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Cross-Resistance to Ionizing Radiation in a Murine Leukemic Cell Line Resistant to *cis*-Dichlorodiammineplatinum(II): Role of Ku Autoantigen

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ABSTRACT

cis-Dichlorodiammineplatinum(II) (CDDP; cisplatin) is commonly used in combination with ionizing radiation (IR) in the treatment of various malignancies. In vitro, many observations suggest that acquisition of CDDP resistance in cell lines confers cross-resistance to IR, but the molecular mechanisms involved have not been well documented yet. We report here the selection and characterization of a murine CDDP-resistant L1210 cell line (L1210/3R) that exhibits cross-resistance to IR because of an increased capacity to repair double-strand breaks compared with parental cells (L1210/P). In resistant cells, electrophoretic mobility shift assays revealed an increased DNA-end binding activity that could be ascribed, by supershifting the retardation complexes with antibodies, to the autoantigen Ku. The heterodimeric Ku protein, composed of 86-kDa (Ku80) and 70-kDa (Ku70) subunits, is the DNA-targeting component of DNA-dependent protein kinase (DNA-PK), which plays a critical role in mammalian DNA double-strand breaks repair. The increased Ku-binding activity in resistant cells was associated with an overexpression affecting specifically the Ku80 subunit. These data strongly suggest that the increase in Ku activity is responsible for the phenotype of cross-resistance to IR. In addition, these observations, along with previous results from DNA-PK^{-} mutant cells, provide evidence in favor of a role of Ku/DNA-PK in resistance to CDDP. These results suggest that Ku activity may be an important molecular target in cancer therapy at the crossroad between cellular responses to CDDP and IR.

cis-Dichlorodiammineplatinum(II) (CDDP) is commonly used in combination with ionizing radiation (IR) in the treatment of various malignancies, such as head and neck tumors. Significant correlations between the response to CDDP and the subsequent response to IR have been reported in patients receiving CDDP-based regimens followed by radiotherapy (reviewed in Coughlin and Richmond, 1989). In vitro, many observations suggest that acquisition of CDDP resistance in cell lines often confers cross-resistance to IR (Wallner and Li, 1987; De Pooter et al., 1991; Hill, 1991). Interestingly, De Pooter et al. (1991) demonstrated that although resistance to CDDP might have unfavorable consequences for IR, the reverse was not true, because increased sensitivity to CDDP was found when resistance against IR was induced in the same cells. Finally, significant cross-resistance between IR and CDDP was shown in one series of early passage human tumor cell lines (Schwartz et al., 1988). However, molecular mechanisms operating in cancer cells resistant to both treatments have not been well documented so far.

In this respect, the DNA-dependent protein kinase (DNA-PK) is of particular interest because several studies demonstrated that this protein is involved in the cell responses to both IR and CDDP. DNA-PK is a nuclear serine/threonine protein kinase composed of a large, 460-kDa catalytic subunit (DNA-PKcs), and a DNA binding subunit, the Ku autoantigen (a dimer of the Ku70 and Ku80 proteins) (Gottlieb and Jackson, 1993). Ku binds to DNA double-strand ends and other discontinuities in the DNA (Blier et al., 1993; Falzon et al., 1993) and recruits the catalytic subunit of the complex (Gottlieb and Jackson, 1993). The active DNA-PK complex then acquires the capacity, at least in vitro, to phosphorylate many DNA-bound proteins in the vicinity (for review, see Anderson et al., 1995). A large number of studies demon-

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ABBREVIATIONS: CDDP, *cis*-dichlorodiammineplatinum(II) (cisplatin); DNA-PK, DNA-dependent protein kinase; DNA-PKcs, DNA-dependent protein kinase catalytic subunit; IR, ionizing radiation; DSB, double-strand break; NM, nitrogen mustard; NER, nucleotide excision repair; ICL, interstrand cross links or linking; DEB, DNA-end binding; CHO, Chinese hamster ovary; TBST, Tris-buffered saline/Tween 20; FAR, fraction of activity released; EMSA, electrophoretic mobility shift assay.

strated that rodent cell lines mutated for either component of DNA-PK are hypersensitive to ionizing radiation (IR) because of a decreased ability to repair DNA double-strand breaks (DSBs) (for review, see Jeggo, 1997). Further evidence that DNA-PK is involved in DNA DSB repair is provided by the hypersensitivity to IR of knock-out mice for Ku80 (Nussenzweig et al., 1996; Zhu et al., 1996) or Ku70 (Ouyang et al., 1997). Thus, these experiments clearly identified DNA-PK as a crucial component of a nonhomologous mechanism of DSB rejoining in mammalian cells (for review, see Jeggo, 1997). In addition, these mutant cells also exhibit significant hypersensitivity to CDDP and to the nitrogen mustards (NMs) melphalan and mechlorethamine (Caldecott and Jeggo, 1991; Tanaka et al., 1993). The hypersensitivity to CDDP of a rodent mutant cell line that lacks the Ku80 subunit seems to be related to the DNA-PK defect, because upon stable transfection with the human Ku80 gene (*XRCC5*), enhanced resistance to CDDP was regained together with bleomycin resistance and Ku DNA end-binding activity (Muller et al., 1998a). In contrast to IR, the mechanism(s) by which DNA-PK participates in the cellular response to CDDP remains only partially understood. We have recently demonstrated a role for DNA-PK as a positive modulator in vivo of the nucleotide excision repair (NER) process, the main pathway involved in the repair of CDDP intrastrand cross-links (Muller et al., 1998a). In addition, it has been suggested that DNA-PK might participate in the repair of interstrand cross-links (ICL) that are removed from DNA in mammalian cells by the combined actions of NER and recombination processes (Caldecott and Jeggo, 1991; Chaney and Sancar, 1996; Bessho et al., 1997). Thus, DNA-PK seems to be involved in the cellular response to IR and CDDP through DNA-repair-mediated mechanism(s).

The aim of the present study was to determine whether regulation of Ku/DNA-PK activity might be involved in the acquisition of cellular resistance to IR after CDDP exposure. To test this hypothesis, we have selected a CDDP-resistant L1210 cell line that displays significant cross-resistance to IR. We demonstrated here that this phenotype is associated with enhanced DNA-end binding (DEB) activity involving the Ku heterodimer.

Materials and Methods

Cell Lines and Culture. The CDDP-resistant L1210/R cell line was obtained by chronic exposure of L1210/P (a gift from Dr. S. Cros, Institut de Pharmacologie et de Biologie Structurale, Toulouse, France) to stepwise increasing concentrations of CDDP (a kind gift of Roger Bellon, groupe Rhône-Poulenc Rorer, Montrouge, France) over a period of 9 months. The resistance phenotype was stable, even in the absence of CDDP for several months. The HeLa S3 cell line was obtained from the stock of the European Molecular Biology Laboratories (Heidelberg, Germany). The Chinese hamster ovary (CHO)-AA8 and the corresponding mutant CHO-V3 cell lines were kindly provided by Dr. G. Whitmore (Ontario Cancer Institute, Toronto, Ontario, Canada) (Peterson et al., 1995). L1210/P, L1210/3R, and HeLa cells were cultured in suspension in RPMI 1640 (Gibco BRL) supplemented with 10% fetal calf serum, 2 mM glutamine, penicillin (2×10^5 U/liter), and streptomycin (50 mg/liter). All cells were maintained at 37°C in a 5% CO_2 humidified atmosphere.

Cell Treatment and Cytotoxicity Studies. Cellular CDDP, mechlorethamine, and melphalan toxicities were determined by the colorimetric 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) metabolic dye assay (Sigma, St. Louis, MO) as described elsewhere (Canitrot et al., 1997). Ionizing radiation toxicity was determined by clonogenic assay as follows: A total of 10^3 cells/ml was resuspended in RPMI medium supplemented with 10% fetal calf serum, 0.9% methylcellulose (Stem Cell Technologies, Vancouver, British Columbia, Canada; TEBU). Five hundred cells were seeded into 35-mm Petri dishes and irradiated at 1 Gy/min with a cobalt source (Centre d'Études et de Recherche de Toulouse-l'Office National d'Études et de Recherches Aérospatiales et de l'Espace, Toulouse, France). The dishes were then incubated at 37°C in a humidified 5% CO_2 atmosphere for 8 days. Colonies of more than 50 cells were counted by microscope. IC_{50} values represent the drug concentration or the γ-ray dose leading to 50% cell survival.

Cell Extracts. Whole-cell protein extracts were prepared according to Manley et al. (1983) with the minor modifications described previously (Wood et al., 1988). Protein concentrations were determined by the method of Bradford (1976) using the Bio-Rad protein assay dye (Bio-Rad Labs., Hercules, CA).

Band Shift Assay. The probe was prepared as follows: an aliquot of a 123-basepair DNA ladder (Gibco BRL, Gaithersburg, MD) was digested with *Ava*I (Gibco BRL) to generate 123-basepair monomers. After purification from agarose gel, fragments were end-labeled with [α-^{32}P]dCTP using DNA polymerase I Klenow fragment (Gibco BRL). End-labeled probes were separated from unincorporated nucleotides by chromatography through Sephadex G-50 (Sigma). The band-shift assay was performed as described previously (Zhang and Yaneva, 1992). Briefly, radiolabeled DNA (4 ng, 100,000 cpm) was incubated with extracts (3 μg) in 20 μl of binding buffer (20 mM Tris · HCl, pH 7.5, 100 mM NaCl, 1 mM dithiothreitol, 5 mM $MgCl_2$, 0.2 mM EGTA, and 5% glycerol) in the presence of 1 μg of circular plasmid as a nonspecific competitor at 30°C for 30 min. The samples were electrophoresed on a 5% polyacrylamide gel at 4°C for 2 h at 100 V. The gel was dried on Whatman paper and autoradiographed with Kodak X-OMAT films (Eastman Kodak, Rochester, NY). The regions of the gel containing the free probe and the retardation complexes were quantified by scintillation counting. The supershift experiments were performed as described previously (Muller et al., 1998c). The antibodies used were purified on protein A sepharose from human autoimmune antisera Hi (kindly provided by Dr. Y. Takeda, Medical College of Georgia, Augusta, GA) and AF (a generous gift from Dr. E. M. Tan, Scripps Research Institute, La Jolla, CA). Human sera Hi and AF specifically recognize Ku70 alone or Ku70 and Ku80, respectively (Francoeur et al., 1986). The human serum Sa that contains anti-ribonucleoprotein antibodies (provided by Dr Y. Takeda, Augusta, GA) was used as a control. Monoclonal 30F3 antibody was provided by Dr. M. Le Romancer (Institut National de la Santé et de la Recherche Médicale U10, Hopital Bichat, Paris, France)

Western Blot Analysis. Protein extracts from rodent cells (100 μg) and HeLa cells (20 μg) as a control were resolved by SDS-polyacrylamide gel electrophoresis and transferred to a nitrocellulose membrane (Amersham, Arlington Heights, IL) by semidry electroblotting. After checking for homogeneous transfer by Red Ponceau staining, the membrane was blocked with Tris-buffered saline/Tween 20 (TBST)-milk (20 mM Tris · HCl, pH 7.6, 137 mM NaCl, 0.2% Tween, and 5% nonfat dry milk). The membrane was hybridized overnight at 4°C with TBST-milk containing either 500-fold diluted AF human antiserum, 5,000-fold diluted 18.2 monoclonal anti-DNA-PKcs antibody (a generous gift from Prof. T. H. Carter, St. John's University, New York, NY) or 10,000-fold diluted monoclonal antiactin antibody (Interchim, Montlucon, France). The membrane was then rinsed with TBST at room temperature and hybridized with TBST-milk containing peroxidase-conjugated goat antihuman antibodies (Jackson ImmunoResearch Laboratories, West Grove, PA). The membrane was then extensively washed with TBST and developed for antibody binding by the enhanced chemiluminescence procedure carried out according to the manufacturer's recommendations (Du Pont-NEN, Boston, MA).

DNA-PK Activity Assays. Kinase assays were performed as described previously (Finnie et al., 1995) with some modifications

(Muller et al., 1998a). Each sample was assayed in the presence of either DNA-PK-specific peptide substrate (SQE peptide: EPPL**SQE**AFADLLKK) or a negative control peptide (SEQ peptide: EPPL**SEQ**AFADLLKK). DNA-PK activity was expressed in counts per minute incorporated in the SQE peptide or in the SEQ peptide for a given extract.

Kinetics of DSB Repair. Kinetics of DSB repair assay was performed using pulsed-field electrophoresis (CHEF DRIII; BioRad) in the megabase size region as reported previously (Foray et al., 1997). The repair kinetics data were presented as the percentage of fraction of activity released (FAR) remaining at indicated times, as previously described (Foray et al., 1997). The FAR data were fitted to the variable repair half-time (VHRT) model (Foray et al., 1996)

Results

In Vitro Drug and γ-Ray Sensitivity of L1210 Cell Lines. The L1210 cell line (L1210/P) was adapted in vitro to stepwise increasing concentrations of CDDP for about 1 year, leading to a subline termed L1210/3R, which was resistant to 3 μg/ml CDDP. The resistance was stable, even in the absence of CDDP for several months (data not shown). As judged by the IC_{50} value obtained from MTT metabolic dye assays (Table 1), L1210/3R cells were 16-fold resistant to CDDP compared with the L1210/P cell line and exhibited cross-resistance to the DNA cross-linking agents mechlorethamine and melphalan (3.6- and 2.3-fold, respectively; see Table 1). We then investigated whether L1210/3R cells were cross-resistant to IR. As shown in Fig. 1, L1210/3R were resistant to IR by a factor of 2-fold compared with L1210/P cells (see Table 1 for IC_{50}). Again, this resistance was stable, even in the absence of CDDP for several months (data not shown). In contrast, L1210/3R cells were not cross-resistant to the monofunctional alkylating agent *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine, which induces DNA damage mainly processed by the base excision-repair pathway (Table 1).

DSB Repair Kinetics. Repair of DSBs has been shown to play a key role in the radiosensitivity of mammalian cells. We investigated whether, compared with L1210/P cells, the resistance of L1210/3R cells to IR was related to an increased capacity to repair DSBs. The kinetics of DSB repair was studied by measuring the percent of FAR remaining as a function of the time, after an exposure to 30 Gy. As shown in Fig. 2, the capacity to repair DSBs was more rapid in the resistant L1210/3R than in the parental L1210/P cells. After the irradiation, the percentage of FAR remaining dropped to 50% in 30 min for the L1210/3R cells, whereas a longer time (60 min) was necessary to observe the same effect in the L1210/P cells. Because Ku/DNA-PK represents one of the major protein complexes involved in DSBs repair in mammalian cells, this result raises the possibility of an involvement of this complex in the cross-resistance phenotype of L1210/3R cells to IR.

Activity and Expression of the Ku/DNA-PKcs. We first investigated the kinase activity of the whole DNA-PK complex in L1210/P and L1210/3R cells as well as in CHO-AA8 and CHO-V3 as control. As shown in Fig. 3A, the DNA-PK activity was comparable in the two parental cell lines L1210/P and CHO-AA8, although, as expected, it was absent in the DNA-PKcs mutant cell line (CHO-V3). No significant differences in DNA-PK activity were observed between protein extracts from resistant and sensitive L1210 cells. Accordingly, DNA-PKcs was expressed at comparable levels in CHO-AA8, L1210/P, and L1210/R cells and was absent in CHO-V3 cells (Fig. 3B). The activity of the regulatory subunit of DNA-PK, the Ku heterodimer, was then assessed. Ku DEB activity can be detected easily by using double-stranded DNA fragments in an electrophoretic mobility shift assay (EMSA) (Zhang and Yaneva, 1992). Independent cell extracts were used; a representative experiment is shown in Fig. 4A. The DEB activity was increased by 5- to 6-fold in cell extracts from resistant cells compared with extracts from the parental

TABLE 1

Sensitivity pattern of L1210 cell lines grown in vitro to different genotoxic treatments

Values reported are mean values from at least three independent experiments ± S.E. See *Materials and Methods* for details.

Cell lines (L1210)	Genotoxic Treatments				
	CDDP[a]	MCE[a]	MLP[a]	MNNG[a]	γ-Rays[b]
	μM				*Gy*
P	2.0 ± 0.9	2.1 ± 0.9	7.5 ± 1.5	16.7 ± 4.6	2.8 ± 0.5
3R	32.7 ± 2.9	7.5 ± 2.6	17.5 ± 2.5	15.0 ± 2.4	5.5 ± 1.0

MCE, mechlorethamine; MLP, melphalan; MNNG, *N*-methyl-*N*′-nitro-*N*-nitrosoguanidine.

[a] IC_{50} values determined with the MTT metabolic dye assay.

[b] ID_{50} values determined with the clonogenic assay.

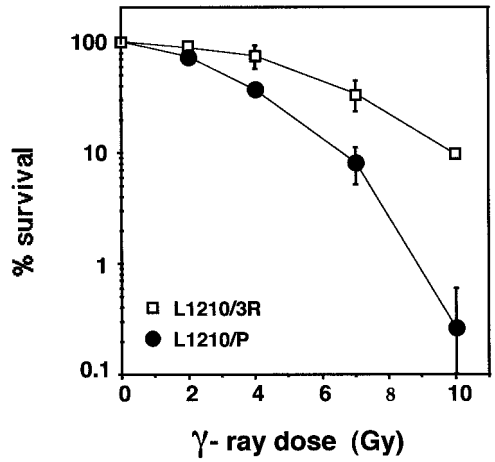

Fig. 1. Ionizing radiation response of L1210/P and L1210/3R cells. L1210/P (P, □) and L1210/3R (3R, ■) were subjected to γ-radiation and cell survival was determined by clonogenic assay. Plotted values represent mean values from three experiments ± S.E. IC_{50} values were 2.84 ± 0.49 Gy and 5.52 ± 1.02 Gy for L1210/P and L1210/3R, respectively.

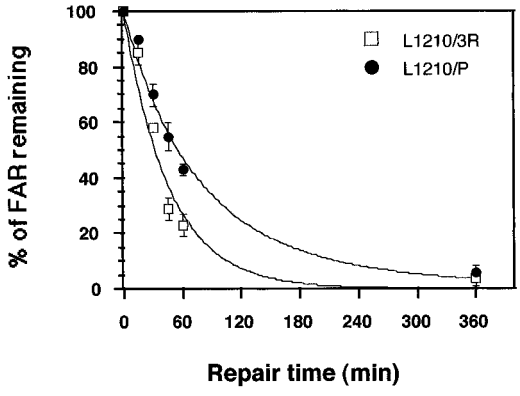

Fig. 2. DSB repair kinetics in L1210/P and L1210/3R cells. Percentage of FAR plotted up to 6 h after irradiation for L1210/3R (□) and L1210/P (●) cells irradiated at 4°C (30 Gy). Each data point is the mean ± S.E. of at least three replicate experiments.

L1210/P cells. As described previously (Zhang and Yaneva, 1992; Falzon et al., 1993), Ku was considered the most likely causal protein for the formation of these DEB complexes. To verify this hypothesis, we evaluated the presence or absence of Ku70 and Ku80 proteins in the DEB complexes, by using various antibodies directed against these proteins in EMSA supershift experiments. Antibodies purified from two independent human sera able to recognize Ku70 (Fig. 4B, lane S1) or both Ku70/80 subunits (Fig. 4B, lane S2) were used. In the presence of these antibodies, DEB complexes were completely supershifted and retarded in the gel, as shown in Fig. 4B. Moreover, the S0 control antiserum (which contained anti-ribonucleoprotein autoantibodies) has no effect on the migration of the DEB complexes (lane S0). Thus, this set of experiments indicates that the DEB complexes corresponded to the Ku heterodimer.

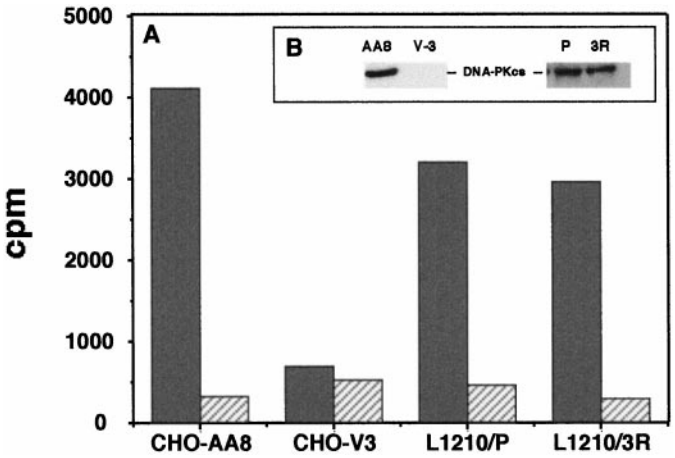

Fig. 3. Activity and expression of the DNA-PKcs in L1210/P and L1210/3R cells. A, protein extracts from L1210/P (P) and L1210/3R (3R) cells were analyzed by the DNA-PK pull-down assay in the presence of SQE (■) or SEQ (▨) p53 peptide as indicated in *Materials and Methods*. Protein extracts from AA8 or V3 cells were used as positive and negative controls, respectively. Mean of at least two experiments. B, Western blot analyses of DNA-PKcs expression in L1210/P (P) and L1210/3R (3R) cells. CHO-AA8 and CHO-V3 cell extracts were added as positive and negative controls, respectively.

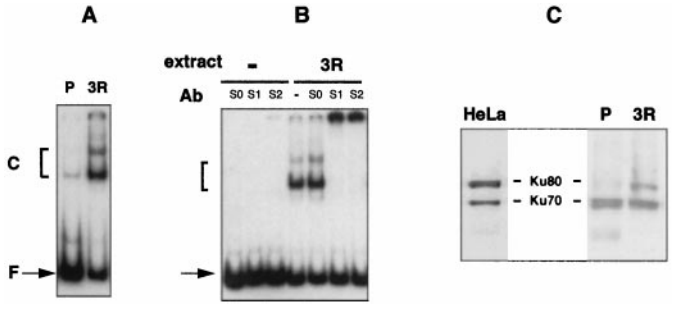

Fig. 4. Activity and expression of the Ku heterodimer in L1210/P and L1210/3R cells. A, cell extracts (3 μg) obtained from L1210/P (P) or L1210/3R (R) cell lines were incubated with linear labeled probe in the presence of closed circular plasmid DNA as a nonspecific competitor. The electrophoretic mobility of the protein DNA-complexes were analyzed as described in *Materials and Methods*. F, position of the free probe; C, position of protein DNA-complexes consisting of DEB activity. B, EMSA reactions were carried out without extracts (left) or with L1210/3R protein extracts (3R; right), as in A, except that antibodies were added before incubation. S_0, Sa control human antiserum; S_1 and S_2, Hi and AF human autoimmune antisera reacting with Ku70 alone and Ku70/Ku80, respectively. C, Western blot analyses of Ku80 and Ku70 protein expression in L1210/P (P), L1210/3R (3R), and HeLa (H) cell extracts. Ku proteins were probed with AF antiserum as described in *Materials and Methods*. Given the large variation of anti-Ku antibody reactivities between human and murine protein extracts, HeLa and L1210 cell extracts were resolved in the same gel, but the autoradiographs presented resulted from two different time exposures.

Western blot analyses were then performed to determine whether variations in Ku protein expression could account for those variations observed in DEB activity between resistant and sensitive L1210 cell lines. As shown in Fig. 4C, an increase in Ku80 protein level was observed in L1210/3R compared with L1210/P cells, as detected by antibodies purified from human autoimmune antisera (AF). Similar results were obtained using a monoclonal antibody (30F3) raised against the Ku80 protein (data not shown). The apparent discrepancy in expression levels between Ku70 and Ku80 in L1210 cells compared with HeLa cells, in which similar signal intensities were found, is likely to result from a better cross-reactivity of antibodies purified from human AF serum with murine Ku 70 than with murine Ku80.

Discussion

Molecular mechanisms operating in cancer cells resistant to IR after CDDP exposure have not been well documented thus far. In the present article, we report that the Ku antigen is likely to play a role in this phenotype.

The L1210/3R cells exhibited a stable cross-resistance to IR because of an increased capacity to repair DSBs compared with parental cells. According to the literature, the increase in Ku DEB activity in resistant cells is likely to explain this phenotype. Indeed, the extreme radiosensitivity and DSB repair deficiency found in Ku80 (Smider et al., 1994; Taccioli et al., 1994) or Ku70 (Lee et al., 1995) rodent mutant cell lines, as well as in the Ku80 (Nussenzweig et al., 1996; Zhu et al., 1996) or Ku70 (Ouyang et al., 1997) knock-out mice, clearly identify Ku as a major component of the DSB's repair apparatus in mammalian cells. In our model, the enhanced Ku DEB activity in L1210/3R cells was not associated with an increase in kinase activity of the whole DNA-PK complex (Fig. 3A). Thus, our results suggest that the level of expression of DNA-PKcs, which is not modified in resistant cells (Fig. 3B), is the limiting component of the holoenzyme activity. Recent evidence suggests that enhanced Ku DEB activity alone may account for the observed increase in DSB repair. Indeed, it has been reported that Ku, independently of DNA-PKcs, plays a direct role in the repair of DSBs by stimulating DEB by mammalian ligases (Ramsden and Gellert, 1998). First, we investigated the potential inducibility of Ku80 expression by using CDDP treatment after an incubation of sensitive L1210 cells with the drug (1 h at doses corresponding to the IC_{50} and IC_{90} values). No change in Ku80 expression was noticed until 48 h postincubation time as determined by Western blot analysis and EMSA (data not shown). Thus, the increased Ku DEB activity of the resistant cells seemed dependent on a stable overexpression of the Ku80 protein. It has been reported previously that the individual Ku subunits are degraded when not dimerized, because the Ku 70 protein was undetectable in Ku80 mutants (Satoh et al., 1995; Singleton et al., 1997). In addition, both subunits need to be present at stoichiometric levels to form stable complexes in an EMSA (Wu and Lieber, 1996). The differential signal obtained by Western blotting experiments between Ku70 and Ku80 is probably caused by the respective

recognition property of the primary antibodies used. However, additional mechanisms, such as post-translational modifications affecting Ku affinity for DNA termini, have been reported (Quinn et al., 1992). We cannot exclude that an additional post-translational modification of the Ku70 subunit participates in the increase in Ku DEB activity.

Our data show for the first time that an increase in Ku DEB activity is associated with a stable phenotype of resistance to acute γ-ray radiation, with this increase in Ku DEB activity being observed after CDDP selection. An increasing body of evidence from our laboratory and others suggest that the increase in Ku activity is likely to be directly involved in CDDP resistance rather than reflecting random genetic changes occurring after exposure to CDDP over 9 months. Indeed, it is now clearly established that rodent cells mutated for either component of DNA-PK, in addition to being hypersensitive to IR, also exhibit sensitivity to CDDP (Caldecott and Jeggo, 1991; Tanaka et al., 1993; Muller et al., 1998a). Moreover, resistance to CDDP was restored by transfection of a functional human Ku80 (*XRCC5*) gene in a Ku80 minus cell line (Muller et al., 1998a). At the molecular level, the role of Ku/DNA-PK in CDDP resistance can be considered to involve different, nonexclusive DNA-repair-mediated mechanisms. We have recently demonstrated a regulatory function of Ku/DNA-PK in the NER process in vivo but not in vitro (Muller et al., 1998a). Accordingly, the increase in Ku DEB activity might facilitate the NER activity in L1210/3R cells and therefore contribute to CDDP resistance. In fact, the resistant cells exhibit enhanced NER activity as measured with an in vitro cell-free repair assay (F. Frit, P.C., J. M. Barret and B.S., unpublished observations). Modulation of Ku levels of expression in vivo, with either dominant negative constructs or antisense strategies, will be necessary to demonstrate the facilitating effect of Ku on NER activity in L1210/3R cells. Such approaches are currently under investigation in our laboratory. In addition, an increase in Ku DEB activity might also enhance the repair of ICL, because this subset of adducts is believed to be processed by mechanisms requiring both excision and recombination steps (Chaney and Sancar et al., 1996; Bessho et al., 1997). In accordance, the DNA-PK deficient cells are hypersensitive to the NMs melphalan and mechlorethamine (Caldecott and Jeggo, 1991; Tanaka et al., 1993). The ICL of DNA is generally thought to be responsible for the cytotoxicity of these drugs. Interestingly, the L1210/3R cells also exhibited significant cross-resistance to these two drugs (Table 1). In addition to the well described role of DNA-PK in the cellular response to CDDP, there are further arguments suggesting that an increase in Ku DEB activity is probably not an additional, independent event occurring during the selection of the resistant cell line. First, we observed an increase in Ku DEB activity in another CDDP-resistant L1210 cell line that has been selected in vivo in tumor-bearing mice (Geran et al., 1972; Calsou et al., 1993; our data not shown). However, we could not obtain further evidence that this cell line exhibited cross-resistance to IR and increased DSB repair activity, because adaptation to in vitro growth conditions resulted in rapid loss of the resistance phenotype (Geran et al., 1972; our data not shown). Second, we have reported previously that Ku DEB activity was increased in lymphocytes from patients that exhibited a chronic lymphocytic leukemia resistant to NMs compared with sensitive ones (Muller and Salles, 1997; Christodoulopoulos et al., 1998; Muller et al., 1998b). Interestingly, these resistant samples also exhibited significant cross-resistance to the radiomimetic agent, neocarzinostatin (Muller and Salles, 1997). Thus, along with the present study, these results suggest that the increase of Ku DEB activity might be an important feature in cancer cells resistant to IR after CDDP or NM selection and might participate in the emergence of cells resistant to these two chemotherapeutic agents. An increase in Ku expression and activity has been observed in two model systems (rodent cells and human primary tumor cells) exhibiting a basal level of Ku expression lower than the level usually observed in established human cell lines (Muller and Salles, 1997; Muller et al., 1998b; present study). Thus, one may suppose that an increase in Ku expression associated with CDDP resistance would not be observed in cells exhibiting constitutively very high basal levels of Ku expression. According to this hypothesis, the only study that has examined the expression of Ku 70 and Ku 80 in a human CDDP-resistant ovarian cell line revealed no change in comparison with the CDDP-sensitive parental cell line (Henkels and Turchi, 1997).

The relation between CDDP and radiation resistance in vitro, as demonstrated in the present study, is particularly interesting, given the results of clinical trials with CDDP regimens and radiotherapy. Consequently, it would be of great interest to investigate the Ku binding activity in clinical samples resistant to CDDP, particularly when associated with radioresistance. Such studies would be of great clinical interest because, once the role of Ku is demonstrated, modulation of its activity by antisense or dominant-negative constructs might contribute to improving the efficacy of cancer therapy.

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