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Cell Kinetics in the Spleen of Ehrlich Ascites Tumor-Bearing Mice

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Summary. During the period of 18 days after transplantation the uptake of ^{3}H-thymidine into DNA was significantly increased in the spleen of Ehrlich ascites tumor-bearing Swiss and NMRI mice. Adenosine deaminase activity was within the control limits in tumor-bearing Swiss mice and significantly increased for the tumor-bearers of the NMRI strain. However, the increase in the DNA content, which is associated to splenic hyperplasia, was evident only for the Swiss strain. The time course of the retention of ^{3}H-DNA revealed that the half life of spleen cells ranged from 41 to 46 h in normal and tumor-bearing Swiss mice and normal NMRI mice. For tumor-bearing NMRI mice the rate of cell depletion was significantly increased ($t_{1/2} = 32$ h) compared with that found in normal NMRI mice ($t_{1/2} = 44$ h). The mean survival time was 23.0 days for the tumor-bearing Swiss mice and 24.6 days for the NMRI mice. These data suggest that the immune responses were similar in both strains of mice, although cell kinetics was different in the spleen of tumor-bearing Swiss and NMRI mice.

Key words: DNA synthesis — adenosine deaminase activity — ^{3}H-DNA depletion.

Tumor-bearing organisms present an increase in DNA content and DNA synthesis and an increase in activity of DNA polymerase, TdR kinase and TMP kinase in the liver and spleen (Shirasaka and Fujii, 1975; Morgan and Cameron, 1973). It was suggested that tumor tissues are capable to release some growth-stimulating substances that enhance the activity of DNA-synthetizing enzymes and DNA synthesis of various tissues (Shirasaka and Fujii, 1975). We report here the results of studies on cell proliferation and cell kinetics in the spleen of Ehrlich ascites tumor-bearing Swiss and NMRI mice. It was found that the spleen DNA content of tumor-bearing NMRI mice was slightly decreased compared with that observed in normal NMRI mice. Thus, the aim of the present study was to find an explanation for the absence of splenic hyperplasia in tumor-bearing NMRI mice.

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Materials and Methods

Conventional male Swiss mice (randomly bred), weighing 28 ± 2 g, from the Medical and Pharmaceutical Institute (Cluj-Napoca, Rumania) and conventional male mice of the NMRI strain (randomly bred), weighing 28 ± 2 g, obtained from the Cantacuzino Institute (Bucharest, Rumania) were used throughout the experiments. The animals received a standard diet. Groups of 10 mice were i.p. injected with 4×10^6 Ehrlich ascites tumor cells, a hyperdiploid line.

Mice received intravenously 7 or 10 μCi of ^{3}H-thymidine (5 Ci/mmole) (Amersham, England). Animals were killed by cervical dislocation. The spleen DNA content was determined by using the diphenylamine colour reaction, as previously described (Suciu et al., 1976). The ^{3}H-radioactivity was counted in a liquid scintillation spectrometer (Intertechnique ABAC SL 40), as previously indicated (Suciu et al., 1975). Adenosine deaminase activity was determined according to Hovi et al. (1976). Spleen cells were brought into suspension by gentle teasing of donor spleens in cold 0.25 M sucrose. 0.3 mM $CaCl_2$ (1:10, w/v). Cell suspension was centrifuged at 1000 × g for 10 min (0° C). The spleen cells were homogenized in 0.15 M phosphate buffer (pH 7.1) (1:5, w/v). The homogenate was centrifuged at 3000 × g for 10 min (0° C). The reaction mixture contained 1.8 ml of 0.15 M phosphate buffer (pH 7.1), 0.1 ml of 1.0 mM adenosine (NBC, Cleveland, Ohio) and 0.05 ml of tissue extract. The reaction was followed by recording the decrease in optical density at 265 nm (30° C) with the aid of a Specord UV VIS (Carl Zeiss Jena) double-beam spectrophotometer (cuvette 1 cm). One unit of adenosine deaminase activity was defined as the amount of enzyme extracted from the splenic tissue (corresponding to 1 mg spleen DNA) which caused a decrease in optical density of 0.010/min.

Statistical evaluation of data was made according to Student's "t" test.

Results

The spleen of tumor-bearing Swiss mice showed an increase in DNA content (Table 1) and an increased uptake of ^{3}H-thymidine into DNA (Table 2). The adenosine deaminase activity was depressed up to 6 days after the tumor transplantation and then increased above the control level (Table 3). The DNA content of tumor-bearing NMRI mice was slightly decreased up to 18 days after the tumor inoculation (Table 1). On the contrary, the uptake of ^{3}H-thymidine (Table 2) and adenosine deaminase activity (Table 3) were increased throughout the period of observation. As reported by Hovi et al. (1976), a parallelism has been found between the uptake of ^{3}H-thymidine into DNA and adenosine deaminase activity of dividing lymphoid cells. In our experiments this parallelism is evident only for the NMRI strain (Table 2 and 3).

The results suggest that cell proliferation was increased in the spleen of both Swiss and NMRI mice, but the depletion was accelerated in the cell population of the NMRI mice (Table 1, 2, and 3). This assumption received an experimental confirmation by determining the time course of the retention of ^{3}H-DNA in the spleen of normal and tumor-bearing mice (Fig. 1 and Table 4). In tumor-bearing mice the labelling was carried out 4 days after the tumor transplantation. No significant difference was found between the $t_{1/2}$ values of normal and tumor-bearing Swiss mice (Table 4). On the contrary, the tumor-bearers of the NMRI strain presented an accelerated depletion of ^{3}H-DNA radioactivity in the spleen. It therefore seems probable that the absence of hyperplasia and the decreased DNA content observed in tumor-bearing NMRI mice (Table 1) can be correlated to an increased depletion rate of the spleen cell population. However, as can be seen from Figure 2, the survival of tumor-bearing Swiss and NMRI mice was not significantly different.

Table 1. Spleen DNA content of Ehrlich ascites tumor-bearing mice. Groups of 10 mice were killed at different time intervals after the tumor transplantation. In the control groups (day 0) the DNA content was 3.24±0.37 mg/organ for the Swiss strain and 5.76±0.56 mg/organ for the NMRI mice

Days	Swiss strain		NMRI strain	
	% DNA	$p<$	% DNA	$p<$
0	100.0±11.4		100.0±9.7	
1	120.5± 8.2	NS[a]	93.5±9.5	NS
2	125.7± 9.8	NS	86.9±6.3	NS
3	151.3± 3.1	0.01	81.7±9.9	NS
4	160.8±10.4	0.01	103.4±8.1	NS
5	174.8±12.1	0.01	81.1±8.3	NS
6	151.9±11.7	0.02	97.6±7.9	NS
7	150.6±11.0	0.02	101.6±8.8	NS
8			86.3±5.7	NS
9	131.1± 9.7	NS		
10			81.4±9.0	NS
11	132.8± 3.1	0.05		
12			76.4±3.8	0.05
13	109.8±10.2	NS		
14			80.2±7.7	NS
15	123.5± 6.1	NS		
16			99.0±8.6	NS
17	149.2±10.7	0.02		
18			81.5±7.0	NS

[a] NS, not significant

Table 2. Specific activity of DNA in the spleen of Ehrlich ascites tumor-bearing mice. Seven μCi of ^{3}H-thymidine were administered i.v. one hour before killing. The specific activity in the control groups (day 0) was 23235±2220 dpm/mg DNA for the Swiss mice and 13549±1480 dpm/mg DNA for the NMRI mice

Days	Swiss strain		NMRI strain	
	% Specific activity	$p<$	% Specific activity	$p<$
0	100.0± 9.5		100.0±10.8	
1	89.6±10.4	NS[a]	96.4± 5.3	NS
2	127.3± 9.5	NS	128.8± 9.6	NS
3	129.6± 7.6	0.05	146.5±11.3	0.02
4	127.8± 5.3	0.05	123.7± 9.4	NS
5	146.7± 6.2	0.01	156.2± 8.8	0.01
6	113.4± 8.2	NS	190.4±12.5	0.001
7	136.9± 4.8	0.01	179.1± 9.2	0.001
8			151.0± 5.3	0.01
9	145.9±12.3	0.02		
10			141.9± 8.2	0.02
11	146.3±14.1	0.05		
12			124.6± 7.4	NS
13	123.3± 6.5	NS		
14			174.5±11.3	0.001
15	116.0±10.9	NS		
16			116.9± 6.8	NS
17	61.1± 6.0	0.02		
18			114.3± 3.7	NS

[a] NS, not significant

Table 3. Adenosine deaminase activity in the spleen of Ehrlich ascites tumor-bearing mice. The activity in the control groups (day 0) was 28.3 ± 2.9 units (see materials and methods) for the Swiss strain and 13.1 ± 1.6 units for the NMRI mice

Days	Swiss strain		NMRI strain	
	% Activity	$p<$	% Activity	$p<$
0	100.0 ± 10.2		100.0 ± 12.2	
1	102.5 ± 8.7	NS[a]	88.3 ± 10.6	NS
2	78.3 ± 9.2	NS	103.2 ± 6.8	NS
3	56.1 ± 4.8	0.01	146.5 ± 10.3	0.02
4	74.6 ± 4.1	0.05	116.4 ± 9.7	NS
5	77.6 ± 5.3	NS	154.8 ± 4.3	0.01
6	81.6 ± 6.4	NS	138.6 ± 7.1	0.05
7	122.8 ± 3.9	NS	118.3 ± 11.4	NS
8			136.6 ± 9.1	0.05
9	94.8 ± 11.7	NS		
10			167.0 ± 5.4	0.001
11	113.1 ± 8.2	NS		
12			134.4 ± 4.8	0.05
13	125.4 ± 3.8	0.05		
14			178.9 ± 12.2	0.01
15	108.9 ± 10.5			
16			149.7 ± 10.9	0.02
17	115.2 ± 8.1	NS		
18			119.6 ± 9.8	NS

[a] NS, not significant

Table 4. $t_{1/2}$ values[a] corresponding to the experimental data included in Figure 1

Swiss strain			NMRI strain		
Normal mice	Tumor-bearing mice	$p<$	Normal mice	Tumor-bearing mice	$p<$
46.4 ± 2.1	41.4 ± 1.6	NS[b]	44.2 ± 2.8	32.4 ± 2.3	0.01

[a] Analysis of experimental data was made according to Puck (1966)

$$t_{1/2} = \left(\frac{0.301}{\log N_0/N}\right) \triangle t$$

$t_{1/2}$ = the apparent depletion time constant (h)
$\triangle t$ = elapsed time (h) between labelling and sacrifice
N_0 and N were defined in Figure 1 (50 determination for each curve)

[b] NS, not significant

Discussion

The present results indicate that the absence of splenic hyperplasia in Ehrlich ascites tumor-bearing NMRI mice was associated with an accelerated depletion in the splenic population. The kinetic data reported in Figure 1 and Table 4 are based on the assumption that the depletion of the previously labelled DNA

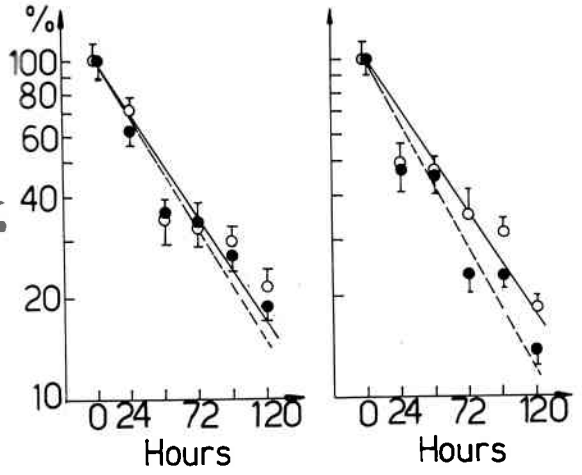

Fig. 1. Time course of the retention of ^{3}H−DNA in the spleen of normal (○) and Ehrlich ascites tumor-bearing (●) mice of the Swiss (left) and NMRI (right) strains. Ten μCi of ^{3}H-thymidine were administered i.v. and the animals (groups of 10 mice) were killed within 120 h after labelling. For the tumor-bearing mice the labelling was carried out 96 h after transplantation. N_0 = dpm/DNA/organ at one h after labelling. N = dpm/DNA/organ at different time intervals after labelling. N_0 was 61459±9218 for normal Swiss mice and 96462±9650 for tumor-bearing animals. The similar data of the NMRI strain were 78145±8520 in the control group and 106417±10190 for tumor-bearing mice

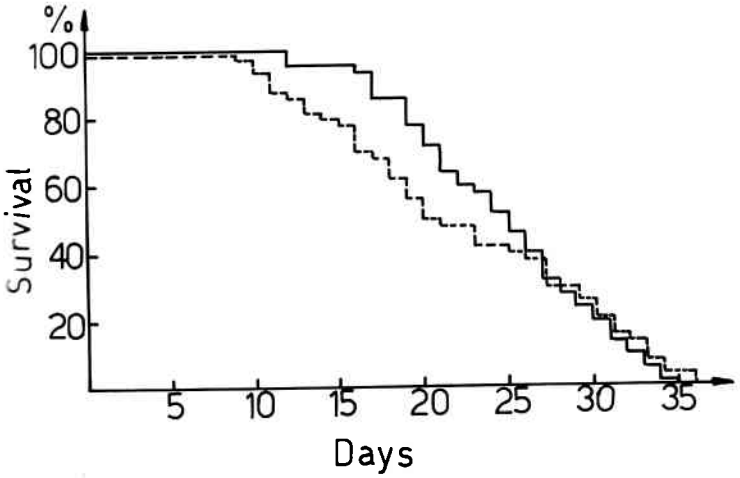

Fig. 2. Survival of Ehrlich ascites tumor-bearing Swiss (———) and NMRI (———) mice. Groups of 50 mice were intraperitoneally injected with 4×10^6 Ehrlich ascites tumor cells. The mean survival time was 23.0±1.1 days for the Swiss strain and 24.6±0.8 days for the NMRI mice

corresponds to the loss of labelled cells from the spleen, and that reincorporation of ^{3}H-thymidine is negligible (Nygaard and Potter, 1960; Sanders et al., 1963; Suciu et al., 1975, 1976). Since no difference has been found between the loss of ^{125}I-IUdR-labelled DNA and ^{3}H-TdR-labelled DNA from the spleen of mice, this assumption is probably correct (Joel et al., 1977). In contrast with ^{125}I-IUdR, in most tissues ^{3}H-TdR can be reincorporated into DNA after release from dead cells (Hughes et al., 1964; Ertl et al., 1970), but this effect is almost absent in the spleen (Joel et al., 1977).

The results of this work and previously published data suggest that the increase in the DNA content observed in the spleen of tumor-bearing animals (Shirasaka and Fujii, 1975; Morgan and Cameron, 1973) which is associated to splenic hyperplasia (Siegler and Koprowska, 1962; Konda et al., 1973), is evident when the splenic population presents a half life of about 41—46 h. Thus, the Ehrlich ascites tumor-bearing NMRI mice appear as an exception, because they show lower $t_{1/2}$ values (32 h) and the absence of splenic hyperplasia.

It has been observed that adenosine deaminase activity is strongly suppressed in cases of severe combined immunodeficiency (Giblett et al., 1972; Dissing and Knudsen, 1972; Carson et al., 1977; Ullman et al., 1976). In our experiments adenosine deaminase activity was within the normal limits in tumor-bearing Swiss mice and significantly increased in tumor-bearing NMRI mice (Table 3). From this point of view, the data suggest that the immune system was not impaired in tumor-bearing Swiss and NMRI mice. In addition, since no difference was found between the mean survival time of tumor-bearing Swiss and NMRI mice (Figure 2), the immune responses were probably similar in both strains of mice.

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