NOTES

TRANSIENT DELAYED-TYPE HYPERSENSITIVITY INDUCED BY LIGHTLY LIPID-CONJUGATED BSA AND ITS CONVERSION INTO SUSTAINED NATURE BY CYCLOPHOSPHAMIDE TREATMENT IN GUINEA PIGS

Injection of bovine serum albumin (BSA) heavily conjugated with dodecanoic acid, a saturated straight chain fatty acid, has been found to induce strong sustained delayed-type hypersensitivity (DTH) in guinea pigs. DTH was specific for BSA and was not accompanied with detectable immune response to the lipid moieties (Coon and Hunter, 1973). Since it was able to induce strong DTH and no detectable antibody production without using adjuvant, this lipid-conjugated BSA (D-BSA) seems to be suitable for the investigation of regulatory mechanism involved in DTH. In this paper, we will report transient DTH, which was changed into sustained DTH by treatment with cyclophosphamide, induced by a single injection of lightly conjugated D-BSA and discuss possible presence of some suppressive mechanism for DTH.

D-BSA preparations with different degrees of conjugation were obtained in manners similar to those described by Coon and Hunter (1973) and Dailey and Hunter (1974). The average number of fatty acids conjugated per BSA molecule is indicated by a subscript, e.g., D₅₅BSA.

Groups of five guinea pigs (Hartley strain, female, 350 to 500-g weight) were injected intradermally in the rear footpads with D-BSA in 0.1 ml of PBS to give a total dose of 50 μ g D-BSA per animal. The animals were skin tested by intradermal injection on both sides of shaved flank with 50 μ g of BSA in 0.1 ml of PBS. Delayed-type skin reactions (24-hr reactions) were read by measuring the thickness of foci of induration with a dial gauge caliper (Ozaki Seisakusho Co., Tokyo). The results were expressed in the specific increase in skin thickness, i.e., the reading 24 hr after the injection minus that before the injection at the same skin site. The animals were bled by cardiac puncture immediately after measuring the 24-hr reactions. Anti-BSA antibody titers of the sera were determined by passive hemagglutination with 0.5% suspension of sheep red blood cells conjugated with BSA with chromium chloride (Jandle and Simons, 1957) in microtiter plates.

Figure 1 shows the kinetics of the DTH responses to D₅₅BSA (BSA heavily conjugated with the lipid) and D₂₈BSA (BSA lightly conjugated with the lipid). Twenty-four-hour skin reaction to BSA and circulating anti-BSA antibody titer were tested every week for 3 weeks after sensitization. When 50 μ g doses of D₅₅BSA were injected into the footpads of guinea pigs, sustained DTH was

Fig. 1. Kinetics of delayed-type hypersensitivity response after sensitization with D₅₅BSA, BSA heavily conjugated with dodecanoic acid (—○—) or D₂₈BSA, BSA lightly conjugated with the lipid (—△—). Groups of five guinea pigs weighing 350 g were injected with 50 μ g of D₅₅ or D₂₈BSA in PBS. The animals were skin tested by intradermal injection of 50 μ g of BSA one 2 and 3 weeks after sensitization. The mean increase in skin thickness in 24 hr $\pm$ standard error is shown.

induced; DTH was detected in one week and remained positive for 3 weeks. This is in agreement with the data reported by Coon and Hunter (1973). In another experiment, DTH was detected 10 weeks after sensitization performed under similar conditions (data are not shown). Entirely different kinetics of DTH was obtained by injection of the same dose (50 μ g) of D₂₈BSA. In one week, DTH was observed to a similar level to that induced by D₅₅BSA. Thereafter, DTH was depressed and disappeared in 3 weeks. Both skin reactions induced by D₅₅BSA and D₂₈BSA were of typical delayed-type. The reactions were small in 6 hr but became larger with induration and erythema in 24 hr. They were not hemorrhagic. No obvious differences were observed between the DTH reaction induced by D₅₅BSA and that by D₂₈BSA. Histological observations of the skin reaction in 24 hr revealed severe infiltration consisting of predominant mononuclear cells with a minimal number of leukocytes in the dermis even in the center of the reaction focus.

No circulating antibody was detected by passive hemagglutination for 3 weeks after injection of either antigen. Therefore, the depression of DTH induced by BSA lightly conjugated with the lipid does not seem to depend on "blocking" antibodies which have been well documented in tolerance to tumors

Fig. 2. Effects of the treatment with cyclophosphamide (CY) on the kinetics of delayed-type hypersensitivity response after sensitization with D_{34} BSA (BSA lightly conjugated with dodecanoic acid). Groups of five guinea pigs weighing 400 to 500 g were injected intraperitoneally with one dose of CY (200 mg/kg). Three days later, CY-treated (—▲—) or CY-untreated (—△—) animals were injected with 50 μ g of D_{34} BSA in PBS. The animals were skin-tested with 50 μ g of BSA one and 3 weeks after sensitization. The mean increase in skin thickness in 24 hr $\pm$ standard error is shown.

and allograft transplants (Hellström, and Alison, 1972; Voison, Kinsky and Duc, 1972) as well as in that to protein antigens (Crowle and Hu, 1969). The possibility of depression of DTH mediated by a minimal amount of antibody, however, can not be excluded.

Figure 2 shows the effect of cyclophosphamide (CY, Shionogi Co., Osaka) on the kinetics of transient DTH induced by BSA lightly conjugated with the lipid. CY was injected intraperitoneally in a single dose of 200 mg/kg 3 days before sensitization. The treated and untreated animals were sensitized with 50 μ g of BSA lightly conjugated with the lipid (D_{34} BSA). The animals were skin tested with 50 μ g BSA one and 3 weeks after sensitization. In the CY-untreated animals, transient DTH similar to that induced by D_{28} BSA was observed. DTH was demonstrable in one week and became very weak in 3 weeks. In the CY-treated animals, on the other hand, DTH was demonstrated in one week in a slightly higher degree than that in untreated animals and the enhanced response persisted for 3 weeks. Similar enhancement by CY of DTH induced by lipid-conjugated protein (dodecanoyl lysozyme) was observed also in mice in this laboratory (Kojima et al., Unpublished data).

As described above, transient DTH was enhanced by a treatment with CY

3 days before sensitization and converted into DTH of sustained nature. This result suggests that the transient nature of DTH induced by lightly conjugated D-BSA does not simply depend on a lower extent of stimulation for DTH induction but depends on some suppressive mechanism for DTH.

Suppressor cells in guinea pigs have been reported for DTH of the Jones-Mote type and contact sensitivity. These cells removed by passing through an anti-guinea pig gamma globulin-column were found to be sensitive to the treatment with CY (Neta and Salvin, 1973, 1974; Katz et al., 1974; Katz, Parker and Turk, 1974, 1975). The treatment with CY is known to deplete B cells in the lymphoid organs in guinea pigs (Turk and Poulter, 1972; Scheper et al., 1975). Suppressor cells similar to those demonstrated in the Jones-Mote type and contact skin reactions might be involved in the transient DTH response described in this paper.

ACKNOWLEDGEMENT

The authors wish to express their appreciation to Prof. Y. Watanabe, Department of Zoology, Tokyo Kyoiku University for his valuable advice. They also thank Mrs. K. Miyanomae for her technical assistance and Miss M. Kimura for her assistance in preparation of the manuscript.

This study was supported in part by a Grant-in Aid for Developmental Scientific Research from the Ministry of Education (No. 057366).

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(Received: July 7, 1976)