Host Immune Rejection of TA3 Ascites Carcinoma Cells Following Administration of a Water Soluble Carbodiimide[1]

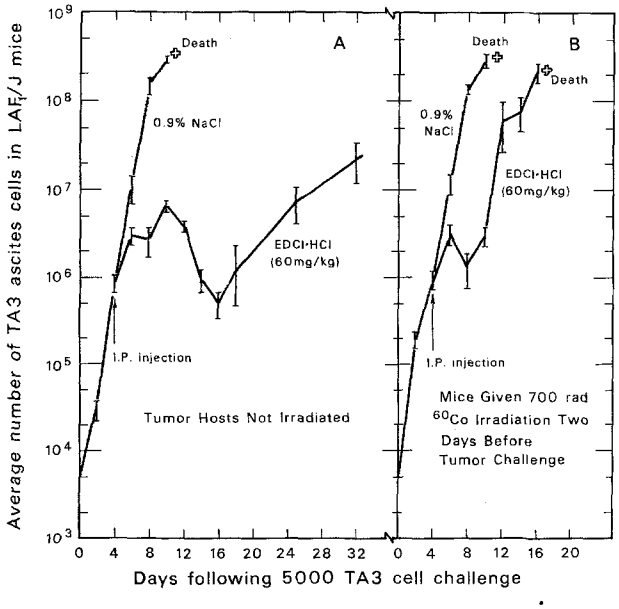
T. S. Tenforde, R. A. Fawwaz and R. A. Grossman[2]

Lawrence Berkeley Laboratory, Biology and Medicine Division, Building 74, University of California, Berkeley (California 94720, USA), 27 August 1975.

Summary. Regression of TA3 ascites carcinoma tumors occurred following i.p. injection of 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide · HCl. An immunological mechanism of drug action was indicated by the fact that no significant antitumor activity was demonstrable in mice that had previously received an immunosuppressive 700 rad dose of ^{60}Co radiation.

Several reagents that chemically alter the cellular surface have been demonstrated to enhance tumor immunogenicity, with a resultant host rejection of the modified cells[3]. Effective methods for increasing tumor immunogenicity include enzymatic cleavage of sialic acid residues from the cell surface[4–6], and chemical blockade of membrane sulfhydryl[7,8] and amino[9] functional groups. In a recent communication, the water soluble carbodiimide salt 1-ethyl-3-(3′-dimethylaminopropyl)carbodiimide · HCl (EDCI · HCl), which is highly reactive with electrically charged groups[10] and antigenic structures[11] at the cell surface, was demonstrated to promote the lysis of cultured neuroblastoma C1300 and carcinoma TA3 cells by humoral antibodies[12]. Based on tests of the relative chemotherapeutic effectiveness of this compound in immunocompetent and immunosuppressed tumor-bearing mice, evidence is now presented that administration of EDCI · HCl results in host immune rejection of TA3 ascites tumor cells.

Methods. The TA3 adenocarcinoma ascites tumor used in these studies is a hypotetraploid subline of the TA3/Ha tumor[13–15]. Tumors were maintained by the weekly i.p. injection of 10^5 cells into recipient isogeneic LAF_1/J mice (The Jackson Laboratories, Bar Harbor, Maine).



Growth kinetics of TA3 ascites tumors in LAF_1/J hosts following i.p. injection of 60 mg/kg EDCI · HCl or 0.9% NaCl solution. Tumor growth is shown for non-irradiated LAF_1/J hosts (panel A), and for hosts that have received an immunosuppressive 700 rad whole-body dose of ^{60}Co radiation (panel B). Each point of a TA3 tumor cell growth curve was determined as the mean ± standard error of the cell numbers measured in the peritoneal cavities of 5 mice.

Growth kinetics of TA3 ascites tumors were measured by determining the total number of cells in the peritoneal cavities of LAF_1/J mice as a function of time after tumor transplantation. The total population of TA3 cells per mouse was determined from the product of peritoneal volume and ascites tumor cell concentration. Volume was measured by the isotope dilution technique[16] using an i. p. injection of ^{131}I-labeled human serum albumin (Mallinkrodt, St. Louis, Mo.: 0.5 mCi/cm^3). Cell concentration was measured with a Model B Coulter Counter (Coulter Electronics, Hialeah, Florida).

The carbodiimide derivative EDCI · HCl (Ott Chemical Co., Muskegon, Michigan) was administered as a single 60 mg/kg i. p. dose in an injection volume of 0.2 ml of 0.9% NaCl solution. This dose level did not produce weight loss or mortality in either normal or TA3 tumor-bearing LAF_1/J mice. Control tumor-bearing mice received an i. p. injection of 0.2 ml of 0.9% NaCl solution. The chemical properties of EDCI · HCl[17], and the potential role of structural features of this drug in achieving cell surface modification and an antitumor effect[18], have previously been discussed.

Immunosuppression of LAF_1/J mice was carried out by administering a single 700 rad whole-body dose of ^{60}Co radiation at an exposure dose rate of 18 R/min.

Results. As summarized in the Table, when a single 60 mg/kg i. p. dose of EDCI · HCl was administered to

[1] Research support received from United States Atomic Energy Commission Contract No. W-7405-eng-48 with the Lawrence Berkeley Laboratory.

[2] University of California at Los Angeles Medical School, Los Angeles, California 90024, USA.

[3] M. D. Prager and F. S. Baechtel, Meth. Cancer Res. *9*, 339 (1973).
[4] B. H. Sanford, Transplantation *5*, 1273 (1967).
[5] R. L. Simmons and A. Rios, Science *174*, 591 (1971).
[6] L. Weiss, J. natn. Cancer Inst. *50*, 3 (1973).
[7] C. A. Apffel, B. G. Arnason and J. H. Peters, Nature, Lond. *209*, 694 (1966).
[8] M. D. Prager, I. Derr, A. Swann and J. Cotropia, Cancer Res. *31*, 1488 (1971).
[9] J. S. L. Lin and W. H. Murphy, Cancer Res. *29*, 2163 (1969).
[10] T. Tenforde, Adv. biol. med. Phys. *13*, 43 (1970).
[11] C. McNeil, E. Sax, K. J. Nielsen and J. D. Wahlen, Proc. Soc. exp. Biol. Med. *139*, 1144 (1972).
[12] R. A. Fawwaz, T. S. Tenforde and W. H. Mehlberg, Cancer Res. *35*, 679 (1975).
[13] T. S. Hauschka, Trans. N.Y. Acad. Sci. *16*, 64 (1953).
[14] T. S. Hauschka, L. Weiss, B. A. Holdridge, T. L. Cudney, M. Zumpft and J. A. Planinsek, J. natn. Cancer Inst. *47*, 343 (1971).
[15] S. Friberg, jr., J. natn. Cancer Inst. *48*, 1477 (1972).
[16] L. S. Kelly, J. D. Hirch, G. Beach and N. L. Petrakis, Proc. Soc. exp. Biol. Med. *94*, 83 (1957).
[17] T. Tenforde, R. A. Fawwaz, N. K. Freeman and N. Castagnoli, jr., J. org. Chem. *37*, 3372 (1972).
[18] T. S. Tenforde, R. A. Fawwaz and N. Castagnoli, jr., J. Pharm. Sci., in press.

Survival of TA3 tumor-bearing mice following injection of EDCI · HCl

Tumor inoculum[a] (No. Cells)	Injected compound[b]	Host irradiation[c] (rad dose)	No. of mice	No. of cures[d]	Mean survival time (days ± SD)
5000	0.9% NaCl	0	35	0	11.5 ± 1.7
5000	60 mg/kg EDCI · HCl	0	65	8	33.5 ± 12.9[e]
5000	0.9% NaCl	700	35	0	11.1 ± 0.8
5000	60 mg/kg EDCI · HCl	700	35	0	14.1 ± 3.5

[a] TA3 cells injected i.p. on Day 0. [b] I.p. injections were made on Day 4. [c] Tumor hosts were irradiated 2 days before Day 0. [d] Mice surviving on Day 60 were considered cured. [e] The difference in mean survival time of drug-treated and control non-irradiated mice was statistically significant at the level $p < 0.001$.

LAF_1/J mice on the 4th day following challenge with 5000 TA3 ascites cells, a 3-fold increase was observed in the mean survival time of drug-treated mice relative to 0.9% NaCl solution-injected control mice. Complete tumor regression and indefinite host survival occurred in 12% of the tumor-bearing mice injected with EDCI · HCl. When this compound was administered to mice that had received an immunosuppressive 700 rad whole-body dose of ^{60}Co radiation 2 days prior to injection of a 5000 TA3 cell challenge (6 days prior to drug injection), no significant increase in mean survival time of the irradiated, drug-treated mice was observed relative to that of control mice.

TA3 ascites tumor growth kinetics, and the response to EDCI · HCl injection, were also studied in non-irradiated and irradiated LAF_1/J mice. When EDCI · HCl was administered as a single 60 mg/kg i. p. dose to non-irradiated mice on the 4th day after a 5000 TA3 cell challenge, tumor growth kinetics demonstrated a distinct 3-phase response (Figure A): a) For 6 days following drug injection, tumors underwent net growth, although the average growth rate was reduced relative to that of tumors in 0.9% NaCl solution-injected control mice. b) The tumors then regressed in average size by approximately an order of magnitude during the next 6 day period. c) A slow, but progressive, net tumor growth ensued during the following 2 to 3 weeks, at which point the drug-injected mice expired except for approximately 10% whose tumors exhibited complete regression.

As shown by TA3 growth kinetics plotted in Figure B, administration of a single 60 mg/kg dose of EDCI · HCl into irradiated mice led to a reduced rate of tumor growth for a period of 4 days post-injection. Tumor growth then resumed at a rate identical to that of control tumors. Irradiated mice receiving EDCI · HCl expired, on the average, only 3 to 5 days later than control mice.

Discussion. Data presented here demonstrate that injection of the carbodiimide derivative EDCI · HCl leads to regression of TA3 tumors in immunocompetent, but not in radiation immunosuppressed LAF_1/J mice. Similar results have previously been described with TA3 tumors following in vivo administration of neuraminidase, an enzyme that cleaves *O*-glycosidically linked sialic acid residues from the cellular surface[4,19,20]. The molecular mechanisms involved in EDCI · HCl action are not defined by the present experimental results; however, the possibility can be ruled out that reaction of this compound with cell surface components promotes the cytotoxic activity of host immune agents present at the time of drug injection. The experimental basis for this conclusion is the fact that no humoral or cell-bound antibodies directed against the TA3 tumor can be detected in isogeneic LAF_1/J mice at 4 days following a 5000 TA3 cell challenge, i.e. at the time of EDCI · HCl injection[12].

Two alternative mechanisms of EDCI · HCl action are suggested by TA3 growth kinetics, which demonstrate tumor regression does not occur until 6 days following drug injection (Figure A). The first of these mechanisms is a non-specific stimulation of the host reticuloendothelial system by EDCI · HCl, with a resultant heightening of immune defenses against the TA3 tumor. In this context, it has been reported that treatment of spleen cells with bacterial endotoxin in vivo or in vitro elicits a cytotoxic reaction against TA3/Ha tumors[21,22]. A comparable mechanism of EDCI · HCl antitumor action does not appear to be operative, however, since preliminary in vitro studies in our laboratory have indicated that incubation of spleen cells with EDCI · HCl does not render them cytotoxic to TA3 cells.

A second possible mechanism of EDCI · HCl action that is compatible with the observed TA3 growth kinetics in drug-injected mice is an increase of tumor immunogenicity resulting from the reaction of EDCI · HCl with cell surface components, thereby leading to host immune recognition of the carbodiimide-modified cells and a subsequent production of cytotoxic antibodies. An increase in tumor immunogenicity following reaction with EDCI · HCl could result from the exposure of tissue antigens or tumor neoantigens that are normally not expressed at the TA3 cell surface. Alternatively, complexes formed by covalent linkage of EDCI · HCl with TA3 cellular surface groups may serve as haptenic antigen determinants, in response to which the host produces soluble or cell-bound antibodies that are cytotoxic to the carbodiimide-modified cells. In order to clearly define the mechanism by which EDCI · HCl increases tumor immunogenicity, a determination must be made of the specificity of antibodies produced in response to challenge with carbodiimide-modified cells.

[19] B. H. SANFORD and J. F. CODINGTON, Tissue Antigens *1*, 153 (1971).
[20] L. S. KELLY, Proc. Am. Ass. Cancer Res. *12*, 63 (1971).
[21] J. GROHSMAN and A. NOWOTNY, J. Immun. *109*, 1090 (1972).
[22] C. YANG and A. NOWOTNY, Infect. Immun. *9*, 95 (1974).