

## TOLERANCE OF HEXAVALENT CHROMIUM INDUCED BY FREUND'S INCOMPLETE ADJUVANT IN GUINEA PIGS

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**Abstract.** Tolerance of hexavalent chromium was induced by one injection of an emulsion of  $K_2Cr_2O_7$  and Freund's incomplete adjuvant, followed by a series of 3 weekly injections of a chromium emulsion containing Freund's complete adjuvant. Guinea pigs receiving only the 3 weekly injections of the  $K_2Cr_2O_7$  combined with the complete adjuvant, developed good degrees of delayed hypersensitivity reactions upon intradermal challenge with  $K_2Cr_2O_7$ , 2 weeks after completing the third dose of the series. These data were interpreted to emphasize the importance of an animal's first contact with an antigen with regard to the pattern of sensitization which develops.

Human tolerance of chemical sensitizing agents has been recognized since 1928, when Frei demonstrated the failure of humans, who had previously received intravenous injections of neoarsphenamine, to achieve sensitization to the drug intradermally (9). This same reaction was later observed in guinea pigs given neoarsphenamine (8). In the case of 2,4-dinitrochlorobenzene (DNCB), similar immunologically specific tolerance of allergic contact dermatitis was achieved by previous regular administrations of the drug (3). Temporary desensitization has also occurred in animals already sensitive to DNCB following a moderately large intravenous dose of the drug (4).

Still a third way of inducing immunologic unresponsiveness has been found by exposing fetal guinea pigs to an antigen, which causes blockage of delayed hypersensitivity reactions to the antigen in adult life (1).

The value of Freund's complete adjuvant in the experimental induction of delayed hypersensitivity with many antigen systems is well known. Its superiority to Freund's incomplete adjuvant in this capacity is also accepted. Recently we re-

ported a method for the induction of delayed hypersensitivity to hexavalent chromium in the guinea pig which involved a series of subcutaneous injections of antiemulsion of hexavalent chromium together with Freund's complete adjuvant (5). Inadvertently, a group of animals received their first dose of chromium combined with Freund's incomplete adjuvant. Upon realizing this, the usual series of sensitizing doses was begun 1 week later, with the complete adjuvant in the emulsion. Surprisingly, none of the animals showed any degree of delayed hypersensitivity upon challenge. This report describes the apparent immunologic unresponsiveness encountered in an experiment conducted to reduplicate the original observation.

### METHOD

Ten albino guinea pigs weighing 300 to 500 g were given one subcutaneous injection in the nape consisting of 0.5 cc incomplete Freund adjuvant (Difco) with 0.5 cc of  $3.4 \times 10^{-3}$  M of  $K_2Cr_2O_7$ . After 1 week, a series of 3 subcutaneous injections in the nape was begun at weekly intervals. The emulsion in the series consisted of 0.5 cc complete Freund adjuvant (Difco) and 0.5 cc of  $3.4 \times 10^{-3}$  M of  $K_2Cr_2O_7$ .

Another set of albino guinea pigs of similar weight received only the series of 3 injections consisting of 0.5 cc complete Freund adjuvant (Difco) and 0.5 cc of  $3.4 \times 10^{-3}$  M of  $K_2Cr_2O_7$ . All animals were challenged 2 weeks after the third dose of the series. Reactions were interpreted at 48 hours, on an arbitrary scale as follows: 0 = no reaction or barely perceptible trace of erythema; + = well defined erythematous patch with no induration; ++ = large erythematous patch with induration; +++ = large erythematous plaque with induration and vesiculo-pustule formation.

## RESULTS

Of the 10 animals whose first contact with hexavalent chromium was in conjunction with the incomplete adjuvant, only one animal showed a 1+ reaction at 48 hours. The remaining 9 animals failed to show any reaction upon challenge testing at 48 hours.

Four of the 10 animals which received the complete adjuvant+hexavalent chromium emulsion showed 2+ reactions and the remaining 6 animals showed 1+ reactions at 48 hours upon challenge testing.

## DISCUSSION

Many investigators have compared hypersensitivity reactions in guinea pigs following sensitization protocols for a variety of antigen systems differing only in the use of Freund's complete adjuvant rather than incomplete adjuvant. Freund maintained that the incomplete adjuvant did not induce delayed hypersensitivity, and that it augmented the immediate type of sensitization. It has subsequently been shown, however, that the incomplete adjuvant-antigen emulsion facilitates both forms of sensitization to different degrees, and that the addition of mycobacteria (complete adjuvant) not only increases the antibody and sensitization responses, but seems to shift potentiation from the immediate type of sensitivity to the delayed form.

Nelson & Boyden (7) using protein antigens in Freund's incomplete adjuvant in the guinea pig, did not achieve a delayed type of sensitivity. At a test on day 7, they reported in some animals weak skin reactions that were delayed in onset, but transient. By 14 days the skin reactions were of a pure Arthus type showing no delayed component. They did achieve delayed hypersensitivity with the same antigens using complete adjuvant.

Magnusson & Kligman (6), using potassium dichromate 1%, studied delayed hypersensitivity by comparing the efficacy of one emulsion prepared with Freund's complete adjuvant and one prepared with Freund's incomplete adjuvant. Twenty-one of 25 animals were sensitized using the complete adjuvant, 10 of 25 were sensitized using the incomplete adjuvant. In another experiment using the same concentration of potassium

dichromate without any adjuvant at all only 1 of 25 guinea pigs became sensitized.

Our experiment essentially confirms the earlier demonstration by Bowser & Baer (2) that tolerance of a contact sensitizer can be induced in the guinea pig by previous injection of the same sensitizer in Freund's incomplete adjuvant. These investigators used 3-*n*-pentadecylcatechol, a component of poison ivy extract. When injected subcutaneously with Freund's complete adjuvant almost all animals became sensitive; injection with incomplete adjuvant induced a state of immunologic unresponsiveness to contact sensitivity. Dosage levels inducing unresponsiveness were of the same order of magnitude as those inducing sensitivity. We are reporting the same phenomenon with another sensitizer of a quite different class.

The results in our experiment are unusual in that only one dose of antigen combined with incomplete adjuvant, followed subsequently by a series of injections with the same concentration of antigen but combined with complete adjuvant, not only failed to result in the consistently good degrees of sensitization usually produced by our protocol, but seemed to evoke a state of immunologic unresponsiveness to the antigen. It is as if the first contact with the antigen was all-important in setting the pattern of immunologic response, in that a regular sensitizing series using complete adjuvant following this first exposure no longer produced the characteristically good degrees of delayed hypersensitivity reactions to hexavalent chromium. The only difference in this first exposure to antigen, as compared with the usual protocol, is the combination with incomplete adjuvant instead of complete adjuvant. The exact modes of action of Freund's complete or incomplete adjuvant are little understood, and hypothesizing within this realm has many alternatives. It seems that, in the guinea pig, almost any exposure to a sensitizer which fails to sensitize will induce tolerance. Thus the cellular mechanism of tolerance induction, whatever it may be, must have an exquisite sensitivity.

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