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Suppressor cells in antigenic competition in contact allergy in mice

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Summary. The effects of cyclophosphamide (CY), thymectomy and splenectomy on the antigenic competition in contact hypersensitivity between dinitrofluorobenzene (DNFB) and picryl chloride (PCl) was investigated. Contact sensitivity to PCl was suppressed by a prior painting of DNFB. When these two sensitizers were painted 7 days apart and CY was injected 3 days after the painting of DNFB, the antigenic competition did not occur. On the other hand, when CY was injected 3 days before painting of DNFB, the antigenic competition was partially abolished.

The antigenic competition was seen in mice 2 weeks after adult thymectomy and in splenectomized mice. However, antigenic competition did not occur in mice 6 weeks after thymectomy. These results suggest that some thymus-derived cells may be involved in the antigenic competition in contact sensitivity.

INTRODUCTION

Antigenic competition is known to be a widely recognizable immunological phenomenon. However, the mechanism of the phenomenon cannot be explained in a single way (Pross & Eidinger, 1974). In the antibody response, two possible mechanisms have been proposed. One is the competition at the level of macrophages. This was proposed by Brody & Siskind (1972), Taussig & Lachmann (1972) and Feldmann & Schrader (1974). The other is the participation of suppressor cells or suppressive factors, as was suggested by Gershon & Kondo (1971) and Thomas, Roberts & Talmage (1975).

Antigenic competition also occurs in the delayed-type hypersensitivity (DTH) to skin sensitizing agents (Wallington & Verrier Jones, 1974; Nakano, 1977). In a previous paper (Nakano, 1977), it was shown that the pretreatment of mice with 2,4-dinitrofluorobenzene (DNFB) interfered with the subsequent sensitization with picryl chloride (PCl), and that 2,4-dinitrobenzenesulphonic acid sodium salt (DNBS) inhibited the development of effector cells to DNFB, but this did not affect the competitive effect of DNFB on the response to PCl. It was also observed that antigenic competition still occurred when the second sensitizer was painted on a site remote from the first sensitizer. These results seem to suggest that suppressor cells or suppressive factors may be involved in antigenic competition in contact sensitivity.

This paper is concerned with the effect of cyclophosphamide (CY) on antigenic competition, since CY-treatment is known to deplete the activity of some types of suppressor cells (Turk & Parker, 1973; Polak & Turk, 1974; Polak, Geleik & Turk, 1975). The results to be presented show that CY limits the effect of antigenic competition on the delayed-type

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hypersensitivity. The effects of splenectomy and thymectomy on antigenic competition were also investigated.

MATERIALS AND METHODS

Animal

Closed colony ICR mice of both sexes were used. These mice were 7–11 weeks old at the time of sensitization. Five mice were included in each experimental group unless otherwise stated.

Sensitization

Picryl chloride (PCl, Tokyo Kasei Co., Tokyo, Japan) and 2,4-dinitrofluorobenzene (DNFB, Katayama Chemical Co., Osaka, Japan) were used as sensitizers for contact sensitivity. These sensitizers were dissolved in absolute ethanol. Sensitization was performed by painting with 0·05 ml of 5% (w/w) PCl or DNFB in ethanol on clipped-backs or rear footpads of mice using a 1 ml-syringe.

Challenge

PCl or DNFB was dissolved in olive oil (1%, w/w) and 0·1 ml of each solution was smeared on both sides of right ear of mice with cotton wool (1 × 5 × 10 mm).

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. The degree of DTH to contact sensitizers in mice was assessed by the degree of ear swelling 24 h after challenge (Nakano, 1977). The average ear thickness of 30 normal 7–11 weeks old mice used in these experiments was 21·5 ± 0·2 ($\times 10^{-2}$ mm).

Tolerance induction

In order to induce tolerance to DNFB, 15 mg of 2,4-dinitrobenzenesulphonic acid sodium salt (DNBS, Tokyo Kasei Chemical Co., Tokyo, Japan) in saline was injected intravenously (i.v.) into mice 7 days before sensitization with DNFB.

Cyclophosphamide

CY (Endoxan) was a gift from Schionogi Pharm., Co. (Osaka, Japan) and dissolved in saline immediately before used and was injected intraperitoneally (i.p.) at the dose of 5 mg/mouse.

Thymectomy and splenectomy

Thymectomy and splenectomy were preformed under the anaesthesia with Nembutal (Sodium pentobarbital, Abott Lab., North Chicago, Ill.). At the end of experiments, all the thymectomized mice were autopsied and no thymic remnants were found in these mice.

Statistical analysis

The means and standard errors were based on the percent ear swelling of each mouse. P values were calculated using the Student's t-test. All the comparisons reported were significant ($P \leqslant 0{\cdot}05$).

RESULTS

Effect of CY on contact sensitivity

The effect of CY on DTH to DNFB was investigated and the results are shown in Fig. 1. Mice were sensitized with 5% DNFB on their backs and 5 days later challenged with 1% DNFB on their ears. These mice showed significant ear swelling at 24 h after challenge (positive control; Group B). Effect of CY was examined by injecting CY (5 mg/mouse) intraperitoneally 3 days before or 3 days after the sensitization with DNFB. The DTH to DNFB in the mice injected with CY before sensitization was higher than that in the mice which were not injected with CY (Group C). On the contrary, the DTH to DNFB in the mice injected with CY 3 days after sensitization was completely depressed (Group D). Thus, CY produced either augmenting or suppressing effects according to the timing of its administration.

Three groups of mice (Group E, F, G in Fig. 1) were injected i.v. with 15 mg of DNBS 7 days before sensitization with DNFB. Two out of these groups were also injected with CY 3 days before or 3 days after sensitization with DNFB. All the mice were sensitized with DNFB. DNBS completely suppressed the DTH to DNFB (Group E). When mice were injected with CY 3 days after DNBS, the suppressive effect of DNBS disappeared (Group F). In Group G, CY was administered 10 days after

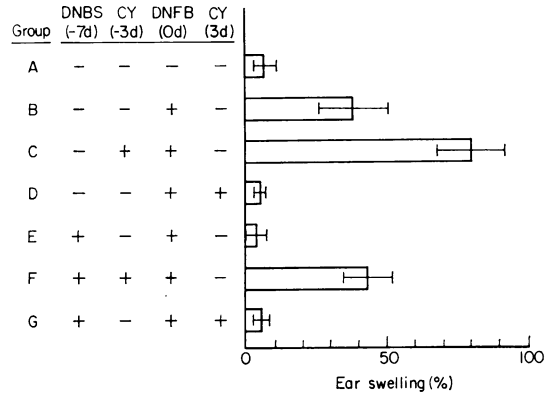

Figure 1. Effect of CY on contact sensitivity to DNFB. Seven groups of mice were treated as indicated in the figure. The numbers in parentheses show the day of treatment. DNFB was painted on the back of mice. All the mice were challenged with DNFB at day 5. The length of each column represents the mean ear swelling of five mice at 24 h after challenge. Each horizontal bar represents the standard error of the mean.

DNBS (i.e. 3 days after DNFB). The reactivity of these mice was not increased. This strongly suggested that DNBS did not affect the precursor of effector cells but induced suppressor cells interfering with the generation of effector cells.

Effect of CY on antigenic competition

As shown in a previous paper (Nakano, 1977), painting of 5% DNFB on the back of mice 7 days before sensitization with 5% PCl painted at the same site resulted in a significant depression of the development of DTH to PCl (Groups B and D in Fig. 2). The effect of CY on this phenomenon was examined. Mice were injected with CY either 3 days before or 4 days after painting of DNFB. All the mice were sensitized with PCl 7 days after the DNFB, painting. Results are shown in Fig. 2. The mice injected with CY 4 days after DNFB painting responded as well to PCl as the mice sensitized only with PCl (Groups C and E). In the mice injected with CY 3 days before DNFB, the suppressive effect of DNFB was partially reduced (Groups D and F).

Thus, CY administered after painting of DNFB completely repressed the suppressive effect of DNFB on the DTH to PCl. As shown in Fig 1, CY also inhibited the development of effector cells to DNFB. These results may imply that certain cell populations other than effector cells participate in antigenic competition in the contact sensitivity.

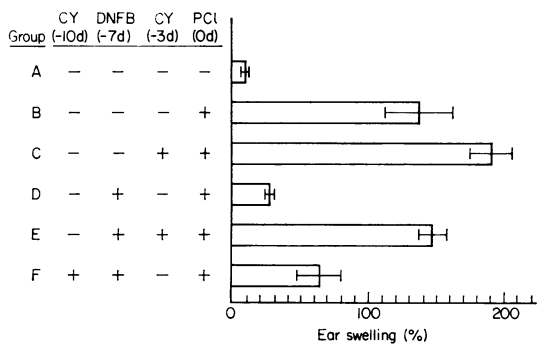

Figure 2. Effect of CY on antigenic competition between DNFB and PCl. Six groups of mice were treated as indicated in the figure. The numbers in parentheses show the day of treatment. DNFB and PCl were painted on the back of mice. All the mice were challenged with PCl at day 5. Each column represents the mean ear swelling of five mice at 24 h after challenge. Each horizontal bar represents the standard error of the mean.

Effect of thymectomy on antigenic competition

The occurrence of antigenic competition in mice which were painted with DNFB immediately before the sensitization with PCl was shown in a previous paper (Nakano, 1977). The effect of thymectomy on the antigenic competition was examined in this schedule. Mice were thymectomized at the age of

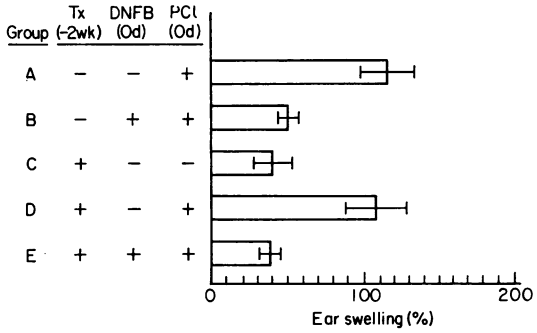

Figure 3. Effect of thymectomy performed 2 weeks before used for antigenic competition. Five groups of mice were treated as indicated in the figure. The numbers in parentheses show the week (wk) or the day (d) of treatment. DNFB and PCl were painted on the back of mice. All the mice were challenged with PCl at day 5. Each column represents the mean ear swelling of mice at 24 h after challenge. Each horizontal bar represents the standard error of the mean. Groups B and E consisted of three and six mice, respectively. The others consisted of five mice.

5 weeks and 2 or 6 weeks later, these thymectomized mice were painted on their backs with DNFB. Immediately afterwards, sensitization with PCl on the same site was performed. As shown in Fig. 3, antigenic competition between DNFB and PCl was observed when mice thymectomized 2 weeks before were used (Groups B and E). Antigenic competition, however, did not occur when mice used 6 weeks after thymectomy (Groups C and F in Fig. 4). In both experiments the response to PCl in DNFB-untreated mice was unaffected by thymectomy.

Thus, antigenic competition seems to be attributable to relatively short lived T cells whose population tends to decrease after thymectomy.

Effect of thymectomy on tolerance induction

Mice were thymectomized at the age of 5 weeks. Six weeks later, thymectomized mice were injected with 15 mg of DNBS intravenously. One week thereafter, they were painted on their backs with DNFB. Unoperated mice and thymectomized mice not receiving DNBS injections served as controls. As shown in Fig. 5 treatment with DNBS could suppress, irrespective of thymectomy, the level of the DTH to DNFB (Group F). Thus, thymectomy did not interfere with the induction of tolerance to DNFB by DNBS; in contrast, the suppressor cells in antigenic competition seemed to be eliminated by thymectomy (Fig. 5).

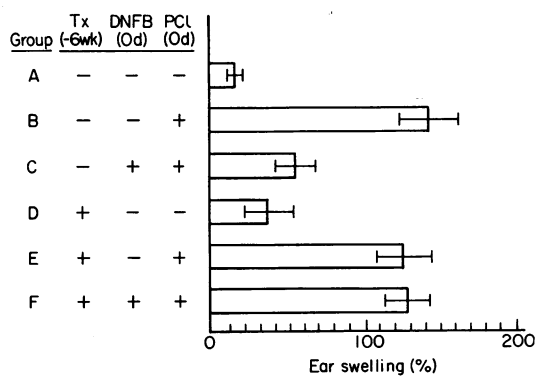

Figure 4. Effect of thymectomy performed 6 weeks before used for antigenic competition. Six groups of mice were treated as indicated in the figure. The numbers in parentheses show the week (wk) or the day (d) of treatment. DNFB and PCl were painted on the back of mice. All the mice were challenged with PCl at day 5. The length of each column represents the mean ear swelling at 24 h after challenge. Each horizontal bar represents the standard error of the mean. Group E consisted of four mice. The others consisted of five mice.

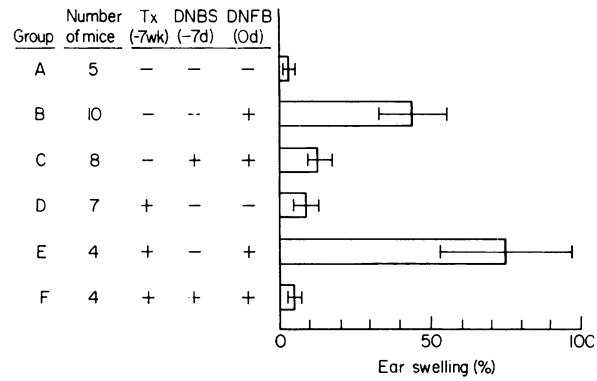

Figure 5. Effect of thymectomy performed 6 weeks before used for tolerance induction to DNFB. Six groups of mice were treated as indicated in the figure. The numbers in parentheses show the week (wk) or the day (d) of treatment. DNFB was painted on the back of mice. All the mice were challenged with PCl at day 5. The length of each column represents the mean ear swelling of mice at 24 h after challenge. Each horizontal bar represents the standard error of the mean.

Effect of splenectomy on antigenic competition

The results shown hitherto indicate that the suppressive effect of DNFB on the response to PCl is abrogated by CY. This seems to suggest that DNFB causes the development of nonspecific suppressor cells for DTH. Localization of some type of suppressor cells mainly in spleen was shown in the experiment of Scott (1974) that the reduced DTH to sheep erythrocytes induced by the pretreatment with *Propionibacterium acnes* (*Corynebacterium parvum*) was restored by splenectomy. Therefore, the effect of splenectomy on antigenic competition was investigated. Four groups of mice were splenectomized. Six days later, two groups of mice were painted with DNFB. Immediately thereafter, all the mice were sensitized with PCl. Results are shown in Fig. 6. Antigenic competition still occurred in the splenectomized mice whether DNFB and PCl were painted on the same footpad (Group C) or on the opposite footpad (Group D). In the following experiments, splenectomy was performed 3 days after the painting of DNFB. These mice were sensitized with PCl 4 days thereafter. Results are shown in Fig. 7. The mice painted with competing DNFB showed depressed DTH to PCl despite splenectomy.

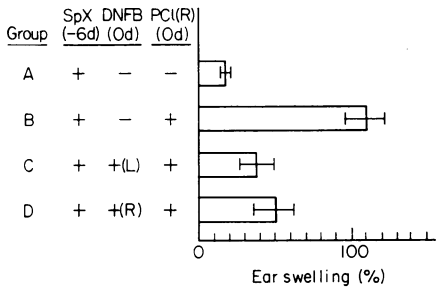

Figure 6. Effect of splenectomy on antigenic competition. Four groups of mice were treated as indicated in the figure. The numbers in parentheses show the day of treatment. DNFB was painted on the right (R) or left (L) footpads of mice. PCl was painted on the right (R) footpads of mice. All the mice were challenged with PCl at day 5. The length of each column represents the mean ear swelling at 24 h after challenge. Each horizontal bar represents the standard error of the mean. Group A consisted of four mice. The others consisted of five mice.

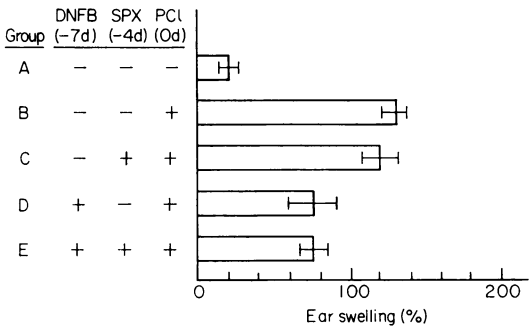

Figure 7. Effect of splenectomy on antigenic competition. Five groups of mice were treated as indicated in the figure. The numbers in parentheses show the day of treatment. DNFB and PCl were painted on the right footpad of mice. All the mice were challenged with PCl at day 5. The length of each column represents the mean ear swelling at 24 h after challenge. Each horizontal bar represents the standard error of the mean. Groups B and E consisted of three and six mice respectively. The others consisted of five mice.

Thus, splenectomy did not prevent antigenic competition whether the operation was performed before or after painting with the competing sensitizer.

DISCUSSION

Recently several suppressor cell systems affecting humoral or cell-mediated immunity have been reported. CY is reported to inactivate some type of suppressor cells, e.g. suppressor T-cells causing tolerance to contact sensitizing agent (Polak & Turk, 1974) and suppressor cells generated by the contact sensitizing agent (Turk, Parker & Poulter, 1972; Zembala & Asherson, 1976). In a previous paper, Nakano (1977) showed that although there was a greater degree of cross-reactivity between anti-DNP and anti-TNP antibody (Little & Eisen, 1969), there was no cross-reactivity between DNFB and PCl in contact sensitivity and that antigenic competition did occur between these sensitizing agents. In the present study the effect of CY on antigenic competition between DNFB and PCl was investigated. It was found that CY was effective in preventing antigenic competition, if CY was administered 4 days after the painting of competing sensitizer DNFB.

It seems likely from the present study that the antigenic competition in contact sensitivity is not caused by the competition between effector cells of different specificities but attributable to some type of suppressor cells induced by the competing sensitizer. The finding that antigenic competition was observed even in the mice made tolerant to the competing sensitizer and that the antigenic competition was reversed by the administration of CY support the possibility of the intervention of suppressor cells in antigenic competition.

Some authors showed that suppressor cells are located preferentially in spleen in comparison with lymph nodes (Gershon *et al.*, 1971; Scott, 1974). Scott demonstrated that the DTH to sheep erythrocytes reduced by the pretreatment with *Corynebacterium parvum* was restored by splenectomy. However, the putative suppressor cells responsible for the antigenic competition in the present study were shown to be present in some site other than the spleen.

Fig. 4 demonstrates that antigenic competition does not occur in the adult-thymectomized mice. This may appear that thymus-derived and relatively short-lived cells function as suppressors. Asherson *et al.* (1976) showed that the loss of suppressive activity in tolerized mice by adult-thymectomy was not due to the removal of relatively short-lived T cells but to the removal of the source of thymus hormone which might increase the activity of suppressor cells (Asherson, *et al.*, 1976). Suppressor cells in antigenic competition might also be dependent on thymus hormone and not belong to

a short-lived population which are thymus-derived. Moreover, we cannot conclude that suppressor cells were exclusively T cells. Recent studies have revealed that even B cells may play a suppressive role in delayed hypersensitivity. Turk *et al.* (1972) and Parker (1973) showed in guinea-pigs that CY-treatment increased the intensity of contact sensitization and Jones-Mote reaction which were modulated by a B-cell response. Parker, Katz & Turk (1975) showed directly by the transfer of cells separated by anti-guinea-pig IgG-coated column that B cells suppressed the T-cell function in Jones-Mote reaction. Zembala *et al.* (1976) demonstrated in mice that suppressor B-cells which suppress the function of effector T-cells specific for PCl were produced after the painting of PCl. On the contrary, Phanuphak, Moorhead & Claman (1974) and Zembala & Asherson (1974) demonstrated that suppressor cells produced by tolerance induction in mice were T cells.

The results obtained in the present study strongly suggest that the suppressor in antigenic competition is not generated as a result of the differentiation of effector cells. Suppressor cells reported by Zembala *et al.* (1976) were also generated after the painting of PCl. However, these suppressor cells may be different from the suppressor in the antigenic competition in our experiment, since the latter seems to be generated earlier than the former after PCl-painting. As shown in Fig. 3, the antigenic competition does occur even if the competing agent was given shortly before the sensitizing agent on the same day (Nakano, 1977). This indicated that the suppressor must be produced to function soon after the painting of competing sensitizer. On the other hand, Zembala's suppressor cells were reported to be produced between 5 days and 12 days after the painting of PCl.

In our experimental system, adult-thymectomy did not interfere with the induction of tolerance to DNFB by DNBS, in contrast, the suppressor cells in antigenic competition seemed to be eliminated by adult-thymectomy. This seems to indicate that the suppressor involved in the tolerance and that in the antigenic competition are derived from different cell populations. However, Asherson & Zembala (1974) showed that the picrylsulphonic acid-induced suppressor factor in tolerance depressed nonspecifically DTH to oxazolone when normal mice were injected with peritoneal cells armed with suppressor factor and challenged with a mixture of PCl and oxazolone. In our experimental system, we also cannot exclude the possibility that the suppressor involved in tolerance may have some effect causing the suppression of antigenic competition.

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