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A STUDY OF SECONDARY RESPONSE IN ALLERGIC CONTACT SENSITIVITY IN THE GUINEA-PIG

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Abstract. Guinea-pigs were sensitized by painting on 2 consecutive days with a toxic dose of DNCB. After 35 days the animals were divided at random into 5 groups, after which those in 4 groups were given a new toxic dose of DNCB, a so-called secondary sensitization. Two tests respectively of 10 μg and 20 μg of DNCB per cm^2 of skin area were applied to the animals in groups 1, 2, 3 and 4 on respectively the 6th, 8th, 10th and 14th day following secondary sensitization. The 5th group served as a control group and was tested at the same time. The degree of hypersensitivity was assessed on the basis of macroscopic changes in the test reactions, and the lymphoid-cell infiltration, which was assessed by a method called the "lymphoid-cell response in epidermis". No change in the degree of hypersensitivity, corresponding to the well-known secondary response of humoral antibodies after reinjection of a specific antigen, could be demonstrated in this investigation in contact allergy in guinea-pigs.

A problem regarding contact allergy that is especially important from a clinical point of view is, whether the degree of hypersensitivity changes spontaneously or can be influenced by renewed more or less intense contact with an allergen.

Such studies are rendered more difficult, however, since our opportunities of grading contact hypersensitivity are limited. Usually this type of hypersensitivity is assessed with regard to the intensity of an epicutaneous test reaction. Here, variations in the visible changes are greater in man and sufficient for practical use; but they are substantially less pronounced in the guinea-pig, i.e. the animal generally used for experimental investigations of contact allergy. By testing the different amounts of antigen per unit area attempts have been made to increase the possibility of grading hypersensitivity; nonetheless the method must be regarded as comparatively crude.

A source of error is also introduced into the studies on the above-mentioned problem when the same individual is tested repeatedly, inasmuch as the antigen supplied through the test may influence the further degree of hypersensitivity.

The investigation reported on here was made on guinea-pigs which were sensitized with 2.4-dinitrochlorobenzene (DNCB) and subsequently exposed to a new epicutaneous contact with the same antigen. The problem was whether renewed intense antigen contact in an animal previously sensitized causes rapid onset and more enhanced contact allergy, i.e. if a form of "secondary response" can be demonstrated in this delayed type of allergy. The intensity of the test reactions has been assessed in this investigation in part macroscopically and in part with regard to lymphoid-cell infiltration, which affords a greater possibility of grading the strenght of the reaction (5).

MATERIAL AND METHODS

Animals. Albino guinea-pigs were used whose initial weight at the primary sensitization was about 300 g.

Antigen. For sensitization, 2.4-dinotrochlorobenzene (DNCB) was used in a 20% acetone solution. 0.02 ml of this solution was applied over an area of about 1 cm^2 of clipped skin, on the 1st day on one shoulder and on the 2nd day on the other shoulder. This will be termed in what follows the primary sensitization. In some groups of animals a renewed application of the same solution was made 35 days later to a shoulder area not previously employed: secondary sensitization.

Testing was made with DNCB in a 2% alcohol solution on the flank skin which had previously been clipped and shaved electrically. On a demarcated area, 10 μg of DNCB per cm^2 were applied with a pipette to one test area and 20 μg of DNCB per cm^2 to the other. The tests were assessed after 24 hours.

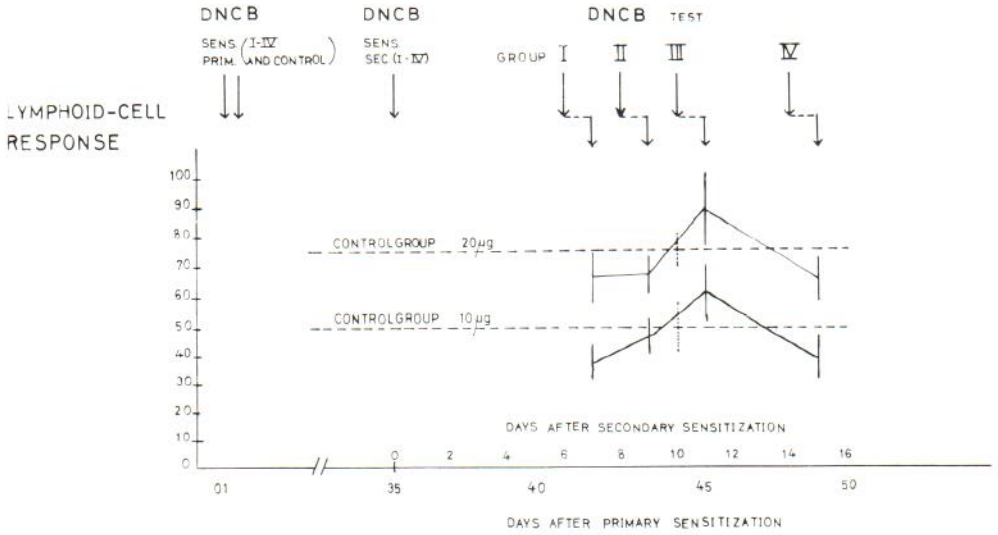

Fig. 1. Sensitization and testing schedule, with summary of test reactivity assessed by the "lymphoid-cell response in epidermis" method.

Methods of assessment. Macroscopic inspection (naked eye) was graded as follows: 0 = negative or doubtful reaction; + = redness, often somewhat uneven; + + = pronounced redless, possibly with slight infiltration; + + + = pronounced redless and manifest infiltration.

Assessment with so-called lymphoid-cell response (for details see 5). In principle, 1000 cells are counted in the epidermis, and epithelial cells differentiated from lymphoid cells. Thus the percentage of lymphoid cells infiltrated into the epidermis is obtained. The lymphoid-cell response is equivalent to the per mille lymphoid cells in a reaction area minus the per mille lymphoid cells in the normal skin of the same animal.

Histological technique. After macroscopic assessment the animals were killed and pieces of skin from the test area and from the normal skin were stamped out carefully avoiding traumatization. The preparations were fixed in carbonate buffered formalin with an addition of 0.7% saline. After dehydration in alcohol and xylol the preparations were embedded in paraffin. Sections 5 μ thick were used throughout and at least 3 sections were discarded for each section used (to avoid counting the same cells). After deparaffinization Harris' haemotoxylin and eosin were employed for staining.

Experimental procedure. All the animals were kept in a separate quiet environment with good ventilation and temperature regulated between 23–25°C. They received the same standardized diet, and the experiments were made during the winter. All the animals were subjected to primary sensitization on day 0 and 1. After 35 days they were all weighed. Then they were divided at random into 5 groups, but so that the weight distribution was about the same in all the groups. The mean weight was then about 500 gr.

All the animals in 4 of the groups were subjected to secondary sensitization on day 35 by applying the same amount of antigen to the skin as in the primary sensitization. The 5th group served as the control group and was not sensitized again. Before testing, the animals were put in separate well-cleaned cages and the flank skin was very carefully clipped and shaved with electric machines. Tests were then applied to the animals in groups 1, 2, 3 and 4 on respectively the 6th, 8th, 10th and 14th day after the secondary sensitization. The control animals were tested at the same time, i.e. 44 days after the primary sensitization (Fig. 1). All the tests were assessed after 24 hours by a person with great experience, but who was not aware of the amounts of antigen or the experimental conditions. Immediately after the macroscopic assessment the animals were killed. Pieces of skin were taken from the test sites and also normal skin for histological preparation and microscopy.

RESULTS

Tables I–IV shows the test results for the 4 groups of guinea-pigs which, 35 days after the primary sensitization, received an antigen dose for secondary sensitization.

As is evident from the tables the mean value in all the groups for the test reactions, assessed according to lymphoid-cell response in epidermis, was considerably lower for 10 μg per cm^2 than for the higher test dose of 20 μg per cm^2. However, there were quite appreciable individual variations between the animals in each group. The macroscopic inspection showed a similar tendency.

Table I. *Guinea-pigs tested 6 days after a second sensitization (group 1)*

The degree of the contact reactivity of two tests on each animal, assessed by macroscopic examination (naked eye) and lymphoid-cell response 24 hour after application of respectively 10 μg and 20 μg DNCB cm^2

Animal	10 μg / cm^2			20 μg / cm^2		
	Naked eye	Lymphoid cell response in epidermis		Naked eye	Lymphoid cell response in epidermis	
1	+ +	(71–26)	45	+ + +	(73–26)	47
2	+ +	(69–20)	49	+ +	(61–20)	41
3	+	(40–11)	29	+ +	(79–11)	68
4	+ +	(73–11)	62	+ + +	(109–11)	98
5	+ +	(82–18)	64	+ +	(102–18)	84
6	+	(51–19)	32	+	(67–19)	48
7	+	(33–15)	18	+ + +	(120–15)	105
8	+	(69–32)	37	+ +	(85–32)	53
9	+	(33–22)	11	+ +	(73–22)	51
	13/9 = 1.5	Mean	38.56	20/9 = 2.2	Mean	66.11
		S.E.	6.08		S.E.	7.97

Fig. 1 gives a survey, taken from the tables, of the mean values and standard error of the mean for the lymphoid-cell response. The lower continuous line joins the mean values for 10 μg and the upper for 20 μg tests in the respective animal groups. The same tendency is observed for both test doses, namely that group 3, which was tested on day 10 and assessed on day 11, shows higher mean values than the other groups tested on the 6th, 8th and 14th day respectively after secondary sensitization. When a comparison is made between lymphoid-cell response between the control group (group 5) that was only primarily sensitized and the animal groups that were also secondarily sensitized, then the animals in group 3 continue to show a higher mean value, whereas the mean value of the other groups was lower, irrespective of which test dose was applied. The differences, however, are not significant.

DISCUSSION

When dinitrochlorobenzene (DNCB) is applied in sufficient amount and concentration to the skin, it causes in man, as in the guinea-pig, contact allergy. Individual variations in the degree of hypersensitivity are common, and among guinea-pigs, strains have been described that are very difficult to sensitize to certain contact allergens (1, 10). Spontaneous changes in the degree of

Table II. *Guinea-pigs tested 8 days after a second sensitization (group 2)*

Animal	10 μg / cm^2			20 μg / cm^2		
	Naked eye	Lymphoid cell response in epidermis		Naked eye	Lymphoid cell response in epidermis	
10	+ +	(83–17)	66	+ + +	(87–17)	70
11	+	(43–15)	28	+	(68–15)	53
12	+	(60–23)	37	+ +	(95–23)	72
13	+	(66–39)	27	+ +	(81–39)	42
14	+ +	(70–32)	38	+ + +	(115–32)	83
15	+	(43–16)	27	+ + +	(111–16)	95
16	+ +	(91–21)	70	+ +	(97–21)	76
17	+ +	(92–21)	71	+ +	(59–21)	38
18	+ +	(79–23)	56	+ +	(87–23)	64
19	+ +	(76–25)	51	+ + +	(107–25)	82
	16/10 = 1.6	Mean	47.1	23/10 = 2.3	Mean	67.5
		S.E.	5.68		S.E.	5.82

Table III. *Guinea-pigs tested 10 days after a second sensitization (group 3)*

Animal	10 μg / cm²			20 μg / cm²		
	Naked eye	Lymphoid cell response in epidermis		Naked eye	Lymphoid cell response in epidermis	
20	+ +	(73–34)	39	+ + +	(96–34)	62
21	+ + +	(133–22)	111	+ + +	(175–22)	153
22	+ +	(78–15)	63	+ + +	(107–15)	92
23	+ +	(52–15)	37	+ + +	(112–15)	97
24				+ +	(85–19)	66
25	+ +	(114–33)	81	+ +	(61–33)	28
26	+ +	(65–23)	42	+ +	(94–23)	71
27	+ +	(95–44)	51	+ + +	(135–44)	91
28	+ +	(106–28)	78	+ + +	(143–28)	115
	17/8 = 2.1	Mean	62.75	24/9 = 2.7	Mean	86.11
		S.E.	9.15		S.E.	11.83

hypersensitivity have been reported. According to Frey (3) contact sensitivity to DNCB decreased in guinea-pigs after 90 days in a number of animals, but more frequently remained unchanged in those animals which from the outset showed a high degree of hypersensitivity.

In a number of investigations it could be established that an epidermal desensitization was possible in contact allergy in guinea-pigs. Thus, by daily application of DNCB in subtoxic doses to DNCB-sensitized guinea-pigs it can be shown that there is both a macroscopic and microscopic decrease in sensitivity, or even its abolition, in comparison with the non-desensitized control group (6, 9). Lowney (8) sensitized guinea-pigs with 2 contact allergens and was able to establish, by desensitizing with one antigen in small doses given for a long time, that the effect was specific.

Clinical experience shows, however, that a person whose allergic contact eczema has been recently cured who is again exposed to contact with the same antigen will develop a more pronounced eczematous reaction. Chase (2) was able to show experimentally that following extracutaneous sensitization according to a special method, a subsequent application of the same antigen caused distinct exacerbation of hypersensitivity. The extracutaneous sensitization, however, also causes humoral antibodies; it has also been demonstrated (7) that the test reactions have a different histological picture, depending on whether the animals were sensitized cutaneously or extracutaneously.

Both the primary and secondary sensitization were obtained in this investigation by application of the antigen to the epidermis. The amount of antigen administered on each occasion was, ac-

Table IV. *Guinea-pigs tested 14 days after a second sensitization (group 4)*

Animal	10 μg / cm²			20 μg / cm²		
	Naked eye	Lymphoid cell response in epidermis		Naked eye	Lymphoid cell response in epidermis	
29	+	(45–24)	21	+ +	(91–24)	67
30	+ +	(67–21)	46	+ + +	(117–21)	96
31	+	(43–22)	21	+ +	(58–22)	36
32	+ +	(61–29)	32	+ +	(63–29)	34
33	+ +	(29–25)	4	+ + +	(86–25)	61
34	+ +	(74–28)	46	+ + +	(108–28)	80
35	+	(45–19)	26	+ +	(74–19)	55
36	+ +	(92–19)	73	+ +	(102–19)	83
37	+ +	(110–28)	82	+ + +	(101–28)	73
	15/9 = 1.7	Mean	39.00	22/9 = 2.5	Mean	65.00
		S.E.	8.49		S.E.	6.97

Table V. *Guinea-pigs tested after a primary sensitization (group 5; control group)*

Animal	10 μg / cm²			20 μg / cm²		
	Naked eye	Lymphoid cell response in epidermis		Naked eye	Lymphoid cell response in epidermis	
38	+	(71–18)	53	+ +	(84–18)	66
39	+ +	(45–21)	24	+ +	(96–24)	75
40	+ +	(77–26)	51	+ + +	(125–26)	99
41	+ +	(87–34)	53	+ + +	(135–34)	101
42	+ +	(103–26)	77	+ +	(76–26)	50
43	+ +	(96–19)	77	+ + +	(89–19)	70
44	+	(52–33)	19	+ + +	(92–33)	59
45	+	(57–33)	24	+ +	(105–33)	72
46	+ +	(87–17)	70	+ + +	(92–17)	75
	15/9 = 1.7	Mean	49.78	23/9 = 2.7	Mean	66.7
		S.E.	7.98		S.E.	5.57

cording to previous experience with this guinea-pig strain, sufficient to cause a mean relatively high degree of hypersensitivity. With the well-known secondary response of humoral antibodies the highest titres are obtained earlier than after primary stimulation.

The times for testing in this investigation were chosen with regard to the fact that the latency period between sensitization with DNCB and positive test reactions is usually seldom seen before the 6th day (4). Moreover, in a preliminary experiment it had not been possible to convincingly establish any earlier influence on the test reactions.

Besides assessing the degree of hypersensitivity with regard to the macroscopic changes, a microscopic examination was also made of the tested skin. The characteristic lymphoid-cell infiltration into the corium and epidermis in allergic contact eczema, which is fully developed after 24–48 hours, was utilized for the quantitative estimation of the test reactions. In the epidermis of the guinea-pig the lymphoid cells can be directly counted and related to the number of epithelial cells in the corresponding area of the epidermis. In the epidermis of normal skin there are also a limited and somewhat varying number of lymphoid cells. A more exact measure of lymphoid-cell infiltration in epidermis, which a certain amount of antigen produces, is therefore obtained by subtracting the relative number of lymphoid cells in normal epidermis from the corresponding number in the test reaction in the same animal. This method of assessment, which is called "lymphoid-cell response in epidermis" has proved in earlier investigations to be a serviceable measure of the intensity of the allergic contact reaction (5).

The somewhat stronger reactions in the animals tested on the 10th day after the secondary sensibilization do not differ significantly from the tests in the other groups, but the tendency corresponds with earlier observations after primary sensitization, where a maximum in the test reactions was reached at the same point of time (5). The differences that were obtained between the doubly sensitized animal groups and the control group are insignificant and do not lead to any conclusions.

Thus in this investigation, when the degree of hypersensitivity in allergic contact dermatitis was assessed according to macroscopic changes and lymphoid-cell infiltration in the test reactions, it may be stated that, after secondary stimulation, it was not possible to demonstrate any secondary response of the type observed in immunity elicited by humoral antibodies.

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