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EXPERIMENTAL INTRADERMAL GRANULOMA FORMATION*

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ABSTRACT

Experimental induction of an intradermal granulomatous hypersensitivity reaction in the primarily immunized guinea pig was studied by means of light microscopy during the course of the immune response. Animals were sensitized to dinitrophenylated human serum albumin (DNP_{10}-HSA), and challenged with this antigen covalently linked to Sepharose 2B beads by the cyanogen bromide reaction. At appropriate intervals, the dermal changes produced by this complex antigen were compared with those produced by DNP_{10}-HSA or soluble antigen, Sepharose 2B beads alone, and DNP_{10}-HSA plus Sepharose 2B in the same animal. The complex antigen produced lesions characteristic of granulomatous hypersensitivity 5 and 11 days beyond the time of the skin test. The others showed the characteristic responses of hypersensitivity, foreign-body reaction, and a mixture of delayed-hypersensitivity and foreign-body reaction, but the intensity of the reactions had diminished by 5 days and had disappeared after 11 days. These results indicate that a dermal granulomatous hypersensitivity reaction may be differentiated from a foreign-body reaction. They also suggest that locally retained antigen interacts with sensitized lymphocytes to contribute to granuloma formation.

Many disease states are characterized by the presence of granulomatous inflammation [1], but experimental methods are required to determine the factors governing the formation of granulomata. Earlier investigations involving the use of *Schistosoma mansoni* ova suffer from the disadvantage that the antigen is complex [2,3].

Previous studies have shown that a soluble protein antigen covalently linked to an insoluble support, if given intravenously, will lead to pulmonary granulomata in an appropriately sensitized guinea pig [4,5]. The aim of the present investigation was to develop an intradermal model for granuloma formation. This approach has further advantages in that several different injections can be performed in an individual animal, and the ease of observation and examination of the lesions is facilitated.

The method used was the intradermal injection into sensitized guinea pigs of dinitrophenylated human serum albumin (DNP_{10}-HSA) covalently linked to Sepharose 2B beads, followed by histologic examination of the dermal lesions at timed intervals.

MATERIALS AND METHODS

Human serum albumin (Pentex, Inc., Kankakee, Ill.) was substituted with the hapten, dinitrophenyl, using 2,4-dinitrobenzene-sulfonic acid as starting material [6]. The substituted material contained 10 moles of DNP per mole of HSA. The covalent linkage of DNP_{10}-HSA to Sepharose 2B (Pharmacia, Uppsala, Sweden) was carried out by the cyanogen bromide technique [7]. The efficiency of conjugation was 46%, and the final reagent contained 20 μgm of DNP_{10}-HSA on 2,500 beads.

Strain 2 guinea pigs were immunized by foot-pad injection with 200 μgm of DNP_{10}-HSA emulsified in an equal volume of Freund's complete adjuvant (2 mgm/ml of *Mycobacterium tuberculosis* H37Ra, Difco Laboratories, Detroit, Mich.). The emulsion was distributed equally among the four foot pads. Some animals were boosted with 50 μgm of DNP_{10}-HSA in saline given subcutaneously 2 weeks following the primary immunization. Control animals received only adjuvant. A total of 24 guinea pigs were used in this study. At appropriate intervals, each animal was inoculated intradermally at four separate sites with the following:

Site 1: DNP_{10}-HSA in saline (soluble antigen)
Site 2: Sepharose 2B, alone
Site 3: DNP_{10}-HSA plus Sepharose 2B
Site 4: DNP_{10}-HSA-Sepharose 2B (complex antigen)

Each injection was delivered in a volume of 0.1 ml with or without 2,500 beads of Sepharose 2B and a total quantity of 20 μgm of DNP_{10}-HSA. The skin tests were administered either 10 or 14 days after primary immunization, or 14 days following a depot booster injection with soluble antigen. After either immunization schedule, the subsequent results were comparable. Groups of three animals were injected and killed 24 hr, 36 hr, 4-5 days, and 11 days following injection. The skin injection sites were removed, fixed immediately in neutral, buffered, 4% formalin, processed by standard histologic methods, and stained with hematoxylin and eosin.

RESULTS

The typical, delayed-type hypersensitivity skin reaction was assessed by the presence of induration and erythema. Positive, macroscopic reactions occurred in sensitized animals at those sites injected

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with soluble or complexed antigen. Unsensitized animals did not show comparable reactions at any of the injection sites at any time.

The character and degree of dermal cellular infiltration, and the appearance of granulomata were evaluated microscopically. A granulomatous hypersensitivity reaction was defined as a circumscribed lesion consisting mainly of macrophages, but also containing variable numbers of lymphocytes, multinucleated giant cells, and polymorphonuclear leukocytes. A foreign-body reaction was defined as a circumscribed lesion consisting mainly of polymorphonuclear leukocytes, but with variable numbers of multinucleated giant cells, macrophages, and lymphocytes.

Figures 1-4 are examples, in sequence, of a delayed-hypersensitivity dermal reaction, a foreign-body reaction, a mixture of delayed and foreign-body reactions, and granulomatous inflammation of the hypersensitivity type.

In all animals primarily immunized, the macroscopic form of the delayed-hypersensitivity dermal reaction appeared after day 1. This was predominantly a mononuclear cell infiltrate of lymphocytes and macrophages, in a pericapillary and stromal distribution. This response was found at skin site 1 (Fig. 1) indicative of an immune response to the antigen. After 36 hr, only microscopic evidence of this lesion was seen for the initial 5 days of the test period after the skin test.

The gross and microscopic changes of the foreign-body type response to the Sepharose 2B beads alone 24 hr after the skin test, site 2 (Fig. 2), diminished markedly after day 5, and this was unrelated to the immune status of the host. At this site (Fig. 2), the beads were colorless, with a faintly visible periphery, and they were circumscribed by polymorphonuclear leukocytes and monocuclear inflammatory cells. These were largely lymphocytes and macrophages. Focal stromal necrosis was found.

The dermis at sites 3 and 4 (Figs. 3, 4) demonstrated a mixture of inflammatory reaction patterns. The histopathologic and macroscopic changes at skin site 4 were prominent at 5 days after the skin test, and they were observed for the 11 days of the test period. In contrast, site 3 failed to yield a similar degree of microscopic dermal inflammation beyond day 5. However, the inflammatory reaction at site 3 exceeded that which appeared at site 2 in this period of time.

Site 3 (Fig. 3) contained an intradermal, mixed response of delayed hypersensitivity and foreign-body-type 36 hr after the skin test, and following primary immunization with antigen. The injection at this site was soluble DNP_{10}-HSA plus Sepharose 2B beads. The beads were rimmed by mononuclear inflammatory cells, including lymphocytes and macrophages, and occasional polymorphonuclear leukocytes. At this time, compared to Figure 2, the acute inflammatory changes had subsided. This local cellular response had some characteristics of granulomatous inflammation. However, after day 5 following the skin test, this tissue reaction diminished, in a comparable manner to sites 1 and 2.

Site 4 (Fig. 4) possessed an intradermal reaction of delayed and granulomatous hypersensitivity 5 days after the skin test, following primary immunization with antigen. The injection at this site consisted of the complex antigen DNP_{10}-HSA-Sepharose 2B. The periphery of the bead was surrounded by mononuclear cells, mainly macrophages and lymphocytes, and a multinucleated giant cell. This hypersensitivity type of granulomatous inflammatory lesion was prominent from day 5, and it persisted for the 11 days of observation. No comparable lesions were found at any of the other skin sites during this time interval.

DISCUSSION

These results indicate that the hypersensitivity type of granulomatous inflammation may be experimentally produced by the dermal injection of a soluble protein antigen coupled to an inert matrix, and that this may be distinguished from a foreign-body reaction.

The delayed-hypersensitivity reaction results from the interaction of sensitized lymphocytes and antigen. The activation of lymphocytes leads to cell division and, with the production of soluble factors, the recruitment of additional lymphocytes and macrophages. The characteristics of a foreign-body reaction imply a nonspecific host response to tissue injury. However, in both circumstances, the precise mechanisms which account for these cellular effects are not known. The administration to a sensitized animal of a foreign body in association with soluble antigen would be expected to produce a mixed reaction of delayed hypersensitivity and the response to foreign material. These histologic features were, in fact, observed at skin site 3 (Fig. 3). However, the intensity of the inflammatory reaction subsided after 5 days of observation. When an otherwise soluble antigen is rendered insoluble by covalent linkage to an inert support, the fully developed lesion has the distinctive features of a hypersensitivity granuloma, as seen at site 4 (Fig. 4).

Within the context of this morphologic and temporal study, dermal granulomatous inflammation of the hypersensitivity type was observed, and it was persistent, for the time span of the test period of 11 days. Alternatively, the explanation is also valid that the extension in time of delayed hypersensitivity to a soluble, strong, protein antigen that is bound to an insoluble, inert support such as Sepharose 2B may assume this morphologic expression. Thus, the histopathologic distinctions cannot be absolutely identified between granulomatous hypersensitivity and a modification of delayed hypersensitivity due to the prolonged removal of antigen in the presence of a foreign-body response.

The mechanism of granuloma formation is obscure, but at least two factors may be operative in

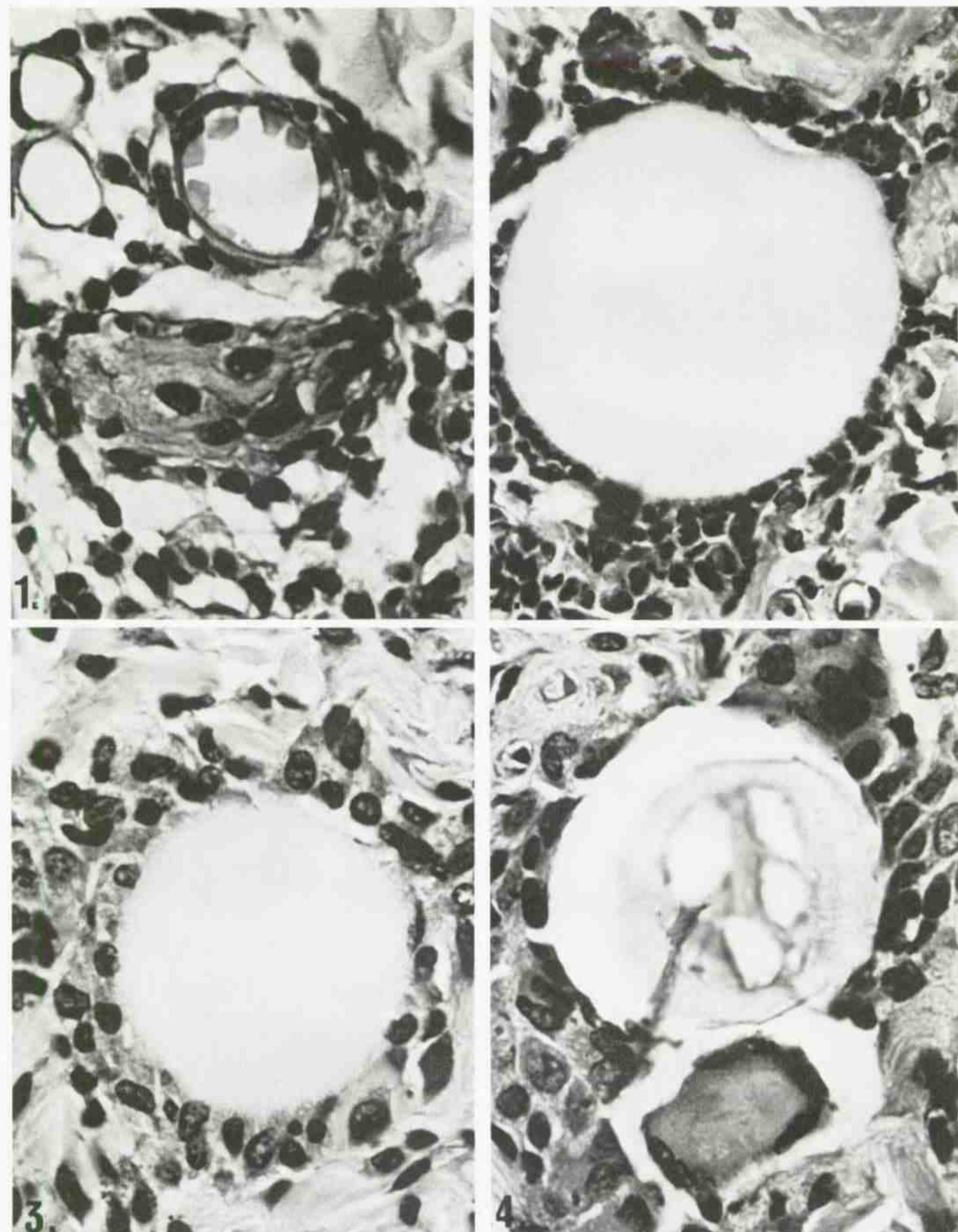

FIG. 1: Delayed-hypersensitivity reaction to DNP_{10}-HSA (soluble antigen) 36 hr after skin test and primary immunization with antigen. This and all subsequent figures magnified × 400.

FIG. 2: Foreign-body-type reaction to the Sepharose 2B beads alone 24 hr after skin test, unrelated to the immunization history of the animal.

FIG. 3: Mixed reaction of delayed hypersensitivity and foreign body 36 hr after skin test with soluble DNP_{10}-HSA plus Sepharose 2B beads, and following primary immunization with antigen.

FIG. 4: Reaction of delayed and granulomatous hypersensitivity, 5 days after skin test with the complex antigen, DNP_{10}-HSA–Sepharose 2B, and following primary immunization with antigen.

the model presented in this paper. First, the location of antigen at the bead surface may alter its local concentration and the presentation of antigenic determinants to sensitized lymphocytes. Second, the covalent linkage of antigen to an insoluble support may delay or prevent its removal.

Whatever the requirements for the induction of granulomata, this experimental model allows for

the investigation of several of its aspects. The effects of antigenic presentation or concentration can be evaluated at different times after various doses or routes of sensitization. Furthermore, it is possible to produce delayed-type-hypersensitivity dermal reactions in normal recipients by the local transfer of sensitized lymphocytes [8,9]. This model of granuloma formation would therefore permit analysis of the relative roles played by antibody or lymphocytes, T-thymus dependent or B-bone marrow dependent, with such a local transfer technique.

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