Europ. J. Cancer Vol. 15, pp. 837–842.

0014-2964/79/0601-0837 $02.00/0

Spleen Cell Kinetics in Mice Bearing the Landschütz Ascites Tumour*

JOHN BRAZIL and HUGH McLAUGHLIN

Department of Pathology, University College, Dublin 2, Ireland

Abstract—*The proliferative response of splenic lymphocytes in mice bearing the Landschütz ascites tumour has been examined* in vitro *and correlated with the changes occurring in the growth characteristics of the tumour. Between* 8 *and* 12 days *after tumour transplantation the spleen increased markedly in size and this has been shown to be associated with an increased proliferative activity of the splenic lymphocytes. The activity rapidly diminished as the spleen atrophied. Maximal tumour cell DNA synthetic activity also occurred* 12 days *after transplantation corresponding to the attainment of maximum tumour cell numbers. Although the volume of the ascites fluid continued to increase after this time the absolute number of tumour cells fell in parallel with a reduction in DNA synthesis. The close temporal relationship between the changes in both spleen cell and tumour cell populations suggests that factors influencing cell growth are acting simultaneously on both cell populations.*

INTRODUCTION

In animals receiving an intraperitoneal injection of Landschütz ascites tumour cells an increase in spleen size was noted reaching a maximum at 12 days. This was followed over the next 2 weeks by a decrease in spleen size and splenic atrophy. It has been shown in another model that this phenomenon is not due to pooling of blood and presumably reflects a proliferation of lymphoid cells within the spleen [1]. We set out to confirm this earlier report in the present model and to study the *in vitro* growth characteristics of splenic lymphocytes in tumour bearing animals at varying intervals following tumour inoculation. The spontaneous uptake of tritiated thymidine by spleen cells at different times was assessed and this was compared to the uptake by tumour cells taken from the same animals.

MATERIALS AND METHODS

Mice

Eight to twelve weeks old animals of both sexes were obtained from a closed colony of random-bred Schofield albino mice maintained at this laboratory.

Tumour

The Landschütz ascites carcinoma is propagated by weekly serial passage in female mice. Tumour cells for experiment were collected into phosphate buffered saline (PBS) and washed three times. A cell count was carried out in a haemocytometer and the cell viability determined by trypan blue vital staining. Only cell suspensions of >90% viability were used. Tumour growth was initiated in the experimental animals by intraperitoneal injection of 10^7 viable tumour cells in 1 ml of PBS. This dose has been previously shown to kill 100% of injected animals within 30 days of transplantation, 50% of the animals dying within 23 days [2]. Animals receiving tumour cells killed with 10% formol saline for 30 min and washed in P.B.S. served as controls.

Spleen weights

Spleens were excised intact from 8 normal, 24 tumour-bearing and 24 control mice at 4 day intervals after injection. Care was taken to remove any adherent adipose tissue, and the organs were placed in PBS. They were then weighed on a Mettler 6HT balance after removing excess moisture with tissue paper.

Accepted 21 August 1978.
*This work has been supported by a grant from the Medical Research Council of Ireland.

Spleen haemoglobin

After weighing, the spleens were disrupted in a constant volume of distilled water in a Dounce homogeniser and the homogenates subjected to repeated freezing and thawing to lyse the cells. The cell debris was spun down and the supernatants assayed for haemoglobin using the cyan-methaemoglobin method and a Coulter haemoglobinometer measuring absorbance at 540 nm.

Tumour cell culture

Tumour cells were recovered into HBSS and treated in the same manner as the spleen cells recovered at the same time.

Radioactivity measurement

After incubation the cells were resuspended and transferred onto filter paper discs. Acid precipitable radioactivity was measured in a Philips automatic scintillation counter using PPO/POPOP as scintillant.

RESULTS

Spleen weights

The changes in spleen weight following intraperitoneal injection of equal quantities of live and formalin-killed tumour cells are illustrated in Fig. 1. In animals receiving live cells, spleen weights increased to 2–3 times the normal value between 8 and 12 days after transplantation. This was followed by a progressive decrease in weight so that by the 24th day the size of the organ was only one-third normal. No significant changes were observed in the spleen weights of control mice receiving killed cells.

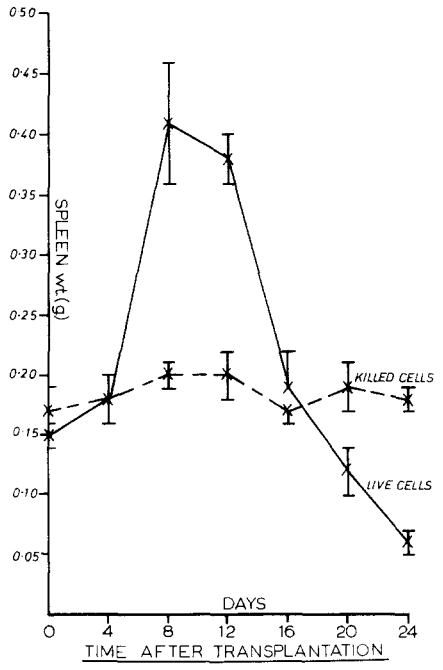

Fig. 1. Changes in spleen weight following intra-peritoneal injection of 10^7 viable or formalin-killed Landschütz ascites tumour cells.

Spleen haemoglobin

A 50% rise in spleen haemoglobin content followed intra-peritoneal injection of both live and killed tumour cells (Fig. 2). There was no significant difference between tumour-bearing animals and controls and the spleen haemoglobin content values returned to normal in both cases within 12 days of injection.

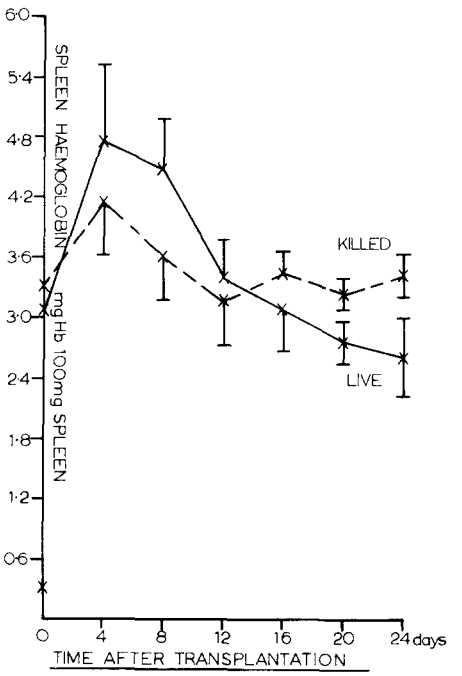

Fig. 2. Variation in spleen haemoglobin content after intra-peritoneal injection of 10^7 viable or formalin-killed Landschütz ascites tumour.

Spleen cell culture

Maximal spontaneous thymidine incorporation by unstimulated spleen cells occurred within 24 hours of their removal from the host animal (Table 1). Consequently an 18 hour pulse period was chosen for the culture experiments.

Figure 3 illustrates the change in DNA synthetic activity of spleen cells observed in tumour bearing animals. Following tumour transplantation the DNA synthetic activity of spleen cells increased, reaching a peak of five times the normal value 12 days later. By the sixteenth day, however, this had fallen to only twice normal. Due to the progressive atrophy of the spleen after this time it was not possible to

recover sufficient cells to determine whether this trend continued.

Table 1. *Uptake of tritiated thymidine by* 2×10^5 *normal murine spleen cells in unstimulated cultures at different culture periods expressed as* counts/min ±S.E.

Incubation time (hr)	counts/min	±	S.E.
12	2463	±	210*
18	3347	±	283
24	3485	±	365
42	2783	±	204
48	2869	±	225
66	1642	±	122

*Each value = mean of triplicate determinations carried out on each of 4 animals.

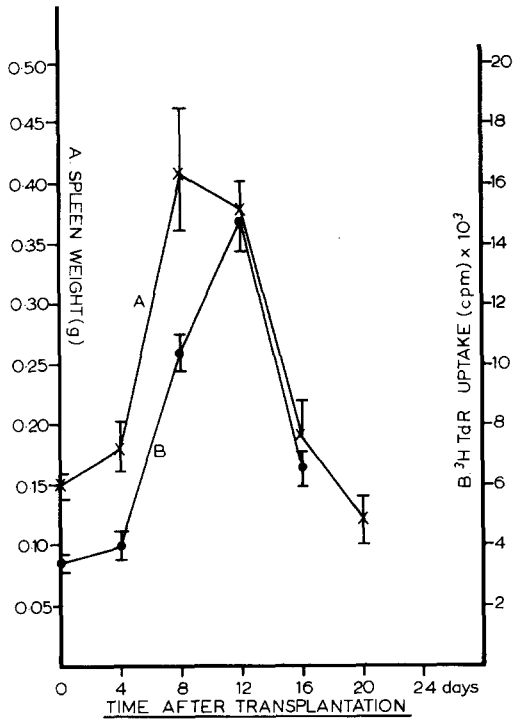

Fig. 3. *Spleen weight changes correlated with* in vitro *tritiated thymidine* (^{3}*HTdR*) *uptake after transplantation of* 10^7 *viable Landschütz ascites tumour cells.*

Tumour cell quantitation in vivo

The variation in tumour cell numbers in the ascites fluid during the course of tumour growth was similar to that previously reported (2). The close relationship between the size of the tumour cell population and the DNA synthetic activity of those cells is shown in Fig. 4. No evidence of tumour cell escape outside the peritoneal cavity other than limited growth at the size of injection was observed.

Tumour cell culture

The incorporation of tritiated thymidine by

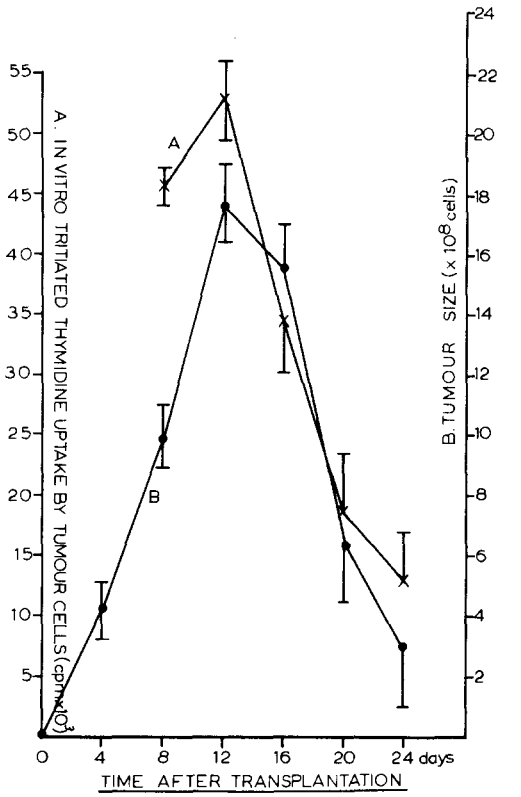

Fig. 4. *Variation in tumour cell numbers and* in vitro ^{3}*HTdR uptake after transplantation of* 10^7 *viable Landschütz ascites tumour cells.*

tumour cells was considerably greater than that of an equal number of spleen cells (Fig. 5). Both however reached peak values at 12 days post-transplantation after which the fall in spleen cell activity was paralleled by a similar decrease in tumour cell DNA synthetic activity. The determination of the *in vitro* DNA synthetic activity of tumour cells recovered within a week of transplantation was not possible because an insufficient number of cells were recoverable at this stage of tumour growth.

DISCUSSION

In mice bearing Landschütz ascites tumour, mean spleen weight increased progressively over a twelve day period and this was followed by a progressive decrease in weight and atrophy. The weight changes observed have been related to changes in the lymphoid population of the spleen as total haemoglobin content did not show comparable changes. A direct relationship between spleen size and *in vitro* DNA synthetic activity of spleen cells has been demonstrated. Studies on DNA synthesis by tumour cells similarly showed a direct relationship between synthetic activity and total tumour cell count.

Similar spleen weight changes have previously been reported following transplantation of both the Landschütz and the Ehrlich tumour [1] although the cause is uncertain. In both cases, however, it had returned to normal before maximal splenomegaly occurred.

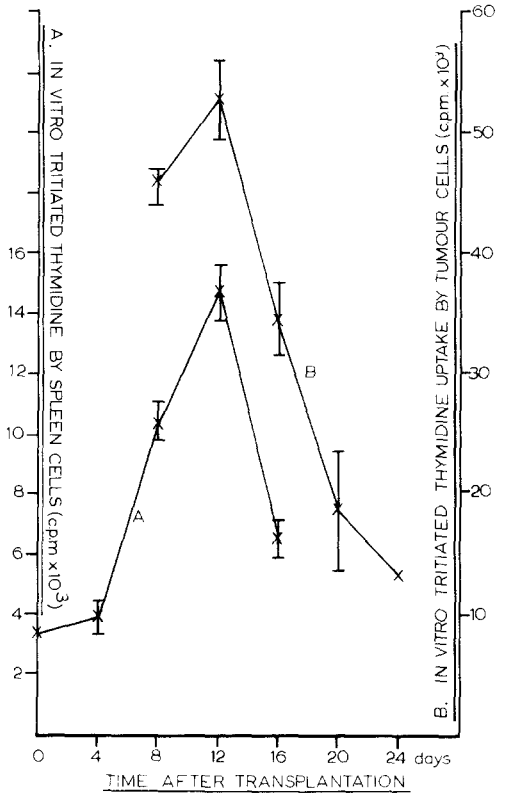

Fig.5. Changes in ^{3}HTdR uptake by spleen cells and tumour cells after transplantation of 10^7 viable Landschütz ascites tumour cells.

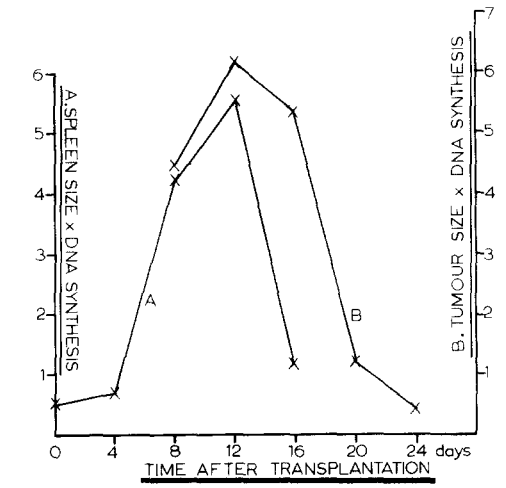

Fig. 6. Changes in "total" DNA synthesis by both spleen and tumour, i.e. allowing for the changes occurring in the size of each population (arbitrary units).

ascites tumours [1, 4] although splenomegaly did not invariably occur [5]. This may be due to contaminant passenger viruses within the tumour [6]. The initial increase in splenic haemoglobin content observed with this tumour has also been noted with the Ehrlich

Spleen cell culture

Spleens were removed aseptically from 4 tumour-bearing animals at 4 day intervals post-transplantation and teased apart with forceps into a plastic Petri dish containing Hanks' balanced salt solution (HBSS). The cell suspensions were washed with Tris–ammonium chloride solution to lyse the contaminating erythrocytes, washed three times in HBSS, and finally resuspended in RPMI 1640 + 25 mM HEPES (Gibco) containing 10% foetal calf serum with streptomycin and penicillin. Cell counts and viablilities were determined using a haemocytometer and trypan blue vital staining. The single cell suspensions contained predominantly lymphocytes (>90% by visual inspection).

Volumes of 0.1 ml of each cell suspension containing 2×10^6 cells per ml were placed in triplicate in the wells of flat bottomed Cooke microtitre plates together with 0.1 ml of tissue culture medium and 1 μCi of tritiated thymidine (specific activity 5 Ci/mmole, Radiochemicals, Amersham) added to each of the wells, the plates sealed and then incubated at 37°C for 18 hr.

Tumour cell quantitation in vitro

This was performed as previously described [2, 3]. Sheep red blood cells were labelled with Cr^{51} and injected intra-peritoneally into live tumour-bearing animals. These animals were sacrificed 10 min later and a known volume of ascites fluid recovered into 3.8% trisodium citrate solution. Radioactivity measurements were carried out in a Philips gamma counter which together with the concentration of tumour cells in the ascites fluid determined visually in a haemocytometer, allowed quantitation of the total tumour load viz.

Radioactivity injected (counts/min/ml)/ radioactivity recovered (counts/min/ml) × tumour cell count (cells/ml) = total number of tumour cells.

Tumour cell quantitation was performed at intervals of 4 days after transplantation.

Altered immunological responsiveness following tumour transplantation has been repeatedly demonstrated both *in vitro* and *in vivo*. The response of spleen cells taken from tumour-bearing mice to mitogens such as

phytohaemagglutinin and concanavalin A has been shown to be depressed as tumour growth proceeds [7, 8] although in this latter study Kapoor and Chowdhury observed a return to normal response levels at an intermediate phase of tumour growth but which again diminished as the animals neared death [8]. The number of B lymphocytes in the spleen has recently been shown to increase over the first 7 days of growth of the Ehrlich ascites tumour but no data for later changes was reported [9]. The response of tumour-bearing mice to antigens such as sheep red blood cells is diminished and factors within the ascites fluid lead to an impairment of humoral immunity [5, 10, 11]. Cellular immune responses as evidenced by the ability to mount a graft-vs-host response did not appear to be affected [10], although prolonged survival of skin allografts in animals bearing ascites tumours has been reported [12, 13]. The impairment of macrophage function in tumour bearing animals has also been repeatedly demonstrated (reviewed in [14]).

Several mechanisms have been postulated by which such immune depression might be brought about. Antigenic competition by the tumour may result in unresponsiveness to other antigens. Badger *et al.* have shown that this is not the mechanism involved in immunosuppression mediated by cell-free tumour ascites fluid [15]. Hršak and Marotti have pointed out that given the weak antigenicity of most murine ascites tumours this is unlikely [10]. Immunosuppressive factors released either by the tumour or by reactive host cells ther by the tumour or by reactive host cells have been reported. The murine leukaemia L1210 has been shown to release a lymphokine-like substance with macrophage migration inhibition factor-like (MIF) activity [16]. Ascites fluids from patients with cancer metastatic to the peritoneum have been shown to depress immune responses in mice in a manner similar to that of the immunoregulatory alpha-globulin [15].

The reduction observed in spleen cell DNA synthetic activity may be due to the release of such factors but it is also possible that the poor nutritional state of the host plays a role since the tumour cells suffered a marked concurrent reduction in activity. This latter phenomenon has been observed in many murine ascites tumours and seems to be due to a lengthening of the cell cycle throughout the total cell population [17] though reduced growth fractions have also been reported [18]. Anoxia [19], the accumulation of toxic metabolites [20], and the action of tumour-specific chalones [21] have been suggested as possible mechanisms. Anoxia or toxic metabolites would also be expected to depress the activity of other rapidly proliferating cell populations within the host, including spleen cells involved in an immune response.

The close relationship between the four parameters studied in the rates of DNA synthesis by both tumour and spleen cells and the changes in the size of each population suggests a common cause for the observed phenomena. It appears likely that the initial rise in spleen cell activity reflects an active immune response which is aborted by either the unavailability of vital nutrients, the accumulation of toxic metabolites, or the release of non-specific suppressive factors from the tumour. Whichever mechanism is operative, it seems the tumour cells are influenced to a similar extent.

REFERENCES

1. S. Thunold, Local cellular changes and splenic weight changes in mice with Ehrlich's ascites carcinoma. *Acta path. microbiol. scand.* **72,** 542 (1968).
2. J. Brazil and H. McLaughlin, *In vivo* growth characteristics of the Landschütz ascites tumour. *Irish J. med. Sci.* **147,** 134 (1978).
3. J. Brazil and H. McLaughlin, The modification of the growth characteristies of the Landschütz ascites tumour by neuraminidase. *Europ. J. Cancer* **14,** 757 (1978).
4. A. E. Stuart and A. M. el Hassan, Pathology of the Landschütz ascites tumour. *Brit. J. Cancer* **18,** 551 (1964).
5. I. Kamo and N. Ishida, Immune responses in tumour-bearing mice. *Gann* **62,** 453 (1971).
6. V. Riley, F. Lilly, E. Huerto and D. Bardell, Transmissible agent associated with 26 types of mouse neoplasma. *Science* **132,** 545 (1960).

7. W. H. Adler, T. Takiguchi and R. T. Smith, Phytohaemagglutinin unresponsiveness in mouse spleen cells induced by methylcholanthrene sarcomas. *Cancer Res.* **31,** 864 (1971).
8. Q. S. Kapoor and T. K. Chowdhury, Changes in the mitogenic transformation of murine lymphocytes during the progressive growth of anaplastic carcinoma. *J. reticuloendothel. Soc.* **21,** 385 (1977).
9. G. Keeb and P. K. Lala, Effects of Ehrlich ascites tumour transplantation in mice on the distribution of cells capable of forming haemopoietic colonies *in vitro*. *Europ. J. Cancer* **14,** 331 (1978).
10. I. Hrsak and T. Marotti, Immunosuppression mediated by Ehrlich ascites fluid. *Europ. J. Cancer* **9,** 717 (1973).
11. K. N. Yamazaki and H. Umezawa, Immunosuppression induced with cell-free fluid of Ehrlich carcinoma ascites and its fractions. *Gann* **64,** 83 (1973).
12. R. E. McCarthy, Modification of the immune response of mice to skin homografts and heterografts by Ehrlich ascites carcinoma. *Cancer Res.* **24,** 915 (1964).
13. R. E. McCarthy, J. N. Coffin and S. K. Gates, Selective inhibition of the secondary immune response to mouse skin allografts by cell-free Ehrlich ascites carcinoma fluid. *Transplantation* **6,** 737 (1968).
14. K. James, The influence of tumour cell products on macrophage function *in vitro* and *in vivo*. In *The Macrophage and Cancer Proceedings of the EURES Symposium*. 12–14 September, 1977. (Edited by K. James, W. McBride and A. E. Stuart) p. 225. Edinburgh (1977).
15. A. M. Badger, S. R. Cooperband, K. Merluzzi and A. H. Glasgow, Immunosuppressive activity of ascitic fluid from patients with cancer metastatic to the peritoneum. *Cancer Res.* **37,** 1220 (1977).
16. J. M. Mansfield, W. M. Shannon, S.-E. Yen and J. H. Wallace, Production of factors with immunosuppressive activity by a murine lymphoblastoid tumour cell line. *Proc. Soc. exp. Biol.* (N.Y.) **154,** 341 (1977).
17. I. F. Tannock, A comparison of cell proliferation parameters in solid and ascites Ehrlich tumours. *Cancer Res.* **29,** 1527 (1969).
18. J. W. Harris, F. Meyskeens and H. M. Patt, Biochemical studies of cytokinetic changes during tumour growth. *Cancer Res.* **30,** 1937 (1970).
19. I. F. Tannock and G. G. Steel, Tumour growth and cell kinetics in chronically hypoxic animals. *J. nat. Cancer Inst.* **45,** 123 (1970).
20. P. K. Lala, Influence of local environment on the growth parameters of the Ehrlich ascites tumour. *Europ. J. Cancer* **8,** 197 (1972).
21. P. Bichel, Specific growth regulation in 3 ascites tumours. *Europ. J. Cancer* **8,** 167 (1972).