Delayed Hypersensitivity in BALB/c and Charles-River Mice Injected at Birth with a Single Dose of 7,12-Dimethylbenz (α) Anthracene (DMBA)

Reduction of humoral antibody response and cell-mediated immunity have been observed in animals given chemical carcinogens either at birth or later in life[1-6], and in mice treated with several oncogenic viruses[7-12].

We have recently reported that Swiss mice injected at birth with a single dose of 7,12-dimethylbenz (α) anthracene (DMBA) show an impaired primary immune response to sheep erythrocytes[13]. The present study was designed to study further the relationship between DMBA given at birth and immune response, as far as cell-mediated immunity is concerned.

New-born BALB/c and Charles-River mice of our colony were s.c. injected with a single dose of 100 µg of DMBA (Eastman Organic Chemicals) in 0.05 ml of purified olive-oil. Untreated litters were kept as controls. At 21 days of age, the animals were separated according to sex.

Groups of 21- to 30, and 90- to 100-day old DMBA treated and untreated mice were painted twice, 7 days apart, on both sides of the ears with a 3% solution in olive-oil of the skin-sensitizing agent 2-phenyl-4-ethoxy-methylene-5-oxazolone (oxazolone) or with olive-oil. 24 and 48 h after the second painting the thickness of the ears was measured with a Panter's micrometer and the results were expressed in units of 10^{-3} cm.

Table I shows that after oxazolone there is not significant difference in ear thickness between DMBA treated and untreated 21- to 30-day-old mice. Since these results may be interpreted as due to a condition of unresponsiveness of the young mice to oxazolone, the ear thickness of the control mice painted with oxazolone or olive-oil was compared. A significant ear swelling was observed only in mice sensitized with oxazolone. Therefore it was concluded that contact sensitivity can be induced in young mice of our strains.

Table II presents the results obtained in 90- to 100-day-old mice and the incidence of observed tumours. It is clear that there is a statistically significant reduction in ear swelling after oxazolone in the animals treated with DMBA, as compared with those not given DMBA.

The experiments reported here indicate that DMBA given at birth does not induce a reduction of contact sensitivity to oxazolone in young 21- to 30-day-old animals. On the contrary, it seems to reduce this type of cell-mediated immune response in animals sensitized 90 to 100 days after birth and DMBA treatment.

Table I. Average ear thickness in 21- to 30-day-old BALB/c and Charles-River mice treated at birth with DMBA

Strain	Treatment			Ear thickness[a]			
				24 h	Significance	48 h	Significance
BALB/c	DMBA	Oxaz.	(8)	23.57 ± 2.40	$0.5 > P > 0.4$	24.69 ± 1.36	$0.2 > P > 0.1$
	–	Oxaz.	(9)	24.72 ± 3.16		26.16 ± 2.22	
	DMBA	Oil	(5)	22.50 ± 1.00	$0.2 > P > 0.1$	24.30 ± 0.75	$0.5 > P > 0.4$
	–	Oil	(4)	21.38 ± 1.10		23.63 ± 1.60	
Charles-River	DMBA	Oxaz.	(11)	22.00 ± 1.79	$0.2 > P > 0.1$	23.13 ± 1.39	$0.05 > P > 0.02$
	–	Oxaz.	(10)	23.00 ± 1.02		24.50 ± 1.02	
	DMBA	Oil	(5)	21.20 ± 3.05	$0.4 > P > 0.3$	22.00 ± 2.42	$0.5 > P > 0.4$
	–	Oil	(5)	19.80 ± 1.03		21.00 ± 1.27	

[a] The results are expressed in units of 10^{-3} cm as absolute values.

Table II. Average ear thickness and tumour incidence in 90- to 100-day-old BALB/c and Charles-River mice treated at birth with DMBA

Strain	Treatment			Ear thickness[a]				Total No. of tumour-bearing Mice with:	
				24 h	Significance	48 h	Significance	Malignant lymphoma	Other tumours
BALB/c	DMBA	Oxaz.	(10)	12.08 ± 7.44	$0.02 > P > 0.01$	11.98 ± 6.26	$0.05 > P > 0.02$	3	1[b]
	–	Oxaz.	(10)	18.88 ± 2.81		17.20 ± 3.78		–	–
	DMBA	Oil	(6)	0.54 ± 0.64	$0.1 > P > 0.05$	1.35 ± 1.14	$0.2 > P > 0.1$	1	–
	–	Oil	(6)	1.97 ± 1.52		2.75 ± 1.53		–	–
Charles-River	DMBA	Oxaz.	(20)	9.61 ± 5.43	$0.001 > P$	12.07 ± 5.41	$0.01 > P > 0.001$	7	1[c]
	–	Oxaz.	(7)	20.50 ± 5.02		18.73 ± 3.02		–	–
	DMBA	Oil	(8)	0.84 ± 1.52	$0.8 > P > 0.7$	1.43 ± 1.40	$P > 0.9$	1	1[d]
	–	Oil	(4)	1.13 ± 2.36		1.45 ± 2.45		–	–

[a] The results are expressed in units of 10^{-3} cm as increase in thickness. [b] Subcutaneous Rabdomyosarcoma. [c] Mucous adenocarcinoma of glandular stomach. [d] Lung adenoma.

It has recently been shown that thymus-dependent cells play an essential role in the response to oxazolone[15]. We have previously indicated[14] that DMBA given at birth acts on the thymus, and consequently mainly on the long-lived thymus-dependent lymphoid cell population carrying immunological memory[16,17], that in rodents have a potential life span of some months[18]. Therefore, if some thymus cells have escaped from the effect of the carcinogen, we can assume that at 20 to 30 days of age, at least part of the thymus-dependent cell population is present and DMBA fails to exert an inhibitory effect on cellmediated immunity.

Between 90 to 100 days, i.e. up to an age corresponding approximately to one-third of the life span of a mouse, the long-lived thymus-dependent lymphocytes could be almost completely eliminated by the effect of DMBA given at birth, and by the involution of the cells derived from those which escaped the effect of the carcinogen. Their depletion explains the failure of the adult mice given DMBA at birth to show a normal delayed hypersensitivity response.

We have previously shown that DMBA injected at birth in mice greatly reduces the primary immune response prior to the appearence of the tumours[13]. The present results indicate that such treatment also reduces cell-mediated immunity. The reduction of both humoral and cell-mediated immune responses is thus compatible with the suggestion that there is a relationship between immunodepression and the high frequency of tumours that DMBA treatment induces[13,14,19].

Riassunto. Una iniezione neonatale di 100 μg di DMBA in topi BALB/c e Charles-River deprime la risposta immunitaria, a tipo ipersensibilità ritardata, in animali di 90–100 giorni di età, mentre non pare agire come fattore deprimente in topi valutati 20–30 giorni dopo la nascita ed il trattamento oncogeno.

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Further Studies on Bovine Red Cells Having a Different Glycoprotein Coat

In a previous study[1] antigenic properties of bovine neuraminic acid (NA) containing erythrocyte mucoid preparations have been reported, using reagents for the blood group antigens in all bovine blood group-systems, whereas immunization procedures with the same glycoproteins were less successful[2]. A next step was the investigation of some heterophilic receptors of the NA-free mucoid[3]. Finally, a correlation was found between the thickness of the outer NA-containing glycoprotein layer of the red cells (rbc) and different forms of their agglutinability with special regard to 'incomplete' antibodies[4].

NA-containing receptors – like MN in human rbc – have not been detected, except myxovirus-receptors[1]. A comprehensive review of different receptors (virus, biological, serological and pharmacological), where NA is involved, has been given elsewhere[5]. Recently a contribution has been made[6] involving NA containing blood group receptors in the bovine isoantigen system. It was observed that NA is involved in the specificity of the F-antigen in bovine rbc. The assumption is based on the following experimental data: a) The F-antigen is inactivated by neuraminidase and b) in F/F homocygote rbc-stroma the NA-content is larger than in F/V rbc and in the latter larger than in V/V rbc.

In spite of these convincing data, however, the following should be taken into consideration: 1. Obviously the F-antigen having NA is not part of the outer mucoid layer and accordingly deeper in the membrane because otherwise a) F should be partly removed by proteolytic enzymes and b) the corresponding antibody should be an agglutinating one because of the superficial 'outside' localization of the antigen c) the mucoid should be a better inhibitor (it inhibits only weakly).

2. Accordingly the F-antigen could belong to the NA-containing glycolipid fraction of the stroma. This would imply a) removal by glycolipid extraction (methanol/chloroform) b) crossreaction of the anti-F with other

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