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*BRIEF COMMUNICATION*

# Tolerance to a Defined Chemical Hapten Produced in Adult Guinea-Pigs after Thymectomy

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**Summary.** Specific immunologic tolerance to the picryl hapten was induced in thymectomized adult guinea-pigs by a single intravenous injection of picrylated erythrocyte stromata, demonstrating that the lymphoid system may be rendered tolerant independently of the thymus. This dual unresponsiveness, involving circulating antibody as well as contact delayed reactivity to the hapten, cannot be differentiated from tolerance similarly induced in normal animals.

## INTRODUCTION

The thymus is now well known to have a central controlling influence over the development of an immunologically competent population of lymphoid cells in several species of laboratory animals. Although attention has been focussed primarily on the striking retardation in immunological development which results from the removal of the thymus in neonatal animals (Miller, 1963), the thymus may have equally important functions in the adult animal. Thus, the thymus has been shown to mediate recovery from immunological depression produced in adult mice either by total X-irradiation (Miller, 1962b) or by the administration of rabbit antiserum to mouse lymphocytes (Monaco, Wood and Russel, 1965). A possible relationship of the thymus to some states of immunological tolerance was first suggested by Miller (1962a), who demonstrated that neonatally thymectomized mice grafted with foreign thymus tissue readily accept grafts of thymus donor-type skin, but not of other types. More specifically, Claman and Talmadge (1963) have shown the thymic dependency of the recovery of the adult mouse from a state of experimentally induced specific immunologic tolerance, while Isakovic, Smith and Waksman (1965) have reported that thymectomized irradiated rats are specifically tolerant after receiving thymus tissue grafts from tolerant donors. No experimental evidence, however, has yet clarified the relationship of the thymus to the induction or development of tolerant states in the immunologically mature animal. The present work was carried out to determine whether the presence of the thymus was necessary or important for the induction of immunological tolerance in young adult guinea-pigs.

## MATERIALS AND METHODS

*Thymectomy and induction of tolerance*

Initially sixteen female Hartley strain guinea-pigs of an average weight 350 g were surgically thymectomized and the removed organ histologically identified as thymus by routine stained section. Five days later, ten of the animals were subjected to a single

intrajugular injection of 2 ml of a suspension of 2·5 mg of guinea-pig erythrocyte stromata to which 90 μg of the chemical hapten, picryl chloride (PCl), had been chemically coupled *in vitro*. Using this procedure, we have shown membrane-coupled hapten (MCH) to produce specific immunologic tolerance in 75 per cent of treated animals (Battisto and Bloom, 1966). The remaining six thymectomized guinea-pigs were left as controls.

*Attempted sensitization*

After a rest period of 14 days following the intravenous pretreatment described above, the pretreated animals as well as the controls were given seven daily intradermal injections of PCl in 1 per cent ethanol solution (2·5 μg/0·1 ml inoculum), a standard sensitization procedure in our laboratory.

*Test for delayed response to contact*

Eight days following the last injection of the sensitization series, skin tests for delayed reactivity to contact were carried out in both pretreated and control groups by cutaneous applications of the allergen PCl in olive oil at separate sites on the back in concentrations of 1 and 0·33 per cent by weight. Skin reactions were read 24 and 48 hours later and animals graded in reactivity according to the degree of erythema and induration.

*Test for circulating antibody response*

To determine whether intravenous pretreatment of thymectomized guinea-pigs with membrane-coupled hapten produced hapten-specific tolerance involving the absence of circulating antibody, the animals of both groups were test bled 10 days after the last sensitizing injection and the sera screened for antibody by passive cutaneous anaphylaxis (PCA). Aliquots of 0·1 ml of all sixteen sera were injected intradermally into the backs of 250-g Hartley guinea-pigs. Reactions were elicited 18 hours later by intravenous injection of these animals with 5 mg of picrylated casein in 2 ml of a 0·5 per cent Evans blue dye solution and the diameters of the reactions recorded 30 minutes later. Sera reactive in the undiluted state were diluted 1 : 3 and 1 : 10 in saline and were re-tested.

## RESULTS

The results of the tests for delayed response to contact after the intradermal series of challenges given to both pretreated and control animals demonstrate that eight of ten animals pretreated with intravenous injections of picrylated stromata were rendered tolerant to the hapten. These animals showed no reactions to the weaker concentration of PCl in olive oil (Table 1). At the 1 per cent application site, four of the unresponsive animals showed no reactivity, while four showed trace reactions. Two animals of the test group failed to become tolerant and showed significant erythema, although no induration, at both test sites. Of the control group, all six animals showed significant erythematous reactions to weak as well as strong solutions of hapten and two highly-reactive animals showed noticeable induration at the reaction site of the 1 per cent application.

The intravenously pretreated animals proved equally tolerant with respect to circulating antibody production. Of the ten animals pretreated with intravenous MCH, seven showed no PCA reactivity in their sera, two showed moderate serum reactivity and only one produced a high antibody titre (Table 1). In contrast to the test group, four of the six control animals responded with high antibody titres, one animal had a moderate titre and one did not react. In keeping with previously reported evidence of the independence of

TABLE 1

INDUCTION OF IMMUNOLOGICAL TOLERANCE IN ADULT THYMECTOMIZED GUINEA-PIGS BY PRETREATMENT WITH MEMBRANE-COUPLED PICRYL HAPTEN (MCH)

| Treatment prior to attempted sensitization | Contact delayed responses | | | PCA antibody responses | | |
|---|---|---|---|---|---|---|
| | None* (tolerant) | Moderate reactivity | High reactivity | None† (tolerant) | Moderate reactivity | High reactivity |
| Thymectomy and i.v. MCH | 8/10‡ | 2/10 | 0/10 | 7/10 | 2/10 | 1/10 |
| Thymectomy only (controls) | 0/6 | 4/6 | 2/6 | 1/6 | 1/6 | 4/6 |

* In the grading of delayed responses, tolerant animals are those non-reactive to either 500 μg (1 per cent) or 167 μg (0·33 per cent) applications of PCl; moderate reactors show erythema at both sites; high reactors show erythema at both sites with induration at the 500 μg site.

† In the grading of antibody responses, tolerant animals are those whose sera show no reactivity; moderate reactors have sera reactive up to 1 : 3 dilution; high reactors have sera reactive at 1 : 10 dilution or above.

‡ Numbers represent the number of animals in the subgroup listed out of the total number of animals in the group.

delayed-type hypersensitivity and the formation of circulating antibody (Battisto and Chase, 1965), we observed that one animal rendered tolerant for delayed sensitivity to picryl chloride possessed a high titre (1 : 10 or above) of PCA antibody.

*Specificity of the tolerant state*

The specificity of the tolerant state for picryl chloride was demonstrated by subjecting the animals of both groups to sensitization with an unrelated antigen, bovine γ-globulin (BGG). Test and control animals received intradermal injections of 2·5 μg BGG into all four footpads and were tested intradermally with the antigen 3 weeks later. As has been previously reported in normal guinea-pigs tolerant to PCl (Battisto and Bloom, 1966), both test and control groups of animals responded to BGG sensitization with equal frequency and intensity.

*Tenacity of the tolerant state*

A further procedure was carried out on animals of the test group to examine the tenacity of the tolerant state we had induced in the thymectomized guinea-pigs. The four animals from the test group in which no trace of reactivity had been elicited were each injected with an emulsion of Freund's complete adjuvant and picrylated guinea-pig erythrocyte stromata and then painted weekly with 1 per cent PCl in olive oil on the skin. In normal guinea-pigs this vigorous method of combined sensitization produces exquisite degrees of reactivity (Chase, 1954). All four of the guinea-pigs thus treated in the present experiment remained tolerant.

## DISCUSSION

Of several theories proposed to explain artificially induced specific immunological tolerance, one often expressed describes the mechanism of tolerance as an interference with the lymphoid cell's capacity to respond to an immunologic stimulus (cf. Medawar, 1961). This has been supposed to be dependent upon contact of the cell with antigen or hapten either in a special form, at a special site *in vivo* or at a special stage of the cell's development. The effectiveness of microgram quantities of membrane-coupled hapten for the induction of tolerance in adult guinea-pigs, as shown in our laboratory (Battisto and Bloom, 1966), provides evidence that tolerogenic antigen or hapten need not be present in soluble form. Results of the present experiment in particular indicate that the thymus is unnecessary for induction or for maintenance of the tolerant state in adult animals. We

are unable to substantiate the view (Isakovic *et al.*, 1965) that tolerance may require entry of injected antigen into the thymus and its persistence there at a critical site. On the contrary, our findings show that the peripheral lymphoid tissue in normal animals can be rendered tolerant independently of the thymus. Nonetheless, it seems clear that the thymus is responsible for seeding the lymphoid tissues with immunologically competent cells (Miller, 1963), and that a thymic humoral factor may aid in the development or potentiation of their immunologic competence (Osoba and Miller, 1964; Osoba, 1965). The question, however, of whether the thymus possesses a special relationship to immunologic tolerance which is distinct from that maintained by other central lymphoid tissues remains problematic.

## REFERENCES


BATTISTO, J. R. and BLOOM, B. R. (1966). 'Mechanism of immunologic unresponsiveness: A new approach.' Federation Symposium on *Immunologic Tolerance to Defined Antigens and Hapten*, Atlantic City, New Jersey, *Fed. Proc.*, **25**, 152.

BATTISTO, J. R. and CHASE, M. W. (1965). 'Induced unresponsiveness to simple allergenic chemicals.' *J. exp. Med.*, **121**, 591.

CHASE, M. W. (1954). 'Experimental sensitization with particular reference to picryl chloride.' *Int. Arch. Allergy*, **5**, 163.

CLAMAN, H. N. and TALMADGE, D. W. (1963). 'Thymectomy: prolongation of immunological tolerance in the adult mouse.' *Science*, **141**, 1193.

ISAKOVIC, K., SMITH, S. B. and WAKSMAN, B. H. (1965). 'Immunologic tolerance in thymectomized, irradiated rats grafted with thymus from tolerant donors.' *Science*, **148**, 1333.

MEDAWAR, P. B. (1961). 'Theories of immunological tolerance.' *Ciba Foundation Symposium on Cellular Aspects of Immunity*, p. 134. Little-Brown, Boston.

MILLER, J. F. A. P. (1962a). 'Role of the thymus in transplantation immunity.' *Ann. N.Y. Acad. Sci.*, **99**, 340.

MILLER, J. F. A. P. (1962b). 'Immunological significance of the thymus of the adult mouse.' *Nature (Lond.)*, **195**, 1318.

MILLER, J. F. A. P. (1963). 'Role of the thymus in immunity.' *Brit. med. J.*, **ii**, 459.

MONACO, A. P., WOOD, M. L. and RUSSEL, P. S. (1965). 'Adult thymectomy: effect on recovery from immunologic depression in mice.' *Science*, **149**, 433.

OSOBA, D. (1965). 'The effects of the thymus and other lymphoid organs enclosed in millipore diffusion chambers on neonatally thymectomized mice.' *J. exp. Med.*, **122**, 633.

OSOBA, D. and MILLER, J. F. A. P. (1964). 'The lymphoid tissues and immune responses of neonatally thymectomized mice bearing thymus tissue in millipore diffusion chambers.' *J. exp. Med.*, **119**, 177.