PLASMA LEVELS OF A VIRAL PROTEIN AS A DIAGNOSTIC SIGNAL FOR THE PRESENCE OF MAMMARY TUMOR: THE EFFECT OF TUMOR REMOVAL*

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We have adopted the murine mammary tumor model to explore the feasibility of using plasma concentrations of a viral protein to assess the presence and status of a solid tumor. As a necessary prelude, a convenient affinity chromatography procedure was devised (1) for the isolation in good yields of the 52,000 dalton glycoprotein (gp52)[1] of the mouse mammary tumor virus (MMTV). The availability of the pure protein made it possible to develop (1) a radioimmune assay (RIA) sufficiently sensitive and accurate for measurement of gp52 in the plasma at levels down to 0.1 ng/100 μl.

Application of this assay in a study (2) of tumor-free and tumor-bearing individuals revealed the following features of the relation between mammary tumors and plasma antigen: (*a*) tumor-bearing animals, male or female, show markedly elevated (100–1,000 ng/ml) levels of gp52 as a free soluble protein in the plasma and the mean concentration increases with average tumor size; (*b*) the presence of another malignancy (leukemia) does not result in any change of this protein in the blood; (*c*) mammary tumor tissue located (by transplantation) outside the mammary gland is also detected by high plasma gp52; (*d*) low (2–10 ng/ml) plasma levels of gp52 are found in tumor-free mice, whether they originate from strains characterized by high or low frequencies of spontaneous mammary tumors; (*e*) tumor-free lactating females exhibit the normally low levels of plasma gp52 despite the fact that their milk contains an average of 20,000 ng/ml of this protein; and finally, (*f*) the circulatory clearance time of gp52 in tumorous animals is sufficiently rapid (a half-life of 4–6 h) to suggest a requirement for continued replenishment to maintain the high levels observed.

The rapid clearance time and the other features noted offer the intriguing possibility that plasma levels of gp52 may be useful as specific and responsive indicators of the presence and extent of murine mammary neoplasia. It is the purpose of the present investigation to subject this expectation to further test by examining the effect of surgical removal of tumor on the gp52 levels in the

* This study was supported by U. S. Public Health Service grant CA-02332, the Virus Cancer Program Contract NO1-1-1010, and Contract NO1-CM-67081 of the Division of Cancer Treatment of the National Cancer Institute.

[1] *Abbreviations used in this paper:* gp52, 52,000 dalton glycoprotein; MMTV, mouse mammary tumor virus; RIA, radioimmune assay.

circulation. If the tumor is the sole or principal source of the plasma gp52, excision should lead to a drop in its level. If the expected decrease does occur, an opportunity is provided that is of even greater interest for monitoring disease status. Animals can be followed postoperatively for periods sufficient for the occurrence of relapse to determine when and how the gp52 returns to its high presurgical levels.

In particular, the present investigation was designed to answer the following questions: (*a*) What is the effect of tumor excision on the circulating level of gp52? (*b*) Can regrowth or new primaries during the postoperative period be detected by rises in the gp52 plasma levels? (*c*) More importantly, can the behavior of the gp52 level signal the recurrence of a tumor before it is detectable by palpation? (*d*) When individual mice are compared during periods of relapse, are the gp52 levels per unit mass of developing tumors the same or different from one mouse to another?

The data obtained provide answers to these questions and offer evidence that the plasma levels of gp52 do provide information on tumor status that is of both diagnostic and prognostic value.

Materials and Methods

Mouse Strain. MMTV-infected hybrid (BALB/c ×- DBA/8) F_1 mice, designated by $CD8F_1$ were obtained and used, as previously described (2, 3), as a convenient source of autochthonous spontaneous mammary tumors. $CD8F_1$ females were selected for all the experiments described. It should be noted that the use of animals with single tumors does not eliminate the likely appearance of a new primary tumor in one of the nine other apparently nondiseased breasts during the course of the experiments.

Surgical Procedures. Three surgical procedures were used, all under general anesthesia (sodium pentobarbitol). One is a "sham" operation consisting of an incision around the tumor, which is left in place. Another is a "strip" or enucleative removal. Here a longitudinal incision is made through the skin and the subcutaneous tissue adjacent to the tumor. The tumor-bearing skin flap is then mobilized laterally and the tumor enucleated from its subcutaneous attachment by blunt dissection. After the area is sponged, the skin flaps are closed with metallic clips. This procedure often 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.

Finally, we have the "radical" excision in which the skin is incised in an elliptical fashion around the tumor leaving a margin of clearly uninvolved normal skin attached to the tumor. Mobilization of skin, tumor, and fat pad is begun cephalad and carried toward the inguinal or axillary region with en bloc dissection of the regional lymph nodes. Radical surgery, in contrast to strip enucleation, is often locally curative.

Blood Samples. Approximately 500 μl were removed about every 2 wk from the retro-orbital venus plexus, using heparinized tubes. Plasmas were separated from cells by low speed centrifugation, frozen rapidly in a dry ice methanol bath, and then stored at −70°C until subjected to radioimmunoassay.

Radioimmunoassay (RIA) of MMTV gp52. The MMTV gp52, purified by concanavalin A affinity chromatography and iodinated with ^{125}I-Bolton-Hunter reagent, was used for RIA as previously described (1). The assay is a blocking radioimmunoassay in which delayed addition of the labeled antigen is employed to maximize the sensitivity of the measurement.

Determination of Tumor Weight. The weights of tumors were determined using one of the following alternative procedures: (*a*) When the tumors were surgically removed their weights were determined gravimetrically. (*b*) If left in place, tumors were measured at each palpation with calipers (two perpendicular measurements). The weights were then estimated (4) in milligrams from the relation $0.4 (L \times W^2)$, where L and W represent, respectively, the length and width of the tumor in millimeters.

Results

The collaborative experiments to be described had a useful double-blind component. All of the surgical procedures and diagnostic palpations for tumor presence were carried out at the Catholic Medical Center of Brooklyn and Queens. All of the blood samples were coded and then sent for gp52 analysis to the laboratories at the Institute of Cancer Research of Columbia University. The virtually complete concordance under these circumstances between the clinical evaluations and the gp52 levels added further confidence to the validity of the conclusions deduced.

The Effect of Tumor Removal on Plasma gp52. We first performed a limited experiment designed to test the feasibility of the whole approach by probing the following issues: (*a*) Does the gp52 plasma level in fact drop on tumor removal? (*b*) If it does fall, how far and how fast? (*c*) What happens postsurgically to the gp52 levels with particular reference to tumor recurrence? (*d*) Does the particular surgical procedure used have an effect on the subsequent behavior of the plasma gp52?

15 tumor-bearing animals were divided into three groups of 5 each and subjected to the surgical procedures described in Materials and Methods. These include the sham control operation, which leaves the tumor in place, the strip method, which is a simple enucleation of the tumor, and the radical procedure intended to achieve a local "cure." Blood samples for gp52 analysis were taken 1 day before surgery and postsurgically at 2-wk intervals. Here we may note a restraint that limits the amount of information attainable in actual practice with the mouse system. To insure the quantitative reliability of our gp52 assays we performed a three point titration with varying amounts of plasma, which requires a sample size of between 200 and 500 μl at each bleeding. To avoid imposing an unacceptable strain on the animals in the postsurgical period, blood samples were taken no more often than every 2 wk, even though a more frequent sampling would have been prognostically more informative in view of the comparatively rapid progress of this disease in the mouse.

The gp52 plasma levels were followed for a period of 3 mo after surgery, and the findings in the individual mice are detailed in Fig. 1 as a semilog plot of the plasma concentration of gp52 versus time. Solid symbols indicate the presence of a palpable tumor, and the open symbols signify that no tumor could be detected by palpation at the time the blood sample was taken.

The solid horizontal line at 2.5 ng/ml in Fig. 1 marks the level of gp52 observed (2) in normal mice. The dashed line, corresponding to a level of 40 ng/ml, of Fig. 1 was drawn for its comparative convenience in this experiment. It will be noted that all animals having values significantly above 40 ng/ml had palpable tumors, whereas those with plasma concentrations below this level were free of tumors detectable by palpation. This fact alone establishes the value of the gp52 level as a signal for the presence of tumor. However, it should be noted immediately that the particular cut-off value of 40 ng/ml cannot be taken as an absolute invariant that can serve to distinguish tumorous from nontumorous individuals for all groups of animals and for all samples of reagents. In the first place, the size of the usable sample (eight animals in all) in this experiment is far too inadequate to establish a valid value of this parameter. In addition, we

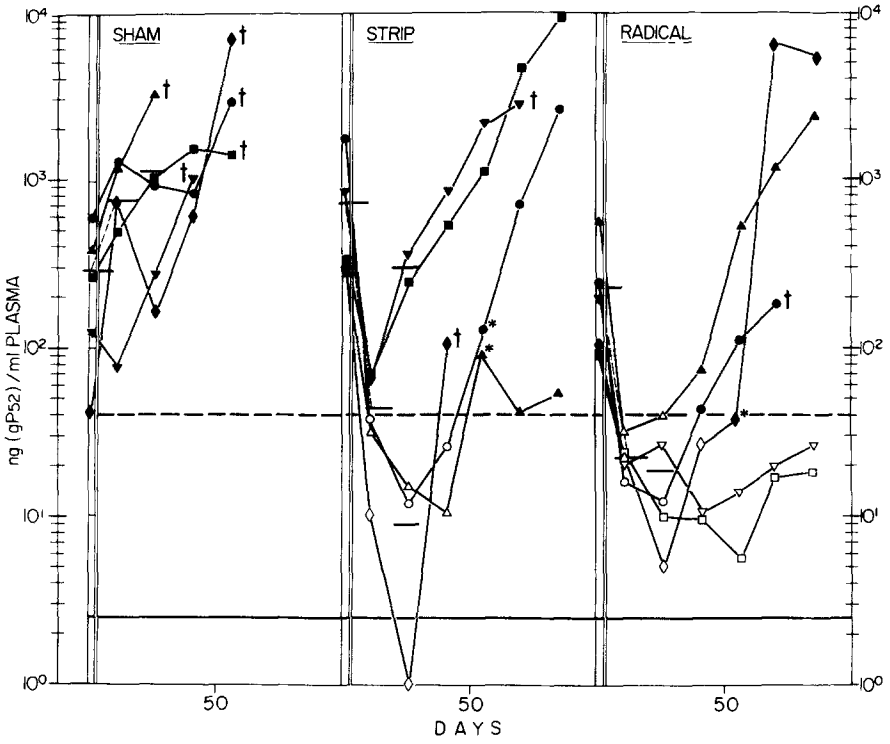

FIG. 1. The concentration of gp52 in the plasma level of individual mice is presented both before and after surgery. The three groups are divided according to type of surgery performed: sham (tumor left in place), strip (tumor is removed), and radical (tumor, fat pad, local lymph nodes, and surrounding skin are removed). Short solid horizontal lines depict mean concentrations before and after surgery. The dotted line represents an approximate division point betwen tumor-bearing and nontumor-bearing animals with respect to gp52 plasma concentration. The lower solid line represents the mean concentration for 20 normal CD8 mice (10 males and 10 females). Filled symbols indicate the presence of a tumor, while open symbols indicate tumor-free animals. A (†) is used to signify an animal's death, while an (*) is used where tumors were clinically detected 4 days after a plasma determination. The double vertical line marks the time of surgery.

find it necessary to monitor the quantitative properties of every preparation of gp52 and its ^{125}I derivative by a complete titration curve before they can be reliably used in a RIA. It has been our experience that it is not difficult to establish a reliable cut-off gp52 level with a given set of reagents, but we find that the actual value will range between 15 and 40 ng/ml.

A striking feature of the data described in Fig. 1 is the clear-cut difference between the control sham group and the two in which the tumors were surgically removed. The average gp52 concentration in the controls rose from the presurgical level of 190 ng/ml to over 600 ng/ml in the first 2 wk after surgery. Then the average values continued to increase above 1,000 ng/ml until all of this group succumbed to the disease by the 8th wk of the experiment. Two of the control individuals experienced moderate transient drops, but none fell below the 40 ng/ml characteristic of the tumor-free state. This is in sharp contrast to the strip and radical groups in which the tumors were removed at surgery. Both

of these experienced sharp drops in the first 2 wk after surgery, amounting to declines in both instances of between one and two orders of magnitude in the plasma concentrations of gp52. This decline continued in the next 2 wk in those animals that had not suffered relapses in the first 2 postsurgical wk.

The fall in gp52 after removal of tumor obviously provides a positive answer to the first question posed and supports the expected relation between tumor and plasma antigen deduced from our initial study (2). It would appear that the principal source of the gp52 found in the plasma of tumorous animals is the tumor itself.

The excellent agreement between the clinical and immunological diagnostic decisions was made even more gratifying by an apparent exception. One mouse, having a gp52 level well below the 40 ng/ml level, was diagnosed by palpation as positive for a tumor mass. However, a more detailed examination revealed the presence of a nonmalignant cyst.

Several other noteworthy features emerged from these experiments. Despite the limitations imposed by the necessarily infrequent blood sampling, the gp52 levels yielded some information on clinical status before it became evident from physical examination. Two animals (starred in Fig. 1) of the strip group were diagnosed as positive on the basis of gp52 levels at the fourth postsurgical bleeding although no tumor could be detected at that time on palpation. However, in both instances tumors became palpably evident within the next week. A similar situation held true for two animals of the radical group, both of which were close to the 40 ng/ml level and were tumor free by palpation at the time. The one that was at that stage by the second postsurgical bleeding became tumor positive by palpation at the next bleeding. The other (starred at the fourth postsurgical bleeding of the radical group in Fig. 1) developed a palpable tumor within 3–4 days after this sample was taken. It will be seen from Fig. 1 that two animals of the radical surgery group were the only ones to be cured by the surgical procedures. These were also the only ones in which the gp52 plasma concentration remained well below the 40 ng/ml for the entire duration of the experiment.

Increases in the plasma gp52 levels were observed in all relapses whether they were due to recurrence at the original surgical site or to new primary tumors elsewhere. It may be seen from Table I that the surgical procedure influenced the probability of relapse at the original site, but did not affect the incidence of new primaries. Thus, regrowths occurred in three out of the five animals subjected to strip surgery and only one such occurred in the radical surgery group. The number of new primaries were identical in each.

Variations in gp52 Levels Versus Tumor Size. In our previous study (2) we noted that there were excellent positive correlations between average tumor size and the means of the corresponding plasma concentrations of gp52. However, it was evident from the wide scatter of the gp52 levels around the mean values that factors other than tumor mass were playing a role. The experiments of Fig. 1 and Table I provided us with an opportunity of examining this question in greater detail since we automatically accumulated information on tumor mass and gp52 concentration for each mouse that suffered relapse. Some representative outcomes are described in Fig. 2 in which both tumor sizes (dotted lines) and gp52 concentrations (solid lines) are plotted as a function of time after surgery

TABLE I
Disease Status at the End of the Observation Period

Type of surgery	Number of animals	Percent totally cured*	Number of regrowths	Percent cured at the site of surgery‡	New primaries	Percent bearing new primary tumors
Sham	5	0	0	0	0	0
Strip	5	0	3	40	2	40
Radical	5	2	1	80	2	40

Tumor regrowths, development of new primary tumors, and "cures" are presented for each form of surgery. While no regrowths or new primaries were detected in the control (sham) animals, it should be noted that their original spontaneous tumors continued to grow in all cases resulting in their ultimate deaths.

* Animals developing neither a regrowth nor a new primary tumor during the course of the observation period.

‡ The percent of animals free of regrowth at the initial site of surgery.

for the individual mice indicated by the numbers placed next to the corresponding curves. First, it should be mentioned that in all mice studied, including those not detailed in Figs. 2 and 3, increase of tumor mass as the disease progresses was invariably accompanied by a rising level of gp52. The quantitative relation between the two, however, fell into three classes. One of these, in which the increases in tumor size and antigen levels paralleled each other quite well, is exemplified by numbers 18, 38, 24, and 19 of Fig. 2 A. Fast growing tumors are accompanied by rapid rises in plasma gp52, whereas slow growing ones are matched by very gradual increases in this antigen. A second type is shown by number 63 of Fig. 2 B in which the tumor grew faster than would be expected from the rate of increase in the plasma gp52. Finally, the obverse situation appears to hold in the case of number 89 of Fig. 2 A.

Another informative way to look at the relationships between tumor and antigen in individual mice is shown in Fig. 3 where the gp52 plasma concentration is plotted against tumor mass. The positive slopes and the shapes observed indicate an approximately linear relation between tumor growth and increasing levels of plasma gp52. However, the differences in slopes suggest that the amount of gp52 produced per unit increment in tumor mass differs from individual to individual, a result not unexpected from the rather wide scatter around the mean for each tumor size range (2).

Further Evaluation of gp52 Plasma Levels as Diagnostic and Prognostic Indicators of Tumor Status. The principle purpose of the experiment described in Figs. 1–3 and Table I was to establish whether tumor excision is followed by a fall in gp52. The subsequent behavior of the gp52 in response to new tumors predicts the potential usefulness of plasma gp52 as a diagnostic device for mammary neoplasia. However, the numbers of animals involved were quite small, and it was clearly necessary to check the validity of the diagnostic implications by setting up a more extensive experiment with a larger number of tumorous animals, all treated alike. On the basis of our preliminary experience we elected to employ a strip surgical removal of the initial tumor, leaving in most cases microfoci of tumor cells for subsequent regrowth of the original tumor. If new primaries arose elsewhere during the course of the experiment,

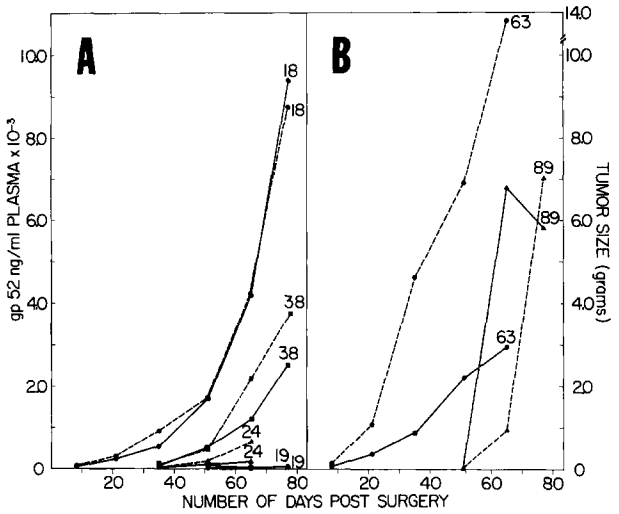

FIG. 2. Tumor size (dashed lines) and gp52 concentration in plasma (solid lines) are both presented as a function of days after surgery. Each mouse is represented with two curves, one for tumor size and one for gp52 level. These can be compared by matching mouse numbers. Mice presented in (A) demonstrate a close correspondence between increasing tumor size and gp52 level, while (B) contains a few mice in which increases in tumor growth and antigen level were disproportionate.

these would be removed by radical surgery. This strategy is designed to maximize the amount of information gained on the relations of the gp52 level to recurrence at the original site.

45 animals were bled 1 or 2 days before surgery, at 9 days after surgery, and at approximately 2-wk intervals thereafter. The gp52 levels were determined for all 45 animals at each time point. Examination for tumors by palpation and their measurements were made about every 3rd day, always including the day of the bleeding.

We present in Fig. 4 a semilog plot of gp52 levels versus time for 10 of the 45 mice. These individuals were chosen because they exemplify the types of relationships observed between the gp52 levels and tumor behavior. Here open symbols refer to animals free of palpable tumors and completely or partially filled symbols indicate the presence of tumors at the times indicated. Again, all animals experienced a 10–100-fold drop in plasma gp52 levels immediately after surgical removal of the tumor. However, as in the first experiment, three strikingly different postsurgical patterns are identifiable in the curves of Fig. 4. One group (numbers 1–4) are survivors that remain tumor free throughout the experimental period, a feature reflected in their gp52 plasma concentrations, that kept below the 15 ng/ml level. Although only four survivors are described in Fig. 4, there were in all 12 such tumor-free survivors with corresponding low levels of gp52. The second group consists of tumorous animals that were still alive at the end of the experiment. Some (e.g., number 5) experienced a relapse late (at the fifth postsurgical bleeding) and others (e.g., numbers 6 and 7) at intermediate times. In all such cases, recurrence was signaled by a rise in the gp52 levels followed by further increases paralleling the progressive regrowth of

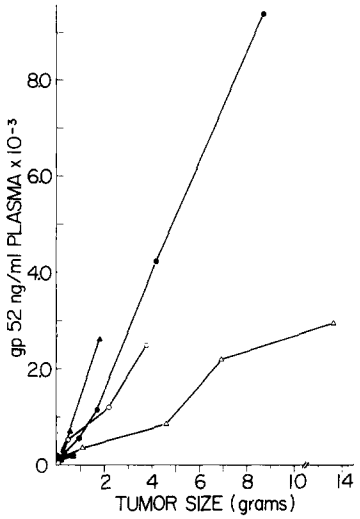

FIG. 3. gp52 levels are presented as a function of tumor size for six mice. Each postoperative individual is represented by a different symbol. Differences in the slopes of these lines indicate differences in the amount of gp52 detected per unit mass of tumor.

the tumor. In all, 14 of the 45 mice showed this pattern of surviving the experiment despite their increasing tumor loads. The third group, exemplified by numbers 8–10 of Fig. 4, are characterized by early recurrence and death, events clearly foreshadowed by a sharp rise in plasma gp52 at the second postsurgical bleeding. There were 16 mice that showed this kind of aggressive tumor response.

The final clinical evaluations taken after 106 days, when the experiment was terminated, are summarized in Table II. The strip surgery eliminated the disease for the period of observation in 12 animals and did not in 31. Two animals were omitted from the experiment because of premature deaths not attributable to tumor recurrence.

It is evident from the 10 mice shown in Fig. 4 that gp52 levels provide information on the clinical status and the progress of the disease in the three types represented. Elevations of gp52 above 30 ng/ml are invariably associated with the appearance of tumors. Only those that maintained levels below this value remained tumor free. The general validity of this pattern is confirmed by the data on all 45 mice which are presented more compactly in Fig. 5 as a semilog scatter plot of gp52 plasma concentrations versus time after surgery. Solid circles and open circles indicate tumor-bearing and tumor-free animals, respectively. Circles containing an X identify tumor-free animals that will develop a palpable tumor by the next bleeding. The gp52 mean values at the various time intervals are indicated by the horizontal heavy zigzag lines. All gp52 averages in the postsurgical periods are based on the values obtained from the tumor-free members of the group at each time period. Only the presurgical average value is determined from animals with tumors. Although not identifying individual mice, this method of presentation furnishes an overall view of tumor status and gp52 levels and how these change with time within the tumor-free population.

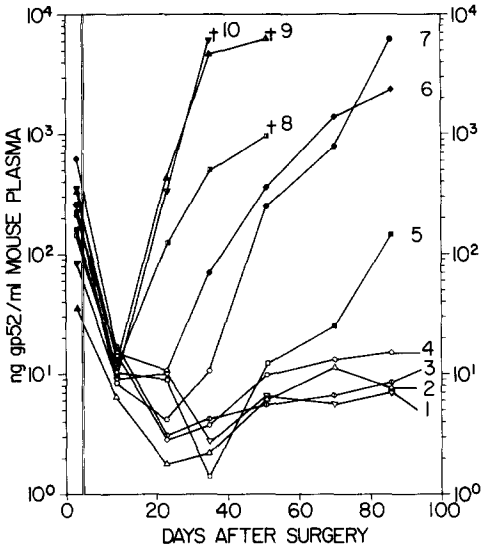

FIG. 4. gp52 levels have been presented as a semilog plot versus time after strip surgery. 10 (numbered 1 through 10) of the 45 mice (each represented by a separate symbol) were chosen to exemplify the types of relationships observed between gp52 levels and the tumor status of individual postoperative animals. Filled or partially filled symbols indicate the presence of tumor, while open symbols indicate the absence of a detectable regrowth. The double vertical line indicates the time of surgery and a (†) signifies death.

The average gp52 plasma concentrations underwent the customary 10–100-fold fall in the first 9 days after surgery. This decrease continues for the next 4 wk and is then followed by a slight rise. The distribution with respect to the means of the gp52 values in the postsurgical intervals have diagnostic and prognostic significance. Thus, all animals with tumors exhibit gp52 levels well above the mean, not a single one falling below these levels. Further, those that suffer relapse before the next bleeding interval (crossed circles) tend to cluster near or above the mean values, whereas those that remain disease free for the next interval (open circles) tend to be found below the mean.

Table III summarizes these diagnostic features in greater detail by tabulating for each time interval the probabilities of an animal having a palpable tumor as a function of its plasma concentration of gp52. The first 9-day postsurgical period is omitted since the time interval is too short for the development of a tumor detectable by palpation. The three gp52 levels used correspond to the means of the tumor-free animals at each time interval, twice this mean, and 30 ng/ml which corresponds to twice the highest mean observed in the nontumorous groups. The 30 ng/ml level alone is used for the presurgical values, and all but one (at 25 μg/ml) of these had gp52 levels above 30 ng/ml. Since the last group (at 86 days) had the highest tumor-free mean of 15 ng/ml, we used 15 and 30 ng/ml as the basis of comparison.

If the gp52 level of any animal fell at 30 ng/ml or above, the probability that it had a tumor was a virtual certainty at all the intervals examined. There were only two exceptions in the 94 assays of nontumorous animals with gp52 values above 30 μg/ml. One was a mouse that died before the next bleeding and the other developed a tumor by the next bleeding. If the gp52 values fell above twice

TABLE II
Clinical Evaluation 106 Days after Strip Surgery

Number of tumor recurrences	Percent recurrence	Number of deaths*	Number of tumor-bearing survivors	Number of tumor-free survivors	Percent cured‡
31	68.9	19	14	12	26.7

The disease status of 45 mice have been evaluated during 106 days after "strip" surgery. The number of cures, recurrences, tumor-bearing survivors, and deaths are recorded.

* Note: two deaths were not directly attributable to tumor recurrence.

‡ Cured refers to mice free of disease at the end of the observation period.

the mean (at a given interval) the probabilities of having a palpable tumor ranged from 0.80 to 1.00. Finally, in all the intervals, if an animal had a gp52 value below the mean, the probability of finding a detectable tumor was zero. There were no exceptions to this rule in the 59 assays falling below the mean.

The prognostic values of gp52 levels are examined in Table IV which records the probabilities of tumor recurrence. Those with gp52 values above the mean have a much greater chance of relapsing in the next 2 wk than those with values below the mean. The last column of Table III calculates the probability that a given animal at each time interval either has a tumor or will get one in the next 2-wk interval. As might be expected, the relationship to the gp52 value is even stronger here than for either one alone. Thus, animals above the mean have probabilities ranging from 0.63 to 1.00 of either having a tumor or relapsing with one by the time of the next bleeding.

Table IV considers only the short term likelihoods of a relapse by predicting its occurrence in the next 2-wk interval. It was of interest to examine whether gp52 levels possessed long range prognostic value and in particular, whether presurgical levels of gp52 could identify those individuals that would be likely candidates for tumor-free survival. This question is examined in Table V where we tabulate the probabilities of surgical cures as a function of plasma gp52 levels before surgery. For comparison we also include similar probabilities based on the different tumor size classes presented at surgery. There were eight mice with gp52 plasma concentrations below 100 ng/ml at surgery, and six (or 75%) of these are to be found among the 12 tumor-free survivors. The probability of survival without disease drops markedly from 0.75 to 0.18 and 0.14 in the groups that had between 100 and 3,400 ng/ml of gp52 in their plasmas just before surgery. Rather surprisingly, the probability of tumor-free survival does not vary significantly with the size of the tumor removed despite the fact that the tumor masses range from less than 200 mg to over 2 g.

It would appear that gp52 plasma levels at surgery are considerably superior to tumor mass as a prognostic indicator of a successful surgical cure. While this is not an outcome that could have been predicted on any a priori grounds, it was logically conceivable in view of the marked variations observed in gp52 plasma concentrations per unit mass of tumor.

Discussion

Our immediate aims were to determine the effect of tumor excision on the plasma gp52 and to follow the subsequent behavior of the antigen in the

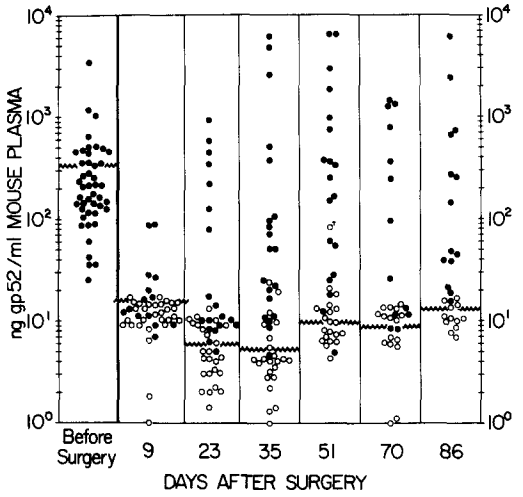

FIG. 5. gp52 levels for each of the 45 mice receiving strip surgery are presented as a semilog scatter plot of gp52 concentration versus time after surgery. Filled symbols indicate the presence of tumor while open symbols indicate that animals were tumor free. Symbols with an (×) represent nontumor-bearing animals which will suffer recurrence before the next bleeding. A (†) is utilized to designate the death of one apparently tumor-free animal that possessed an abnormally high gp52 level and was a suspect for a metastatic growth. The mean gp52 levels are indicated by heavy zigzag horizontal lines. Mean values after surgery are determined from the nontumorous individuals only. The double vertical line denotes the time of surgery.

postoperative period with particular emphasis on its relation to tumor recurrence. The sharp decrease in the plasma gp52 which attends tumor removal identifies the tumor as the principal source of the gp52 required to maintain the high levels characteristic of a tumorous animal. The low levels of gp52 found after surgery made it possible to achieve our second objective by providing the opportunity to examine the usefulness of gp52 as a detector of relapse. The data obtained suggest that the plasma concentrations of this antigen do in fact measure tumor status as reflected by the following findings: (*a*) All animals with tumors possessed gp52 plasma concentrations well above the mean values (6.4–15.7 ng/ml) of the tumor-free animals. (*b*) All tumor recurrences were identified by increases in gp52 levels, and some were detected 4–7 days before they were found by palpation. (*c*) In any given interval, the probability for relapse in the next 2 wk is considerably higher for those animals with gp52 levels above the mean of the tumor-free individuals. (*d*) Tumor regrowths were accompanied by continued increases in plasma gp52 concentrations at rates which usually matched the speed of tumor development. (*e*) The only animals that remained tumor free at the end of the 106-day experimental period were those that maintained their gp52 levels at or below 15 ng/ml. (*f*) Finally, and most remarkably, levels of gp52 at the time of surgery are superior to tumor loads as prognostic indicators of a successful long-term surgical cure.

Despite the restrictions imposed by the limited frequency with which blood samples can be drawn, clinically useful information can be obtained on tumor status in the mouse by following plasma concentrations of gp52. It would appear

TABLE III
Antigen Level as an Indicator of Tumor Status

Days relative to surgery	gp52 values	Probability of having a tumor
	ng/ml	
−1	≥30.0	1.00
23	≥30.0	1.00
	>13.2	0.80
	≤6.6*	0.00
35	≥30.0	1.00
	≥12.8	0.93
	≤6.4*	0.00
51	≥30.0	0.93
	≥21.0	0.88
	≤10.5*	0.00
70	≥30.0	1.00
	≥18.2	1.00
	≤9.1*	0.00
86	–	–
	≥30.0	0.91
	≤15.0*	0.00

* These are the mean values of the plasma gp52 concentrations determined from the tumor-free animals.

that our goal of developing a systemic measure of mammary neoplasias has been achieved.

It is obviously of pressing importance to exploit this new methodology by determining whether the gp52 assay can be used for monitoring the effectiveness of adjuvant chemotherapy. It is conceivable that the data thus generated will provide a more rapid and informed estimation of the therapeutic effectiveness of new and varied drug combinations than the methods presently in use. The fact that gp52 levels at surgery appear to provide better indications than tumor mass for the future outcome supports the hope that gp52 concentrations will likewise serve as earlier indicators of ultimate success with other therapeutic modalities. It also suggests the desirability of testing the effect of presurgical chemotherapeutic suppression of the plasma gp52 to levels below 100 ng/ml in the hopes of improving the probability of long-term tumor-free survival.

We have already noted in our previous study (2) that although there is a correlation between plasma gp52 and tumor weight, there are clearly factors other than simple tumor mass which play a role in determining the plasma gp52 concentrations, a feature which is clearly illustrated by the results of the present investigation described in Figs. 2 and 3. It is obviously of urgent interest to identify the reasons underlying the variations in gp52 observed per milligram of tumor, particularly since the aggressiveness of a tumor may be positively related to this ratio. One possible explanation for apparently abnormally high rates of gp52 production is the existence of metastatic lesions which would go

TABLE IV
Probability Analysis of gp52 Antigen Level as an Indicator of Present and Future Tumor Status

Number of days postsurgery	gp52 values relative to mean*	Probability of relapse in the next 2 wk	Probability of having a tumor or relapsing in next 2 wk
9	>15.7	0.78	0.78
	≤15.7	0.19	0.19
23	>6.6	0.35	0.72
	≤6.6	0.11	0.11
35	>6.4	0.33	0.97
	≤6.4	0.05	0.05
51	>10.5	0.22	0.86
	≤10.5	0.08	0.08
70	>9.1	0.18	0.63
	≤9.1	0.22	0.22
86	>15.0	0.67	1.00
	≤15.0	0.00	0.00

At each time interval the animals are divided into those with gp52 levels above the average and those with concentrations below the average. Probabilities are then estimated for these two groups of suffering a relapse. The last column uses the means of Table III to calculate the combined probabilities at each time interval of either having a tumor or relapsing in the next time interval.

* Means are determined from the tumor-free mice as nanograms per milliliter.

undetected by the physical examinations employed. It will not be difficult to obtain the relevant information on this issue since in this model natural metastases almost always initially involve the lungs. Whatever the cause, the existence of this variability makes it evident that serial assays of individuals for plasma gp52 will continue to provide the most illuminating information on disease status and its future probable outcome.

The overall purpose of our earlier investigations (1, 2) and the present one was to see whether a solid tumor associated with a viral agent will release into the plasma a viral protein that could serve as a useful monitor of disease status. The $CD8F_1$ mouse mammary tumor model was chosen because it has already proven (3) its value as a predictive guide for exploring adjuvant chemotherapeutic modalities for the most effective management of human breast cancer. It seems likely that availability of a specific and sensitive systemic monitor of disease status during therapy will serve to augment the future usefulness of the $CD8F_1$ experimental model.

The ultimate reason for studying the mouse mammary tumor model stems from the hope that it will generate the technology required for the attempt to identify a correspondingly useful particle-related protein in human breast cancer. That this is not an unreasonable expectation is suggested by our previous studies which have shown that human breast tumors contain particles exhibit-

TABLE V

Probabilities of Surgical Cures Determined from Antigen Levels and Tumor Size at Surgery*

	gp52 level (ng/ml) at surgery			
	(25–100)	(100–300)	(300–3,400)	
Probability of being free of disease‡	0.75	0.18	0.14	
	Tumor size classes (mg) at surgery			
	(69–200)	(200–300)	(300–500)	(500–2,300)
Probability of being free of disease	0.23	0.36	0.27	0.20

The probabilities of being cured by "strip" surgery are determined from the gp52 levels at surgery and compared with similar estimations based on the size of the tumor removed.

* Cure means that the animal remained free of tumors at the end of the 106-day postsurgery observation period.

‡ Probability of being free of disease is calculated by determining the number of surviving mice in each category (mice remaining tumor free at the end of the observation period) and dividing by the total number of mice in each category.

ing many of the diagnostic features of the mouse mammary tumor virus. In addition to similar sizes and densities, these include the possession of a reverse transcriptase associated with a characteristically large 70S RNA (5, 6) which in turn shares detectable sequence homologies (7, 8) with the RNA of the mouse mammary tumor virus. In addition, evidence has appeared recently (9) suggesting that breast cancer patients display cellular hypersensitivity responses to a protein of the mouse mammary tumor virus. All of this, along with the information reported here on the mouse mammary tumor model, makes this approach towards a specific diagnostic test in the human disease sufficiently plausible to warrant continued efforts.

Summary

We have previously shown (1, 2) that mice with mammary tumors can always be identified by their very high plasma levels of gp52, a 52,000 mol wt glycoprotein of the mouse mammary tumor virus (MMTV). The present investigation demonstrates that the tumor is the principal source of the plasma gp52 since surgical excision is invariably followed in the first 9 days by a sharp decreasing (10–100-fold) of the gp52 levels. Control animals in which the tumors were left in place by a "sham" surgical procedure maintained their high levels of gp52, which continued to increase as the disease progressed.

The behavior of the gp52 after surgical removal suggests that gp52 plasma concentrations are diagnostically and prognostically informative, as indicated by the following findings: (*a*) All tumor recurrences were correctly diagnosed by increases in gp52 levels, and some were detected 4–7 days before they were found by palpation. (*b*) Tumor regrowths were accompanied by continued increases in plasma gp52 concentrations at rates that usually matched the speed of tumor development. (*c*) The only animals that remained tumor free at the termination of the experiment were those that maintained their gp52 levels at or below 15

ng/ml. (*d*) The probability of a tumor-free animal relapsing within 2 wk is much higher if its gp52 level is above the mean. (*e*) More remarkably, the plasma levels of gp52 at the time of surgery are superior to the sizes of the tumors removed as prognostic indicators of eventual surgical "cures."

The availability of a specific and sensitive systemic measure of disease status should augment the usefulness of the murine mammary tumor model by catalyzing a more rapid acquisition of information on the therapeutic effectiveness of the new and varied drug combinations being tested for adjuvant chemotherapy.

We wish to express our appreciation to L. Stolfi, L. Markovitz, and V. D'Elia for their excellent technical assistance.

Received for publication 28 December 1976.

References

1. Ritzi, E., A. Baldi, and S. Spiegelman. 1976. The purification of a gs antigen of the murine mammary tumor virus and its quantitation by radioimmunoassay. *Virology*. 75:188.
2. Ritzi, E., D. S. Martin, R. L. Stolfi, and S. Spiegelman. 1976. Plasma levels of a viral protein as a diagnostic signal for the presence of tumor: the murine mammary tumor model. *Proc. Natl. Acad. Sci. U. S. A.* 73:4190.
3. Martin, D. S., R. A. Fugmann, R. L. Stolfi, and P. E. Hayworth. 1975. Solid tumor animal model therapeutically predictive for human breast cancer. *Cancer Chemotherapy Rep.* 5:89.
4. Attia, M. A. M., and D. W. Weiss. 1966. Immunology of spontaneous mammary carcinomas in mice. V. Acquired tumor resistance and enhancement in strain A mice infected with mammary tumor virus. *Cancer Res.* 26:1787.
5. Axel, R., S. C. Gulati, and S. Spiegelman. 1972. Particles containing RNA-instructed DNA polymerase and virus-related RNA in human breast cancers. *Proc. Natl. Acad. Sci. U.S.A.* 69:3133.
6. Spiegelman, S., R. Axel, and J. Schlom. 1972. Virus-related RNA in human and mouse mammary tumors. *J. Natl. Cancer Inst.* 48:1205.
7. Axel, R., J. Schlom, and S. Spiegelman. 1972. Presence in human breast cancer of RNA homologous to mouse mammary tumour virus RNA. *Nature (Lond.)*. 235:32.
8. Vaidya, A. B., M. M. Black, A. S. Dion, and D. H. Moore. 1974. Homology between human breast tumour RNA and mouse mammary tumour virus genome. *Nature (Lond.)*. 249:565.
9. Black, M. M., R. E. Zachrau, A. S. Dion, B. Shore, D. L. Fine, H. P. Leis, Jr., and C. J. Williams. 1976. Cellular hypersensitivity to gp55 of RIII-murine mammary tumor virus and gp55-like protein of human breast cancers. *Cancer Res.* 36:4137.