Plasma Levels of a Viral Protein during Adjuvant Treatment: Reflection of Murine Mammary Tumor Status and Therapeutic Effect

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ABSTRACT

The mouse mammary tumor and its associated virus, mouse mammary tumor virus, were chosen to test the possibility of using plasma levels of a M, 52,000 virally glycoprotein (gp52) as a means for monitoring changes in tumor status during surgical adjuvant cyclophosphamide:doxorubicin (Adriamycin):5-fluorouracil treatment. Analysis of tumor recurrence and plasma gp52 concentrations during the postoperative period demonstrated that both parameters were significantly decreased in the group receiving cyclophosphamide:doxorubicin:5-fluorouracil treatment. This observation suggests that plasma gp52 levels may be a useful alternative measure of therapeutic effect during surgical adjuvant treatment. A retrospective analysis of gp52 plasma levels and tumor status of individuals during treatment has revealed the following associations. (a) An early sharp postsurgical elevation in plasma gp52 level was associated with subsequent death of treated animals. (b) Maintenance of postsurgical gp52 levels at a low level (≤4.2 ng/ml) during and after treatment was characteristic of tumor-free survivors. (c) A gradual rise in plasma gp52 level accompanied CAF-delayed tumor recurrence. gp52 levels increased in all treated animals 2 wk prior to detectable tumor regrowths, resulting in a statistically significant increase in mean gp52 level (2.2 to 5.4 ng/ml). However, the magnitude of this increase was small for the majority of animals with tumor regrowths, and greater, more definitive elevations in plasma gp52 levels were only detected at the time of frank tumor recurrence. In addition, comparisons of early mean gp52 levels (8 to 10 days after surgery) for controls and for animals receiving various forms of alternative treatment have indicated that differences in gp52 levels reflect subsequent differences in recurrence rates.

The present data, obtained during surgical adjuvant treatment of BALB/c × DBA/8 F1 mice and viewed in a retrospective fashion, demonstrate that plasma gp52 concentrations reflected therapeutic effects.

INTRODUCTION

The need for a systemic means of monitoring mammary disease status and, ultimately, evaluating the course of mammary disease during therapy has led us to evaluate plasma levels of a viral protein as a possible diagnostic marker for the presence of tumor and the progression of disease in a murine mammary tumor model. As an initial step, the envelope gp52 of MMTV was purified and utilized in the development of a sensitive and accurate RIA (1). Two subsequent investigations provided the following important insights into the relationship of plasma gp52 levels to the presence of mammary tumors. (a) All mammary tumor-bearing mice were identified with markedly elevated plasma antigen levels (100 to 1000 ng/ml), while tumor-free mice maintained low (2 to 10 ng/ml) antigen levels.

(b) Antigen levels in plasma were found to rise concomitantly with increases in tumor size (although the ratio of these parameters varied from one spontaneous tumor to another). (c) Following tumor excision, a sharp decrease of 10- to 100-fold in the gp52 plasma levels demonstrated that the tumor was the principal source of plasma gp52, and (d) all tumor recurrences following surgical removal of tumor were coordinately reflected by elevations in gp52 plasma levels (2, 3). The capability to detect tumor regrowth following surgery and the subsequent accurate assessment of tumor status in individual mice achieved during the progression of mammary disease (3) have indicated that the gp52 level of plasma is a useful marker for the presence of disease. An initial study of gp52 levels during CAF treatment of tumor-bearing BALB/c × DBA/8 F1 (hereafter called CD8F1) mice has indicated that decreased plasma gp52 levels provide a measure of therapeutic effect for animals bearing significant tumor loads (4).

In the present study, gp52 levels of animals with minimal residual disease have been obtained serially during surgical adjuvant CAF treatment to determine if changing levels of gp52 in plasma correspond to changes in tumor status. The CD8F1 mouse model of spontaneous breast cancer was chosen for this investigation, since it has been previously demonstrated to be therapeutically predictive for human breast cancer management (5). This evaluation of therapeutic effect of surgical adjuvant CAF chemotherapy using serial determinations of MMTV gp52 levels in plasma has focused upon answering the following questions. (a) During surgical adjuvant treatment with CAF, will changing levels of plasma gp52 reflect changes in tumor status, or will the chemotherapy interfere with the use of gp52 as a marker for animals with minimal tumor load? (b) If viral antigen levels of the control and CAF-treated group are compared, will a lowering of gp52 levels in the treated population provide a relative measure of therapeutic effect? (c) Will antigen levels for treated individuals remain low with tumor-free survival, rise with tumor recurrence, or continue to elevate with disease progression? (d) Can early gp52 levels, those determined 8 to 10 days after surgery (i.e., early in the course of chemotherapy), provide some insight in judging the subsequent success of a therapeutic protocol?

The data presented herein demonstrate that MMTV gp52 does provide a useful systemic measure of therapeutic effect for animals with minimal residual disease. Plasma gp52 levels were decreased in all animals following surgical tumor removal. Maintenance of this decreased level was associated with decreased tumor recurrence in mice receiving surgical adjuvant CAF treatment. Subsequent increases in plasma gp52 levels could be detected 2 wk prior to detectable regrowth of mammary tumors. However, although the elevation of plasma gp52 that was detectable 2 wk prior to the appearance of palpable tumor was statistically significant, the magnitude of this signal of impending tumor regrowth was small, and larger elevations in gp52 levels were detected only at the time of frank tumor recurrence.
MATERIALS AND METHODS

Mouse Strain

MMTV-infected hybrid CD8F1 mice bearing spontaneous, autochthonous mammary tumors were used for all experiments and have been described previously (3, 5).

Surgical Procedure

The procedure used has been routinely referred to as a "strip" or enucleative removal of tumor (3). A longitudinal incision is made through the skin and the s.c. tissue adjacent to the tumor. The tumor-bearing skin flap is then mobilized laterally, and the tumor is enucleated from its s.c. attachment by blunt dissection. After the area is sponged, the skin flaps are closed with metallic clips. This procedure leaves behind microscopic tumor foci that usually regrow and, thereby, furnishes an excellent experimental model of minimal residual disease for the study of tumor recurrence during alternative adjuvant treatments. Sham and radical surgical procedures have been described previously (3).

Blood Samples

Approximately 500 µl were removed from the retroorbital venous plexus, using heparinized tubes. Plasmas were separated from cells by low speed centrifugation (1760 x g for 15 min), frozen rapidly in a dry ice methanol bath, and then stored at -70°C for radioimmunoassay.

RIA of MMTV gp52

The MMTV gp52, purified by concanavalin A affinity chromatography from virus of RIIII mouse milk and iodinated with 125I-labeled Bolton Hunter reagent, was used for RIA as described previously (1). The antiserum used for assay was a polyclonal rabbit serum that specifically reacted with MMTV gp52 but did not react with up to 25 µg of C-type viral antigen [purified virus produced in murine leukemia virus (Rauscher)-infected JLSV-9 cell cultures] (1) and did not react with other radiolabeled MMTV structural proteins (6). The assay used was a blocking radioimmunoassay in which delayed addition of the labeled antigen was used to maximize the sensitivity of the measurement. The sensitivity of this gp52 assay was 0.5 to 1.0 ng/ml which exceeded the sensitivity of previous whole particle assays (1). This assay was performed in the presence of the protease inhibitor Trasylol (500 kallikrein inhibitor units/ml). Use of Trasylol and diisopropyl fluorophosphosphate demonstrated that proteases did not interfere with the assay (2). The assay buffer (0.1 M potassium phosphate buffer, pH 7.5) contained 0.1% bovine serum albumin, 0.001 M EDTA, and 500 kallikrein inhibitor units of Trasylol per ml. As an assessment of assay variation for CAF treatment studies, plasma samples from control and CAF-treated mice were obtained from mice bearing significant mammary tumor loads (4). Determinations obtained from triplicate assays of 39 plasma samples of all animals present at an intermediate point in CAF therapy were analyzed. The standard error of the mean for 92% of these different mouse plasmas was less than 10%. The maximum SE, detected for only a single sample, was 14% (4).

Determination of Tumor Weight

The weights of tumors were determined using one of the following alternative procedures. (a) When tumors were surgically removed their weights were determined gravimetrically. (b) For tumor regrowths, tumors were measured at each palpation with calipers (two perpendicular measurements). The weights were then estimated with the formula, mg = 0.5 (L x W²), where L and W are respectively, the length and width of the tumor in mm (7).

Statistical Methods

The x² test has been utilized to test the significance of treatment on tumor recurrence. Differences in recurrence have been held significant at P ≤ 0.05. A two-tailed Mann-Whitney U test, a nonparametric test which makes minimal assumptions about the group distribution of data, has been utilized to test for population differences in plasma gp52 levels and group differences in time of tumor recurrence (8). The Mann-Whitney U test has been held significant at P ≤ 0.05 (two-tailed test).

Surgical Adjuvant Therapy

CAF Treatment. Thirty mice with spontaneous mammary tumors (300 to 2900 mg) were divided into two groups so that mice with approximately equal size tumors were represented in each group. Tumors were removed from all animals using "strip" surgery. Subsequent to tumor removal, one group received (i.p.) CAF injections [cyclophosphamide (50 mg/kg):doxorubicin (Adriamycin) (2 mg/kg):5-fluorouracil (50 mg/kg)] at 7-day intervals from Day 3 to Day 59 (CAF therapy is a proven treatment for both mouse and human mammary tumors (9–11)). Control animals received surgery and saline injections. Both control and CAF-treated mice were bled for measurement of plasma gp52 level at 4 days prior to surgery, at 10 days postsurgery, and at approximately 2-wk intervals thereafter for 107 days. Since previous results indicated that bleeding itself temporarily diminished plasma gp52 and that CAF-treated animals upon bleeding required an average of 13.2 days to recover to pretreatment gp52 levels, a bleeding interval of 14 days was used in the present study. Animals were evaluated for the presence of tumor by weekly palpation.

Thymosin Treatment. Thymosin obtained from Dr. Cleland (Hoffmann-LaRoche) was administered as a possible immunotherapeutic adjuvant to surgery. Thymosin has been reported to be active in patients with cellular immunodeficiency (12). Forty-five spontaneous tumor-bearing mice were subjected to "strip" surgery. These mice with "minimal tumor load" were divided into 3 groups of 15 mice each. Each group received ten injections (i.p.) at 2- to 3-day intervals from 1 wk prior to surgery to 14 days after surgery. Group one animals (controls) received saline. Group two animals received 0.1 mg of thymosin per mouse per injection. Group three animals received 1.0 mg of thymosin per mouse per injection. Animals were bled for determination of viral antigen level and evaluated for tumor recurrence both prior and subsequent to the termination of therapy.

RESULTS

The collaborative experiments to be described had a useful double-blind component. All of the surgical procedures, administration of adjuvant therapy, and diagnostic palpations for tumor presence were carried out in a laboratory which routinely evaluates the efficacy of new surgical adjuvant treatments, whereas all of the blood samples were coded and sent for gp52 analysis to a separate virology laboratory.

Tumor Recurrence during CAF Surgical Adjuvant Therapy. Mice receiving CAF adjuvant treatment and controls subjected only to "strip" surgery have been palpated to compare the effects of CAF treatment on tumor recurrence prior and subsequent to the termination of treatment. The results of palpation for both treated and control mice have been presented as the percentage of mice free of tumor recurrence in Fig. 1. The use of CAF treatment as an adjuvant to surgery resulted in a significantly greater number of tumor-free mice at all times during the course of treatment in comparison to saline-treated controls [x² test ranged from P ≤ 0.001 (Day 28) to P ≤ 0.05 (Day 69)]. Following the termination of treatment, tumor recurrence increased sharply in CAF-treated animals, and eventually the difference in the incidence of recurrence between the two groups no longer was significant. Therefore, the marked therapeutic effect was most evident as a delay in the time required for tumor recurrence. The period of time between tumor removal and detectable recurrence differed markedly for control and CAF-treated animals. Comparison of the average times for tumor recurrence, 24 days for control animals and 67 days for CAF-treated animals (P ≤ 0.002), further illustrates the effectiveness of this treatment.
in each control animal, simultaneous increases in tumor mass and gp52 level were noted. These increases indicate that elevated gp52 levels of controls were a reflection of recurrence and subsequent tumor growth. Between Days 39 and 53, the average antigen level of controls dropped sharply (Fig. 2). This drop in antigen level can be understood by analyzing the upper portion of Fig. 2. In the control group there was a large decrease in the percentage of survivors between days 39 and 53. The loss of these animals bearing the largest tumors and highly elevated gp52 levels accounts for the decrease in average antigen levels of the remaining controls. After Day 53, control antigen levels rose again in association with continued tumor growth in surviving mice.

Antigen levels for CAF-treated animals behaved quite differently. For these animals, average antigen level remained low for 39 days (2.61 to 10.1 ng/ml) and only increased slightly to 25.7 ng/ml during the latter days of the treatment period. At all times during surgical adjuvant treatment, significantly lower plasma gp52 levels were detected in CAF-treated animals ($P \leq 0.002$ to $P \leq 0.02$). Large increases in the antigen level of CAF-treated animals were only detected at late times (80 to 120 days) following the termination of therapy. The maintenance of lower average antigen levels in treated mice provided an indication of therapeutic effect in the treated group which reflected both a low incidence and a relative delay in tumor recurrence.

**Fate of Individual Mice during and after Therapy.** An evaluation of disease status for controls and CAF-treated mice was conducted at 107 days following surgery. While 100% of controls had suffered a tumor regrowth or a new primary tumor, only 73% of CAF-treated animals had regrowths, and new primaries were not detected. A very marked difference was noted in the number of deaths that occurred due to frank tumor enlargement before the termination of therapy on Day 59. While 60% of control animals had died prior to Day 59, only 7% of CAF-treated animals died. The lower number of deaths in the CAF-treated group led to a higher proportion of tumor-bearing survivors (47 versus 7%) and a higher proportion of tumor-free survivors (27 versus 0%). At 107 days postsurgery, 80% of control mice had died from progressively growing tumor regrowths, while 72% of the CAF-treated mice still were alive. For purposes of clarity, individual patterns of changing plasma gp52 levels will be considered separately for mice which died before 107 days and those surviving at 107 days.

**Plasma gp52 Levels prior to Death.** To retrospectively compare changes in tumor status with changes in plasma levels of viral antigen, plasma gp52 levels from four representative individuals (two controls and two treated animals) that died during the observation period are presented as a semilog plot of antigen level versus time after surgery in Fig. 3. These animals demonstrate the following pattern of changing antigen levels. A marked decrease in gp52 concentration is noted after surgery; a subsequent rise in gp52 level occurs with onset of tumor; a progressive increase in gp52 level occurs with growth of tumor; and subsequently death occurs at an elevated gp52 level. This pattern of changing plasma gp52 levels was detected for all treated and control animals that died within the 107-day observation period. An early sharp elevation in antigen level was associated with subsequent death for both treated and control animals. Ten of 16 deaths occurred within 67 days of surgery following a rapid rise in gp52 levels. The only noteworthy difference between treated and control animals was the degree of antigen elevation detected. The single CAF-treated animal that died during the period of administration of chemotherapy

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**Fig. 1.** Tumor recurrence during CAF surgical adjuvant therapy. Palpation has been utilized to determine the percentage of animals remaining free of tumor regrowth. At each point in time, this percentage is indicated for both CAF-treated and untreated animals during the postoperative period. Arrows indicate the times of CAF administration and untreated animals during the postoperative period. Arrows indicate the times of CAF administration. The following numbers of surviving animals were present at the times indicated in control and CAF-treated groups, respectively: Day 0, 15 and 15; Day 39, 12 and 15; Day 53, 7 and 14; Day 81, 5 and 13; and Day 107, 3 and 11.

**Fig. 2.** Plasma gp52 levels and animal survival during treatment. The percentages of animals remaining alive in the control (□) and CAF treatment group (▲) have been compared on each bleeding data prior and subsequent to surgery (●). Plasma gp52 levels of controls (□) and CAF-treated animals (▲) have been presented as average plasma concentrations before and after “strip” surgery (●). Bars, SE. CAF was administered at approximate 7-day intervals from Days 3 to 59. The time of surgery is indicated by a dashed vertical line.

**Plasma Levels of gp52 during Surgical Adjuvant CAF Treatment.** The mean concentration of plasma gp52 before surgery and at sequential times during the postoperative period in CAF-treated and control animals is presented in the lower portion of Fig. 2. The percentage of surviving animals has been presented at each point in time in the upper portion of Fig. 2 in order to permit assessment of the effect of animal deaths (particularly in the control group) on plasma gp52 concentrations.

Measurement of gp52 levels in both groups prior to surgery revealed an elevated but statistically similar average level of gp52 (control, 128 ng/ml, and CAF treated, 164 ng/ml). With surgical removal of tumor, antigen levels dropped sharply in both groups. By Day 10 (i.e., 7 days after the first course of CAF chemotherapy), antigen level had fallen to an average of 16.7 ng/ml for controls and 2.61 ng/ml for CAF-treated animals. As depicted in Fig. 2, gp52 levels of controls increased sharply in subsequent samples during the treatment period, reaching a high point (985 ng/ml) at 39 days after surgery. This rapid increase in antigen level of controls correlated with the high incidence of tumor recurrence (Fig. 1) and unchecked increase in tumor mass with time. Following tumor recurrence
had a lower antigen level than any of the nine control animals which died during the treatment period. It is conceivable that chemotherapeutic toxicity contributed to the death of this mouse. Those animals that died after the termination of therapy as a result of progressively growing tumor regrowths also displayed elevated gp52 levels with the onset of tumor. The ability to monitor disease status, for individuals in which CAF treatment failed, indicates that gp52 levels provide similarly useful information with respect to changing tumor status in both the presence and absence of CAF treatment.

**Plasma gp52 Levels in CAF-treated Survivors.** To examine the behavior of viral gp52 concentrations prior to regrowth, viral antigen concentrations are presented as a scatter plot for treated individuals at the time of regrowth and at three succeeding bleedings in Fig. 4. All treated animals suffering a regrowth are presented at the time of regrowth and at one bleeding prior to regrowth. Fewer animals are presented at two and three bleedings prior to regrowth because a sufficient time interval was not available to obtain these bleedings from animals with early regrowth (within 6 wk of surgery). gp52 levels at one bleeding prior to regrowth are significantly increased over levels at two bleedings prior to regrowth (P ≤ 0.009; Mann-Whitney U test). The following gp52 levels were obtained prior to and at the time of regrowth: 2.1 ± 0.4 (SE) ng/ml (—6 wk); 2.2 ± 0.4 ng/ml (— 2 wk); and 24.3 ± 7.0 ng/ml (regrowth). An approximate 2-fold increase in gp52 levels 2 wk prior to regrowth was associated with tumor recurrence.

To further examine the relationship between changing plasma levels of viral antigen and changes in tumor status during treatment, gp52 concentration has been plotted versus time after surgery for both tumor-free and tumor-bearing CAF-treated survivors. These individual patterns of change are presented in Fig. 5 for treated mice surviving at the end of the 107-day observation period. The four tumor-free CAF-treated mice that are presented are the only control or CAF-treated animals that suffered neither a new primary tumor nor a regrowth during the entire 107-day observation period. In these tumor-free animals, antigen level fell sharply and remained low at every bleeding throughout the observation period (<4.2 ng/ml). A solid horizontal line has been drawn in Fig. 5 at the upper limit of gp52 concentration obtained at any point in time in plasma from a tumor-free survivor. The first major point to be made from serially monitoring treated survivors is that upper levels of tumor-free survivors establish an approximate gp52 threshold for tumor-free survival. This threshold level was crossed by all animals experiencing tumor regrowth. The second point is that, while all animals experienced a sharp decrease in gp52 level following surgery, the rates of antigen increase prior to regrowth differed for individual animals. Further increases in plasma antigen levels reflected continued tumor growth in all tumor-bearing survivors.

**Plasma Levels of gp52, an Early Indication of Therapeutic Effect.** The use of the CD8F1 mouse model as a means of
VIRAL gp52, LEVELS REFLECTING THERAPEUTIC EFFECT

evaluating alternative surgical adjuvant therapies provided the opportunity to consider if changes in the initial gp52 levels of treated mice, those obtained at the first bleeding following surgery, might provide an indication of subsequent therapeutic effects as classically measured by changes in tumor recurrence. To test this hypothesis, both initial gp52 levels and subsequent tumor incidence have been compared in three different experiments for animals receiving alternative forms of treatment. The following alternative forms of therapy have been evaluated: (a) alternative surgical technique; (b) combination chemotherapy (CAF); and (c) immunotherapy (thymosin treatment). The details of therapeutic protocols have been presented in “Materials and Methods.” Plasma gp52 levels early after surgery (8 to 10 days) are presented for each experimental group subjected to alternative forms of surgery or surgery plus adjuvant therapy in Table 1 alongside the percentage of mice suffering a regrowth. For the purpose of this comparison, evaluation of tumor recurrence was conducted at a period in the course of therapy where the success or failure of a given therapeutic protocol could be judged.

The first experiment assessed alternative surgical techniques. In this case, results in Table 1 indicate the following hierarchy of postsurgical antigen levels: sham > strip > radical. In comparison, the incidence of mice with tumor recurrence at 21 days demonstrates the same hierarchy of therapeutic effect. In this experiment comparing alternative surgical procedures, a lower gp52 level following surgery correlated with an increase in the degree of therapeutic success.

While this therapeutic effect was expected for the surgical procedures used, the ability of early gp52 levels to reflect this effect encouraged further comparative analysis. In the second experiment using CAF treatment, early gp52 levels of treated animals have again been compared to controls. The results obtained indicate a significantly lower antigen level in the group receiving CAF treatment (P ≤ 0.002). This comparatively lower gp52 level at 10 days following surgery proved to be a reflection of the significantly lower percentage of animals with tumor noted at 39 days after surgery.

In the final experiment, pre- and postsurgical administration of two different doses of thymosin, a drug previously demonstrated to enhance T-cell-dependent immune competence (12–15), did not result in a protective effect (i.e., there were no significant differences in the number of regrowths). Correspondingly, the results presented in Table 1 indicate that initial gp52 levels did not vary significantly when controls and treated animals were compared 8 days after surgery. In this experiment, the failure to reduce viral antigen level following thymosin administration reflected the failure of this protocol to significantly reduce the incidence of animals with tumor at 21 days following surgery.

Therefore, for each of the alternative treatments tested, a comparison of early gp52 levels provided an indication of the relative therapeutic success or failure which was detected subsequently.

DISCUSSION

Early studies which detected gp52 in mouse milk, mouse mammary tumor tissue, and blood (2, 16–19) provided a basic understanding of MMTV protein expression in both tumor-free and tumor-bearing mice of different strains. We have attempted to obtain more clinically pertinent information by serially measuring plasma gp52 levels in therapeutic settings where this viral protein could later be retrospectively evaluated, as a marker for changing tumor status. Earlier studies demonstrated that increases in plasma gp52 levels occurred either prior to or coincident with tumor regrowths following surgery, and that further elevations in gp52 levels were correlated with continued tumor growth (3). These prior results indicated that plasma gp52 levels were responsive indicators of changing tumor status. The nearly 1:1 correspondence between plasma gp52 levels and tumor status following surgery alone (3) and the more recent finding that CAF treatments did not interfere with the use of gp52 levels as a measure of therapeutic effect in mice bearing significant tumor loads (4) encouraged the present investigation of gp52 levels as a measure of therapeutic effect for surgical adjuvant CAF-treated animals.

The present study has clearly addressed the possibility that CAF chemotherapy might interfere with the use of plasma gp52 levels to monitor postsurgical disease status. The results obtained herein for animals with minimal residual disease following strip surgery have demonstrated clearly that the assessment of tumor status gained during CAF treatment is as good as or better than that obtained in the absence of chemotherapy. The following findings support this conclusion, answer many of our initial questions, and illustrate how changing viral antigen levels of treated mice reflected the success or failure of treatment for each experimental group, as well as each individual. (a) When controls and CAF-treated animals were compared, decreased concentrations of plasma gp52 in CAF-treated animals were associated with a decrease in tumor recurrence and animal deaths during the postoperative period. (b) When treated individuals were monitored, a sharp early postoperative elevation of gp52 level was associated with subsequent death during the observation period. This death resulted from rapidly growing tumors. (c) The maintenance of plasma gp52 levels at a low level (≤4.2 ng/ml) during and after treatment was associated with tumor-free survival for treated animals. (d) A gradual elevation of gp52 level upon regrowth of tumor reflected the delay in tumor recurrence afforded by CAF treatment. (e) Initial gp52 levels of plasma, those determined 8 to 10 days after surgery and initiation of treatment, provided an early indication of subsequent therapeutic effect.

The retrospective evaluation presented in Fig. 4 for gp52

Table 1 Early gp52 levels and therapeutic effect

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Early gp52 levels (ng/ml mouse plasma)</th>
<th>Therapeutic effect (%)</th>
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<tbody>
<tr>
<td></td>
<td>post Surgery</td>
<td>with tumor</td>
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<tr>
<td>Alternative surgery</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sham</td>
<td>779.0 ± 236 6</td>
<td>100</td>
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<tr>
<td>Strip</td>
<td>44.0 ± 11.3 (P = 0.004)</td>
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<td>Radical</td>
<td>24.0 ± 2.8 (P = 0.004)</td>
<td>0 (P = 0.01)</td>
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<td>CAF chemotherapy</td>
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<tr>
<td>Surgery (control)</td>
<td>17.0 ± 6.5</td>
<td>83</td>
</tr>
<tr>
<td>Surgery + CAF</td>
<td>26.0 ± 0.6 (P = 0.002)</td>
<td>27 (P = 0.01)</td>
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<td>Thymosin immunotherapy</td>
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<tr>
<td>Surgery (control)</td>
<td>8.9 ± 1.8</td>
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<tr>
<td>Surgery + 0.1 mg thymosin</td>
<td>6.4 ± 1.1</td>
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<tr>
<td>Surgery + 1.0 mg thymosin</td>
<td>9.1 ± 4.5</td>
<td>42</td>
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</tbody>
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*Plasma gp52 levels were determined at 8 days following surgery in thymosin and alternative surgery protocols, whereas 10-day levels were obtained for the CAF protocol.

*Mean ± SE for gp52 concentrations.
levels at prior bleedings and at the time of regrowth has indicated that a significant but small increase in mean gp52 level is detected 2 wk prior to regrowth. However, a much more substantial elevation in gp52 level was noted at the time of frank tumor recurrence (coincident with detection by palpation). Although a small elevation in gp52 level was detected prior to regrowth, whether or not it can be used as a general diagnostic signal of future tumor regrowths can only be determined by future prospective studies in which large numbers of animals, subdivided objectively on the basis of gp52 levels, are used to predict risk of tumor regrowth. The results presented herein demonstrate a correspondence between tumor status during therapy and viral antigen levels of plasma that approach 1:1 and are most encouraging to the virologist; however, to the therapist, these results also point out some limitations of this viral marker which are general difficulties in our search for effective systemic markers for solid tumors. The degree of gp52 elevation, as well as the time interval prior to regrowth, is important in determining if gp52 offers advantages over frank tumor analysis as a measure of tumor status and therapeutic effect. The inability to detect a more substantial rise in gp52 level at an earlier point in time may indicate some limitations for gp52 as a marker, as well as indicate a more general feasibility problem in the clinical use of tumor-associated markers, since viral gp52, shed as both a soluble and particulate antigen from the tumor cell surface, should provide one of the most opportune situations for release and detection of a tumor-associated antigen (20).

The ability of early viral antigen levels to provide an indication of subsequent therapeutic effect offers the hope of providing a screening mechanism for evaluating therapeutic protocols. The results in Table 1 indicated that the degree of depression of early gp52 levels was inversely correlated with subsequent tumor recurrence rate. The failure of the pilot study with thymosin alone to affect tumor incidence was not unexpected and demonstrates a need to optimize the use of this agent in a combined regimen; however, the lack of a significant difference in early gp52 levels correctly reflected the lack of a demonstrable therapeutic effect.

One of the goals of our studies is to achieve a more effective adjuvant therapy. Since metabolic modulation by one drug may influence the effectiveness of a second drug, our ability to evaluate the therapeutic benefit of adding one component to another is essential to the development of more effective combination chemotherapy treatment. For example, the recent demonstration that tamoxifen arrests human mammary tumor cells in the G1 stage of the cell cycle suggests that, in combination chemotherapy, tamoxifen may modify the efficacy of other proven or experimental chemotherapeutic protocols (21). In this case, the many current surgical adjuvant trials using tamoxifen and additional chemotherapeutic protocols (22, 23) argue strongly that a need exists for an alternative means of evaluating therapeutic effect. If changes in gp52 levels reflect therapeutic effect, as noted in these studies, then changes in gp52 levels could be used as a systemic means of assessing if drugs are synergistic, antagonistic, or more effective than existing combination chemotherapy. It is hoped that this alternative means of assessing antitumor effect may contribute to our understanding of the mechanisms of drug action, as well as aid in the search for more effective forms of adjuvant therapy.

These studies which have tested the feasibility of using a viral diagnostic marker to obtain clinically relevant information during treatment engender the hope that similarly useful diagnostic markers will be identified in human tumor systems. When such markers are identified, it is anticipated that the experience gained in this murine system will prove invaluable as a model for studying this approach to the management of human cancers.

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