Clin. exp. Immunol. (1990) **81**, 507–509

Low-dose exposure to antigen induces sub-clinical sensitization

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(Accepted for publication 11 April 1990)

SUMMARY

We examined the effects of a small initial sensitizing dose of antigen (dinitrochlorobenzene, DNCB) on the subsequent response to a second, defined sensitizing stimulus. The second stimulus was actually the regimen of four doses of DNCB (3·125, 6·25, 12·5, and 25 μg; total 46·9 μg) normally used as the elicitation challenge. In two separate experiments 13 and 18 control subjects received an initial 'challenge' with the four doses to induce sensitivity, and 4 weeks later their responses were determined with a second, elicitation challenge. Two groups of 12 and 15 experimental subjects received an inital dose predicted to induce clinically detectable sensitivity in 50% or 25%, respectively. Four weeks later, their responsiveness was determined with quantitative challenge and the subjects who gave no response received a further challenge 4 weeks later. Their responses, compared with those from the control subjects, were augmented, indicating that sub-clinical priming of the immune system had indeed occurred.

Keywords contact hypersensitivity DNCB sub-clinical human

INTRODUCTION

Our previous work on the immune response to epicutaneous application of 2,4-dinitrochlorobenzene (DNCB) has defined certain of the dose–response relations of the immune system (Friedmann *et al.*, 1983; Moss *et al.*, 1985). We found that as sensitizing dose of antigen increases, the proportion of subjects showing clinically detectable reactivity to an elicitation challenge increases. Furthermore, as sensitizing dose increases there is a proportionate increase in the degree of reactivity of sensitized subjects. These observations indicate that the process of sensitization entails a continuous augmentation of the afferent mechanisms of the immune system. However, the question arises of what has happened to the immune system in subjects who show no clinically detectable response to the elicitation challenge. Has the sensitizing stimulus not been perceived at all, i.e. a null event, or has the immune system been activated at a sub-clinical level? In the latter case, it would be expected that the initial sensitizing dose had primed the system initiating immunological memory. From this it could be predicted that a second sensitizing stimulus would induce an augmented or boosted response. In the present study, the approach we adopted made use of the fact that the challenge regimen of four DNCB doses (3·125, 6·25, 12·5, and 25 μg; total 46·9 μg) is itself a moderately potent sensitizing stimulus. Thus the degree of reactivity of control subjects sensitized by application of the challenge regimen could be determined with a second, elicitation challenge 4 weeks after the first sensitizing exposure. Responses could be compared with those from subjects given the same regimen who had first received a low potency sensitizing stimulus. We report here the results of two experiments showing that low-dose sensitizing doses induce sub-clinical sensitization.

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SUBJECTS AND METHODS

Subjects

Healthy volunteers and patients with minor non-inflammatory skin lesions were recruited from our Outpatient Department. All gave informed consent and the study was approved by the local Ethics Committee.

Sensitization and challenge

The general protocol employed was to induce sensitivity by application of a dose of DNCB to a 3-cm diameter circle on the forearm, followed by occlusion for 48 h. Four weeks later the presence of clinically detectable sensitivity was elicited by a challenge of four doses of DNCB (3·125, 6·25, 12·5, and 25 μg), each applied on 1-cm patch test felts (A1 test). After 48 h, the response at each challenge site was evaluated clinically as: 0, no detectable response; 1, erythema only; 2, erythema with induration; and 3, as for 2 but with vesicle formation. The responses were also quantified as increase in skin-fold thickness measured with Harpenden calipers at each site before and 48 h after challenge (Friedmann *et al.*, 1983).

The challenge regimen (total 46·9 μg of DNCB) is a moderately potent sensitizing stimulus and some subjects

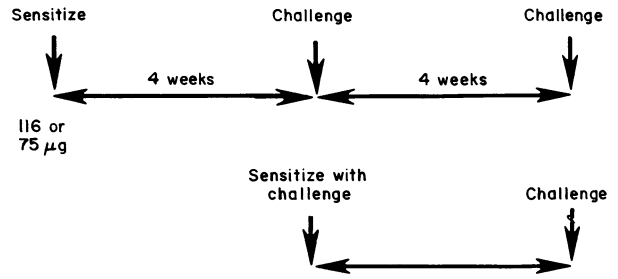

Fig. 1. Protocols for sensitization and challenge. Experimental subjects received an initial dose of 116 or 75 μg of DNCB. Four weeks later they were challenged and subjects who were unresponsive were given a further challenge after 4 more weeks. Control subjects received the challenge regimen to induce sensitization and their degree of reactivity was determined 4 weeks later with an eliciting challenge.

received the 'challenge regimen' as their initial, sensitizing exposure (see below). The degree of sensitivity was measured 4 weeks later by quantifying responses to a second application of the challenge regimen.

Experimental protocols

The approach used was to attempt to sensitize experimental subjects with a low dose of DNCB. Four weeks later their reactivity was determined with a challenge (Fig. 1). The subjects who showed no detectable responses were given a further challenge after 4 more weeks. Their responses were compared with those from control subjects sensitized by application of the challenge regimen, and then given a second, eliciting challenge 4 weeks later.

Experiment 1

Thirteen control subjects (eight women, five men) received the challenge regimen applied to the upper, inner arm for 24 h. Four weeks later their degree of sensitivity was determined by measurement of the responses elicited by a second application of the challenge regimen. Responses were quantified with calipers at 48 h as described above.

Twelve experimental subjects received an initial dose of 116 μg DNCB. From our previous work (Friedmann *et al.*, 1983) this was the estimated 50% effective sensitizing dose. Four weeks later they were challenged to determine sensitivity (Fig. 1). Six subjects (four women, two men) gave no detectable response and they were given a second challenge after 4 more weeks (Fig. 1) and the responses quantified as described above. Comparison with responses from control subjects would reflect effects of the first, apparently ineffective sensitization.

Experiment 2

The overall design was similar to that of experiment 1, with two modifications that reduced the sensitizing potency of both the challenge regimen and the initial sensitizing dose. Firstly, the challenge regimen was applied for only 12 h, secondly, the 'subclinical' sensitizing dose was reduced to 75 μg DNCB—the dose estimated as the 25% effective sensitizing dose.

Eighteen control subjects (all male) received the challenge regimen to induce sensitization (Fig. 1) and 4 weeks later their responses were quantified with a second, eliciting challenge.

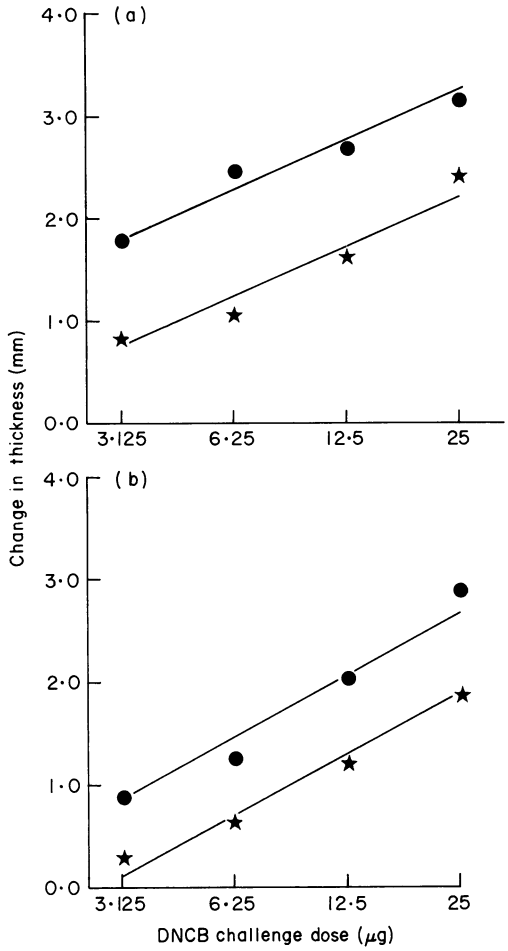

Fig. 2. (a) Experiment 1: augmentation of responsiveness by 'subclinical' sensitization. Subjects given an initial sensitizing dose of 116 μg DNCB were found 4 weeks later to be unresponsive to the first challenge. They received a second challenge to determine their degree of reactivity (●). Control subjects were sensitized by application of the challenge regimen (★). (b) Experiment 2: as experiment 1, but the experimental subjects received 75 μg as the initial sensitizing dose (●). Control subjects (★).

Fifteen experimental subjects (all male) received 75 μg DNCB as sensitizing dose. Four weeks later they were challenged. The 11 unresponsive subjects were given a second challenge after 4 more weeks and responses measured and compared with those of the control subjects.

The results were compared using a split unit analysis of variance (Armitage & Berry, 1984). In experiment 1, 12 subjects were female (eight control and four experimental) and sex differences were allowed for in the analysis. In experiment 2 all subjects were male.

RESULTS

Experiment 1

Of the subjects who received only the challenge regimen as sensitizing stimulus, 12 out of 13 (92%) responded to the elicitation challenge. These 12 responders provided the reference for comparison with the experimental group (Fig. 2a). Of the experimental subjects who received the initial dose of 116 μg,

six out of 12 (50%) showed no response at all to the first challenge. However, their responses to the second challenge were greater than those from the controls ($P < 0.07$; mean experimental − control = 1·125; 95% confidence interval = −0·05, 2·30) (Fig. 2a). The dose–response curves remained parallel.

Experiment 2

Findings were essentially the same as in experiment 1. Of the subjects who received only the challenge regimen as sensitizing stimulus, 15 out of 18 (83%) responded to the elicitation challenge. Of the experimental group, 11 out of 15 (73%) were unresponsive at the first challenge. However, their responses to the second challenge were greater than those of controls ($P < 0.06$; mean experimental − control = 0·765; 95% confidence interval = −0·06, 1·56) (Fig. 2b).

Although the mean responses for the sub-clinically sensitized subjects were much greater than those from the controls, the significance of the differences are not great because of the large inter-subject variations seen in this system.

DISCUSSION

The present observations confirm our earlier finding that application of low doses of a potent antigen induces clinically detectable sensitivity in only a proportion of subjects (Friedmann *et al.*, 1983). We have also shown that the regimen used for elicitation or challenge (total dose 46·9 μg on four 1-cm patch test felts) is a moderately potent sensitizing stimulus. Moreover, the challenge regimen (46·9 μg) is a more potent sensitizing stimulus than 116 μg. This is because the total area of application of the former is less (3·1 cm^2 compared with 7·1 cm^2), hence the concentration in μg/cm^2 is greater. Our previous studies have shown that with areas greater than 0·7 cm^2 (a 1-cm diameter patch), concentration of antigen is the major determinant of sensitization (Rees & Friedmann, 1990). We showed that if concentration is kept constant, changing the area and hence the total dose applied, made very little difference to sensitivity (White *et al.*, 1986; Rees & Friedmann, 1990).

The major finding of the present study is that subjects given a low initial sensitizing stimulus, e.g. 75 or 116 μg, and who show no response to a first eliciting challenge then go on to give augmented responses to a second challenge. This indicates that the initial dose of DNCB had in fact stimulated the induction of 'immunological memory' at a sub-clinical level. With the immune system primed thus, the sensitizing effect of the first challenge is much greater because it is actually augmenting the existing sensitivity—an anamnestic response well known in the humoral immune response.

These observations complement our previous work showing that activation of the clinically detectable immune response to DNCB is a graded, continuous process which, as with many other physiological systems, shows a direct proportionality to the logarithm of sensitizing dose (Friedmann *et al.*, 1983; Moss *et al.*, 1985). We now suggest that this graded response extends down below the level at which it can be detected clinically; in other words, sub-clinical sensitivity occurs.

From these observations various aspects of the response at the cellular level can be inferred. It is known that following application of antigen to skin, antigen-bearing Langerhans cells appear first in the dermis (Silberberg-Sinakin *et al.*, 1976; Bergstresser, Toews & Streilein, 1980; Carr *et al.*, 1984) and then in the regional lymph node (Macatonia, Edwards & Knight, 1986; Okamoto & Kripke, 1987; Kinnaird *et al.*, 1989). The numbers of such cells is proportional to the concentration of antigen applied (Macatonia *et al.*, 1986). The antigen-bearing Langerhans cells activate specific T lymphocytes, inducing clonal proliferation within the lymph node. Normally, when the stimulus of antigen presentation is strong enough, the clonal expansion continues to a point at which the specific T cells leave the node and enter the circulation in appreciable numbers. Presumably this is when sensitivity becomes detectable as a response to antigen challenge. However,when the first dose of antigen is low the numbers of Langerhans cells reaching the lymph node will be low, the stimulus to T cells low and the clonal expansion small. Hence, egress of T cells to the circulation will be too low to be detectable with a challenge. Nevertheless, the expanded clone of T cells will get off to a quicker start when a second stimulus with that antigen is offered.

There are still may questions regarding the factors that regulate all the steps in the afferent limb of the immune response. One question of particular interest is what determines the limits of clonal expansion and hence magnitude of sensitization induced by an encounter with antigen.

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