

LOCAL REACTIONS TO FREUND'S COMPLETE
ADJUVANT IN MICE OF HIGH
AND LOW-CANCER LINES AT DIFFERENT AGES

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Investigation of the magnitude of local reactions in mice to Freund's complete adjuvant showed that in mice of low-cancer line C57BL reactions after 2, 12, and 20 days were more marked than in mice of the high-cancer lines C3H, C3HA, and BALB. Results were also obtained indicating that the reactivity apparatus responsible for the local reaction to Freund's complete adjuvant, especially for the second phase of its course, matures in low-cancer lines of mice earlier in the course of development than in high-cancer lines.

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Considerable importance is attached to the state of immunologic reactivity of the host in the pathogenesis of tumors, and many investigators pay particular attention to the so-called cellular immunity [3-6, 8-10].

It may be asked whether reactions taking place with the participation of cells can serve as a criterion of initial resistance to the development of tumors. This class of reactions includes, for example, allergic reactions of delayed type to certain antigens (tuberculin, tularin, etc.), and reactions to Freund's complete adjuvant and to various other complete and incomplete antigens.

In the present investigation local reactions to Freund's complete adjuvant were studied in mice differing in their initial resistance to tumor development, at different age periods.

EXPERIMENTAL METHOD

Experiments were carried out on 42 mice belonging to lines C57BL, with high resistance to the development of spontaneous tumors, and C3H, C3HA, and BALB, susceptible to spontaneous mammary gland carcinoma.

Experiments to study the special features of local reactions to Freund's complete adjuvant were carried out on mice of three age groups: 1 month, 2 months, and 10 months.

Freund's complete adjuvant was prepared by the method described previously [1]. It was injected into the plantar pads of the forelimbs or of one of the hind limbs in a dose of 0.04 ml. The severity of the local reaction to the adjuvant was assessed after 2, 12, and 20 days.

Four degrees of severity of the reactions were distinguished: I) erythema of the plantar pad and part of the medial surface of the leg; II) erythema of the plantar pad and of the whole leg, with clearly defined edema, thus increasing the volume of the paw by approximately 1.5 times; III) an increase in the volume of the limb by 2-2.5 times; IV) limb volume increased by 20 times or more. The increase in volume of the limb was estimated from the difference in the volume of fluid displaced from a measuring glass when the intact limb and the limb into which the adjuvant had been injected were immersed in turn in the glass up to the knee joint.

Statistical analysis of the results was carried out by methods recommended by Oivin [2].

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TABLE 1. Reaction to Freund's Complete Adjuvant in Mice of Different Lines ($M \pm m$)

Age of mice (in months)	Line of mice	No. of mice	Time of reaction (in days)					
			2-3	P	12	P	20	P
1	C57BL	18	$0,9 \pm 0,17$		$1,9 \pm 0,23$		$2,6 \pm 0,33$	
	C3H	19	$0,1 \pm 0,07$	<0,001	$0,4 \pm 1$	<0,001	$0,4 \pm 0,35$	<0,001
	BALB	6	$0,0 \pm 0,00$	<0,001	$0,7 \pm 0,15$	<0,001	$0,5 \pm 0,05$	<0,05
2	C57BL	28	$2,22 \pm 0,15$		$2,6 \pm 0,21$		$3,4 \pm 0,16$	
	C3H	18	$1,4 \pm 0,01$	<0,001	$1,4 \pm 0,27$	<0,001	$1,4 \pm 0,63$	<0,001
	BALB	7	$1,5 \pm 0,23$	<0,019	$1,3 \pm 0,43$	<0,02	$0,6 \pm 0,31$	<0,001
	C3HA	5	$1,4 \pm 0,05$	<0,01	$1,4 \pm 0,05$	<0,001	$1,1 \pm 0,10$	<0,001
10	C57BL	15	$3,3 \pm 0,05$		$3,1 \pm 0,23$		$3,3 \pm 0,17$	
	C3H	14	$1,6 \pm 0,11$	<0,001	$1,7 \pm 0,27$	<0,001	$1,2 \pm 0,07$	<0,001
	BALB	4	$1,8 \pm 0,47$	<0,001	$1,3 \pm 0,42$	<0,01	$1,8 \pm 0,85$	<0,02
	C3HA	8	$3,3 \pm 0,42$	<0,05	$2,5 \pm 0,16$	<0,05	$1,6 \pm 0,20$	<0,001

EXPERIMENTAL RESULTS

After injection of Freund's complete adjuvant into animals aged 1 month the reaction was absent on the second day in most C3H and BALB mice, and was slight in the C57BL mice; later (12-20 days) it was well-defined in the C57BL mice and slight in C3H and BALB mice. The differences in the course of the local reaction to Freund's complete adjuvant between C57BL mice and the other lines specified above were statistically significant at both early and late periods (Table 1).

These results are in agreement with those obtained by Japanese workers [7], who investigated the course of the local reaction to Freund's complete adjuvant over a period time in rats and distinguished a first phase of this reaction (before 8 days) and a second phase (after 8 days).

In the mice of all these lines at the age of 2 months the first and second phases of the reaction to Freund's adjuvant were well-marked, but in C57BL mice the intensity of the reaction, especially of its second phase, was much greater. The differences between the magnitudes of the reactions in C57BL mice compared with C3H, C3HA, and BALB mice were statistically significant (Table 1).

Similar results were obtained in mice aged 10 months.

The fact will be noted that with an increase in age of the mice of all lines, the intensity of the local reaction to Freund's complete adjuvant also increased, although it was much less marked in the C57BL mice than in mice of the high-cancer lines.

These results show that during development of the organism the formation of systems responsible for the first and, in particular, the second phase of the reaction to Freund's complete adjuvant, when injected into the plantar pad, takes place more slowly in mice of high-cancer lines than in those of low-cancer lines. In mice of the low-cancer line C57BL, belonging to the first age group (1 month), the differences between the intensities of the first and second phases of the reaction to the adjuvant were statistically significant ($P < 0.02$). The writer is inclined to explain this fact by the earlier maturation of the mechanisms of reactivity responsible for the second phase of the reaction compared with the mechanisms of reactivity responsible for the first phase.

In mice of low-cancer lines these differences were less marked and were not statistically significant. Furthermore, in mice of high-cancer lines the immunologic apparatus responsible for the local reaction to Freund's complete adjuvant (especially its second phase) does not in general reach the same level of development as in C57BL mice. Evidently in mice resistant to tumor development there are greater potential opportunities for the realization of immunologic reactions involving the participation of cells than in mice with low resistance.

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