Vaginal immunization of rats with a synthetic peptide from human immunodeficiency virus envelope glycoprotein

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Local secretory immunity in the vagina may confer a degree of protection against heterosexual transmission of human immunodeficiency virus (HIV). Since the vagina has been shown to respond to local immunization, we have undertaken intravaginal immunization of rats with a 20-mer peptide (amino acid residues 102 to 121) of the HIV-1 envelope glycoprotein (gp120). The peptide was administered in combination with an ‘absorption enhancer’, lysophosphatidyl glycerol (LPG), which has previously been shown to promote the absorption of intravaginally administered peptides, while exerting only mild effects on epithelial membrane integrity. Intravaginal immunization with LPG and the peptide induced serum and vaginal wash IgA and IgG antibody responses which were enhanced in comparison to those after immunization with the peptide alone. Serum antibodies induced by both subcutaneous and intravaginal immunization were able to recognize recombinant HIV-1 gp120. However, the rat antiserum displayed no neutralizing activity against the virus. These results demonstrate that LPG is an effective immunological adjuvant for intravaginally administered peptide antigens. An alternative absorption enhancer, bestatin (BES), was not effective as an immunological adjuvant when administered intravaginally and blocked the adjuvant activity of LPG when BES and LPG were used in combination.

Although the majority of individuals infected with the human immunodeficiency virus (HIV) acquire their infection mucosally through heterosexual contact (Forrest, 1991), the majority of HIV vaccine research has concentrated on parenteral immunization. Parenteral immunization is generally associated with the induction of serum IgG, but little or no secretory IgA (Mestecky & McGhee, 1987). Recently, it has been suggested that HIV vaccine research should focus on the stimulation of protective immunity at the most common site of exposure, the vagina (Forrest, 1991).

Antibodies to the major antigenic components of HIV-1 (Belec et al., 1989a) and HIV-2 (Belec et al., 1989b) have been demonstrated in the genital secretions of infected individuals. Wolfsy et al. (1986) suggested that antibodies in genital secretions may be able to reduce transmission of HIV, but this remains to be proven. The important protective functions of secretory IgA and its capacity to neutralize different viruses have been well documented (Mestecky & McGhee, 1987). Passive transfer studies have recently confirmed the role of secretory IgA in protection against influenza virus infection (Renegar & Small, 1991). Furthermore, experimental studies in mice have indicated that local antibodies in the genital tract are responsible for increased resistance to local infection with herpes simplex virus type 2 (HSV-2) (McDermott et al., 1990). An animal model for assessing heterosexual transmission of HIV has been established (Miller et al., 1989) and should allow the ability of secretory immunity to protect against genital transmission of HIV to be evaluated (Miller & Gardner, 1991).

Mucosal immunity is most efficiently induced by local presentation of antigens and experimental evidence confirms that the vagina is capable of mounting an immune response to locally administered antigens (Parr & Parr, 1985). Studies in humans have shown that specific IgA and IgG antibodies are secreted into the cervico-vaginal mucus following vaginal or cervical immunization (Waldman et al., 1971; Ogra & Ogra, 1973).

In the studies described here, we intravaginally immunized rats with a synthetic peptide representing a region of the envelope glycoprotein (gp120) from HIV-1\textsubscript{HIV}. The use of defined peptide epitopes for vaccine
development against HIV has recently been discussed by Berzofsky (1991). The peptide approach potentially allows the inclusion of epitopes that induce protective responses, while omitting those which may be responsible for harmful responses. Epitopes that have been shown to induce antibodies reported to enhance HIV infection (Robinson et al., 1988) may be omitted. A recent study in macaques has confirmed that a peptide vaccine comprising selected regions of the envelope glycoprotein is capable of inducing protective immunity against simian immunodeficiency virus (SIV) (Shafferman et al., 1991).

The peptide (ADP 740-8) used here contained 20 amino acids from HIV-1 gpl20, corresponding to residues 102 to 121. Hence, the chosen peptide contained the majority of the epitope (residues 112 to 126) reported to induce virus-neutralizing antibodies recognizing HIV gpl20 (Neurath et al., 1990). The peptide was administered intravaginally in combination with 'absorption enhancers', one of which, lysophosphatidyl glycerol (LPG), has previously been shown to promote the vaginal absorption of peptides (Richardson et al., 1992).

Groups of six female Wistar BKW rats (200 g) were immunized with peptide ADP 740-8, obtained from the Medical Research Council AIDS Reagent Project, National Institute for Biological Standards and Control, Potters Bar, U.K. Four groups (no. 1 to 4) of rats were immunized intravaginally with 200 μg of peptide in acetate buffer pH 5-5 daily for 4 days on two occasions separated by 3 weeks. The groups were immunized as follows: group 1, peptide alone; group 2, peptide with 0-5% (w/v) LPG; group 3, peptide with 0-01% (w/v) bestatin (BES); and group 4, peptide with a combination of 0-5% (w/v) LPG and 0-01% (w/v) BES. The absorption enhancers LPG and BES were co-dissolved with the peptide, and the immunogens were administered absorbed into tampons.

In addition, a fifth group (no. 5) of rats was immunized subcutaneously (s.c.) with 200 μg of peptide in Freund's adjuvants. The animals were immunized s.c. on two occasions, initially with peptide in complete Freund's adjuvant and 3 weeks later with peptide in incomplete Freund's adjuvant.

Vaginal wash and serum samples were taken from all animals in groups 1 to 5 at 3, 6 and 9 weeks after the booster immunization. Rat vaginas were diluted and 100 μl of sterile saline pH 6 was aspirated in and out of the lumen using a pipette. Plastic pipette tips were rounded by brief exposure to a flame to prevent possible epithelial abrasion. On each occasion, vaginal wash samples were collected over 4 consecutive days, centrifuged to remove cell debris and pooled for assay. Blood samples were collected from the tail veins, and serum was collected and stored frozen at −20°C.

The levels of IgA and IgG in the serum and vaginal wash samples were measured by ELISA as described previously (O'Hagan et al., 1991), except that peptide ADP 740-8 was adsorbed to microtitre plates at 10 μg/ml in 0-05 M-carbonate buffer pH 9-9 and sheep anti-rat IgA horseradish peroxidase conjugate (Serotec) was used at a dilution of 1:5000.

The results for the serum and vaginal wash samples are expressed in Table 1 as antibody units calculated from a standard curve obtained by diluting the samples from group 5, which showed the highest levels of peptide

<table>
<thead>
<tr>
<th>Group</th>
<th>Immunization</th>
<th>Serum IgG and IgA</th>
<th>Vaginal wash IgG and IgA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Week 3</td>
<td>Week 6</td>
</tr>
<tr>
<td>1</td>
<td>Peptide alone</td>
<td>IgG 591 (132)</td>
<td>620 (108)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA 1617 (229)</td>
<td>594 (103)</td>
</tr>
<tr>
<td>2</td>
<td>Peptide + LPG</td>
<td>IgG 4731 (599)</td>
<td>3258 (889)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA 2211 (441)</td>
<td>1635 (372)</td>
</tr>
<tr>
<td>3</td>
<td>Peptide + BES</td>
<td>IgG 679 (181)</td>
<td>449 (153)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA 1152 (281)</td>
<td>706 (182)</td>
</tr>
<tr>
<td>4</td>
<td>Peptide + LPG + BES</td>
<td>IgG 396 (101)</td>
<td>648 (170)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA 557 (161)</td>
<td>725 (196)</td>
</tr>
<tr>
<td>5</td>
<td>Peptide s.c.</td>
<td>IgG 10000 (1407)</td>
<td>8598 (1321)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>IgA 10000 (1209)</td>
<td>6691 (924)</td>
</tr>
<tr>
<td></td>
<td>Preimmunization values</td>
<td>IgG 258 (72)</td>
<td>707 (163)</td>
</tr>
</tbody>
</table>
specific antibodies. The highest levels of antibodies found in the samples from group 5 were assigned a value of 10000 antibody units. All serum and vaginal wash samples were assayed at four different dilutions. An unpaired Student's t-test was used to compare the means for each study group at the different sample times and to assess statistical significance. Results were considered statistically significant at \( P < 0.05 \).

Vaginal immunization with peptide ADP 740-8 and LPG induced significantly enhanced levels of serum IgG and vaginal wash IgA and IgG antibodies for the full 9 week duration of the study (Table 1). Furthermore, at week 9, the levels of vaginal wash IgA antibodies in the animals immunized intravaginally with ADP 740-8 and LPG were comparable to those induced by s.c. immunization (Table 1). Vaginal immunization with ADP 740-8 and BES did not result in enhanced serum or vaginal wash IgG or IgA antibody responses. In fact, BES appeared to interfere with the effects of LPG when they were combined (Table 1).

These results showed that LPG is an effective immunological adjuvant for an intravaginally administered peptide antigen. LPG has been used previously as an absorption enhancer for vaginal administration of insulin in rats (Richardson et al., 1992). Although its mechanism of action has not been accurately defined, LPG is surface-active at low concentrations and the mechanism of absorption enhancement is likely to be similar to that of alternative surfactants (O'Hagan & Illum, 1990). However, an important consideration is the low level of membrane toxicity reported for LPG in comparison to alternative absorption enhancers (Richardson et al., 1992).

Previous studies in rodents involving intravaginal immunization have reported only low levels of IgA antibodies in vaginal wash samples and IgG responses as being generally absent (Parr et al., 1988; Thaparr et al., 1990). In addition, intravaginal immunization with sheep erythrocyte membrane proteins in immune-stimulating complexes (iscoms) induced only low levels of IgG and IgA antibodies in vaginal wash samples when compared to the levels induced by s.c. immunization (Thaparr et al., 1991). The levels of vaginal wash antibodies induced by intravaginal immunization with ADP 740-8 and LPG were comparable to those induced by s.c. immunization of ADP 740-8 with Freund's adjuvant (Table 1). Although we have not directly demonstrated that the antibodies in vaginal wash samples are of local origin, we are measuring 'local' antibodies in response to local immunization.

BES is an inhibitor of aminopeptidases, the enzymes mainly responsible for the degradation of peptides and proteins on mucosal surfaces, and has been used as an absorption enhancer (O'Hagan & Illum, 1990). In addition, BES has also been shown to be an immunological adjuvant (Umezawa, 1984). However, BES was not an effective adjuvant following intravaginal administration and appeared to inhibit the adjuvant effect of LPG (Table 1). There may be some physical interaction between LPG and BES or, alternatively, BES may interact directly with the peptide to inhibit its transmucosal transport.

Serum samples taken from both groups 2 and 5 at 9 weeks after booster immunization were screened for antibodies which could bind to recombinant gp120 using an antigen-capture ELISA as described previously (Moore et al., 1989). Recombinant gp120 (rgp120) (HIV-1MB; BH10 clone) was expressed in and purified from CHO cells by Celltech for the MRC AIDS Directed Programme; some of its properties have been described elsewhere (Moore et al., 1990). Briefly, rgp120 was captured via its C terminus by sheep antibody D7324 (Aalto Bioreagents). The serum samples were added, and bound rat antibodies were detected using alkaline phosphatase-conjugated sheep anti-rat IgG. Serum sam-

Fig. 1. The binding of rat antiserum to gp120. The rat antiserum was raised by s.c. immunization with peptide ADP 740-8 (a) or by intravaginal immunization with peptide ADP 740-8 and LPG (b). The results are shown as bound antibody, expressed as the absorbance in ELISA, against the dilution of serum from four individual rats (■, □, ●, ○).
pies from both groups 2 and 5 recognized rgpl20 (Fig. 1a and b).

The ability of rat serum samples to neutralize HIV was determined in an established neutralization assay. Briefly, cell-free HIV (10^3 TCID_{50} infectious virus/100 μl) was incubated with rat antisera (dilution of 1:20) for 1 h at 37 °C and then assayed for infectivity on C8166 cells as described previously (McKeating et al., 1989). No virus neutralization activity could be detected (data not shown).

The ability of antisera induced by immunization with peptides from the region of gp120 represented by ADP 740-8 (residues 102 to 121) to recognize gp120 is consistent with earlier findings (Neurath et al., 1990; Davis et al., 1990; Ahmar et al., 1991). However, the lack of neutralization activity in the antiserum contrasts with the findings of Neurath et al. (1990), who reported low titres of neutralizing antibodies following repeated immunization (five times) of rabbits with a peptide (residues 102 to 126) in Freund’s adjuvant. It is possible that additional immunizations of rats with ADP 740-8 would have resulted in the induction of antibodies with detectable neutralizing activity. Nevertheless, a recent report by Vahlne et al. (1991) has shown that a similar peptide (residues 102 to 126) from gp120 induces antibodies in monkeys that recognize gp120, but that there is no virus-neutralizing activity.

Although a local secretory immune response occurs in the female genital tract of many species (Parr & Parr, 1985), little is known about the uptake, processing and recognition of antigens at this site. Lymphoid aggregates have been observed in the adventitia adjacent to the urethra and the vagina in mice (Parr & Parr, 1990), but their relevance to mucosal immunity is unknown. Thus, IgA may not be the most important immunoglobulin in vaginal secretions. Indeed, the protective antibody response induced in mice by local immunization with HSV-2 is of the IgG isotype (McDermott et al., 1990). Nevertheless, in humans exposed via the genital tract to pathogenic organisms, a local IgA immune response is elicited (O'Reilly et al., 1976; Merriman et al., 1984). It remains to be determined whether IgA is more important than IgG in the female reproductive tract and whether species differences exist.

It has been shown that parenterally administered whole inactivated SIV vaccine does not protect macaques against SIV infection or ameliorate disease after genital challenge with SIV, but does delay disease in animals exposed intravenously (Sutjipto et al., 1990). It is hoped that approaches to vaccination involving mucosal administration of antigens may be more effective at blocking genital mucosal transmission of SIV and HIV.

It has been claimed that peptide vaccines could exploit the phenomenon of ‘original antigenic sin’ to steer the immune response towards protective epitopes following exposure to whole virus proteins or virus particles (Berzofsky, 1991). Whether this phenomenon can be exploited by mucosal immunization with peptides remains to be determined. Nevertheless, the results described indicate that peptides can be used to elicit local antibodies in the genital tract which are capable of recognizing gp120. Alternative peptide immunogens may induce local antibodies capable of neutralizing HIV, but this remains to be investigated. In addition, peptide immunogens may also be used locally to ‘prime’ the immune response so that a secondary response is elicited on exposure to the virus. Hence, the ability of HIV to infect mucosal Langerhans’ cells could be restricted, or infected cells could be eliminated before they transport the virus to the local lymph nodes.

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References


O'REILLY, R. J., LEE, L. & WELCH, B. G. (1976). Secretory IgA

O'HAGAN, D. T. & ILLUM, L. (1990). Absorption of peptides and proteins from the respiratory tract and the potential for the


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