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Immunization against Chlamydial Genital Infection in Guinea Pigs with UV-Inactivated and Viable Chlamydiae Administered by Different Routes

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Female guinea pigs were immunized with viable or UV light-inactivated chlamydiae (agent of guinea pig inclusion conjunctivitis), belonging to the species *Chlamydia psittaci*, by intravenous, subcutaneous, oral, or ocular routes. All animals were then inoculated vaginally with viable chlamydiae to determine the extent of protection against challenge infection induced by the various regimens. The course of genital infection was significantly reduced in intensity in all groups of animals except the unimmunized controls and those animals immunized orally with inactivated antigen. Guinea pigs immunized with viable antigen were more likely to develop resistance to challenge infection and, in general, had a significantly greater degree of protection than animals immunized with inactivated antigen. No one route seemed superior in producing a protective response. Animals in all groups demonstrating protection developed serum and secretion immunoglobulin G antibody responses to chlamydiae. Lymphocyte proliferative reactions to chlamydial antigen were variable among groups. Immunoblot analysis of serum and secretions indicated a wide range of antibody specificities, but most protected animals produced antibodies to the major outer membrane protein, lipopolysaccharide, and the 61-kilodalton protein. No definitive associations could be made between the increased ability of immunization with viable organisms to produce resistance to challenge infection and a particular immune parameter. These data indicate that viable chlamydiae given by various routes are able to induce a strong immune response which can provide resistance against reinfection in some cases or at least reduce the degree of infection to a greater degree than inactivated antigen. However, complete resistance to genital tract infection may be difficult to obtain and alternate immunization strategies may have to be developed.

Genital infections caused by *Chlamydia trachomatis* remain a major problem despite the availability of effective chemotherapeutic agents. This is particularly true in females, in that irreversible tubal damage may be produced before the infection is diagnosed and treated. Thus, it would be most appropriate to develop an immunization procedure to prevent infection from occurring.

We have been studying the basic immune mechanisms responsible for resolving a primary chlamydial genital infection and providing resistance to reinfection by using guinea pigs infected with the *C. psittaci* agent of guinea pig inclusion conjunctivitis (GPIC). Despite the fact that this organism is classified as *C. psittaci*, the disease in guinea pigs has been demonstrated to closely resemble human chlamydial genital infections with regard to pathogenesis, pathology, and immunology (1, 20). We have found that both antibody and cell-mediated immune (CMI) mechanisms are required for resolution of the infection (21, 27) as well as resistance to challenge infection (22, 26). Of major concern, however, was our finding that complete resistance to reinfection following a primary infection was relatively short in duration. By 2 months after resolution of the primary infection, animals became reinfected upon challenge but had a markedly less severe infection as well as one of shorter duration. This "partial" immunity persisted for up to about 2 years (2, 25). The loss of complete resistance was associated with the decline of immunoglobulin G (IgG) antibodies in serum and IgG and IgA in genital secretions as well as a decline in CMI response as determined by lymphocyte proliferation responses to GPIC antigen (25). Humans, too, can become reinfected, and some evidence suggests that immunity does develop but is also short-lived (9). Thus, the problem becomes how one can induce a long-lasting protective immune response in the genital tract when a primary infection, usually the strongest immunizing event, does not itself elicit long-term protection. To date, no effective vaccines against genital infections with any of the genital pathogens have been developed in humans or animal models. To further complicate the situation, it has been suggested, although not proven in humans or in models of ascending infection, that delayed-type hypersensitivity mechanisms may actually exacerbate the disease process in the fallopian tubes (17, 18). There is certainly precedent for this phenomenon in chlamydial infections of the eye (28, 29).

In this study, we explored several different routes of immunization with either viable or inactivated antigen in the guinea pig model to determine whether immunity to reinfection could be elicited. The results of challenge infection were correlated to levels of different immune parameters prior to challenge. These parameters included total anti-GPIC and component-specific antibody responses in serum and secretions and lymphocyte proliferation to GPIC as a measure of cell-mediated immunity.

MATERIALS AND METHODS

Experimental animals. Female Hartley strain guinea pigs, weighing 450 to 500 g, were obtained from Sasco Laboratories, Omaha, Nebr. All animals were housed individually in cages covered with fiberglass filters and were given food and

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water ad libitum. The room was maintained on a 12:12 light:dark cycle.

Infection of guinea pigs with chlamydiae. The GPIC agent for infection was grown in HeLa cell cultures (7). Aliquots of host cell-free chlamydiae were frozen in a sucrose-phosphate buffer (2-SP) at −70°C until needed. Animals were infected by intravaginal inoculation of 0.05 ml of the GPIC suspension which contained 10^7 to 10^8 inclusion-forming units (IFU) (25). Animals were monitored for the course of the infection by assessing the percentage of chlamydial inclusions on a vaginal scraping stained with Giemsa and by the isolation of organisms in McCoy cell cultures from cervical swabs (25). The cultures were not quantified by were merely scored as positive or negative for chlamydiae. The latter method has been found to be the more sensitive of the two assay systems (25).

Immunization of guinea pigs with GPIC. Viable organisms for immunization were grown in McCoy cells and were stored cell-free in 2-SP at −70°C until needed. For injection, the organisms were diluted in phosphate-buffered saline (PBS). Inactivated GPIC was prepared by treating McCoy cell-grown and Percoll-purified elementary bodies with UV light (G30T8 UV lamp, model J225, Blak Ray) at a distance of 10 cm for 3 h. When such preparations were assessed for viable organisms in cell culture, none were detected. The UV-inactivated chlamydiae were suspended in PBS for immunization purposes.

Intravenous immunization was accomplished by injecting methoxyflurane-anesthetized guinea pigs in one of the superficial veins on the dorsal aspect of the pinna of the ear by using a 30-gauge, 0.5-in. needle bent at a 45° angle. Care was taken with the live material to swab the area with 70% ethanol after the injection. Each animal received either 4×10^7 IFU of viable organisms or 100 μg of inactivated GPIC, each contained in 0.1 ml of PBS.

Animals were injected subcutaneously in two sites in the inguinal area with 0.25 ml of PBS each for a total of 2×10^8 IFU of viable GPIC or 500 μg of inactivated GPIC. In one experiment, animals were immunized subcutaneously with 500 μg of inactivated GPIC suspended in an equal volume of Freund complete adjuvant. For the booster inoculations, the GPIC antigen was suspended in Freund incomplete adjuvant. Animals were injected with 0.25 ml of the suspension at four sites in the axial and inguinal areas.

Oral immunization was performed by anesthetizing guinea pigs with methoxyflurane and passing a piece of Tygon tubing (outside diameter, 2 mm; length, 8 cm) connected to an 18-gauge, 1.5-in. needle about 6 cm into the oral cavity. Each animal received 0.5 ml of PBS containing either 2×10^8 IFU of viable chlamydiae or 500 μg of inactivated organisms.

In one experiment, guinea pigs were immunized by a single ocular infection with 5×10^6 IFU as previously described (23).

All animals (except those ocularly immunized) were given a primary inoculation followed by two booster inoculations at 2-week intervals and were challenged 2 weeks after the last immunization by intravaginal inoculation. During the immunization period all animals, particularly those given live organisms, were monitored for the development of ocular (except the ocular-immunized group) or genital infections. If animals were positive for either, they were eliminated from the study.

Determination of antibody levels. Sera and genital secretions were obtained from guinea pigs as previously described (22, 27) and stored at −20°C until all specimens in the experiment were collected. Serum IgG was measured by an enzyme-linked immunosorbent assay using HeLa-grown GPIC elementary bodies as the antigen and peroxidase-labeled rabbit anti-guinea pig IgG (heavy- and light-chain specific) (ICN ImmunoBiologicals, Lisle, Ill.) (23). IgA in genital secretions was determined by a similar assay except that rabbit anti-guinea pig IgA (α-chain specific) (ICN) was used, followed by peroxidase-labeled goat anti-rabbit IgG (heavy- and light-chain specific) (ICN) (23).

Immunoblot analysis. Sodium dodecyl sulfate-polyacrylamide gel electrophoresis, electrophoretic transfer of chlamydial proteins to nitrocellulose membranes, and immunoblotting assays were performed as previously described (2). Nitrocellulose membranes were blocked by using a solution containing 150 mM NaCl–10 mM Tris–0.5% (wt/vol) nonfat dry milk (Carnation), pH 7.4. Guinea pig serum specimens were diluted 1:500 to 1:1,000. Antibody binding was localized by using rabbit anti-guinea pig IgG (heavy- and light-chain specific) (Miles Laboratories, Inc., Naperville, Ill.) followed by radioiodinated goat anti-rabbit IgG (Cooper Biomedical, Inc., West Chester, Pa.) and autoradiography.

Individual immunoreactive bands were judged to be present or absent in a blinded fashion. Molecular weights were assigned to minor bands by measuring their migration on autoradiograms and comparing them to a plot of migration versus molecular weight. Major bands of known molecular mass (39-kilodalton [kDa] major outer membrane protein [MOMP], 61 kDa, 15 kDa) were used as standards.

Determination of cell-mediated immune response. CMI activity in immunized animals was assessed by the proliferative response of peripheral blood lymphocytes to GPIC antigen and concanavalin A (Sigma Chemical Co.) 2 to 3 days before challenge infection (25). Four milliliters of blood was obtained by cardiac puncture, and the blood was mixed with sodium citrate. The blood was diluted with 3 volumes of RPMI 1640 and centrifuged at 400 × *g* for 40 min over Histopaque (specific weight, 1.077 g/ml) (Sigma). Peripheral blood mononuclear cells were collected from the interface, washed, and placed into microculture at 2×10^5 cells per well in RPMI 1640 containing 10% fetal calf serum, 50 nM 2-mercaptoethanol, 2 mM glutamate, and penicillin (100 U/ml) and streptomycin (100 mg/ml). UV-inactivated HeLa-grown GPIC antigen (7) was added to triplicate wells at 16 μg/ml, and concanavalin A was similarly added at 2.5 μg/ml. Cultures were labeled with 1 μCi of [^{3}H]thymidine per ml over the final 24 h of a 5-day incubation at 37°C in 5% CO_2. The results were expressed as the mean counts per minute of cultures from five animals.

RESULTS

Course of challenge infections. In order to determine whether protection against a genital tract infection could be elicited with an inactivated antigen, an immunizing regimen was used which incorporated the use of Freund complete and incomplete adjuvants with UV-inactivated GPIC in the immunization of five guinea pigs. Another group of five guinea pigs was given a single ocular infection with GPIC as a positive control, since it has been previously shown that this would induce protection against a genital infection (14; R. G. Rank, unpublished data). A third group remained unimmunized to serve as an infection control.

Both immunized groups demonstrated a significant degree of protection when compared to challenged unimmunized controls ($P < 0.001$, according to a two-factor [days, treatment] analysis of variance with repeated measures on one

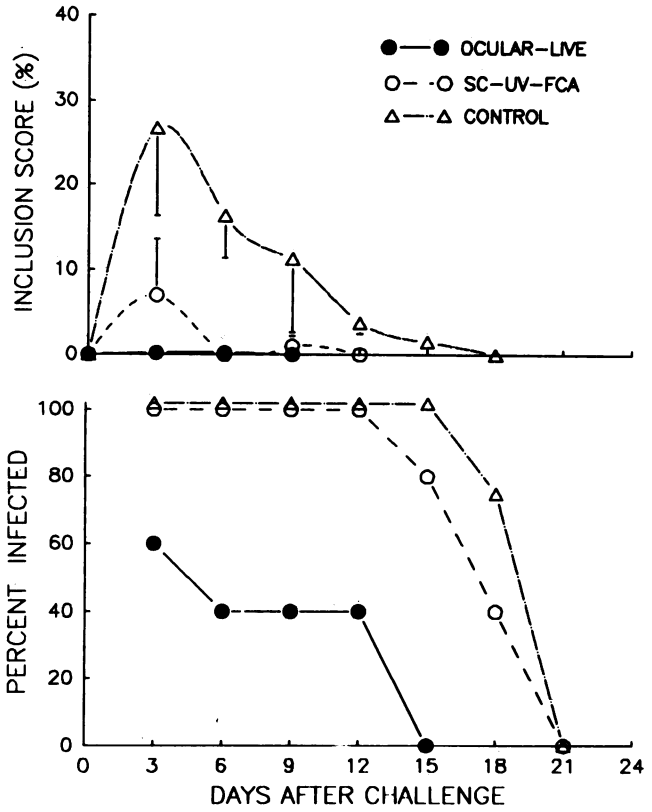

FIG. 1. Results of challenge infection of animals immunized with UV-inactivated GPIC in Freund complete adjuvant (FCA) or by a single prior ocular infection. (A) Coures of challenge infection as measured by inclusion score. (B) Course of infection as determined by percentage of animals infected as assessed by isolation of GPIC from cervical swabs. SC, subcutaneous.

factor [days]) when the course of their infections was assessed by the percentage of inclusion-bearing cells in vaginal scrapings (Fig. 1). When the infection course was monitored by isolation of chlamydiae from cervical swabs, two of the five ocular-infected animals were resistant to reinfection, and the infections of the other three animals were resolved by day 15. All animals immunized with adjuvant and inactivated antigen became infected upon challenge and did not resolve until day 21 even though the infection was less intense as judged by inclusion scores. Thus, the length of the infection in the group immunized with inactivated antigen was unaltered despite the development of high titers of serum IgG (>10,240), secretion IgG (5,120) and IgA (1,470), and strong lymphocyte proliferation reactions to GPIC antigen (5,741 cpm) in guinea pigs immunized with inactivated antigen.

Since the data from this first experiment suggested that immunization with inactivated antigen was able to provide a degree of protection but that actual infection at a different site was somewhat more effective, we conducted a series of experiments to compare the effectiveness of different routes of immunization with either viable or UV-inactivated antigen without adjuvant. Each route was assessed in two to three experiments with five animals per group. The data from all experiments are summarized in Fig. 2 (inclusion scores) and 3 (chlamydial isolation). Intravenous, subcutaneous, and subcutaneous-oral immunization with inactivated antigen were able to significantly ($P < 0.001$) reduce the level of the

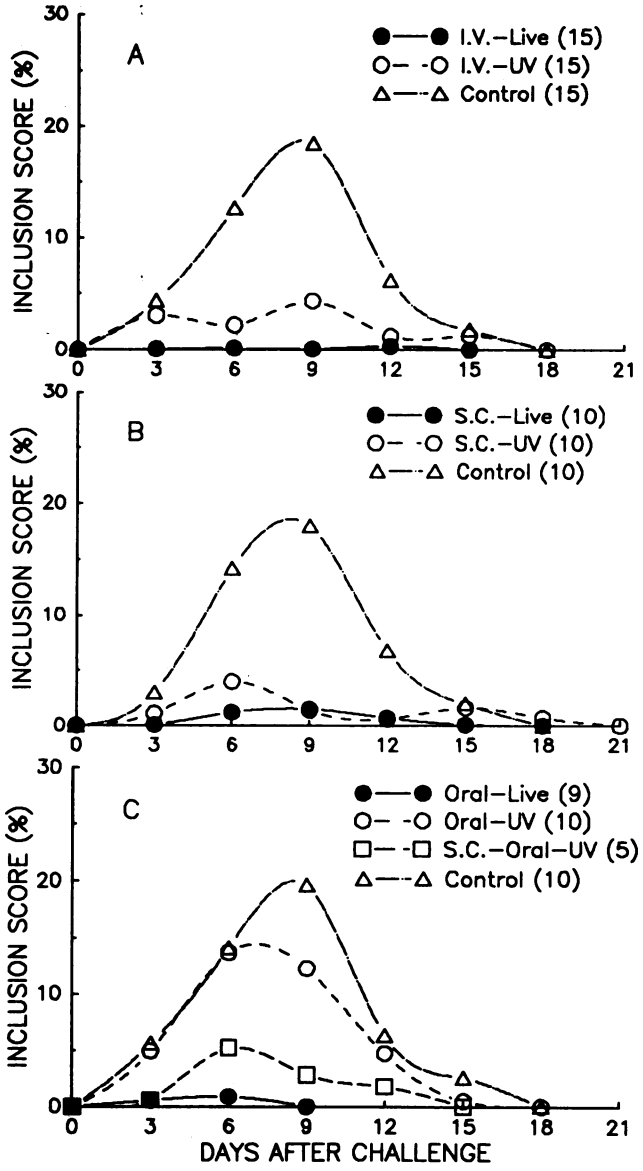

FIG. 2. Course of infection in groups of animals immunized with either viable or UV-inactivated GPIC by various routes as measured by inclusion score. I.V., Intravenous; S.C., subcutaneous.

challenge infection when compared to that of controls (Fig. 2). Oral immunization with inactivated antigen, in general, did not produce any protective effect, although two animals had negative inclusion scores upon challenge. Immunization with viable chlamydiae by all routes significantly shortened the course and markedly lowered the intensity of the challenge infection. In many animals demonstrating protection, no inclusions could be detected at any time point during the observation period.

When cervical swabs were assayed for viable chlamydiae, few animals were found to be uninfected after challenge. All animals immunized with inactivated antigen became reinfected, and despite the low inclusion scores in many animals, no differences were seen between immunized and control guinea pigs with regard to the length of the challenge infection (Fig. 3). Only animals receiving viable organisms demonstrated resistance to reinfection. Of these, intrave-

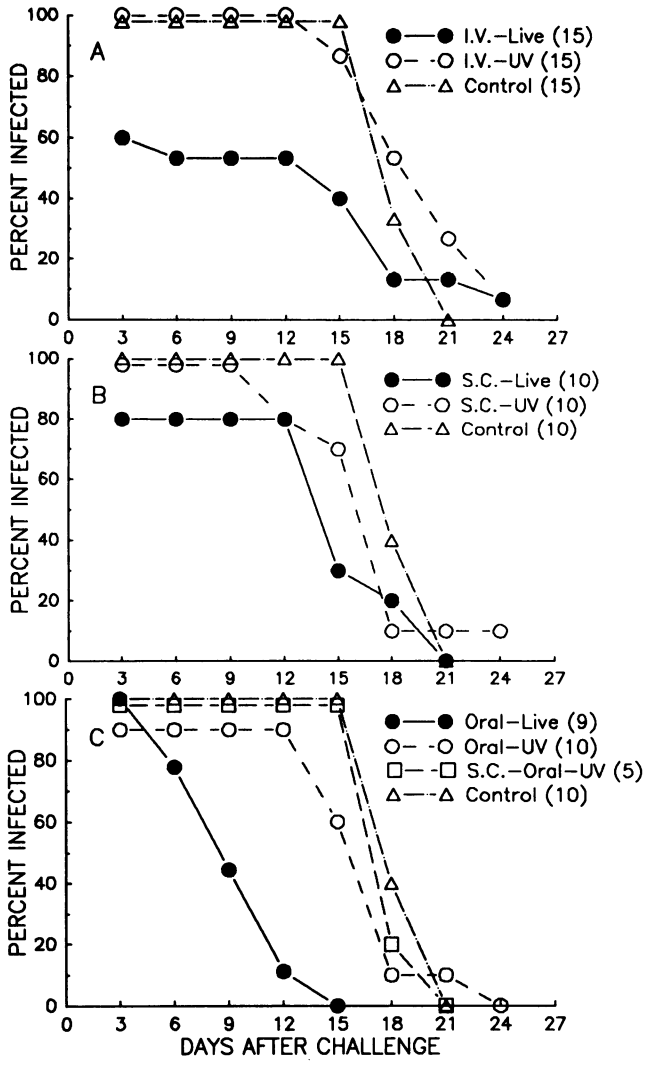

FIG. 3. Course of infection in groups of animals immunized with either viable or UV-inactivated GPIC by various routes as measured by isolation of GPIC from cervical swabs. I.V., Intravenous; S.C., subcutaneous.

nous inoculation of viable GPIC produced resistance in 6 of 15 guinea pigs. Animals immunized orally with live organisms resolved the challenge infection more quickly than control guinea pigs or guinea pigs immunized with inactivated antigen. However, some animals immunized with viable GPIC remained infected as long as unimmunized controls.

The difference in protective capacity of the live versus inactivated vaccines was analyzed by determining the length of infections, as determined by isolation of GPIC, of animals receiving live or inactivated preparations. This information was combined for all animals immunized with viable organisms and all animals immunized with inactivated organisms, regardless of the route, and the percentage of each group with infections of n days in length was determined (Fig. 4). Animals immunized with live organisms had significantly shorter infections (8.1 ± 6.6) than those animals immunized with inactivated organisms (15.2 ± 4.3) or unimmunized controls (16.0 ± 1.5) ($P < 0.0001$ according to a one-tailed t test).

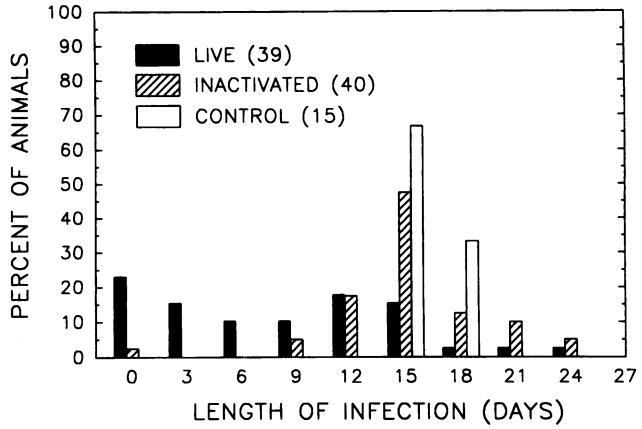

FIG. 4. Length of infections of guinea pigs immunized with inactivated or live organisms or those remaining untreated. The total number of animals for each group is given in parentheses, and each bar represents the percentage of that total which had infections of n days. Infection was determined by isolation of organisms from cervical swabs obtained at 3-day intervals.

Immune parameters. Various immune parameters were determined on all animals 1 or 2 days prior to challenge infection in an attempt to correlate protection or resistance with a particular immune response. Figure 5 represents a single experiment in which five animals each were immunized with viable or inactivated antigen by intravenous, subcutaneous, or oral routes. All groups with the exception of the group immunized orally with inactivated antigen produced high serum and secretion IgG antibody responses. IgA antibody levels in genital secretions were low throughout except for animals immunized subcutaneously with inactivated antigen. When peripheral blood lymphocytes were tested for proliferation to GPIC antigen, the best responses were seen in subcutaneously immunized guinea pigs, either with live or inactivated antigen. Although results were not shown in Fig. 5, the two animals in the group immunized orally with inactivated antigen which were immune were the only animals in that group to have elevated serum and secretion IgG as well as lymphocyte proliferation responses. It is interesting that nonspecific lymphocyte proliferation as measured by the response to concanavalin A was significantly greater ($P < 0.05$ according to a one-factor analysis of variance with Scheffe test for groups with significant differences) in the animals infected subcutaneously when compared to all groups except for intravenously infected guinea pigs (data not shown).

When a correlation matrix (Pearson's) was performed on data from 89 animals included in this study for which antibody and proliferative responses were available, the only parameter which correlated with low inclusion scores upon challenge was the level of serum IgG antibody. Thus the higher the titer of serum IgG antibody to GPIC, the lower the inclusion scores in challenged animals. Although the level of secretion IgG was strongly correlated to that of serum IgG, no association was seen between the level of infection and the titer of secretion IgG.

Antigen-specific antibody response. The antigen-specific antibody response in serum was also assessed prior to challenge infection to determine if the presence or absence of an antibody response to a particular antigen could be associated with protection or susceptibility to challenge infection. Each serum was evaluated for the presence of IgG

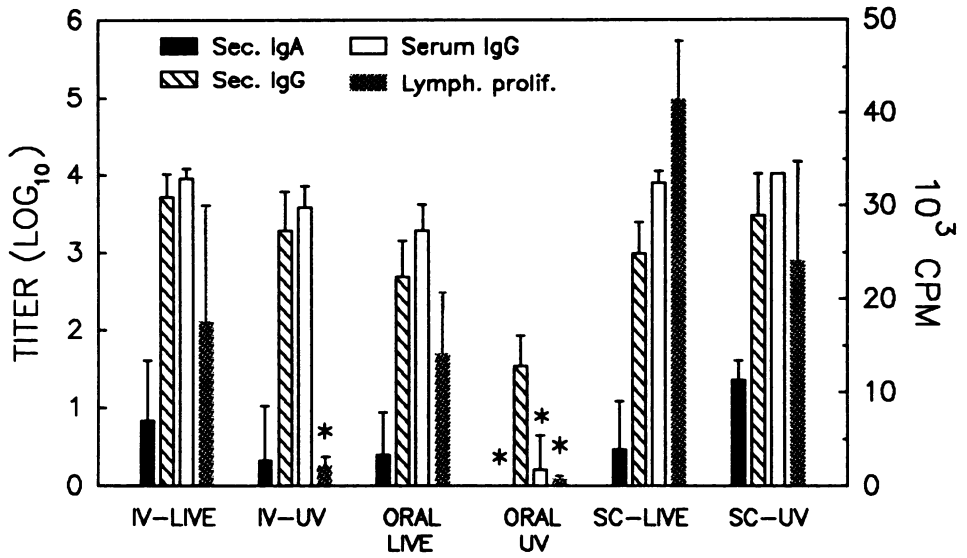

FIG. 5. Immune response parameters from guinea pigs immunized by different routes with either live or UV-inactivated GPIC. Data are from a single experiment so that comparisons among groups can be more readily identified. *, Significant difference of at least $P < 0.05$ according to a one-factor analysis of variance with a Scheffe test for significant differences. Sec., Secretion; I.V., intravenous; S.C., subcutaneous; Lymph. prolif., lymphocyte proliferation.

antibody to each of 13 different antigens. With the exception of most guinea pigs immunized orally with inactivated antigen which had negligible antibody responses, all animals produced serum antibodies to the 61-kDa protein and a very high percentage responded to MOMP and lipopolysaccharide (LPS) (Table 1). The 61-kDa protein is an outer membrane protein analogous to the cysteine-rich outer membrane protein of *C. trachomatis* (6) although a Sarkosyl-soluble protein comigrates with it in preparations of whole elementary bodies. The 61-kDa Sarkosyl-soluble protein is probably the same as the 57-kDa delayed-hypersensitivity protein reported by Morrison et al. (11). To differentiate between the Sarkosyl-soluble and Sarkosyl-insoluble moieties appearing in the 61-kDa area, some sera in each group were assessed for antibody activity to the two Sarkosyl fractions by immunoblot analysis. Virtually all animals responded to the 61-kDa protein in both soluble and insoluble fractions (data not shown) as has been seen before (2). Most animals also responded to the 47-kDa moiety. The serum antibody responses to other antigens were variable among and within the different immunization regimens.

One interesting observation is that sera from guinea pigs immunized orally with viable GPIC were consistently positive only in their reactions to MOMP, LPS, and the 61-kDa protein. Moreover, the two protected animals in the group immunized orally with inactivated GPIC were the only animals in that group to respond to the 61-kDa protein (both animals), MOMP (1 animal), and LPS (both animals).

Similar results were obtained upon determination of the secretion IgG antibody response although not a large number of animals were tested. Antibodies to MOMP, LPS, and the 61-kDa protein were present in the majority of the animals. Even the animals immunized orally with inactivated GPIC had a high response rate to the 61-kDa protein and about a 50% response rate to MOMP. The remainder of the reactions among all immunization groups to other antigens was variable.

DISCUSSION

The data presented in this study indicate that immunization of guinea pigs with UV-inactivated GPIC elementary

TABLE 1. Serum IgG antibody responses to specific chlamydial antigens as determined by immunoblot analysis

Antigen	% of animals responding to antigen (no. of animals tested)[a]							
	Ocular (5)	i.v.-live (15)	i.v.-UV (15)	s.c.-live (10)	s.c.-UV (10)	Oral-live (9)	Oral-UV (10)	s.c.-oral (5)
84 kDa	100	87	80	80	90	33	10	0
72 kDa	20	53	33	50	50	11	0	0
61 kDa	100	100	100	100	100	100	20	100
52 kDa	20	47	0	50	0	11	0	0
47 kDa	100	100	93	60	100	89	50	80
MOMP	100	100	100	90	100	100	10	80
35 kDa	0	47	93	40	90	11	0	20
33 kDa	100	73	7	40	20	11	0	20
31 kDa	40	73	20	40	50	11	0	0
27 kDa	80	100	87	80	90	22	0	40
19 kDa	0	100	87	0	90	11	0	40
15 kDa	100	47	53	100	80	67	70	40
LPS kDa	100	100	93	100	100	100	20	100

[a] Regimens: i.v., intravenous; s.c., subcutaneous.

bodies is able to provide a significant measure of protection against a challenge infection in the genital tract. This protection is, however, only reflected in a decreased intensity of the challenge infection and not in an increased resistance to infection nor in an abbreviated infection course; i.e., all animals given inactivated antigen became infected, and these infections were unaltered in duration compared to controls. The route of immunization did govern to some extent the development of protection against challenge. Both intravenous and subcutaneous routes were effective in inducing a protective immune response as judged by a decrease in inclusion scores, but oral immunization conferred protection in only a small proportion of animals. Only when oral immunization was preceded by a subcutaneous primary injection did immunity result. The lack of significant protection by oral immunization with inactivated organisms was somewhat unexpected since induction of an immune response with an inert antigen via a mucosal pathway has been shown to elicit a good response at other mucosal sites (10).

Since it has been previously observed by others that prior genital, ocular (8, 14), or oral (16) infection results in resistance to reinfection at a distant site in a certain percentage of animals, we compared the effect of inoculation of guinea pigs with viable GPIC by the intravenous, subcutaneous, ocular, and oral routes to immunization with inactivated antigen via these same routes (except the ocular route). Interestingly, injection of viable organisms by the intravenous and subcutaneous routes resulted in 40 and 20%, respectively, of the animals being completely resistant to reinfection. Although oral inoculation of live GPIC did not elicit resistance to reinfection, the course of infection in most animals was considerably shorter than in control animals or animals immunized with inactivated antigen by any route. A smaller percentage of animals in each group had challenge infections of normal duration. Thus, immunization with viable organisms produced a significantly stronger immunity than immunization with inactivated antigen. Murray and Radcliffe (15) reported similar findings in attempts to immunize against ocular GPIC infection. In their studies, Formalin-inactivated antigens were found to be ineffectual in inducing immunity as judged by inclusion scores, while intraperitoneal injection of viable GPIC was able to reduce the level of subsequent ocular challenge infection. Moreover, Taylor et al. (30) also found that orally administered live but not UV-inactivated *C. trachomatis* serovar L_2 was able to induce a protective response against serovar B challenge in their primate model for trachoma.

When the immune response of the immunized guinea pigs was determined prior to challenge, a correlation was seen between the level of serum IgG and reduction in infection intensity, but no factors were found which could be associated with the increase in incidence of resistance to infection and the shortened duration of infection seen in animals given viable GPIC. Both intravenous and oral route groups given inactivated antigen had significantly lower lymphocyte proliferation reactions, but inactivated antigen administered subcutaneously induced a very strong proliferative response with no apparent difference in the kinetics of the challenge infection. The strongest T-cell response occurred in the group injected subcutaneously with viable GPIC. This group also had an increase in the nonspecific T-cell proliferative response, but the resulting infection course upon challenge was not remarkably different from the other groups given viable organisms.

The only factor positively identified with a protective response, as measured by inclusion scores, was the ability of the immunization regimen to induce the production of serum IgG. That serum antibody plays an important role in immunity to reinfection has been demonstrated previously when we were able to elicit a reduction in infection intensity by the passive transfer of immunoglobulin from immune animals to naive guinea pigs (24). Moreover, animals deficient in antibody but competent in CMI response to GPIC became reinfected upon challenge with infections resembling a primary GPIC genital infection (22).

It is quite likely that antibody in secretions is responsible for reducing the level of infection in all animals demonstrating some degree of protection. As would be expected, antibodies to a wide range of antigens were detected by immunoblot. The only antibody responses which were consistent among protected animals were directed against MOMP, LPS, and the 61-kDa protein(s), which would suggest that protective epitopes are contained on these moieties. Monoclonal antibodies to MOMP have been shown to neutralize infectivity both in vivo (31, 32) and in vitro (5, 19). We have demonstrated that MOMP, purified by using a nondenaturing detergent extraction, is capable of inducing a protective response against GPIC genital infection in guinea pigs (B. E. Batteiger, R. G. Rank, and L. S. F. Soderberg, unpublished data).

The reasons for the difference in protective capacity of live- versus inactivated-antigen immunization regimens are not clear, especially since UV inactivation does not alter the ability of the organism to enter the host cell or resist lysosomal fusion (4, 12). It is certainly possible that the live infection generates quantitatively more antigen by nature of replication, but one might expect that a difference should be reflected in the level of antibody and lymphocyte proliferative responses, which was not the case. More likely possibilities are that either different antigens are expressed by the dividing organism or that macrophage processing of a viable organism with the ability to replicate intracellularly results in a qualitatively different immune response, particularly with respect to the T-cell response. This concept is not without precedent. Müller and Louis (13) have reported that protective T-cell clones could be generated in vitro by using viable *Leishmania* organisms as the antigen, but they could not be induced with inactivated antigen. In fact, T-cell clones elicited with inactivated antigen were more likely to induce an immunopathological but not protective response.

The significance of this study is that a certain degree of immunity can be produced by immunization with inactivated antigen and to a somewhat greater extent with viable chlamydiae. Aside from the impracticality of immunizing with viable chlamydiae in human subjects, an important point to consider is that none of the regimens tested could reliably prevent challenge infection. In fact, it should be noted that actual genital infection, which should be the strongest immunizing event, results only in complete immunity to reinfection for a very short period of time, with animals becoming reinfected if challenged as soon as 50 days after resolution of a primary infection. Therefore, it is reasonable that an objective other than complete prevention of genital infection should be pursued. Since the major morbidity associated with genital infection is the development of salpingitis with resultant tubal obstruction, an appropriate immunization strategy would be the prevention of ascending infection but not necessarily the prevention of lower-genital-tract infection. The feasibility of this approach has been suggested by a study of recurrent gonococcal infection (3). Women who had pelvic inflammatory disease (PID) and were reinfected in the lower genital tract with the same

serotype had no recurrent PID. However, women who were reinfected with a different serotype had a 50% incidence of PID. Thus, the primary infection was able to prevent salpingitis upon challenge infection but not able to prevent infection of the lower genital tract. Since the data in the guinea pig model indicate that the level of infection can be reduced in immunized animals for relatively long periods of time, this strategy is a reasonable possibility.

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