evidence indicates that lovastatin inhibits the increased activity of the -889T/T IL-1A promoter induced by LPS. The anti-inflammatory property of both salicylate and lovastatin on the IL-1 α promoter in AD may play roles in the treatment of AD. Finally, it is noted that the NCBI database entry for the sequence including IL1A (NT_022135), based on newer ESTs, shows that "-889" actually lies at +12 relative to the start of transcription.

Conclusion

Cytosine/thymine conversion increases activity of the interleukin-1A promoter in glial cells, in the presence or absence of A β and LPS.

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Fig 1. Activity of the IL-1A (T/T, h2) promoter is 15% higher than the IL-1A (C/C, wt) promoter Transfected cell extracts were prepared and analyzed by using a luciferase assay. Measurements were expressed in relative levels, adjusted to untreated control cells, of luciferase adjusted for β -galactosidase levels. The activity of the IL-1A (T/T, h2) promoter was significantly higher than the activity of IL-1A (C/C, wt) promoter. *, $p < 0.05$. The data are representative of at least three independent experiments performed in triplicate with similar results that are expressed as relative luciferase units and the SEM of triplicate samples.

Fig 2. LPS-stimulated and A β -stimulated IL-1A promoter activity in transfected SVG cells

Transfected cell extracts were prepared and analyzed by using a luciferase assay. Measurements were expressed in relative levels, adjusted to untreated control cells, of luciferase adjusted for β -galactosidase levels. **(a)** LPS (10 ng/ml) enhanced the activity of IL-1A (T/T, h2) promoter more strongly in comparison to the IL-1A (C/C, wt) promoter. ***, $p < 0.001$. **(b)** A β_{25-35} (Ab25_35, 50 μ M) A β_{1-40} (Ab1-40, 50 μ M) significantly enhanced the activity of the IL-1A (T/T, h2) promoter more strongly in comparison to the IL-1A (C/C, wt) promoter. *, $p < 0.05$. The data are representative of at least three independent experiments performed in triplicate with similar results that are expressed as relative luciferase units and the SEM of triplicate samples.

Fig 3. Pretreatment with salicylate or lovastatin inhibits luciferase activity in transfected SVG cells stimulated with A β

Transfected cell extracts were prepared and analyzed by using a luciferase assay. Measurement were expressed in relative levels, adjust to untreated control cells, of luciferase adjusted for β -galactosidase levels. **(a)** Salicylate (asp, 3 mM) pretreatment markedly decreased luciferase activity of SVG cells cultured in the presence of A β_{25-35} (Ab). **, $p < 0.01$. **(b)** Lovastatin (10 μ M) 24-hr pretreatment in SVG cells cultured in the presence of A β_{25-35} (Ab) also significantly attenuates their luciferase activity. *, $p < 0.05$. Both salicylate and lovastatin did not affect cell viability by using MTT assay. All data are representative of at least three independent experiments performed three times with similar results and expressed as relative luciferase units and the SEM of triplicate samples.

Figure 4. Lovastatin pretreatment decreases luciferase activity in LPS-stimulated, transfected SVG cells

Transfected cell extracts were prepared and analyzed by using a luciferase assay. Measurement were expressed in relative levels, adjust to untreated control cells, of luciferase adjusted for β -galactosidase levels. Transfected SVG cells were pretreated with lovastatin (lova, 10 μ M) for 24 hours and then cultured in the presence of LPS (10 ng/ml). ***, $p < 0.001$. Pretreatment with lovastatin was able to abrogate luciferase activity in SVG cells stimulated with LPS (10 ng/ml). Lovastatin did not affect cell viability. Data are representative of at least three independent experiments performed three times with similar results and expressed as relative luciferase units and the SEM of triplicate samples.