8-Oxoguanine DNA Glycosylase-1 Augments Proinflammatory Gene Expression by Facilitating the Recruitment of Site-Specific Transcription Factors

Xueqing Ba,*,† Attila Bacsi,*,[1] Jixian Luo,* Leopoldo Aguilera-Aguirre,* Xianlu Zeng,† Zsolt Radak,*,[2] Allan R. Brasier,‡,x and Istvan Boldogh*,‡

*Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, Galveston, TX 77555; †Key Laboratory of Molecular Epigenetics, Institute of Genetics and Cytology, Northeast Normal University, Changchun 130024, China; ‡Sealy Center for Molecular Medicine, University of Texas Medical Branch at Galveston, Galveston, TX 77555; and xDepartment of Internal Medicine, University of Texas Medical Branch at Galveston, Galveston, TX 77555

[1]Current address: Institute of Immunology, Medical and Health Science Center, University of Debrecen, Debrecen, Hungary.

[2]Current address: Research Institute of Sport Science, Faculty of Physical Education and Sport Science, Semmelweis University, Budapest, Hungary.

Address correspondence and reprint requests to Prof. Istvan Boldogh, Department of Microbiology and Immunology, University of Texas Medical Branch at Galveston, 301 University Boulevard, Galveston, TX 77555-1070. E-mail address: sboldogh@utmb.edu

This work was supported by National Institute on Environmental Health Sciences Grant R01 ES018948 (to I.B.), National Institute of Allergy and Infectious Diseases Grant AI062885 (to A.R.B. and I.B.), National Heart, Lung, and Blood Institute Proteomic Center Grant N01HV00245 (to I.B.; Dr. A. Kurosky, Director), National Institute on Environmental Health Sciences Grant P30 ES006676, International Science-Technology Collaboration Foundation Grant 20120728 of Jilin Province in China (to X.B.), and by the European Union and European Social Fund Grant TAMOP 4.2.2.A-11/1/KONV-2012-2023 (to A.B.). A.B. is a Janos Bolyai Fellow, which is supported by the Hungarian Academy of Sciences. L.A.-A. is an Environ- mental Toxicology Research Training Fellow, which is supported by National Institute on Environmental Health Sciences Grant T32 ES007254.

Abbreviations used in this article: AO, antioxidant; AP, apurinic/apyrimidinic; BALF, bronchoalveolar lavage fluid; BER, base excision repair; ChIP, chromatin immunoprecipitation assay; Gro, growth-regulated protein; IIR, innate immune response; Luc, luciferase; MEF, mouse embryo fibroblast; NAC, N-acetyl-L-cysteine; OGG1, 8-oxoguanine DNA glycosylase-1; 8-oxoG, 8-oxo-7,8-dihydroguanine; Pol, polymerase; RNAi, RNA interference; rOGG1, recombinant 8-oxoguanine DNA glycosylase- 1; ROS, reactive oxygen species; siRNA, small interfering RNA; Sp1, specificity protein 1; SR, superrepressor; TFIID, transcription initiation factor II-D.

Abstract

Among the insidious DNA base lesions, 8-oxo-7,8-dihydroguanine (8-oxoG) is one of the most abundant, a lesion that arises through the attack by reactive oxygen species on guanine, especially when located in cis-regulatory elements. 8-oxoG is repaired by the 8-oxoguanine glycosylase 1 (OGG1)–initiated DNA base excision repair pathway. In this study, we investigated whether 8-oxoG repair by OGG1 in promoter regions is compatible with a prompt gene expression and a host innate immune response. For this purpose, we used a mouse model of airway inflammation, supplemented with cell cultures, chromatin immunoprecipitation, small interfering RNA knockdown, real-time PCR, and comet and reporter transcription assays. Our data show that exposure of cells to TNF-α altered cellular redox, increased the 8-oxoG level in DNA, recruited OGG1 to promoter sequences, and transiently inhibited base excision repair of 8-oxoG. Promoter-associated OGG1 then enhanced NF-kB/RelA binding to cis-elements and facilitated recruitment of specificity protein 1, transcription initiation factor II-D, and p-RNA polymerase II, resulting in the rapid expression of chemokines/cytokines and inflammatory cell accumulation in mouse airways. Small interfering RNA depletion of OGG1 or prevention of guanine oxidation significantly decreased TNF-α–induced inflammatory responses. Taken together, these results show that nonproductive binding of OGG1 to 8-oxoG in promoter sequences could be an epigenetic mechanism to modulate gene expression for a prompt innate immune response.

The genomes of living organisms are continuously exposed to reactive oxygen species (ROS) derived from the normal metabolism, as well as from pathophysiological processes caused by exposure to physical, chemical, or infectious agents. ROS inflict oxidative damage on various macromolecules, in- cluding proteins, lipids, and nucleic acids (both DNA and RNA) (1). Repair of DNA lesions is crucial for maintaining genomic in- tegrity, whereas other oxidatively damaged macromolecules un- dergo degradation (2). One of the most abundant oxidized DNA lesions is 8-oxo-7,8-dihydroguanine (8-oxoG), as guanine has the lowest oxidation potential among the four bases in DNA (3). The repair of 8-oxoG is important because it can mispair with adenine, which results in a G:C to T:A transversion during replication, leading to mutations (4). In mammalian cells the enzyme cat- alyzing the excision of the 8-oxoG base from the DNA duplex is 8-oxoguanine DNA glycosylase-1 (OGG1; human and mouse) (5, 6), which is a functional analog of the Escherichia coli for- mamidopyrimidine–DNA glycosylase protein (5).

The loss or functional deficiency of OGG1 has been linked to mutagenesis and carcinogenesis and is thought to contribute to the aging process and age-associated diseases (7, 8). Intriguingly, the supraphysiological levels of 8-oxoG in Ogg1$^{-/-}$ mice do not impair embryonic development, and the mice have a normal life span and show no marked pathological changes or tumor induction (9–11). Moreover, Ogg1$^{-/-}$ mice are highly resistant to inflam- mation induced by ROS, LPS, or allergens. These mice express lower levels of proinflammatory chemokines/cytokines (e.g., MIP-1α, TNF-α, IL-4, IL-6, IL-10, IL-12, and IL-17), and thus the homing of inflammatory cells to the site of injury is decreased (12,13). Our previous work showed that decreased OGG1 expression and 8-oxoG

repair in the airway epithelium resulted in a lower allergic inflammatory response after ragweed pollen challenge of sensitized mice, as shown by decreased expression of Th2 cyto- kines, eosinophilia, and airway hyperresponsiveness (14). These results imply that 8-oxoG base and/or OGG1 could play a key role in proinflammatory gene expression and inflammatory processes. It is well documented that transcriptional regulation of proin- flammatory genes is highly regulated by ROS-mediated signaling. Previous studies have addressed the role of ROS in the posttrans- lational modifications of transcription factors (e.g., NF-kB, AP-1) (15–17); however, the possibility that oxidative damage to cis-elements may affect the binding of sequence-specific transcription factors and assembly of the transcription machinery has attracted less attention. Intriguingly, vertebrate genome evolutionarily has a high GC content in the promoter regions of RNA polymerase (Pol) II–transcribed genes, despite guanine's vulnerability to be oxidized to the mutagenic 8-oxoG and subjected to DNA repair. For instance, a genomic-wide survey revealed that 72% of promoters belong to a class with high CpG content (18). Also, the consensus binding sites for many transcription factors (e.g., specificity protein 1 [Sp1] and NF-kB) are guanine-rich (17, 19). We thus speculate that the high GC content in promoter regions is an advantage for tran- scriptional regulation due to nonproductive binding of OGG1 to 8-oxoG under conditions of oxidative stress.

In the present study we show that TNF-α –induced ROS increased 8-oxoG levels in the genome, including promoter region(s), and also binding of OGG1 to the CXC motif chemokine ligand 2 promoter (mouse and human). Bound OGG1 enhanced Cxcl2 ex-pression via facilitating the recruitment of transcription initiation factor II-D (TFIID), NF-kB/RelA, Sp1, and p-RNA Pol II. OGG1 depletion decreased transcription factor binding

to the promoter and TNF-α–mediated innate immune responses (IIRs) in a mouse model of airway inflammation. These data strongly suggest a role of OGG1 in transcriptional activation of proinflammatory genes in response to oxidative stress.

Materials and Methods

Reagents and Abs

TNF-α was purchased from PeproTech (Rocky Hill, NJ); N-acetyl-L-cysteine (NAC) Tris, NaCl, EDTA, EGTA, Nonidet P-40, sodium pyrophos- phate, glycerophosphate, Na_3VO_4, NaF, aprotin/leupeptin/PMSF, $NaDodSO_4$, and paraformaldehyde were from Sigma-Aldrich (St Louis, MO). Abs to RelA, Sp1, TFIID, GAPDH, and control IgG were from Santa Cruz Bio- technology (Santa Cruz, CA), and Ab to p-RNA Pol II was from Covance (Princeton, NJ). Anti-OGG1 and anti-Flag Abs were from Epitomics (Bur- lingame, CA) and Sigma-Aldrich, respectively.

Animals, challenge, and evaluation of inflammation

Six- to 8-wk-old OGG1-proficient or -deficient female BALB/c mice (~20 g; Harlan Sprague Dawley, San Diego, CA) were TNF-α challenged intra- nasally with or without antioxidant (AO) pretreatment. OGG1 depletion was performed as described below, and AO treatment was achieved by i.p. injection of NAC (320 mg/kg) plus ascorbic acid (400 mg/kg) 1 h before TNF-α challenge. RNA from the upper one third of lungs was extracted as described previously (20). Expression of inflammation-related cytokines and chemokines was analyzed by plate-based quantitative PCR arrays (SABiosciences, Valencia, CA) using pooled cDNA from each group as template (n = 5). To evaluate inflammation, bronchoalveolar lavage fluids (BALF) were collected 16 h after challenge, processed, cytospin slides were stained with Wright–Giemsa, and the number of neutrophils was counted as we previously described (21). All experiments were performed according to the National Institutes of Health Guidelines for the Care and Use of Laboratory Animals.

The protocol used was approved by the University of Texas Medical Branch Animal Care and Use Committee (no. 0807044A).

Cell cultures

HEK 293 cells were maintained in DMEM high-glucose medium, and MLE- 12 (an immortalized type 2 mouse lung epithelial cell line) was cultured in RPMI 1640 medium, per the instructions of the American Type Culture Collection. $Ogg1^{-/-}$ and $Ogg1^{+/+}$ mouse embryo fibroblasts (MEFs) (10) were provided by Dr. Deborah E. Barnes (Imperial Cancer Research Fund, Clare Hall Laboratories, South Mimms, U.K.) and cultured in DMEM/Ham's F-12 medium (22). All media were supplemented with 10% FBS (Atlanta Biologicals, Lawrenceville, NJ), penicillin (100 U/ml; Life Technologies- BRL, Gaithesburg, MD), and streptomycin (100 μg/ml; Life Technologies- BRL). Cells were regularly tested for mycoplasma contamination.

Depletion of OGG1 expression

To deplete OGG1 from mouse lungs, Stealth RNA interference (RNAi; (Invitrogen Life Technologies, Carlsbad, CA, catalog no. MSS237431) was used. Under mild anesthesia, parallel groups of mice were treated with Stealth RNAi to Ogg1 (or control RNAi) intranasally, and depletion of Ogg1 mRNA in airway epithelial cells was determined by real-time PCR (20) using primers purchased from Integrated DNA Technologies (Newark NJ, catalog no. Mm.PT.56a.30885470). Small interfering RNA (siRNA) transfection of cultured cells was performed using the N-TER nanoparticle transfection system (Sigma-Aldrich) per the manufacturer's instructions. siRNA to downregulate mouse Ogg1

(catalog no. M-048121-01-005) and human OGG1 (catalog no. M-005147-03-0005) were purchased from Dharmacon (Pittsburg, PA). Depletion of the target proteins was determined by Western blotting.

RNA extraction and real-time PCR analysis

RNA was extracted from mouse lungs, HEK 293 cells, and MLE-12 cells using an RNeasy kit (Qiagen, Valencia, CA) per the manufacturer's in- structions. Total RNA (1 μg) was reverse-transcribed using a SuperScript III first-strand synthesis system (Invitrogen Life Technologies). Inflammation- related cytokine and chemokine quantitative PCR arrays were analyzed as suggested by the manufacturer (SABiosciences). mRNA levels of individual genes in cultured cells were determined using primers purchased from Integrated DNA Technologies: mouse Cxcl2, forward, 5'-CTCCTTTCCAGGTCAGTTAGC-3', reverse, 5'-CAGAAGTCATAGCCACTCTCAA-3'; mouse Gapdh, forward, 5'-CTCATGACCACAGTCCATGC-3', reverse, 5'-CACATTGGGGGTAGGAACAC-3'; human CXCL2, forward, 5'-CACACTCAAGAATGGGCAGA-3', reverse, 5'-CTTCAGGAACAGCCACCAAT-3'; human GAPDH, forward, 5'-CTGGAGAAACCTGCCAAGTA-3', reverse, 5'-TGTTGCTGTAGCCGTATTCA-3'. Real-time-PCR was performed in an ABI 7000 thermal cycler. Relative expression levels of target genes were calculated by the DDCt method as we described pre- viously (20).

Reporter and expression constructs

The mouse Cxcl2 promoter (–571 to +81) was cloned from the MLE-12 genome and inserted into reporter vector pGL4.2 (Promega, Madison, WI) using restriction enzyme

sites KpnI and BglII to generate the construct Cxcl2-luciferase (Luc). The proximal Sp1 binding site (−109 to −100) deletion mutation was designated as Cxcl2-Sp1-del-Luc. The inserted DNA was confirmed by sequencing. The plasmid pRL-SV40 encoding Renilla luciferase driven by an SV40 promoter (Promega) was used as an internal control. The expression plasmid (pcDNA3) encoding IkBa superrepressor (IkBa-SR; resistant to phosphorylation by IKK), a mutant form of IkBa (S32A/S36A) was used to block NF-kB shuttling from the cytoplasm to nucleus. The cDNA of IkBa-SR (~1000 bp) was cloned into pcDNA3 at the BamHI restriction site. The pCMV-N-Flag-OGG1 expression plasmid encoding N-terminal Flag-tagged human OGG1 was constructed as described in our previous report (23).

Transfection and dual reporter luciferase assay

Transfection was performed using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instruction. Cells were seeded in 24-well plates and incubated in growth medium without antibiotics overnight, then transfected with Cxcl2-Luc (400 ng/well) and pRL-SV40–encoding Renilla luciferase (40 ng/well). After 4 h, the medium was replaced with complete medium. Twelve hours later, the transfected cells were stimulated with TNF-α for various time intervals (0, 1, 3, 6 h) with or without the ROS scavenger NAC, and then lysed. Firefly and Renilla luciferase activities were measured using the dual luciferase assay system (Promega) with a GloMax microplate luminometer (Promega). The transcriptional activity of Cxcl2 was represented by the firefly luciferase activity normalized to that of Renilla.

Coimmunoprecipitation and Western blotting

HEK 293 cells (1×10^7) were transfected with a Flag-OGG1 expression plasmid, then stimulated with TNF-α as described above and lysed in lysis buffer (50 mmol/l Tris [pH 7.5], 150 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 1% Nonidet P-40, 2.5 mmol/l sodium pyrophosphate, 1 mmol/l glycerophosphate, 1 mmol/l Na_3VO_4, 1 mmol/l NaF, and 20 μg/ml aprotin/leupeptin/PMSF). Cell lysates were centrifuged at 4˚C, 13,000 x g for 30 min and the supernatants were incubated with 30 μl protein G– Sepharose (Millipore, Billerica, MA) at 4˚C for 1 h. The precleared supernatants were incubated with Ab against Flag for 12 h and then protein G–Sepharose for 3 h with continuous rotation. The immunoprecipitates were then washed with lysis buffer and resolved by SDS–PAGE. After the proteins were transferred to nitrocellulose membranes, the membranes were washed with TBST (20 mmol/l Tris base, 500 mmol/l NaCl, 0.05% Tween 20 [pH 7.5]) and blocked with 10% nonfat dry milk and then incubated for 1 h with primary Abs at 1:1000 dilution and subsequently with HRP-conjugated secondary Abs at a 1:4000 dilution (SouthernBiotech, Birmingham, AL). The signals were detected using the ECL Plus chemi- luminescent detection system (GE Life Sciences, Bucking Hampshire, U.K.).

Chromatin immunoprecipitation assay

Chromatin immunoprecipitation (ChIP) assays were performed as described previously (24) with slight modifications. Briefly, Flag-OGG1–transfected HEK 293 cells or MEFs (Ogg1-/- and Ogg1+/+) were stimulated with TNF-α for 30 min. The cells were harvested and the ChIP assays were performed using Abs against Flag or NF-kB/RelA.

ChIP reagents were used according to the recommended protocol from Millipore. Cells (1×10^6) were crosslinked with 1% paraformaldehyde and sheared with 10-s pulses using a GEX 130 ultrasonic processor (Cole-Parmer, Vernon Hills, IL) equipped with 2-mm tip and set to 30% of maximum power. One milliliter of the 10-fold–diluted reaction mixture was incubated with or without Abs and then immunoprecipitated with protein A– or G–agarose (Millipore) blocked with salmon sperm DNA. Before adding Abs (Flag, NF-kB/RelA) and agarose beads, one tenth of the dilution was directly subjected to DNA extraction and used as input. The precipitates were washed extensively with washing buffers, de-crosslinked, and subjected to regular or real-time PCR. Primers for amplification were: human CXCL2 promoter, forward, 5'-ATTCGGGGCAGAAAGAGAAC-3', reverse, 5'-ACCCCTTTTATGCATGGTTG-3'; mouse Cxcl2 promoter, forward, 5'-GAAGGGCAGGGCAGTAGAAT-3', reverse, 5'-TGAAGTGTGGCTGGAGTCTG-3'. For regular PCR analyses, 35 cycles were applied to amplification from ChIP products.

Assessment of intracellular ROS levels

Amplex UltraRed (10-acetyl-3,7-dihydroxyphenoxazine; Invitrogen) reagent specifically reacts with H_2O_2 in the presence of HRP to generate a stable product, resorufin. Amplex red assays were carried out as we previously described (20) with minor modifications. Briefly, after TNF-α addition (0, 5, 10, 15, 25, 30, 60 min) equal numbers of cells were washed with PBS (pH 7.4), harvested, and sonicated (3 x 30 s) in reaction buffer containing the Amplex UltraRed. Mixtures were incubated for 5 min, cell debris was removed (1 min at 13,500 x g), and changes in resorufin fluorescence were determined at 560 and 620 nm (excitation and emission, respectively) by using a BioTek FLx800

fluorometer. To establish the standard curve, increasing concentrations of H_2O_2 (0–1000 nM) were used. Resorufin formation in cell extracts was inhibited by addition of catalase (5 U/ml; Sigma-Aldrich).

Assessment of protein cysteine oxidation

To determine whether OGG1 undergoes redox changes, DCP-Bio1 reagent (KeraFAST, Boston, MA) was used. Briefly, cells were transfected with FLAG-OGG1 plasmid, and then exposed to TNF-α for 0, 15, 30, 60, and 90 min. Cells were lysed in lysis buffer (50 mmol/l Tris [pH 7.5], 150 mmol/l NaCl, 1 mmol/l EDTA, 1 mmol/l EGTA, 1% Nonidet P-40, 2.5 mmol/l sodium pyrophosphate, 1 mmol/l glycerophosphate, 1 mmol/l Na_3VO_4, 1 mmol/l NaF, and 20 μg/ml aprotin/leupeptin/PMSF) containing 100 μM DCP-Bio1 (25). Cell extracts were clarified and the supernatants were incubated with 30 μl protein G–Sepharose (Millipore) at 4˚C for 1 h. The precleared supernatants were then incubated with Ab against Flag for 12 h and added to protein G–Sepharose for 3 h with continuous rotation. The immunoprecipitates were washed with lysis buffer and resolved by SDS-PAGE. Proteins were transferred to nitrocellulose membranes, blocked with 5% nonfat dry milk in TBST for 1 h, and then incubated with streptavidin-conjugated HRP for 1 h. OGG1 reacted with DCP-Bio1 was detected by ECL. Band intensities were quantitated by densitometry using ImageJ (version 1.44) software (National Institutes of Health), and percentage changes in levels of oxidative-modified OGG1 was calculated using Microsoft Excel.

Protein–protein interaction assays

Physical interaction between OGG1 and transacting factors was analyzed as we described previously (26). Briefly, Ni-NTA-agarose beads (Qiagen) were incubated with His-OGG1 protein (6 pmol) (Cytoskeleton) in interaction buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, 0.05% Tween 20 [pH 7.5]). After 60 min incubation at 4˚C, His-OGG1–bound beads were washed three times, and equimolar, nontagged NF-kB/RelA (6 pmol; human RelA, Abnova, catalog no. H00005970-P02) was added. To elucidate the interaction of OGG1 with Sp1, His-tagged Sp1 (ProteinOne) was used. His-Sp1 (6 pmol; catalog no. P1034) was added to Ni-NTA for 60 min and washed, and then nontagged OGG1 (6 pmol; Cytoskeleton) was added in interaction buffer. After incubation for 60 min at 4˚C, samples were washed, proteins were eluted with Laemmli buffer (0.125 M Tris- HCl, 4% SDS, 20% glycerol, 10% 2-ME [pH 6.8]) and then subjected to SDS-PAGE and analyzed by Western blotting.

Assessment of 8-oxoG level

8-oxoG in genomic DNA was assessed by determining the levels of OGG1- sensitive sites using an OGG1 FLARE comet assay (Travigen, Gaithersburg, MD) (14, 27). Briefly, MLE-12 cells with or without TNF-α challenge were embedded in agarose and lysed, detergent was removed by washing, and recombinant OGG1 (rOGG1) protein was added in the digestion buffer and then the DNA was subjected to alkaline electrophoresis. Fifty cells were evaluated for each data point, using the comet assay IV version 4.2 system (Perceptive Instruments, Suffolk, U.K.).

To determine whether TNF-α challenge generates 8-oxoG in the Cxcl2 promoter region, MLE-12 cells were challenged with TNF-α for various lengths of time (0, 15, 30,

60, and 90 min), and genomic DNA was extracted (QIAamp DNA kit; Qiagen). Genomic DNA (20 ng) was subjected to real-time PCR to amplify the proximal region of the Cxcl2 promoter, using the primers used in ChIP assays (as described above).

To examine the 8-oxoG levels in the proximal region of the Cxcl2 pro- moter, 2 μg genomic DNA from each sample was incubated with or without rOGG1 (Novus Biologicals) in a 100 μl reaction (50 mmol/l Tris-HCl [pH 7.4], 150 mmol/l NaCl, 1 mmol/l EDTA, 2 mmol/l DTT, 1 mg/ml BSA, and 5 ng rOGG1) at 37˚C for 20 min. After digestion, DNA was subjected to phenol/chloroform extraction, and 20 ng DNA from each sample was used as a template to amplify the proximal region of the Cxcl2 promoter as described above. The base excision activity of rOGG1 was determined using a 40-mer oligonucleotide containing an 8-oxoG at position 19 and labeled at the 3' end with Cy5 (5'-AGAGAAGAAGAAGAAGAA/8oxodG/AGATGGGTTATTCGAACTAGC /3Cy5Sp/-3), as we previously described (28).

Statistical analysis

Statistical analysis was performed using a Student t test to analyze changes at the mRNA and protein levels. Data from mouse treatment groups were analyzed by ANOVA, followed by Bonferroni post hoc analyses for least significant difference. The data are presented as the means ± SEM. Differences were considered to be statistically significant at $p < 0.05$.

Results

OGG1 depletion decreased TNF-α–induced inflammation in mouse lungs

To address the role of OGG1 in the regulation of proinflammatory genes, mice expressing OGG1 (proficient) or depleted in OGG1 (deficient) were challenged intranasally with TNF-α. One hour after the challenge, the lungs were excised, RNAs extracted, and cDNAs synthesized and subjected to analysis by real-time PCR arrays for cytokine and chemokine expression. TNF-α challenge upregulated the expression of inflammatory mediators, including CXCLs, CCLs, ILs, and TNF-α. For instance, *Cxcl1* mRNA was increased 75.9-fold and that for *Tnfa* 23-fold (Fig. 1A). Unexpectedly, OGG1 depletion from the airway epithelium significantly decreased TNF-α –induced proinflammatory gene expression. For example, the extent of Cxcl1 and Tnfa mRNA induction decreased to 23.6- (by ~70%) and 2.84-fold (by ~90%), respectively, compared with that of OGG1-expressing mice (Fig. 1B). Ogg1 mRNA expression in airway epithelial cells was decreased by 85 ± 6.3% (data not shown) after application of Ogg1 Stealth RNAi, similar to what we have shown previously (14). In selected experiments mice were treated with AOs (NAC plus ascorbic acid, as described in Materials and Methods). Pretreatment with AOs significantly decreased the expression of proinflammatory mediators after TNF-α challenge (Fig. 1C), consistent with previous observations (16, 29). AOs alone had no effect on the expression of inflammatory medi- ators (data not shown).

The chemokines CXCL1 and CXCL2 (homologs of human growth-regulated protein [Gro] α and β, respectively) are potent attractants of neutrophils, so we further examined their mRNA levels by real time RT-PCR using individual primer pairs. The results not only confirmed our PCR array data (Fig. 1A), but they showed even higher

increases in mRNA levels for CXCL1 (281 ± 73-fold, Fig. 1D, left panel) and CXCL2 (535 ± 73-fold; Fig. 1D, right panel) in TNF-α –challenged lungs. As expected from the robust increase in Cxcl1 and Cxcl2 expression, TNF-α challenge induced extensive neutrophilia (>8 x 10^5 neutrophils/ml BALF) in the lungs. Intriguingly, OGG1 deficiency significantly decreased Cxcl1 (by ~74%, Fig. 1D, left panel) and Cxcl2 mRNA levels (by ~80%, Fig. 1D, right panel), in line with the number of neutrophils in the BALF (Fig. 1E). Pretreatment of mice with AOs decreased both Cxcl1 and Cxcl2 mRNA levels (Fig. 1D) and the number of recruited neutrophils in the BALF (Fig. 1E). These results suggested that IIR mediated by TNF-α are ROS- and/or OGG1-dependent.

OGG1 augments TNF-α –induced mRNA expression of CXCL2

To explore the molecular mechanism by which OGG1, a DNA repair enzyme, modulates the expression of proinflammatory genes we used a mouse airway epithelial cell (MLE-12) model as in our previous studies (16, 29), with Cxcl2 as a representative proinflammatory cytokine gene. Addition of TNF-α (20 ng/ml) to the medium resulted in a rapid increase in the level of Cxcl2 mRNA, which reached a maximum (>500 ± 44-fold) at 30 min and markedly decreased by 90 min (Fig. 2A). A similar time course of CXCL2 (Grob) gene expression was observed in HEK 293 cells in response to TNF-α treatment (Fig. 2B). Transfection of MLE-12 and HEK 293 cells with siRNA to Ogg1 (or OGG1) lowered the increase in Cxcl2 and CXCL2 mRNA levels by ~85 and ~70%, respectively (Fig. 2C, 2D), which was proportional to the OGG1 protein levels in siRNA-treated cells (Fig. 2E). Moreover, to con- firm OGG1-dependent expression of Cxcl2, we used Ogg1-/- and Ogg1+/+ MEFs. Intriguingly, the increase in Cxcl2 mRNA levels after TNF-α

exposure was ~8-fold higher in Ogg1+/+ MEFs than in Ogg1+/+ MEFs (Fig. 2F). The ROS scavenger NAC significantly decreased the TNF-α –induced increase in Cxcl2 mRNA (Fig. 2G, 2H) as well as intracellular ROS levels (Fig. 2I, 2J) levels in both cell types after 30 min of exposure, suggesting an essential role of ROS in regulating Cxcl2 and GROb gene expression. Our previous work also showed an ROS-dependent increase in the proinflammatory cytokines IL-1β and IL-8 at 60 min after TNF-α challenge (16). The combined results strongly suggest that ROS generated via TNF-α and OGG1 expression have crucial roles in upregulation of the proinflammatory gene Cxcl2.

OGG1 is implicated in transcriptional activation from the CXCL2 promoter

To determine whether OGG1 benefits the expression of CXCL2 via transcriptional activation of the CXCL2 promoter, we constructed a reporter plasmid by inserting the mouse Cxcl2 promoter (–571 to +81) into the vector pGL4.2. Immediately adjacent to the TATA box there are two NF-kB and one Sp1 binding site (Fig. 3A). The proximal Sp1 binding site deletion mutant was constructed by deleting the consensus nucleotides (–109 to –100). Time course studies in which we used the luciferase activity assay revealed an increase in activation of the Cxcl2 promoter from 1 h on, and >10-fold after 6 h of TNF-α treatment (Fig. 3B). No significant increase in luciferase activity was detected in HEK 293 cells trans- fected with empty vector plasmid pGL4.2 at any time after the addition of TNF-α (data not shown).

Overexpression of IkB-SR, an NF-kB repressor, decreased Cxcl2 transcription at 6 h after challenge nearly to the basal level, implying that NF-kB is an essential factor to enhance the transcriptional activation of Cxcl2 (Fig. 3C), in line with our previous

observations (16). We also examined whether the proximal Sp1 consensus sequence is involved in the activation of the Cxcl2 promoter. A dual reporter assay of Cxcl2 promoter deleted in its Sp-1 binding site revealed that activation of the mutant Cxcl2 promoter reached only ~50% of that of the wild-type promoter in response to TNF-α exposure (Fig. 3D). These results imply the involvement of Sp1 in the transcriptional activation of Cxcl2. To address whether OGG1 is implicated in activation of the Cxcl2 promoter, we downregulated OGG1 expression in HEK 293 cells before transfection with reporter plasmids. In OGG1-depleted cells TNF-α induced significantly less luciferase activity than in control siRNA-transfected HEK 293 cells (Fig. 3E). Treatment with NAC before TNF-α addition decreased Cxcl2 promoter activation by ~50% (Fig. 3F), indicating the involvement of ROS. These results are in line with those showing a decrease in Cxcl2 mRNA levels in NAC-treated cells (Fig. 2G, 2H). Taken together, these results imply that in response to TNF-α –induced ROS, OGG1 plays a role in the transcriptional activation of *Cxcl2* driven by NF-kB.

OGG1 binds to CXCL2 promoter and facilitates the recruitment of NF-kB/RelA

To explore whether OGG1 facilitates *Cxcl2* transcription based on its binding to the promoter, HEK 293 cells were transfected with a Flag-OGG1 expression plasmid and ChIP assays were performed. A 30-min treatment with TNF-α was sufficient to induce an impressive increase in the CXCL2 mRNA level; therefore, ChIP assays were carried out at 30 min after challenge. The results summarized in Fig. 4A show the amplification of the 282-bp fragment (which is the proximal region of the CXCL2 promoter, containing Sp1 and NF-kB binding sequences) in the OGG1-associated chromatin precipitate from TNF-α

–treated (but not untreated) cells. As a positive control, Ab to NF-kB/RelA also pulled down these promoter sequences from cells treated with TNF-α, whereas the negative controls (without incubation with Flag or NF-kB/RelA Ab) did not show detectable amplification.

To investigate whether OGG1 expression affects the binding of NF-kB/RelA to the CXCL2 promoter region, we used OGG1- depleted HEK 293 cells as well as Ogg1-/- and Ogg1+/+ MEF cells. ChIP assays were performed using an NF-kB/RelA Ab. siRNA silencing of OGG1 expression decreased the level of NF-kB/RelA associated with the CXCL2 promoter in the ChIP product by ~70% compared with that in control siRNA-transfected HEK 293 cells (Fig. 4B). Additionally, TNF-α treatment induced a <2-fold increase in NF-kB/RelA–bound Cxcl2 promoter in Ogg1-/- MEF cells, whereas there was a nearly 5-fold increase in NF-kB/RelA pulldown DNA in Ogg1+/+ MEF cells (Fig. 4C). Taken together, these data suggest that OGG1 interacts with the promoter and facilitates the subsequent recruitment of NF-kB/ RelA.

Binding of OGG1 to 8-oxoG in the Cxcl2 promoter in TNF-α –treated cells is nonproductive

In the human and mouse CXCL2 promoters, Sp1 (GGGGCGGGGC) and NF-kB (GGGAATTTCC) binding sites are guanine-rich, which predicts the generation of 8-oxoG upon ROS due to TNF-α exposure (Fig. 2I). 8-oxoG is repaired during OGG1–base excision repair (BER) (30), and it is conventionally thought that its efficient removal from DNA benefits sequence fidelity of promoter and transcription. To investigate this possibility, we first performed FLARE comet assays as described in Materials and

Methods. As expected, the 8-oxoG levels in TNF-α–treated MLE-12 cells were significantly higher than those in untreated cells (Fig. 5A). Fig. 5B shows representative comet moment images of DNA with/without rOGG1 (its excision activity is shown in Fig. 5C) digestion from cells exposed to TNF-α for 30 min.

To examine whether 8-oxoG accumulates in the Cxcl2 promoter region, we extracted genomic DNA from TNF-α –treated MLE-12 cells at various time points (0, 15, 30, 60, and 90 min). A 240-bp fragment of the Cxcl2 promoter that contains Sp1 and NF-kB binding sites was amplified by real-time PCR. Unexpectedly, the amplifiable amount of Cxcl2 promoter was increased after 30 and 60 min of TNF-α treatment (Fig. 5D) despite the oxidative stress (Fig. 2I). To test whether 8-oxoG is present in the Cxcl2 promoter, we used rOGG1 protein to release 8-oxoG from genomic DNA. We observed that OGG1 digestion significantly decreased the amplifiable amount of Cxcl2 promoter sequences (assayed after a 15-, 30-, and 60-min exposure to TNF-α; Fig. 5E) when compared to the amount of the Cxcl2 promoter fragment from TNF-α untreated cells. Interestingly, after 90 min of TNF-α exposure the amplifiable amount of Cxcl2 promoter sequence was similar to that at time 0 (Fig. 5E). NAC treatment before TNF-α addition decreased 8-oxoG levels in the DNA and promoter region (data not shown). These results imply that 8-oxoG is formed in the proximal region of the Cxcl2 promoter, which was not removed by OGG1 in response to TNF-α treatment. To obtain insight into modification of OGG1, which could account for its decreased repair activity, we used the DCP-Bio1 reagent (see Materials and Methods) that specifically reacts with oxidized cysteine (sulfenic acid) residues (25). Results in Fig. 5F show that OGG1 oxida- tively modified at cysteine residues was the highest at 15 min and then gradually decreased at 30 and 60 min

after TNF-α addition. Quantitation of band densities showed that DCP-Bio1–reacting OGG1 represent 14.8 ± 4.27% of total OGG1 at 15 min. Its level decreased to 11.2 ± 1.9 and 9.7 ± 3.22% at 30 and 60 min, respectively, after TNF addition (Fig. 5F, right panel). Cysteine modification has been shown to transiently decrease the repair activity of OGG1 (31, 32), which may explain the observed increase in 8-oxoG level and binding of OGG1 to the proximal region of the Cxcl2 promoter (Figs. 4A, 5D). These data, combined with the ChIP results, suggested that in TNF-α –treated cells, binding of OGG1 to the promoter is nonproductive in terms of its 8-oxoG excision activity, as such activity would be expected to decrease the amount of fragment amplified, rather than increasing it.

OGG1 interacts with transcription factors in TNF-α–treated cells

The results from the above studies showed that OGG1 is non-productively bound to 8-oxoG in the Cxcl2 promoter in response to TNF-α treatment (Fig. 5A), raising the possibility that it interacts with proteins in the transcription complex on the Cxcl2 promoter. To test this hypothesis, HEK 293 cells were transfected with Flag-OGG1 expression plasmid, followed by treatment with TNF-α, and coimmunoprecipitation assays were performed. The results showed that a 15-min TNF-α treatment resulted in increased interactions of OGG1 with Sp1, TFIID, and p-RNA Pol II. Interestingly, an increased interaction between OGG1 and NF-kB/RelA was observed from 30 min on, and it lasted up to 60 min after TNF-α addition (Fig. 6A). Given that a 15-min TNF-α challenge was not sufficient to increase Cxcl2 mRNA levels (Fig. 3A, 3B), these results imply that NF-kB/RelA is indeed a limiting factor for the expression of CXCL2, as shown previously (16, 29). At 90 min after the addition of TNF-α, all these transcription factors were markedly decreased in the OGG1-

associated complex and coin- cided with a low Cxcl2 mRNA level (Fig. 3A, 3B). As shown in Fig. 6B, NAC pretreatment significantly lowered the interaction of OGG1 with NF-kB/RelA and p-RNA Pol II.

Next, we explored a possible physical interaction between OGG1 and NF-kB/RelA and Sp1. To do so, we carried out protein–protein interactions assays as described in Materials and Methods. Results summarized in Fig. 6C and 6D show that OGG1 binds both NF-kB/RelA and Sp1. Quantitation of eluted OGG1 and comparison with input NF-kB indicated a nearly equimolar binding of NF-kB/RelA to OGG1 and OGG1 binding to Sp1, suggesting that these transacting factors are directly interacting with OGG1 protein. From these results and those showing cysteine oxidation in OGG1 (Fig. 5F), one may propose that oxidatively modified OGG1 binds to promoter sequences and interacts with transacting factors in TNF-α–exposed cells. To test this hypothesis we examined whether NF-kB/RelA and/or Sp1 interacts with cysteine sulfenic acid–containing OGG1. To do so, Flag-OGG1–expressing cells were exposed to TNF-α, lysed in the presence of DCP-Bio1, and coimmunoprecipitation assays were performed using NF-kB/RelA or Sp1 Abs. As shown on Fig. 6E and 6F, OGG1 was present both in NF-kB/RelA and Sp1 immune complex. More importantly, OGG1 was modified at cysteine (Fig. 6E, 6F) suggesting that oxidative modification is required for its physical interactions with transacting factors in TNF-α –exposed cells.

Discussion

ROS generated by biological, chemical, and physical agents, as well as ligand–receptor interactions or combinations thereof, in- duce cell signaling and modify proteins, lipids, and nucleic acids. 8-oxoG is the most common oxidative lesion in DNA because guanine has the lowest ionization potential among the DNA bases (1, 4). 8-oxoG preferentially accumulates in the guanine-rich ge- nomic regions, including those in the enhancer/promoter regions of genes. It is expected that 8-oxoG is repaired to avoid mutations and to maintain the sequence fidelity of promoters for efficient binding of sequence-specific transcription factors. In this study, we made unexpected discoveries suggesting that an association of OGG1 with proximal promoter regions and its interaction with Sp1, NF-kB, TFIID, and p-RNA Pol II play a fundamental role in TNF-α –induced expression of proinflammatory chemo- kines.

These results are highly unexpected, as OGG1 is thought to be a canonical DNA BER enzyme for the removal of 8-oxoG (and Fapy G) owing to its DNA glycosylase–apurinic/apyrimidinic (AP) lyase activity. The repair activities initiated by OGG1 are complex and are modulated by posttranslational modifications, including phosphorylation (33) and acetylation (23), as well as by its inter- actions with other repair and nonrepair proteins (34). Recent reports also showed that redox changes affecting cysteines significantly decrease the activity of OGG1 (31, 32). Binding of OGG1 to 8-oxoG in promoter regions plays a role in the enhancement of transcription from target genes has never been described.

We have previously documented that TNF-α increases intra- cellular ROS and genomic 8-oxoG levels, with a peak increase at 15 min (16, 35). We also showed that

TNF-α –induced phosphorylation of NF-kB/RelA at Ser^{276} (p–NF-kB/RelA) and its nuclear translocation are tightly associated with ROS signaling, and that inhibition of TNF-α–induced ROS blocks the activities of kinases and decreases NF-kB/p-RelA levels (16, 35). Importantly, we showed that p–NF-kB/RelA stable enhanceosome formation with p300 and p-RNA Pol II and binding to the IL-8 promoter are ROS-dependent, and that the chemically unrelated AOs dimethyl sulfoxide, NAC, and/or vitamin C decreased TNF-α –induced IL-8 expression (16, 35, 36).

In the present study we document that TNF-α induced a robust oxidative stress-dependent expression of CXCL and CCL proinflammatory mediators and neutrophilia in mouse lungs, which was inhibited by AOs (NAC plus ascorbic acid). Intriguingly, the TNF-α challenge-induced IIR was nearly prevented by OGG1 depletion in the lung epithelium. Likewise, AOs or siRNA to Ogg1 mRNA significantly decreased proinflammatory gene expression in cul- tured human and mouse cells.

TNF-α has been shown to signal via distinct cell surface receptors TNFR1 and TNFR2 (37, 38). For example, upon binding to TNFR1, the liganded receptor aggregates to serve as a scaffold to sequentially recruit death domain–containing adaptor proteins, TNFR1-associated death domain, TNFR1-associated death domain– associated factor 2, and the receptor-interacting protein (39). The activated complex in turn recruits kinases, including MAPKs and IkB kinase, and induces ROS for posttranslational modification and activation of transcription factors. Therefore, the effects of Ogg1 depletion on the TNF-α –induced IIR are extremely intrigu- ing, whereas the effect of AOs may be explained by a lack of ROS signaling.

Taking into consideration the similar inhibitory impacts of ROS scavengers shown

in the present study and in previous studies (16, 35), the effect of OGG1 depletion on the IIR and the lack of known interactions of OGG1 with TNF-α signaling intermediates, we speculate that the observed phenomena could be associated with oxidative damage to guanines and lack of OGG1 BER of 8-oxoG in the promoter regions. It is documented that a large number of promoters driving the transcription of redox-sensitive genes have cis-regulatory elements containing runs of guanines (e.g., NF-kB and Sp1 binding sites) (17, 19). Thus, it is reasonable to expect that oxidative damage to guanines and BER of 8-oxoG will hamper the binding of transcription factors to their DNA con- sensus sequences.

Our results from real-time PCR analysis of the Cxcl2 promoter showed that 8-oxoG level had increased transiently, suggesting that BER of 8-oxoG did not initiate immediately after TNF-α exposure. These data imply that 8-oxoG in the promoter region appears not to be an obstacle to the binding of transcription fac- tors. Indeed, substitution of the 5ϑ guanine in guanine runs for 8-oxoG has no significant effect on Sp1 binding in vitro (40). Another study showed that a 5ϑ 8-oxoG in a guanine run actually increased NF-kB/p50 binding to its consensus DNA sequence, wheras the substitution of later guanines for 8-oxoG decreased p50 binding (41). Binding of Sp1, NF-kB, and others to 8-oxoG– containing promoters could have significance, because under ox- idative stress conditions in a double helix, containing a p-stacked array of heterocyclic bp is favorable for the migration of charge over molecular distance, which results in the selective oxidation of 5’ guanine in runs of guanines (42, 43). Alternatively, our data strongly suggest that a TNF-α–induced ROS burst leads to oxidation of guanine and OGG1 as well as a nonproductive binding of OGG1 to its substrate. Consequently, this complex is beneficial for the binding of transcription factors as well as mRNA

synthesis. Indeed, OGG1 depletion significantly decreased TNF-α–induced gene expression.

Our ChIP analysis showed that OGG1 binds to the Cxcl2 pro- moter, and it appears that its presence is essential for the re- cruitment of transcription factors. To prove this point, we show that amplification by real-time PCR from a 240-bp (MLE-12 cells) and a 282-bp (HEK 293 cells) fragment of the Cxcl2 promoter was increased in OGG1-expressing cells after TNF-α addition, compared with amplification of those from untreated cells. Our data also showed that TNF-α–induced oxidative stress increases 8-oxoG levels in the promoter, as digestion of extracted DNA with rOGG1 before real-time PCR significantly decreased amplification from the Cxcl2 promoter. These results imply that after TNF-α addition, OGG1 still binds 8-oxoG in the Cxcl2 promoter, but its excision activity is inhibited. The mechanism by which BER activity of OGG1 is inhibited is not known, but we speculate that TNF-α –induced cellular redox changes may indeed hamper OGG1's 8-oxoG glycosylase/AP lyase activity. In support of this possibility, elegant studies showed that oxidation of cysteine residues, of which eight are present in both human and mouse OGG1, could be responsible for its decreased 8-oxoG excision activity (31, 32). Cysteine sulfenic acid is a well-established oxidative stress-induced modifier of enzymatic activities and disulfide bond formation, and it is key in protein folding (44). To obtain insight into oxidative modification of OGG1 at cysteines in TNF-α–exposed cells we used DCP-Bio1 reagent that specifically reacts with cysteine sulfenic acid (25). We observed that a significant portion (~15%) of OGG1 contained cysteine sulfenic acid, suggesting that OGG1 bound to 8-oxoG in G-rich promoter sequences is inactivated via cysteine oxidation. The direct interaction of OGG1 with trans-acting factors supports our

coimmunoprecipitation data and our hypothesis of the role of OGG1 in transcriptional initiation of genes. It may thus be suggested that OGG1 nonproductively binds 8- oxoG in the promoter sequence under oxidizing conditions such as those induced by TNF-α, and it promotes transcriptional activation of proinflammatory genes. The reestablishment of normal cellular redox status (by ~90 min, as shown in the present study and in our previous studies; Ref. 16) coincided with a sharp decrease in Cxcl2 mRNA levels after TNF-α challenge. Therefore, we speculate that OGG1's glycosylase/AP lyase activity is reestablished at this time, and DNA repair intermediates (e.g., AP sites) formed during the BER of 8-oxoG in the promoter could be associated with the loss of (defects in) cis-elements and dislocation of transcription factor and thus termination of transcription.

Our data also show that OGG1 depletion decreased proinflam- matory mediator expression in cultured cells and the IIR in mice after TNF-α challenge. Moreover, TNF-α –induced binding of NF-kB/RelA to the Cxcl2 promoter was significantly lower in Ogg1-/- than in Ogg1+/+ cells, and Sp1, TFIID, and p-RNA Pol II were present in the OGG1 immunoprecipitation complex. We also show that OGG1 depletion decreased TNF-α –induced binding of NF-kB/RelA to the Cxcl2 promoter. These results are consistent with the finding that we have made by using in vitro binding assays. We found a direct and equimolar interaction between OGG1 and NF-kB as well as Sp1, which is in line with presence of OGG1 in the immunoprecipitation complex. We furthered these observations by showing that NF-kB and Sp1 interact with a glycosylase-inactive cysteine-modified OGG1. The significance of these intriguing ob- servations is yet to be revealed, and identification of the OGG1 amino acid residues that interact with Sp1 and NF-kB/RelA will be the focus of future investigations.

Finally, our study identified OGG1-dependent expression of Cxcl2 (the homolog of human GROb), which is the most highly expressed among an array of chemokines and cytokines. A proposed role of OGG1 in transcriptional initiation of proinflammatory mediators is shown in Fig. 7. Expression of CXCL2 may be in- duced by oxidative stress, by chemical and physical agents, by viral, bacterial, and fungal infections, or by ligand–receptor interactions, and it is chemotactic for polymorphonuclear leukocytes and he- matopoietic stem cells. Also, increased expression of CXCL2 is characteristic of senescent cells and aged tissues, and it has been associated with chronic inflammatory states such as obesity, aging processes, and age-associated diseases, including diabetes (types 1 and 2), Alzheimer disease, and others. Moreover, enhanced IL-8 expression has been shown to promote cancer progression, and it is a poor prognostic indicator in therapy of a variety of human malignancies. All inflammatory processes in acute and chronic diseases are associated with an increased oxidative state and formation of 8-oxoG in DNA, especially in guanine-rich promoters. Although it needs to be proven, we propose that continuous formation of 8-oxoG and OGG1 binding to promoter sequences could stimulate chronic inflammatory processes and could be an epigenetic mechanism to modulate gene expression in response to oxidative stress. In support of this hypothesis, mice lacking OGG1 activity show increased resistance to inflammation induced by oxidative stress, LPS endotoxins, and various allergenic proteins (12, 13). Given the rate-limiting role of OGG1 in the expression of CXC chemokines and TNF-α itself, it could be proposed that modulation of OGG1 activity might be a therapeutic target in the resolution of inflammatory processes that impair organ integrity and promote aging processes, as increased OGG1 activity would be expected to decrease the expression of proinflammatory genes and recruitment of

inflammatory cells.

Acknowledgments

We thank Drs. Sankar Mitra (University of Texas Medical Branch) and Miral Dizdaroglu (Chemical Science and Technology Laboratory, National Institute of Standards and Technology, Gaithersburg, MD) for scientific advice. Dr. David Konkel's (Department of Biochemistry and Molecular Biology, University of Texas Medical Branch) scientific input and critical editing of the manuscript are greatly appreciated.

Disclosures

The authors have no financial conflicts of interest.

References

1. Radak, Z., and I. Boldogh. 2010. 8-Oxo-7,8-dihydroguanine: links to gene expression, aging, and defense against oxidative stress. Free Radic. Biol. Med. 49: 587–596.
2. Beal, M. F. 2002. Oxidatively modified proteins in aging and disease. Free Radic. Biol. Med. 32: 797–803.
3. Steenken, S., and S. V. Jovanovic. 1997. How easily oxidizable is DNA? One-electron reduction potentials of adenosine and guanosine radicals in aqueous solution. J. Am. Chem. Soc. 119: 617–618.
4. Dizdaroglu, M. 1985. Formation of an 8-hydroxyguanine moiety in deoxyribonucleic acid on gamma-irradiation in aqueous solution. Biochemistry 24: 4476–4481.
5. Nishimura, S. 2002. Involvement of mammalian OGG1(MMH) in excision of the 8-hydroxyguanine residue in DNA. Free Radic. Biol. Med. 32: 813–821.
6. Bjorâs, M., L. Luna, B. Johnsen, E. Hoff, T. Haug, T. Rognes, and E. Seeberg. 1997. Opposite base-dependent reactions of a human base excision repair en- zyme on DNA containing 7,8-dihydro-8-oxoguanine and abasic sites. EMBO J. 16: 6314–6322.
7. David, S. S., V. L. O'Shea, and S. Kundu. 2007. Base-excision repair of oxidative DNA damage. Nature 447: 941–950.
8. Ames, B. N., M. K. Shigenaga, and T. M. Hagen. 1993. Oxidants, antioxidants, and the degenerative diseases of aging. Proc. Natl. Acad. Sci. USA 90: 7915–7922.

9. Minowa, O., T. Arai, M. Hirano, Y. Monden, S. Nakai, M. Fukuda, M. Itoh, H. Takano, Y. Hippou, H. Aburatani, et al. 2000. Mmh/Ogg1 gene inactivation results in accumulation of 8-hydroxyguanine in mice. Proc. Natl. Acad. Sci. USA 97: 4156–4161.

10. Klungland, A., I. Rosewell, S. Hollenbach, E. Larsen, G. Daly, B. Epe, E. Seeberg, T. Lindahl, and D. E. Barnes. 1999. Accumulation of premutagenic DNA lesions in mice defective in removal of oxidative base damage. Proc. Natl. Acad. Sci. USA 96: 13300–13305.

11. Arai, T., V. P. Kelly, O. Minowa, T. Noda, and S. Nishimura. 2006. The study using wild-type and Ogg1 knockout mice exposed to potassium bromate shows no tumor induction despite an extensive accumulation of 8-hydroxyguanine in kidney DNA. Toxicology 221: 179–186.

12. Li, G., K. Yuan, C. Yan, J. Fox, III, M. Gaid, W. Breitwieser, A. K. Bansal, H. Zeng, H. Gao, and M. Wu. 2012. 8-Oxoguanine-DNA glycosylase 1 defi- ciency modifies allergic airway inflammation by regulating STAT6 and IL-4 in cells and in mice. Free Radic. Biol. Med. 52: 392–401.

13. Mabley, J. G., P. Pacher, A. Deb, R. Wallace, R. H. Elder, and C. Szabó . 2005. Potential role for 8-oxoguanine DNA glycosylase in regulating inflammation. FASEB J. 19: 290–292.

14. Bacsi, A., L. Aguilera-Aguirre, B. Szczesny, Z. Radak, T. K. Hazra, S. Sur, X. Ba, and I. Boldogh. 2013. Down-regulation of 8-oxoguanine DNA glyco- sylase 1 expression in the airway epithelium ameliorates allergic lung inflam- mation. DNA Repair (Amst.) 12: 18–26.

15. Brasier, A. R. 2006. The NF-kB regulatory network. Cardiovasc. Toxicol. 6: 111–130.

16. Jamaluddin, M., S. Wang, I. Boldogh, B. Tian, and A. R. Brasier. 2007. TNF-α-induced NF-kB/RelA Ser^{276} phosphorylation and enhanceosome formation is mediated by an ROS-dependent PKAc pathway. Cell. Signal. 19: 1419–1433.

17. Sen, C. K., and L. Packer. 1996. Antioxidant and redox regulation of gene transcription. FASEB J. 10: 709–720.

18. Saxonov, S., P. Berg, and D. L. Brutlag. 2006. A genome-wide analysis of CpG dinucleotides in the human genome distinguishes two distinct classes of pro- moters. Proc. Natl. Acad. Sci. USA 103: 1412–1417.

19. Kadonaga, J. T., K. R. Carner, F. R. Masiarz, and R. Tjian. 1987. Isolation of cDNA encoding transcription factor Sp1 and functional analysis of the DNA binding domain. Cell 51: 1079–1090.

20. Aguilera-Aguirre, L., A. Bacsi, A. Saavedra-Molina, A. Kurosky, S. Sur, and I. Boldogh. 2009. Mitochondrial dysfunction increases allergic airway inflam- mation. J. Immunol. 183: 5379–5387.

21. Boldogh, I., A. Bacsi, B. K. Choudhury, N. Dharajiya, R. Alam, T. K. Hazra, S. Mitra, R. M. Goldblum, and S. Sur. 2005. ROS generated by pollen NADPH oxidase provide a signal that augments antigen-induced allergic airway inflam- mation. J. Clin. Invest. 115: 2169–2179.

22. Bacsi, A., G. Chodaczek, T. K. Hazra, D. Konkel, and I. Boldogh. 2007. In-creased ROS generation in subsets of OGG1 knockout fibroblast cells. Mech. Ageing Dev. 128: 637–649.

23. Bhakat, K. K., S. K. Mokkapati, I. Boldogh, T. K. Hazra, and S. Mitra. 2006. Acetylation of human 8-oxoguanine-DNA glycosylase by p300 and its role in 8-oxoguanine repair in vivo. Mol. Cell. Biol. 26: 1654–1665.

24. Tian, B., J. Yang, and A. R. Brasier. 2012. Two-step cross-linking for analysis of protein-chromatin interactions. Methods Mol. Biol. 809: 105–120.

25. Kaplan, N., N. Urao, E. Furuta, S. J. Kim, M. Razvi, Y. Nakamura, R. D. McKinney, L. B. Poole, T. Fukai, and M. Ushio-Fukai. 2011. Localized cysteine sulfenic acid formation by vascular endothelial growth factor: role in endothelial cell migration and angiogenesis. Free Radic. Res. 45: 1124–1135.

26. Boldogh, I., G. Hajas, L. Aguilera-Aguirre, M. L. Hegde, Z. Radak, A. Bacsi, S. Sur, T. K. Hazra, and S. Mitra. 2012. Activation of ras signaling pathway by 8-oxoguanine DNA glycosylase bound to its excision product, 8-oxoguanine. J. Biol. Chem. 287: 20769–20773.

27. Olive, P. L., D. Wlodek, and J. P. Banáth. 1991. DNA double-strand breaks measured in individual cells subjected to gel electrophoresis. Cancer Res. 51: 4671–4676.

28. German, P., P. Szaniszlo, G. Hajas, Z. Radak, A. Bacsi, T. K. Hazra, M. L. Hegde, X. Ba, and I. Boldogh. 2013. Activation of cellular signaling by 8-oxoguanine DNA glycosylase-1-initiated DNA base excision repair. DNA Repair (Amst.) 12: 856–863.

29. Nowak, D. E., B. Tian, M. Jamaluddin, I. Boldogh, L. A. Vergara, S. Choudhary, and A. R. Brasier. 2008. RelA Ser^{276} phosphorylation is required for activation of a subset of NF-kB-dependent genes by recruiting cyclin-dependent kinase 9/ cyclin T1

complexes. Mol. Cell. Biol. 28: 3623–3638.

30. Mitra, S., T. Izumi, I. Boldogh, K. K. Bhakat, J. W. Hill, and T. K. Hazra. 2002. Choreography of oxidative damage repair in mammalian genomes. Free Radic. Biol. Med. 33: 15–28.

31. Bravard, A., M. Vacher, B. Gouget, A. Coutant, F. H. de Boisferon, S. Marsin, S. Chevillard, and J. P. Radicella. 2006. Redox regulation of human OGG1 activity in response to cellular oxidative stress. Mol. Cell. Biol. 26: 7430–7436.

32. Bravard, A., M. Vacher, E. Moritz, L. Vaslin, J. Hall, B. Epe, and J. P. Radicella. 2009. Oxidation status of human OGG1-S326C polymorphic variant determines cellular DNA repair capacity. Cancer Res. 69: 3642–3649.

33. Dantzer, F., L. Luna, M. Bjørås, and E. Seeberg. 2002. Human OGG1 undergoes serine phosphorylation and associates with the nuclear matrix and mitotic chromatin in vivo. Nucleic Acids Res. 30: 2349–2357.

34. Hegde, M. L., P. M. Hegde, K. S. Rao, and S. Mitra. 2011. Oxidative genome damage and its repair in neurodegenerative diseases: function of transition metals as a double-edged sword. J. Alzheimers Dis. 24(Suppl. 2): 183–198.

35. Vlahopoulos, S., I. Boldogh, A. Casola, and A. R. Brasier. 1999. Nuclear factor-kB-dependent induction of interleukin-8 gene expression by tumor necrosis factor alpha: evidence for an antioxidant sensitive activating pathway distinct from nuclear translocation. Blood 94: 1878–1889.

36. Jamaluddin, M., B. Tian, I. Boldogh, R. P. Garofalo, and A. R. Brasier. 2009. Respiratory syncytial virus infection induces a reactive oxygen species-MSK1-phospho-Ser-276 RelA pathway required for cytokine expression. J. Virol. 83:

10605–10615.

37. Smith, C. A., T. Farrah, and R. G. Goodwin. 1994. The TNF receptor superfamily of cellular and viral proteins: activation, costimulation, and death. Cell 76: 959–962.

38. Chen, G., and D. V. Goeddel. 2002. TNF-R1 signaling: a beautiful pathway. Science 296: 1634–1635.

39. Hsu, H., H. B. Shu, M. G. Pan, and D. V. Goeddel. 1996. TRADD-TRAF2 and TRADD-FADD interactions define two distinct TNF receptor 1 signal trans- duction pathways. Cell 84: 299–308.

40. Ramon, O., S. Sauvaigo, D. Gasparutto, P. Faure, A. Favier, and J. Cadet. 1999. Effects of 8-oxo-7,8-dihydro-2ϑ-deoxyguanosine on the binding of the tran- scription factor Sp1 to its cognate target DNA sequence (GC box). Free Radic. Res. 31: 217–229.

41. Hailer-Morrison, M. K., J. M. Kotler, B. D. Martin, and K. D. Sugden. 2003. Oxidized guanine lesions as modulators of gene transcription. Altered p50 binding affinity and repair shielding by 7,8-dihydro-8-oxo-2ϑ-deoxyguanosine lesions in the NF-kB promoter element. Biochemistry 42: 9761–9770.

42. Saito, I., M. Takayama, T. Nakamura, H. Sugiyama, Y. Komeda, and M. Iwasaki. 1995. The most electron-donating sites in duplex DNA: guanine-guanine stacking rule. Nucleic Acids Symp. Ser. 1995: 191–192.

43. Hall, D. B., R. E. Holmlin, and J. K. Barton. 1996. Oxidative DNA damage through long-range electron transfer. Nature 382: 731–735.

44. D'Autréaux, B., and M. B. Toledano. 2007. ROS as signalling molecules:

mechanisms that generate specificity in ROS homeostasis. Nat. Rev. Mol. Cell Biol. 8: 813–824.

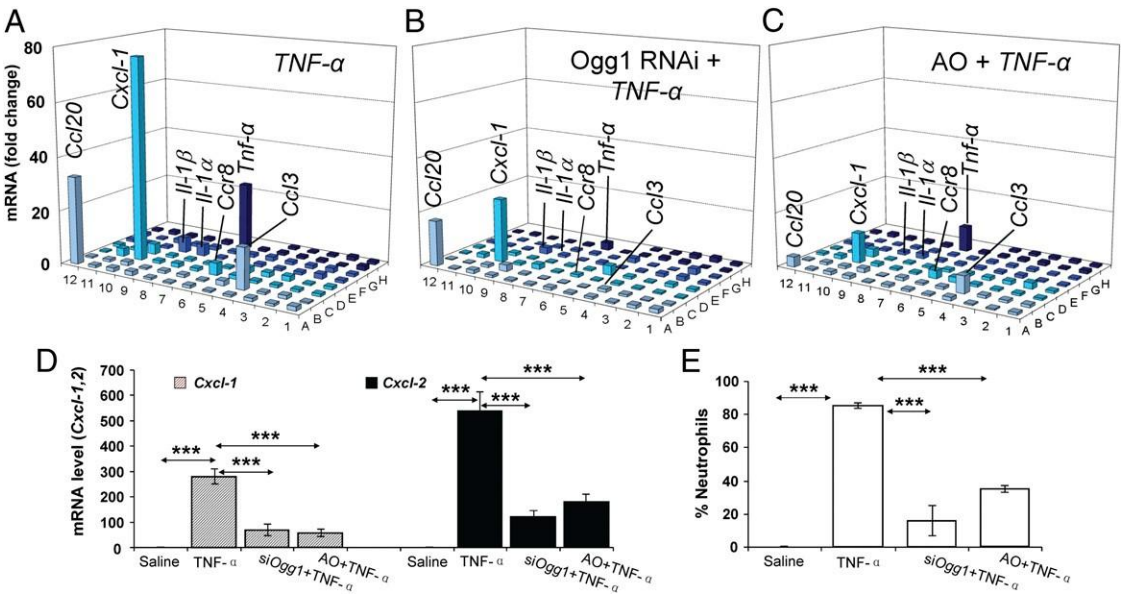

FIGURE 1. Effect of OGG1 on expression of cytokines and chemokines induced by TNF-α . (A) TNF-α –induced expression of mRNAs for proin- flammatory mediators. (B) OGG1 deficiency decreases TNF-α –induced expression of proinflammatory chemokine and cytokine mRNAs. (C) Effect of AO pretreatment on TNF-α –induced expression of proinflammatory mediators. In (A)–(C), groups of mice proficient or deficient in expressing OGG1 in the airway epithelium were challenged intranasally with TNF-α . One hour later, lungs were excised and RNA was extracted. Pooled cDNA from each group (n = 5) was used as a template to perform plate-based inflammation-related PCR arrays. (D) Changes in Cxcl1 (left panel) and Cxcl2 (right panel) mRNA levels as determined by real-time PCR in individual lung RNA extracts. OGG1 depletion and AO pretreatment are described in Materials and Methods. (E) Effect of OGG1 depletion and AO on the number of neutrophils in BALF of TNF-α –challenged mice. Mice were TNF-α treated and lavaged at 16 h (see Materials and Methods). The percentage of neutrophils was determined as in Materials and Methods (n = 5). *p < 0,05, **p < 0.01, ***p < 0.001.

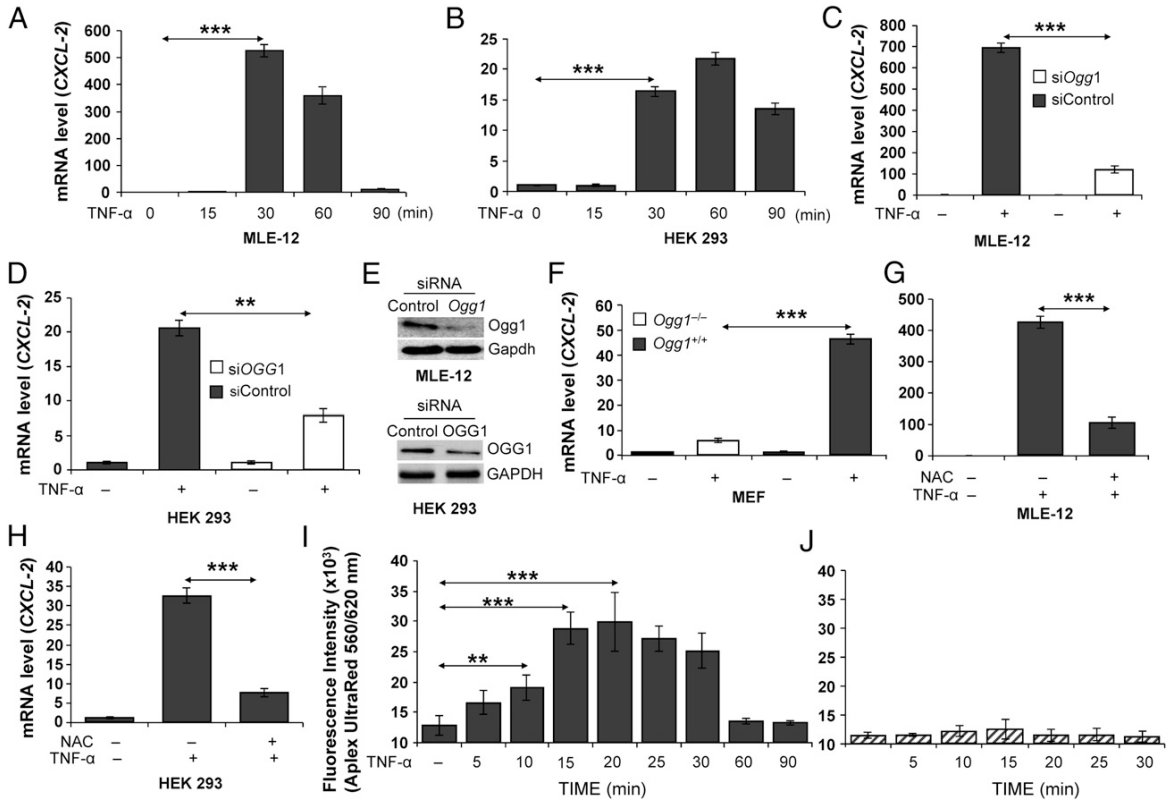

FIGURE 2. TNF-α –induced expression of Cxcl2 mRNA is decreased in OGG1-depleted cells. (A and B) Time course of Cxcl2 mRNA expression upon TNF-α exposure. MLE-12 (A) and HEK 293 (B) cells were TNF-α treated for various lengths of time, RNA was extracted, and real-time PCR was performed. (C and D) OGG1 depletion by siRNA lowers the increase in Cxcl2 mRNA levels upon TNF-α treatment. OGG1-depleted and control cells [MLE-12 cells in (C), HEK 293 cells in (D)] were exposed to TNF-α for 30 min, RNA was extracted, and real-time PCR was performed. (E) OGG1 protein levels after siRNA silencing of Ogg1. MLE-12 (upper panels) and HEK 293 (lower panels) cells were transfected with siRNA to Ogg1, lysed, and Western blot analyses were performed. (F) MEFs lacking OGG1 activity express low levels of Cxcl2 mRNA after TNF-α exposure. Ogg1+/+ and Ogg1-/- MEF cells were treated with TNF-α for 30 min, RNA was extracted, and real-time PCR was performed. (G and H) The AO pretreatment decreases TNF-α – induced expression of the Cxcl2 gene. MLE-12 (G) and HEK 293 (H) cells were challenged with TNF-α for 30 min with or without NAC, RNA was extracted, and real-time PCR was performed. (I) Kinetic changes in cellular ROS levels in TNF-α –exposed cells as determined by Amplex UltraRed assays. (J) NAC pretreatment decreases ROS levels in TNF-α –exposed cells (n = 3-5). **$p < 0.01$, ***$p < 0.001$.

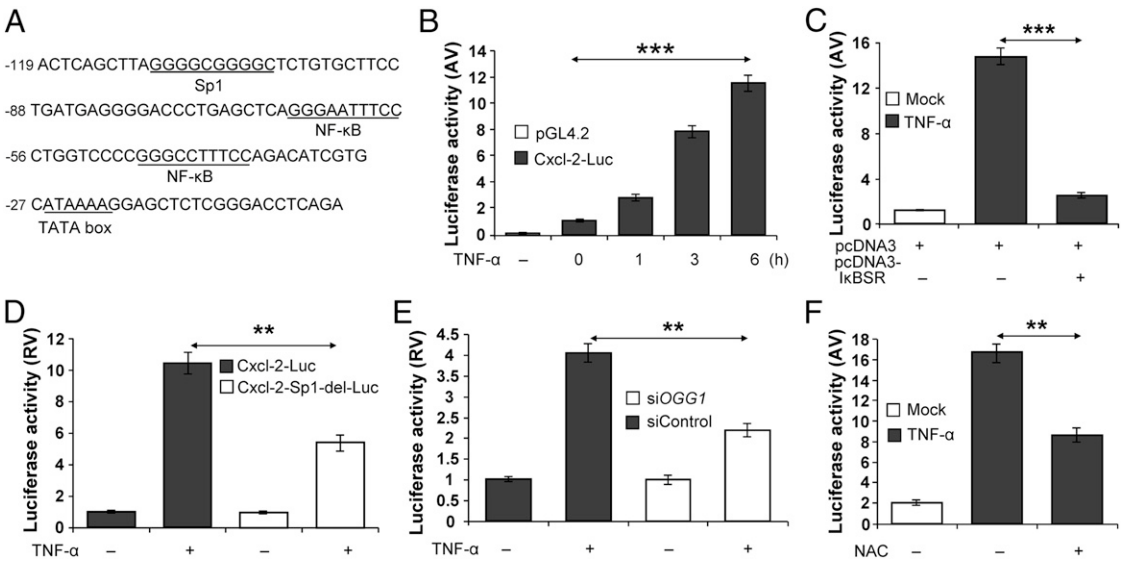

FIGURE 3. OGG1 expression enhances TNF-α –induced activation of the CXCL2 promoter. (A) Diagram of the proximal region of the mouse Cxcl2 promoter (containing the TATA box, two NF-kB binding sites, and one Sp1 binding site). (B) TNF-α exposure activates the Cxcl2 promoter. HEK 293 cells were transfected with a reporter plasmid (Cxcl2-Luc) or control vector (pGL4.2) and then challenged with TNF-α for the time intervals indicated. Dual reporter assays were performed as in Materials and Methods. (C) Activation of the Cxcl2 promoter by TNF-α is inhibited by an NF-kB superrepressor (IkBa-SR). HEK 293 cells were transfected with the reporter plasmid Cxcl2-Luc with or without overexpression of IkBa-SR (pcDNA3-IkBa-SR), then challenged with or without TNF-α for 6 h. (D) Deletion of the Sp1 consensus sequence decreases TNF-α –induced Cxcl2 promoter activation. HEK 293 cells were transfected with report plasmids Cxcl2-Luc or Cxcl2-Sp1-del-Luc and then mock or TNF-α challenged for 6 h. Luciferase activity in cells without TNF-α challenge was taken as 1. (E) OGG1 depletion inhibits TNF-α –induced Cxcl2 promoter activation. HEK 293 cells were transfected with siRNA to OGG1 (or control) and transfected with reporter plasmid. After a 12-h recovery, cells were treated with or without TNF-α for 6 h. Luciferase activity in cells without TNF-α challenge was taken as 1. (F) AO pretreatment decreased TNF-α –induced Cxcl2 promoter activation. HEK 293 cells were transfected with the reporter plasmid Cxcl2-Luc, treated with NAC as in Materials and Methods, and then mock or TNF-α challenged for 6 h. n = 4–6. **p < 0.01, ***p < 0.001. AV, absolute value; RV, relative value.

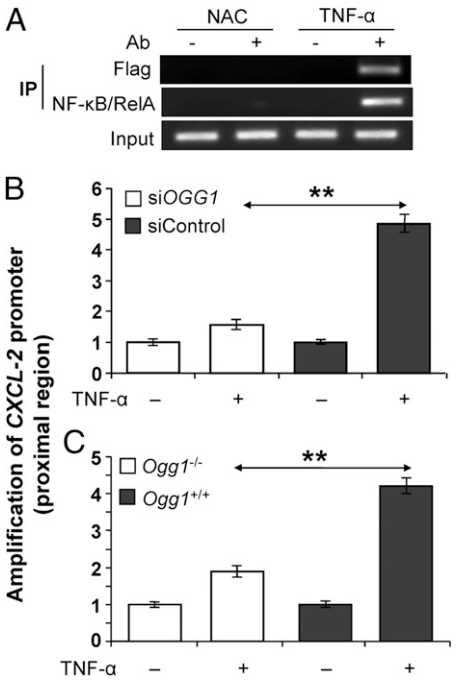

FIGURE 4. OGG1 binds to the CXCL2 promoter and facilitates NF-kB/RelA recruitment. (A) TNF-α increases binding of OGG1 and NF-kB/ RelA to the CXCL2 promoter. HEK 293 cells were transfected with Flag- OGG1 plasmid and then treated with or without TNF-α for 30 min. ChIP assays were performed using Abs against Flag and NF-kB/RelA. The pulled-down CXCL2 promoter was detected by PCR amplification and agarose electrophoresis (amplification from samples without Ab incuba- tion served as negative controls). A representative set of experiment is shown out of three. (B) OGG1 depletion decreases the association of NF- kB/RelA with the CXCL2 promoter. HEK 293 cells were transfected with siRNA (or control siRNA) to OGG1 and then treated with or without TNF-α for 30 min. (C) Lack of OGG1 activity in MEFs hampers the binding of NF-kB/RelA to Cxcl2 promoter sequences. Ogg1-/- and Ogg1+/+ MEFs were treated with or without TNF-α for 30 min. In (B) and (C), ChIP assay was performed using Ab to NF-kB/RelA. Quantitative amplification of the CXCL2 promoter from the ChIP products of different cells was compared by real-time PCR. Amplification from the ChIP products was normalized to that from input genomic DNA, and the value for untreated cells was taken as 1. n = 3–4. **$p < 0.01$.

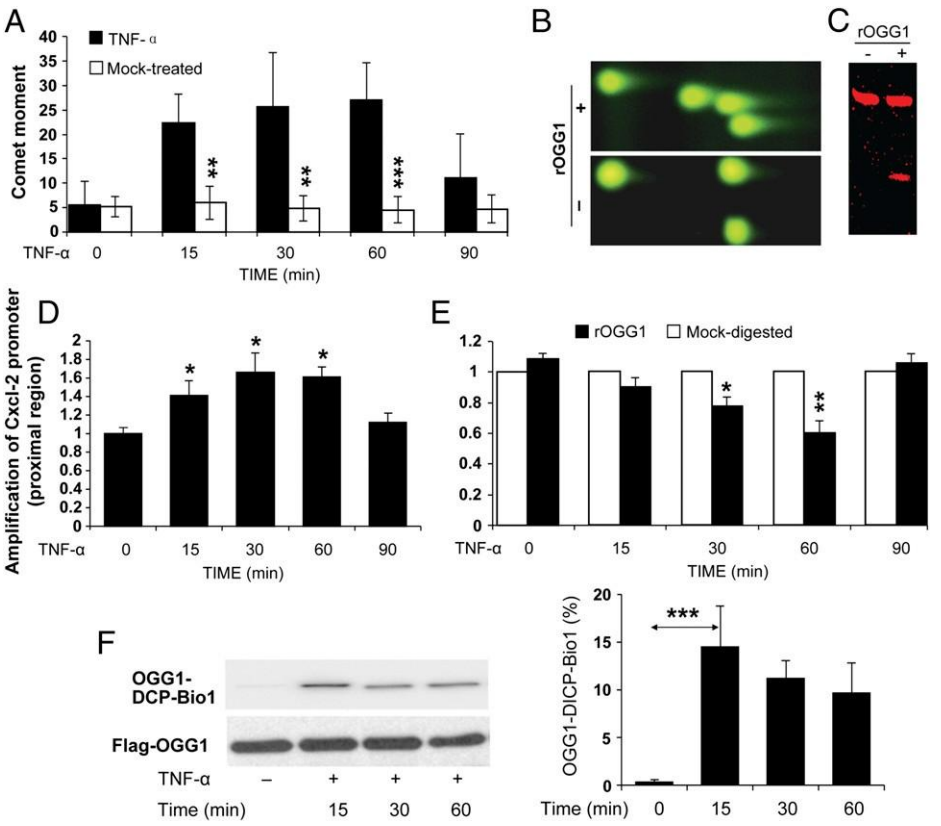

FIGURE 5. TNF-α treatment increases the integrity of the Cxcl2 promoter. (A) Accumulation of 8-oxoG in genomic DNA in TNF-α –treated cells. MLE-12 cells were challenged with or without TNF-α for 0, 15, 30, 60, and 90 min. OGG1 FLARE comet assays were performed as in Materials and Methods. (B) Representative images show comet moments of DNA with or without rOGG1 digestion (after 30 min of TNF-α exposure). (C) 8-oxoG excision activity of rOGG1. The activity of OGG1 was determined using Cy5-labeled 8-oxoG probe (as in Materials and Methods). (D) The amplifiable amount of Cxcl2 promoter is increased in response to TNF-α challenge. MLE-12 cells were exposed to TNF-α for time intervals indicated. Genomic DNA was extracted and real-time PCR was performed to determine amount of proximal 240-bp-long region of Cxcl2 promoter. (E) 8-oxoG accumulates in Cxcl2 promoter. MLE-12 cells were exposed to TNF-α as in (A), the extracted DNA was digested with rOGG1, and then a 240-bp-long region of Cxcl2 promoter was amplified by real-time PCR. (F) Oxidative modifications at cysteines of OGG1 in TNF-α –exposed cells as shown by DCP-Bio1, a sulfenic acid reacting reagent (left upper panel). Right panel shows percentage of oxidatively modified OGG1 at cysteines. n = 3–4. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

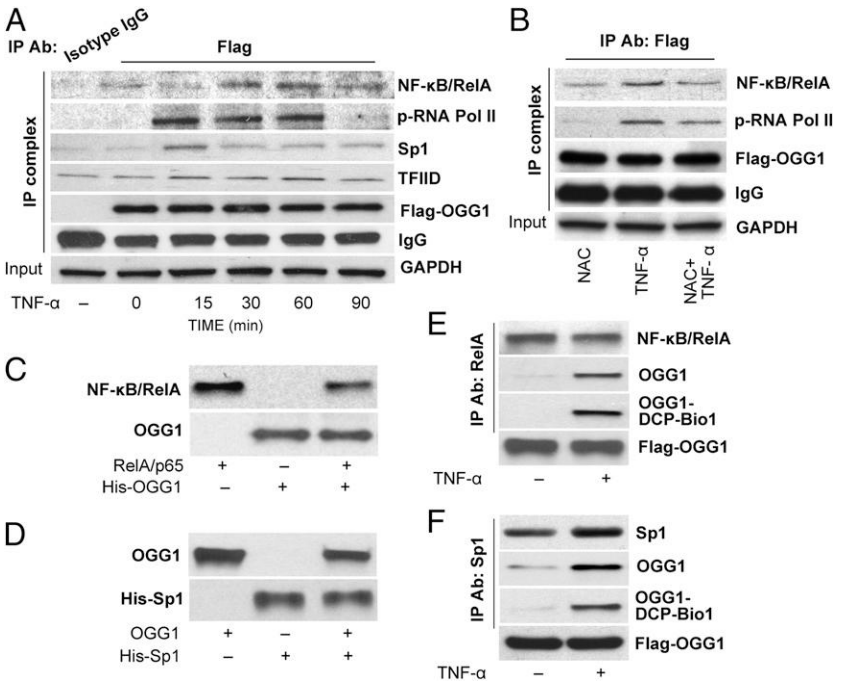

FIGURE 6. OGG1 interacts with general and site-specific transcription factors. (A) Interactions of OGG1 with NF-kB/RelA, p-RNA Pol II, Sp1, and TFIID in response to TNF-α challenge. HEK 293 cells were transfected with a Flag-OGG1–expressing plasmid and 24 h later cells were treated with TNF-α for the indicated time intervals. Coimmunoprecipitation was performed using an Ab against Flag. (B) AO decreased the interaction of OGG1 with NF-kB/ RelA and p-RNA Pol II. HEK 293 cells were transfected with the Flag-OGG1–expressing plasmid and subjected to TNF-α challenge for 30 min with or without NAC pretreatment. Coimmunoprecipitation was performed to analyze the interaction of OGG1 with NF-kB/RelA and p-RNA Pol II. Shown are representative results of three independent experiments. (C) Physical interaction between NF-kB/RelA and OGG1. His-OGG1 was immobilized to NTA- agarose beads, washed, and then incubated with equimolar nontagged RelA in interaction buffer for 30 min (see Materials and Methods). Bound proteins were eluted and analyzed by Western blotting. (D) Protein–protein interaction between OGG1 and Sp1. Assays were carried out as in (C) except His-Sp1 was NTA-agarose immobilized. (E) NF-kB/RelA interacts with oxidatively modified OGG1 at cysteine. Flag-OGG1–expressing cells were TNF-α exposed and lysed in buffer containing DCP-Bio1. Coimmunoprecipitation was performed using Ab to NF-kB/RelA. OGG1 associated with RelA was analyzed for cysteine oxidation as in Materials and Methods. (F) Oxidatively modified OGG1 at cysteines interacts with Sp1. Flag-OGG1–expressing cells were TNF-α exposed and lysed in buffer containing DCP-Bio1. Coimmunoprecipitation was performed using Ab to Sp1. OGG1 associated with Sp1 was analyzed for cysteine oxidation as in Materials and Methods. n = 3–4.

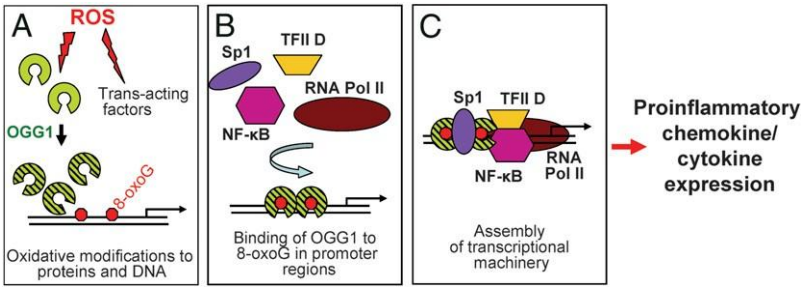

FIGURE 7. A model of OGG1-driven transcriptional initiation of proinflammatory mediators. (A) Oxidative modification to guanine and OGG1 as well as activation of trans-acting factors by ROS. (B) Nonproductive bind- ing of OGG1 to 8-oxoG in promoter region of proin- flammatory genes. (C) Assembly of transcriptional ma- chinery facilitated by OGG1.