VIROLOGY **225,** 213–215 (1996)
ARTICLE NO. 0589

SHORT COMMUNICATION

Topically Applied Human Recombinant Monoclonal IgG_1 Antibody and Its Fab and $F(ab')_2$ Fragments Protect Mice from Vaginal Transmission of HSV-2

L. ZEITLIN,*,[1] K. J. WHALEY,*,† P. P. SANNA,‡ T. R. MOENCH,† R. BASTIDAS,§ A. DE LOGU,‡ R. A. WILLIAMSON,§ D. R. BURTON,§,¶ and R. A. CONE*,†,[2]

*Department of Biophysics, The Johns Hopkins University, Baltimore, Maryland 21218; †ReProtect LLC, Baltimore, Maryland 21286; and Departments of ‡Neuropharmacology, ¶Molecular Biology, and §Immunology, The Scripps Research Institute, La Jolla, California 92037

Received May 7, 1996; accepted August 19, 1996

A recombinant human anti-herpes simplex virus monoclonal IgG_1 antibody and the corresponding Fab and $F(ab')_2$ fragments were tested for efficacy in preventing vaginal transmission of HSV-2 infection in a well-established mouse model for genital herpes. IgG_1, Fab, and $F(ab')_2$ were approximately equally protective; vaginal delivery of 1–5 ng provided approximately 50% protection, and vaginal delivery of 400 ng completely protected mice from genital herpes infection ($P \ll 0.001$). These results suggest that topical applications of human monoclonal antibodies may be useful in developing new methods for preventing sexually transmitted disease. © 1996 Academic Press, Inc.

Despite widespread promotion of condoms and other methods for preventing unintended pregnancies and sexually transmitted disease (STD), in the United States alone there are an estimated 3 million unintended pregnancies and 12 million new cases of STD each year (see *1*). These pandemics of unintended pregnancies and STD infections indicate an urgent need for improved methods of prophylactic contraception (*2*). Since antibodies are natural protective agents abundant in mucus secretions, and since they are potent and specific, monoclonal antibodies (MAbs) have recently been suggested for topical application to genital surfaces for contraception and the prevention of STDs (*1, 3*). Indeed, using mouse MAbs, we have recently confirmed that vaginal delivery of anti-rabbit sperm MAbs to the rabbit vagina can prevent pregnancy in the rabbit (*4*). Similarly, we have shown that topical delivery of a mouse anti-herpes simplex virus type 2 (HSV-2) MAb can prevent transmission of HSV-2 infection in the mouse via the vagina (*3*), as well as prevent transmission via genital skin and the rectum (unpublished results). For human applications, human MAbs may be desirable to reduce the likelihood of undesirable immune responses. With the advent of the ability to clone human MAbs from combinatorial libraries (reviewed in *5, 6*), the production of human MAbs against pathogens has become straightforward (*7–9*). Generation of a human anti-HSV MAb, HSV8, has recently been described (*8, 10*); this MAb binds glycoprotein D and is a potent neutralizer *in vitro.* Systemic delivery of HSV8 has been shown to protect nude mice from intradermal and corneal transmission of HSV-1 (*10*). We report here the results of efficacy trials testing topical delivery of HSV8 for preventing vaginal transmission of HSV-2 in a mouse model (*3, 11*). We also tested Fab and $F(ab')_2$ fragments derived from this monoclonal antibody.

Whole HSV8 antibody (*10*), was produced in CHO cells maintained in CHO serum-free medium (GIBCO-BRL; Gaithersburg, MD); antibody was purified from the supernatant by affinity chromatography with an immobilized chicken anti-human antibody according to standard techniques (*12*). Human IgG_1 purified from myeloma serum was obtained from Chemicon (Temecula, CA) and used as a control. Proteolytic digestions of HSV8 to obtain Fab and $F(ab')_2$ fragments were carried out as described by Harlow and Lane (*12*). Electrophoresis performed under nondenaturing conditions confirmed the purity of these fragments (not shown). To increase the susceptibility of mice to vaginal transmission of HSV-2 (*11*), we treated female, 8- to 12-week-old C57Bl/6 mice (Harlan; Frederick, MD) subcutaneously (100 μl, 2.5 mg/dose) with a long-acting progestin (Depo-Provera; Upjohn, Kalamazoo, MI) 7 days prior to the day of viral inoculation. Antibodies or antibody fragments were delivered in 10 μl of phosphate-buffered saline to the vagina approximately 20 sec prior to delivery of a 10 μl HSV-2 inoculum containing 10^5 $TCID_{50}$. This inoculum is approximately 10

[1] Current address: ReProtect LLC, Baltimore, MD 21286.
[2] To whom correspondence and reprint requests should be addressed at Thomas C. Jenkins, Department of Biophysics, The Johns Hopkins University, 3400 N. Charles Street, Baltimore, MD 21218. Fax: (410) 516-6597.

0042-6822/96 $18.00
Copyright © 1996 by Academic Press, Inc.
All rights of reproduction in any form reserved.

Halozyme EX2159
Merck v. Halozyme
PGR2025-00052

ID_{50} when infection is assayed by observation of visible lesions and 30 ID_{50} when assayed by culture of vaginal lavage (see below). HSV-2, strain G, was obtained from Virotech International (Rockville, MD). The sample was thawed, refrozen in aliquots at −70°, and thawed and diluted with media (Bartels Tissue Culture Refeeding Medium; Baxter) just prior to use. These thawed aliquots had a titer of 5×10^8 $TCID_{50}$/ml on human newborn diploid fibroblasts (Baxter; Issaquah, WA). Mice were assessed for infection by observation of visible lesions and by detection of viral shedding from the vagina as determined by culture of vaginal lavages (*3*) taken 3 days after inoculation (infected animals shed peak amounts of virus on Day 3; unpublished results). Mice were examined daily for 17 days after viral inoculation for visible lesions (perineal hair loss, reddening, and swelling); mice displaying lesions were sacrificed immediately to prevent undue suffering. Vaginal lavages were obtained by pipetting 20 μl of medium (Bartels Tissue Culture Refeeding Medium; Baxter) in and out of the vagina 10 times; lavages were diluted to 0.1 ml, and placed on target cells (human newborn foreskin diploid fibroblasts; Baxter). Cytopathic effect (CPE) was scored 2 days later. A total of 168 mice were inoculated. Statistical significance was determined by Fisher's exact test (*13*).

Figure 1 illustrates the efficacy of the three molecules for preventing vaginal transmission of HSV-2 infection as assayed by detection of vaginally shed virus (Fig. 1A) and by observation of visible lesions (Fig. 1B). At the higher doses, IgG_1, Fab, and $F(ab')_2$ were approximately equally effective on a mass basis, suggesting a slightly lower potency for the Fab on a molar basis. At lower doses, although the data are subject to experimental scatter, the Fab appeared to be up to an order of magnitude less effective than IgG on a molar basis. Complete protection against vaginal transmission of HSV-2 infection was achieved at doses of 400 ng antibody or antibody fragment ($P < 0.001$, compared to human IgG_1 or PBS controls), while approximately 1–5 ng provided 50% protection.

To our knowledge, these experiments are the first to demonstrate the protective efficacy of topical passive immunization with a human MAb or Fab fragment for preventing infection by a human STD pathogen *in vivo*. This MAb is one of the most potent anti-HSV MAbs described to date. Similar to the anti-HSV mouse MAb (III-174; *14*) used in our previous study (*3*), this human MAb is believed to neutralize by a postattachment mechanism (*8*); however, this human MAb is approximately 10 times more potent than III-174, which required 10 ng to provide 50% protection (*3*).

It is noteworthy that Fab was nearly as potent as IgG. Multivalent binding can potentially greatly increase the functional affinity, or avidity, of an antibody relative to the affinity of a monovalent Fab fragment (*15*), if steric constraints allow for multivalent binding. The absence of a dramatic functional difference between the human IgG and Fab in this study is probably due to steric constraints that prevent multivalent binding; electron micrographs by Stannard *et al.* suggest that glycoprotein D is deeply recessed in the virion's glycocalyx (~10 nm; *16*).

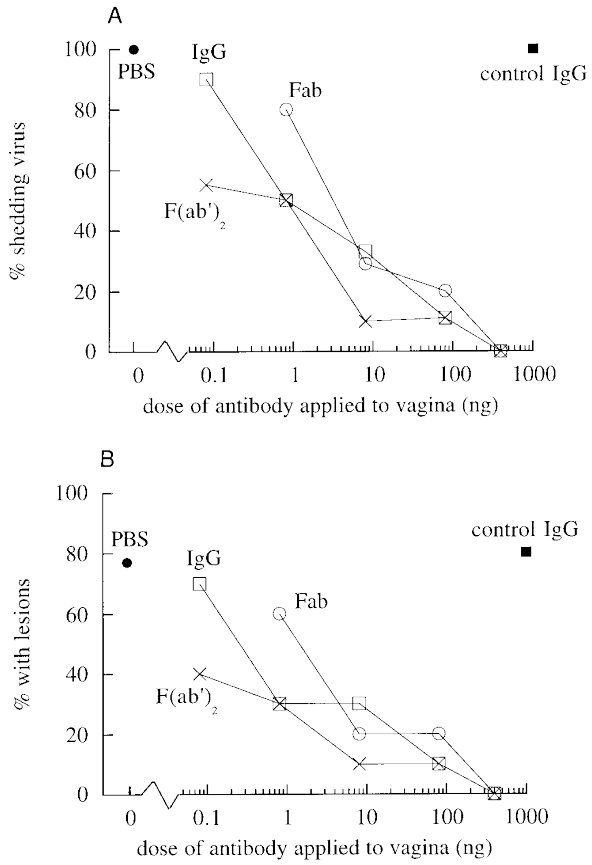

FIG. 1. Percentage infected animals as a function of vaginal dose of human anti-HSV monoclonal IgG_1, Fab, $F(ab')_2$. (A) Percentage of mice shedding virus ($7 \leq n \leq 10$; $n = 18$ for phosphate-buffered saline with no antibody; PBS). $n < 10$ in some instances because some animals could not be scored due to fungal overgrowth of the target cells. (B) Percentage of mice with visible lesions ($n = 10$, except for PBS, $n = 18$). For clarity, standard deviation is not shown; for all data points (A and B) the standard deviation ≤15%.

Since human MAb Fab fragments completely protected mice from HSV-2 infection, it appears that effector functions mediated by F_c, such as complement and antibody-dependent cellular cytotoxicity (ADCC), are not required for topically applied protection. In addition, since antibody alone was capable of preventing infection, these results suggest that vaccines that succeed in stimulating a strong mucosal vaginal antibody response, independent of a cell-mediated response, may be capable of preventing vaginal transmission of genital herpes.

The potency and specificity of human MAbs may help create improved methods for preventing STDs. Indeed, the human MAb used in this study is on the order of 1000 times more potent for preventing HSV-2 infection in the mouse than nonoxynol-9, the spermicidal deter-

gent now used in most vaginal contraceptives. The duration of protection by a topically applied monoclonal is limited by the residence time of antibodies in the vagina. The residence time in the human vagina is unknown; however, we found the residence half-life in the mouse vagina to be approximately 5 hr (*17*). Since the mouse vagina has about 20 times the surface area-to-volume ratio of the human vagina, we expect that several days of protection may be provided by a single dose of antibody in humans. For longer-term protection, vaginal devices that provide sustained release of antibodies may protect for weeks to months; we found antibody-releasing vaginal devices to be capable of releasing antibody in the mouse vagina for as long as a month (*18*) and capable of protecting mice from a vaginal HSV-2 inoculum for as long as a week (*19*).

The advent of methods for producing antigen-specific human MAbs by screening combinatorial libraries (*5*), for enhancing their affinity *in vitro* (*20, 21*), and for producing these MAbs in plants at a fraction of the cost of traditional techniques (*22, 23*) makes new methods for preventing unintended pregancies and STDs both technically and economically feasible.

ACKNOWLEDGMENTS

We thank Roman Rozenshteyn and Maria Samson for technical assistance. Partially supported by NIH Grants AI37582 (P.P.S.), AI33292 (D.R.B.), and GM43873. L.Z. was a Howard Hughes Medical Institute Predoctoral Fellow.

REFERENCES

1. Cone, R. A., and Whaley, K. J., *Am. J. Reprod. Immunol.* **32,** 114–131 (1994).
2. ICPD. International Conference on Population and Development. United Nations. Cairo, Egypt. September 5–13, 1994. Section 12.10.
3. Whaley, K. J., Zeitlin, L., Barratt, R. A., Hoen, T. E., and Cone, R. A., *J. Infect. Dis.* **169,** 647–649 (1994).
4. Castle, P. E., Whaley, K. J., Hoen, T. E., Moench, T. R., and Cone, R. A., *Biol. Reprod.,* in press.
5. Burton, D. R., and Barbas, C. F., *Adv. Immunol.* **57,** 191–280 (1994).
6. Winter, G., Griffiths, A. D., Hawkins, R. E., and Hoogenboom, H. R., *Annu. Rev. Immunol.* **12,** 433–455 (1994).
7. Williamson, R. A., Burioni, R., Sanna, P. P., Partridge, L. J., Barbas, C. F., and Burton, D. R., *Proc. Natl. Acad. Sci. USA* **90,** 4141–4145 (1993).
8. Burioni, R., Williamson, R. A., Sanna, P. P., Bloom, F. E., and Burton, D. R., *Proc. Natl. Acad. Sci. USA* **91,** 355–359 (1994).
9. Sanna, P. P., Williamson, R. A., DeLogu, A., Bloom, F. E., *et al., Proc. Natl. Acad. Sci. USA* **92,** 6439–6443 (1995).
10. Sanna, P. P., DeLogu, A., Williamson, R. A., Hom, Y. L., *et al., Virology* **215,** 101–106 (1996).
11. Whaley, K. J., Barratt, R. A., Zeitlin, L., Hoen, T. E., and Cone, R. A., *J. Infect. Dis.* **168,** 1009–1011 (1993).
12. Harlow, E., and Lane, D., Antibodies: A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 1988.
13. Matthews, D. E., and Farewell, V., Using and Understanding Medical Statistics, pp. 20–38. Karger, Basel, Switzerland, 1985.
14. Para, M. F., Parish, M. L., Noble, A. G., and Spear, P. G., *J. Virol.* **55,** 483–488 (1985).
15. Crothers, D. M., and Metzger, H., *Immunochemistry* **9,** 341–357 (1972).
16. Stannard, L. M., Fuller, A. O., and Spear, P. G., *J. Gen. Virol.* **68,** 715–725 (1987).
17. Sherwood, J. K., Zeitlin, L., Chen, X., Whaley, K. J., Cone, R. A., and Saltzman, W. M., *Biol. Reprod.* **54,** 264–269 (1996).
18. Radomsky, M. L., Whaley, K. J., Cone, R. A., and Saltzman, W. M., *Biol. Reprod.* **47,** 133–140 (1992).
19. Sherwood, J. K., Zeitlin, L., Whaley, K. J., Cone, R. A., and Saltzman, W. M., *Nature Biotechnol.* **14,** 468–471 (1996).
20. Barbas, C. F., Hu, D., Dunlop, N., Sawyer, L., Cababa, D., Hendry, R. M., Nara, P. L., and Burton, D. R., *Proc. Natl. Acad. Sci. USA* **91,** 3809–3813 (1994).
21. Yang, W-P., Green, K., Pinz-Sweeney, S., Briones, A. T., Burton, D. R., and Barbas, C. F., *J. Mol. Biol.* **254,** 392–403 (1995).
22. Hiatt, A., *Nature* **344,** 469–470 (1990).
23. Ma, J. K., Hiatt, A., Hein, M., Vine, N. D., Wang, F., Stabila, P., van Dolleweerd, C., *et al., Science* **268,** 716–719 (1995).