Temporary Disappearance ("Eclipse") of LPC-1 Plasmacytoma M Component Synthesis following Tumor Cell Transfer[1]

Carmelita G. Frondoza,[2] Sudhir M. Trivedi, and Richard L. Humphrey

Oncology Center, The Johns Hopkins University School of Medicine, Baltimore, Maryland 21205

ABSTRACT

The early phase of LPC-1 plasmacytoma development was studied by *in vivo* labeling with [6-^{14}C]arginine using its M component (immunoglobulin G 2a,κ) as marker. At a time when M component was not detected or faint by protein staining of electrophoretograms, significant labeling of M component was detectable by autoradiography. Labeling of the M component was fairly constant for the first 10 hr but was markedly decreased from Days 1 to 7. Nadir (0 to 3% of initial 30-min value) was observed on Day 3. Recovery of M component labeling to the 30-min level was complete by Day 13. This period of marked reduction or "eclipse" in newly synthesized M component was shortened by 2 days when mice were pretreated with pristane or cyclophosphamide prior to tumor cell transfer. The eclipse period was also 2 days shorter in athymic BALB/c-*nu/nu* mice than in normal BALB/c mice. The eclipse period corresponds to the classical "lag" following tumor cell transfer before tumor growth can be detected by conventional methods. The sensitivity of the [6-^{14}C]arginine pulse permits the *in vivo* detection of small numbers of tumor cells (as few as 10^6 cells) over the early time periods after transfer. Modification of eclipse by manipulating host and/or tumor cells may elucidate the accompanying cellular and biochemical events.

INTRODUCTION

The early phase of tumor development occurring immediately after transfer of transplantable tumor cells has been difficult to study since conventional methods usually are not sensitive enough to detect the presence of less than 10^7 tumor cells (5, 7, 8). Standard techniques of measuring the increase in tumor mass (16, 17) and tumor-associated biomarker concentration, *e.g.*, M component (2, 6), characterize the more advanced stages of tumor growth and are not suitable to describe the earlier period immediately after tumor cell transfer. Recently, Rohrer *et al.*, (4, 15) utilized an "*in vitro-in vivo*" system, using MOPC 315 grown in Millipore diffusion chambers implanted in normal syngeneic mice, which permitted analysis of the early period after tumor cell transfer. They demonstrated progressive time-dependent shift from nonsecreting lymphocytoid surface IgA-bearing cells to plasmacytoid IgA-secreting cells. This process of tumor cell "differentiation" was suggested by an inverse relationship between IgA expression and plasmacytoma stem cell frequency (15). The promotion or suppression of this tumor cell differentiation process was altered by stimu-

lating host helper or suppressor T-cells (14). These studies showed that the differentiation of tumor precursor cells can be regulated, not only by inherent tumor cell properties but by host factors as well.

The studies reported here analyze the period immediately following LPC-1 plasmacytoma cell transfer in its syngeneic host (BALB/c mice). The *in vivo* [6-^{14}C]arginine pulse-labeling technique which we described earlier (3, 9) was used to follow newly synthesized LPC-1 tumor-associated protein (IgG 2a,κ and M component). This radiolabeling procedure is sensitive enough to detect as few as 10^6 cells, and the labeling period is very short (<30 min) and hence suitable for examining the early phase of tumor development. We report here the detection of a temporary disappearance ("eclipse") of LPC-1 plasmacytoma M component synthesis following cell transfer, as revealed by this *in vivo* [6-^{14}C]arginine-labeling technique.

MATERIALS AND METHODS

Animals. Female BALB/c mice weighing 20 to 25 g obtained from Microbiological Associates, Bethesda, Md. were given standard Charles River chow (RMH 1000) and water *ad libitum*. BALB/c athymic *nu/nu* mice (17 to 20 g) were the generous gift of Dr. Michael Potter, NIH. The mice were maintained under aseptic conditions in a Biohood and given sterilized chow (NIH 31; Zeigler Bros., Gardners, Pa.) and acidified water (0.02 ml of N HCl/500 ml distilled water).

Tumors. LPC-1 plasmacytoma, an IgG 2a,κ producer originally isolated by Dr. Potter, was procured from Litton Bionetics, Inc., Rockville, Md. and was maintained by serial passage in female BALB/c mice. Tumor tissue was excised and cut into small pieces in Roswell Park Memorial Institute Tissue Culture Medium 1630 with 10% fetal calf serum (Microbiological Associates). The tissue fragments were further minced with a scalpel blade and passed through sterile gauze pads. Single-cell suspensions in 0.2% trypan blue dye (0.2% dye in 0.9% NaCl solution) were counted in a hemocytometer. The volumes were adjusted to 10^6 viable cells/ml of medium. The tumor line was maintained by the transfer of 10^6 viable tumor cells/ml/ mouse s.c. every 2 weeks.

Chemical Reagents. CY[3] was obtained from Mead-Johnson & Co. (Evansville, Ind.). Pristane (2,6,10,14-tetramethylpentadecane) was purchased from Aldrich Biochemical Co. (Milwaukee, Wis.).

Isotopes. [6-^{14}C]Arginine, 25.9 mCi/mmol in 0.1 N HCl, obtained from New England Nuclear (Boston, Mass.), was neutralized with 0.1 N NaOH and then diluted with an appropriate volume of 0.9% NaCl solution. The labeled amino acid (40 μCi/mouse) was administered i.p.

Collection of Blood Samples. Blood was collected from the

[2] To whom requests for reprints should be addressed, at Oncology Center, The Johns Hopkins University School of Medicine, 600 North Wolfe Street, Baltimore, Md. 21205.

[3] The abbreviation used is: CY, cyclophosphamide.

retroorbital plexus using sterilized heparinized micropipets 24 hr following isotope administration. The micropipets were centrifuged, and the plasma obtained was frozen at −20° until used.

Electrophoresis and Radioautography. Plasma (1 μl) was electrophoresed on Pol-E film (1% agarose/5% sucrose in 0.075 M barbital buffer, pH 8.6; Pfizer Diagnostics Division, Clifton, N. J.) at 100 V for 50 min. Subsequently, the films were soaked in 5% trichloroacetic acid containing 0.2% Ponceau S solution for 20 min. Excess stain was removed by washing with 5% acetic acid. The films were dried at 72° and radioautographed using Kodak SB54 negatives for up to 4 weeks. The negatives were developed with Model M6A-N Kodak RP X-OMAT processor. The electrophoretograms and radioautograms were scanned at 525 nm with a Model 415 Clifford densitometer. The relative distribution of protein components (*i.e.*, albumin, γ-globulins, and M component) and radioactivity were determined based on the densitometric tracings. The M component region for each electrophoretic run was defined by unlabeled purified LPC-1 M component, placed in top and bottom wells of each membrane, and used as guide strips. This permitted the arbitrary assignment of radioactivity (from the radioautograms) and protein concentration (from the Ponceau S-stained membranes) to the M component, even when the M component concentration or radioactivity was too low to reveal a homogenous band.

For the purpose of comparing different times of isotope administration, the labeling of M component was expressed as percentage of the 30-min values.

RESULTS

M Component Labeling *in Vivo* with [6-¹⁴C]Arginine: Detection of Eclipse Phenomenon

BALB/c mice were given injections of 10^8 tumor cells, followed by i.p. injections of [6-¹⁴C]arginine at various time intervals. Plasma obtained 24 hr after isotope injection was electrophoresed and radioautographed. The radioautograms were scanned, and the percentage of incorporation of radioactivity into M component was determined. The results obtained for the control animals are exhibited by Chart 1, *open circles*. There was only a very low level of M component detected (<2%) by Ponceau S staining over the time period from Days 0 to 7 following cell transfer (Chart 1*B*). However, there was significant radioactive labeling of M component detected during the first 10 hr after tumor cell inoculation (Chart 1*A*). By Days 1 to 7, marked reduction in M component labeling was observed. Nadir (3% of the initial 30-min value) was noted on Day 3. Recovery of M component labeling to the 30-min level was complete by Day 13, coincident with the earliest detectable development of ascites. The period of marked reduction in the labeling of newly synthesized M component was called eclipse phenomenon.

"Permanent Eclipse"

The eclipse period was prolonged indefinitely when CY (125 mg/kg) was given 3 hr following tumor cell injection (CY, Day 0; Chart 1, *closed circles*) with no recovery in M component synthesis (Chart 1*A*, *closed circles*) and no measurable M component by protein staining (Chart 1*B*, *closed circles*). This
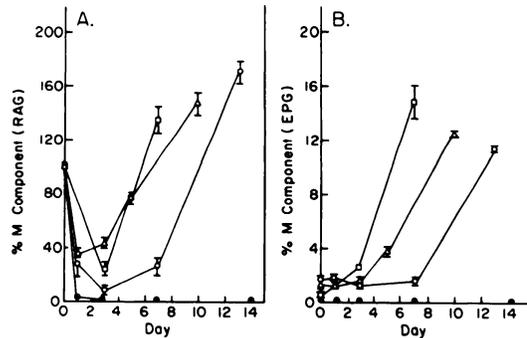

Chart 1. *A*, LPC-1 M component synthesis after tumor cell transfer as detected by [6-¹⁴C]arginine radioautogram (*RAG*), expressed as percentage of total radioactivity in protein; *B*, LPC-1 M component after tumor cell transfer as detected by Ponceau S protein-staining electrophoretogram (*EPG*), expressed as percentage of total protein. BALB/c mice were given 10^8 LPC-1 cells [control (○)]. A second group was pretreated i.p. with CY, 300 mg/kg, one day prior to tumor cells [CY, Day −1 (△)]. The third group was given CY, 125 mg/kg i.p., 3 hr after tumor cells [CY, Day 0 (●)]. The last group of mice was pretreated i.p. with 1 ml of pristane 21 days prior to an identical dose of tumor cells [pristane, Day −21 (□)]. Forty μCi of [6-¹⁴C]arginine were given i.p. on the days indicated following cell transfer. Plasma was obtained 24 hr after isotope administration. *Points*, mean from 3 mice; *bars*, ± S.E.

group did not develop ascites or any palpable tumor mass, and lived indefinitely (>6 months). The permanent eclipse period in this case corresponds to complete CY cure of tumor.

Modification of the Eclipse Period

Potter *et al.* (11, 12) have demonstrated that the success of plasmacytoma tumor induction and transfer is enhanced by priming the host with oil preparations, *e.g.*, pristane. Other studies (10) have shown altered plasmacytoma induction and transfer rates with the use of rabbit anti-mouse thymocyte serum, thymectomy, total body X-irradiation, CY, and azathioprine. Recent findings further showed that the early period of tumor cell differentiation can be regulated by interaction of tumor cells with host helper and suppressor T-cell populations (14). To determine if similar manipulations of host factors would alter the duration of eclipse, mice were pretreated with pristane or CY prior to tumor cell transfer. We also compared the eclipse period in athymic (BALB/c-*nu/nu*) mice with that of its normal thymus-bearing BALB/c counterpart.

Pristane Priming. As seen in Chart 1*A* (*open squares*), mice given i.p. injections of 1 ml pristane 21 days prior to cell transfer had a 2-day shorter eclipse period and had an earlier recovery of M component synthesis than did controls (Chart 1*A*, *open circles*). The total M component shown by Chart 1*B* (*open squares*), detectable by protein staining, also appeared sooner and reached higher levels than did controls (Chart 1*B*, *open circles*).

CY Pretreatment. Similar shortening in eclipse period was observed when mice were pretreated i.p. with CY, 300 mg/kg, 1 day before tumor cell transfer (Chart 1*A*, *open triangles*). In addition, nadir occurred earlier (Day 1, 35% of 30-min value; Chart 1*A*, *open triangles*), and did not reach as low a level as did untreated mice (3% of 30-min value; Chart 1*A*, *open circles*). The total M component in these CY-pretreated mice (Chart 1*B*, *open triangles*) measured by protein staining appeared earlier and reached significantly higher levels than did controls (Chart 1*B*, *open circles*).

Athymic (*nu/nu*) BALB/c Mice. The significance of thymic cells on the duration of eclipse was also investigated. Athymic

BALB/c-*nu/nu* mice, reported to be deficient in T-cell population (13), were given i.p. injections of 10^7 LPC-1 cells followed by [6-^{14}C]arginine at various time intervals. Eclipse was shortened to 2 days, with an earlier recovery of M component labeling, and a plateau was reached by Day 7 (Chart 2A, open circles). Athymic mice developed higher concentrations of M component, as measured by protein staining (Chart 2B, open circles), than did normal mice (Chart 2B, closed circles).

DISCUSSION

In the present paper, we demonstrate with [6-^{14}C]arginine technique the transient disappearance or eclipse of LPC-1 M component synthesis after injection of LPC-1 cells into syngeneic BALB/c mice. The eclipse period appears to correspond to the classical "lag" following tumor cell transfer before growth can be detected (16, 17). It is comparable to the period of differentiation described by Rohrer et al. (14, 15), where early after cell transfer there is preferential loss of plasmacytoid M component-producing cells. The subsequent period of M component synthesis recovery seems to parallel the reported progression of precursor lymphoid cells into plasmacytoid immunoglobulin producers (14, 15).

These experiments demonstrate that eclipse of M component synthesis could be shortened and reduced in severity by pretreatment of host mice with pristane, CY, or by the absence of the thymus gland (athymic *nu/nu* mice). It has been established that mineral oil and its constituents enhance plasmacytoma tumor development (11, 12). The mechanism of oil-induced tumor enhancement is not known but seems to be associated with granuloma formation and participation of adherent cell populations (1). In comparison, CY causes both myelosuppression and immunosuppression with atrophy of lymphoid tissue and impairment of antibody, as well as cell-mediated responses (4). It appears that either providing a suitable environment (*e.g.*, pristane priming), or possible reduction of host resistance (*e.g.*, CY pretreatment), shortens the duration of eclipse phenomenon.

Recent studies indicated that some plasmacytoma cells, *e.g.*, MOPC-315, exhibit carrier-specific stimulation and suppression similar to antigen-induced normal B-cell differentiation (14). The shorter eclipse period noted in athymic mice in these experiments further suggests the possible role of noncarrier, primed thymic cells (*e.g.*, T-cells and/or their products) in the regulation of tumor development. This would suggest that these tumor cells still retain some capacity to be influenced by the presence of a normal T-cell system.

These studies have demonstrated that the sensitivity of the *in vivo* [6-^{14}C]arginine pulse-labeling technique permits study of the early events following tumor cell transfer. It is apparent that these early events determine the success or failure of tumor growth, and a better understanding of these early events may provide new insight into how tumor establishment can be altered. Modification of the eclipse period by host manipulation prior to tumor cell transfer is further evidence that host factors can influence tumor growth. In addition, measurement of M component synthesis by [6-^{14}C]arginine has the advantage of monitoring a different metabolic function of the tumor cell from those measurements of DNA synthesis that traditionally have been used to study cell kinetics. This pulse-labeling technique monitors all of the tumor cells that are metabolically active in M component synthesis and secretion, whereas DNA synthesis is likely to be occurring in less than 15% of the cells in this tumor. Attempts are presently being made to correlate M component synthesis with tumor growth kinetics, to identify the possible subsets of tumor cells participating in the early events immediately following tumor cell transfer, and to clarify the host factors that may regulate tumor development.

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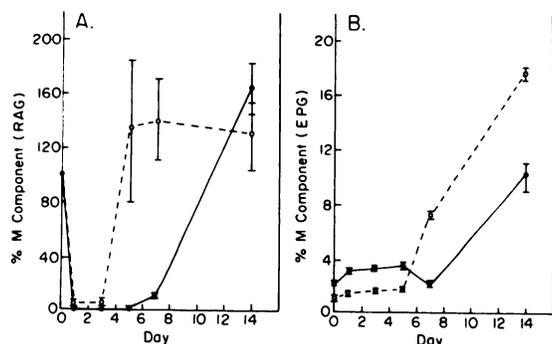
Chart 2. *A,* comparison of LPC-1 M component synthesis after tumor cell transfer as detected by [6-^{14}C]arginine (*RAG*) in normal and athymic (*nu/nu*) BALB/c mice, expressed as percentage of total radioactivity in protein; *B,* comparison of LPC-1 M component after tumor cell transfer, as detected by Ponceau S staining (*EPG*) in normal and athymic BALB/c mice, expressed as percentage of total protein. Athymic (O) and normal (●) mice were given injections i.p. with 10^7 LPC-1 cells, followed at the indicated intervals i.p. with 40 μCi [6-^{14}C]arginine. Plasma was obtained 24 hr after isotope. *Points,* mean from 3 to 6 mice; *bars,* S.E.

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