The anticancer drug, cisplatin, increases the naturally occurring cell-mediated lysis of tumor cells

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Summary. It has been proposed that a component of the antitumor potential of the chemotherapeutic agent, cisplatin, resides in the host's ability to respond to cisplatin-treated tumor cells. Here we report that tumor cells that are normally resistant to lysis mediated by naturally occurring cytotoxic cells showed an increased sensitivity to lysis mediated by murine spleen cells or human peripheral blood monocytes and lymphocytes when cisplatin was added at the beginning of the lytic assay. This was shown for the lysis of both murine and human tumor cells. The pretreatment of tumor cells, but not effector cells with cisplatin caused an increase in lysis in the presence of murine spleen cells or human peripheral blood leukocytes, indicating that the effect of cisplatin is to reduce resistance to lysis by these effector cells. The lysis of tumor cells by naturally occurring cytotoxic cells was blocked by antibodies specific for tumor necrosis factor. In addition, the ability of cisplatin to increase lysis was seen with cells that are sensitive to natural cytotoxic cells, but not with cells that are sensitive to natural killer cells. These results suggest that the effector cells that mediate the lysis of these tumor cells in the presence of cisplatin are likely to be natural cytotoxic cells. The ability of cisplatin to increase the lysis of tumor cells by naturally occurring cytotoxic cells indicates that these cells may be a host defense mechanism that contributes to the anticancer potential of cisplatin.

Introduction

Cisplatin (cis-diamminedichloroplatinum II) is a member of a relatively new class of anticancer drugs representing metal coordination complexes. It has been shown to be effective in experimental animal tumor model systems [15, 24, 25, 29, 32, 33] and in the clinical treatment of a variety of human cancers [3, 10–12]. Although the primary mechanism of the antitumor activity of cisplatin probably resides in its ability to inhibit DNA synthesis [27], it has been suggested that a component of the antitumor activity of cisplatin may arise from a host immunological reaction against cisplatin-treated cells [22]. In this regard it has been shown that cisplatin has a reduced antitumor activity in immunologically depressed (i.e., X-irradiated) mice [23]. This finding, along with the observation that the treatment of cells

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with cisplatin can result in the increased appearance of surface antigens [31], has led to the hypothesis that treatment of tumor cells with cisplatin increases the expression of tumor cell-surface-associated antigens and thereby permits the immunological recognition and subsequent rejection of cisplatin-treated cells. Although it is possible that cisplatin-treated cells may interact more effectively with components of the immune system, it is also possible that cisplatin effects the interaction of tumor cells with host defense mechanisms that are not formally part of the immune system.

Over the past several years our analyses of the interactions between host defense mechanisms and tumors have demonstrated a role for naturally occurring cytotoxic cell activity as an antitumor surveillance mechanism [4, 6, 8, 16, 19, 20]. Because immunologically depressed animals (e.g., X-irradiated mice) also have reduced levels of naturally occurring cytotoxic cell activity, it is possible that the diminished antitumor activity of cisplatin in such animals could, in part, result from a reduction in the activity of these cells. For this reason we analyzed the effect of cisplatin on the natural cell-mediated lysis of tumor cells. The results of this analysis demonstrate that cisplatin specifically increases the lysis of tumor cells, mediated both by the murine spleen cells and by human peripheral blood monocytes and lymphocytes, and lends support to the hypothesis that there is a host component involved in the antitumor activity of cisplatin.

Materials and methods

Cell lines. The cloned, transformed cell lines, B/C-BPDE-F(C).3, B/C-BPDE-F(G).1, and B/C-BPDE-F7.1A, were derived from the nontransformed, contact-inhibited Balb/c mouse cell lines B/C-N [18], following the *in vitro* exposure of B/C-N cells to benzo[*a*]pyrenedihydrodiol epoxide and selection of cells that expressed contact non-inhibition in vitro [5, 7]. B/C-BPDE-F(C).3, B/C-BPDE-F(G).1, and B/C-BPDE-F7.1A are tumorigenic in syngeneic normal and nude mice. The YAC-1 cell line was originally adapted to in vitro culture from a T-cell lymphoma of an A/Sn mouse, induced by Molony murine leukemia virus [13]. The cloned human cell line, K-562 is an undifferentiated myeliod cell line originally derived from a patient with chronic myelogenous leukemia [17, 30]. The uncloned human cell lines, HTS-1 and DL-5, were established as *in vitro* cultures from different patients with

adenocarcinomas of the endometrium, and the uncloned human cell line, DL-4, was established as an *in vitro* culture from a patient with an adenocarcinoma of the ovary. The HTS-1, DL-4, and DL-5 cell lines were isolated in our laboratory. All cell lines were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal calf serum, L-glutamine, penicillin and streptomycin [6].

The in vitro assay of naturally occurring cytotoxic activity. Varying numbers of Balb/c spleen cells from untreated mice or human leukocytes from normal adult peripheral blood were mixed with 10^4 ^{51}Cr-labelled target cells in 96-well microtiter plates containing 0.1 ml/well RPMI-1640 medium supplemented as previously described [9], and referred to as supplemented RPMI-1640 medium. Cisplatin diluted in supplemented RPMI-1640 was added (0.05 ml/well, at 3 × the final concentration) at the beginning of the assay. Control wells contained only ^{51}Cr-labelled targets or ^{51}Cr-labelled targets and cisplatin in 0.1 ml supplement RPMI-1640 medium. These wells were used to determine the amount of ^{51}Cr spontaneously released from targets. The amount of ^{51}Cr release (i.e., target lysis) was determined 14–16 h after targets and effectors were mixed. This was true whether the targets were sensitive to lysis mediated by natural cytotoxic (NC) or natural killer (NK) cells. The percentage specific ^{51}Cr release was calculated by the following formula:

Specific lysis (%) =
$$\frac{^{51}\text{Cr release from sample} - \text{spontaneous } ^{51}\text{Cr release}}{\text{total } ^{51}\text{Cr} - \text{spontaneous } ^{51}\text{Cr release}} \times 100$$

where ^{51}Cr release is measured in cpm. In experiments where either effector cells or tumor cells were pretreated with cisplatin, they were treated for 2 h in supplemented RPMI-1640 medium containing 2 µg/ml cisplatin. When tumor cells were pretreated they were labelled with ^{51}Cr before being treated with cisplatin. After treatment with cisplatin the cells were washed three times before they were used in the lytic assay. The pretreatment of targets with cisplatin did not affect the spontaneous release of ^{51}Cr. For all targets the spontaneous release of ^{51}Cr ranged between 19% and 30% of the total ^{51}Cr incorporated. In experiments where cisplatin was added, the spontaneous release of ^{51}Cr from targets was determined in the presence of cisplatin. The addition of cisplatin increased the spontaneous release of ^{51}Cr from labelled targets by 0–5% above that released in the absence of cisplatin. This indicates that these tumor cells are resistant to the cytotoxic effects of cisplatin, as measured by the release of ^{51}Cr. All assays were performed in triplicate. The standard error of the mean percentage specific lysis for assays performed on the same day was less than 5%. In addition, the standard error of the mean percentage specific lysis for assays performed on different days was less than 10%. Murine spleen cells were obtained from 8–10-week-old Balb/c mice. Spleen cells were dissociated in phosphate-buffered saline, pH 7.2, and clumps were allowed to settle at unit gravity. Suspended single cells were collected, centrifuged at 200 *g* for 10 min, resuspended in supplemented RPMI-1640 medium, and counted. Human peripheral blood was collected in tubes containing EDTA as the anticoagulant. The monocyte/lymphocyte-enriched fraction was separated from whole blood with the use of Sepracel-MN (Sepratech

Corp., Oklahoma City, Okla) by using the method provided by the Sepratech Corp. After separation, the monocyte/lymphocyte fraction was centrifuged at 200 *g* for 10 min, resuspended in supplemented RPMI-1640 medium, and counted.

Reagents. Rabbit serum containing antibody to recombinant human tumor necrosis factor (TNF) and rabbit serum containing antibody to recombinant murine TNF were obtained from the Cetus Corp. (Emeryville, Calif). When anti-TNF antibody was added to the assay of naturally occurring cell-mediated lysis, enough to block 150 units of TNF was added to each well. Cisplatin was obtained from Bristol Lab. (Syracuse, N.Y.)

Results

Cisplatin increases the murine spleen-cell-mediated lysis of resistant murine transformed cells

The cloned murine cell lines, B/C-BPDE-F(C).3, B/C-BPDE-F(G).1, and B/C-BPDE-F7.1A, are transformed (i.e., express contact-noninhibition in vitro) and tumorigenic when injected into normal mice. As shown in Fig. 1 A, B, C, these cell lines are resistant to lysis mediated by murine spleen cells (i.e., less than 10% specific lysis at a spleen-cell-to-target ratio of 80:1). The addition of cisplatin results in a marked increase in the spleen-cell-mediated lysis of these cells. The addition of cisplatin (1–2 µg/ml) causes a small increase in the amount of ^{51}Cr that is spontaneously released from the target cells (see Materials and methods), however, because this is subtracted when the percentage specific lysis is calculated (see Materials and methods) the increased lysis shown in Fig. 1 (and other figures), which results from the addition of cisplatin, is mediated by the effector cells. Although 1 µg/ml cisplatin did not increase the spleen-cell-mediated lysis of B/C-BPDE-F(C).3 cells, it did increase the spleen-cell-mediated lysis of both B/C-BPDE-F(G).1 and B/C-BPDE-F7.1A cells. The addition of 2 µg/ml cisplatin increased the lysis of all three cell lines. The percentage specific lysis of B/C-BPDE-F(C).3, B/C-BPDE-F(G).1, and B/C-BPDE-F7.1A cells, at a spleen-cell-to-target ratio of 5:1, in the presence of 2 µg/ml cisplatin, is greater than the percentage specific lysis of these cells at a spleen-cell-to-target ratio of 80:1 when no cisplatin was added. Thus by comparing the number of spleen cells required to give an equivalent amount of lysis, B/C-BPDE-F(C).3, B/C-BPDE-F(G).1, and B/C-BPDE-F7.1A cells are at least 16 times more sensitive to lysis mediated by murine spleen
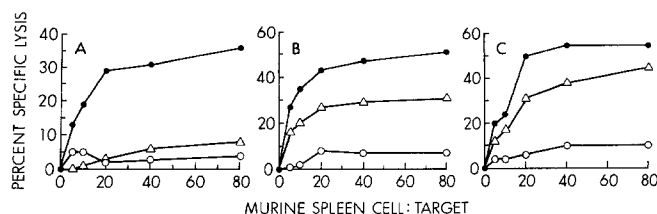

Fig. 1. Cisplatin increases the murine spleen-cells-mediated lysis of murine targets. **A** B/C-BPDE F(C).3 targets, **B** B/C-BPDE F(G).1 targets, **C** B/C-BPDE F7.1A targets. The percentage specific lysis as a function of the effector-to-target ratio was determined in the absence of cisplatin (O), or in the presence of cisplatin at a concentration of 1 µg/ml (△), or 2 µg/ml (●)
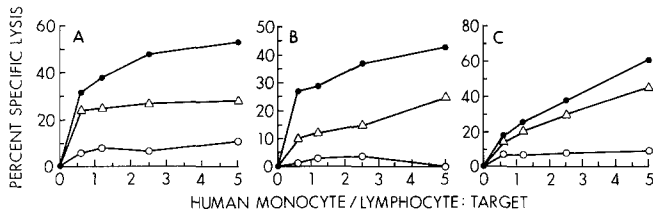
Fig. 2. Cisplatin increases the human peripheral blood monocyte/lymphocyte-mediated lysis of murine targets. **A** B/C-BPDE F(C).3 targets, **B** B/C-BPDE F(G).1 targets, **C** B/C-BPDE F7.1A targets. The percentage specific lysis as a function of the effector-to-target ratio was determined in the absence of cisplatin (O), or in the presence of cisplatin at a concentration of 1 μg/ml (△), or 2 μg/ml (●)
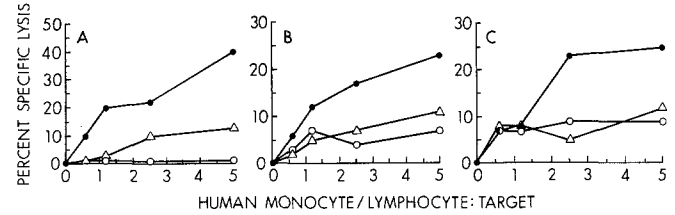
Fig. 3. Cisplatin increases the human peripheral blood monocyte/lymphocyte-mediated lysis of human targets. **A** DL-5 targets, **B** HTS-1 targets, **C** DL-4 targets. The percentage specific lysis as a function of the effector-to-target ratio was determined in the absence of cisplatin (O), or in the presence of cisplatin at a concentration of 1 μg/ml (△), or 2 μg/ml (●)

cells in the presence of 2 μg/ml cisplatin than in the absence of cisplatin.

Cisplatin increases the human monocyte/lymphocyte-mediated lysis of resistant murine transformed cells

We have previously shown that human peripheral blood monocytes, present in the monocyte/lymphocyte-enriched fraction of separated whole blood (see Materials and methods) are able to recognize and lyse murine cell lines that are sensitive to murine NC-mediated lysis [8]. The human monocytes that mediate this lysis are similar to murine NC effector cells [8]. As shown in Fig. 2A, B, C these murine cell lines are resistant to lysis by the human peripheral monocyte/lymphocyte-enriched fraction of whole blood, as they are to murine spleen-cell-mediated lysis. The addition of 1–2 μg/ml cisplatin increases the sensitivity of these tumorigenic cells in a dose-dependent manner. Cisplatin at 1 μg/ml increases the sensitivity of the cells at least eightfold (on the basis of the number of effector cells required to produce an equivalent level of lysis) above the value without cisplatin, and 2 μg/ml increases the sensitivity at least an additional eightfold above that of 1 μg/ml cisplatin for both the B/C-BPDE-F(C).3 and B/C-BPDE-F(G).1 cell lines and approximately twofold for the B/C-BPDE-F7.1A cell line.

Cisplatin increases the human monocyte/lymphocyte-mediated lysis of resistant human tumor cells

As shown in Fig. 3A, B, C all three cell lines were more sensitive to lysis mediated by human effector cells in the

presence of cisplatin than they were when no cisplatin was added. Although these cell lines are somewhat less sensitive to lysis mediated by peripheral blood leukocytes in the presence of cisplatin than the murine cell lines lysed by the same effector cells (see Fig. 2), the cell lines DL-5 (Fig. 3A) and HTS-1 (Fig. 3B) are at least eightfold more sensitive to effector-cell-mediated lysis in the presence of 2 μg/ml cisplatin as compared to results when no cisplatin was added. The cell line, DL-4 (Fig. 3C), is approximately fourfold more sensitive to lysis by peripheral blood monocytes and lymphocytes in the presence of 2 μg/ml cisplatin.

Cisplatin decreases the tumor cell resistance to lysis by naturally occurring cytotoxic cells

Because in the previous experiments cisplatin was added at the beginning of the assay, we could not know if the increased lysis was a result of an increase in the activity of the effector cells or a decrease in the resistance of the tumor cells. For this reason effector cells or tumor cells were pretreated for 2 h with cisplatin then washed extensively before they were used in the lytic assay. As shown in Tables 1 and 2, the pretreatment of effector cells did not increase the lysis of the tumor cells. In contrast, when the tumor cells were pretreated with cisplatin they were more sensitive to lysis by naturally occurring cytotoxic cells, indicating that the increased lysis in the presence of cisplatin was a result of decreased resistance to lysis by naturally occurring cytotoxic cells.

Table 1. The pretreatment of targets with cisplatin increases their sensitivity to lysis by murine spleen cells

Target	Pretreatment (2 μg/ml cisplatin)		Specific lysis (%) at a spleen-cell:target ratio of		
	Targets	Spleen cells	5:1	20:1	80:1
B/C-BPDE F(C).3	−	−	0	4	12
	−	+	0	5	10
	+	−	12	24	36
B/C-BPDE F(G).1	−	−	3	8	14
	−	+	5	6	16
	+	−	15	31	55
B/C-BPDE F7.1A	−	−	0	0	6
	−	+	0	5	8
	+	−	9	20	45

Table 2. The pretreatment of targets with cisplatin increases their sensitivity to lysis by human monocytes and lymphocytes

Target	Pretreatment (2 μg/ml cisplatin)		Specific lysis (%) at a mono/lymphocyte: target ratio of		
	Targets	Spleen cells	0.3 : 1	1.2 : 1	5 : 1
DL-5	–	–	0	4	4
	–	+	0	1	3
	+	–	8	21	40
HTS-1	–	–	0	7	12
	–	+	4	8	13
	+	–	0	13	25
DL-4	–	–	6	5	9
	–	+	5	6	10
	+	–	11	16	30
B/C-BPDE F(C).3	–	–	5	6	11
	–	+	8	12	14
	+	–	16	30	46
B/C-BPDE F(G).1	–	–	1	2	0
	–	+	2	5	7
	+	–	9	18	31
B/C-BPDE F7.1A	–	–	2	3	5
	–	+	1	8	10
	+	–	14	41	57

Cisplatin increases the NC-, but not NK-mediated lysis of tumor cells

Since this lytic activity is present in normal, unimmunized mice and humans and because the activity is directed at a variety of human and murine cell lines it is unlikely that it is mediated by cells of the immune system (e.g., cytotoxic T cells). Because the effector cells are from untreated individuals it is also unlikely that the effector cells are activated macrophages. It is, however, possible that the increased lytic activity is mediated by NK or NC cells. NK activity, like NC activity, is not dependent on prior stimulation and is capable of lysing different cell types of both human and mouse origin. For this reason we determined the effect of cisplatin on the human and murine NK-mediated lysis of
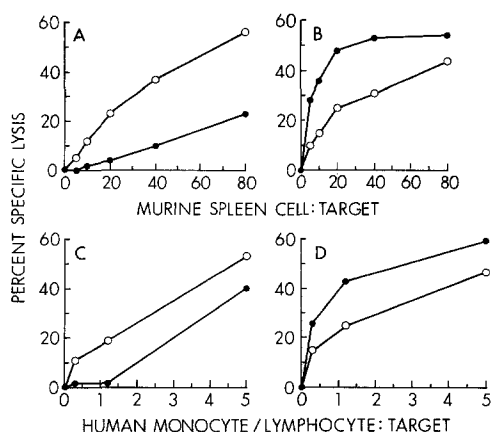

Fig. 4. Cisplatin increases the lysis of cells sensitive to NC-mediated lysis. **A** YAC-1 targets, **B** 10ME targets, **C** K-562 targets, **D** 10ME targets. The percentage specific lysis as a function of the effector-to-target ratio was determined in the absence of cisplatin (○), or in the presence of cisplatin at a concentration of 2 μg/ml (●)

NK-sensitive cell lines and the NC-mediated lysis of NC-sensitive cells. The cell line, YAC-1, is a murine cell line and K-562 is a human cell line (see Materials and methods). While YAC-1 is sensitive to lysis mediated by murine NK cells it is resistant to lysis mediated by murine NC effector cells [4, 16, 28]. K-562 is similar to YAC-1, in that it is sensitive to human NK-mediated lysis but resistant to NC-mediated lysis. The cell line, 10ME is sensitive to both murine and human NC-mediated lysis but is resistant to NK-mediated lysis [16]. As shown in Fig. 4B, D, 10ME is sensitive to murine as well as human NC-mediated lysis even in the absence of cisplatin. However, when cisplatin is added at 2 μg/ml there is an increase in the lysis of 10ME that is mediated by either murine spleen cells or human peripheral blood monocytes and lymphocytes. In contrast, the lysis of the NK-sensitive cell line, YAC-1, by murine spleen cells is reduced in the presence of cisplatin (Fig. 4A). And, as shown in Fig. 4C, the addition of cisplatin also reduces the lysis of the NK-sensitive cell line, K-562, by human peripheral blood monocytes and lymphocytes.

The cell line 10ME, when lysed by NC effector, shows a lag of 4–6 h before the release of ^{51}Cr is detected [4]. In contrast, the lysis of YAC-1 and K-562 begins within 30 min of the addition of effector cells. Although the kinetics of the effector-cell-mediated release of ^{51}Cr from NK-sensitive or NC-sensitive cells can be used to distinguish NK and NC effector cells, both NK and NC activity can be measured 14–16 h after the addition of effector cells; if cells are sensitive to NK-mediated lysis they will be sensitive whether their sensitivity is measured after 4–6 h or after 14–16 h. The addition of cisplatin does not alter the kinetics of the release of ^{51}Cr from these targets. In addition, the kinetics of the release of ^{51}Cr from the murine and human tumor cells in the presence of effector cells and cisplatin is similar to that of 10ME, in that there is a 4–6 h lag followed by a linear release of ^{51}Cr (data not shown).
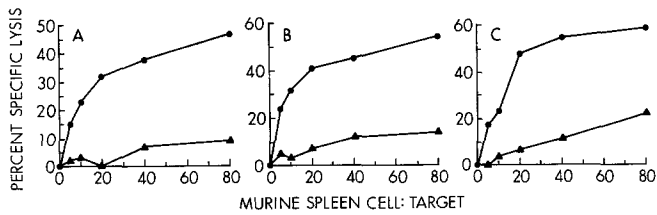
Fig. 5. Anti-murine TNF antibody inhibits the murine spleen-cell-mediated lysis of murine targets in the presence of cisplatin. **A** B/C-BPDE F(C).3 targets, **B** B/C-BPDE F(G).1 targets, **C** B/C-BPDE F7.1A targets. The percentage specific lysis as a function of the effector-to-target ratio was determined in the presence of cisplatin at a concentration of 2 μg/ml (●) or in the presence of cisplatin at a concentration of 2 μg/ml and anti-murine TNF antibody (▲)
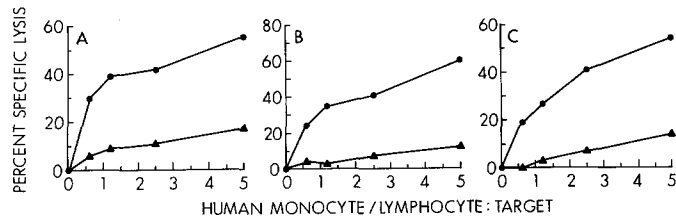

Fig. 6. Anti-human TNF antibody inhibits the human peripheral blood monocyte/lymphocyte-mediated lysis of murine targets in the presence of cisplatin. **A** B/C-BPDE F(C).3 targets, **B** B/C-BPDE F(G).1 targets, **C** B/C-BPDE F7.1A targets. The percentage specific lysis as a function of the effector-to-target ratio was determined in the presence of cisplatin at a concentration of 2 μg/ml (●) or in the presence of cisplatin at a concentration of 2 μg/ml and anti-human TNF antibody (▲)

Antibody to tumor necrosis factor (TNF) blocks the increase in naturally occurring cell-mediated lysis that results from the addition of cisplatin

We have previously shown that both murine and human NC activity is mediated by TNF [8, 21], and that anti-TNF antibodies can block the NC-mediated lysis of NC-sensitive cells [8, 21]. In order to determine whether the lysis of tumor cells by murine and human effector cells in the presence of cisplatin was dependent on TNF, anti-TNF antibody was added to the assays of murine and human effector cell activity. As shown in Fig. 5 A, B, C, the presence of rabbit antiserum, containing antibodies to murine TNF inhibits the cisplatin-augmented lysis of murine target cells by murine effector cells. Similarly, the addition of rabbit antibodies specific for human TNF blocks the lysis of these targets that is mediated by human effector cells in the presence of cisplatin (Fig. 6 A, B, C). Although lysis is dependent on TNF, TNF was not detected in the medium in which effector cells lysed tumor cells. This indicates that TNF is not released and is consistent with our findings that NC effectors use TNF, but do not secrete TNF (unpublished). Since neither cytotoxic T cells nor NK cells use TNF to mediate lysis, these results also support the hypothesis that the effector cells that mediate the lysis of these tumor cells in the presence of cisplatin are NC cells.

Discussion

It has been proposed that a component of the anticancer potential of the chemotherapeutic drug, cisplatin, resides in the ability of the host to respond to autochthanous tu-

mors in the presence of cisplatin [22, 23]. Cisplatin is known to increase a number of immune-related activities. Mice treated with cisplatin mount an enhanced splenic plaque-forming cell response to sheep erythrocytes and pneumococcal polysaccharide in vitro [2] and an enhanced responsiveness to tumor cells in vivo [14, 26] as well as in vitro [26]. In addition to its effect on immune activity, cisplatin has also been shown to stimulate spontaneous human monocyte-mediated cytotoxicity directly [14].

The data presented here indicate that naturally occurring cytotoxic cell activity may be a host antitumor defense mechanism whose efficacy is also increased in the presence of cisplatin. This is based on our findings that tumor cells that are normally resistant to lysis by naturally occurring cytotoxic cells show an increased sensitivity to lysis mediated by these cells in the presence of cisplatin. Cisplatin, when used clinically, is usually given at a dose that results in plasma concentrations of approximately 2.5 μg/ml [1]. Although cisplatin alone can be cytotoxic in vitro, cisplatin in the range of 1–2 μg/ml and under the conditions of the assays used here has a very low toxicity for the cells used in this study. Similarly, all of the tumor cell lines used in this study are relatively resistant to lysis mediated by naturally occurring cytotoxic cells. While these cells are not lysed by naturally occurring cytotoxic cells alone or cisplatin alone, the combination of cisplatin and either murine or human naturally occurring cytotoxic cells results in at least a 16-fold increase in the lysis of murine tumor cells (Fig. 1 and 2) and a minimum fourfold increase in the lysis of human tumor cells (Fig. 3). These increases are determined by comparing the number of effector cells required to give an equivalent level of target lysis in the presence and absence of cisplatin. We believe this to be a more accurate way of measuring the increase in lysis than simply comparing the percentage specific lysis at a single effector-to-target ratio. This is based on the observation that the relationship between the level of target lysis and the ratio of effectors to targets is not linear; with the possible exception of very low effector-to-target ratios, the percentage specific lysis does not double when the effector-to-target ratio is doubled. However, even if the increase in sensitivity is determined by a direct comparison of the percentage specific lysis at a given effector-to-target ratio, it is obvious that cisplatin increases the sensitivity of these cells to lysis mediated by naturally occurring cytotoxic cells. Even if cisplatin alone were to be more cytotoxic under other conditions (e.g., increased times of exposure or when cytotoxicity is measured by a reduction in cloning efficiency rather than by the release of ^{51}Cr) the rate of this cytotoxicity must be increased by naturally occurring cytotoxic cells.

The experiments in which either effector cells or tumor cells are pretreated with cisplatin (Tables 1 and 2) indicate that the increase in sensitivity to lysis by naturally occurring cytotoxic cells probably results from a decrease in resistance to these effector cells. We have previously shown that some tumor cells resist lysis by a protein-synthesis resistance mechanism [4]. A similar resistance mechanism may be operative in the tumor cells used here.

That the effectors that mediate the lysis of these tumor cells in the presence of cisplatin may be NC effector cells is shown by the following: (a) the lytic activity is present in normal, unimmunized mice and humans; (b) the lytic activity is capable lysing a variety of cell lines and is not

species-specific (Fig. 2); (c) the lysis of cells that are sensitive to NC-mediated lysis is increased in the presence of cisplatin (Fig. 4B, D), while the lysis of NK-sensitive cells is decreased in the presence of cisplatin (Fig. 4A, C); (d) the lytic activity is mediated by TNF (the effector molecule that is used by NC effector cells), but is not dependent on the release of TNF; (e) the kinetics of the release of ^{51}Cr from labelled tumors cells in the presence of cisplatin is similar to that of NC-sensitive cells.

The fact that NK activity is reduced in the presence of cisplatin (Fig. 4A, C) may also be of clinical significance. If the tumors that are being treated are sensitive to NK-mediated lysis the presence of cisplatin could reduce the ability of these tumors to be rejected by NK effector cells. This would be true whether cisplatin decreased NK effector cell activity or increased the resistance of NK-sensitive cells to the NK lytic mechanism.

Although these data strongly suggest that NC effectors are involved in the lysis of tumor cells in the presence of cisplatin, and that the effect of cisplatin can be seen when only targets are treated with cisplatin (Tables 1 and 2), we cannot formally exclude the possibility that the addition of cisplatin affects the lytic activity of other effector cells such that they express NC-like activity. Regardless of the type of effector cells that mediate lysis, the results presented demonstrate that naturally occurring cytotoxic cells of the host have the potential to increase the rate or level of killing of tumor cells in the presence of cisplatin. These cells along with other host responses, which are known to be increased in the presence of cisplatin [2, 14, 26], may contribute to the overall anticancer effect of cisplatin.

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