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Dermal Granulomatous Hypersensitivity in Q Fever: Comparative Studies of the Granulomatous Potential of Whole Cells of *Coxiella burnetii* Phase I and Subfractions

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Dermal granulomatous reactivity to Q fever antigens in guinea pigs has been described as a model for vaccine reactions seen in previously sensitized humans. This model has now been applied to study the ability of subfractions of *Coxiella burnetii* to produce granulomas. Q fever organisms in phase I, trichloroacetic acid-soluble and -insoluble fractions, and the extract and residue of chloroform-methanol extraction were tested for their relative ability to elicit and immunize for dermal granulomatous reactions and specific lymphocyte proliferative responses in guinea pigs. The results suggest that a determinant(s) causing granulomas can be removed by chloroform-methanol extraction of phase I whole cells. The chloroform-methanol residue elicited strong delayed-type hypersensitivity without subsequent granuloma formation. The chloroform-methanol residue appears to possess a determinant(s) for lymphocyte stimulation equivalent to that of whole phase I organisms.

Granulomas are part of the natural pathology of Q fever in humans. We have recently described a model for granulomatous skin reactions to whole cells of *Coxiella burnetii* in immune guinea pigs which allows us to study the immunological basis of granulomatous hypersensitivity (2). This model affords an opportunity to gain new insights into the antigenic nature of Q fever rickettsiae as well as to study the immunological basis of one form of granulomatous hypersensitivity. In our previous study, the time course of induration after skin tests with phase I *C. burnetii* suggested granuloma formation. This was confirmed by histological and electron microscopic analyses of the infiltrate, but no in vitro correlates of cellular immunity were reported. In this study, we address the relative ability of different strains and fractions of *C. burnetii* in phase I to induce and elicit skin and in vitro lymphocyte reactions in guinea pigs.

MATERIALS AND METHODS

Antigens and immunizations. The immunizing and test antigens were a Formalin-inactivated phase I Henzerling strain vaccine prepared for human use (6), a cobalt-irradiated phase I Ohio strain, the soluble component obtained by extraction with trichloroacetic acid (TCA) of phase I Henzerling strain rickettsiae (4), the residue of such extraction (5), and the extract and residue of treatment of Ohio strain rickettsiae with chloroform-methanol (7). TCA fractions were kindly supplied by Ralph Wachter. Immunizations consisted of injecting an equivalent amount of each antigen (10 μg [dry weight]) emulsified in Freund incomplete adjuvant into the four footpads of guinea pigs.

Guinea pigs. Female Hartley guinea pigs of ca. 350 to 500 g were obtained from Simonsen and maintained as previously reported (2). They were used in groups of five or six animals per treatment.

Skin test procedure. Dermal delayed-type hypersensitivity (DTH) testing consisted of injecting 0.1 ml of an appropriate dilution of each antigen preparation into the shaved flank skin of guinea pigs and measuring the diameter and intensity of erythema and induration, determined with a skin calliper, as in previous studies (3).

LT testing. For lymphocyte transformation (LT) testing, lymphocyte cultures of guinea pig heart blood were performed using a whole-blood technique as previously described (3). The same antigen preparations used for immunization were added to cultures at two or more serial 10-fold dilutions. Phytohemagglutinin was used as a nonspecific mitogen control. The cultures were incubated for 4 days, labeled with 0.02 μCi of [^{14}C]thymidine (New England Nuclear Corp.), incubated overnight, harvested, and counted by standard procedures. The LT data are expressed as the geometric mean counts per minute (± standard error) of four replicate cultures.

Histology. Routine histological studies were performed as reported previously (2).

RESULTS

Elicitation of DTH by different antigens. As a test of their ability to elicit DTH and granuloma-

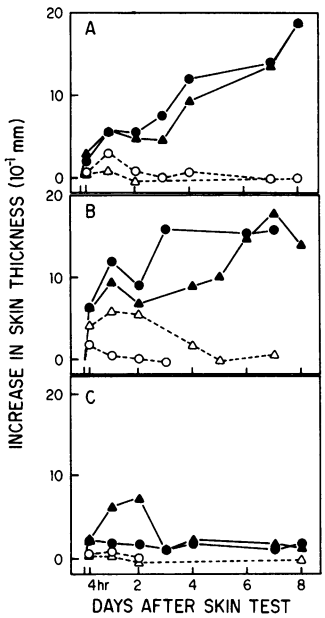

FIG. 1. Relative ability of different skin test materials to elicit DTH and granulomatous responses in Freund complete adjuvant-phase I vaccine-immune guinea pigs 4 weeks after immunization. Solid symbols, Immune animals; open symbols, control animals. Skin tests: (A) 60 ng of phase I Ohio *C. burnetti* (●) and 60 ng of phase I Henzerling vaccine (▲); (B) 1:100 fraction of TCA residue (●) and 10 μg of soluble antigen (▲); (C) 50 ng of chloroform-methanol extract antigen (●) and 50 ng of CMR antigen (▲).

tous responses, the various antigens were used for skin tests in phase I Henzerling strain-immune guinea pigs. The two strains of *C. burnetii* in phase I, Henzerling and Ohio, were equivalent in their ability to elicit late reactions (Fig. 1A). TCA-soluble and residue fractions prepared from phase I rickettsiae were compared and showed a significant degree of early (day 1) reactivity along with significant late reactivity (Fig. 1B). The soluble antigen has a high degree of nonspecific early reactivity in controls at the 10-μg dose.

The chloroform-methanol fractions obtained from phase I Ohio strain rickettsiae differed from all other preparations tested. The extract from chloroform-methanol treatment was devoid of either early or late reactivity, whereas the chloroform-methanol residue (CMR) elicited strong reactions at days 1 and 2 typical of DTH (Fig. 1C). The reactions to CMR were more erythematous compared with the reactions to all other preparations. No late reactions were observed to CMR at this dose. Only when the dose of CMR was increased 100-fold over that used here, very intense DTH followed by local necrosis was seen, similar to that obtained with tuberculin purified protein derivative in Freund complete adjuvant-immune guinea pigs (data not shown).

LT studies. Whole-blood lymphocyte cultures were obtained from groups of animals immunized with phase I Ohio strain rickettsiae or CMR. The cultures were stimulated with each antigen at multiple doses. Over a 5-log range of doses tested, CMR and Henzerling and Ohio strain antigens were virtually identical on a weight basis in their ability to elicit LT. Figure 2 illustrates the response to a constant amount (20 ng per culture) of each preparation. The CMR-immune animals showed equivalent LT reactivity to each of the antigens tested. These data show that CMR contains the antigenic component responsible for both induction and elicitation of LT reactivity in guinea pigs.

Histological examination of the reactions to the various preparations observed in these studies revealed no significant differences in the character of the granulomas from that previously described (2). The TCA-soluble and -insoluble fractions also elicited similar histological changes, but both chloroform-methanol fractions failed to show granulomatous changes by this technique.

DISCUSSION

The propensity for Q fever vaccines to induce granulomas or sterile abscesses in sensitive recipients has been a major impediment to the widespread use of these vaccines. In our previous study, we presented an animal model in immune guinea pigs which reproduced the gross and histological characteristics of the human reactions (2). In the present study, we have used this assay to test the ability of various prepara-

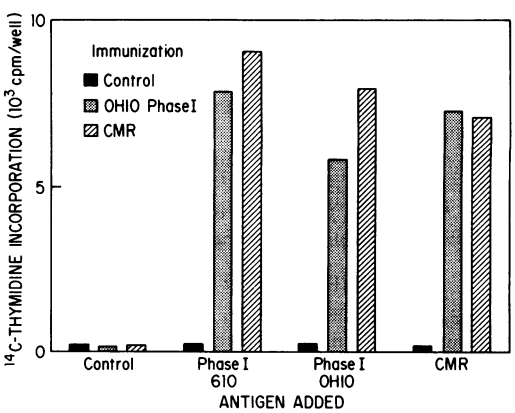

FIG. 2. Lymphocyte proliferative responses of guinea pigs immunized with phase I Ohio strain organisms and CMR and stimulated with phase I Ohio and phase I Henzerling strain antigens and CMR from Ohio strain antigen 4 weeks after immunization.

tions of *C. burnetii* to induce or elicit DTH (days 1 to 3) and granulomatous (days 8 to 10) skin reactions.

When various preparations were used as skin tests in phase I-immune animals, a consistent granulomatous response was seen to each of two different phase I strains, Ohio and Henzerling, and to both the TCA-soluble antigen and the residue of such extraction. In contrast, the chloroform-methanol extraction procedure led to different results. In addition to a lessened toxicity apparent in previous animal studies (7), CMR elicited high-grade DTH in immune animals at doses devoid of late granulomatous reactivity. Previous studies of phenol-extracted soluble antigen have shown that a nontoxic hapten could be extracted from a TCA-soluble antigen, but this antigen lacked protective immunogenicity (1). To date, CMR appears to retain protective potential in mouse challenge experiments (7). The LT studies that use CMR suggest that the lymphocyte-stimulating determinant of phase I rickettsiae is present in CMR for both the priming and elicitation of LT, providing an in vitro correlate for the DTH reactions seen with this product.

In selecting a skin test reagent, it must be kept in mind that the most adverse reactions in humans to vaccines are of a granulomatous type. If granulomas can occur without preceding DTH, skin testing with CMR might not predict such reactions. Although granulomatous changes in the guinea pig model occur without preceding DTH, experience to date with humans has demonstrated that virtually all granulomatous reactors to skin testing have had significant prior DTH (2a). Adverse reactions consisting of painful arms and local erythema were accurately predicted by the DTH component of the reaction and could be avoided by screening with a reagent-like CMR. The reading of skin tests for only late granulomas is likely to result in the unnecessary vaccination of immune individuals.

In conclusion, the results show that CMR, a derivative of phase I rickettsiae, is capable of eliciting strong DTH in guinea pigs without granuloma formation. This finding makes CMR a promising candidate for application as a skin test antigen in humans. In the future, we will study the fractions chloroform-methanol extract and CMR obtained from the phase II as well as the phase I organisms to test the phase specificity of these findings.

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