

Induction of contact sensitivity and antigenic competition by the intravenous administration of contact sensitizers

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Summary. The effect of route of administration of contact sensitizers on the induction of antigenic competition between dinitrofluorobenzene (DNFB) and picryl chloride (PCI) and between oxazolone (Ox) and PCI was investigated. Antigenic competition (depressed contact sensitivity to PCI) was induced not only by painting with DNFB but also by the administration of DNFB via various routes, such as intravenous (i.v.), oral or subcutaneous routes. Antigenic competition was abolished by the treatment with cyclophosphamide (CY) 4 days after i.v. injection of competing DNFB or Ox, and also by thymectomy 6 weeks prior to the injection of DNFB. These results suggest that thymus-derived suppressor cells may be involved in the antigenic competition induced by the i.v. injection of DNFB.

Primary contact sensitivity (CS) was induced by painting of DNFB or Ox and also by i.v. injection of Ox. Although an i.v. injection of DNFB into normal mice did not induce CS, it induced a strong CS in the mice pretreated with CY 2 days prior to the injection of DNFB. Examination on the effect of dose of contact sensitizers on the induction of antigenic competition, primary CS and tolerance by painting or by i.v. injection of DNFB or Ox showed that the degree of

antigenic competition has a closer relationship with CS, than with unresponsiveness.

INTRODUCTION

The induction of contact sensitivity and of tolerance is known to be dependent on the anatomical sites of the administration of contact sensitizer. It is reported that DNFB which induces strong contact sensitivity when painted on mice, is also a potent tolerogen when it is administered intravenously (i.v.) into animals (Claman, 1976). Hapten-conjugated lymphocytes or macrophages are reported to induce contact sensitivity when they are introduced subcutaneously (s.c.) into animals (Greene, Sugimoto & Benacerraf, 1978), while hapten-conjugated cells induce tolerance when they are injected i.v. (Miller & Claman, 1976; Ptak & Rózycka, 1977; Ptak, Rózycka, Askenase & Gershon, 1980). It has recently been shown that Langerhans cells in the skin play a critical role in the induction of contact sensitivity (Toews, Bergstresser & Streilein, 1980; Ptak *et al.*, 1980).

The dose of contact sensitizer applied is one of the important factors which regulate the degree of contact sensitivity. First, Asherson, Perera & Thomas (1979) recently reported that contact sensitivity to oxazolone (Ox) was induced by feeding of a large dose of the contact sensitizer, while painting or feeding a low dose

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of Ox induced poor responses and reduced the response to a standard immunizing dose. Second, reduced response was induced by painting with a supraoptimal dose of contact sensitizer (Sy, Miller & Claman, 1977).

We have reported that painting with a contact sensitizer induces a depressed response to another sensitizer (Nakano, 1977) and that this phenomenon (antigenic competition) is mediated by suppressor cells (Nakano & Nakano, 1978). In this paper, we describe the effect of route and dose on the induction of antigenic competition and also on the induction of the primary response and tolerance. It will be shown that antigenic competition can be induced not only by painting but also by various other routes of administration of contact sensitizers.

MATERIALS AND METHODS

Animals

Closed colony ICR mice of both sexes were used. These mice were 5–7 weeks old at the beginning of experiments.

Contact sensitizers

Picryl chloride (PCI, Tokyo Kasei Co., Tokyo, Japan), 2,4-dinitro-1-fluorobenzene (DNFB, Tokyo Kasei) and 4-ethoxymethylene-2-phenyl-oxazolone (Ox, British Drug Houses Chemicals, Poole) were used.

Preparation of contact sensitizers before application to mice

Depending on the routes of administration, the methods of preparation of contact sensitizers varied as described:

Painting on the backs of mice and subcutaneous (s.c.) injection. A solution of DNFB or Ox was made by dissolving the contact sensitizer in absolute ethanol and was painted on or injected into mice in a volume of 0.025 or 0.05 ml.

Intravenous (i.v.) injection. A solution of DNFB was made by dissolving 57 mg of DNFB in 40 ml of phosphate-buffered saline (PBS) at 60° by vigorously stirring with a magnetic bar and then was immediately cooled to 4°. This solution was further diluted appropriately with PBS, if necessary. Diluted or undiluted solution was injected in a volume of 0.4 ml. In the case of the i.v. injection of Ox, a solution of Ox was made

by dissolving Ox in ethanol and was injected into mice in a volume of 0.025 ml, using a 0.25 ml syringe. Precautions were taken so as not to apply contact sensitizers to the skin or to perivascular tissues.

Oral administration. A solution of DNFB was made by dissolving the contact sensitizers in absolute ethanol. Then, 1 volume of this solution was mixed with 9 volumes of olive oil, 0.5 ml of the final solution was administered orally into mice, using a small feeding catheter.

At the time of the experiments, doses of contact sensitizers were expressed in terms of a combination of percent (w/w) and volume of solutions. In this paper, however, doses of contact sensitizers are shown in terms of weight for clarity and convenience of description. For comparison with our earlier publications (Nakano, 1977; Nakano & Nakano, 1978), it should be noted that 0.05 ml of 5% (w/w) DNFB in ethanol contains 2.0 mg of DNFB and that 0.05 ml of 3% Ox in ethanol contains 1.2 mg of Ox.

Sensitization

In the test for the ability of contact sensitizers to induce antigenic competition and tolerance, a procedure which is called 'sensitization' was employed for several days (in most cases, 7 days) after the pretreatment with contact sensitizers. Sensitization was performed by painting with either 0.05 ml of 5% (w/w) PCI in ethanol or 0.05 ml of 1% (w/w) DNFB in ethanol or 0.05 ml of 3% of Ox (w/w) in ethanol on clipped backs of mice using a 0.25 ml syringe.

Challenge

PCI, DNFB or Ox was dissolved in olive oil at a concentration of 1% (w/w). As Ox is difficult to dissolve in olive oil directly, it was dissolved first in acetone to 5% and then dispersed in olive oil to a final concentration of 1%. One tenth millilitre of each solution was painted on both sides of the right ear with cotton wool. Challenge was performed 5 days after the sensitization of mice.

Measurement of ear thickness

Ear thickness of mice was measured with a dial thickness gauge (Ozaki MFG Co., Tokyo, Japan) before and 24 h after challenge. The degree of ear swelling was expressed as follows;

$$\text{Ear swelling (\%)} = \frac{E_2 - E_1}{E_1} \times 100$$

where E_1 and E_2 represent the ear thickness before and 24 h after challenge, respectively. The degree of contact sensitivity (CS) was assessed by the degree of ear swelling at 24 h after challenge (Nakano, 1977). The average ear thickness of thirty normal 6–7 week-old mice used in these experiments was 22.5 ± 0.3 ($\times 10^{-2}$ mm).

Cyclophosphamide (CY) and thymectomy

CY (Endoxan) was a gift from Shionogi Pharmaceutical Co. (Osaka, Japan). It was dissolved in saline immediately before use and was injected intraperitoneally (i.p.) at a dose of 5 mg/mouse which is equivalent to 195 mg/kg body weight on average. Thymectomy was performed under anaesthesia (Somnopenityl, sodium pentobarbital, Pitman-Moore, Washington Crossing, N.J.).

Statistical analysis

The means and standard errors (SE) were based on the percent ear swelling of each mouse. *P* values were calculated using Student's *t*-test and *P* values smaller than 0.05 were considered to be significant.

RESULTS

Time course of the induction of antigenic competition by the i.v. injection of contact sensitizers

We have recently reported that prepainting with DNFB on the backs of mice suppressed the subsequent induction of CS to PCI (Nakano, 1977). It was of interest to determine whether DNFB can induce antigenic competition when it is given to mice via routes other than painting. For this purpose, mice were either injected i.v. or painted on their backs with 600 μ g of DNFB 7, 3, or 1 day(s) before sensitization with PCI. Mice in one group were sensitized with PCI only. The degree of CS was assessed 5 days after sensitization. Results are shown in Fig. 1. An i.v. injection of DNFB suppressed CS to PCI. The degree of suppression was largest when DNFB was injected 7 days before sensitization with PCI. DNFB given i.v. 3 or 1 day(s) before sensitization with PCI did not suppress CS to PCI, whereas the painting of DNFB 3 days before sensitization significantly suppressed CS to PCI.

Antigenic competition induced with 600 μ g of DNFB given via various routes

An experiment was designed to compare the degree of

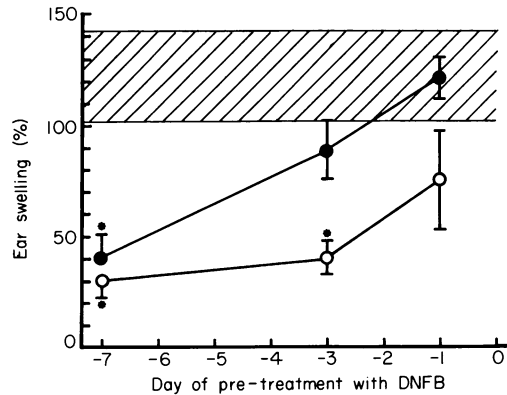


Figure 1. Time course of the induction of antigenic competition by i.v. injection of DNFB. Six groups of mice were injected i.v. with 600 μ g of DNFB (●) or painted with 600 μ g of DNFB (○) various days before sensitization with PCI (day 0). Five days after sensitization, mice were challenged with PCI. Data are presented as mean ear swelling (\pm SE) of five mice 24 h after challenge. The shaded band covers the upper and lower limits of the standard errors of the mean ear swelling of mice sensitized with PCI. *, Significantly different from the control group ($P < 0.05$).

competition by DNFB when this sensitizer was given via various routes. Four groups of mice were given 600 μ g of DNFB by painting, s.c. injection, i.v. injection or oral administration. Seven days later, all the mice were sensitized with PCI and were challenged with PCI 5 days after sensitization. Figure 2 shows that a significant suppression of CS to PCI was induced by painting, s.c. injection or i.v. injection of DNFB. An

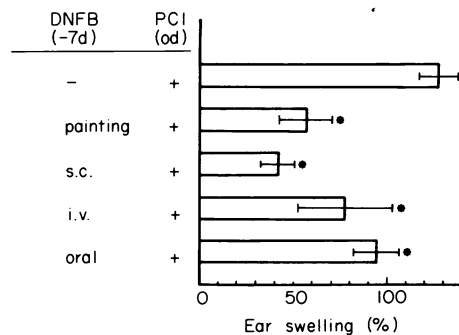


Figure 2. Degree of antigenic competition induced with 600 μ g of DNFB given via various routes. Mice were given DNFB at day -7 via various routes as indicated in the figure. All the mice were sensitized with PCI on their backs at day 0 and were challenged with PCI at day 5. Data are presented as mean ear swelling (\pm SE) 24 h after challenge. *, Significantly different from the control group ($P < 0.05$).

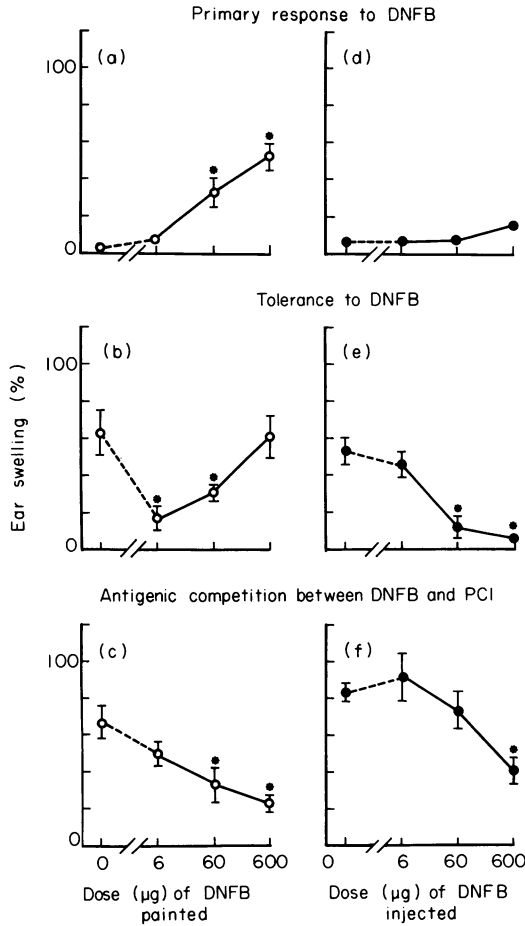


Figure 3. Dose response of DNFB on primary response, tolerance and antigenic competition induced with DNFB given by painting or i.v. injection. *Primary response* (panels a and d); mice were either painted (a) or injected i.v. (d) with various doses of DNFB. Five days later, all the mice were challenged with DNFB. *Tolerance* (panels b and e); mice were either painted (b) or injected i.v. (e) with various doses of DNFB. Seven days later, all the mice were sensitized with DNFB and were challenged with DNFB 5 days after sensitization. *Antigenic competition* (panels c and f); mice were either painted (c) or injected i.v. (f) with various doses of DNFB. Seven days later, all the mice were sensitized with PCI and were challenged with PCI 5 days after sensitization. Data are presented as mean ear swelling (\pm SE) 24 h after challenge. *, Significantly different from the respective control groups ($P < 0.05$).

oral administration of DNFB also caused suppression, but to a lesser extent. It was thus shown that DNFB given via routes other than painting could induce antigenic competition.

Dose response of DNFB or Ox on antigenic competition, primary response and tolerance

Experiments were performed to investigate the dose response of DNFB or Ox on antigenic competition, primary CS and tolerance when these contact sensitizers were either painted or injected i.v. For the examination of antigenic competition, mice were painted or injected i.v. with 6, 60 or 600 µg of DNFB or Ox. In a

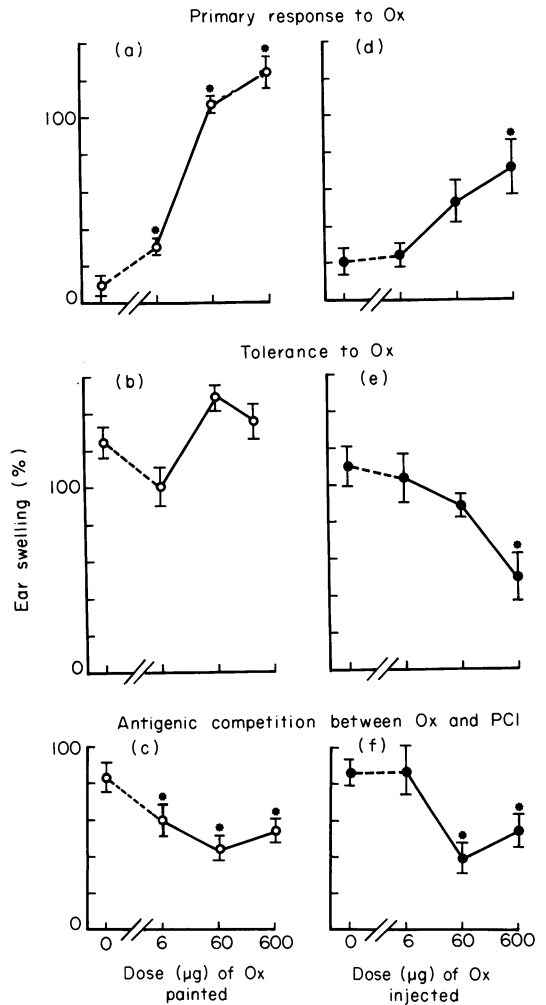


Figure 4. Dose response of Ox on primary response, tolerance and antigenic competition induced with Ox given by painting or i.v. injection. Experimental protocols are the same as described in Fig. 3, except that Ox was used instead of DNFB. Data are presented as mean ear swelling (\pm SE) 24 h after challenge. *, Significantly different from the respective control groups ($P < 0.05$).

preliminary experiment, more than 600 μg of DNFB was found to be toxic to mice when it was given i.v. All the mice were sensitized with PCI 7 days later and were challenged with PCI 5 days after sensitization. Results are shown in panels c and f of Figs 3 and 4. Antigenic competition was strongest when the highest dose of DNFB was administered. The suppressive effect of Ox on CS to PCI was greatest when 60 μg of Ox was administered.

In the next experiment, we compared the degree of antigenic competition with that of primary response. Mice were given DNFB or Ox by i.v. injection or by painting; 5 days later, all mice were challenged with DNFB or Ox, respectively. Results are shown in panels a and d of Figs 3 and 4. DNFB caused CS when it was given by painting. Ox caused CS when it was given by painting or by i.v. injection. From these results, it seems likely that the degree of antigenic competition has a close relationship with the degree of primary response, except that the i.v. injection of DNFB induced poor or no CS, whereas it caused strong antigenic competition. The fact that effector cells are generated by the i.v. injection of DNFB will be shown later.

In order to examine inducibility of tolerance, mice were given DNFB either by painting or i.v. injection and 7 days later, were sensitized with DNFB. Five days thereafter, they were challenged with DNFB. Results are shown in panels b and e of Fig. 3. DNFB given via both routes induced tolerance, although the optimal doses for the induction of tolerance differed; i.e. the lowest dose was most suppressive when DNFB was painted, while the highest dose was most suppressive when it was given i.v. The induction of tolerance with Ox was examined in the same way as described above for the induction of tolerance to DNFB. Results are shown in panels b and e of Fig. 4. Only the i.v. injection of the highest dose of Ox induced tolerance. Thus, it was shown that the degree of antigenic competition had no relationship to that of tolerance.

Evidence for the generation of effector cells by the i.v. injection of DNFB in the mice pretreated with CY

The finding that the i.v. injection of Ox can induce CS (Fig. 4) raised the possibility that effector cells can be induced by the i.v. injection of DNFB. To test this possibility, an experiment was performed in the mice pretreated with CY which is known to eliminate suppressor cells without impairing the function of effector cells in mice (Sy *et al.*, 1977; Asherson *et al.*, 1979).

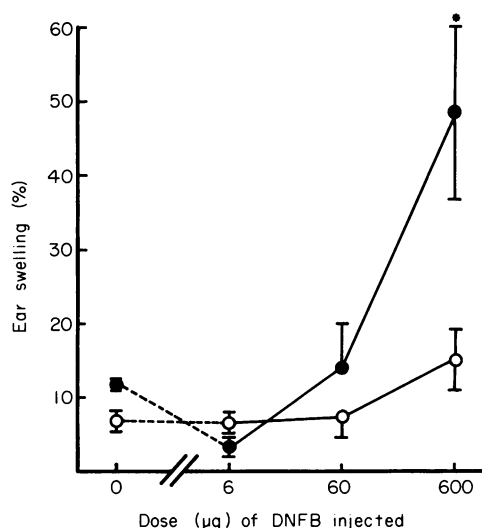


Figure 5. Effect of CY on primary response induced by i.v. injection of DNFB. Mice were either untreated (o) or treated (●) i.p. with 5 mg of CY. Two days later, mice were injected i.v. with various doses (0–600 μg) of DNFB. Five days thereafter, all the mice were challenged with DNFB. Data presented as mean ear swelling (\pm SE) 24 h after challenge. *, Significantly different from the corresponding groups which were not pretreated with DNFB ($P < 0.05$).

Mice which had been either untreated or treated with 5 mg of CY 2 days previously, were injected i.v. with 6, 60 or 600 μg of DNFB. Five days later, all the mice were challenged with DNFB. Figure 5 shows that significant CS was induced by the i.v. injection of DNFB in the mice pretreated with CY.

Effect of CY on antigenic competition induced by the i.v. or oral administration of contact sensitizers

We have previously reported that treatment with CY abolished the antigenic competition induced by painting of DNFB (Nakano & Nakano, 1978). An experiment was undertaken to see if antigenic competition, induced by giving sensitizers via routes other than painting, could be abolished by the treatment with CY. Eight groups of mice were either given 600 μg of DNFB or Ox, i.v. or orally, or were uninjected. Four days later, four groups of mice were given 5 mg of CY i.p. All the mice were sensitized with PCI 3 days after the injection of CY and were challenged with PCI 5 days after sensitization. Figure 6 shows that CY abolished the antigenic competition induced by i.v. or oral administration of contact sensitizers. This indicates

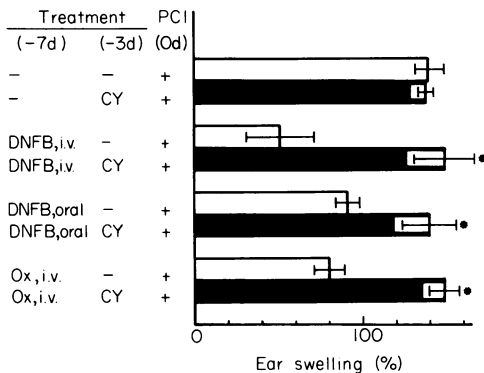


Figure 6. Effect of CY on antigenic competition induced by i.v. or oral administration of contact sensitizers. Eight groups of mice were treated at days -7 and -3 as indicated in the figure. The dose of contact sensitizers administered was 600 µg. All the mice were sensitized with PCI at day 0 on their backs and were challenged with PCI at day 5. Data are presented as mean ear swelling (±SE) 24 h after challenge. *, Significantly different from the corresponding control groups which were pretreated with contact sensitizers on day -7, but not injected with CY on day -3 ($P < 0.05$).

that antigenic competition induced by i.v. injection or oral administration of contact sensitizers might be mediated by suppressor cells.

Effect of adult thymectomy on the induction of antigenic competition or tolerance by the i.v. injection of DNFB

Antigenic competition between DNFB and PCI was previously shown not to occur in the mice thymectomized 6 weeks previously (Nakano & Nakano, 1978). In the following experiment, we examined the effect of

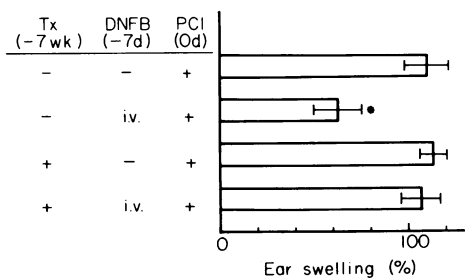


Figure 7. Effect of adult thymectomy on the induction of antigenic competition by i.v. injection of DNFB. Four groups of mice which were either thymectomized or not at -7 weeks were treated with 600 µg of DNFB at day -7 as indicated in the figure. All the mice were sensitized with PCI on their backs at day 0 and were challenged with PCI at day 5. Data are presented as mean ear swelling (±SE) 24 h after challenge. *, Significantly different from the corresponding control groups which were not pretreated with DNFB on day -7 ($P < 0.05$).

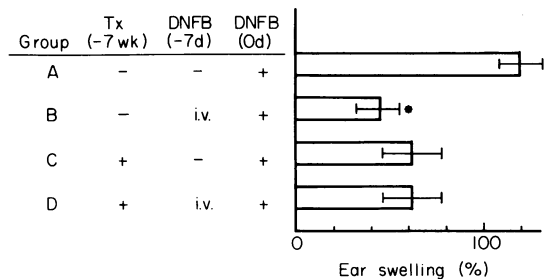


Figure 8. Effect of adult thymectomy on the induction of tolerance by i.v. injection of DNFB. Four groups of mice which were either thymectomized or not at -7 weeks, were treated with 600 µg of DNFB at day -7 as indicated in the figure. All the mice were sensitized with DNFB on their backs at day 0 and were challenged with DNFB at day 5. Data are presented as mean ear swelling (±) 24 h after challenge. *, Significantly different from the corresponding control groups which were not pretreated with DNFB on day -7 ($P < 0.05$).

adult thymectomy on antigenic competition induced by i.v. injection of DNFB. Mice which had been thymectomized 6 weeks previously, at the age of 5 weeks, were injected with 600µg of DNFB i.v. Seven days later, sensitization was done with PCI. All the mice were challenged with PCI 5 days after sensitization. As shown in Fig. 7, antigenic competition was abolished by adult thymectomy. Thus, antigenic competition induced by i.v. injection of DNFB seems to be attributable to relatively short-lived T cells whose population tends to decrease after thymectomy.

The effect of adult thymectomy on the induction of tolerance was investigated. Mice which had been thymectomized 6 weeks previously were injected with 600 µg of DNFB i.v. Seven days later, they were sensitized with DNFB and were challenged 5 days after sensitization. As shown in Fig. 8, the level of CS to DNFB was reduced significantly in normal mice by the pre-injection of DNFB (groups A and B). However, the level of CS to DNFB was not reduced by the injection of DNFB in thymectomized mice (groups C and D), although the degree of CS to DNFB in the mice which was not pre-injected with DNFB was lowered by thymectomy (groups A and C). Thus, the i.v. injection of DNFB did not induce antigenic competition or tolerance in thymectomized mice.

DISCUSSION

In this paper, we have shown that antigenic competition can be induced not only by painting contact sensitizers but also by the administration of contact

sensitizers via various routes, such as i.v., s.c. or oral routes. The results, that antigenic competition is abolished by CY and thymectomy (Figs 6 and 7) suggest that some type of suppressor cells may be involved in the antigenic competition induced by the i.v. injection of DNFB. However, conclusive evidence for participation of suppressor cells in the present phenomenon requires a transfer experiment using an inbred strain of mice.

Recently, the auxiliary cells which appear after painting with a supraoptimal dose of DNFB were shown to be sensitive to CY and thymectomy (Sy, Miller, Moorhead & Claman, 1979). It is possible that these cells may be involved in the present phenomenon. However, the phenomenon of antigenic competition and suppression by a supraoptimal dose of DNFB differ markedly in respect of the specificity of the phenomena. That is, the suppression by a supraoptimal dose of DNFB is antigen-specific and the antigenic competition is non-specific. Since it is shown that macrophages incubated with antigen-specific T suppressor factor liberated a non-specific factor when exposed to antigen (Ptak, Zembala, Hanczakowski-Rewicka & Asherson, 1978), the possibility cannot be excluded that the mechanism of antigenic competition induced by i.v. injection of DNFB could involve an antigen-specific factor(s).

Cross-reactivity between DNP-specific and TNP-specific antibodies is well documented (Little & Eisen, 1969). We did not observe any cross-reactivity between DNP and TNP determinants in primary CS (Nakano, 1977). However, we do not know yet whether there is a cross-reactivity between them in tolerance induction. There is a possibility that the depressed CS to PCI, which is induced by the i.v. injection of DNFB, could be due to cross-tolerance by DNFB. Therefore, we tried to see if the induction of tolerance and of antigenic competition can be segregated in the thymectomized mice, but to the contrary, it was shown that both tolerance and antigenic competition were abolished in the mice thymectomized 6 weeks prior to the injection of DNFB. We cannot, therefore, exclude the possibility that DNP-specific suppressor cells induced by the i.v. injection of DNFB may partially depress CS to PCI. However, this possibility does not seem likely, since i.v. injection of Ox also depressed CS to non-cross-reacting PCI (Fig. 4).

The conditions for the induction of CS and of tolerance have been investigated by some authors, and it was found that the anatomical site where contact sensitizers are applied is one of the important factors

in determining whether tolerance or CS is induced. For example, painting of DNFB caused strong CS, whereas an i.v. injection of DNFB induced tolerance (Claman, 1976). TNP-conjugated macrophages induced CS when injected s.c. (Greene, Sigimoto & Benacerraf, 1978), whereas TNP-conjugated macrophages caused tolerance when injected i.v. (Ptak & Różycka, 1977). Another important factor is whether or not contact sensitizers are presented on epidermal Langerhans cells. Ptak *et al.* (1980) showed that haptened skin Langerhans cells elicit CS, even if they are injected i.v. Toews *et al.* (1980) have clearly shown that the density of Langerhans cells on skin is critical in determining whether CS or tolerance is induced by painting. We made an unexpected observation when we employed Ox: strong CS to Ox was induced when mice were injected i.v. with Ox (Fig. 4). Because Langerhans cells are not likely to be involved in this response, it is possible that there may be other antigen-presenting cells in spleen. Ptak *et al.* (1980) have suggested that dendritic cells in spleen may also have an antigen-presenting capacity in CS.

In the present study, DNFB did not induce CS when it was injected i.v. in normal mice. However, it was shown that DNFB has the ability to induce CS by i.v. injection in CY-pretreated mice (Fig. 5). On the action of CY, two possibilities can be considered at present. One is that the i.v. injection of DNFB may favourably induce suppressor cells which prevent the generation of effector cells. The other is that CY may enhance the ability of a limited number of effector cells to cause CS by increasing the number of circulating monocytes which play a role in the expression of delayed hypersensitivity (Milon & Marchal, 1978).

In connection with Langerhans cells, the fact that antigenic competition can be induced even when contact sensitizers were applied to mice to bypass Langerhans cells, suggests that these cells may not be indispensable to the induction of antigenic competition, although the mechanism, leading to antigenic competition after i.v. injection of DNFB or Ox, requires further investigation.

In the present study, results on the dose response of the induction of antigenic competition seem to indicate that the degree of antigenic competition has a close relationship with that of primary response. The finding that i.v. injection of DNFB did not induce primary response, does not seem to agree with this statement. However, it was shown that DNFB has a capacity to sensitize mice (Fig. 5) even when given i.v., although the capacity of mice to develop and/or

express CS may be blocked by a mechanism involving suppressor cells.

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