Evidence for Absence of Toxicity of T101 Immunotoxin on Human Hematopoietic Progenitor Cells Prior to Bone Marrow Transplantation


ABSTRACT

T101-ricin A-chain immunotoxin is a hybrid molecule made up of the T101 monoclonal antibody bound to the A-chain of ricin. It specifically destroys cells expressing the cell surface T65 antigen. We have designed a preclinical study to evaluate its possible use for the in vitro treatment of T-cell hematological cancers prior to autologous bone marrow transplantation. The data presented here show that conditions previously defined to produce high tumor cell killing, i.e., a 20-hr incubation at 37°C in the presence of T101-ricin A-chain immunotoxin up to 10^{-7} m in a 10 mM ammonium chloride solution, do not affect the in vitro proliferative capacity of human hematopoietic stem cells studied by means of semisolid medium cultures (granulocyte-macrophage progenitors, burst-forming units-erythrocyte) and continuous liquid cultures (pre-granulocyte-macrophage progenitors). Therefore, autologous bone marrow transplantation with T101-ricin A-chain immunotoxin-treated graft should be feasible.

INTRODUCTION

Monoclonal antibodies have been used recently in an attempt to purge leukemic bone marrows, collected in remission, of their few residual tumor cells (21, 27). Indeed, many of the obstacles limiting their use in vivo, such as the presence of circulating antigens, antigenic modulation, reactivity with normal cells, or immune response to murine antibody, may be circumvented through the carefully controlled manipulation of the reagents in vitro. However, these techniques require important bone marrow processing and the use of heterologous complement, the non-specific toxicity of which may result in the loss of normal hematopoietic stem cells.

Monoclonal antibodies may be now used as carriers of various cytotoxic agents. Specific procedures to conjugate to antibodies chemotherapeutic agents (1, 10), lipid vesicles loaded with either drugs or toxins (17), or plant toxins (28) such as ricin A-chain (2, 11, 15, 16, 20, 25) are presently available.

We report here the results of a preclinical study designed to evaluate the possible toxicity on human hematopoietic stem cells of T101-IAT,3 consisting of the A-chain subunit of ricin linked to the T101 monoclonal antibody, in view of its possible application to the in vitro treatment of bone marrow in patients with T-cell cancers prior to autologous bone marrow transplantation.

MATERIALS AND METHODS

Immunotoxin T101. T101-IAT (Centre de Recherches Clin-Midy-Sanofi, Montpellier, France) is an hybrid molecule made up of the A-chain subunit of ricin linked to the T101 monoclonal antibody described by Royston. T101-IAT contains a mean of 2.5 A-chains per antibody molecule (4). T101, an IgG2a globulin, reacts with the T65 antigen present on the surface of all normal T-cells, most thymocytes, T-cells of acute lymphocytic leukemias, and leukemic T-cell lines (22) and also on B-cells of chronic lymphocytic or prolymphocytic leukemias (23). The T101 binds the T65 differentiation antigen which allows the A-chain of ricin to cross the membrane. Then the A-chain penetrates the cytoplasm, inactivates ribosomes, and blocks protein synthesis irreversibly, inducing cell death (16).

Patient Population and Bone Marrow Collection. Bone marrow samples were harvested by aspiration from iliac spines in patients either with T-cell cancers in complete remission or with solid tumors without bone marrow involvement at the time of collection in the surgery room for autologous bone marrow transplantation.

Bone Marrow Treatment with T101-IAT. Volumes of 50 or 100 mL of freshly aspirated marrow were used for the following in vitro studies. The total bone marrow (i.e., without any cell separation) was incubated in a 150-mL bag (GAMBRO DF 1000, Heschingen, West Germany) at 37°C in a 10 mM ammonium chloride solution with increasing dosages of T101-IAT, expressed as final A-chain concentration in molarity, with gentle continuous shaking. Marrow samples were drawn at various intervals (1 to 20 hr) for hematopoietic stem cell assay.

Bone Marrow Freezing Technique. After the incubation period, bone marrow was mixed with an equal volume of freezing medium consisting of TC 199 with glutamine and salts of Earle's medium (Gibco/Biocult, Glasgow, Scotland), 20% dimethyl sulfoxide (Assistance Publique, Paris, France), and 10% matched human serum. The final concentrations were 10% for dimethyl sulfoxide and 5% for serum. This mixture was then frozen according to previously described techniques (6). Bone marrow was frozen in the vapor phase of liquid nitrogen in a Nicool biological freezing system (Air Liquide, Grenoble, France). The freezing rate was 9°C/min until the release of the heat of fusion at which time the temperature inside the freezing chamber was sharply diminished. A particular attention was devoted to the third slope in order to keep a cooling rate slower than ~5°C/min after the transition phase (7, 13). The marrow was then stored in the gas phase of liquid nitrogen at ~196°C. After thawing in a waterbath at 37°C, specimens were drawn for in vitro assays of the cryopreserved stem cells.

Culture of CFUc. CFUc assay was performed in semisolid agar by a modification of the technique of Pike and Robinson (19). The basic medium was McCoy's Medium 5A without serum (Gibco/Biocult) supplemented with 30% decomplemented FCS (Gibco/Biocult). CSA was sup-
plied by 10% human placental conditioned medium (kindly provided by Dr. R. Berthier, CEN, Grenoble, France), produced according to the method of Schlunk and Schleyer (24). The same batches of FCS and human placental conditioned medium were used for all of the studies.

The mononuclear bone marrow cells were isolated over a Ficoll-sodium metrizoate gradient (d = 1.077, Lymphoprep; Nyegaard, Oslo, Norway). They were cultured in 35- x 10-mm Petri dishes (Coming), using 2 x 10^5 bone marrow cells/ml of medium containing equal volumes of 0.6% agar and McCoy’s Medium 5A 2x (Gibco/Biocult) to achieve a final serum concentration of 15%. Three Petri dishes were plated for each assay.

The cultures were incubated for 10 days at 37°C in a humidified 5% CO2 atmosphere. The colonies were examined on the Petri dishes by means of an inverted microscope, and counts were made of colonies containing more than 50 cells. The results were expressed in terms of the number of colonies per ml of marrow.

Culture of BFUe. BFUe assay was performed in methylcellulose by a modification of the technique of Iscover and Sieber (14). Mononuclear cells (2 x 10^6) isolated as above were cultured in triplicate in 0.9% methylcellulose in α medium (Gibco/Biocult) containing 20% FCS, 1% bovine serum albumin (Sigma), 20% NCTC (Eurobio, Paris, France) and 1 unit porc erythropoietin (CNTS, Paris, France). Cultures were incubated for 14 days at 37°C in a humidified 5% CO2 atmosphere. Colonies were examined on the Petri dishes through an inverted microscope, and counts were made of colonies containing more than 40 cells. The erythroid nature of the cells in colonies was confirmed by examination of picked colonies stained with May-Grunwald-Giemsa. The results were expressed in terms of the number of colonies per ml of marrow.

Continuous Liquid Cultures. Long-term liquid cultures were established using a modification (8) of the technique of Dexter et al. (5). Bone marrow buffy coat cells (10^6) from normal donors were suspended in 1 ml of α medium (Gibco/Biocult) supplemented with 20% FCS. Five ml of this suspension were put into Falcon 25-ml tissue culture flasks, and the suspension was incubated at 37°C in a 5% CO2 atmosphere. Cultures were maintained by weekly demineralization with addition of fresh medium. Under these conditions, an uniform adherent layer spread within 3 to 4 weeks. Then, this primary culture was depopulated of all suspension cells and irradiated at 30 Gy. A new allogenic inoculum of bone marrow cells was added onto the adherent layer. Cultures were maintained by replacing all the medium weekly, without demidepopulation. At the same time, suspension cells were assayed for CFUc as described above.

RESULTS

The sensitivity of normal progenitors was evaluated by their ability to proliferate. Results were expressed in terms of the percentage of stem cells recovered per ml of manipulated marrow, by comparison with nonmanipulated marrow. The results shown represent the mean ± S.D. of 3 to 9 determinations for each point. Sequential studies with incubation of the marrow for periods up to 20 hr were done for each individual patient. In these experiments, the mean number of colonies detected in controls were, respectively, for CFUc and BFUe: 124 ± 38 and 149 ± 52/2 x 10^5 mononuclear cells or 1420 ± 340 and 1603 ± 228/ml bone marrow. These values represent what is usually expected for normal marrow in our experience.

Conditions previously shown to produce high tumor cell killing were tested (5). Table 1 shows the recovery of CFUc and BFUe after the incubation of the marrow at 37°C for a period up to 20 hr in the presence of 10 mM NH4Cl. As indicated, NH4Cl does not alter the viability of CFUc or BFUe. Moreover, a slight increase in CFUc and BFUe is observed. To test the influence of these experimental conditions further on the sensitivity of progenitor cells to cryopreservation, we then compared the recovery following freezing and thawing of incubated marrow to the recovery of cryopreserved nonincubated marrow. CFUc recovery of marrow incubated for 20 hr with 10 mM NH4Cl dropped to 33 ± 9%. BFUe were somewhat more sensitive with a recovery of 32 ± 16%.

Marrows were then incubated in those same conditions with increasing doses of T101-IAT, ranging from 10^-6 to 10^-4 M. Results are shown on Table 2. No effect of T101-IAT on progenitor cells proliferation was observed after a 4-hr incubation up to 10^-6 M. After a 20-hr incubation, toxicity was seen at the 10^-5 M level. BFUe progenitors were more sensitive to T101-IAT than was CFUc. After cryopreservation, CFUc and BFUe recovery again was reduced but in a way similar to that observed previously for marrows either nontreated or NH4Cl incubated.

The ability of T101-IAT treated marrow to maintain myelopoiesis in continuous marrow culture was tested. Frozen bags of marrows incubated with 10^-6 M T101-IAT for 20 hr at 37°C in a 10 mM NH4Cl solution were maintained over the primary irradiated allogenic adherent layer. No impairment in CFUc generation (Table 3) was seen in the T101-IAT-treated group. Moreover, a small but significant improvement in the extent of CFUc production was evident at Weeks 1, 2, and 3, as compared to
erythrocytes and granulocytes do not bind T101-IAT nonspecifically into DMA synthesis. T101-IAT itself has no significant effect on the in vitro proliferation of committed progenitor cells. Moreover, the slight decrease of CFUc and BFUe within 1 hr, immediately followed by a secondary increase, may be attributed to a triggering into DNA synthesis. T101-IAT itself has no significant toxicity up to 10^{-6} m. These results are consistent with those of Teatle and Royston (26), demonstrating the absence of T65 antigen on normal CFUc and BFUe. They also confirm the results of Casellas et al. (4) showing the lack of nonspecific toxicity of T101-IAT on the Daudi cell line (T65 negative) up to 10^{-7} m. The slight sensitivity of BFUe may be related to the absence of T-cells, the presence of which could be required for their in vitro proliferation in semisolid media culture (18).

The use of T101 immunotoxin A-chain may also be extended to the setting of allogenic bone marrow transplantation, through the elimination of T-cells for prevention of graft-versus-host disease (12).

Monoclonal antibodies as carriers for toxins are a promising tool for a selective removal of leukemic cells which may significantly increase the clinical effectiveness of antibodies in vitro, without any nonspecific toxicity.

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REFERENCES


**Table 3**

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<th>Wk in culture</th>
<th>CFUc/ml culture</th>
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<tr>
<td></td>
<td>T101-IAT-treated frozen marrows</td>
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<tr>
<td>0</td>
<td>938 ± 691^a</td>
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<tr>
<td>1</td>
<td>663 ± 811</td>
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<td>705 ± 593</td>
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<td>27 ± 10</td>
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^a Mean ± S.D. of 3 separate experiments.
T101-RICIN IMMUNOTOXIN AND HUMAN PROGENITOR CELLS


7. Douay, L., Gorin, N. C., and Duhame1, G. Rôle critique de la vitesse de
renforcement aur-delé de la libération de la chaleur de fusion, pour la
conservation des cellules souches hématopoïétiques. C. R. Acad. Sci. Paris,

8. Douay, L., Gorin, N. C., Najman, A., Laguens, M. C., and Duhame1, G. Conti-
nuou1. human bone marrow culture. Sensitivity to chemotherapeutic agents of
probable pluripotential stem cells. Application to autologous bone marrow

9. Francis, G. E., Guimaraes, J. E. T., Granger, S., Bemey, J. J., Tidman, I. V.,
Wing, M. A., Janossy, G., and Hoflbrand, A. V. Distinct T lymphocyte subjects
affect granulocy-monocytic differentiation and proliferation. Stem Cells, 19: 76–
87.

10. Ghose, T., and Naim, A. H. Antibody-linked cytotoxic agents in the treatment
1978.

Koprowski, H. Antibody-directed cytotoxic agents: use of monoclonal antibody
to direct the toxin of toxin A chains to colorectal carcinoma cells. Proc. Natl.

Schey, S., Goldstein, G., Janowsky, G., and Hoflbrand, A. V. Allogenic bone
marrow transplantation: the monitoring of granulocyte-macrophage colonies
following the collection of bone marrow mononuclear cells and after the
subsequent in vitro cytolysis of OKT4 positive lymphocytes. Br. J. Haematol.,

M., Najman, A., and Duhame1, G. Delayed kinetics of recovery of haemato-
poiesis following autologous bone marrow transplant. The role of exces-
sively rapid marrow freezing rates after the release of fusion heat. Eur. J.

14. Isacove, N. N., and Sible1, F. Erythroid progenitors in mouse bone marrow
detected by macroscopic colony formation in culture. Exp. Hematol., 3: 32–
43, 1975.

15. Jansen, F. K., Blythman, H. E., Cartiere, D., Casellas, P., Diaz, J., Gros, P.,
Hennequin, J. R., Paolucci, F., Pau, B., Poncelet, P., Richer, G., Salhi, S. L.,
Vidal, H., and Volein, G. A. High specific cytotoxicity of antibody-toxin hybrid

16. Jansen, F. K., Blythman, H. E., Cartiere, D., Casellas, P., Gros, O., Gros, P.,
Laurent, J. C., Paolucci, F., Pau, B., Poncelet, P., Richer, G., Vidal, H., and
Volein, G. Immunotoxins: hybrid molecules combining high specificity and

17. Leserman, L. D., Barbel, J., Kourilsky, F., and Weinstein, J. N. Targeting to
cells of fluorescent liposomes covalently coupled with monoclonal antibody or
protein A (Lond.), 288: 602, 1980.

18. Nathan, D. G., Chess, L., Hillman, D. G., Clarke, B., Breard, J., Merzlier, E.,
and Housman, D. E. Human erythroid burst forming unit: T cell requirement

19. Pike, B. L., and Robinson, W. A. Human bone marrow colony growth in agar

20. Raso, V., and Griffin, T. Specific cytotoxicity of a human immunoglobulin-

21. Ritz, J., Sallat, S. E., Bost, R. C., Lipton, J. M., Nathan, D. G., and Schlossman,
S. E. Autologous bone marrow transplantation in CALLA positive ALL following
in vitro treatment with J5 monoclonal antibodies and complement. Blood, 58
(Suppl. 1), 1981.

22. Royston, I., Majda, J. A., Baird, S. M., Meserve, B. L., and Griffiths, J. C.
Monoclonal antibody specific for human T lymphocytes: identification of normal

23. Schule, T., and Schu, M. The influence of culture conditions on the
production of a colony-stimulating activity by human placenta. Exp. Hematol.,

24. Seon, B. K. Specific killing of human T leukemia cells by immunotoxins prepared
with ricin A chain and monoclonal anti T cell leukemia antibodies. Cancer Res.,

25. Sferrazza, P., and Scriver, C. Radial outgrowth patterns in vitro from bone

26. Taette, R., and Royston, I. Human T cell antigens defined by monoclonal

Cline, M. J. Autotransplantation after in vitro immunotherapy of lymphoblastic

28. Wells, J., Junquas, S., Dujardin, P., Le Pecq, J-B., and Tursz, T. Properties of
immunotoxins against a glycolipid antigen associated with Burkitt’s lymphoma.
Evidence for Absence of Toxicity of T101 Immunotoxin on Human Hematopoietic Progenitor Cells Prior to Bone Marrow Transplantation


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