STUDIES ON THE BONE MARROW AND SPLENIC ERYTHROPOIESIS IN MICE BEARING EHRLICH ASCITES CARCINOMA

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ABSTRACT

The effect(s) of transplanted tumours on the host's erythropoiesis has been studied in Ehrlich ascites carcinoma. Results indicate that the mice become progressively anemic with the advancement of the tumour. Both the total nucleated cells and erythroid precursors in the bone marrow were decreased significantly. On the other hand, elevated erythropoiesis was observed in the spleen. While the serum iron concentration was decreased, total iron binding capacity was elevated and the stainable iron stores in the reticulo-endothelial organs were abundant in tumour-bearing animals. It is probable that diminished bone marrow erythropoiesis and impaired iron metabolism have contributed considerably to the etiology of this anemia.

INTRODUCTION

ANEMIA is a major hematological abnormality during the course of malignant tumorigenesis both in human[1] and experimental animals[2]. A number of studies have been performed to elucidate the mechanism of anemia and other hematologic abnormalities in a variety of malignant tumours[3]. But the results that have been obtained by earlier workers are contradictory and therefore inconclusive and this leaves the problem as a complex one.

The present study has been designed to elucidate the relationship, if any, between the erythropoietic activity and anemia in mice bearing transplanted Ehrlich ascites carcinoma under controlled experimentation.

MATERIALS AND METHODS

Experimental Animals

The tumour was maintained by serial transplantation in 4–6 week old male strain A mice (average body weight 23–25 grams) by i.p. inoculation of 1×10^7 Ehrlich ascites carcinoma (EAC) cells in sterile physiologic saline. The animals were sacrificed on the 10th (mid phase) and 18th (late phase) day of tumour for experimental studies.

Hematologic Measurements

Blood was obtained by cardiac puncture for routine haematologic measurement on day 10 and day 18 after tumour transplantation. Total counts of erythrocytes, hematocrit percentage, and determination of hemoglobin concentration were performed by standard methods[4]. The mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) and the mean corpuscular hemoglobin concentration (MCHC) of blood samples were calculated according to the standard procedures. The reticulocyte percentage of the peripheral blood was determined by counting at least 1000 red cells stained with brilliant crystal blue[4].

Marrow and Splenic Cellularity

The numbers of total nucleated cells per femur and tibia were determined by flushing the bones with physiologic saline and counting the suspension by hemocytometer, subsequent to the lysis of mature red cells with 3% glacial acetic acid. The percentage of erythroid precursors per femur and tibia were scored from methanol—fixed smears of the cell suspension stained with Leishman's stain (pH 6·8). Total number of erythroid precursors/femur and tibia were calculated from the total nucleated cellularity and the percentage of erythroid precursors.

The number of splenic nucleated cells were determined by hemocytometer after mincing the organ in cold physiologic saline and sequential passage through 20, 22 and 23 gauge needles and subsequent lysis of the mature erythrocytes by 3% glacial acetic acid treatment. The percentage of splenic erythroid precursors and total number of erythroid precursors/spleen were calculated as in medullary measurements.

Serum Iron and Total Iron binding Capacity (TIBC)

Serum iron and TIBC were measured from the pooled serum specimens by standard procedure[5]. To measure the non-circulating stored iron in the bone marrow and reticulo-endothelial organs, smears and histological sections were stained for prussian blue reaction[6].

RESULTS

Hematologic Values

It is evident from Table I that the tumour bearing mice become progressively anemic with the advancement of the disease, with circulating erythrocyte,

TABLE I

Hematologic values[a] in control and tumour bearing mice

	Days of Tumour bearing	
	10	18
Hemoglobin (gm/dl)		
Control	13·4±0·18	13·6±0·33
EAC[b]	10·8±0·30**	9·6±0·29**
RBC × 10^6/μl		
Control	6·4±0·17	6·1±0·21
EAC	4·8±0·18**	4·4±0·19**
Hematocrit (%)		
Control	45·4±1·77	44·8±0·62
EAC	40·2±2·10**	36·9±1·53**
Mean Corpuscular Volume		
Control (fl)	70·9±1·30	71·3±0·67
EAC	83·4±1·92**	83·1±1·38**
Mean Corpuscular Hemoglobin (pg)		
Control	20·9±0·74	21·2±0·81
EAC	22·5±0·87	21·6±0·61
Mean Corpuscular hemoglobin concentration (%)		
Control	29·5±0·59	29·8±0·44
EAC	27·0±1·02*	26·0±0·89*
Reticulocyte (%)		
Control	2·4±0·52	2·3±0·34
EAC	4·2±1·02**	4·1±0·86**

* $P < 0·01$; ** $P < 0·001$; [a] Mean ± S.D.;
[b] Ehrlich ascites Carcinoma.

hematocrit and hemoglobin level declining by approximately 38%, 27% and 29% respectively in comparison to the control values. The anemia is characterized by a 16% increase in MCV ($P < 0·01$) which indicate the anemia as slightly macrocytic. The mean corpuscular hemoglobin was almost the same in experimental and control animals throughout the period of observation. The mean corpuscular hemoglobin concentration (MCHC), however, showed 14% decrease ($P < 0·01$) in experimental animals within 3 weeks after tumour transplantation. A moderate reticulocytosis of 78% ($P < 0·001$) was also noticed in the tumour-bearing animals in both mid (day 10) and late (day 18) phases of the tumour.

Marrow and Splenic Cellularity

Total femoral nucleated cellularity of the experimental animals was significantly depressed by 25% and 38% of the control levels on day 10 and day 18 respectively (Table II). However, in case of the tibia, although the nucleated cellularity in tumour bearing mice was similar to the control value, there was 15% reduction ($P < 0·05$) at the late phase of the tumour. Concomitantly, there was a significant mean decrease in the number of erythroid precursors of the femur and tibia of the experimental animals which was evident both at the mid (60%) and late (85%) phases of the tumour. In contrast, the total number of nucleated cells/spleen was increased by about 24% and 65% at 1 week and 3 week of tumour, together with a 2-fold elevation in total number of erythroid precursors/spleen at the 3rd week (Table II). As a result, there was a 2·7-fold increase in the spleen weight.

Iron Metabolism

Coincidentally, with the fall in hematocrit, the serum iron concentration decreased by 20%, and 28% increase in TIBC was observed in experimental animals. As a result, the unsaturated iron binding capacity (UIBC) of transferrin was elevated (Table III). The tumour was creamy-white and there was no indication of any form of hemorrhage in the area surrounding the tumour. Though the non-circulating iron pool was decreased in the bone marrow of experimental animals, stainable iron was plentiful in sections of liver and spleen, particularly the latter in both the groups (Table III).

DISCUSSION

Anemia associated with various malignant diseases has been characterized and classified into the group of "anemia of chronic disorders"[7,8] The anemia of this group is mild and the erythrocytes are normochromic and normocytic. Biochemically, it is associated with several manifestations of altered iron metabolism, namely, low serum iron and TIBC, increased reticulo-endothelial iron stores and decreased number of sideroblasts in the bone marrow[7,8]. In our experiments, we have also observed that the tumour-bearing animals become progressively anemic as the disease advances and it simulated the anemia of chronic disorders as it occurs in human patients in that it is mild, serum iron concentration was decreased and storage iron pool was plentiful. But it differed from the said group in that the erythrocytes were slightly macrocytic rather than normocytic, and the TIBC was not decreased, on the other hand, was increased. The anemia observed in our experiments, therefore, was different from the anemia of chronic disorders in human cancer patients.

TABLE II

Bone Marrow and Splenic Cellularity in Control and Experimental Mice[a]

	Total nucleated cellularity (A, B $\times 10^6$; C $\times 10^8$)		Erythroid precursors (A, B $\times 10^6$; C $\times 10^7$)	
	10 day	18 day	10 day	18 day
A. *FEMUR*				
Control	22·4±3·39	23·1±1·20	3·9±0·37	4·0±0·20
EAC	16·6±1·32*	14·0±2·35**	1·4±0·23*	0·6±0·18***
% of change over control	−25	−38	−63	−84
B. *TIBIA*				
Control	12·5±2·59	12·7±2·40	2·7±0·56	2·9±0·32
EAC	12·4±1·79	10·8±1·68	1·0±0·19	0·3±0·06***
% of change over control	−0·8	−15	−60	−87
D. *SPLEEN*				
Control	1·9±0·15	2·1±0·26	1·4±0·18	1·6±0·24
EAC	2·4±0·33	3·5±0·64*	1·6±0·01	3·3±0·52*
% of change over control	+24	+67	+17	+107

[a] Mean ±S.D. ; * $P<0·05$; ** $P<0·01$; *** $P<0·001$

TABLE III

Serum and Tissue Iron in Control and EAC

	No. of Mice	Serum Iron (μg/dl)	TIBC* (μg/dl)	% Saturation	UIBC** (μg/dl)	Stainable Iron		
						Bone-marrow	Liver	Spleen
Control								
A	20	220	410	53	190	++	++	++
B	26	260	463	56	203	++	++	++
Tumour								
A	32	194	533	36	339	−	++	+++
B	26	185	584	31	399	+	++	+++

* Total iron binding capacity. ** Unsaturated iron binding capacity.

Total iron binding capacity is an indirect measure of serum transferrin concentration[9]. The elevated TIBC in experimental animals is, therefore, indicative of the increased transferrin concentration in the plasma of tumour-bearing animals. However, increased TIBC coupled with low serum iron concentration has been regarded as a pathological indication of iron deficiency[10]. But as the R-E iron stores were abundant (at least not exhausted) it is unlikely that the anemia that we have observed was due to iron deficiency. On the contrary, it is highly suggestive that defective reutilization of iron from R-E stores to the erythropoietic regions was the causative factor behind elevated unsaturated iron binding capacity.

In patients with malignant tumours, diminished erythropoiesis frequently contribute to the production of anemia[11]. In our experiments, we have noticed erythropoietic hypoplasia of both the femur and tibia

in the tumour-bearing animals. However, the mechanism of this hypoproliferative marrow activity is poorly understood. In our studies, the tumour was confined to the peritoneal cavity and we rarely observed any tumour-infiltration into the bone marrow. Therefore, we suggest that the tumour cells probably elaborate biologically active substance(s) which singularly or in combination act on the host's organic system and cause an adverse effect on the control mechanism of erythropoiesis.

In contrast to the depressed bone marrow erythropoietic response noted in tumour-bearing animals, the number of total splenic nucleated cells were elevated 1·5-fold on day 18, at which time nearly 2-fold increase in erythroid precursors levels were documented. During normalcy, murine spleen usually account for approximately 2–3% of total red cell production[12]. And as our experimental animals were subjected to intense erythropoietic challenge caused by the decreased marrow activity, the spleen become hyperactive to produce compensatory erythropoiesis. This elevated splenic erythropoiesis, therefore, could perhaps account for the moderate reticulocytosis that was observed in tumour-bearing animals.

In spite of elevated splenic erythropoiesis, persistent anemia was observed. It is possible, therefore, that this elevation was not adequate to compensate for the diminished bone marrow activity. Hemorrhage and hemolysis within the tumour have frequently contributed to the etiology of anemia in some tumour-bearing mice[13]. But lack of indication of any form of hemorrhage within the vicinity of tumour in the present study suggests that such a process did not operate here. It has been reported that premature destruction of erythrocytes occur in certain forms of experimental tumours[14]. Whether this is also true in the present case requires further investigation.

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