Direct Gene Transfer for Treatment of Human Cancer

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Immune function has been studied extensively in the search for treatments for cancer. Malignant tumors are able to escape detection by the immune system, even in immunocompetent hosts. The mechanisms that allow this to occur are not well defined. Studies suggest that the process by which tumor cells grow confers an advantage to clones that are less immunogenic.[1] Numerous investigations have been conducted to determine factors that enhance immune recognition and destruction of foreign proteins. Factors that affect the immune response include cellular mediators such as cytotoxic T cells and macrophages,[2-10] humoral effectors, and cytokines, including tumor necrosis factor-α (TNF-α), interferon, interleukin-2 (IL-2), IL-4, granulocyte colony-stimulating factor (G-CSF), and granulocyte-macrophage colony-stimulating factor (GM-CSF).[11-20]

Expression of histocompatibility antigens has also been found to be a critical element in immune recognition and rejection. Measurements of cells isolated from fresh tumors reveal that there is either defective expression or a lack of expression of the class I major histocompatibility (MHC) proteins[21-26] in a large number of cases. When expression of histocompatibility antigens is induced through the introduction of MHC genes into tumor cell lines, there is a specific cytolytic T cell reaction that causes an increase in the immunogenicity of the cells.[27-31]

The knowledge gained from immunological studies of tumorigenicity and immune reactivity, although incomplete, has provided some promising new therapies for the treatment of malignancies. Unique methods have been used to enhance immune reactivity against malignant cells. These include treating tumor cells directly to increase their immunogenicity, administering systemic immune enhanc-

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ing or tumor suppressing reagents, and introducing anti-tumor reactive cells. Tumor regression and cell destruction have been demonstrated in studies employing agents such as those described above.[32–35]

At the University of Michigan, we have developed a novel approach for the treatment of malignancies. In this method, a vector that expresses the class I histocompatibility antigen, HLA-B7, is transferred *in vivo* directly into tumor cells using intratumoral injection or catheter delivery. This method differs from previous trials which have used *ex vivo* treatment of cells to introduce genes into the host. Direct injection has many advantages over *ex vivo* treatment. It eliminates the risk of contaminating the *in vitro* cell cultures, reduces the number of manipulations to the cells, avoids the need to establish a cell line from each tumor, eliminates concerns about alterations of cellular phenotype in culture and can significantly decrease the time between diagnosis and treatment.

Direct injection was initially tested in animal models using both retroviral vectors and DNA/liposome complexes.[36,37] In a mouse model, β-galactosidase was introduced into tumors using direct injection, *in vivo*. A cDNA clone encoding the mouse class I MHC, H-2K was also used for these studies. These vectors were transferred into tumors grown in mice. Both types of vectors, retroviral or DNA/liposome complexes encoding allogeneic MHC, were found to signal the immune system to mount a response. Specific cytolytic activity to the antigen was noted. The most important result of these studies, however, was the observation that transfer of the histocompatiblity gene into tumor cells caused a general immune reaction against untransfected autologous tumor. This was demonstrated by a marked regression in tumor growth, and in some cases, complete elimination of the tumor. This response was T-cell mediated, and lysis was inhibited by antibodies to self-MHC, indicating that this treatment induced an immune response to previously unrecognized tumor antigens.[38,39] The DNA/liposome complex offered the highest degree of safety and transfection efficiency, and has now been tested against metastatic melanoma in a human phase I clinical trial at the University of Michigan. A second phase I trial which encompasses other types of cancer is ongoing.

In the phase I study that was recently completed, 5 patients with advanced melanoma received the treatment, a complex composed of the DNA vector and [N,N′,N′-(dimethylaminoethane) carbamoyl] cholesterol/dioleoyl phosphatidylethanolamine/3b (DC-Chol/DOPE) liposome through direct intratumoral injection.[39] In addition, one patient was treated at a distant site through catheter-mediated delivery.[40] The goals of this study were to determine expression of the antigen in the treated cells, identify the presence of an immunological response against the foreign histocompatibility antigen and ascertain the safety of the procedure.

Polymerase chain reaction (PCR) analysis of tissue biopsies that were collected from each patient 3–7 days after treatment demonstrated the expression of HLA-B7 DNA and mRNA in 4 of the 5 treated patients. Protein expression was confirmed in 1–10% of tumor cells surrounding the site of injection in all 5 patients. No toxicities or adverse reactions were observed at any point in the study, and there were no anti-HLA-B7 antibodies. The lack of antibody response to HLA-B7 may result from the absence of class II stimulation, required for helper T cells to induce B cell activation. Plasmid DNA was not detected in post-treatment blood samples by PCR analysis.[39] This testing indicates that the plasmid was confined to the injected area, and did not enter the blood system. Additional data collected from this study demonstrated an increase in HLA-B7-reactive cytotoxic T lymphocyte (CTL) precursors and an

increase in CTLs that were specific for autologous tumor. One patient had complete regression of injected subcutaneous nodules. In addition, several uninjected subcutaneous nodules as well as two pulmonary lesions regressed, suggesting that a systemic response occurred.

A second phase I clinical trial is currently being conducted. This study incorporates an improved vector, a more efficient liposome, two methods of gene delivery (direct intratumoral and catheter), and will be tested on several different types of cancers which have demonstrated their ability to express the gene. A new cationic liposome formulation, 1,2-dimyristyloxypropyl-3-dimethyl-hydroxyethyl ammonium bromide (DMRIE)/DOPE, replaced the DC-Chol/DOPE that was used in the first clinical trial. DMRIE is an improvement over the DC-Chol because it provides increased transfection efficiency (up to 10-fold higher) and does not aggregate at high concentrations. This second property allows for higher doses of DNA/liposome to be administered without causing toxicity to the host.

The vector was also modified to enhance the expression of the HLA-B7 on the surface of the cell. The structure of the plasmid was altered to improve the overall expression of the histocompatibility antigen. In addition, in order for expression of class I MHC proteins to occur, the genes must associate with β-2 microglobulin inside the cell and be co-transported to the surface. Several types of tumors, particularly melanoma, have a defect in the mechanism that is responsible for the production of endogenous β-2 microglobulin. This defect in cell function prevents the HLA-B7 antigen from being expressed on the cell. To overcome this problem, the modified vector includes the gene for β-2 microglobulin.

During the initial clinical trial, one patient was treated via catheter delivery to a distant site of metastasis without complication.[40] This second trial will test this method of gene delivery in a controlled setting. A catheter-based approach will allow genes to be delivered to selected sites in the microcirculation of the tumor providing the opportunity for more selective delivery, particularly in cancers other than melanoma. Potential inactivation of the DNA/liposome complex by serum is also reduced when delivered by this method. This will expand the potential utility of the therapy.

The results of these clinical trials will not only provide information regarding the clinical efficacy of this treatment, but will improve our knowledge of tumor immunology. The results of testing in animals and the first clinical trial suggest that the anti-tumor response is T cell dependent and potentially mediated by the more effective presentation of antigens to cytolytic T cells within the tumor mass. It is also postulated that allostimulation may trigger the release of cytokines locally which can augment T cell proliferation and inflammation within the parenchyma of the tumor.

The treatment of human malignancy through the direct injection of recombinant genes is a promising new treatment for cancers which have previously been resistant to current therapies. The ability to stimulate an immune response against autologous tumor by genetically altering cells *in vivo* with a minimum of manipulation offers the opportunity of developing an effective treatment that would be widely available to patients. Additionally, the use of a nonviral delivery system for the introduction of the gene reduces the risk that is posed by the use of certain viral vectors and problems of immune response directed against the vector. We are now in the process of developing improved catheters that will allow for more targeted delivery and are testing other genes and combination therapies that will augment the gene therapy treatment.

SUMMARY

Genetic instability within malignant cells gives rise to mutant proteins which can be recognized by the immune system. Recognition of tumor-associated antigens by T lymphocytes could thus contribute to the elimination of neoplastic cells. We have developed a molecular genetic intervention for the treatment of malignancies based upon the knowledge that foreign major histocompatibility complex (MHC) proteins expressed on the cell surface are efficient at stimulating an immune response. Expression of this foreign MHC gene within tumors induced a cytotoxic T cell response to the introduced gene. More importantly, the immune system recognized tumor-specific antigens on unmodified tumor cells as foreign. Growth of the tumors diminished, and in many cases, there was complete regression. This research provides evidence that direct gene transfer *in vivo* can induce cell-mediated immunity against specific gene products, and offers the potential for effective immunotherapy for the treatment of cancer and infectious diseases in man. Our laboratory conducted a phase I clinical trial to determine the safety and efficacy of this treatment in humans. These studies suggest that direct gene transfer provides a safe and feasible approach for the treatment of human cancer. More recent developments using combination gene therapy and the initiation of a second human trial with improvements on this technology have been implemented. Finally, we have begun to define mechanisms of resistance to immune recognition by established malignancies.

REFERENCES

1. KLEIN, G. & E. KLEIN. 1977. Proc. Natl. Acad. Sci. USA **74:** 2121.
2. OLDHAM, R. K. 1983. Cancer Metastasis Rev. **2:** 323.
3. HERBERMAN, R. B. 1985. Concepts Immunopathol. **1:** 96.
4. ROSENBERG, S. A. 1988. Immunol. Today **9:** 58.
5. YRON, I., T. A. WOOD, P. SPIESS & J. A. ROSENBERG. 1980. J. Immunol. **125:** 238.
6. ROSENBERG, S. A., P. SPIESS & R. LAFRENIERE. 1986. Science **223:** 1318.
7. SPIESS, P. J., J. C. YANG & S. A. ROSENBERG. 1987. J. Natl. Cancer Inst. **79:** 1067.
8. MARCELLETTI, J. & P. FURMANSKI. 1978. J. Immunol. **120:** 1.
9. RALPH, P. I. NAKOINZ & D. REMICK. 1988. J. Exp. Med. **167:** 212.
10. ALEXANDER, P. 1973. Natl. Cancer Inst. Monogr. **39:** 127.
11. OLD, L. J. 1985. Science **230:** 630.
12. POWEL, M. B., B. S. CONTA, M. HOROWITZ & N. H. RUDDLE. 1985. Lymphokine Res. **4:** 13.
13. LINDAHL, P., P. LEARY & I. GRESSER. 1973. Proc. Natl. Acad. Sci. USA **20:** 2785.
14. LINDAHL, P., I. GRESSER & M. G. TOVEY. 1976. Proc. Natl. Acad. Sci. USA **73:** 1284.
15. OLD, L. J. 1987. Nature **326:** 330.
16. CLARK, S. C. & R. KAMEN. 1987. Science **236:** 1229.
17. LOTZE, M. T., E. GRUM, A. MAZUMDO, J. L. STRAUSSER & S. A. ROSENBERG. 1984. Cancer Res. **41:** 4420.
18. JOHNSON, C. S. *et al.* 1990. Cancer Res. **50:** 5682.
19. PHILLIPS, J. H. & L. L. LANIER. 1986. J. Exp. Med. **164:** 814.
20. MULE, J. J., J. A. KROSNICK & S. A. ROSENBERG. 1989. J. Immunol. **142:** 726.
21. HOLDEN, C. A., A. R. SADISEN & D. M. MCDONALD. 1983. J. Am. Acad. Dermatol. **9:** 867.
22. ISAKOV, N., S. KATZAV, M. FELDMEN & S. J. SEGAL. 1983. J. Natl. Cancer Inst. **71:** 139.
23. SCHMIDT, W., G. ATFIELD & H. FESTENSTEIN. 1979. Immunogenetics **9:** 405.
24. FUNA, K., A. F. GAZDAR, J. D. MINNA & R. I. LINNOLIA. 1986. Lab. Invest. **55:** 186.
25. LAMPSON, L. A., C. A. FISHER & J. P. WHELAN. 1983. J. Immunol. **130:** 2471.
26. TANAKA, K., K. J. ISSELBACHER, G. KHOURY & G. JAY. 1985. Science **228:** 26.

27. HUI, K., F. GROSVELD & H. FESTENSTEIN. 1984. Nature **311:** 750.
28. WALLICH, R., N. BULBUC, G. J. HAMMERLING, S. KATZAO, S. SEGAL & M. FELDMAN. 1985. Nature **315:** 301.
29. LJUNGGREN, H-G. & K. KARRE. 1986. J. Immonogenet. **13:** 141.
30. HAMMERLING, G. J. *et al.* 1986. J. Immunogenet. **13:** 153.
31. ROSENBERG, S. A., M. T. LOTZE, L. M. MUUL *et al.* 1987. N. Engl. J. Med. **316:** 889.
32. JOHNSON, C. S., S. M. THURLOW & P. FURMANSKI. 1989. Leukemia **3:** 91.
33. ROSENBERG, S. A. *et al.* 1990. N. Engl. J. Med. **323:** 570.
34. FEARON, E. R., D. M. PARDOLL, T. ITAYA, P. GOLUMBEK, H. I. LEVITSKY, J. W. SIMONS, H. KARASUYAMA, B. VOGELSTEIN & P. FROST. 1990. Cell **60:** 397.
35. TEPPER, R. I., P. K. PATTENGALE & P. LEDER. 1989. Cell **57:** 503.
36. NABEL, E. G., G. PLAUTZ & G. J. NABEL. 1990. Science **249:** 1285.
37. FELGNER, P. L. *et al.* 1987. Proc. Natl. Acad. Sci. USA **84:** 7413.
38. PLAUTZ, G. E. *et al.* 1993. Proc. Natl. Acad. Sci. USA **90:** 4645.
39. NABEL, G. J. *et al.* 1993. Proc. Natl. Acad. Sci. USA **90:** 11307.
40. NABEL, E. G. *et al.* 1994. Hum. Gene Ther. **5:** 1089.