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The Effect of the Immune Status of the TAR Mouse on the Growth and Metastasis of Tumour Xenografts

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Abstract—*Mice thymectomised at 3–4 weeks of age and subsequently given whole-body irradiation (9 Gy) under cytosine arabinoside cover (TAR mice) provide an alternative model to the athymic nude (nu+/nu+) mouse for studying the biological characteristics of tumour xenografts. In the present study we have evaluated the repopulation events in the bone marrow and spleen following whole body irradiation of TAR mice, and analysed immune competence up to 98 days following irradiation. Repopulation of both bone marrow and spleen was evident in the weeks following whole body irradiation, and an initial increase in the relative proportion of T-lymphocytes present in the spleen was followed by a decrease in the percentage of lymphocytes expressing T-cell markers, which remained below the level observed in control mouse spleen cell preparations. TAR mice exhibited a decreased ability to respond to a non-specific T-cell mitogen and to elicit a T-cell dependent antibody response to influenza viral antigen. Both TAR and control mice possessed macrophages which could be activated to the tumouricidal state, and natural killer activity of TAR mice was enhanced greater than 3-fold above control values. The ability of TAR mice to accept tumour xenografts decreased with the increasing time interval between irradiation and subcutaneous implantation of tumour cells, and (in some instances) spontaneous regression was observed. In addition, a hamster tumour cell line possessing high metastatic potential in its syngeneic host was shown to metastasise to the regional lymph node, lungs, liver, kidneys and spleen of TAR mice from a cell inoculum implanted subcutaneously immediately after irradiation; however, with increasing time between irradiation and inoculation of tumour cells tumour metastasis decreased. The ability of TAR mice to support the growth and metastasis of tumour xenografts would appear to inversely correlate with the increase in natural killer cell activity following irradiation.*

INTRODUCTION

IMMUNODEFICIENT animals have provided a valuable system for the study of human tumours *in vivo*; in particular congenitally athymic nude (nu+/nu+) mice have been used extensively for the growth of human tumours from fresh biopsy tissue and cultured cell lines [1, 2]. Tumours arising in nude mice have been shown to maintain their morphological characteristics as well as many of their biological and biochemical properties [3, 4]. The inability to allow maturation of T-lymphocyte precursors in the thymus, results in these animals being severely restricted in their ability to mediate T-lymphocyte-dependent immune responses; however, the level of innate resistance mediated by natural killer (NK) cells is elevated [5, 6].

There are several disadvantages in using nude mice for such studies, including their susceptibility to infection, the high cost of purchase, housing and maintenance of these animals, and also the limitations of the model when used to study certain biological characteristics of tumours, such as tumour dissemination and metastasis [7]. A promising alternative mouse system found to be suitable for the growth of tumour xenografts is the thymectomised, whole-body irraditaed (TAR) mouse first described by Steel *et al.* [8]. These mice are thymectomised at 3–4 weeks of age and injected with cytosine arabinoside before whole body irradiation (9 Gy). Cytosine arabinoside is thought to essentially augment bone marrow recovery following

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irradiation [9] and protect the gut from the lethal effects of irradiation [10]; TAR mice do not require a bone marrow graft after irradiation, and are therefore highly immunosuppressed [8].

TAR mice have been used previously to study the effect of chemotherapeutic agents on tumour growth [11, 12], and comparative studies would indicate that they are not only more receptive to the growth of cultured tumour cell lines than congenitally athymic nude mice, but also show an increased incidence of tumour metastasis [8]. Although previous observations would suggest that TAR mice recover their ability to reject tumour xenografts during the months following irradiation [8], the time-course of events and immune status of TAR mice has not been evaluated. In the present study the degree of immunosuppression and the nature of returning immunocompetence in TAR mice was investigated, and the influence of immune status on the growth of primary tumour xenografts and spontaneous metastases observed.

MATERIALS AND METHODS

Preparation of TAR mice

Three- to four-week-old female CBA mice were purchased from the University of Sheffield Animal House, or from OLAC, Shaws Farm, Bicester. Thymectomy was performed under ether anaesthesia according to the method of Steel *et al.* [8]. Four to six weeks after, thymectomised mice were injected with 200 mg/kg of cytosine arabinoside, and 2 days later given 9 Gy whole body irradiation using a Marconi 250 kV experimental X-ray unit. Sham thymectomised/non-irradiated mice were used as controls in all studies unless otherwise stated.

Histological studies

The sternum and part of the spleen were fixed in neutral buffered formalin. The bone was decalcified by immersion in ethylene diamine–tetra-acetic acid (EDTA); paraffin sections were prepared and stained with haematoxylin and eosin. Normal marrow, packed with haemopoietic cells, was designated 100% cellularity, and deviations from this were expressed as a percentage residual cellularity as estimated simultaneously by 2 observers (JRG and AMB) using direct microscopy and a television monitor. The spleen histology was assessed by judging the degree of lymphocyte cellularity of the lymphoid aggregates. T-Lymphocytes normally occupy a position adjacent to penicillar arteries while B-lymphocytes are peripheral to them: these are designated T and B zones respectively. The cellularity of bone zones was estimated on a 4 point scale (4+ being normal) by observation as above. On completing the assessment for marrow and spleen, the individual results were compared: no major differences were present and minor ones were re-examined, and an agreed table of cellularity was compiled.

Mitogen stimulation assay

Spleen cells were cultured in 96-well round-bottomed microtitre plates at 4×10^4 cells per well in 0.2 ml RPMI + 10% foetal calf serum (FCS), containing 12.5 μg PHA. After 72 hr incubation at 37° C in a 5% CO_2 atmosphere, cultures were pulsed with 0.5 μ *cis*-(^{3}H)-thymidine (Amersham International, Bucks, England). Four hours later the cells were removed from the wells using a cell harvester (Scatron, Lier, Norway) and (^{3}H)-thymidine uptake was determined by liquid scintillation counting.

Influenza virus antibody titres

Mice were infected intranasally with a stock laboratory strain of influenza virus—A/Port Chalmers/1/73 (H3N2). Two weeks after infection blood samples were assessed for serum antibody levels using the haemagglutination inhibition (HI) test. HI titrations were carried out using standard methods previously described [13].

Cytotoxicity assays

Four hours and 18 hr ^{51}Cr release assays were used to measure natural killer and macrophage activity respectively. These assays were performed as previously described [14].

The percentage cytotoxicity was calculated by the formula:

$$\% \text{ Cytotoxicity} = \frac{(\text{test release}) - (\text{spontaneous release})}{100 - (\text{spontaneous release})} \times 100.$$

Preparation of spleen cells

Mouse spleens were removed aseptically into ice-cold PBS, then passed through a 120-gauge stainless steel mesh. Five ml of the cell suspension was then layered onto 4 ml of lymphocyte separation medium and centrifuged for 30 min at 400***g*** at room temperature. The cells at the interface were collected, washed × 3 in RPMI + 10% newborn calf serum (NBCS) then counted, and stored at 4° C until used.

Preparation of macrophages

Macrophages were activated by injecting 0.375 mg of *Corynebacterium parvum* intraperitoneally (i.p.). Ten days later cells were harvested from the peritoneum and washed × 3 in Hank's balanced salt solution (HBSS). The cells were then counted, resuspended in RPMI + 10% NBCS and used as effector cells in a 18 hr ^{51}Cr release assay; resident (non-activated) macrophages were harvested from TAR and control mice injected i.p. with PBS.

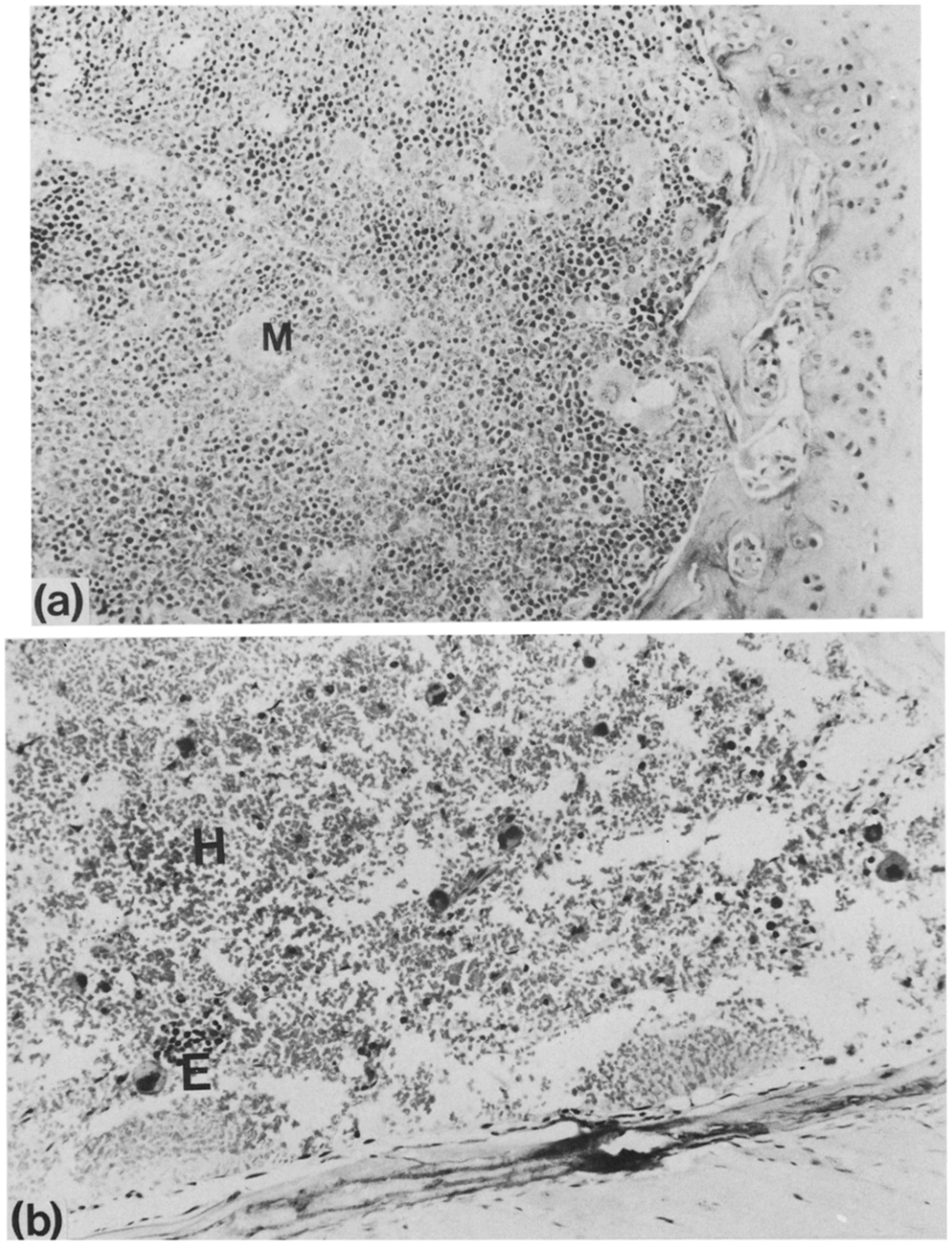
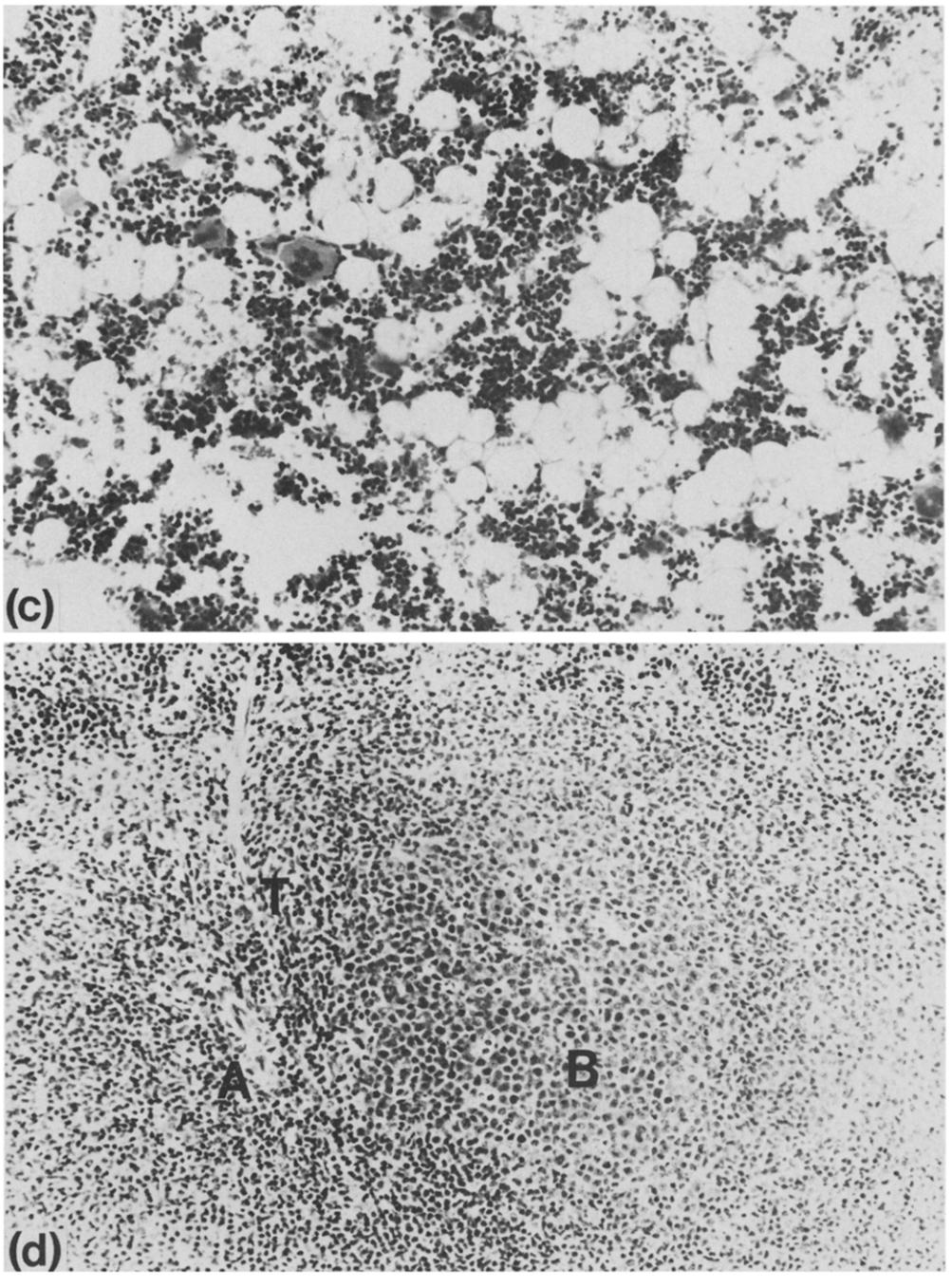


Fig. 1. (*a*) *Marrow, Day* 0 (*M: megakaryocytes*) (*b*) *Marrow, Day* 1 (*H: haemorrhage, E: surviving erythroblasts*) (*c*) *Marrow, Day.* 42. *Partial recovery* (*d*) *Spleen, Day* 0 (*A: arteriole, T: T-cell area, B: B-cell area*) (*e*) *Spleen, Day* 1. *Marked lymphoid cell depletion* (*f*) *Spleen, Day* 42. *Greater repopulation of B than T.*


(c)
T
A
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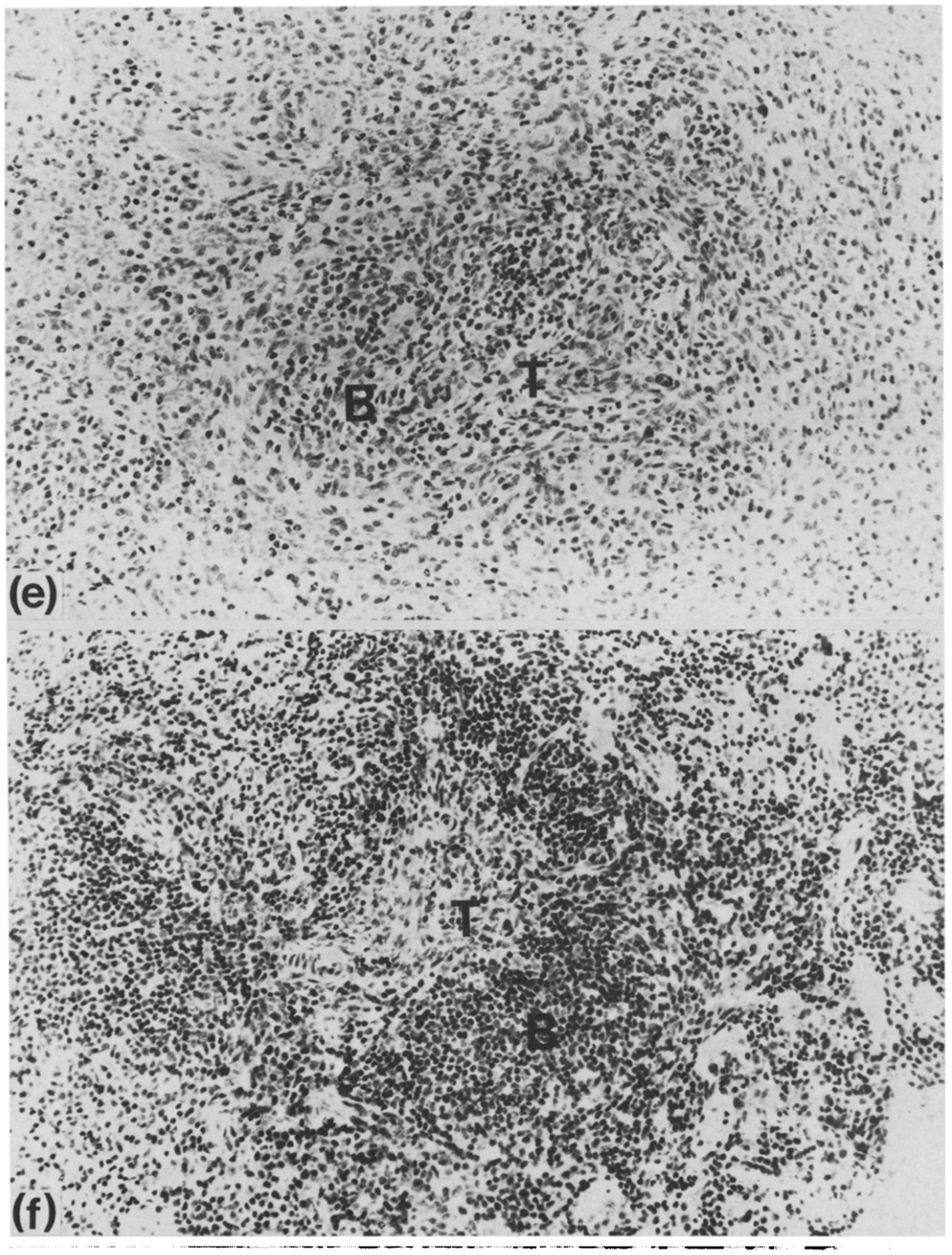
B
T
(e)
T
B
(f)

Effector cells were allowed to adhere for 2 hr at 37° C, then non-adherent cells were removed by washing before adding the target cells. Cytocentrifuge preparations were used to evaluate the percentage macrophages by non-specific esterase staining.

Identification of T-lymphocyte subsets

The mouse T-cell monoclonal antibodies Thy 1.2, Lyt-1 and Lyt-2 were purchased directly conjugated to FITC from Becton Dickinson. Spleen cells (1×10^6) were stained with 100 μl of antibody at an appropriate dilution for 20 min at room temperature. The cells were then washed 3 times in phosphate buffered saline and analysed by flow cytometry on a FACS 420, gated to exclude non-viable cells.

Tumour cell lines—experimental procedures

The YAC-1 mouse lymphoma cell line used as a target for mouse NK activity was donated by Dr. R.B. Herberman (National Cancer Institute, Bethesda), and cultured in RPMI + 10% FCS. The P815 mastocytoma line was provided by Dr. W. Johnson (Smith, Kline and French Laboratories, Philadelphia), and was also mainained as a suspension culture in Eagles minimal essential medium + 10% FCS.The SW 742 colon carcinoma cell line was cultured as a monolayer in RPMI + 10% FCS, and was provided by Dr. G. Poste (Smith, Kline and French Laboratories, Philadelphia).

The Met B cell line was derived from a lung metastasis of a herpes virus type 2 transformed cell line (HSV-3-2-26) implanted subcutaneously into male Syrian hamsters [15]. This line was grown as a monolayer in Eagles minimal essential medium supplemented with 10% FCS. Cells were harvested using 0.1% trypsin, washed twice in RPMI + 10% NBCS and finally resuspended in RPMI at the required concentration for injection. Three weeks later the animals were sacrificed and examined post-mortem for the presence of metastases with histological confirmation of the findings. In the syngeneic host this cell line is metastatic to the regional lymph node, lungs and kidneys from a subcutaneous tumour cell implant [16].

RESULTS

Effect of thymectomy and irradiation on the bone marrow and spleen histology

Marrow sections from control and thymectomised mice were normal throughout the period of observation. Thymectomy followed by irradiation resulted in subtotal ablation of the marrow by day 1 with empty areas, haemorrhage and debris taking the place of haemopoietic cells; only a few megakaryocytes were recognisable. Substantial recovery then occurred over the ensuing weeks (Table 1, Fig. 1). The spleens of control mice (sham thymectomised/non-irradiated) remained histologically normal throughout the experiment. TAR animals showed an immediate loss of splenic haemopoiesis, and of small lymphocytes from lymphoid aggregates; both B and T areas were similarly affected. Over the following 42 days there was some recovery of lymphocyte numbers in B-zones but not T-zones, resulting in an apparent halo around penicillar arteries (Fig. 1). Thereafter, T-zone repopulation became established (Table 1), and splenic haemopoiesis was re-established gradually over a time scale similar to marrow recovery.

Table 1. Histology of spleen and marrow in TAR mice day 1 to day 112

Day*	Group	Spleen‡ B-Zones	Spleen‡ T-Zones	Marrow cellularity %§
1	Control†	++++	++++	100
	TAR	+	+	5
7	TAR	++	+	5
17	TAR	+	+	30
42	TAR	+++	+	60
56	TAR	+++	+++	60
70	TAR	++	++	100
112	TAR	ND	ND	70

*Time in days after irradiation.
†Normal control mice and sham thymectomised-irradiated mice were assessed at regular time intervals up to day 112 of the experiment.
‡Arbitary scale of + to ++++ (as assessed by 2 observers).
§Total cell population expressed as a percentage of control mice.

Quantitation of splenic lymphocyte depletion and recovery in TAR mice

Splenic lymphocytes were prepared from both control and TAR mice at time points over the 70 days following irradiation. The number of viable cells was assessed (by trypan blue exclusion), and the results were expressed by calculating the lymphocyte count for the TAR group as a percentage of the counts for control mice (Fig. 2). This plot indicated that drastic lymphocyte depletion occurred after irradiation, which was followed by a gradual but steady recovery until by day 70 over 60% of the total lymphocyte population had been recovered. Spleen cell suspensions were stained with fluorescence-labelled monoclonal antibodies specific for the Thy 1.2, Lyt-1 and Lyt-2 surface antigens expressed on murine T-cells, and analysed by flow cytometry. Initially, an increase in the proportion of Thy 1.2-positive lymphocytes was recorded (days 1 and 7 post-irradiation); however, by day 14, and at subsequent time points up to day 70, a marked decrease in the proportion of Thy 1.2-positive cells was observed (Table 2). A similar

Table 2. T-Lymphocyte contents of cells recovered from the spleens of TAR and control mice

Day post-irradiation	Percentage lymphocyte staining* Thy-1.2 Control	Thy-1.2 TAR	Lyt-1 Control	Lyt-1 TAR	Lyt-2 Control	Lyt-2 TAR
1	27 ± 0†	54‡	29 ± 4†	49‡	10 ± 1†	11‡
7	14 ± 9	60	26 ± 6	58	10 ± 2	30
14	28 ± 1	19	34 ± 1	26	13 ± 1	14
42	43 ± 8	18	24 ± 1	7	12 ± 1	3
56	49 ± 1	14	44 ± 5	19	18 ± 2	11
70	30 ± 1	10	40 ± 7	15	14 ± 3	6

*Spleen cells were stained using monoclonal antibodies directly conjugated to fluorescein and analysed on a FACS-420 gated for viable cells.
†Mean ± standard deviation (S.D.) for 2–4 mice.
‡Pools of 4–6 mouse spleens

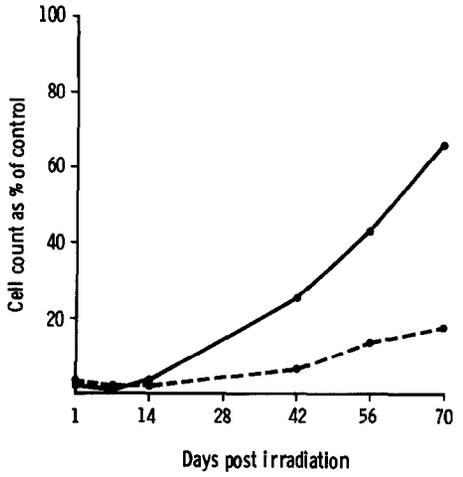

Fig. 2. Number of lymphocytes recovered from the spleen of TAR mice expressed as a percentage of the control cell counts. Recovery of total lymphocytes (a) are shown as percentage of control total lymphocytes (—). The recovery of T-cells is also shown as a percentage of the control T-cells (b) (---). Results are for 4–6 control and TAR mice.

time-course of events was shown using Lyt-1 and Lyt-2 monoclonal antibodies, although the initial increase in Lyt-2 staining was only apparent on day 7 post-irradiation.

The number of T-cells present in the spleen of control and TAR mice was calculated from the total lymphocyte number and the percentage of these cells staining with the Thy 1.2 antibody. T-Lymphocyte depletion and recovery was assessed by expressing the T-lymphocyte count for the TAR group as a percentage of the T-lymphocyte count of the control group (Fig. 2). The overall number of T-lymphocytes was low, with only 6% recovery of Thy 1.2 antigen-positive cells on day 42 and 17% on day 70.

T-Lymphocyte function in TAR mice

The mitogenic response of spleen cells recovered from TAR and control mice was assessed 56 and 70 days post-irradiation. Whereas spleen cells from control mice demonstrated a mitogen response to PHA (SI = 11.24), lymphocytes prepared from the spleens of TAR mice were unable to demonstrate an appreciable response; the maximum SI obtained in 4 experiments was 3 (Table 3a).

The ability of TAR mice to demonstrate a T-cell-dependent antibody response to influenza viral antigen was assessed 4 months post-irradiation. The results (Table 3b) indicate that whereas all 4

Table 3. T-cell responses in TAR and control mice

(a) PHA stimulation assay

Day post-irradiation	Individual assays	Stimulation index* Control	TAR
56	1	24†	1‡
	2	21	3
70	1	21	2
	2	11	2

*Three-day cultures of spleen cells stimulated with 2.5 μg PHA were labelled with (^{3}H)-thymidine for 4 hr, and the stimulation index (SI) calculated by the formula:

$$SI = \frac{^3\text{H Uptake (counts) of lymphocytes stimulated with PHA}}{^3\text{H Uptake (counts) of unstimulated lymphocytes}}.$$

†Individual mice.
‡Pools of 3 mice.

(b) T-cell-dependent antibody response

Animal number	Serum HI antibody titre Control	TAR*
1	80	< 10
2	120	< 10
3	80	20
4	160	< 10

*Four months after irradiation.

Two weeks after intranasal infection with influenza virus, serum antibody levels were estimated using the haemaglutination inhibition (HI) assay (levels < 10 are not detectable by this test).

Table 4. Macrophage activity in TAR mice at Day 28 post-irradiation

E : T ratio*	Control PBS†	Control CP‡	Tar PBS	Tar CP
12 : 1	ND	47	ND	47
6 : 1	−4.0	43	−0.7	46
3 : 1	−0.6	37	−4.3	39

Macrophage tumouricidal activity was assessed in an 18-hr ^{51}Cr release assay using P815 mastocytoma cells as targets.
*E T : ratio, effector-to-target ratio.
†PBS Phosphate buffered saline given i.p. 10 days prior to harvesting peritoneal exudate cells.
‡CP *C. parvum* given i.p. 10 days prior to harvesting peritoneal exudate cells.

control mice were able to produce significant serum antibody (HI titres = 80–160), only 1 TAR mouse had detectable antibody (HI titre = 20).

Tumouricidal activity of activated macrophages

Activated and resident (control) macrophages from TAR (28 days post-irradiation) and control mice were assayed for tumouricidal activity against the P815 mastocytoma target cell line in an 18 hr ^{51}Cr release assay. The results (Table 4) show that the ability of macrophages to respond *in vivo* to *C. parvum* is not impaired in TAR mice, and comparable cytotoxic activity against P815 cells was observed with macrophages from TAR and control animals. Resident macrophages from TAR and control mice were not tumouricidal for P815 targets.

Natural killer cell activity in TAR mice

Spleen cells prepared from TAR mice on days 28, 42, 56 and 98 post-irradiation consistently demonstrated a significant increase in cytotoxicity against YAC-1 targets compared with spleen cell preparations from control mice (Fig. 3); all effector-to-target cell ratios (6 : 1–50 : 1) showed a greater than 3-fold increase in natural killing above control spleen cell values.

Growth and regression of SW 742 tumours in TAR mice

SW 742 colon carcinoma cells were injected subcutaneously into TAR mice at times following irradiation, and tumour takes and growth rates recorded. It was observed that the tumour take was 100% when cells were injected subcutaneously into TAR mice 1 day post-irradiation. However, when cells were injected 42 and 98 days post-irradiation the take rate fell to 60% and 0% respectively (Fig. 4). It was subsequently observed that complete regression of developing tumours occurred in mice receiving a tumour cell inoculum on days 42 and 70 post-irradiation.

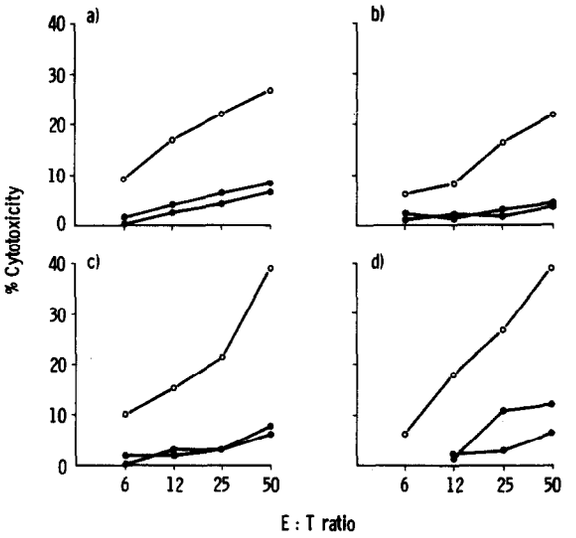

Fig. 3. NK activity for TAR and control mice in the weeks following whole-body irradiation. Cytotoxic activity of spleen cells was measured in a 4-hr ^{51}Cr release assay using YAC-1 lymphoma cells as targets. Both control and TAR mice were assessed at day 28 (a), 42 (b), 56 (c), and 98 (d) post-irradiation. Results are for individual control mice (●) and for pooled spleen cell preparations from TAR mice (○).

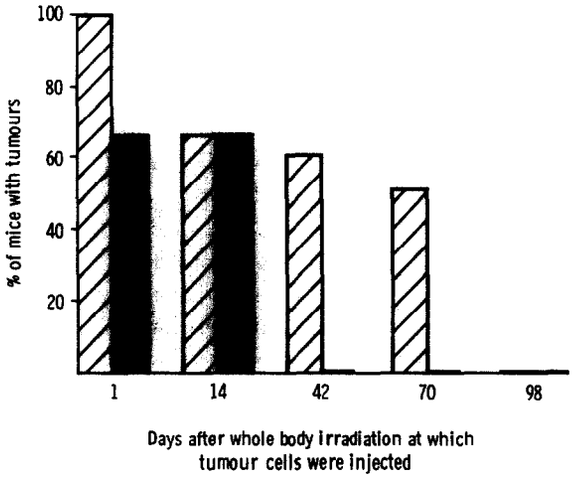

Fig. 4. Tumour takes in TAR mice inoculated at varying times after whole-body irradiation. Each group of 5 or 6 mice received subcutaneous inoculations of 2 × 10^6 human colon carcinoma cells (SW 742) 1, 14, 42, 70 or 98 days after irradiation. Each group was monitored for the appearance and regression of tumours. The results show the percentage of mice with tumours at 14 days (▨) and 98 days (■) after inoculation for each group.

Growth and metastases of Met B in TAR mice

The Met B subline spontaneously metastasises in its syngeneic host and was shown to grow progressively when 1 × 10^5 cells were injected subcutaneously (s.c.) into TAR mice; the tumour take rate was 100%. Met B cells were injected at various time intervals after irradiation of the mice, and the animals examined for tumour metastases at post-mortem when s.c. tumours were 10–15 mm dia.

Table 5. *MET B tumour metastases in TAR mice*

Day*	Individual mice	Assessment of metastases to: Lung	Liver	Kidney	Regional lymph-node	Spleen
1	1	++++†	+++	+++	+++	++
	2	++++	+++	+++	++	–
	3	++++	+++	+++	+++	–
14	1	+++	–	–	+++	–
	2	+++	–	–	+	–
	3	++	–	–	–	–
	4	+	–	–	–	–
42	1	+++	–	–	–	–
	2	++	–	–	–	–
	3	+	–	–	–	–
70	1	++	–	–	–	–
	2	+	–	–	–	–
	3	+	–	–	–	–
	4	+	–	–	–	–
	5	–	–	–	–	–
98	1	+	–	–	–	–
	2	+	–	–	–	–
	3	–	–	–	–	–
	4	–	–	–	–	–
	5	–	–	–	–	–
	6	–	–	–	–	–
	7	–	–	–	–	–

*Days after irradiation at which tumour cells were injected.
†Arbitrary scale of – (no macroscopic metastases) to ++++ (gross macroscopic metastases).

Table 5 shows the distribution of metastases and demonstrates the effect of delaying the subcutaneous inoculation 1–98 days after irradiation. Those mice inoculated on day 1 showed more widespread secondary tumour growth than previously observed in the syngeneic host. A delay of 14 days before injection of Met B cells resulted in pulmonary and some nodal metastases, although longer intervals resulted in pulmonary metastases alone in reducing quantities.

DISCUSSION

The present study evaluates the immune status of TAR mice at time intervals after whole-body irradiation.

Cellular depletion and recovery after thymectomy and irradiation was observed in the spleen and bone marrow of TAR mice. High-dose irradiation (9 Gy) given to thymectomised cytosine arabinoside-primed mice resulted in destruction of almost all lymphoid cells in the bone marrow and spleens. Recovery from irradiation was evident by the repopulation pattern observed at both sites. Within 2 months following irradiation B-cell areas of the spleen had recovered the majority of their cellularity, but in addition there was a considerable repopulation of the periarteriolar sheath, where T-cells are normally located. Similar T-like cells have been reported in the periarteriolar sheath of nude mouse spleens [17], although their precise identity is unknown. Lymphoid cells expressing T-cell markers were present in the spleen of mice given whole-body irradiation, although repopulation of the spleen was accompanied by a relative decrease in the percentage of T-cells identified with specific monoclonal antibody, which this population established at 8–14% of the recoverable lymphoid cell population at 6 weeks post-irradiation. This finding may reflect the presence of radio-resistant T-lymphocytes, or bone marrow-derived thymic lymphocytes expressing T-cell markers. T-Lymphocyte functional ability was impaired, as shown by the reduced response of spleen cells from TAR mice to mitogen stimulation with PHA, and their reduced ability to mediate a T-cell-dependent antibody response, which was barely detectable. The presence of lymphocytes expressing phenotypic T-cell markers in the spleens of TAR mice does not constitute a population of T-cells with detectable functional activity.

It has previously been reported that in nude mice, high NK cell activity may influence the tumour take-rate and metastases of xenografts [18, 19]. Young nude mice, or older animals injected with cyclophosphamide, possess reduced NK activity, which is correlated with an improved tumour take-rate and an increase in the incidence of metastases [20]. In the present study the level of activity in

TAR mice would appear to correlate inversely with both tumour take-rate and metastases. The reason for the observed increase in NK activity is not clear, although one possible explanation is the concentration effect of pre-thymic bone marrow-derived lymphocytes in the spleen, resulting in higher relative numbers of NK effector cells being present. Alternatively, an increase in cytolytic activity of individual NK effectors, possibly due to *in vivo* activation, cannot be ruled out. Macrophages may also play a role in controlling tumour growth and metastases, and their activation was readily induced in TAR mice, thus constituting an additional or alternative mechanism capable of mediating antitumour activity.

The results of the present study also show the tumour take-rate and metastases of xenografts implanted subcutaneously into TAR mice at time intervals following irradiation. The human colon carcinoma cell line SW 742 gave a high take-rate in TAR mice when cells were implanted immediately after whole-body irradiation, but this decreased as the interval between irradiation and inoculation of tumor cells increased, and paralleled the repopulation events observed in the bone marrow and spleen. This confirms the earlier report by Steel *et al.* [8] that TAR mice recover their ability to reject tumour xenografts in the months following irradiation. Although no specific immunological mechanism has been ascribed to these events, evidence presented here of increasing NK cell activity, in addition to the presence of macrophages capable of being activated to the tumouricidal state, would suggest that innate immune mechanisms play a decisive role in the host defence against tumour growth.

The ability of a metastatic hamster tumour subline to form secondary tumour deposits in the regional lymph node, lungs and other organs also decreased with the time interval between whole body irradiation and inoculation of tumour cells. Evidence from other workers would suggest that NK status of the host is a factor determining blood-borne spread of tumour cells [21, 22], as this could, in part, account for the reduced metastasis observed in animals injected with Met B tumour cells 14 days or more following whole-body irradiation. The profile of tumour metastases in the TAR mouse is similar, but not identical to, the tumour spread observed in the syngeneic host [16]. In TAR mice inoculated with Met B tumour cells directly after irradiation, metastases were detected not only in the regional lymph node and lungs, but also in the kidney, liver and in 1 animal in the spleen, suggesting that the TAR mouse may have a reduced capacity to resist metastases compared with the syngeneic host, or that tissue differences between the species produces a different 'soil' for secondary tumors to develop. The TAR mouse is highly immunosuppressed directly following irradiation, and at this stage is receptive to primary tumour growth and metastases. That natural immunity affects metastases in nude mice has been documented [18, 20], and the results presented here would support the premise that immunosuppression of non-specific and specific effector limbs of the immune response is necessary for tumour metastases to occur. A consideration in studying tumour xenografts in TAR mice is the growth rate of the primary tumour implant. Although fast-growing tumours would show a high tumour take-rate when implanted immediately after whole-body irradiation, slow-growing tumours would probably be influenced by host defence systems in the weeks following irradiation, resulting in regression of the primary, and possibly secondary, tumours. In this instance, further immunosuppression of the TAR mouse might be advocated at an appropriate time after irradiation.

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REFERENCES

1. Shimasoto Y, Kamoya T, Nagai K, *et al.* Transplantation of human tumours in nude mice. *JNCI* 1976, **56**, 1251–1260.
2. Kiessling R, Klein E, Wigzell H. Cytotoxic cells with specificity for mouse Maloney leukaemia cells. Specificity and distribution according to genotype. *Eur J Immuol* 1975, **5**, 112–117.
3. Povlsen CO, Fialkow PJ, Klein E, Klein G, Rygaard J, Weiner J. Growth and antigenic properties of a biopsy-derived Burkitt's lymphoma in thymusless (nude) mice. *Int J Cancer* 1973, **11**, 30–39.
4. Sordat B, Fritsche R, Mach JP, Carrel S, Ozzello L, Cerotini JC. Morphologic and functional evaluation of human solid tumours serially transplanted in nude mice. In: Rygaard J, Povlsen CO, eds. *Proceedings of the First International Workshop on Nude Mice.* Karger-Basel, 1974, 395–404.
5. Hanna N. Expression of metastatic potential of tumour cells in young nude mice is correlated with low levels of NK cell-mediated cytotoxicity. *Int J Cancer* 1980, **26**, 675–680.

6. Kiessling R, Wigzell H. An analysis of the murine NK cell as to structure, function and biological relevance. *Immunol Rev* 1979, **44**, 166–208.
7. Hanna N, Fidler IJ. The role of natural killer cells in the destruction of circulating tumour emboli. *JNCI* 1980, **65**, 801–809.
8. Steel GG, Courtney VD, Rostom AY. Improved immune-suppression techniques for the xenografting of human tumours. *Br J Cancer* 1978, **37**, 224–230.
9. Millar JL, Blackett NM, Hudspith BN. Enhanced post-irradiation recovery of the haemopoietic system in animals pretreated with a variety of cytotoxic agents. *Cell Tissue Kinet* 1978, **11**, 543–550.
10. Phelps TA. Cytarabin (Ara-C) induced radioresistance of mouse jejunal stem cells following a single or fractionated doses of irradiation. *Int J Radiat Oncol Biol Phys* 1980, **6**, 1671–1676.
11. Steel GG, Courtenay VD, Pekham MJ. The response to chemotherapy of a variety of tumour xenografts. *Br J Cancer* 1983, **47**, 1–13.
12. Steel GG. Therapeutic response of human tumour xenografts in immune-suppressed mice. In: Sordat B, ed. *Immune-Deficient Animals. 4th International Workshop on Immune-Deficient Animals in Experimental Research.* Karger-Basel, 1984, 395–404.
13. Goodeve AC, Potter CW, Clark A, Jennings R, Schild GC, Yetts R. A graded dose study of inactivated surface antigen influenza B vaccine in volunteers: reactogenicity, antibody response and protection to challenge virus infection. *J Hygiene* 1983, **90**, 107–115.
14. Ali SA, Hawrylowicz CM, Peel J, Griffith C, Rees RC. Human spleen cells mediating natural killing: altered natural cytotoxicity of spleen effector cells from patients with carcinoma. *J Cancer Res* 1983, **106**, 202–209.
15. Duff R, Doller E, Rapp F. Immunological manipulation of metastases due to herpes transformed cells. *Science* 1973, **180**, 79–83.
16. Walker JR, Rees RC, Teale DM, Potter CW. Properties of a herpes virus-transformed hamster cell line. I. Growth and culture characteristics of sublines of high and low metastatic potential. *Eur J Cancer Clin Oncol* 1982, **18**, 1017–1026.
17. Bamat J, Sordat B, Lees RK, Zaech P, Ceredig R, MacDonald HR. Development and localization of 'T-like' cells in the nude mouse. In: Sordat B, ed. *Immune-Deficient Animals. 4th International Workshop on Immune-Deficient Animals in Experimental Research.* Karger-Basel, 1984, 25–29.
18. Hanna N. Role of natural killer cells in control of cancer metastasis. *Cancer Metast Rev* 1982, **1**, 45–63.
19. Hanna N, Fidler IJ. Expression of metastatic potential of allogeneic and xenogeneic neoplasms in young nude mice. *Cancer Res* 1981, **41**, 438–444.
20. Hanna N, Schneider M. Enhancement of tumour metastasis and suppression of natural killer cell activity by β-estradiol treatment. *J Immunol* 1982, **130**, 974–980.
21. Gorelik E, Wiltrout KH, Okumura K, Habu S, Herberman RB. Role of natural killer cells in the control of metastatic spread and growth of tumour cells in mice. *Int J Cancer* 1982, **30**, 107–112.
22. Habu S, Fukue H, Shimamura K, *et al. In vivo* effects of anti-asialo GMI. I. Reduction of natural killer cell activity and enhancement of transplantable tumour growth in nude mice. *J Immunol* 1981, **117**, 34–38.