Direct gene transfer with DNA–liposome complexes in melanoma: Expression, biologic activity, and lack of toxicity in humans

(gene therapy/human clinical trials/immunotherapy/major histocompatibility complex)

GARY J. NABEL*[†‡§], ELIZABETH G. NABEL[†], ZHI-YONG YANG*[†‡], BERNARD A. FOX[¶],
GREGORY E. PLAUTZ[†‡ǁ], XIANG GAO**, LEAF HUANG**, SUYU SHU[¶], DAVID GORDON[††],
AND ALFRED E. CHANG[¶]

*Howard Hughes Medical Institute, Departments of [†]Internal Medicine, [‡]Biological Chemistry, [ǁ]Pediatrics, [¶]Surgery, and [††]Pathology, University of Michigan Medical Center, 1150 West Medical Center Drive, 4510 Medical Science Research Building I, Ann Arbor, MI 48109-0650; and **Department of Pharmacology, University of Pittsburgh School of Medicine, West 1351 Biomedical Science Tower, Pittsburgh, PA 15261

Communicated by J. L. Oncley, September 8, 1993 (received for review August 23, 1993)

ABSTRACT Direct gene transfer offers the potential to introduce DNA encoding therapeutic proteins to treat human disease. Previously, gene transfer in humans has been achieved by a cell-mediated *ex vivo* approach in which cells from the blood or tissue of patients are genetically modified in the laboratory and subsequently returned to the patient. To determine the feasibility and safety of directly transferring genes into humans, a clinical study was performed. The gene encoding a foreign major histocompatibility complex protein, HLA-B7, was introduced into HLA-B7-negative patients with advanced melanoma by injection of DNA–liposome complexes in an effort to demonstrate gene transfer, document recombinant gene expression, and determine the safety and potential toxicity of this therapy. Six courses of treatment were completed without complications in five HLA-B7-negative patients with stage IV melanoma. Plasmid DNA was detected within biopsies of treated tumor nodules 3–7 days after injection but was not found in the serum at any time by using the polymerase chain reaction. Recombinant HLA-B7 protein was demonstrated in tumor biopsy tissue in all five patients by immunochemistry, and immune responses to HLA-B7 and autologous tumors could be detected. No antibodies to DNA were detected in any patient. One patient demonstrated regression of injected nodules on two independent treatments, which was accompanied by regression at distant sites. These studies demonstrate the feasibility, safety, and therapeutic potential of direct gene transfer in humans.

We have developed a protocol which relies upon the direct transmission of recombinant genes into established tumors *in vivo* to genetically modify them as they grow *in situ*. In murine models, introduction of a gene encoding a foreign major histocompatibility complex (MHC) protein (class I) *in vivo* signals the immune system to respond to the foreign antigen (1, 2). More importantly, when this gene is transduced into established tumors *in vivo*, a cytolytic T-cell response is also generated against unmodified tumor cells by increasing the efficacy of the local immune response. This approach has led to significant reductions in tumor growth and, in some cases, complete remission in mice (2). Based on these studies, we received approval from the Recombinant DNA Advisory Committee of the National Institutes of Health to conduct a human clinical protocol using direct transfer of a human transplantation antigen gene in cutaneous melanoma. We now describe the results of this human clinical study.

MATERIALS AND METHODS

Clinical Protocol and Study Design. Five patients with stage IV melanoma refractory to all available therapies were enrolled

based on guidelines of the clinical protocol (3, 4) and admitted to the Clinical Research Center. Informed written consent was obtained according to The Committee to Review Grants for Clinical Research and Investigation Involving Human Beings of the University of Michigan Medical School, the Recombinant DNA Advisory Committee of the National Institutes of Health, and the Food and Drug Administration.

A cutaneous tumor nodule was identified for treatment, and its borders were measured. Control (untreated) nodules were quantitated by computerized tomography immediately prior to the procedure. At each treatment, group I ($n = 3$) received one injection of ≈ 0.2 ml of DNA–liposome complex (0.29 μg of DNA) into the tumor (cumulative dose, 0.86 μg), and group II ($n = 3$) received three injections (0.2 ml each) at one session within the same nodule (cumulative dose, 2.58 μg). All patients received a total of three treatments with a 2-week interval between treatments. Patient 3 received three courses of treatment (group I, group II, and pulmonary catheterization), with an interval of 9 weeks between group I and II.

Vector Production and Analysis. A eukaryotic expression vector plasmid, pHLA-B7, was prepared by insertion of an HLA-B7 cDNA into the Rous sarcoma virus (RSV)-β-globin plasmid (1, 5). The β-globin gene was removed by digestion with *Hind*III and *Bgl* II, treated with calf intestinal alkaline phosphatase, phenol and chloroform extracted, ethanol precipitated, and treated with the Klenow fragment of *Escherichia coli* DNA polymerase I to generate the plasmid backbone. An insert was prepared as a *Bam*HI–*Sal* I fragment of pLJ-HLA-B7 (1), kindly provided by Alan Korman (Institut Pasteur, Paris), which was treated with Klenow enzyme and ligated to the fragment from RSV-β-globin. The resultant plasmid contains pBR322, the RSV enhancer, and the simian virus 40 polyadenylylation sequence, similar to RSV-β-globin (5).

Preparation and Administration of DNA–Liposome Complex. DNA–liposome complexes were mixed immediately prior to injection by adding 0.1 ml of lactated Ringer's solution into a sterile vial of HLA-B7 plasmid DNA (20 μg/ml; 0.1 ml). An aliquot of this solution (0.1 ml) was added at room temperature to 0.1 ml of 150 μM dioleoyl phosphatidylethanolamine/3b- [N-(N',N'-dimethylaminoethane) carbamoyl]cholesterol liposome (6) in lactated Ringer's solution in a separate sterile vial. DNA and liposome vials were prepared in accordance with Food and Drug Administration guidelines and quality control procedures. Plasmids were grown and purified in the absence of ethidium bromide or penicillin derivatives by using a commercially available column chromatography method (Promega). After incubation for 15 min at room temperature, an additional 0.5 ml of sterile

---

Abbreviations: CTL, cytolytic T lymphocyte; MHC, major histocompatibility complex; RSV, Rous sarcoma virus.
§To whom reprint requests should be addressed.

lactated Ringer's solution was added to the vial and mixed. The DNA–liposome solution (0.2 ml) was injected through a 22-gauge needle into the patient's melanoma nodule under sterile conditions at the bedside after administration of local anesthesia (1% lidocaine). The injection was performed once per treatment session (group I, patients 1, 2, and 3A) or three times per treatment session (group II, patients 3B, 4, and 5). In patient 3, the DNA–liposome solution (0.6 ml) was also delivered into the pulmonary artery of the posterior segment of the right lower lobe on two separate occasions, 2 weeks apart, with percutaneous delivery by right heart catheterization from the right femoral vein.

Biochemical and Hemodynamic Monitoring. To monitor the potential toxicities of the DNA liposome treatment, biochemical, hematological, and hemodynamic parameters were evaluated. Vital signs and cardiac rhythm were monitored and subjective complaints of patients were sought and recorded.

Analysis of HLA-B7 Gene Expression. To confirm recombinant HLA-B7 gene expression within treated tumor nodules, core needle biopsy samples of the injected tumor were obtained 3–7 days after gene transfer. Genomic DNA was isolated from biopsy material (1), and PCR for the HLA-B7 gene was performed (7, 8) with the primers 5'-AGT-GCC-CAG-GGC-TCT-GAT-GTG-TCT-CTC-ACA-3' (sense; HLA-B7) and 5'-ACC-ACA-GAA-GTA-AGG-TTC-CTT-CAC-AAA-GAT-3' (antisense; simian virus 40 polyadenylylation signal) to generate a 301-bp fragment (Fig. 1 *A* and *C*).

For the RNA analysis, these primers were used after reverse transcription with oligo(dT). For analysis of plasmid in blood, a different set of primers was used, 5'-CTA-CGT-GGA-CGA-CAC-CCA-GTT-CGT-G-3' (sense) and 5'-AGG-GTG-GCC-TCA-TGG-TCA-GAG-ATG-G-3' (antisense), yielding a 525-bp fragment (Fig. 1*B*). RNA was analyzed by PCR after DNase digestion and incubation with reverse transcriptase as described (9). Southern blot hybridization of PCR products from the DNA and RNA analysis (Fig. 1 *A* and *C*) was performed with a probe to internal sequence, derived by digestion of pHLA-B7, described above, with *Pvu* II and *Bgl* II by standard methods (8). The control using human embryonic kidney 293 cells transfected with plasmids *in vitro* served to establish the conditions for DNase digestion (Fig. 1*B*). Under these conditions, no PCR signal was detected in the absence of reverse transcription.

Immunologic Analysis. Limiting dilution analysis of the HLA-B7 cytolytic T-lymphocyte (CTL) response was performed by a modification from a previous protocol (10) comparing autologous Epstein–Barr virus-transformed B cells to a subline transduced with an HLA-B7 retroviral vector (1). CTL function against melanoma cells was assayed as described (11).

RESULTS

Five patients who satisfied the entry criteria (3) were treated in the Clinical Research Center at the University of Michigan
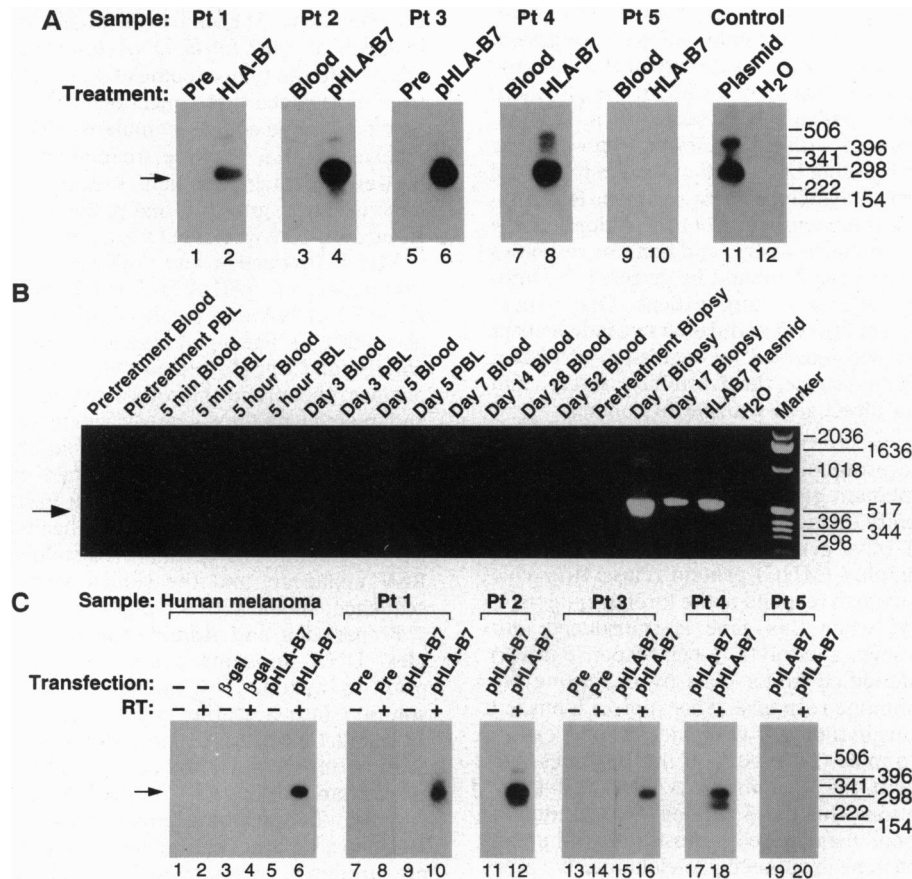

FIG. 1.   Gene transfer and expression of foreign MHC gene in human melanoma. (*A*) Detection of plasmid DNA in melanoma nodules after direct gene transfer with the DNA-liposome complex. Nucleic acids were isolated from injected nodules and analyzed by PCR (1). Size markers are indicated at right in base pairs. Samples were taken at the indicated times, and DNA was extracted according to standard methods. The sensitivity of the PCR analysis is ≈1 copy of recombinant gene per 10^5 genomes (7). Pt, patient. (*B*) Localization of plasmid DNA at the injection site. PCR analysis was performed on DNA extracted from biopsy samples obtained either pretreatment (Pre) or 3–7 days posttreatment (HLA-B7) or from blood samples at the indicated times following gene transfer in patient 3A. PBL, peripheral blood lymphocytes. (*C*) Confirmation of gene expression in tumor nodules transduced by direct HLA-B7 gene transfer. Recombinant HLA-B7 mRNA was detected by using a reverse transcriptase–PCR technique on nucleic acids from biopsy samples. Total RNA was incubated in the presence (+) or absence (−) of reverse transcriptase (RT) and analyzed (8).

Medical Center from June 4, 1992 to February 11, 1993. Clinical profiles of these patients are presented in Table 1. In each case, these patients suffering from melanoma exhibited progressive disease (stage IV) unresponsive to all conventional forms of therapy. Each patient tolerated the treatment well with no acute complications.

A sample of the treated tumor nodules was obtained by a core needle biopsy 3–7 days following the intratumoral injection with HLA-B7 DNA–liposome complexes. This tissue was analyzed for the presence of plasmid DNA, mRNA coding for HLA-B7, and the expression of HLA-B7 protein. In four of five patients, pHLA-B7 DNA was detected within the injected nodule (Fig. 1A). Blood samples obtained immediately before and multiple times after injection were analyzed for the presence of plasmid DNA by PCR. In the five patients, plasmid DNA was not detected at any time in the blood following gene transfer by PCR (Fig. 1B; sensitivity, <2 pg/ml).

To determine whether the recombinant HLA-B7 gene was expressed in transduced tumors, mRNA was analyzed. In four of five tumor biopsies, RNA coding for HLA-B7 was detected by PCR after incubation with reverse transcriptase but not in its absence (Fig. 1C), suggesting that RNA encoding the gene product was synthesized *in vivo*. In the single case where mRNA was not detected (patient 5), an inhibitor of the PCR was present (data not shown), as was observed in the DNA PCR in this patient (Fig. 1A).

HLA-B7 protein expression was confirmed within these biopsy samples by immunochemical staining of the samples with monoclonal antibody against this gene product (Fig. 2) or by indirect immunofluorescence of dissociated tumor cells (Fig. 2 legend; data not shown). In all cases, the recombinant protein was detected by these methods at frequencies ranging from 1–10% of tumor cells near the site of injection (Fig. 2 and legend). These data also confirmed that the failure to detect DNA and RNA in patient 5 was technical, related to inhibition of the PCR, since HLA-B7 was immunohistochemically detected in tumor cells of this patient.

Analysis of serum biochemical parameters revealed no pattern of systemic abnormalities in these patients, whether DNA was injected intratumorally or by catheter, including serum markers of liver, renal, pancreatic, and cardiac function (data are available upon request). No changes from baseline in any of the serum biochemical parameters were found in the acute period, days 3–7. Myocardial abnormalities were not detected by analysis of creatine kinase or its isoenzymes in the serum of treated patients (data are available upon request), and no electrocardiographic changes or arrhythmias were noted.

A major question regarding the future potential of this gene-delivery approach was whether an immune response would be generated to DNA. Such a response or the formation of antibodies to DNA could induce resistance to treatment or signal the development of autoimmunity. In this study, no increases in anti-DNA antibodies were detected in patients, and there was no clinical evidence of autoimmune phenomena, as indicated by changes in antinuclear antibodies, C-reactive protein, or other immunologic markers following DNA–liposome treatment (data available upon re-
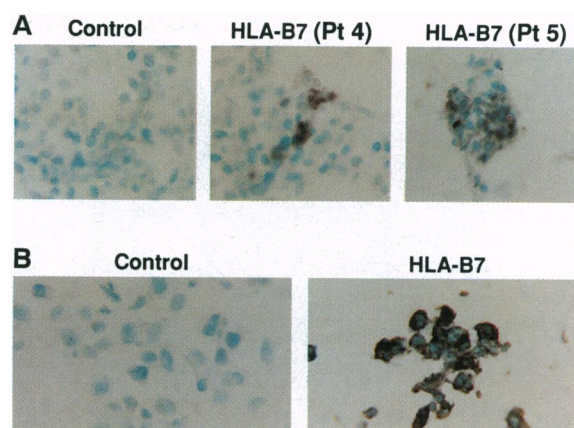

FIG. 2. Immunohistochemical detection of HLA-B7 expression in transduced tumor nodules. (A) A representative immunohistochemical stain on biopsies from patients (Pt) 4 and 5 is shown, with a control (IgG1 monoclonal) or anti-HLA-B7 (ME-1/HB119) antibody (IgG1 isotype) as indicated. Because this antibody can be crossreactive with HLA-B27, expression was also confirmed with another antibody nonreactive with HLA-B27 (MB 40.2/HB59; patient 5). The frequency of positive cells was estimated at ≈1% in both patients, but could be influenced by sampling. (B) Expression of HLA-B7 protein was evaluated in an HLA-B7-positive Epstein–Barr virus-transformed line, JY (12). Additional monoclonal antibodies used to human HLA-B7 were ME-1 (13) and MB 4.0.2. Immunostaining was performed using a horseradish peroxidase-coupled rabbit anti-mouse second antibody (Vector Cloning Systems) (1). Samples were analyzed in some cases by immunofluorescence with the BB7.1 monoclonal antibody (14). Indirect immunofluorescent staining of mechanically dispersed unfixed cells was evaluated in patients 1, 2, 3A, and 3B and revealed a frequency of positive cells of ≈1% for patients 1 and 2 and 1–10% for patient 3, treatments A and B, in the biopsy sample.

quest). These data suggest that DNA is not highly immunogenic *in vivo* and that the immune response is unlikely to limit *in vivo* gene transfer by DNA–liposome complexes or other forms of DNA.

Evaluation of the cell-mediated immune response in patient 1 revealed a 5-fold increase in the frequency of HLA-B7-reactive CTL precursors after treatment (Fig. 3A). The immune response to autologous tumor was also evaluated in two patients where cell lines could be established from the tumor. Tumor-specific CTLs were detected after treatment in both cases, either from tumor-infiltrating lymphocytes (patient 1) or from peripheral blood lymphocytes (patient 3), each of which lysed autologous melanoma specifically in a dose-dependent fashion (Fig. 3 B and C). These responses to autologous tumor cannot be unequivocally attributed to the gene-transfer treatment; however, the ability to detect T cells specific for melanoma in the peripheral blood (patient 3) is unusual and likely to be treatment-related. This finding, taken together with the increased CTL precursor frequency for HLA-B7, suggests that expression of a foreign MHC gene after direct gene transfer altered the reactivity of the immune system in these patients.

Table 1. Clinical profiles of patients and tumors

| Group	Patient	Age and sex	HLA haplotype	Previous treatment	Treatment site
I	1	62, F	A2,3; B44,−,W4	Surgery	Left axillary node
	2	61, F	A1,68; B27, 37, W4	Surgery, radiation	Right inguinal node
	3A	68, M	A1,−; B5, 57, W4	Surgery, chemotherapy, tumor vaccine plus interleukin 2, interferon γ, radiation	Left chest
II	3B	68, M	See 3A	See 3A	Left chest
	4	52, M	A1,−; B8, 17	Surgery, chemotherapy, T cells plus interleukin 2	Left axillary node
	5	65, F	A2,26; B27, 44, W4	Surgery, radiation	Right cervical node
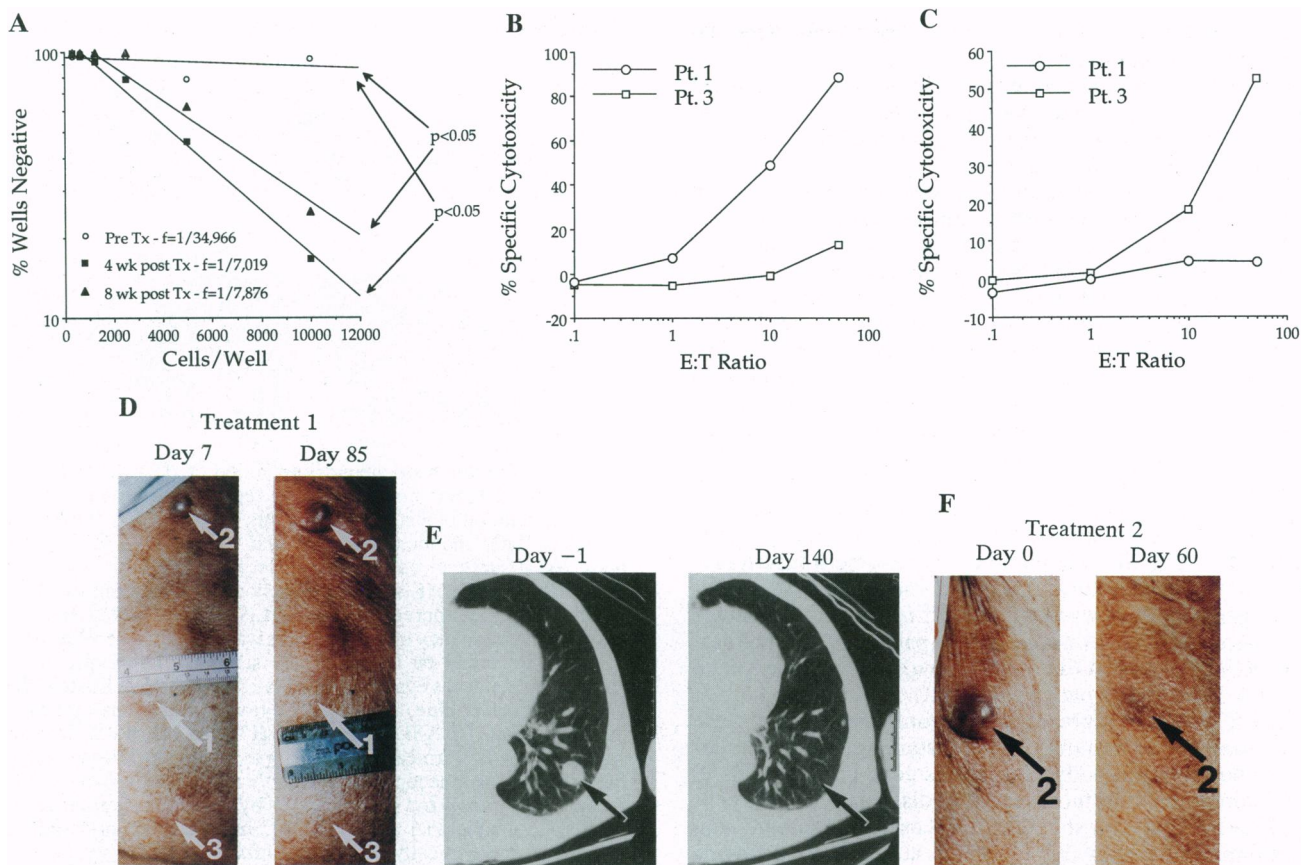
FIG. 3.   CTL response to HLA-B7, antitumor response, and tumor regression in patients. (*A*) Limiting dilution analysis of frequencies (f) of cytotoxic precursors specific for HLA-B7 was performed with patient 1 pretreatment (Pre Tx) (○) or 4 weeks (■) or 8 weeks (▲) posttreatment. Significance ($P < 0.05$) was assessed by χ^2 minimization. (*B* and *C*) Dose–response analysis of CTL activity from patients 1 and 3 tested against melanoma target cells derived from patient 1 (*B*) or patient 3 (*C*) is shown at the indicated effector/target (E:T) ratios. To verify the specificity of this response and to show that these tumor targets were equally susceptible to lysis, lymphokine-activated killer cells were generated separately in the presence of interleukin 2 (1000 units/ml). Lymphokine-activated killer activity (percent specific lysis) against melanoma for patient 1 (*B*) was 1%, 28%, and 58%, and for patient 3 (*C*) was 12%, 68%, and 96%, at effector/target ratios of 1:1, 10:1, and 50:1, respectively. (*D*) Tumor regression in patient 3 at the site of injection and at a distal site is shown. Photographs show a treated nodule (no. 1) and untreated nodules (nos. 2 and 3) at the indicated times after the beginning of treatment 1 in patient 3A. Treatment 1 consisted of single injections at 2-week intervals (three times) in nodule 1. (*E*) Computerized tomographic evidence of tumor regression at a distant pulmonary site is indicated, showing a responsive pulmonary nodule at the indicated times after treatment 1 began. (*F*) Photographs of nodule 2 days before (*Left*) or 60 days after (*Right*) initiation of treatment 2 (patient 3B). Treatment 2 consisted of three injections into nodule 2 at 2-week intervals (three times) and was begun 85 days after the initiation of treatment 1.

With regard to tumor growth, one patient receiving treatment to a cutaneous nodule (patient 3A) showed complete regression of a treated nodule (Fig. 3*D*). In this patient, metastatic lesions at distant sites displayed complete regression over the same time period, including a 3-cm pulmonary nodule (Fig. 3*E*), a 0.5 × 0.5-cm lesion on the lower back, a 1 × 1-cm lesion on the upper back, a 1 × 1-cm lesion on the skin of the upper arm, and a 1 × 1-cm lesion on the skin of the abdomen. In the same patient, at least one distant site showed no response to DNA–liposome treatment, indicating a mixed response; however, this unresponsive distant site in the lung was documented later to be a second primary cancer, adenocarcinoma. In addition, an independent treatment of a second site resulted in a reduction in tumor size (Fig. 3*F*). These data suggest that introduction of the HLA-B7 gene may potentially lead to a therapeutic effect at the treatment site and at distant sites of disease.

DISCUSSION

In this study, a human HLA-B7 gene was introduced into subcutaneous melanoma tumors of HLA-B7-negative patients by direct gene transfer with DNA–liposome complexes. The transferred gene was expressed and was local-

ized to the site of injection, and no apparent toxicity or anti-DNA antibodies were associated with this treatment. In one patient, regression of a treated lesion was observed and was associated with a similar response at several other distant sites of disease.

A variety of genetic abnormalities arise in human cancer which contribute to neoplastic transformation and malignancy. Instability of the genome generates mutations which alter cell proliferation, angiogenesis, metastasis, and tumor immunogenicity. Despite increasing understanding of the molecular basis of cancer, many malignancies remain resistant to established traditional forms of treatment. The definition of tumor-associated genetic mutations, however, has heightened interest in cancer as a target for gene therapy. Indeed, specific cancers, such as melanoma or renal cell carcinoma, are relatively more responsive to modulation of immune function, possibly because the immune system can be induced to recognize mutant gene products which arise in these cells. Conventionally, approaches to immunotherapy have involved the administration of nonspecific immunomodulating agents such as bacillus Calmette–Guérin or cytokines and/or the adoptive transfer of lymphokine-activated killer cells, which has shown promise in animal models (15–20) and in humans (21–24). More recently, molecular genetic inter-

Medical Sciences: Nabel *et al.*

Proc. Natl. Acad. Sci. USA 90 (1993)   11311

ventions have been designed in an attempt to improve the efficacy of immunotherapy (25, 26).

In this study, it is postulated that expression of HLA-B7 in tumor cells caused recognition of the foreign transplantation antigen by the immune system, stimulating the release of cytokines locally which induce a T-cell response against the unmodified tumor (2). In murine models, such local immune stimulation enhances recognition of tumor antigens previously undetected by the immune system and induces tumor lysis by CTLs (2). One patient responded to this treatment on two occasions, with both local and distant tumor regression. Of note, this patient failed to respond to previous immunotherapy with bacillus Calmette–Guérin and interleukin 2 (Table 1 legend), which predated gene transfer by 11 months. Although this response was encouraging, we caution that it is not possible at this stage to conclude that the treatment is consistently therapeutic: additional clinical studies will be required to define its efficacy. It is interesting to note that the patient whose tumors regressed after treatment received injections in cutaneous nodules, whereas in the other patients, metastatic lymph nodes were treated (Table 1). Possibly, lymph node replacement by tumor is associated with a greater degree of immunosuppression, or dermal dendritic cells may provide more efficient antigen presentation and immune stimulation.

Although modified viruses have served as vectors essential for *ex vivo* gene transfer, their ability to recombine or interact with endogenous viruses has raised concerns regarding their safety for *in vivo* gene transfer. Nonviral vectors provide an important and potentially safer alternative. Nonviral vectors include DNA–liposome complexes, which can mediate gene transfer into tissues and facilitate uptake by cells *in vivo* (27, 28). Plasmid DNA containing appropriate regulatory sequences can be utilized to express a variety of different gene products. Both DNA and liposomes may be stored stably for months, and the use of liposomes obviates the need to synthesize viral vectors, establish subclones of producer cell lines, and assess viral titers and the presence of replication-competent helper virus. Plasmid DNA complexed to liposomes has now been employed to transfer genes by injection or catheter into tissues where they stimulate localized biological responses (1, 2, 8, 27, 29–31).

The findings reported here represent a requisite step in the development of direct gene transfer for treatment of human disease. These studies demonstrate that a recombinant gene can be directly introduced into humans *in vivo* and that the gene product is expressed by host cells. The treatment is safe, and no toxicities were observed. The next steps will be to analyze the mechanism of the antitumor response and to improve its efficacy. Now that the safety of this approach has been determined, it is possible to include other genes in addition to MHC genes directed at other molecular targets. For example, such genes might include those coding for cytokines, antioncoproteins, growth factor antagonists, or inhibitors of angiogenesis. Alternatively, gene-transfer approaches could be used in combination with existing treatments such as cytokines or adoptive T-cell therapy. The findings reported here suggest that direct gene transfer warrants further development and is likely to contribute to the treatment of cancer and other human diseases.

1. Nabel, E. G., Plautz, G. & Nabel, G. J. (1992) *Proc. Natl. Acad. Sci. USA* **89**, 5157–5161.
2. Plautz, G. E., Yang, Z., Wu, B., Gao, X., Huang, L. & Nabel, G. J. (1993) *Proc. Natl. Acad. Sci. USA* **90**, 4645–4649.
3. Nabel, G. J., Chang, A., Nabel, E. G., Plautz, G., Fox, B. A., Huang, L. & Shu, S. (1992) *Hum. Gene Ther.* **3**, 399–410.
4. Nabel, G. J., Chang, A., Nabel, E. G., Plautz, G., Fox, B. A., Huang, L. & Shu, S. (1992) *Hum. Gene Ther.* **3**, 705–711.
5. Gorman, C., Padmanabhan, R. & Howard, B. H. (1983) *Science* **221**, 551–553.
6. Gao, X. & Huang, L. (1991) *Biochem. Biophys. Res. Commun.* **179**, 280–285.
7. Stewart, M. J., Plautz, G. E., Del Buono, L., Yang, Z. Y., Xu, L., Gao, X., Huang, L., Nabel, E. G. & Nabel, G. J. (1992) *Hum. Gene Ther.* **3**, 267–275.
8. Sambrook, J., Fritsch, E. F. & Maniatis, T. (1989) *A Laboratory Manual* (Cold Spring Harbor Lab. Press, Plainview, NY), pp. 31–57.
9. Nabel, E. G., Yang, Z., Liptay, S., San, H., Gordon, D., Haudenschild, C. C. & Nabel, G. J. (1993) *J. Clin. Invest.* **91**, 1822–1829.
10. Tzeng, J., Barth, R. F. & Orosz, C. G. (1992) *J. Immunol. Methods* **146**, 177–184.
11. Fox, B. A. & Rosenberg, S. A. (1989) *Cancer Immunol. Immunother.* **29**, 155–166.
12. Ho, I.-C., Yang, L.-H., Morle, G. & Leiden, J. M. (1989) *Proc. Natl. Acad. Sci. USA* **86**, 6714–6718.
13. Ellis, S. A., Taylor, C. & McMichael, A. (1982) *Hum. Immunol.* **5**, 49–59.
14. Brodsky, F. M., Parham, P., Barnstable, C. J., Crumpton, M. J. & Bodmer, W. F. (1979) *Immunol. Rev.* **47**, 3–61.
15. Zbar, B., Bernstein, I. D. & Rapp, H. J. (1971) *J. Natl. Cancer Inst.* **46**, 831–839.
16. Rosenberg, S. A., Mule, J. J. & Spiess, P. J. (1985) *J. Exp. Med.* **161**, 1169–1188.
17. Shu, S. & Rosenberg, S. A. (1985) *Cancer Res.* **45**, 1657–1662.
18. Spiess, P. J., Yang, J. C. & Rosenberg, S. A. (1987) *J. Natl. Cancer Inst.* **79**, 1067–1075.
19. Chou, T., Chang, A. E. & Shu, S. (1988) *J. Immunol.* **140**, 2453–2461.
20. Yoshizawa, H., Chang, A. E. & Shu, S. (1991) *J. Immunol.* **147**, 729–737.
21. Morton, D. L., Eilber, F. R. & Holmes, E. C. (1974) *Ann. Surg.* **180**, 635–643.
22. Rosenberg, S. A., Lotze, M. T., Yang, J. C., Aebersold, P. M., Linehan, W. M., Seipp, C. A. & White, D. E. (1989) *Ann. Surg.* **210**, 474–485.
23. Rosenberg, S. A., Packard, B. S., Aebersold, P. M., Solomon, D., Topalian, S. L., Toy, S. T., Simon, P., Lotze, M. T., Yang, J. C. & Seipp, C. A. (1988) *N. Engl. J. Med.* **319**, 1676–1680.
24. Kradin, R. L., Lazarus, D. S. & Dubinett, S. M. (1989) *Lancet* **i**, 577–580.
25. Rosenberg, S. A., Aebersold, P., Cornetta, K., Kasid, A., Morgan, R. A., Moen, R., Karson, E. M., Lotze, M. T., Yang, J. C., Topalian, S. L., Merino, M. J., Culver, K., Miller, A. D., Blaese, R. M. & Anderson, W. F. (1990) *N. Engl. J. Med.* **323**, 570–578.
26. Rosenberg, S. A. (1992) *Hum. Gene Ther.* **3**, 57–73.
27. Nabel, E. G., Plautz, G. & Nabel, G. J. (1990) *Science* **249**, 1285–1288.
28. Felgner, P. L., Gadek, T. R., Holm, M., Roman, R., Chan, H. W., Wenz, M., Northrop, J. P., Ringold, G. M. & Danielsen, M. (1987) *Proc. Natl. Acad. Sci. USA* **84**, 7413–7417.
29. Nabel, E. G., Yang, Z. Y., Plautz, G., Forough, R., Zhan, X., Haudenschild, C. C., Maciag, T. & Nabel, G. J. (1993) *Nature (London)* **362**, 844–846.
30. Ulmer, J. B., Donnelly, J. J., Parker, S. E., Rhodes, G. H., Felgner, P. L., Dwarki, V. J., Gromkowski, S. H., Deck, R. R., DeWitt, C. M., Friedman, A., Hawe, L. A., Leander, K. R., Marinez, D., Perry, H. C., Shiver, J. W., Montgomery, D. L. & Liu, M. A. (1993) *Science* **259**, 1745–1749.
31. Hyde, S. C., Gill, D. R., Higgins, C. F., Trezise, A. E., MacVinish, L. J., Cuthbert, A. W., Ratcliff, R., Evans, M. J. & Colledge, W. H. (1993) *Nature (London)* **362**, 250–255.