Interleukin-4 Enhances PARP-Dependent DNA Repair Activity *In Vitro*

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Eukaryotic cells possess several DNA repair mechanisms, including homologous recombination and the non-homologous end-joining (NHEJ) system. There are two known NHEJ systems. The major mechanism depends on the catalytic unit of DNA-dependent protein kinase (DNA-PK_{cs}) and DNA ligase IV, and an alternative mechanism (B-NHEJ) depends on poly(ADP-ribose) polymerase (PARP). These systems are upregulated by genotoxic agents. Interleukin 4 (IL-4) is an immunoregulatory cytokine that is secreted by immune cells upon contact with certain genotoxic compounds and is known to regulate several genes encoding components of DNA repair systems in human monocytes. We have investigated the possible effects of IL-4 on the DNA repair process within murine and human cells exposed to selected genotoxic compounds. In a series of experiments, including the comet assay, cell surface annexin V staining, analysis of histone H2AX phosphorylation, and a DNA end-joining assay, we observed that IL-4 decreased DNA damage in murine fibroblasts and human glioblastoma cells exposed to genotoxic agents and increased DNA ligation activity in the nuclei of these cells in a process that depended on PARP. These observations suggest that IL-4 is capable of upregulating the alternative NHEJ DNA repair mechanism in murine and human cells.

Introduction

DNA INTEGRITY in eukaryotic cells is maintained by complex DNA repair systems that depend on the products of multiple genes that are typically upregulated by genotoxic agents (Christmann and Kaina 2013). These complex DNA repair mechanisms show certain levels of preference for repairing particular types of damage to DNA molecules. The most deleterious, double-strand breaks, are repaired by two distinct enzymatic pathways: homologous recombination (HR) and the non-homologous end-joining (NHEJ) system (Shrivastav and others 2008). HR requires a homologous template and uses the undamaged sister chromatid in most cases, whereas NHEJ is also able to join non-homologous ends. The most important proteins required for NHEJ include the catalytic subunit of DNA-dependent protein kinase (DNA-PK_{cs}), the Ku70/80 heterodimer, XRCC4, XLF, Artemis, and DNA ligase IV (Lieber 2010). However, DNA ends can also be joined via NHEJ in a Ku/ligase IV-independent manner by the alternative pathway, which is dependent on poly(ADP-ribose) polymerase-1 (PARP-1), XRCC1, and ligase III and may serve as a backup (B-NHEJ) to the DNA-PK-dependent (D-NHEJ) pathway (Wang and others 2003; Audebert and others 2004).

Interleukin 4 (IL-4) is one of the major immunoregulatory cytokines and is mostly expressed by Th2 lymphocytes, basophils, and mast cells (Gessner and others 2005; Brown 2008; van Panhuys and others 2011). This cytokine is known for its pleiotropic activities that extend beyond the immune system and that are mediated by IL-4 receptors present on different types of cells (Nelms and others 1999). An interesting feature of *IL-4* expression patterns is the upregulation of IL-4 mRNA and protein in lymphocytes, basophils, and mast cells exposed to different toxins (Dastyk and others 1999; Stein and others 2000; Devouassoux and others 2002; Walczak-Drzewiecka and others 2003). It has been proposed that *IL-4* induction is part of an organismal defense mechanism against toxic xenobiotics, which may involve certain IL-4-mediated phenotypic changes, such as goblet cell differentiation, mucus secretion, upregulation of collagen expression in fibroblasts, and CYP450 expression in hepatocytes potentially limiting toxic exposure (Postlethwaite and others 1992; Abdel-Razzak and others 1993; Dabbagh and others 1999; Cohn and others 2002). Among the xenobiotics that have been reported to induce *IL-4* expression are known genotoxic compounds, including HgCl₂, phthalates, polyaromatic hydrocarbons, and anticancer drugs such as cisplatin (Dastyk and others

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1999; Kepley and others 2003; Wang and others 2003; Kim and others 2011; Guo and others 2012). Interestingly, phenotypic changes associated with IL-4-mediated differentiation of monocytes into dendritic cells and macrophages involve the upregulation of several genes encoding components of DNA repair systems (Briegert and Kaina 2007; Bauer and others 2011). The observation that IL-4 is upregulated upon contact of the cell with a genotoxic compound in a manner similar to many components of DNA repair mechanisms and can upregulate genes involved in DNA repair prompted us to investigate possible functional consequences of the presence of IL-4 in the microenvironment of cells exposed to genotoxic agents. As will be shown in this report, IL-4 decreased DNA damage in murine fibroblasts and human glioblastoma cells exposed to genotoxic agents and increased DNA ligation activity in the nuclei of these cells in a process that depended on PARP, suggesting upregulation of the alternative NHEJ pathway by this cytokine.

Materials and Methods

Reagents

Mouse recombinant IL-4 (mr IL-4), human recombinant IL-4 (hr IL-4), and Annexin V-FITC were obtained from ImmunoTools GmbH (Friesoythe, Germany). Mercuric chloride was obtained from Ubichem plc (Eastleigh, Hampshire, England). NU7026 was obtained from Calbiochem. NU7026 was dissolved in anhydrous DMSO and stored in aliquots at -20°C . All compounds were added to cells to a final concentration of 0.25% DMSO (v/v); control cells were incubated with 0.25% DMSO alone.

Cell cultures

3T3 Swiss fibroblasts were maintained in DMEM (Gibco/Invitrogen, Ontario, Canada), and glioblastoma cell lines M059K (DNA-PK proficient) and M059J (DNA-PK deficient) were maintained in DMEM/F12 medium (Gibco/Invitrogen). All media were supplemented with 10% (v/v) fetal bovine serum (PAA Laboratories GmbH, Pasching, Austria) and antibiotics (penicillin and streptomycin; Sigma Chemical Co., St. Louis, MO). All cell lines were cultured as a monolayer in a humidified atmosphere with 5% CO_2 and were routinely tested and confirmed to be mycoplasma free.

Comet assay

3T3 Swiss fibroblasts were grown to subconfluency on 6-well plates. Cell cultures were either untreated or treated with 50 ng/mL mr IL-4 for 6 h and subjected to incubation with 25 μM cisplatin or 25 μM mercuric chloride for 18 h. DNA strand breakage and resealing were assessed using a single-cell gel electrophoresis assay under alkaline conditions as described previously (Wojewodzka and others 2002) with modifications. A total of 50–100 cells were analyzed per sample in 7 independent experiments. The extent of DNA damage is expressed as relative olive tail moment calculated using dedicated software, comet assay software project (CASP) (Konca and others 2003).

Annexin V staining

3T3 fibroblasts were grown on 96-well plates until they reached subconfluence. For experiments of PARP-1 activity

inhibition, fibroblasts were pretreated for 1 h with 10 μM PJ-34. Then, cells were incubated with 5 ng/mL mr IL-4 for 6 h followed by treatment with cisplatin (0–25 μM) for 18 h. Half an hour before the end of the treatment, Hoechst-33342 solution in phosphate-buffered saline (PBS; 1 $\mu\text{g}/\text{mL}$ final concentration) was added to the culture medium. Incubation was stopped with cold PBS followed by a wash with annexin V buffer (10 mM HEPES, 140 mM NaCl, 2.5 mM CaCl_2 , pH 7.4). Then, cells were stained with annexin V-FITC solution for 15 min at room temperature in the dark. Stained cells were washed with annexin V buffer, and the 96-well microplate was immediately analyzed using an ArrayScan VTI HCS Reader (Thermo Fisher Scientific, Inc., Waltham, MA). Acquired images (3,000–6,000 cells/well) were analyzed using Multiparameter Cytotoxicity BioApplication V3 software (Cellomics BioApplications; Cellomics, Inc., Pittsburgh, PA) to quantify the loss of cell membrane asymmetry, which was detected as an increase in phosphatidylserine available for annexin V binding.

H2AX phosphorylation analysis

Fibroblasts were seeded on 96-well plates and propagated until they reached subconfluence. Cells were incubated with mr IL-4 at 5, 25, and 50 ng/mL concentrations for 18 h followed by treatment with bleomycin (0–50 ng/mL). Cells were washed with PBS and fixed with 4% formaldehyde for 20 min at RT. Cells were then permeabilized with 0.25% Triton X-100 in PBS for 5 min prior to blocking in 1% bovine serum albumin (BSA) in PBS for 30 min. After the blocking procedure, cells were stained with rabbit antibodies against γ -H2AX (1:1,000 ab2893; Abcam plc, Cambridge, United Kingdom) at 4°C overnight in a humidified chamber. Primary antibody was visualized with goat anti-rabbit antibody conjugated with Texas Red (1:200 sc-2780; Santa Cruz Biotechnology, Santa Cruz, CA) followed by cell nuclei staining with 1 $\mu\text{g}/\text{mL}$ Hoechst-33342. A 96-well microplate containing immunostained cells using an automated optical imaging instrument, the ArrayScan VTI HCS Reader (Thermo Fisher Scientific, Inc., Waltham, MA). Acquired images (500–2,000 cells/well in triplicate) were analyzed using Target Activation BioApplication V3 software (Cellomics BioApplications; Cellomics, Inc.) at single-cell resolution to quantify nuclear γ -H2AX immunofluorescence.

Nuclear cell extract preparation

Cells were seeded on culture flasks at a particular density to maintain the exponential phase of growth throughout the experiment. Prior to treatment, culture medium was exchanged for fresh medium. 3T3 Swiss fibroblasts and glioblastoma cells were either untreated or treated with IL-4 within a concentration range of 5–50 ng/mL for 6 h. After incubation, cells were washed once with ice-cold PBS, harvested with a cell scraper, and centrifuged. Pelleted cells were lysed with 10 packed cell volumes of cold lysis buffer containing 10 mM HEPES (pH 7.9), 10 mM KCl, 0.1 mM EDTA, 0.4% IGEPAL CA-630, and a cocktail of protease inhibitors containing the Roche protease inhibitor mixture and additionally 10 $\mu\text{g}/\text{mL}$ pepstatin, 10 $\mu\text{g}/\text{mL}$ aprotinin, 10 $\mu\text{g}/\text{mL}$ leupeptin, 100 $\mu\text{g}/\text{mL}$ soybean trypsin inhibitor, 1 mM DTT, 0.5 mM PMSF, 27 mM iodoacetamide, 32 mM benzamidinium hydrochloride, and 50 mM ϵ -amino-*n*-caproic

acid. After 15 min of incubation on ice, cell lysates were centrifuged at 3,000 *g* for 20 min, and the resulting pellet of nuclei was resuspended in 2 volumes of hypotonic buffer containing 20 mM HEPES (pH 7.9), 20 mM KCl, 0.2 mM EDTA, 1.5 mM MgCl₂, and a mixture of protease inhibitors as described above. Subsequently, 1 volume of high salt buffer containing 20 mM HEPES (pH 7.9), 1.6 M KCl, 0.2 mM EDTA, 1.5 mM MgCl₂, and protease inhibitor cocktail was slowly added to the nuclei suspension and vigorously shaken for 1 h at 4°C followed by centrifugation at 10,000 *g* for 20 min at 4°C. The resulting supernatant was dialyzed (Dispo-Biodialyzer 2 kDa MWCO) against dialysis buffer containing 20 mM HEPES (pH 7.9), 1 mM EDTA, 100 mM KCl, 0.5 mM PMSF, 1 mM DTT, and 10% glycerol overnight at 4°C. The resulting nuclear extract was aliquoted and stored at -80°C. The protein concentration was determined using the BCA method (Pierce) and BSA as a standard.

DNA end-joining assay

Two double-stranded DNA probes were generated by annealing complementary oligonucleotides to form duplex DNA with one-base 3' overhanging ends. One DNA probe included infrared dye labeled oligonucleotide 5'-IRD700-GCATGC GGGACGTGAGCGACGTGTGGCAG-3' annealed to 5'-TG CCACACGTGCTCACGTCCCGCATGC-3'. Another DNA substrate consisted of 5'-CTGCCACACGTGCTCACGTCC CGCATGC-3' and 5'-CATGCGGGACGTGAGCGACGTGT GGCAG-3'. The DNA end-joining reaction was carried out in a buffer containing 20 mM HEPES KOH (pH 7.5), 10 mM MgCl₂, 80 mM KCl, 10% PEG8000, 20 ng DNA (10 ng of each DNA substrate), and 2–4 µg of nuclear extract. Incubation was carried out at 37°C for 1 h unless otherwise stated. The reaction was stopped by adding 120 µL of denaturing loading buffer containing 80% formamide (v/v) and 10 mM EDTA and incubated for 5 min at 95°C. Subsequently, 10 µL of each denatured sample was loaded into a 10% denaturing polyacrylamide gel (7 M urea, 2 mM formamide) and run in Tris-borate EDTA buffer at 27 V/cm for 45 min. Gels were scanned with an Odyssey infrared scanner (LI-COR Biosciences, Lincoln, NE) followed by densitometry analysis using the Odyssey software. The joining efficiency was calculated as a percentage of DNA substrate forming dimers and other higher polymers.

Immunodepletion of PARP-1 and polymeric adenosine diphosphate-ribosylated proteins

Depletion of PARP-1 and polymeric adenosine diphosphate-ribosylated (PAR) proteins was performed using the PureProteome™ Protein G Magnetic Beads system (Millipore, Darmstadt, Germany) according to the manufacturer's instructions. Briefly, 100 µL of bead suspension was washed twice with PBS. Then, 5 µg of rabbit polyclonal anti PARP-1 (sc-25780; Santa Cruz Biotechnology, Inc.) or mouse monoclonal anti PAR (sc-71848; Santa Cruz Biotechnology, Inc.) in 200 µL of PBS was added to the beads, and the mixture was incubated at room temperature for 1 h under constant rotation. Subsequently, the beads were washed twice with dialysis buffer containing 20 mM HEPES (pH 7.9), 1 mM EDTA, 100 mM KCl, 0.5 mM PMSF, 1 mM DTT, and 10% glycerol and incubated with 25 µg of nuclear

extract in 100 µL of ice-cold dialysis buffer at 4°C for 6 h with constant rotation. Immunodepleted samples were collected and stored at -80°C. Anti-β-actin antibody was used as a control for the depletion procedure.

PAR protein immunoblotting

Fibroblasts and glioblastoma M059K cells were seeded on 6-well plates and grown to 80%–90% confluency. Cells were treated with IL-4 at a concentration of 5 ng/mL for 6 h, harvested using 5 mM EDTA in PBS, and centrifuged at 250 *g* for 5 min at 4°C. The resulting pellets were lysed in 35 µL RIPA buffer containing a cocktail of inhibitors (Roche, Basel, Switzerland) followed by incubation on ice for 30 min and centrifugation at 10,000 *g* for 20 min at 4°C. Supernatant proteins were subjected to sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE, NuPAGE gradient gel 4%–12%; Invitrogen, Carlsbad, CA) followed by transfer to nitrocellulose membrane. Immunoblotting was performed with mouse anti-PAR antibodies (1:200, sc-71848; Santa Cruz Biotechnology, Inc.) and secondary HRP-conjugated goat anti-mouse antibodies (1:1,000; Santa Cruz Biotechnology, Inc.).

Statistics

The results are presented as the mean ± SEM. Statistical significance was evaluated by ANOVA followed by the Holm-Sidak test using SigmaStat 2.03 software (SPSS, Inc., Chicago, IL).

Results

We first investigated the effect of preincubation with IL-4 on DNA damage in murine fibroblasts exposed *in vitro* to cisplatin and HgCl₂. As shown in Figure 1, cisplatin resulted

FIG. 1. Effect of interleukin 4 (IL-4) on DNA damage repair in murine fibroblasts. 3T3 fibroblasts were exposed to cisplatin (25 µM) and HgCl₂ (25 µM) for 18 h followed by 6 h incubation with mouse recombinant IL-4 (mr IL-4) (50 ng/mL) before harvesting for alkaline comet assay analysis. Gels were stained with ethidium bromide, and the extent of DNA damage was determined by calculating the relative tail moment, where the tail moment in control cells was set to 100%. Data are presented as the mean ± SEM from *n* = 7 independent experiments. ** Denotes a statistically significant difference of *P* < 0.01.

FIG. 2. Effect of IL-4 on phosphorylation of H2AX in bleomycin-treated murine fibroblasts. 3T3 fibroblasts were cultured in the indicated concentrations of mr IL-4 for 18 h followed by exposure to increasing concentrations of bleomycin for 4 h and then fixed and processed for γ -H2AX immunofluorescence. Data are presented as the mean $\pm$ SEM from $n=2-6$ independent experiments. * And ** denote statistically significant differences of $P<0.05$ and $P<0.01$, respectively.

in a more than 2-fold increase in DNA strand breaks detected with the comet assay compared with the control, and the addition of 50 ng/mL mr IL-4 prior to cisplatin resulted in a decrease in the number of cisplatin-induced DNA breaks to a level similar to control. A similar trend was observed in cells incubated with IL-4 and HgCl₂, but the observed difference was not statistically significant. Thus, IL-4 resulted in a decrease in the level of DNA damage observed following exposure to a genotoxic compound. We next tested whether the DNA damage induced by another anticancer drug, bleomycin, would be similarly modulated by the presence of IL-4. To this end, 3T3 fibroblasts were treated with selected concentrations of bleomycin in the presence of an increased concentration of IL-4 and stained for phosphorylated histone H2AX in the nuclei. As shown in Figure 2, bleomycin induced an increase in the phosphorylation of histone H2AX, and the presence of IL-4 resulted in a dose-dependent decrease in the level of histone phosphorylation compared with the control. Next, we decided to test the effect of IL-4 on the ability of nuclear proteins to join DNA strands. For this purpose, fluorescently labeled, double-stranded DNA fragments were incubated with nuclear extracts, and the number of DNA fragments of increased molecular weight was detected following gel electrophoresis under denaturing conditions. In a series of experiments, several characteristics of DNA-joining activity were present in nuclear extracts, including the requirement for ATP and the presence of divalent ions, and PARP activity was determined. As shown in Figure 3A, this enzymatic process occurred with comparable efficiency in the absence and in the presence of ATP. In contrast, DNA joining was inhibited by depletion of poly-ADP-ribosylated proteins from the nuclear extract (Fig. 3B). Next, we investigated the effects of incubating murine fibroblasts with IL-4 on DNA joining activity in nuclear extracts. Cells were incubated with increasing concentrations of IL-4, and nuclear extract was prepared and analyzed using an *in vitro* DNA ligation as-

say. As shown in Figure 3C, the incubation of fibroblasts in the presence of increasing concentrations of mr IL-4 resulted in a dose-dependent increase in observed ligation activity. In parallel experiments, murine fibroblasts incubated with increasing concentrations of IL-4 were analyzed for the presence of poly-ADP-ribosylated proteins using western blot assays. The addition of IL-4 to the culture medium resulted in an increased level of poly-ADP-ribosylation of cellular proteins in murine fibroblasts (Fig. 3D). To extend these observations to a human system and to determine whether the observed effects of IL-4 were DNA-PK-dependent, we employed proficient (M059K) and DNA-PK-deficient (M059J) variants of an established glioma cell line. As shown in Figure 3E, preincubation with hr IL-4 resulted in comparably significant increases in DNA ligation activity observed in nuclear extracts from both M059K and M059J cells suggesting that IL-4 increases ligation activity in a DNA-PK-independent process. Additionally, the western blot experiments showed that the addition of IL-4 to culture media resulted in increased levels of poly-ADP-ribosylation of cellular proteins in M059K glioma cells (Fig. 3F).

To determine whether the presence of IL-4 prevents apoptosis resulting from DNA damage, murine 3T3 fibroblasts were exposed to increasing concentrations of cisplatin in the absence or in the presence of IL-4 and analyzed for annexin V binding to their surface. As shown in Figure 4A in the presence of IL-4, the amount of annexin V binding to apoptotic cells following cisplatin treatment was significantly lower than in the control. To test whether the observed effect of IL-4 depended on PARP activity, cells were exposed to cisplatin in the absence or presence of IL-4 and in the absence or presence of a specific PARP inhibitor, PJ-34. Figure 4B shows that PJ-34 inhibited the decrease in annexin V binding to cells observed in the presence of IL-4 but did not affect the number of apoptotic cells in the control exposed to cisplatin and not to IL-4. Thus, IL-4 decreased DNA damage and apoptosis in murine and human cells in a process that did not depend on DNA-PK activity and involved PARP activity.

Discussion

It has been proposed that IL-4 is involved in mechanisms that defend organisms from toxins to which they are exposed, and the expression of *IL-4* in immune cells is upregulated following contact with different genotoxic compounds. Additionally, studies have shown that IL-4 mediated the upregulation of proteins involved in DNA repair by BER and/or NHEJ process during the differentiation of monocytes into dendritic cells and macrophages (Briegert and Kaina 2007; Bauer and others 2011). Therefore, we sought to explore whether IL-4 was able to trigger modulation of the DNA repair process within murine and human cells exposed to selected genotoxic compounds. We have observed that preincubation with IL-4 resulted in lower numbers of cisplatin- and bleomycin-induced DNA strand breaks (Figs. 1 and 2). The lower level of DNA damage following exposure to a genotoxic agent mediated by IL-4 was associated with a lower number of cells in early stages of apoptosis (Fig. 4A). These observations suggest that either IL-4 triggered cellular responses that prevented the access of cisplatin and bleomycin to DNA or enhanced DNA repair. Interestingly, the effect of

FIG. 3. DNA end-joining activity in nuclear extracts from cells treated with IL-4. **(A)** DNA end-joining activity of nuclear extracts does not require the presence of ATP. A DNA end-joining activity assay was performed as described in the Materials and Methods section either in the absence or in the presence of ATP. DNA end-joining activity was calculated by dividing the optical density of electrophoretic bands representing multimers of DNA substrate by the optical density of bands representing the total DNA substrate. Data are presented as the mean $\pm$ SEM from $n=4$ independent experiments. **(B)** The decrease in DNA end-joining activity upon immunodepletion of polymeric adenosine diphosphate-ribosylated (PAR) proteins and poly(ADP-ribose) polymerase (PARP) from nuclear extracts. Nuclear extracts obtained from 3T3 fibroblasts were treated with either anti-PAR Ab or anti-PARP-1 Ab and Protein G agarose as described in the Materials and Methods section. The DNA end-joining activity nuclear extracts immunodepleted of PAR protein is expressed as a percentage of the activity observed in control nuclear extracts treated with anti- β -actin Ab. Data are presented as the mean $\pm$ SEM from $n=3$ independent experiments. * And ** denote statistically significant differences of $P<0.05$ and $P<0.01$, respectively. **(C)** IL-4 increases the DNA end-joining activity in nuclear extracts from murine fibroblasts. 3T3 fibroblasts were incubated with increasing concentrations of mr IL-4 for 6 h. Cells were harvested and used to prepare nuclear extracts. A DNA end-joining assay was performed as described in the Materials and Methods section. DNA end-joining activity is expressed as a percentage of the activity observed in extracts obtained from control cells. Data are presented as the mean $\pm$ SEM from $n=3$ independent experiments. * And ** denote statistically significant differences of $P<0.05$ and $P<0.01$, respectively. **(D)** Enhancement of DNA end-joining activity by IL-4 in murine fibroblasts corresponds with increasing poly(ADP) ribosylation of cellular proteins. 3T3 fibroblasts were incubated with mr IL-4 (5 ng/mL) for 6 h. Cells were harvested, lysed, and analyzed for the presence of PAR proteins by western blot using an anti-PAR Ab. **(E)** DNA-PK is not required for an increase in DNA end-joining activity following IL-4 treatment of human glioblastoma cells. DNA-PK-proficient (MO59K) and deficient (MO59J) glioblastoma cells were incubated with human recombinant IL-4 (hr IL-4) (5 ng/mL) for 6 h. Cells were harvested and used to prepare nuclear extracts. DNA end-joining assay was performed as described in the Materials and Methods section. DNA end-joining activity is expressed as a percentage of the activity observed in extracts obtained from control cells. Data are presented as the mean $\pm$ SEM of three independent experiments. * And ** denote statistically significant differences of $P<0.05$ and $P<0.01$, respectively. **(F)** Enhancement of DNA end-joining activity by IL-4 in human glioblastoma cells corresponds to an increasing level of poly(ADP) ribosylation of cellular proteins. MO59K cells were incubated with mr IL-4 (5 ng/mL) for 6 h. Cells were harvested, lysed, and analyzed for the presence of PAR proteins by western blot using an anti-PAR Ab.

FIG. 4. Effect of IL-4 on cisplatin-induced apoptosis of murine fibroblasts. **(A)** 3T3 cells were exposed to the indicated concentration of cisplatin (25 μ M) and in the absence or presence of mr IL-4 (5 ng/mL) as described in the Materials and Methods section. Cells were harvested, fixed, stained with annexin V–FITC, and analyzed using a Celloomics HCS Reader as described in the Materials and Methods section. The number of annexin V-positive cells was normalized to the number of cells detected in the untreated control. **(B)** PJ-34 abolished the protective role of IL-4. Cells were pre-treated with PJ-35 (10 μ M) for 1 h prior to incubation with mr IL-4 (5 ng/mL) for 6 h and exposed to cisplatin (25 μ M) for 18 h. Cells were harvested, fixed, stained with annexin V–FITC, and analyzed using a Celloomics HCS Reader as described in the Materials and Methods section. Data are presented as the mean $\pm$ SEM from $n=4$ –8 independent experiments. Significance of the differences between means: * $P < 0.05$ versus mr IL-4 untreated counterparts.

IL-4 was observed in cells treated with bleomycin, which is a radiomimetic anticancer drug that is known to induce double-strand breaks in DNA (Povirk 1996). This type of DNA damage is frequently repaired by the NHEJ mechanism, which does not depend on HR. This may suggest that IL-4 modulates NHEJ activity. We have shown that in murine and human cells, IL-4 mediated a significant and dose-dependent increase in DNA end-joining activity, which mimics the ligation process required to repair the double-strand break type of DNA damage (Fig. 3C, E). Surprisingly, the observed DNA end-joining process did not require ATP (Fig. 3A) and was dependent on poly-ADP-ribosylated proteins (Fig. 3B). These features of the DNA ligation process and the observed IL-4-mediated poly-ADP-ribosylation of cellular proteins (Fig. 3D, F) are consistent with the hypothesis that IL-4 upregulation of DNA end-joining depends on backup NHEJ processes that utilize poly-ADP-ribose as a source of ATP. This is also consistent with the observation that IL-4 upregulated DNA-joining activity in nuclear extracts isolated from DNA-PK-deficient cells (Fig. 3E) because unlike D-NHEJ, the B-NHEJ system does not require DNA-PK activity (Ahmed and others 2010; Wang and others 2003; Perrault and others 2004).

In summary, we have shown that IL-4 modulates the function of murine and human cells exposed to different genotoxic agents. This IL-4-mediated modulation involves the upregulation of DNA repair processes, most likely the PARP-dependent B-NHEJ mechanism, and as a result, apoptotic cell death induced by DNA damaging agents is downregulated.

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Author Disclosure Statement

No competing financial interests exist.

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