ANTIBODY-CYTOTOXIN CONJUGATES AS IMMUNOTHERAPEUTIC AGENTS

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I. INTRODUCTION

The concept of using therapeutic agents bound to tissue-specific carriers can be traced at least as far back as 1906 (1); and since that time several approaches have been pursued to enhance the efficacy of existing antitumor agents. Treatment of tumors with antitumor antibodies conjugated to potent cytotoxic agents offers the possibility of combining the cytocidal effect with the specificity of the antibody. This approach has been successfully applied in experimental systems when the cytotoxic agent is either covalently or non-covalently bound to the antibody. An ideal cytotoxin would i) retain toxicity, ii) retain specificity for tumor membrane antigen, iii) be exceptionally potent iv) upon cell death be degraded or neutralized, v) be nonimmunogenic. The purpose of this study is to outline the methodology that will be used to prepare and test various combinations of antibody-cytotoxin conjugates. The basic questions to be answered in this study are "can antibody-cytotoxin conjugates be effectively produced to selectively kill tumor cells in vitro?" and "are these conjugates effective as therapeutic agents in altering the course of tumor growth in vivo?" In section II evidence is presented on past work with antibody-cytotoxin conjugates as an immunotherapeutic possibility. The tumor systems available for experimentation, experimental format, and methods are then outlined in section III.

II. BACKGROUND LITERATURE

Can antibody-cytotoxin conjugates be produced and are they effective in selectively killing tumor cells ?

A. TOXINS

Diptheria toxin, a single polypeptide of 62,000 daltons, is known to have a lethal effect by inhibiting protein synthesis in susceptible cells. The N-terminal region of 22,000 daltons, designated fragment A (for "activity"), is liberated by proteolytic and reductive treatment (2). This fragment catalyzes the inactivation of the eucaryotic elongation factor (EF-2), by covalently linking the adenosine diphosphate ribose moeity of NAD+ with transferase II, producing an inactive derivative of the elongation factor via the following reaction (3 4):

$$(NAD+) + \text{transferase II} \longrightarrow \text{ADP-ribose-transferase II} + \text{nicotinamide} + (H+)$$

The complementary domain of 39,000 daltons is designated fragment B (for "binding"), and is responsible for attaching the toxin to specific cell surface receptors and is believed to form a channel through which fragment A can penetrate. Alternatively other evidence claims that the toxin attaches to an endocytotic vesicle followed by disruption of the vesicle to release the toxin into the cytosol (5 6 7).

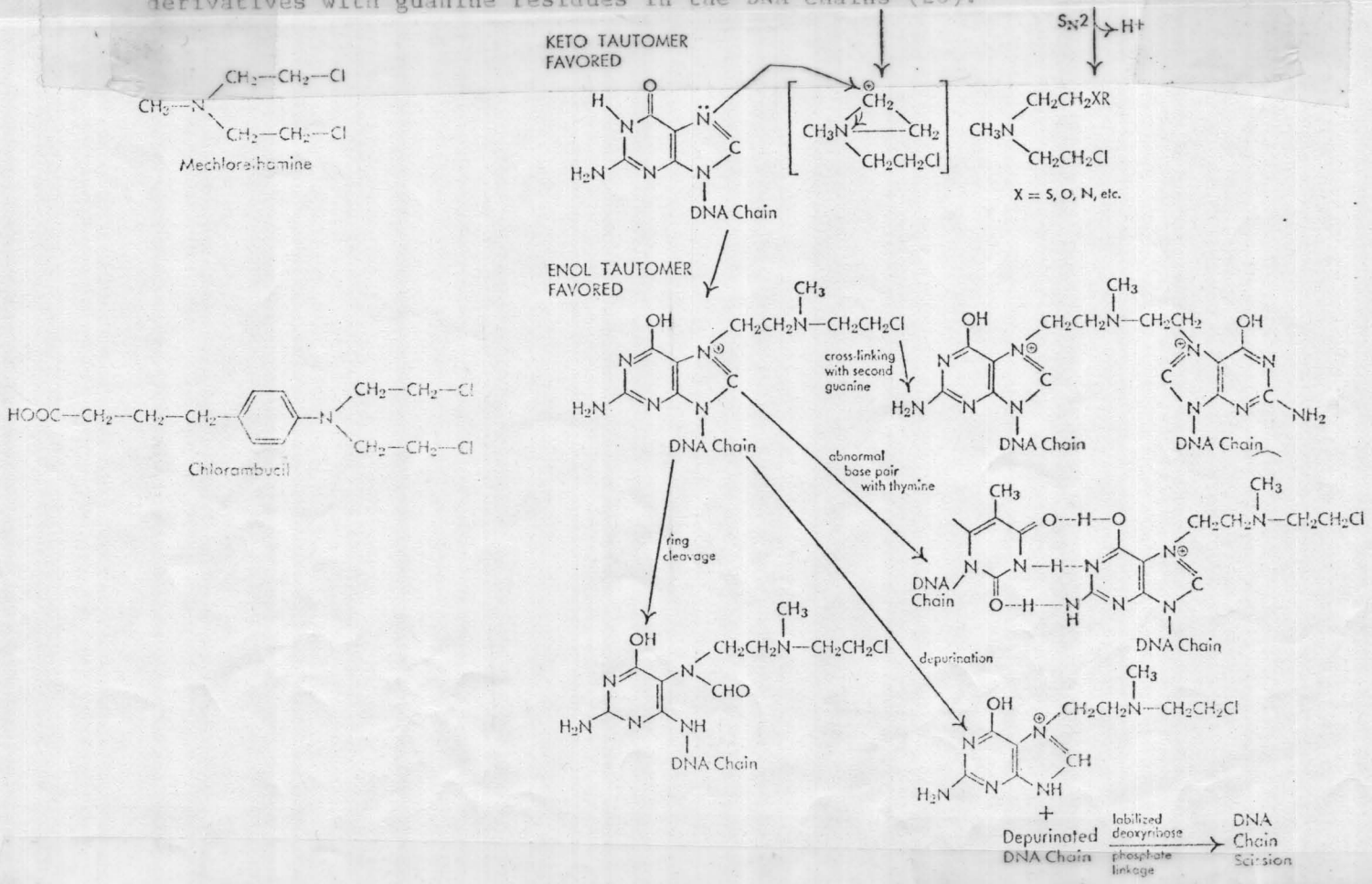
Moolten et al. (8) covalently coupled diptheria toxin to antibodies directed against SV-40-induced antigens and produced a conjugate that exhibited a 2.2-fold greater "specific toxicity" for SV40-transformed tumor cells in vitro compared to a conjugate of normal immunoglobulin. Despite the small differential his conjugate prolonged the life of and occasionally cured hamsters with SV40-induced lymphoma. Their glutaraldehyde method of conjugation was suggested by Thorpe et al. to have formed intra-chain bonds within the toxin molecule, preventing fragment A from dissociating from fragment B (9). Thorpe's method employs a milder oxidation scheme that not only avoids this complication but also prevents the formation of toxin polymers, resulting in a thousand fold increase in cytotoxicity over that of toxin alone (9).

When diptheria toxin is conjugated to antibody, fragment B is inactivated; however, the binding function of fragment B is replaced by that of the cell specific antibody (10). The toxic segment, fragment A, retains its ability to reach the cytoplasm, perhaps by endocytosis (11), and kill the cell. Particularly interesting with regard to the treatment of solid tumor tissue is the toxin's extreme potency, only one or two molecules being required to kill the cell (12). Long term use of diptheria toxin conjugates in vivo could be significantly impeded by the development of host antibody against the conjugate. Moolten et al. (13) found that hamsters responded to the diptheria toxin conjugates with neutralizing antibodies but that this response could be greatly reduced and delayed with the administration of the immunosupressant-antineoplastic cyclophosphamide. This is a possibility that could apply to most cancer patients since Ipsen shows evidence that 55 % of American adults exhibit serum levels of diptheria antitoxin below the 0.01 U/ml. protective threshold (14). Lastly, conjugation of the toxin to IgG has been shown to reduce non-specific toxicity by at least 50% (9).

Phospholipase C, the alpha-toxin of the gas-gangrene bacillus (Clostridium welchii), acts by hydrolyzing the cell surface phospholipids and consequently does not require internalization for toxicity. Moolten et al.(15 16) have successfully conjugated this to rabbit anti-sheep gamma-globulin directed against the Forssman antigen of sheep red blood cells. The conjugate was selectively hemolytic to sheep RBC but not to human RBC, whereas the phospholipase alone was preferentially hemolytic to human RBC. The conjugate failed to hemolyze either human or sheep RBC when normal rabbit globulin was used. These workers also successfully conjugated ribonuclease to antibody directed against HeLa cells. Ribonuclease is virtually non-toxic in an unconjugated form and highly toxic if delivered to the correct intracellular sites. The preparation, containing 0.17-0.50 mg of ribonuclease/ml, was completely effective at inhibiting protein sysnthesis in HeLa cells. A central problem with both phospholipase C and ribonuclease, at least for this group, was purifying and concentrating these toxins.

ALKYLATING AGENTS

Alkylating agents [nitrogen mustard] have in common the property of undergoing chemical reactions that generate highly reactive electrophilic carbonium ions that readily form covalent bonds with various nucleophilic substance, including biologically significant moieties as phosphate, amino, carboxyl, hydroxyl, sulfhydryl, and imidazole groups. Brookes and Lawley (17 18 19), working the interaction of DNA with the alkylating agent, suggest that the purine base guanine, number 7 nitrogen being highly nucleophilic, is the key biological target that is alkylated. The table below illustrates the possible courses of the reaction of the nitrogen mustard and related derivatives with guanine residues in the DNA chains (20):



Chlorambucil, by way of its electron withdrawing substituted phenyl group, has a reduced rate of carbonium ion formation, and as a result can reach distant sites in the body before reacting with elements in the blood and other tissues. While it is possible to covalently bind chlorambucil to antibody it readily forms stable noncovalent complexes with the immunoglobulin and retain its effectiveness (21). The exact mechanism of tumor inhibition by the conjugate remains to be elucidated. Although complexing chlorambucil with immunoglobulin is essential it has been postulated by Ghose et al.(22 23) that a form of synergism occurs between the drug, the antibody, and the cell. Segerling et al.(24) found that upon exposure of target cells to cytotoxic drugs rendered these cells more susceptible to antibody. In addition, Guclu et al.(25) state that "capping" facilitates the transport of cytotoxic drugs across the membrane. Mixtures of the drug and antibody or successive injection of drug and antibody are also effective in the in vivo situation (25 26), as well as in vitro (27 28). Despite its unresolved mode of action, antibody bound chlorambucil is not without its successes. Regression of all metastatic nodules has been reported for a patient with dissiminated melanoma after treatment with chlorambucil linked to goat anti-tumor globulin (29). In another study of patients with inoperable recurrent malignant melanoma, 7 of the 13 treated with chlorambucil-bound xenogeneic antimelanoma globulin showed regression or stabilization of their disease with a median survival of 20 months compared to 3 months for another 11 patients treated with dimethyltriazenoimidazole (DTIC), the most effective and consistent chemotherapeutic agent available for treatment of the disease (30).

ANTIMETABOLITES

Methotrexate, an analog of folic acid, inactivates the enzyme dihydrofolate reductase, which the responsible for the reduction of dihydrofolate to tetrahydrofolate an essential coenzyme. The inhibition caused by methotrexate is quite profound; its kinetics are referred to as "pseudo-irreversible", i.e. the drug cannot be displaced from the enzyme to a significant degree by concentrations of folic acid that are attainable physiologically. Burstein and Knapp have succeeded in conjugating this drug to anti-C3HebFej mouse embryonal ovarian carcinoma antibody using a carbodiimide reagent (31). The study detailed mice challenged with intraperitoneal injections of tumor cells show longer mean survival times when treated 24 and 48 hrs afterwards with the conjugate. Mice treated with a mixture of drug and antibody also showed marked improvement presumably by the same synergetic effect as evidenced with chlorambucil.

ELEMENTAL ISOTOPES

The therapeutic use of boron-10 in the treatment of cancer is based on the nucleus' ability to absorb low-energy thermal neutrons(.025eV), liberating short-range, high-energy fission fragments(2.5MeV) upon capture. Low energy neutrons of this magnitude do not seriously affect tissue since the elements that are involved, carbon, oxygen, nitrogen and hydrogen, do not react with neutrons so much as Boron-10 due to their relatively small nuclear cross

section (Boron-10 = 4000 barns; C,O,N,H < 1 barn; 1 barn = a measure of area = probability of nucleus to capture a slow neutron). The range of the high-energy alpha particle emmitted is less than 10 microns, and as a result will not damage nearby tissue with no B-10 attached to it. There are several requirements for successful neutron-capture therapy, namely: a concentration of B-10 of 15mg / kg of tumor evenly distributed with a source of neutrons of sufficient flux at the site of the Boron for cellular destruction (10^{12} neutrons/cm^2); and specific location of the Boron on the tumor cells as not to damage healthy tissue (32). Hawthorne and Wiersema (33), were able to incorporate Boron-10 in antibody directed against C57 B110 mouse lymphocytes. In an experiment where the B-containing antibody was incubated with both C3H and C57 B110 lymphcytes, subsequently washed with tissue culture media and then subjected to neutron radiation, the C3H cells were unaffected while the C57 B110 cells showed a sharp linear decline in survival with exposure to the neutron radiation.

Radioactive Iodine, specifically I-131, is the most widely used of the radioactive iodine isotopes, owing to its availability and desirable properties. It has a half-life of 8 days and its chemical properties are identical with the stable isotope. Ghose et al.(34) successfully produced a rabbit antiserum to Ehrlich ascites tumor cells which after absorption was shown by immunoflourescence to bind specifically to the tumor cells. Mice challenged with injections of Ehrlich ascites tumor cells which had been incubated with gamma-globulin of the antiserum coupled with I-131 remained healthy whereas those mice inoculated with tumor cells alone or with tumor cells incubated with non-iodinated antiserum or with iodinated non-immune globulin died within fourteen days after injection. Some years later, Ghose and Guclu (35), evidence interesting results of C57BL mice treated with different doses of radio-iodinated rabbit anti-EL4lymphoma antibody, versus mice treated with normal rabbit globulin conjugated with or without the radio-iodine, or mice treated with unconjugated immune, anti-EL4 globulin, after being challenged with an inoculation of 10,000 syngeneic EL4 cells. Although their globulin consisted of only 1% specific anti-EL4 antibody molecules, they obtained a tumor-free survival rate of 200 days in mice treated with radio-labeled conjugate, 72 hours after the EL4 tumor cell injection (1,028uCi/8mg globulin) as opposed to a 16 day median survival rate for control goups. These same "cured" mice were re-challenged with EL4 cells and died 18.8 days thereafter. The suppression of these i.p. injected and free-floating tumor cells has particular relevance to the treatment of leukemia where the cells are more readily accessible to the radio-globulin than the in the case of the solid tumor.

ANTIBODY

Several different methods have been employed in the attempt to generate highly specific antitumor antibody. The most common method involves the immunization of a xenogeneic host followed by an absorption of the serum with normal tissue (36). Another method involves the use of an animal tolerized to normal tissue and subsequently immunized with tumor cells of similar histology (37). A very recent method involves fusing lymphoid cells from a tumor immunized donor with cells from an in vitro adapted myeloma, and isolating the hybrids producing the desired antibody. This

method produces virtually inexhaustible supplies of pure specific, monoclonal antibody, in vitro (38).

Immunological enhancement of tumor growth has been observed in both lymphocytic and non-lymphocytic types of tumor cells. As the amount of antibody administered decreases, tumor growth may be enhanced (39,40). This effect may or may not occur with in vivo applications of the antibody-cytotoxin conjugate depending most likely on the conjugate's ability to kill the tumor cell. There is clear evidence that after antibody has attached to tumor cells it remains there for only a finite period of time, becomes absorbed into the cell and is degraded. Witz (41), using paired-labelled IgG from specific antiserum and non-immune serum, demonstrated preferential degradation of the tumor-specific IgG in ascites tumor cells. Only partially degraded antibody was obtained from detergent lysed cells and since these fragments could not be dissociated from the cells by low pH buffers prior to lysis, it seems that these IgG fragments must have been interiorized. Presumably there will be sufficient numbers of cytotoxin molecules released to exert a lethal effect upon the cell, which may circumvent the antibody-mediated enhancement of tumor growth.

III. EXPERIMENTAL FORMAT AND METHODS

A. Available antibody and tumor systems to be examined

I. Human

a). DHL-1; Esterase positive, phagocytosis positive, hystiocytic lymphoma cell type.

1. Mouse antibody producing hybridoma clones
   i). SAG-6; anti-DHL-1 cell surface IgG; shown by indirect I.F.
   ii). 3D7 subclone 2B9(gamma-1); anti-DHL-1 virus; reacts with viral p28 of 65,000 M.W. membrane associated precursor.
   iii). MEG-8 (parental to NS-1) negative control; produces gamma-1, has anti-SRBC activity.

2. Rabbit anti-human DHL-1 antiserum
   i). Adsorbed rabbit anti-human DHL-1; M.Bieber; binds specifically to DHL-1 and not to B-lymphoblastoid cells. This anti-serum will be further purified by ammonium sulfate precipitation, and Sephadex G-200 gel filtration. The IgG will be tested for binding to DHL-1 cells. The IgG fraction from the preimmunization serum will serve as a negative control.

b). Daudi EBV-positive African Burkitts lymphoma cell line

1. Mouse antibody-producing hybridoma clones
   i). 5 clones available currently being tested for membrane binding activity by indirect I.F.

II. Mouse

a). KKT-2; spontaneous AKR thymic lymphoma cell line.

1. Rat hybridoma clones

i). Rat anti-KKT-2 antibody producing hybridoma clone (work in collaboration with Dr. I. Weissman); The antibody binds to all RadLV induced tumor, and not to normal tissue (thymus,), virus, or viral p30. It reacts with 60,000; 85,000; and 95,000 M.W. Gag precursors which occur as membrane associated antigens.

ii). [rat hyb. clone neg. control]

B. Methods

I. Antibody purification

Hybridoma antibody will be purified by Sephadex G-200 gel filtration, or DEAE ion exchange chromatography. The fractions will be concentrated by Amicon ultrafiltration and dialyzed. Protein concentrations will be assessed by u.v. absorbance at 280nm.

II. Formation of antibody-cytotoxin conjugates.

This section describes briefly the procedures that will be used in IgG-cytotoxin conjugate formation. The IgG/cytotoxin ratio will varied and analyzed for optimization according to the in vitro results.

a). Toxins

1). Diptheria toxin is purified from the filtrate of a culture of Corynebacterium diptheriae by ammonium sulphate fractionation and chromatography on Sephadex G-100 using the procedure of Collier and Kandel (44). The antibody-dipheria toxin conjugate will be prepared and purified according to the procedure of Thorpe et al.(9). For detection and assay, a fraction of the toxin will be radio-labelled with I-125 (42).A mixed chlorambucil anhydride is conjugated to the IgG as a reactive intermediate, which is then separated from unreacted components using Sephadex G-25 filtration. The conjugate will then be concentrated by Amicon ultrafiltration. This reactive intermediate will be heated to activate the mustard groups, and mixed with a desired quantity of toxin to produce a mixture of IgG-toxin conjugates, IgG-IgG polymers, unreacted IgG-chlorambucil and lone toxin and chlorambucil. These products will be separated

on a Sephadex G-150 column, and the fractions will be analyzed for u.v. absorbance (280nm.) and, the presence of toxin associated radioactivity. The fractions containing the desired IgG-chlorambucil-diptheria toxin conjugate are further separated from IgG polymers and unreacted IgG-chlorambucil by ultracentrifugation or by affinity chromatography using an anti-toxin antibody.

2). Phospholipase C conjugation and purification can be accomplished using the procedure of S.H. Zajdel (15). However, since difficulties in concentrating this toxin have been reported, it is believed that this procedure has lower priority.

b). Alkylating Agents

1). The factors governing the specific toxicity of IgG-chlorambucil conjugates has been extensively reviewed by Guclu et al. (45), consequently their coupling procedure will be used here. A 1:140 chlorambucil/protein ratio (weight/weight) yields a product that retains 97% of the original alkylating activity of chlorambucil when conjugated with human IgG at a pH of 2 in the cold. Separation of the conjugate from free chlorambucil is accomplished by Amicon ultrafiltration. For small amounts of material ultracentrifugation will be used. The product is then analyzed spectrophotometrically.

c). Antimetabolites

1). Methotrexate will be conjugated and purified by the procedure of Raso and Schrieber (46). Tritiated methotrexate, a derivative of glutaric acid, is suspended in acetic anhydride which yields a 6-membered anhydride which then reacts readily with the epsilon-amino groups of gamma globulin. The product is mixed with IgG at room temperature for 18 hrs. and is then separated by gel filtration, and analyzed (u.v. absorbance and radioactivity).The final product is then extensively dialyzed and concentrated.

d). Elememtal isotopes

1). Boron-10 conjugation and purification can be accomplished by the procedures of Hawthorne et al. (33,47). A diazonium salt of 1-(4-aminophenyl)-1,2-dicarba-clovo-dodecaborane(12) will be made, which readily binds to the tyrosine, histidine and lysine residues of proteins. The diazonium salt is stirred with IgG in PBS (pH 8.0) at 4 degrees C. After 24 hrs. the mixture is centrifuged, and the supernatant (containing the conjugate) is fractionated over a G-200 column. Fractions containing the conjugate are pooled, dialyzed, and concentrated.

2). I-131, or I-125 conjugation and purification can be done by the chloramine T procedure of Hunter et al. (48). Briefly, IgG

in PBS will be is mixed at 4 degrees C. with I-125 dissolved in .1M NaOH. To this mixture is added chloramine T in PBS. The mixture is agitated for 30 min. after which the reaction is stopped by the addition sodium metabisulfite in PBS. The conjugate is freed from unbound I-125 by repeated dialysis with PBS until no radioactivity is detected in the diffused PBS. The conjugate is isolated by Sephadex G-200 chromatography and monitored by u.v.absorbance and radioactivity.

C. In vitro Assay of Antibody-Cytotoxin Conjugates.

The purpose of these experiments is to evaluate the ability of antibody-cytotoxin conjugates to selectively kill tumor cells in vitro. Viable tumor cells will be incubated with varying quantities of either tumor specific antibody-cytotoxin conjugates or control.

I. In vitro detection methods

a). Tritiated leucine incorporation will be the primary method of assessing cell viability. Cell samples are pulsed for 16 hrs. with 50uCi/ml tritiated leucine and measured for radioactivity as described (43).

b). Trypan Blue or Eosin Red exclusion will visually indicate cell viability. The dye indicates viability for both tumor and non-tumor cells, living cells actively prevent dye incorporation and thus appear clear, while dead cells appear colored.

c). The Chromium-51 release assay may serve as an alternate cell viability assay, however Cr-51 release is dependent upon cell lysis which may not occur appreciably in this system.

d). Fluorescene conjugated anti-tumor conjugate will visually indicate conjugate specificity for tumor cells. Using the procedure by Johnson and Holbrow (49), a fraction of the conjugate can be labelled and mixed with unlabelled conjugate as a visual indicator of tumor cells.

II. The in vitro test categories are as follows:

a). Tumor cells + tumor-specific antibody-cytotoxin conjugate

Methods used: a,b

b). Tumor cells + tumor-specific antibody + cytotoxin

Methods used: a,b

c). Tumor cells + cytotoxin

Methods used: a,b

d). Tumor cells + tumor-specific antibody

Methods used: a,b

e). Non-tumor cells + tumor-specific antibody-cytotoxin conjugate

Methods used: a,b

f). Mixed population of non-tumor cells + tumor cells + tumor-specific antibody-cytotoxin conj.

Methods used: b,d

For the evaluation of the specific toxicity of "simple" cytotoxin-antibody conjugates (e.g. methotrexate-antibody, or I-131 -antibody), the above test categories will suffice. However, the diptheria toxin conjugate is a complex consisting of three molecular species: IgG, chlorambucil, and diptheria toxin. This conjugate is prepared by the linkage of diptheria toxin to an antibody-chlorambucil precursor. The in vitro test categories for testing of the diptheria toxin-chlorambucil-antibody conjugate will consist of categories a-f above, substituting "tumor-specific antibody-chlorambucil" for "tumor-specific antibody". Thus two additional control groups to be included are:

g). Tumor cells + tumor-specific antibody + chloram. + diptheria toxin.

Methods used: a,b

h). Tumor cells + tumor specific antibody

Methods used: a,b

D. In vivo assay of antibody-cytotoxin conjugates.

The conjugate's ability to prevent tumor formation in mice that have been challenged by tumor cell injection will be determined by the in vivo phase of experimentation. Only conjugates showing positive tumor-specific cytotoxicity in the in vitro tests will be examined in the in vivo studies.

I). In vivo methods

a). The in vivo studies of human tumor will be possible by intracranial heterotransplantation in (balb/c) nude mice followed by intracranial treatment by the conjugate. Murine tumor will be studied by intraperitoneal injections and treatment in AKR mice.

b). The L.D. 70 is defined as that number of tumor cells which 70% of mice injected. Groups of mice will be injected with varying tumor cell numbers, generating the dose response curve. Extrapolation from this curve will yield the L.D. 70 for the tumor under study. The L.D. 70 dose of tumor will be used in the subsequent experiments.

c). Tumor challenged mice (human and mouse tumor systems) are separated into four categories that will receive identical treatment regimens at different times following the tumor challenge. These categories are as follows:

1). Treatment concurrent to tumor challenge (t=0)

2). Treatment 24 hrs. following tumor challenge (t=24 hrs.)

3). Treatment 48 hrs. " " " (t=48 hrs.)

4). Treatment 96 hrs. " " " (t=96 hrs.)

Categories 3, and 4 will be run only if results from categories 1, and 2 show conjugate-mediated protection against tumor formation.

d). Each category of mice is divided into groups as follows:

Group No.	Description	No. of mice	Treatment	Injection Quantities
1	Effect of trtmt. against tumor cell injection	15-20	Specific anti-tumor IgG-cyt. conjugate	
2	" "	"	Specific anti-tumor IgG mixed with unbound cytotoxin	
3	" "	"	Non-specific IgG-cyt. conjugate	
4	" "	"	Specific anti-tumor IgG alone	
5	" "	"	Cytotoxin alone	
6	" "	"	Non-specific serum alone	
7	Control	"	saline	

As an initial screen for anti-tumor IgG-cytotoxin conjugates, only groups 1,2, and 7 are run, since the results from these groups are sufficient to determine whether or not the conjugate is responsible for any anti-tumor cytotoxicity. When protection occurs in group 1 but not in groups 2 and 7, the trial will be repeated, running all seven groups for a full analysis. This will determine the precise combination of molecular species that influence the course of tumor growth. A negative result in the screening trial (i.e. no protection in groups 1,2, or 7; or no protection in groups 1 and 7). If no protection is observed in group 1 compared to control groups 2, and 7, then the experiment will be repeated with new IgG-cytotoxin conjugate.

e). The following parameters will be measured for treated and untreated groups of mice.

1). Survival times after injection

2). % survival (number of survivors/total number)

3). Tumor takes, regressors, tumor size with time, and time of tumor appearance.

4). Tumor histology

f). Statistical analysis of survival times of each group using Newman Keuls multi-comparison testing (53) will establish whether or not the survival time means are statistically different from one another. The outcome of this analysis will reflect the degree to which the differences in survival times are due to the difference in treatments.

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July 15, 1974

Mr. Pierre A. Mandel
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All of us at Rensselaer offer our best wishes for your continued success.

Sincerely,

Richard J. Grosh

Richard J. Grosh

RJG:m

UNIVERSITY OF CALIFORNIA, SAN FRANCISCO

BERKELEY • DAVIS • IRVINE • LOS ANGELES • RIVERSIDE • SAN DIEGO • SAN FRANCISCO

SANTA BARBARA • SANTA CRUZ

LANGLEY PORTER NEUROPSYCHIATRIC INSTITUTE

SAN FRANCISCO, CALIFORNIA 94143

July 15, 1986

re: Pierre Mandel

Dear Members of the Committee on Admissions:

I have been asked to submit a letter in support of Pierre Mandel for admission to medical school, and I am pleased to do so, not only because I think so highly of Pierre, but because medicine continues to attract individuals of such high caliber.

I have known Pierre since we were both undergraduate and graduate students at Stanford University. Though I have not had the opportunity to directly evaluate his academic performance as a course instructor, I do feel comfortable sharing my own perceptions of Pierre's suitability for a career in medicine based on a long standing association with him and my own knowledge of the medical profession.

To begin with, I have always been singularly impressed with Pierre's intellectual prowess and his ability to reason and evaluate information in a critical, disciplined fashion. As an undergraduate and graduate student, he was known for his insightful and creative solutions to problems both related to academic coursework as well as to difficulties arising outside the classroom. Early on, he developed a robust commitment to medical science as evidenced by his sustained research efforts in cancer biology and pharmacology. This often required working long hours into the evening and on weekends with little reward other than the excitement of the work itself. Though he experienced some academic difficulty early on, he demonstrated through his performance in more advanced coursework in the biological sciences and his research commitment that he is more than capable of handling the rigorous academic demands of medical school. Pierre has always maintained a healthy exuberance for the scientific method of inquiry and is currently motivated to pursue a career in academic medicine. I believe he could be a thoughtful and productive scholar in any branch of medicine.

Pierre presents a very conscientious and serious demeanor, yet he is equally well embued with a strong sense of compassion and sensitivity for the human condition. This was clearly manifest in his interpersonal relationships and particularly in his clinical work at Stanford Children's Hospital which inspired him even further towards a medical career.

All in all, Pierre's intellectual development and breadth of experience are impressive. He should be considered an exceptional candidate for any medical school in the country. If I can provide any further information in support of Pierre's application, please do not hesitate to contact me.

Sincerely,

Raymond Deicken, M.D., M.S.
Resident III in Psychiatry

STANFORD UNIVERSITY
STANFORD, CALIFORNIA 94305

DEPARTMENT OF BIOLOGICAL SCIENCES

June 17, 1980

Re: Pierre Albert Mandel

Dear Admissions Committee Members:

Mr. Pierre Mandel has asked me to write to you concerning his qualifications for medical school. Mandel took Vertebrate Biology and Organismal Development from me, received grades of A and B, respectively, and ranked approximately 24% and 33% below the tops of those two groups of 250 and 90 preprofessional students. He did a good job in the Vertebrate Biology laboratory in all phases of the course.

Mandel's academic record is very unusual. He had a bad time during a period as an undergraduate, due to parental problems and financial problems. Those sorts of peripheral things led him to get a number of C grades. He has stayed on at Stanford for a fifth year to demonstrate that he does have the capacity to handle medical school academic work. To that end, he has gotten grades of A in physical chemistry, in chemistry lab and the biochemistry course taught to medical students at Stanford Medical School. Assuming that his MCAT scores are consistent with those performances, I see no reason whatsoever to believe that he would be unable to handle the work in medical school. Mandel's primary contact with medicine so far has been work for two years in a cancer biology research laboratory. He has had some direct patient contact, enough to reinforce his desire to enter medicine. He is very interested in basic medical science, so it is unclear whether he will become a pure practitioner or will also engage in some kind of research or teaching.

Mandel is a thin-faced, quiet and serious fellow. His parents were survivors of a German concentration camp. His mother lacked any formal education. He has grown up in an atmosphere respecting education and science, and this is reflected in his thoughtful and serious attitude. Though quiet, he is not shy; to the contrary, he speaks well on a variety of subjects, and I would think would have no difficult communicating as a physician.

In summary, I believe Mandel is better than his grade point average indicates. He has recovered from the poor initial performance and has given every evidence of being able to handle any of the academics that might come his way in medical school. I urge you to give him serious consideration for admission into your next class.

Sincerely,

Norman K. Wessells
Professor
Associate Dean, School of Humanities and Sciences

NKW:gm

BILL FRENZEL
THIRD DISTRICT, MINNESOTA

WASHINGTON OFFICE:
1026 LONGWORTH BUILDING
202-225-2871

STAFF DIRECTOR
RICHARD D. WILLOW

Congress of the United States
House of Representatives
Washington, D.C. 20515

DISTRICT OFFICES:
MRS. MAYBETH CHRISTENSEN, MANAGER
120 FEDERAL BUILDING
MINNEAPOLIS, MINNESOTA 55401
612-725-2173

MISS SANDRA KLUG, MANAGER
3601 PARK CENTER BOULEVARD
ST. LOUIS PARK, MINNESOTA 55416
612-925-4540

June 14,1974

Pierre Albert Mandel
18015 30th Ave. N.
Plymouth, Minnesota 55441

Dear Pierre:

Congratulations on recently being awarded the Rensselaer Medal from Rensselaer Polytechnic Institute, Troy, New York, for outstanding achievement in the study of mathematics and science.

Your achievement in winning this award demonstrates extraordinary initiative and ability. Keep up the good work.

If I can be of help to you in any way, I hope you will let me know.

Best regards,

Bill Frenzel
Member of Congress

BF:sk

VIS IMPORTANT

mille permettra d'éviter, dans la rédaction des
l mariage, des erreurs qui ne pourraient être
jugement et en occasionnant aux familles des
de temps.

ront donc, **dans leur propre intérêt,** présenter
fois qu'il y aura lieu de faire dresser un **acte**
même un **acte notarié.**

TRÈS IMPORTANT

s et l'usage frauduleux des extraits et mentions
de famille exposent leurs auteurs aux sanctions
47 et 148 du Code Pénal (peines de travaux

usage d'un livret de famille où figurent des
s inexactes en raison des changements de l'état
s, afin d'obtenir grâce à cette pièce justificative
culiers, rend son auteur passible de poursuites

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L'ORIGINAL. — Quand cette copie est
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ette pièce doit être établie par l'intéressé qui la
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ou l'usage frauduleux des copies du livret de
des peines prévues à l'art. 161 du Code Pénal,
u 27 août 1948.

VILLE DE PARIS III• Arrondissement

MARIAGE

célébré le sept juin, mil neuf cent cinquante deux

ENTRE : Henri M A N D E L

Né le vingt neuf janvier mil neuf cent vingt deux

Arrond[t] d Lodz dépar[t] d (Pologne)

Profession : Horloger

Domicilié à 3 Villa du Parc Paris-19è

Fils de André
et de Cécile ROSENBLATT } mariés

~~Veuf ou divorcé de~~ xx

ET Denise Georgette R E N S O N

Née le dix sept janvier mil neuf cent trente

Arrond[t] d Paris-3ème dépar[t] d

Profession : Secrétaire

Domiciliée à 6, rue Montmorency Paris-3ème

Fille de Georges
et de Marcelle LOUIS } mariés

~~Veuve ou divorcée de~~ xxxx

Contrat de mariage néant

SIGNATURE DE L'EPOUX, SIGNATURE DE L'EPOUSE,

Mandel. Renson F. Mandel

Délivré le Sept juin mil neuf cent cinquante deux.

Timbre et signature.

RÉPUBLIQUE FRANÇAISE
MAIRIE DU IIIe ARR. DE PARIS

L'Officier de l'Etat civil,

F. Mercier

Réservé aux mentions

— 2 —

ENFANTS

Nom : Mandel

Prénoms : André Georges

Né le dix sept octobre mil neuf cent cinquante deux

à Paris 19

L'Officier de l'Etat civil,

Timbre et signature.

MAIRIE DU 19e ARR. PARIS

Nom : MANDEL

Prénoms : Pierre Albert

Né le Vingt cinq avril mil neuf cent cinquante sept

à Paris 10e

L'Officier de l'Etat civil,

Colette Reday

RÉPUBLIQUE FRANÇAISE — MAIRIE DU 10e Arrt. DE PARIS

Nom : MANDEL

Prénoms : Elizabeth Ann

Né e le Vingt décembre mil neuf cent soixante trois

à Minneapolis, Minnesota

L'Officier de l'Etat civil,

CONSULAT GÉNÉRAL DE FRANCE A CHICAGO U.S.A.

Jean Louis Mandereau

— 3 —

ENFANTS

Nom :

Prénoms :

Né le

à

L'Officier de l'Etat civil,

Timbre et signature.

ASSOCIATION OF AMERICAN MEDICAL COLLEGES
NEW MEDICAL COLLEGE ADMISSION TEST SM

EXAMINEE'S REPORT OF SCORES

11	12	14	13	07	10
BIOLOGY	CHEMISTRY	PHYSICS	SCIENCE PROBLEMS	READING	QUANT.
SCIENCE KNOWLEDGE				SKILLS ANALYSIS	
TEST (SCALED) SCORES					

469-68-4339	OCT. 1980
SOCIAL SECURITY NUMBER	TEST DATE

This report will not be accepted by a medical school. You must have an official AAMC MCAT test report forwarded to the medical school by ACT or through AMCAS.

Reports of your scores have been sent to these coded medical schools as requested on your answer document. ▶

The American College Testing Program
MCAT Records
P.O. Box 451, Iowa City, Iowa 52243
Telephone (319) 337-1305

IMPORTANT: Please forward the second copy (yellow copy) to your health professions advisor.

MANDEL PIERRE A
P O BOX 7205
STANFORD, CA 94305

GENERAL TEST							
TEST DATE MO.	TEST DATE YR.	VERBAL	%	QUANTITATIVE	%	ANALYTICAL	%
06	87	590	80	740	92	580	70

BATCH = 03870088

SUBJECT TEST							
TEST DATE MO.	TEST DATE YR.	CODE	TOTAL SCORE	%	SUBSCORES SS1	SS2	SS3

REPORT OF SCORES

EXAMINEE'S COPY

THIS REPORT IS NOT VALID FOR TRANSMISSION OF SCORES TO AN INSTITUTION.

* The analytical measure was revised October 1981. Percentile ranks are not reported for analytical scores earned before October 1981 because these scores cannot be compared with scores earned on the revised analytical measure.

SPECIAL NOTE: Effective October 1981, the maximum obtainable General Test Score is 800. Any verbal or quantitative scores higher than 800, earned before October 1981, should be interpreted as being equivalent to the 800 maximum.

Graduate school score recipients from registration form only. Additional score report requests will be acknowledged separately.

CODE	SCORES REQUESTED
4840-5	2

1 = Both General and Subject Tests
2 = General Test Only
3 = Subject Test Only

If the code for SCORES REQUESTED shows a "2" and no General Test scores appear on this report or a "3" and no Subject Test scores appear, your score report HAS NOT BEEN SENT to the institution whose code appears on that line. If a delay in ETS processing has caused this situation, your report will be sent to the affected institution as soon as the delayed score(s) are available. If you incorrectly specified which scores should be sent, you may correct it by submitting an Additional Score Report Request Form to ETS.

PIERRE MANDEL
10817 VALIENTE CT
SAN DIEGO CA 92124

See reverse side for Subject Test Codes, subscores names, and information regarding interpretive materials.

TABLE 1

PERCENTAGES OF NEW MCAT EXAMINEES ACHIEVING SCALED SCORE LEVELS AND ASSOCIATED PERCENTILE RANK RANGES BY AREA OF ASSESSMENT

COMBINED APRIL AND OCTOBER 1980 ADMINISTRATIONS

N = 49,646

Lunch

Scaled Score	Biology		Chemistry		Physics		Science Problems		Skills Analysis: Reading		Skills Analysis: Quantitative		Scaled Score
	Percent Achieving Score	Percentile Rank Range	Percent Achieving Score	Percentile Rank Range	Percent Achieving Score	Percentile Rank Range	Percent Achieving Score	Percentile Rank Range	Percent Achieving Score	Percentile Rank Range	Percent Achieving Score	Percentile Rank Range	
15	0.0	99.9	0.1	99.9	0.4	99.7-99.9	0.3	99.8-99.9			0.0	99.9	15
14	0.2	99.9	0.8	99.2-99.9	1.3	98.4-99.6	1.1	98.7-99.7	0.0	99.9	0.4	99.7-99.9	14
13	2.1	97.9-99.8	3.0	97-99.1	2.6	97-98.3	2.6	97-98.6	0.1	99.9	1.3	98.4-99.6	13
12	5.3	94-97.8	4.9	92-96	5.3	91-96	3.6	93-96	2.8	98-99.9	4.2	95-98.3	12
11	10.6	83-93	7.4	85-91	8.8	83-90	7.2	86-92	6.4	92-97	7.6	87-94	11
10	13.8	69-82	11.8	73-84	10.0	73-82	11.7	74-85	15.9	76-91	10.6	77-86	10
9	12.7	56-68	12.8	60-72	11.1	62-72	12.9	62-73	20.7	55-75	10.9	66-76	9
8	15.8	41-55	12.8	47-59	16.0	46-61	13.5	48-61	15.9	39-54	15.3	51-65	8
7	11.7	29-40	14.9	33-46	12.7	33-45	14.9	33-47	10.5	29-38	14.5	36-50	7
6	10.5	18-28	13.4	19-32	12.0	21-32	13.3	20-32	9.3	19-28	13.5	23-35	6
5	6.8	12-17	9.9	09-18	11.6	09-20	9.8	10-19	5.9	13-18	9.8	13-22	5
4	5.4	06-11	6.0	03-08	6.4	03-08	6.2	04-09	4.7	09-12	6.6	06-12	4
3	3.1	02.2-05	1.8	00.5-02	1.6	00.3-02	2.5	00.6-03	2.8	05.0-08	3.2	02.2-05	3
2	1.7	00.5-02.1	0.4	00.1-00.4	0.2	00.1-00.2	0.5	00.1-00.5	2.0	03.0-04.9	1.7	00.5-02.1	2
1	0.4	00.0-00.4	0.0	00.0	0.0	00.0	0.0	00.0	2.9	00.0-02.9	0.4	00.0-00.4	1
	Scaled Score		**Scaled Score**		**Scaled Score**		**Scaled Score**		**Scaled Score**		**Scaled Score**		
	Mean = 8.0 Std. Deviation = 2.58		Mean = 7.9 Std. Deviation = 2.50		Mean = 7.9 Std. Deviation = 2.57		Mean = 7.8 Std. Deviation = 2.51		Mean = 7.7 Std. Deviation = 2.54		Mean = 7.5 Std. Deviation = 2.53		

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office

Address : COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED APPLICANT	ATTORNEY DOCKET NO.
08/248,506	05/24/94	MANDEL	P

E5M1/0520

PIERRE A MANDEL
6116 CALLE MARISELDA
#102
SAN DIEGO CA 92124

EXAMINER	
SHAFER, R	
ART UNIT	PAPER NUMBER
2507	16

DATE MAILED: 05/20/97

This is a communication from the examiner in charge of your application.

COMMISSIONER OF PATENTS AND TRADEMARKS

1. [X] The communication filed 12/13/96, 1/18/97 & 1/21/97 is informal/non-responsive for the reason(s) checked below and should be corrected. **APPLICANT IS GIVEN ONE MONTH FROM THE DATE OF THIS LETTER OR UNTIL THE EXPIRATION OF THE PERIOD FOR RESPONSE SET IN THE LAST OFFICE ACTION (WHICHEVER IS LONGER) WITHIN WHICH TO CORRECT THE INFORMALITY.**

   a. [X] The amendment to claim(s) 1-4, filed 12/13/96, 1/18/97 & 1/21/97 fails to comply with the provisions of 37 C.F.R. 1.121 and is accordingly held to be non-responsive. A supplemental paper correcting the informal portions and complying with the rule is required.

   b. [ ] The paper is unsigned. A duplicate paper or ratification, properly signed, is required.

   c. [ ] The paper is signed by _____, who is not of record. A ratification or a new power of attorney with a ratification, or a duplicate paper signed by a person of record, is required.

   d. [ ] The communication is presented on paper which will not provide a permanent copy. A permanent copy, or a request that a permanent copy be made by the Office at applicant's expense, is required, see M.P.E.P. 714.07.

   e. [X] Other (SEE ATTACHMENT)

2. [ ] In accordance with applicant's request, **THE PERIOD FOR RESPONSE FROM THE OFFICE ACTION DATED _____ IS EXTENDED TO RUN _____ MONTH(S).**
   No further extension will be granted unless approved by the Commissioner. 37 C.F.R. 1.136 (b)

3. [ ] Receipt is acknowledged of papers submitted under 35 U.S.C. 119 which papers have been made of record in the file.

4. [ ] Other

RICKY D. SHAFER
PATENT EXAMINER
ART UNIT 2507

PTOL-327 (rev. 10-79)

NOTICE TO APPLICANT

UNITED STATES DEPARTMENT OF COMMERCE
Patent and Trademark Office
Address: COMMISSIONER OF PATENTS AND TRADEMARKS
Washington, D.C. 20231

SERIAL NUMBER	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/248,506	05/24/94	MANDEL	P

EXAMINER

ESM1/1015

ART UNIT | PAPER NUMBER

2507 | 11

PIERRE A MANDEL
6116 CALLE MARISELDA
#102
SAN DIEGO CA 92124

DATE MAILED: 10/15/96

This is a communication from the examiner in charge of your application.
COMMISSIONER OF PATENTS AND TRADEMARKS

☒ This application has been examined ☐ Responsive to communication filed on _____ ☐ This action is made final.

A shortened statutory period for response to this action is set to expire 3 month(s), _____ days from the date of this letter.
Failure to respond within the period for response will cause the application to become abandoned. 35 U.S.C. 133

Part I THE FOLLOWING ATTACHMENT(S) ARE PART OF THIS ACTION:

1. ☒ Notice of References Cited by Examiner, PTO-892.
2. ☒ Notice of Draftsman's Patent Drawing Review, PTO-948.
3. ☐ Notice of Art Cited by Applicant, PTO-1449.
4. ☐ Notice of Informal Patent Application, PTO-152.
5. ☐ Information on How to Effect Drawing Changes, PTO-1474.
6. ☐ _____.

Part II SUMMARY OF ACTION

1. ☒ Claims 1-5 are pending in the application.
   Of the above, claims _____ are withdrawn from consideration.
2. ☐ Claims _____ have been cancelled.
3. ☐ Claims _____ are allowed.
4. ☒ Claims 1-5 are rejected.
5. ☐ Claims _____ are objected to.
6. ☐ Claims _____ are subject to restriction or election requirement.
7. ☒ This application has been filed with informal drawings under 37 C.F.R. 1.85 which are acceptable for examination purposes.
8. ☐ Formal drawings are required in response to this Office action.
9. ☐ The corrected or substitute drawings have been received on _____. Under 37 C.F.R. 1.84 these drawings are ☐ acceptable; ☐ not acceptable (see explanation or Notice of Draftsman's Patent Drawing Review, PTO-948).
10. ☐ The proposed additional or substitute sheet(s) of drawings, filed on _____. has (have) been ☐ approved by the examiner; ☐ disapproved by the examiner (see explanation).
11. ☐ The proposed drawing correction, filed _____, has been ☐ approved; ☐ disapproved (see explanation).
12. ☐ Acknowledgement is made of the claim for priority under 35 U.S.C. 119. The certified copy has ☐ been received ☐ not been received ☐ been filed in parent application, serial no. _____ ; filed on _____.
13. ☐ Since this application apppears to be in condition for allowance except for formal matters, prosecution as to the merits is closed in accordance with the practice under Ex parte Quayle, 1935 C.D. 11; 453 O.G. 213.
14. ☐ Other

EXAMINER'S ACTION