CHANGES IN THE MYELOGRAM OF MICE WITH THE ASCITIC CARCINOMA OF EHRLICH

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There have been several reports by clinicians of investigations of the bone marrow in patients with carcinoma. These workers have attempted to find a "typical" myelogram of carcinoma, which could be used as a method of diagnosis and prognosis of carcinoma during life. The most pronounced changes in the blood and bone marrow are found in carcinoma of the stomach [1, 2].

In the peripheral blood in the majority of cases there is found a hypochromic anemia, neutrophilia with a shift to the left, and lymphopenia [1, 2, 8]. In the majority of patients there is a high leucocytosis; in some patients, on the other hand, leucopenia. However, the most important feature in this condition is presumably the decline in the hemopoietic and digestive function of the stomach, and disturbance of the metabolic processes in the patient affected by carcinoma is far less important. In carcinoma in other situations the morphological composition of the blood is changed to a smaller degree, or it may even remain within normal limits (carcinoma in an external situation).

In contrast to this, the bone marrow of patients with carcinoma always shows abnormal tendencies, irrespective of the site of the lesion. These abnormalities are most pronounced in carcinomatous lesions of the organs of the gastrointestinal tract, somewhat less so in lesions of other internal organs, and considerably less marked in carcinoma of the skin, tongue and breast. However, the myelogram is always abnormal – even in cases where the cellular composition of the blood of the patient remains normal.

Authors who have studied the myelogram of patients with carcinoma [1, 2, 4, 8, 9, 10] have found that it is not of the same type in all patients. In the bone marrow of the majority of them, for instance, there is found an increase in the promyelocytes, neutrophilic myelocytes and metamyelocytes. In some patients there is an increase in the number of adult neutrophils in the bone marrow [2]. The erythropoietic findings are contradictory: in some patients the number of erythroblasts is increased, and in others reduced [1, 3, 10, 9]. Some authors consider that a suppression of erythropoiesis is characteristic of carcinoma, and that the number of adult erythroblasts greatly exceeds that of the less mature [2, 8], while others consider that the number of erythroblasts sharply increases in carcinoma, and that there is a rise in the number of erythroblasts with basophilic protoplasm and in the number of proerythroblasts [4, 10]. According to the findings of all these workers the number of plasma cells in the bone marrow of patients with carcinoma is somewhat increased. O.V. Khakhaleva [9] considers that in malignant disease there is a most constant and characteristic hyperplasia of the reticular stroma of the bone marrow, while E.A. Fridman [8] found suppression of the reticular tissue of the bone marrow in the majority of patients with carcinoma.

From our point of view, such discrepancies can be explained by the variety of the clinical material. We were unable to find any experimental data on this subject in the literature accessible to us.

TABLE 1

Myelograms of Normal Mice

Animal No.	Hemocyto-blasts	Myeloblasts	Proerythro-blasts	Erythro-blasts	Normo-blasts	Promyelo-cytes	Neutrophil myelocytes	Neutrophil metamyelo-cytes	Stab neutro-phils	Segmented neutrophils	Eosinophilic myelocytes	Eosinophils	Lymphocytes	Plasma cells	Megakaryo-cytes	Reticulo-cytes	Monocytes	Number of mitoses per 2000 cells
	In percent																	
1	2.27	4.71	3.17	14.03	0.23	4.08	8.13	18.44	16.21	16.92	2.59	0.27	8.53	0.05	0.09	0.13	0.15	1
2	2.06	5.03	2.85	20.42	0.18	3.64	8.88	13.83	13.36	16.73	3.03	0.23	9.34	0.01	0.09	0.19	0.14	3
3	3.41	3.57	5.82	18.18	0.38	6.59	9.00	10.20	11.59	16.83	2.69	0.19	11.06	—	0.05	0.24	0.10	1
4	1.84	4.96	6.20	17.28	0.09	4.12	12.55	16.76	12.92	10.84	2.68	0.23	8.71	0.28	0.18	0.31	0.05	2
5	2.23	4.18	3.81	16.20	0.19	3.61	9.51	17.69	15.11	11.74	2.97	0.24	11.54	0.54	0.10	0.25	0.09	1
6	3.49	5.15	6.59	16.49	0.14	3.06	9.79	13.06	18.65	8.75	2.15	0.19	11.98	0.38	—	0.08	0.05	1
Average	2.55	4.60	4.74	17.10	0.20	4.18	9.64	15.00	14.64	13.63	2.68	0.23	10.19	0.21	0.09	0.20	0.10	1.5

The normal myelogram of the mouse has been described by workers studying mouse leukemia [3, 11, 12], but there is not yet the necessary unanimity on the names of the cells and on their interpretation.

The aim of the present investigation was to trace the changes in the myelogram in mice affected by the ascitic carcinoma of Ehrlich.

EXPERIMENTAL METHOD

The experiments were carried out on half-grown male white mice, weighing from 26 to 30 g. The experimental animals were injected intraperitoneally under sterile conditions with 0.2 ml of ascitic fluid taken from mice with the ascitic form of Ehrlich's carcinoma. The strain of ascitic carcinoma of Ehrlich had been kept in the Institute for a long time. The mice were then killed at intervals of one, three, five and seven days after injection of ascitic fluid. At each interval three mice were killed. The bone marrow was extracted from the right and left femora and two respective films prepared, fixed with anhydrous methyl alcohol and stained by the Romanowsky method. As a control six mice were killed and from their bone marrow films were prepared and stained by the same method as for the experimental animals. In producing the myelogram over 2000 bone marrow cells were counted in both films under oil immersion.

EXPERIMENTAL RESULTS

1. The Normal Myelogram of White Mice

We distinguished the following cells in the bone marrow of the mice: hemocytoblasts, myeloblasts, proerythroblasts, erythroblasts (basophilic and polychromatophilic together), normoblasts, promyelocytes, neutrophil myelocytes, neutrophil metamyelocytes (juvenile), stab neutrophils, segmented neutrophils, eosinophilic myelocytes, eosinophils, lymphocytes, plasma cells, megakaryocytes, reticular cells and monocytes. The normal average myelogram of the mice and its individual variations are given in Table 1.

We consider it necessary to give a brief morphological account of the features of certain cells and to describe granulopoiesis in mice.

Formation of nuclei of myelocytes in the bone marrow of mice may occur by the usual way or as a ring type. The great majority of myelocyte nuclei are formed in the latter type. A small, round hole forms in the center of the cell nucleus as a result of mitotic division. The chromosomes in the metaphase are distributed in the form of a ring with an empty space in the center. They are arranged in exactly the same way in the daughter asters in the telophase after separation of the chromosomes. In the later part of the telophase during formation of the nuclei, they

TABLE 2

Myelogram of Mice with Ehrlich's Ascitic Carcinoma

Time after inoculation	Animal No.	Hemocytoblasts	Myeloblasts	Proerythroblasts	Erythroblasts	Normoblasts	Promyelocytes	Neutrophil myelocytes	Neutrophil metamyelocytes	Stab neutrophils	Segmented neutrophils	Eosinophilic myelocytes	Eosinophils	Lymphocytes	Plasma cells	Megakaryocytes	Reticulocytes	Monocytes	Number of mitoses per 2000 cells
		in percent																	
1 day	1	5,40	5.04	3.60	20.08	0.09	3.88	13.98	15.53	11.75	9.28	4.02	0.61	5.21	0.23	0.14	0.23	0.33	9
	2	8.57	3.87	3.66	19.55	0.09	3.33	7.86	13.57	17.05	12.18	3.65	0.28	5.46	0.30	0.14	0.21	0.23	3
	3	5.66	6.39	3.01	12.59	0.09	3.21	20.17	15.09	13.44	9.62	3.82	0.09	6.18	0.13	0.05	0.10	0.37	5
3 days	4	8.51	4.19	3.72	8.17	0.09	5.03	11.27	12.71	15.76	18.37	1.15	0.09	10.15	0.29	0.09	0.16	0.25	4
	5	9.17	4.82	5.52	18.03	—	1.97	6.03	9.93	12.91	18.77	1.82	0.14	10.27	0.14	0.09	0.26	0.13	8
	6	9.87	5.09	4.54	17.94	0.19	2.39	6.81	10.26	13.78	13.59	1.26	0.09	13.25	0.19	0.09	0.30	0.36	4
5 days	7	8.88	5.04	4.81	15.87	—	3.25	3.98	10.95	11.29	15.25	3.20	0.28	16.15	0.71	—	0.15	0.19	6
	8	9.51	3.12	4.00	15.89	—	2.16	9.47	13.18	12.00	14.50	7.19	0.51	7.63	0.23	0.05	0.35	0.21	8
	9	11.67	4.17	2.64	8.21	—	4.81	6.96	13.69	15.95	21.57	2.05	0.09	6.77	0.57	0.10	0.25	0.50	7
7 days	10	7.33	4.53	3.21	9.04	—	5.11	6.31	12.44	17.17	25.55	1.22	0.05	6.19	0.28	0.09	1.2	0.28	8
	11	7.79	4.08	1.72	15.09	0.09	5.93	6.86	16.00	10.61	22.66	2.00	0.36	6.16	0.09	0.18	0.05	0.05	9
	12	8.58	4.62	3.68	8.58	0.04	4.98	10.81	14.09	19.03	16.01	1.64	0.22	6.76	0.44	0.09	0.14	0.15	6

TABLE 3

Summarized Average Myelograms of Mice with Ehrlich's Ascitic Carcinoma

Group of animals	Hemocyto-blasts	Myeloblasts	Proerythro-blasts	Erythroblasts	Normoblasts	Promyelo-cytes	Neutrophil myelocytes	Neutrophil metamyelo-cytes	Stab neutro-phils	Segmented neutrophils	Eosinophilic myelocytes	Eosinophils	Lymphocytes	Plasma cells	Megakaryo-cytes	Reticulo-cytes	Monocytes	Number of mitoses per 2000 cells	Index L/E
	in percent																		
Ascitic carcinoma 1st day	6.54	5.30	3.42	17.41	0.09	3.47	14.00	14.73	14.08	10.36	3.83	0.32	5.62	0.22	0.11	0.18	0.31	5.6	3.1
Ascitic carcinoma 3rd day	9.18	4.70	4.59	14.71	0.12	3.13	8.03	10.96	14.15	16.91	1.41	0.11	11.22	0.21	0.09	0.24	0.24	5.3	3.1
Ascitic carcinoma 5th day	10.02	4.41	3.81	13.32	—	3.41	6.81	12.61	13.08	17.11	4.14	0.29	10.18	0.50	0.05	0.25	0.30	7	3.6
Ascitic carcinoma 7th day	7.90	4.41	2.87	10.90	0.04	5.34	7.99	14.13	15.60	21.41	1.62	0.21	6.37	0.27	0.12	0.46	0.16	7.6	5
Normal	2.55	4.60	4.74	17.10	0.20	4.18	9.64	15.00	14.64	13.63	2.68	0.23	10.19	0.21	0.09	0.20	0.10	1.5	2.93

are in the form of a ring. This process was first described in the bone marrow of rats by Ohno, Kinosita and Ward [13] in myelocyte nuclei.

According to our observations the formation of nuclei by the ring type takes place not only in the myelocytes but also in younger and less differentiated cells. The presence of even a small hole in the center of the nucleus of a cell in all other respects identical with a hemocytoblast is sufficient to identify the cell as a promyelocyte. By microphotography of such cells A.Ia. Krashilina [3] mistakenly quotes them as an example of hemocytoblasts. In the formation of eosinophilic myelocytes the protoplasm inside the nuclear ring may be seen quite clearly to contain a small number of specific eosinophilic granules. Such a picture justifies the hypothesis of the active role of the nucleus in the formation of the specific granules of the granulocytes. The neutrophil granules in mice are very small and can be demonstrated with difficulty even in the fully-formed myelocytes and adult neutrophils, and so in the cells of the promyelocyte type just described it is quite impossible to distinguish them against the background of the basophilic protoplasm.

Later on, the specific granules accumulate in the protoplasm of these cells and the basophilia of the protoplasm fades. Rarely in these cells the nucleus takes the form of an incompletely closed ring; presumably in this case myelocytes are formed with the usual type of nucleus found in all other mammalian forms. Most commonly the promyelocytes and myelocytes develop from myeloblasts and not directly from hemocytoblasts, as described above. They are smaller in size and evidently their path of maturation is shorter.

The two sources of origin of the promyelocytes and myelocytes of the bone marrow of mice account for the considerable variability in the dimensions of these cells. The dimensions of the metamyelocytes vary even more, while those of the stab and segmented leucocytes are relatively constant. We do not agree with A.Ia. Krashilina that there are no stab neutrophils in the bone marrow of mice. This form does exist; as in other mammalia it is distinguished by a definite degree of maturation of the nucleus and protoplasm. In contrast to the nucleus of the metamyelocytes, the ring of the nucleus of the stab forms is considerably narrower, not of the same thickness everywhere, and the nuclear substance is more compact but in it appear characteristic clear spaces in the form of points of light. Usually the ring of the nucleus is twisted into a figure of eight. This is the shape of the stab neutrophil which is given in the atlas of V.N. Nikitin [6].

M.O. Raushenbakh [7] distinguishes stab forms in the bone marrow of mice as a constant group of cells. A group of monoblasts was distinguished quite arbitrarily in the bone marrow of mice by A.Ia. Krashilina. We think that this group was really hemocytoblasts with irregularly-shaped nuclei. A.Ia. Krashilina herself writes that these cells do not resemble the monocytes of the blood.

In contrast to rats, typical basophils are found very rarely in the bone marrow of mice. In all the films examined we saw altogether one typical basophil myelocyte. The cells most often found had indistinctly outlined and washed-out basophilic granules, with a poorly contrasting nucleus. These cells found in the bone marrow of mice and rats are taken by some workers to be basophils [5]. In general, we do not think so. The cells of the erythroblastic series in mice are very clearly distinguished and no doubt arises about them.

2. Myelogram of Mice Affected by the Ascitic Carcinoma of Ehrlich

Until the fifth day from the moment of intraperitoneal injection of ascitic fluid into the mice, the circulation of peritoneal fluid was evidently unaffected. After the fifth day the development of ascites was observed, which must be due to interference with absorption of the peritoneal fluid. After the fifth day, and especially on the seventh day, wholesale death of the ascitic cells was observed. The ascitic fluid was translucent. After the seventh day the pressure of the peritoneal fluid was slightly raised, causing interference with respiration and the circulation of the blood. Two periods may thus be distinguished in the course of the disease. The first – until the seventh day, when all the symptoms are caused by proliferation of carcinoma cells and poisoning of the animal by products of their vital activities and death, and the second – after the seventh day, when the accumulation of a large quantity of fluid in the peritoneal cavity interferes with vitally important functions of the animal – respiration and circulation of the blood, which,in turn, may produce changes in other organs. For example, anoxemia arising as a result of disorder of the respiration and circulation may stimulate erythropoiesis in the bone marrow. However, this effect cannot be considered to be the direct consequence of the carcinoma. Accordingly we decided to investigate the bone marrow in the mice only in the first period of the disease, i.e., until the seventh day inclusive.

The myelogram of the animals at various intervals after injection of ascitic fluid and the mean values expressed as percentages are shown in Tables 2 and 3.

Comparing the mean values of the myelograms of the control and experimental animals (Table 3) it may be seen that the ascitic form of Ehrlich's carcinoma causes suppression of erythropoiesis in mice. This is shown by a gradual fall in the percentage of proerythroblasts and erythroblasts in the bone marrow. The most marked abnormal trend of erythropoiesis was observed in the later stages, i.e., on the seventh day.

The reaction of the cells of the neutrophil series appeared very quickly. In the earliest stages the percentage of immature forms increased at the expense of the mature, and next, conversely an uninterrupted rise in the segmented neutrophils was observed, with simultaneous reversion to normal of the relative metamyelocyte and myelocyte counts after their initial fall. The percentage of stab neutrophils changed only slightly throughout the experiment.

The indices of mitotic activity which we obtained from our investigation are only of relative importance on account of imperfections in the Romanowsky staining method. Nevertheless it can clearly be seen from Table 3 that the number of mitoses calculated per 2000 bone marrow cells after the first 24 hours of the Ehrlich's ascitic carcinoma is already three times greater than normal, and on the seventh day it is more than five times greater. Normally, the erythroblasts and neutrophil myelocytes are responsible for a large part of the mitoses, while in carcinoma it is the proerythroblasts and neutrophil myelocytes and metamyelocytes. Mitoses are met considerably more often among the hemocytoblasts than in the normal marrow.

In order to form a true opinion of the changes in hemopoiesis in carcinoma it was necessary to follow for the same periods of time the changes in the morphological composition of the peripheral blood. For this purpose we made repeated blood counts in four mice and then injected them intraperitoneally with 0.2 ml of ascitic fluid, then after one, three, five and seven days we took blood for analysis from the tip of the tail. The summarized mean results are shown in Table 4.

It can be seen from Table 4 that 24 hours after injection of the fluid the leucocyte count rose, on the third and fifth days it had returned to normal, and on the seventh day it again rose sharply. A moderate anemia was present by the third day. On the seventh day the number of erythrocytes per 1 mm^3 of blood fell to roughly 1,000,000. The leucocytic formula was altered: there was a gradual and significant increase in the percentage of neutrophils with a shift to the left; the percentage of juvenile forms in the blood rose; even myelocytes ap-

TABLE 4

Composition of the Blood of Mice with Ehrlich's Ascitic Sarcinoma. Summarized Mean Values

Time after inoculation	Date of taking blood	Erythrocytes per 1 mm³	Leucocytes per 1 mm³	Leucocytic formula						
				myelocytes	juvenile	stab neutrophils	segmented neutrophils	eosinophils	lymphocytes	monocytes
				in percentages						
1 day	6/8/1957	5,170,000	17,025	–	1.1	3.0	32.0	0.86	57.9	4.7
3 days	6/10/1957	4,570,000	10,125	0.4	0.9	3.1	34.0	1.9	51.8	5.5
5 days	6/12/1957	4,725,000	12,787	0.4	2.2	2.8	47.6	1.0	40.8	4.6
7 days	6/14/1957	4,072,500	22,100	0.1	2.7	4.0	53.3	0.6	35.4	3.4
Normal	6/1/1957 6/6/1957	5,397,500	12,918	–	1.2	3.1	17.0	0.87	68.3	9.0

peared, which are normally not present in the peripheral blood. At the same time the percentage of lymphocytes steadily fell. The percentage of monocytes was also considerably lower in carcinoma than in the normal state.

From a consideration of the changes in the peripheral blood and the simultaneous changes in the myelogram, it must be concluded that ascitic carcinoma in mice causes in the bone marrow some degree of suppression of erythropoiesis and a greatly increased production of neutrophil leucocytes.

The changes observed in the myelogram in mice with ascitic carcinoma are in general agreement with the findings of clinicians on bone marrow hemopoiesis in human carcinoma patients. However, there are differences. In the majority of clinical investigations cited above, for example, the neutrophilic reaction of the bone marrow was almost always characterized by predominance of immature over mature forms. In our experiments this picture was present only in the very beginning, and later the converse relationship held. It is possible that the cause of the difference lies in the different duration of the disease in man and animals and in the peculiarities of the ascitic form of carcinoma. In mice with carcinoma, except in the very earliest stages, erythropoiesis was always suppressed. In clinical observations the findings in this direction are most contradictory.

Attention should be directed to one more interesting fact which has not been found in clinical observations. In the myelograms of mice, starting on the first day after injection of ascitic fluid, the percentage of hemocytoblasts rose sharply. If in the normal myelogram the percentage of hemocytoblasts was 2.55, one day after the injection of ascitic fluid it was 6.54%, after three days – 9.18%, after five days – 10.02%, and after seven days – 7.90%. This reaction is all the more interesting because hemocytoblasts are the least differentiated cells in the bone marrow. In this case it is difficult to account for the reaction by changes in the hemopoietic processes alone, since the percentage of cells formed directly from hemocytoblasts (proerythroblasts, myeloblasts) is not increased.

SUMMARY

The cellular content of the bone marrow and the peripheral blood was studied in white mice in Ehrlich's ascitic carcinoma. Average progressive anemia and a gradually increasing neutrophilic leucocytosis was found in the blood. In 24 hours after the introduction of ascitic fluid the myelogram had already changed. Later the percentage of the hemocytoblasts was greatly increased while that of the proerythroblasts and erythroblasts in the bone marrow decreased. Production of neutrophilic leucocytes was much greater than in normal conditions and showed continuous increase. On the seventh day the mitotic activity of the bone marrow cells was five times greater than normal.

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