Complete Regression of Human Fibrosarcoma Xenografts after Local Newcastle Disease Virus Therapy

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Abstract

We have recently demonstrated that a single local injection of the avian pathogen Newcastle disease virus (NDV; strain 73-T) causes complete regression of human neuroblastoma xenografts in athymic mice (R. M. Lorence, K. W. Reichard, B. B. Katubig, H. M. Reyes, A. Phuang ساب, B. R. Mitchell, C. J. Cascino, R. J. Walter, and M. E. Peeples. J. Natl. Cancer Inst., 86: 1228—1233, 1994). In this report, we tried to determine if this in vivo antineoplastic effect of NDV extends to human sarcomas. Athymic mice with s.c. HT1080 fibrosarcoma xenografts (7—14 mm) were randomly divided into two groups and treated i.t. with a single injection of either 10^7 plaque-forming units of NDV or phosphate-buffered saline. Complete tumor regression occurred in 8 of 10 mice treated with NDV while unabated tumor growth occurred in all 9 mice treated with phosphate-buffered saline (P < 0.001). To determine if complete tumor regression was long lasting, the 8 mice were monitored for 1 year, during which time no tumor recurred. To test the antitumor effects of NDV on tumors derived from a fresh human sarcoma, a similar experiment was performed in athymic mice using TH15145 synovial sarcoma xenografts at their first and second passages. Of 9 mice with TH15145 xenografts, a single i.t. injection of NDV (10^7 plaque-forming units) caused complete regression of 3 tumors and >80% regression in 3 more tumors. In contrast, tumors in all 5 mice treated with phosphate-buffered saline exhibited unabated growth (P < 0.03 for >80% tumor regression). Since HT1080 fibrosarcoma cells express the N-ras oncogene, we explored the effects that transfection of this oncogene has on the sensitivity to NDV. Cultured human fibroblasts that were made tumorigenic following N-ras transfection were found to be 1000-fold more sensitive to NDV than normal fibroblasts in a cytotoxicity assay. Oncogene expression by the HT1080 fibrosarcoma may therefore contribute to the long-lasting complete regression of this sarcoma following a single local injection of NDV.

Introduction

Recently there has been a renewed interest in the potential use of cytolytic viruses to cause selective destruction of tumors (1—3). The rationale for such an approach stems from the numerous anecdotal case reports in the clinical literature describing tumor regression in human cancer patients during virus infection or immunization (4). In an uncontrolled clinical trial, Asada (5) claimed at least some regression of tumors in 79 of 90 cancer patients treated with a wild-type mumps virus (a human paramyxovirus related to the avian NDV^3). In another report, Csatary (6) noted complete remission in a chicken farmer with widely metastatic gastric cancer during a severe outbreak of Newcastle disease within the chicken population. At about the same time, Cassel and Garrett (7) injected NDV into a primary cervical carcinoma and noted marked tumor regression at the site of injection as well as shrinkage of lymph node metastases. Although the mechanism is unclear, Csatary et al. (8) recently claimed that NDV treatment caused partial tumor regression in 8 of 33 patients studied in a small clinical trial.

A potential advantage to the use of NDV in the treatment of cancer is its relative safety for mammals including humans (2). The strain 73-T of NDV (used in this report) had been attenuated by 73 passages in vitro and 13 passages in vivo in murine Ehrlich ascites tumor cells (7). After NDV 73-T was administered in low doses to over 80 human cancer patients in tumor vaccine trials, the only side effect noted was low-grade fever (9, 10). NDV 73-T at doses up to 5 × 10^8 PFUs caused no acute or chronic ill effects in athymic mice (1). Furthermore, in contrast to other oncolytic viruses, NDV 73-T is almost uniquely nonneurotropic since it caused no neuropathic effects after i.c. injection into neonatal mice and it did not replicate in adult rodent brains (7). Even the wild-type NDV produces minimal human disease, mainly mild conjunctivitis and laryngitis (2). However, no such cases of conjunctivitis or laryngitis were noted in any patient given NDV 73-T (9, 10).

There are several possible mechanisms by which NDV may induce tumor lysis (2, 11). We have shown previously that NDV is directly cytotoxic to a wide variety of human cancer cells but not to normal fibroblasts in vitro (11). We also noted that NDV is a potent inducer of tumor necrosis factor alpha and that NDV-infected cells are dramatically more sensitive to lysis by this cytokine than are uninfected cells (12). Finally, results of tumor vaccine trials using oncolysates containing live virus suggest that NDV is an effective immune adjuvant in stimulating antitumor immunity (9, 10, 13).

We have demonstrated previously that a single local injection of NDV strain 73-T causes long-lasting complete regression of human neuroblastoma xenografts in athymic mice (1). In this report we tested if this in vivo antineoplastic effect of NDV extends toward other human tumors including HT1080 fibrosarcoma xenografts. Previously, in vitro results have indicated that this sarcoma line is very sensitive to the cytotoxic effects of NDV (11). Since N-ras expression is critical for HT1080 tumorigenicity (14, 15) we also analyzed the effects that transfection of ras oncogenes into fibroblasts has on sensitivity toward NDV.

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3 The abbreviations used are: NDV, Newcastle disease virus; PFU, plaque-forming units; FBS, phosphate-buffered saline.
Materials and Methods

**Virus Preparation and Cell Lines.** Stocks of purified NDV 73-T were prepared and quantified by plaque assay as described previously (1). The HT1080 human fibrosarcoma cell line was obtained from the American Type Culture Collection (Rockville, MD), and the human fibroblast lines LG1, MSU1.1, DW5PT, and PHDM were gifts from Dr. J. J. McCormick, Michigan State University (East Lansing, MI) (16—18). All cell lines were cultured in Opti-MEM (GIBCO BRL, Grand Island, NY) supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (1).

**Mice.** Six-week-old female athymic homozygous BALB/c-nu/nu mice (Life Sciences, Inc., St. Petersburg, FL) maintained in filter-top cages and provided with sterilized bedding, food, and water were used. The care of these animals and experimental protocols were conducted according to the guidelines established by the Institutional Animal Care Committees, Cook County Hospital and Rush-Presbyterian-St. Luke’s Medical Center.

**Low-Passage Human Tumor Xenografts.** Fresh tumor tissue was obtained from the primary tumor of a 62-year-old woman with a high-grade, biphasic synovial sarcoma of the right wrist (with numerous lung metastases). Within 1 h of surgical removal tumor tissue was removed aseptically from the surgical specimen and transplanted directly into 4 athymic mice with minor modifications according to the methods of Sharkey et al. (19). Briefly, after the tumor tissue was minced into 1-mm tissue pieces, each mouse was given subcutaneously injections with 4 such tissue pieces in 0.3 ml PBS using a 18-gauge needle and a 3-ml syringe. These TH15145 synovial sarcoma tumors at primary passage grew in 3 of 4 mice and reached 7 mm in diameter after approximately 2 months. The second passage of 1 of these tumors into 17 more athymic mice was performed identically as for the first passage. Tumors at second passage grew in 13 of 17 mice. This experimental protocol was conducted according to the guidelines established by the Human Investigation Committee, Rush-Presbyterian-St. Luke’s Medical Center.

**Treatment of s.c. Tumors.** HT1080 fibrosarcoma xenografts were produced as described previously for IMR-32 neuroblastoma xenografts (1). In the first experiment, 10 days after s.c. injection of 10^7 HT1080 cells into athymic mice, all animals exhibited tumors (7—14 mm in diameter). Nineteen animals with tumors [maximal dimension, 9.1 ± 0.5 mm (standard error); mean tumor volume, 125 ± 17 mm^3] were randomly divided into two treatment groups into which one set of mice was treated with PBS and the other set of mice was treated with 10^8 PFU of NDV. s.c. HT1080 fibrosarcoma tumors were passed directly from a surgical specimen from a patient to athymic mice (first passage). For the second passage of tumors, 10 days after s.c. injection of 10^7 HT1080 cells into athymic mice, all animals exhibited tumors (7—14 mm in diameter). Nineteen animals with tumors were again randomly divided into two treatment groups into which one set of mice was treated with PBS and the other set of mice was treated with 10^8 PFU of NDV. s.c. HT1080 fibrosarcoma tumors were passed directly from a surgical specimen from a patient to athymic mice (second passage). Tumors at second passage were used to inoculate athymic mice (third passage).

**Plaque Assay and Cytotoxicity Assay.** These assays were performed as described previously (1, 11, 20). Briefly, for the cytotoxicity assay each cell line to be assayed was plated in rows in triplicate in 96-well plates. After the cells grew to confluence the medium was aspirated and varying dilutions of NDV (5000, 500, 50, 5, and 0 PFU in 0.1 ml PBS) were added to each column of wells. After allowing 30 min for virus adhesion at room temperature the virus suspensions were aspirated and replaced with Opti-MEM and supplemented with 10% heat-inactivated fetal bovine serum and antibiotics (1). After 3 days of incubation at 37°C in an atmosphere of 5% CO2, 95% air, the medium was removed and the cell monolayers were fixed with 100% methanol and stained with 0.2% crystal violet.

**Statistical Analysis.** Tumor responses [presence or absence of regression (complete regression in the case of HT1080 and >80% regression in the case of TH15145)] due to each of the treatments were compared by the 2-tailed Fisher's exact test.

**Results**

**Complete Regression of HT1080 Fibrosarcoma Tumors in Response to NDV.** s.c. HT1080 fibrosarcoma tumors were grown in 19 mice and randomly divided into two groups for i.t. treatment with NDV or PBS. Since previous results with IMR32 neuroblastoma xenografts showed that a dose of 10^8 PFU was optimal (1), this same dose of virus was chosen for administration to one set of mice. At this dose level NDV injection caused 8 of 10 tumors to regress completely (no palpable or visible tumor remained) while rapid tumor growth occurred in all 9 mice treated with PBS (P < 0.001, Fig. 1). For the eight mice that exhibited complete regression no tumor regrowth was seen in the subsequent 12 months.

**Macroscopic and Histological Appearance of NDV-treated HT1080 Tumors.** Fig. 2 shows the macroscopic effects of treating tumors with live NDV. A representative athymic mouse with an untreated 7-mm-diameter HT1080 human fibrosarcoma tumor is shown in Fig. 2A. Eleven days after NDV treatment and shortly before complete regression the same mouse shows nearly total regression of its tumor without any damage to the overlying skin (Fig. 2B). In contrast, treatment of tumors of identical size with PBS (Fig. 2C) was followed by rapid tumor growth, seen 11 days later (Fig. 2D).

In another experiment, including additional mice with HT1080 tumor xenografts, histological sections of tumors were obtained 11 days after treatment while NDV-treated tumors were regressing. NDV treatment led to a total replacement of all viable tumor cells by granulation tissue and a mild-to-moderate mononuclear inflammatory infiltrate without any damage to the overlying epidermis (data not shown). Histological sections of PBS-treated HT1080 tumors showed viable tumor cells with characteristics identical to those seen previously (14).

**Marked Regression of Low-Passage TH15145 Synovial Sarcoma Tumors in Response to NDV.** Since multiple passage in tissue culture (as with the HT1080 cell line) is known to affect the genotypic and phenotypic characteristics of human tumor cells (21), it is important to determine if low-passage human tumors have a similar sensitivity to NDV. To address this question TH15145 synovial sarcoma tumors were passed directly from a surgical specimen from a patient to athymic mice (first passage). Two months after tumor growth in athymic mice this malignancy was passed into another set of athymic mice (second passage). The histology of the first- and second-passage TH15145 synovial sarcoma tumors was identical to that of the tumor in the patient (data not shown). Due to the limited availability of first-passage tumors only one such tumor was treated locally with a single injection of NDV (10^7 PFU); it showed 90% tumor regression by day 43 and 99% tumor regression by day 55, whereas a PBS-injected first-passage tumor grew unabated. After second-passage expansion into a larger number of athymic mice, eight more mice were treated i.t. with NDV. Of these, three mice showed complete tumor regression and two mice showed >80% tumor regression. In contrast, all 4 tumors at second passage and treated i.t. with PBS grew...
unabated. The effects of NDV on 9 low-passage tumors (first and second passages combined) are shown in Fig. 3 (P < 0.03 for >80% tumor regression for NDV treatment versus PBS treatment, Fisher's exact test).

Evidence That ras Oncogene Expression May Increase Sensitivity to NDV. Since N-ras expression is critical for HT1080 tumorigenicity (14, 15) we analyzed the effects that transfection of ras oncogenes has on sensitivity toward NDV. We studied a series of related human fibroblast lines (16–18): LG1 is a normal foreskin fibroblast line; MSU1.1 is an immortalized but nontumorigenic line derived from LG1 after transfection with v-myc; and DW5PT and PH3M are tumorigenic lines derived from MSU1.1 following transfection with N-ras and H-ras, respectively. In a 72-h cytotoxicity assay (Fig. 4) DW5PT (N-ras-transfected) and PH3M (H-ras-transfected, not shown) were 1000-fold more sensitive to NDV than either parental line (LG1 or MSU1.1).

Discussion

We have demonstrated previously that a single local injection of strain 73-T of the avian pathogen NDV causes a complete regression of human neuroblastoma xenografts in athymic mice. In this report we demonstrated that tumors of a different class of malignancy (mesenchymal as opposed to neuroectodermal) showed similar sensitivity to local NDV treatment. Complete regression was observed in 8 of 10 HT1080 fibrosarcoma xenografts and marked regression was seen in 6 of 9 low-passage TH15145 synovial sarcoma xenografts in response to a single i.t. injection of NDV. As with the IMR-32 neuroblastoma tumors treated with NDV, a few of the HT1080 and TH15145 tumors did not respond optimally after one local NDV injection, possibly due to variable leakage of the virus suspension from the tumor site. As shown previously, using neuroblastoma tumors (1), a virus capable of replication was required for tumor regression since HT1080 tumors
of cells to NDV, it cannot be ruled out that some other property of these provide conclusive results. The lack of an effect of transfection with virus susceptibility. Experiments using antisense technology to inhibit transfectants acquired during tissue culture passage is responsible for gemc parental lines from which they were derived (Wi, a normal importantin this regard since v-myc-transfection of normal fibroblasts did
forskin fibroblast; and MSU1.1, an immortalized line derived from Wi (DW5VF;tumorigenic and derived from MSU1.1) and the H-ras- tram contributed to sensitivity toward NDV. We studied a series of related NDV injection in athymic mice.4 Fur petent mice when compared to identical tumors in athymic mice.4 Fur n-ras oncogene expression in the ras transfectants and HT1O8O cells may correlate with increased tumotigenicity. The N-ras-trarafected line (PH3M; tumorigenic and also derived from MSU1.1) were infected line (DW5PT; tumorigenic line derived from Wi after transfection with v-myc; and DW5PT is a tumorigenic line derived from MSU1.1 following transfection with N-ras. Af ter 3 days of virus infection the cell monolayers were fixed with 100% methanol and stained with 0.2% crystal violet (see “Materials and Methods”). Clear wells indicate total destruction of the cell monolayer.

(n = 8) and TH15145 tumors (n = 1) showed unabated tumor growth in response to UV-inactivated NDV (data not shown).

We are beginning to address the applicability of NDV 73-T for human cancer therapy. As shown here low-passage human tumors (which are more representative of actual human tumors than are multipassage tumors) (21) show a similar sensitivity to NDV as do tumors derived from multipassage cell lines (such as HT1O8O). Before any clinical trial, safety is a main concern. The therapeutic effects shown here with the HT1O8O and TH15145 sarcomas were achieved with a quantity of live virus having a high margin of safety because doses up to 500 times greater had no toxicity in athymic mice (1). Most importantly, Cassel et al. (9, 10) have demonstrated a high degree of safety of low doses of NDV 73-T in humans. Additional concerns during preclinical testing are how the immune system may modulate the antitumor effects of NDV and to determine whether systemic therapy is feasible. The antitumor effects of a single local injection of NDV against murine fibrosarcoma and neuroblastoma tumors are enhanced in immunocompetent mice when compared to identical tumors in athymic mice.4 Furthermore we have also noted preliminary antitumor effects after systemic NDV injection in athymic mice.4

Since expression of the N-ras oncogene is important in HT1O8O tumorigenicity (14, 15) we tested if expression of this oncogene may contribute to sensitivity toward NDV. We studied a series of related human fibroblast lines (16–18) and observed that the sensitivity to NDV correlated with increased tumorigenicity. The N-ras-transfected line (DW5PT; tumorigenic and derived from MSU1.1) and the H-ras- transfected line (PH3M; tumorigenic and also derived from MSU1.1) were 1000 times more sensitive to NDV than were either of the nontumorogenic parental lines from which they were derived (LG1, a normal foreskin fibroblast; and MSU1.1, an immortalized line derived from LG1 following transfection with v-myc). Although these results suggest the possibility that N-ras and H-ras expression may increase the sensitivity of cells to NDV, it cannot be ruled out that some other property of these transfectants acquired during tissue culture passage is responsible for virus susceptibility. Experiments using antisense technology to inhibit ras-oncogene expression in the ras transfectants and HT1O8O cells may provide conclusive results. The lack of an effect of transfection with v-myc on sensitivity toward NDV was surprising since we had shown previously that transfection of the related oncogene N-myc into rat neuroblastoma cells markedly increased the sensitivity of these cells toward NDV (20). Perhaps changes associated with tumor cell growth in vivo are important in this regard since v-myc-transfection of normal fibroblasts did not affect tumorigenicity, whereas after N-myc transfection neuroblastoma cells acquired increased metastatic potential (20). This N-myc transfection not only increased the cytotoxic effects of NDV but also increased susceptibility to NDV-mediated rapid cell-to-cell fusion suggesting that the increased sensitivity to NDV may be due to oncogene-induced changes in the tumor cell membrane (1). ras oncogene expression may also increase sensitivity to NDV at an early stage in viral replication (e.g., virus attachment and/or fusion) since newly synthesized viral proteins were detected by indirect immunofluorescence in each ras-transfected line 7 h after infection, but they were not seen in normal fibroblasts even after 2 weeks (22). Most interestingly, similar results of increased sensitivity to NDV have therefore been demonstrated following transfection with two different classes of oncogenes (N-myc, a nuclear oncogene; and N-ras and H-ras, GTP-binding protein-related oncogenes). Expression of each of these oncogenes and increased malignancy in general are related to sialoglycoconjugate expression (23, 24). This may be a possible link to NDV sensitivity since sialic acid is important in NDV attachment to host cells (25, 26). Further characterization of these oncogene-transfected lines, of their parental lines, and of oncogene expression in tumors such as TH15145 may shed additional light on the molecular mechanism of sensitivity to NDV.

In conclusion, N-ras oncogene expression by the HT1O8O fibrosarcoma may contribute toward the long-lasting complete regression of these tumors following a single local injection of NDV. Additionally, sarcoma tissue (TH15145) passed directly from the patient to athymic mice appears to have a similar degree of sensitivity to NDV. Most importantly, these therapeutic effects were achieved with a dose of virus with a high margin of safety in these athymic mice.

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References


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