

# A Lymphopenia-Causing Agent, Probably a Virus, Found in Mice After Injection with Tumor Tissue and with Cell-free Filtrates of Lymphosarcoma T 86157 (MB)

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In a previous hematological study (1) of mice which had been injected subcutaneously with minced tumor tissue of lymphosarcoma T 86157 (MB) in Tyrode's solution, it was found that a leukopenia appeared on the second day after inoculation. This decrease of leukocytes represented a true lymphopenia, which disappeared a few days afterwards. The question arose whether this lymphopenia was caused by the tumor, or whether it had some other excitant. Various substances were therefore injected into mice, to investigate whether they would produce the same phenomenon as the MB tumor did. Also attempts were made to separate the tumor-inducing agent from the substance which caused the lymphopenia, by injections of cell-free filtrates and by injections of tumor into mice of other strains than the F<sub>1</sub>Bd<sup>1</sup> mice.

## EXPERIMENTS

In Tables 1 through 5 some of the results are shown. In Table 1 the blood counts of four normal F<sub>1</sub>Bd mice are presented as controls. In the other four tables the blood counts of mice 48 hours after injection are given.

The tumors and organs were minced with scissors in

Tyrode's solution, and 0.2 cc. of this preparation was injected subcutaneously into F<sub>1</sub>Bd and other mice.

*Experiment 1.*—(Table 2). The following materials were each injected into two F<sub>1</sub>Bd mice: (a) Tyrode's solution; (b) spleen and liver from a normal F<sub>1</sub>Bd mouse; (c) spleen and liver from a normal B mouse. The blood picture of all the mice injected was normal 48 hours after injection.

TABLE 1  
TOTAL AND DIFFERENTIATED WHITE BLOOD COUNTS  
IN NORMAL F<sub>1</sub>Bd MICE

		Mouse number			
		A	B	C	D
Eosinophils	—number	0	0	90	0
	—per cent	0	0	3	0
Stab-cells	—number	0	23	0	0
	—per cent	0	$\frac{1}{2}$	0	0
Polymorphonuclears	—number	1260	846	660	1073
	—per cent	28	18	22	29
Lymphocytes	—number	3015	3619	2130	2627
	—per cent	67	77	71	71
Monocytes	—number	225	211	90	0
	—per cent	5	4 $\frac{1}{2}$	3	0
Unclassified cells	—number	0	0	30	0
	—per cent	0	0	1	0
Totals		4500	4700	3000	3700

TABLE 2  
TOTAL AND DIFFERENTIAL WHITE BLOOD COUNTS IN F<sub>1</sub>Bd MICE INJECTED WITH DIFFERENT MATERIALS

Mouse number		INJECTED MATERIAL							
		Tyrode's solution		Organs from F <sub>1</sub> Bd mouse		Organs from B mouse		Tumor round-cell sarcoma MA	
		1	2	3	4	5	6	7	8
Eosinophils	—number	31	0	39	0	0	75	0	39
	—per cent	$\frac{1}{2}$	0	$\frac{1}{2}$	0	0	1 $\frac{1}{2}$	0	1 $\frac{1}{2}$
Stab-cells	—number	31	0	0	0	19	25	32	0
	—per cent	$\frac{1}{2}$	0	0	0	$\frac{1}{2}$	$\frac{1}{2}$	1	0
Polymorphonuclears	—number	1147	720	1092	1100	722	900	800	598
	—per cent	18 $\frac{1}{2}$	22 $\frac{1}{2}$	14	22	19	18	25	23
Lymphocytes	—number	4929	2416	6591	3700	2983	3775	2340	1963
	—per cent	79 $\frac{1}{2}$	75 $\frac{1}{2}$	84 $\frac{1}{2}$	74	78 $\frac{1}{2}$	77 $\frac{1}{2}$	72	75 $\frac{1}{2}$
Monocytes	—number	31	64	78	150	57	77	64	0
	—per cent	$\frac{1}{2}$	2	1	3	1 $\frac{1}{2}$	1	2	0
Unclassified cells	—number	31	0	0	50	19	75	0	0
	—per cent	$\frac{1}{2}$	0	0	1	$\frac{1}{2}$	1 $\frac{1}{2}$	0	0
Totals		6200	3200	7800	5000	3800	5000	3200	2600

<sup>1</sup> B = C 57 black Little. d = dilute brown Murray-Little. F<sub>1</sub>Bd = F<sub>1</sub>B ♀ × d ♂.

*Experiment 2.*—(Table 2). To investigate whether another round-cell sarcoma would produce a similar lymphopenia, 2 mice were injected with round-cell sarcoma T 90904 (MA) which is not related to tumor MB. No lymphopenia could be demonstrated 2 days after injection.

It seemed then that producing a decrease in lymphocytes was a specific function of round-cell sarcoma MB.

TABLE 3  
TOTAL AND DIFFERENTIAL WHITE BLOOD COUNTS  
IN F<sub>1</sub>Bd MOUSE NO. 10

INJECTED MATERIAL		1ST IN-	2D IN-	3D IN-
		JECTION Filtrate of tumor MB	JECTION Filtrate of tumor MB	JECTION Minced tumor MB
Eosinophils	—number	11	0	68
	—per cent	1	0	2
Metamyelocytes	—number	0	56	34
	—per cent	0	$\frac{1}{2}$	2
Stab-cells	—number	0	0	34
	—per cent	0	0	1
Polymorphonuclears	—number	748	3192	663
	—per cent	68	28 $\frac{1}{2}$	19 $\frac{1}{2}$
Lymphocytes	—number	275	7840	2516
	—per cent	25	70	74
Monocytes	—number	66	112	34
	—per cent	6	1	1
Unclassified cells	—number	0	0	34
	—per cent	0	0	1
Totals		1100	11200	3400

c) Five mice which had been once or twice injected with cell-free filtrates were inoculated subcutaneously with tumor. No lymphopenia was observed, although the growth of the tumor was comparable with that developed in mice not previously injected with cell-free filtrate. It is evident that after injection with a cell-free filtrate of the tumor some immunity was built up relative to the lymphopenia-causing agent but that the growth of the tumor was not affected. The lymphopenia-causing "agent" passed a size 3 Seitz filter, whereas the tumor-producing "agent" did not.

*Experiment 4.*—(Table 4).

a) Minced liver and spleen from mice previously injected with cell-free filtrates were inoculated into 6 mice. Mice Nos. 81 and 82 are used to illustrate this experiment. All 6 mice developed a lymphopenia on the second day after injection. Liver and spleen from these 6 mice again produced lymphopenia when injected into other mice (Nos. 129 and 130). Minced organs from mouse No. 130 produced lymphopenia when injected into 2 mice (Nos. 236 and 239).

b) Heart blood taken from a mouse which had been injected with cell-free filtrate of tumor MB was injected into two mice, which developed lymphopenia after 48 hours.

A lymphopenia-producing "agent" was demonstrated in the spleen, liver, and blood of mice by serial passages of these tissues. The first two mice in this series had been injected with cell-free filtrate of tumor MB. Some of the mice were not killed but no sequelae were seen to develop.

TABLE 4  
TOTAL AND DIFFERENTIAL WHITE BLOOD COUNTS IN F<sub>1</sub>Bd MICE INJECTED WITH  
MINCED ORGANS IN TYRODE'S SOLUTION

Mouse number		INJECTED MATERIAL							
		Organs from normal F <sub>1</sub> Bd mouse		Organs from F <sub>1</sub> Bd mouse injected with filtrate from tumor MB		Organs from F <sub>1</sub> Bd mouse 81		Organs from F <sub>1</sub> Bd mouse 130	
		3	4	81	82	129	130	236	239
Eosinophils	—number	39	0	12	28	140	38	45	54
	—per cent	$\frac{1}{2}$	0	1	4	5	2	3	6
Stab-cells	—number	0	0	0	7	0	0	0	9
	—per cent	0	0	0	1	0	0	0	1
Polymorphonuclears	—number	1092	1100	756	469	1484	1349	795	396
	—per cent	14	22	63	67	53	71	53	44
Lymphocytes	—number	6591	3700	384	182	756	494	555	297
	—per cent	84 $\frac{1}{2}$	74	32	26	27	26	37	33
Monocytes	—number	78	150	48	7	420	19	105	144
	—per cent	1	3	4	1	15	1	7	16
Totals		7800	5000	1200	700	2800	1900	1500	900

It was demonstrated by additional experiments, however, that this was not the case.

*Experiment 3.*—(Table 3).

a) Ten F<sub>1</sub>Bd mice were injected with a cell-free filtrate of tumor MB. All the mice showed a strong lymphopenia on the second day after injection. No tumors were produced, however. Mouse No. 10 is used to illustrate this experiment.

b) Eight of the mice mentioned under experiment 3a were injected for a second time with cell-free filtrate of tumor MB. No lymphopenia could be demonstrated.

*Experiment 5.*—(Table 5).

a) Minced tumor MB was injected into four O 20, two d, and four B mice. In the O 20 and in the B mice a tumor was produced which disappeared. In the d mice no tumors were produced. All mice developed lymphopenia. No lymphopenia was observed in the control animals of the same strains. The lymphopenia in the strains not susceptible to tumor MB was less conspicuous than that which occurred in the F<sub>1</sub>Bd mice.

b) After a second inoculation with tumor MB into the mice of experiment 5a no lymphopenia developed.

c) A cell-free filtrate of the tumor MB produced lymphopenia after the first injection, but none after a second injection.

d) Minced liver and spleen derived from B mouse No. 58 (previously injected with tumor MB) caused lymphopenia when injected into two F<sub>1</sub>Bd mice, Nos. 93 and 94 (Table 6). After four serial liver and spleen transfers in F<sub>1</sub>Bd mice the lymphopenia could still be demonstrated (mice Nos. 289 and 290).

*Experiment 6.*—Tumor MB was cultivated *in vitro* as previously described (1). Cultures containing typical lymphoblast-like cells produced a tumor when injected into F<sub>1</sub>Bd mice.

a) Six mice were inoculated with cultures of lymphosarcoma MB. No lymphopenia developed, whereas the tumor growth was unaffected.

b) A new strain of MB tumor was established in mice injected with cells of sarcoma MB cultivated *in vitro*. This strain maintained its characteristics when transplanted into F<sub>1</sub>Bd mice and even after 34 passages in the mouse did not produce a lymphopenia. Grossly this new tumor did not differ from the original tumor MB. The blood picture of mice bearing the new tumor was the same as that of mice injected with the original tumor MB, except for the absence of a lymphopenia on the second day after inoculation. This result is given as further evidence that the presence of the tumor is not directly related to the production of the lymphopenia observed.

#### SUMMARY

1. A lymphopenia was observed to develop in mice on the second day following injection of

4. Lymphopenia without tumor was also produced by injection of minced organs derived from mice previously injected with cell-free filtrate of sarcoma MB.

5. It is concluded that a filtrable agent, probably a virus, is responsible for the lymphopenia pro-

TABLE 5

TOTAL AND DIFFERENTIAL WHITE BLOOD COUNTS IN MICE OF VARIOUS STRAINS, 48 HOURS AFTER INJECTION WITH MINCED TUMOR MB, AND IN CONTROL MICE OF THE SAME STRAINS

Mice	Treatment	Totals	Lymphocytes (in per cent)	Poly-morpho-nuclears (in per cent)
0 20 mice—	non-injected			
No. 155		6000	64	30
156		7800	61	29
175		2600	73	22
176		2500	73	13
0 20 mice—	injected			
No. 153		3200	48	50
154		2000	60	39
173		1500	55	39
174		1500	52	44
d mice—	non-injected			
No. 185		4800	55	45
186		4100	67	32
d mice—	injected			
No. 183		1900	35	59
184		3400	33	67
B mice—	injected			
No. 58		1600	33	62
59		1500	39	60
60		1200	38	48
61		1100	33	55

TABLE 6

TOTAL AND DIFFERENTIAL BLOOD COUNTS IN B AND F<sub>1</sub>Bd MICE INJECTED WITH DIFFERENT MATERIALS

Mouse	Injected material	Total count	Eosinophils (in per cent)	Polymorpho-nuclears (in per cent)	Lymphocytes (in per cent)	Monocytes (in per cent)
Normal F <sub>1</sub> Bd	Non-injected	± 4000	± ½	± 24	± 71	± 3
B No. 58	Tumor MB	1600	3	62	33	2
B No. 59		1500	0	60	39	1
F <sub>1</sub> Bd No. 93	Organs from	1400	7	47	45	1
F <sub>1</sub> Bd No. 94	mouse No. 58	800	2	69	23	6
F <sub>1</sub> Bd No. 136	Organs from	1000	2	57	37	4
F <sub>1</sub> Bd No. 137	mouse No. 93	1100	2	67	26	5
F <sub>1</sub> Bd No. 240	Organs from	1000	0	39	57	4
F <sub>1</sub> Bd No. 241	mouse No. 137	700	1	64	32	3
F <sub>1</sub> Bd No. 289	Organs from	1400	3	55	36	6
F <sub>1</sub> Bd No. 290	mouse No. 240	2100	3	38	41	18

minced tumor tissue of lymphosarcoma T 86157 (MB).

2. It was shown that a tumor could be produced in mice without a concomitant lymphopenia.

3. Lymphopenia without tumor was produced in mice injected with cell-free filtrate of sarcoma MB.

duced. This virus seems to bear no relationship to the presence of the tumor.

#### REFERENCES

1. DE BRUYN, W. M., KORTEWEG, R., and KITS VAN WAVEREN, E. Transplantable Mouse Lymphosarcoma T 86157 (MB) Studied *in vivo*, *in vitro*, and at Autopsy. *Cancer Research*, 9: 282-293, 1949.