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Experimental Model for Dermal Granulomatous Hypersensitivity in Q Fever

MICHAEL S. ASCHER,[1]* MONIQUE A. BERMAN,[1] DARIEN PARKER,[2] AND JOHN L. TURK[2]

Department of Medicine, University of California, Irvine, California 92717[1] and Department of Pathology, Royal College of Surgeons, London, England[2]

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Q fever has been associated with granulomatous changes in clinical biopsy material obtained from liver and bone marrow. Local reactions to skin testing have been described in previously sensitized humans, but histological studies of such reactions have not been reported. We note that delayed hypersensitivity reactions to whole-cell phase I Q fever vaccine in immunized guinea pigs have a time course of development of induration characteristic of granulomatous hypersensitivity. Histological examination of such skin reactions on day 9 after testing revealed epithelioid cell infiltration and the presence of large numbers of multinucleated giant cells. Prominent in the sections were fragments of disintegrating polymorphonuclear leukocytes having the appearance of leukocytoclasis. Electron microscopic studies confirmed the presence of epithelioid changes in cells of the mononuclear phagocyte series, as well as extensive collagen deposition. This animal system affords a readily reproducible model of dermal granulomatous hypersensitivity and an opportunity to analyze the immunological basis of this reaction.

Q fever is an enzootic disease of sheep and cattle passed to humans through contact with infected placentae and wastes. The clinical illness in humans is usually an undifferentiated fever, but pneumonia and hepatitis are found frequently. The mortality of the uncomplicated infection is low, but chronic infection has been described in humans, leading to endocarditis and high mortality (10, 27).

Since the original description of the histology of Q fever in mice (23), numerous reports have noted granulomatous changes in liver and bone marrow samples obtained from animals and patients (5, 11, 13, 17, 18, 24). Variable degrees of giant cell formation have been described, and blood vessel involvement was claimed in one report (9). Subsequent workers noted a characteristic appearance of the granuloma with a "doughnut" of epithelioid cells around a central adipocyte (7, 21). When liver biopsy material was examined for the presence of rickettsiae, they were found to be present (8).

In addition to these histological descriptions of Q fever infection, one group of workers has reported the results of skin testing with Q fever antigens in rabbits (1). The tests were interpreted as delayed-type hypersensitivity (DTH), but histological studies were not performed. In several studies, human subjects have been immunized with Q fever vaccines, with the development of adverse local reactions in a proportion of recipients (4, 19, 22). In an attempt to avoid such vaccine reactions, skin tests were performed in later studies (14, 15). Two peaks of skin reactivity were described at days 2 and 9, but the two phases of the reaction were not distinguished immunologically, and histological studies were not performed.

As an animal model of this phenomenon, we skin tested immune guinea pigs with Q fever rickettsial antigen and report studies on the histology of the reactions and a preliminary analysis of the immunological basis of the reactions.

MATERIALS AND METHODS

Guinea pigs. Outbred Hartley strain guinea pigs of both sexes weighing 350 to 500 g were used. The animals came from stocks held at the Royal College of Surgeons, London, England, or were purchased from A. Tuck & Son, Ltd., Battlesbridge, Essex, England, or from David Hall, Newchurch, Staffs, England, or Simonsen Laboratories, Gilroy, Calif. They were fed on pelleted diet (F. Dixon & Son, Ware, Herts, England), liberally supplemented with cabbage and hay.

Q fever antigens. The phase I Q fever vaccine was an investigational product kindly supplied by the U.S. Army Medical Research Institute of Infectious Diseases (26).

Immunizations. Guinea pigs were injected with phase I Q fever vaccine emulsified in Freund complete adjuvant (FCA) or Freund incomplete adjuvant (FIA) (vaccines FCA-Q and FIA-Q) at a final concentration of 30 μg/ml of rickettsial protein. A total of 0.4 ml (12 μg) was injected into the four footpads of the guinea pigs.

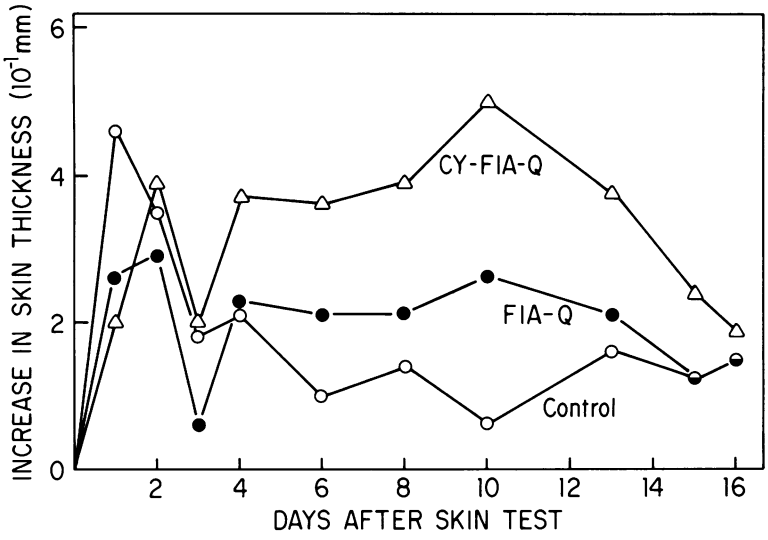

FIG. 1. Effect of CY pretreatment on skin test reactions of control and FIA-Q immune guinea pigs to 60 ng of antigen 2 weeks after immunization. The least significant difference at the 5% significance level is 2.16×10^{-1} mm by analysis of variance.

DTH testing. On day 21 after sensitization, the guinea pigs were injected in a shaved flank with 0.1 ml of a 1:100 dilution of vaccine (60 ng). The reactions were scored for intensity and diameter of erythema, and increase in skin thickness was determined with a skin calliper, as previously described (3). In these studies, measurements were made at 4 and 24 h and daily thereafter for up to 21 days.

CY treatment. Animals were injected intraperitoneally with 250 mg of cyclophosphamide (CY) per kg (Mead Johnson Company, Evansville, Ind.) 3 days before immunization, as previously described (2).

Serum transfer. One or two milliliters of high-titer serum obtained at 4 to 6 weeks from guinea pigs immunized with FIA-Q or FCA-Q was transferred intravenously into normal guinea pigs. One hour later, skin testing was performed as described above.

Histological examination of tissues. Skin test reaction sites were fixed in Bouin solution, sectioned at 5 μm, and stained with hematoxylin and eosin.

Electron microscopic procedures. Skin was prepared for electron microscopy as previously described (29).

Statistical analysis. Animals were tested in groups of five or six. The coefficient of variation of skin test results within groups averaged 65%. Statistical analysis was performed with the analysis of variance. The

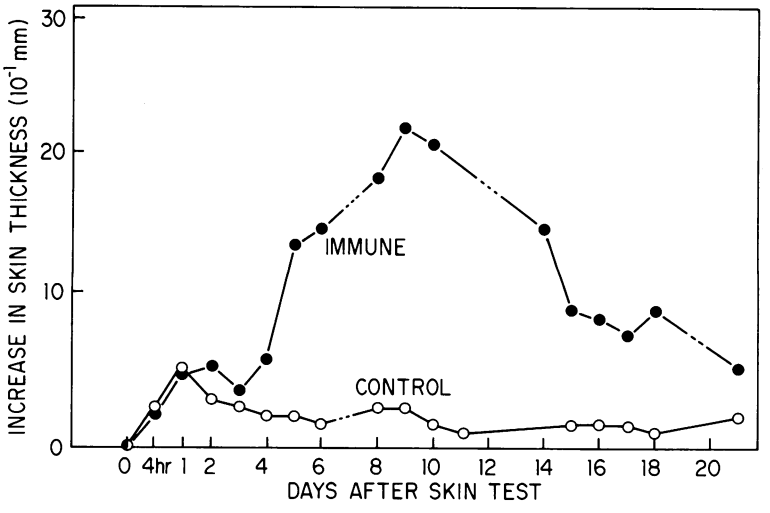

FIG. 2. Skin test reactions of immune and control guinea pigs tested with 60 ng of phase I Q fever vaccine. The least significant difference at the 5% level is 4.87×10^{-1} mm.

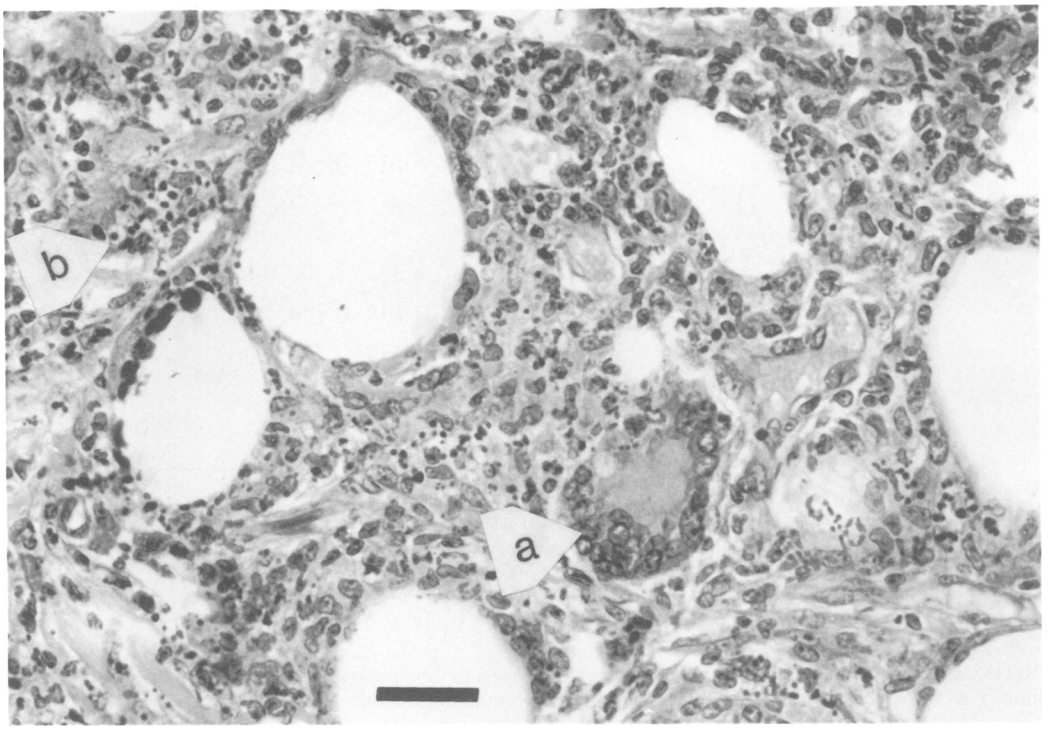

FIG. 3. Hematoxylin- and eosin-stained sections of the skin reaction from an FCA-Q immune guinea pig taken on day 9 after skin testing with 60 ng of vaccine. a, A multinucleated giant cell; b, collections of polymorphonuclear leukocyte nuclear fragments. Bar, 50 μm.

least significant difference at the 5% probability level is 2.16×10^{-1} mm for the data in Fig. 1 and 4.87×10^{-1} mm for the data in Fig. 2.

RESULTS

Guinea pigs were immunized with FIA-Q with or without CY pretreatment and skin tested 2 weeks later. The CY-pretreated group showed significant reactivity beginning on day 4 and persisting through day 10 (Fig. 1). The decrease in reactivity seen on day 1 in immune animals as compared with controls was a consistent finding in this system. When similar groups of animals were held for 6 weeks after immunization with FIA-Q or FCA-Q and skin tested, a fivefold more intense reaction was obtained without the use of CY (Fig. 2). Even at this intensity of the late (day 9) reaction, there was little or no significant early (day 2 to 3) reaction in the immune animals as compared with the controls. To directly assess the role of antibody in this reaction, serum transfers were made into normal controls by using serum obtained at 4 to 6 weeks from animals immunized with FIA-Q or FCA-Q. In no case was any significant early or late skin reaction seen in recipients of such serum transfer (data not shown).

The gross appearance of the skin reactions obtained in these experiments consisted of minimal erythema and induration equivalent in control and immune animals over the first 3 days. There then developed over the next 6 days in immune animals a 5 to 8 mm nodule with minimal erythema but with superficial flaking of the epidermis over the site. Skin test sites were excised at day 9 and examined histologically (Fig. 3). The lesion had numerous epithelioid cells arranged in whorls, multinucleated giant cells (Fig. 3, a), and fragments of nuclei of polymorphonuclear leukocytes (Fig. 3, b). Whole polymorphonuclear leukocytes or nuclear fragments were frequently seen within giant cells. Figures 4 and 5 show the electron microscopic appearance of cells making up the infiltrate of the Q fever skin test. The cell in the field illustrated in Fig. 4 is a typical epithelioid cell (20) with a pale oval nucleus, prominent nucleolus, and rather empty cytoplasm, with swollen rough endoplasmic reticulum. There was no evidence of phagocytic activity in this section, and no rickettsial bodies were identified. Figure 5 shows the ultrastructure of a multinucleated giant cell closely apposed by a mononuclear phagocyte (Fig. 5, a) and surrounded by fragments of polymorphonuclear leukocytes (Fig. 5, b) and bundles of collagen (Fig. 5, c).

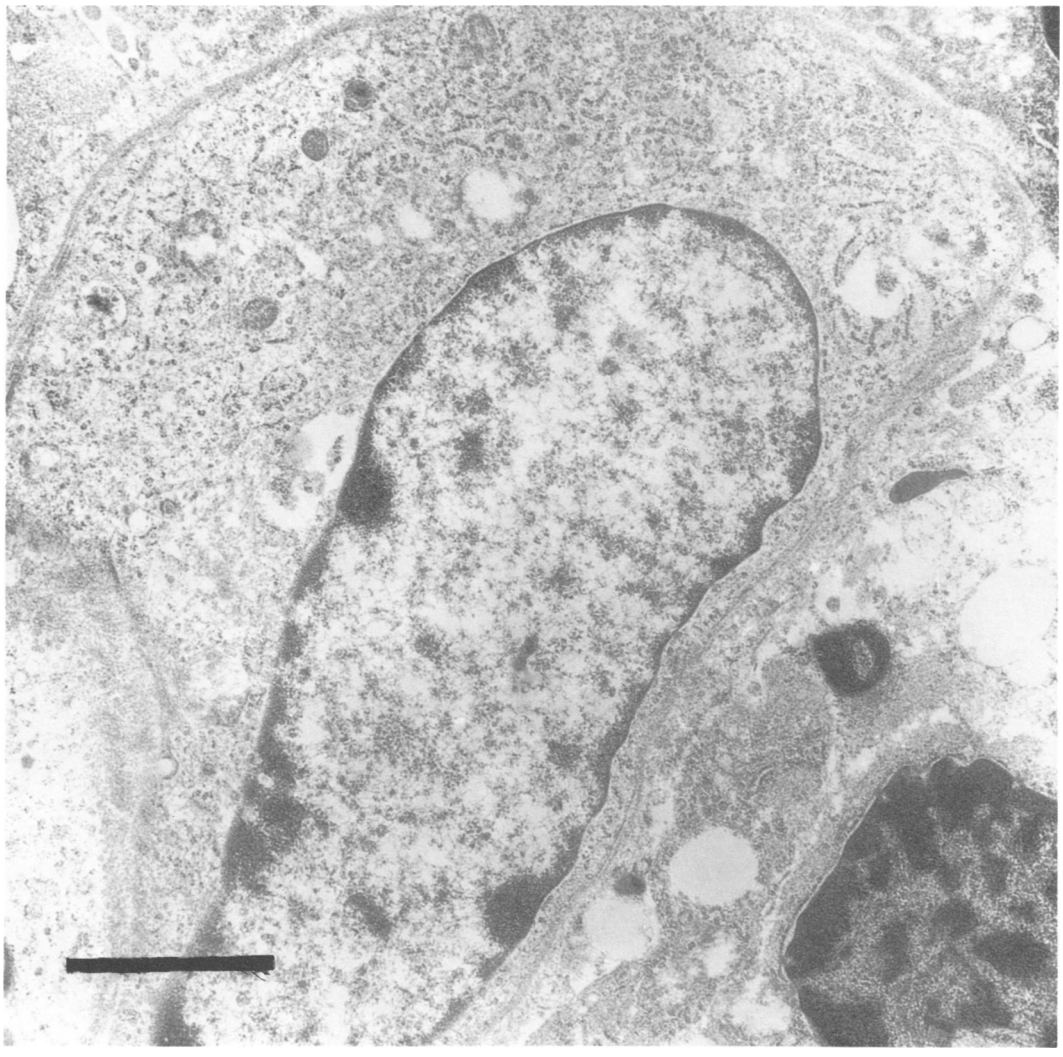

FIG. 4. Electron micrograph of an FCA-Q immune guinea pig skin test site at day 9 after injection of 60 ng of vaccine showing an epithelioid cell with prominent endoplasmic reticulum. Bar, 1 μm.

DISCUSSION

In studies of immunization of humans and animals with Q fever vaccines, cutaneous reactivity with features of both DTH and late reactivity has been noted (1, 15). The immunological basis of the late reactivity has not been determined. In one study, a sterile abscess was aspirated, and, on the basis of a high antibody titer, it was claimed that the reaction was due to active immunity associated with local antibody production (14). We suspect that the late reactions are cell mediated, based on indirect evidence obtained from the use of CY. In other systems, CY produces elevation of DTH and decreased antibody formation (2, 3). In our previous studies, CY enhanced DTH to Q fever vaccine (2), and animals so treated were the first to express the late granulomatous reaction in our laboratory. Both DTH and the late reaction were enhanced by CY pretreatment, suggesting a similar immunological mechanism for the two reactions. Evidence against a role for antibody alone in the mediation of these reactions is based on the finding that CY-treated animals have markedly decreased antibody (2) and on the failure of serum transfer to produce reactions. As in many other systems, direct proof will depend on cell transfer experiments. Since more techniques and reagents are available to characterize cell populations in mice than in guinea pigs we are trying to reproduce the late granulomatous reaction in mice.

Our experiments with Q fever show pure late reactivity without significant DTH and bear a

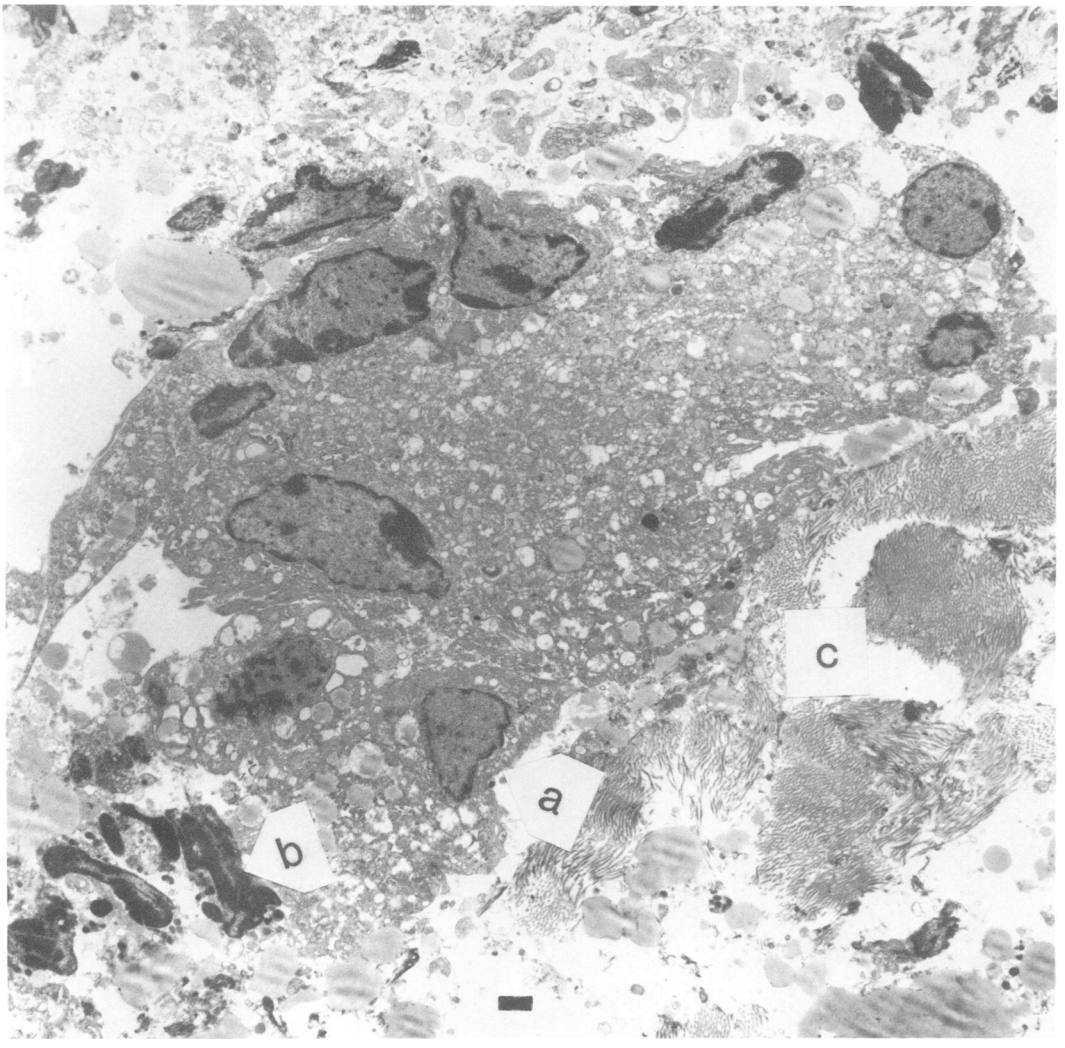

FIG. 5. Electron micrograph of a multinucleated giant cell with a mononuclear phagocyte closely apposed to its surface (a) and surrounded by nuclei of degenerating polymorphonuclear leukocytes (b) and collagen bundles (c). Bar, 1 μm.

striking similarity to the reactions previously described with zirconium lactate in sensitized guinea pigs (30, 31). The Q fever lesions, however, are more intense and reproducible than the zirconium reactions. Other workers have examined granulomatous skin reactions in mycobacterial infections and have reported a similar time course of development of induration to BCG antigen in oil in guinea pigs (12). Q fever vaccine was used as a specificity control, but data with Q fever antigen in sensitized animals were not presented. The published studies on Q fever antigen skin test reactions in sensitized rabbits do not include a description of late reactivity or histology (1). The immunological significance of granulomatous hypersensitivity in Q fever has not been determined. By analogy, two forms of skin reactivity distinguished by their timing are used clinically in the diagnosis of leprosy. Animal studies have also been reported in which two inbred strains of mice express different forms of hypersensitivity and resistance to infection with *Mycobacterium lepraemurium* (6). In this model, resistant mice have reactions characterized by late induration and granulomatous changes on biopsy similar to our findings.

The light-microscopic histological changes in our reactions are epithelioid granulomas with giant cell formation. We note also the presence of "leukocytoclastic" changes (16), which have not been previously noted in the guinea pig. Since we used CY and adjuvants in the immuni-

zation procedure in guinea pigs, our inference relating these reactions to humans and natural infection must be tempered. This inference is based on the presence of similar histological changes in sections of a skin test site from an individual previously infected with Q fever (P. Fiset, personal communication) and from the ability to elicit late reactions in Q fever convalescent guinea pigs (R. Wachter, personal communication). The electron microscopic findings are epithelioid changes in cells of the mononuclear phagocyte series. These cells appear identical to those reported previously in zirconium granulomas in the guinea pig and to those recently reported for BCG in guinea pig lymph nodes (20, 25, 28, 30). The presence of mononuclear phagocytes around the giant cell in Fig. 5 suggests the formation of the latter by fusion of phagocytes.

This model provides a useful assay for further immunological analysis of Q fever granulomatous hypersensitivity. The preparation of vaccine used in these studies is crude, consisting of whole killed rickettsiae. Experiments in progress to determine the antigenic component(s) of the organism responsible for the reaction may allow development of a purified non-granulomagenic reagent for diagnosis of prior sensitivity to Q fever in humans.

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