A humanized monoclonal antibody produced in transgenic plants for immunoprotection of the vagina against genital herpes

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The ability to produce monoclonal antibodies (Mabs) in plants offers the opportunity for the development of an inexpensive method of mucosal immunoprotection against sexually transmitted diseases. To investigate the suitability of plant-expressed Mabs for vaginal preventive applications, we compared a humanized anti–herpes simplex virus 2 (HSV-2) Mab expressed in mammalian cell culture with the same antibody expressed in soybean. We found these Mabs to be similar in their stability in human semen and cervical mucus over 24 h, their ability to diffuse in human cervical mucus, and their efficacy for prevention of vaginal HSV-2 infection in the mouse.

Keywords: applied immunology, agricultural biotechnology, immunization

Epidemic levels of sexually transmitted disease (STD) indicate an urgent need for improved methods of prophylaxis[1]. Prevention is particularly important for incurable infections such as human papilloma virus, herpes simplex virus (HSV), and HIV. Topical passive immunization for infectious disease prevention is being investigated[2]. Antibodies delivered topically in milk protect the entire gastrointestinal tract of infants, and experimental topically applied antibodies prevent infections of the gastrointestinal[3], respiratory[4], vaginal[5,6], and rectal mucosa[7]. With the advent of the ability to humanize murine monoclonal antibodies[8] and clone human monoclonal antibodies (Mabs) from combinatorial libraries[9,10], the production of human Mabs against pathogens has become routine[11]. These Mabs can be highly effective and potent for prevention of infection in vivo[6]. Furthermore, the development of the ability to produce Mabs in transgenic plants[12,13] allows high capacity production (tons) of Mab, and dramatically reduces cost[14]—qualities necessary for a Mab-based STD prophylaxis technology to be economically feasible. Glycosylation patterns in plants differ from those in mammals, and it is unclear whether plant-produced antibodies will protect mucosal surfaces as well as do traditionally produced Mabs. Glycosylation is an important issue for all production systems, but the relative importance of antibody glycosylation depends on the type of antibody product, the intended indication, and the expected mechanism of action. Glycan structure can affect antibody activity[15] and glycans may play a role in immune exclusion mechanisms in mucus.

We compared a humanized anti-HSV glycoprotein B plant-produced antibody (soybean) with the same Mab[16] expressed in mammalian cell-culture (Sp2/0 cells) in a number of assays, including diffusion and stability in mucus as well as prevention of vaginal transmission of genital herpes in the mouse. Prevention is particularly important as HSV infections are incurable. Currently, in the United States, one in five adults is infected with HSV-2 (ref. 17) and morbidity and mortality can occur in immunocompromised individuals and neonates infected during birth. In addition, infection with HSV may facilitate the sexual transmission of HIV[18,19].

Results

Electrophoresis of monoclonals. Plants are capable of high-fidelity assembly of immunoglobulins[12,13]; plant-produced Mab and mammalian cell-culture–expressed Mab were indistinguishable when run on a 4–15% polyacrylamide SDS gel under reducing and nonreducing conditions (Fig. 1).

Fluorocytometric analysis of monoclonals. Both the plant and mammalian expressed monoclonals bound Vero cells infected with HSV in a fluorocytometric analysis[16] in a similar manner (Fig. 2), suggesting that the affinity of the plant monoclonal did not differ from the affinity of the mammalian expressed antibody (5.3×10^{-7} M^{-1})[16].

In vitro neutralization of HSV-2. When the plant and mammalian Mab were tested in a standard neutralization assay, there was no apparent difference in their ability to neutralize HSV-2 (Fig. 3). The Mabs reduced HSV-2–induced cytopathic effect (CPE) by 50% at a concentration of approximately 0.2 μg/ml, and reduced CPE 100% at a concentration of 2 μg/ml. Controls using virus alone (no antibody) resulted in maximal CPE, and controls using antibody alone resulted in no CPE

Stability of monoclonals in human cervical mucus and semen. To determine whether antibody produced in plant is functional after exposure to human reproductive fluids, anti-HSV Mabs were incubated in human cervical mucus and semen, and then tested for neutralizing activity in vitro. Mabs from both production systems had similar stability for 24 h when incubated in cervical mucus and semen at 37°C (Table 1).

Diffusion of monoclonals in human cervical mucus. For antibodies to be able to perform their function on mucosal surfaces, they must be free to diffuse through mucus to bind their antigen[20,21]. To determine if differences in glycosylation affect the ability of plant-produced antibody to diffuse in mucus, we measured the diffusion coefficients (D) of the two Mabs in human midcycle cervical mucus (D_{mucus}) and in water (D_{water}). We found $D_{mucus}/D_{water} = 1.0 \pm 0.3$ for the plant antibody and $D_{mucus}/D_{water} = 1.1 \pm 0.3$ for the mammalian Mab, indicating that neither of these Mabs are slowed in mucus.

In vivo efficacy for the prevention of vaginal HSV-2 transmission. Using a mouse model of vaginal transmission of HSV-2 infection[5,22], we found that vaginal delivery of the plant antibody and mammalian cell-culture–expressed Mab provided similar levels of protection against a vaginal inoculum of HSV-2. Infection was assessed by two methods with different sensitivities: culture of vaginal lavage (Fig. 4A) and observation of lesions (Fig. 4B). When

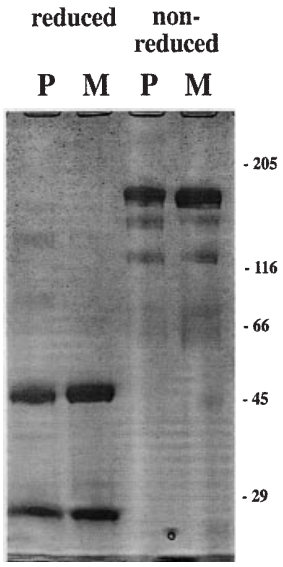

Figure 1. SDS electrophoresis of plant antibody (P) and mammalian (M) anti-HSV antibody on a 4–15% polyacrylamide gel under reduced and nonreduced conditions.

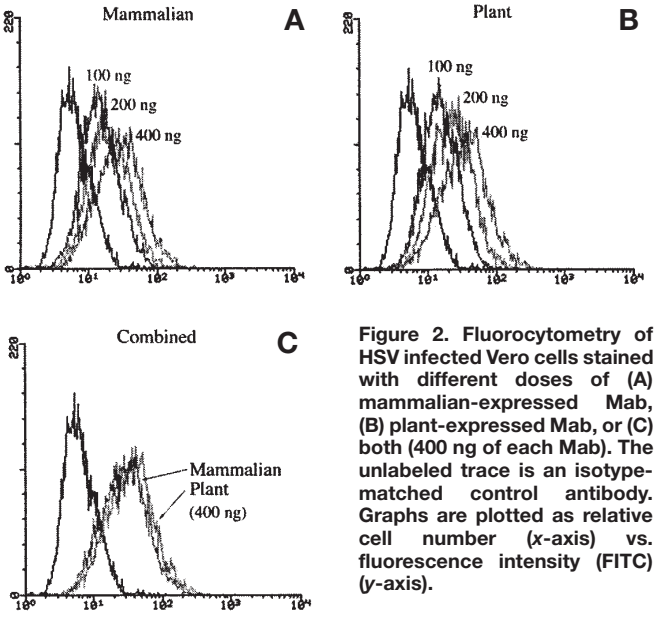

Figure 2. Fluorocytometry of HSV infected Vero cells stained with different doses of (A) mammalian-expressed Mab, (B) plant-expressed Mab, or (C) both (400 ng of each Mab). The unlabeled trace is an isotype-matched control antibody. Graphs are plotted as relative cell number (*x*-axis) vs. fluorescence intensity (FITC) (*y*-axis).

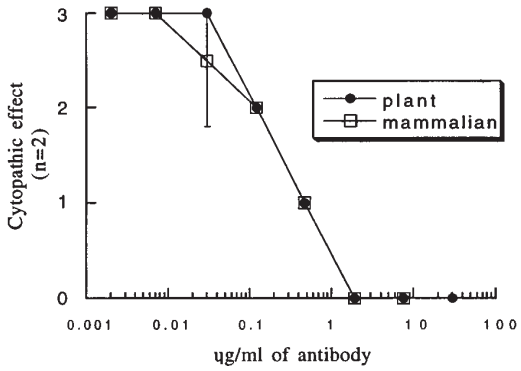

Figure 3. In vitro neutralization of HSV-2. Dilutions of antibody were incubated with 1500 $TCID_{50}$ HSV-2. Cytopathic effect (CPE) was scored at 48 h. 3: greater than 1/2 of target cell monolayer was disrupted; 2: one quarter to one half was disrupted; 1: less than one quarter was disrupted; and 0: no disruption was observed. Two replicates were run for each data point. Error bars indicate standard deviation.

Table 1. Stability of monoclonals in human seminal plasma and cervical mucus at 37°C.

Time at 37°C (h)	Percent of neutralization activity compared with untreated antibody			
	incubated in semen		incubated in cervical mucus	
	mammalian	plant	mammalian	plant
0	100 (25)[a]	100 (25)	100 (50)	100 (50)
1	75 (25)	75 (25)	100 (50)	100 (50)
25	75 (25)	75 (25)	50 (50)	100 (50)

[a]Numbers in parentheses indicate the error inherent in each measurement (a result of assaying antibody by serial dilution).

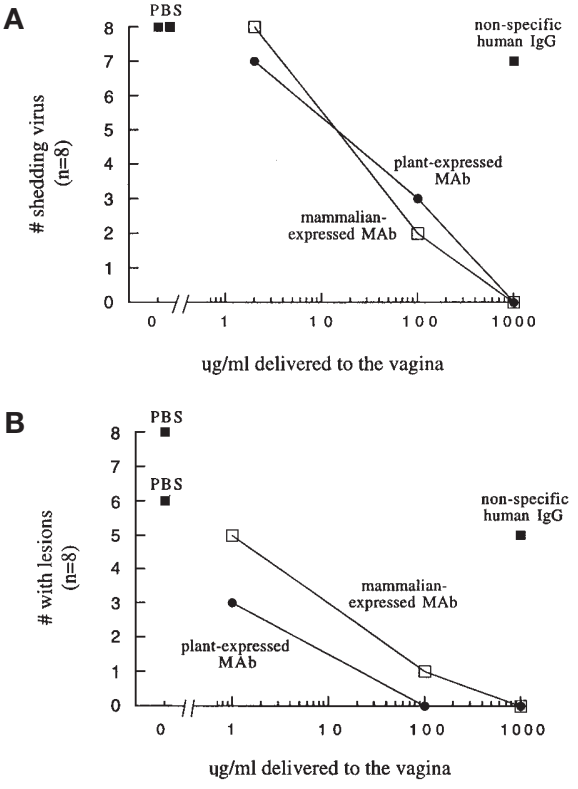

Figure 4. In vivo protection by topically applied Mab against vaginal transmission of HSV-2. (A) Number of mice shedding virus. (B) Number of mice with visible lesions. n = 8 for all groups.

infection was assessed by culture (the more sensitive method [Fig. 4A]), 50% protection was provided with a Mab concentration of 20–30 μg/ml (0.2–0.3 μg dose) and 100% protection was provided with a concentration of 1000 μg/ml (10 μg dose; $p<0.005$ compared with phosphate-buffered saline [PBS] and nonspecific IgG controls). Protection from symptomatic infection (Fig. 4B) required slightly less Mab: 50% protection was provided by a MAb concentration of 1–10 μg/ml (0.01–0.1 μg dose) and 100% protection at a concentration of 100–1000 μg/ml (1–10 μg dose; $p<0.05$ compared with nonspecific IgG control, $p<0.01$ compared with PBS controls).

Discussion

We compared a humanized monoclonal antibody produced in mammalian cell-culture (Sp2/0 cells) with the same antibody produced in plants (soybean) for functionality ex vivo and for immunoprotection against HSV-2 in vivo. This antibody recognizes glycoprotein B of HSV, a protein required for infectivity and implicated in initial binding to the target cell and in fusion of the viral and cellular membranes[23]. This antibody neutralizes HSV in the absence of complement[16], suggesting that its activity may be due to inhibition of binding and/or fusion of the virion to the target cell.

Expression of functional single-chain Fv protein, IgG, and secretory IgA have been achieved in plants; these plant-produced antibodies have antigen binding ability similar to the same protein expressed in bacterial or mammalian expression systems[12,13,24,25]. Mammalian glycans are similar to plant glycans in size and extent of branching; however, the predominant terminal residue in mammals is *N*-acetyl neuraminic acid, a carbohydrate not present in plants[26].

The IgG1 described here has two N-linked glycosylation sites (one per heavy chain)[16]. Glycan composition data and Western blot analyses are consistent with the presence of plant-specific fucose and xylose at the N-linked glycosylation site (D.A. Russell, personal communication). This is not unexpected from tobacco-based antibody production or plant endogenous protein glycosylation[26].

To determine whether plant glycosylation would affect the function of the plant-produced antibody[15], we compared the stability of the monoclonals after incubation in human midcycle cervical mucus and semen. Both monoclonals retained comparable anti–HSV-2 activity after 24 h. Experiments with human polyclonal antibody have demonstrated no loss in activity for sperm-agglutinating antibodies stored in human seminal fluid for 15 days at room temperature[27], or for IgG and SIgA when stored in seminal fluid or cervical mucus supernatant for 48 h at 37°C (ref. 28). Furthermore, monoclonals are stable and capable of binding antigen under the acidic conditions of the human vagina (pH 4–6)[29]. Together, these observations indicate that antibodies are stable in mucosal secretions of the human reproductive tract. We found the plant-produced and mammalian cell-culture–produced monoclonals to have similar efficacy for preventing infection in vivo using a well-characterized mouse model of vaginal transmission of HSV-2 infection[5,6,30].

Mabs, particularly plant-produced antibodies, fulfill many of the qualities desirable in new vaginal microbicides[31,32]. Mabs are efficacious, specific, and potent molecules; in a study evaluating candidate microbicides, an anti-HSV Mab was found to be 100–1000 times more potent on a weight basis than the other agents tested for preventing vaginal transmission of genital herpes infection in mice[30]. Because of their potency, Mabs can be applied in small volumes, allowing this method of woman-controlled protection to achieve the goal of being undetectable to partners[33]. Because of their vaginal residence time[34], Mabs may provide protection even on days when the woman fails to use the method. For longer-term protection, MAbs can also be delivered by sustained release from intravaginal devices[35,36].

Although the generation of a human antiplant antibody response is a potential concern, given our repeated mucosal exposure to plant oligosaccharides in food and personal care products, it is unlikely that any of these antigens will be novel to the mucosal immune system of humans[37,38]. In a recently completed clinical trial with plant-produced antibody for the prevention of oral colonization by *Streptococcus mutans*, no safety problems were found, and no human antiplant antibody responses were observed[39].

We have shown that the ex vivo stability and in vivo efficacy of an antibody expressed in plants is similar to the same antibody expressed in mammalian cell-culture. With the growing fulfillment of the clinical potential of Mabs[40,41], plant-produced antibodies are likely to allow development of inexpensive Mab-based health-care products[42].

Experimental protocol

Monoclonal antibodies. The mammalian antibody tested in this study is a previously described humanized IgG_1 directed against glycoprotein B of HSV[16]. Briefly, heavy and light chain plasmids were linearized and transfected into Sp2/0 mouse myeloma cells by electroporation, and cells were selected for *gpt* expression. Clones were screened by ELISA for human antibody production in the supernatant. Mabs were purified by passing tissue culture supernatant over a protein A-Sepharose CL-4B column (Pharmacia, Uppsala, Sweden) and eluting with 0.2 M glycine-HCl (pH 3.0. Mabs were then neutralized with 1 M Tris-HCl (pH 8.0). The buffer was then exchanged into PBS by passing over a PD10 column (Pharmacia). The antibody-encoding genes were constructed for plant expression in a manner similar to that used for an sFV[43]. This expression cassette uses a general plant promoter (CaMV 35S), a plant signal peptide (Tobacco extensin), and a nopaline synthase polyA addition site. The same cassette was used for both the heavy and light chain. The construction results in the addition of a methionine to the mature heavy chain end, while the light chain is changed to methionine-glutamate, from aspartate[16]. Tobacco cell cultures were developed as by Francisco et al.[43] using kanamycin selection. Expressing lines were defined by ELISA. Transgenic soy plants were developed as in McCabe et al.[44], and screened by ELISA. For antibody production, plants were harvested from the greenhouse at middevelopment and pressed to extract the soluble material. The clarified solution was purified by sequential chromatographic steps via protein A, cation exchange, and ion exchange, with a final storage solution of 20 mM Tris-HCl, pH 8.0.

Electrophoresis of Mabs. Electrophoresis of purified Mabs was performed on 4–15% polyacrylamide gels (Bio-Rad, Hercules, CA). Coomassie blue staining was carried out according to the manufacturer's instructions.

Fluorocytometric analysis of Mabs. Vero cells (ATCC, Manassas, VA) were infected with HSV at 3 plaque-forming units per cell overnight. Cells trypsinized (0.5 mg/ml for 1 min), were washed with PBS, and were resuspended in fluorescence-activated cell sorting (FACS) buffer (PBS/2% fetal calf serum/0.1% NaN_3) at approximately 5×10^6 cells/ml. One hundred microliters of cell suspension were incubated with antibody from plant or mammalian cell culture for 30 min on ice, washed with FACS buffer, and incubated with FITC-labeled goat antihuman antibody for another 30 min on ice. The cells were washed again, resuspended in PBS/1% paraformaldehyde, and analyzed on a FAC-Scan (Becton Dickinson, Franklin Lakes, NJ).

In vitro neutralization assay. A standard neutralization assay was performed[5,30,35,36]. Briefly, fourfold serial dilutions of Mab were incubated with 1500 $TCID_{50}$ HSV-2, strain G (Virotech, Rockville, MD) for 60 min at 37°C in a total volume of 100 μl. The antibody–virus mixture was then placed on target cells (human newborn foreskin diploid fibroblast cells from Bartels, Issaquah, WA) and CPE was semiquantitatively scored at 48 h.

Stability of Mabs in human cervical mucus and semen. Midcycle cervical mucus was obtained by nurse practitioners from donors at the Johns Hopkins University Student Health Center (Baltimore, MD). Semen was collected from healthy male donors by masturbation. All protocols were approved by the Johns Hopkins University Review Board on the Use of Human Subjects. Antibody was mixed (1:1) with cervical mucus or with seminal plasma (1:2) and incubated for 1 h or 24 h at 37°C. Samples were serially diluted in 96-well plates (twofold and fourfold serial dilutions were used for the semen and cervical mucus assay respectively), 250 $TCID_{50}$ HSV-2 was added to each well, the mixture was incubated for 1 h at 37°C, and the dilutions were then placed on target cells (human newborn foreskin diploid fibroblast cells). CPE was scored 2 days later and compared with that of controls that were not exposed to cervical mucus or seminal plasma. Controls

with no antibody resulted in maximal CPE, while controls with cervical mucus or seminal plasma only (no virus) resulted in no CPE. Two replicates were performed for each data point.

Diffusion of Mabs in human cervical mucus. Diffusion of the Mabs was measured by fluorescence recovery after photobleaching as described[20]. Mabs were labeled with FITC (Sigma, St. Louis, MO) and had approximately five fluorophores/antibody molecule. Flat glass capillary tubes (0.3×3.0×50 mm; Vitro Com, Rockaway, NJ) were siliconized (Rain-X; Unelko, Scottsdale, AZ) and filled with 10 parts phosphate-buffered water or human midcycle cervical mucus, 1 part 20 mg/ml bovine serum albumin (to prevent antibodies from binding to the glass), and 1 part Mab. The tubes were allowed to equilibrate overnight. The computer-controlled instrument for photobleaching has been described[45]. Briefly, an attenuated laser beam was focused near the center of the tube, and FITC label was bleached by briefly removing the attenuation (5–30 ms), thereby increasing the light intensity by three to four orders of magnitude. The attenuated beam was then used to measure the time course of recovery of fluorescence in the bleached spot from which the fraction of molecules that were mobile and the diffusion coefficient were determined[46]. In all experiments, the fraction of mobile molecules was near unity.

In vivo model of vaginal transmission. To increase the susceptibility of mice to vaginal transmission of HSV-2 (ref. 22), 8–12-week-old C57Bl/6 female mice (Harlan, Frederick, MD) were treated 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. On the day of inoculation, mice were anesthetized with Metafane (Pitman-Moore, Mundelein, IL), and 10 µl of Mab or PBS was delivered to the vagina with a fire-polished 10 µl pipette (Wiretrol; Drummond, Broomall, PA) approximately 20 s before delivering the virus inoculum in 10 µl of Bartels Tissue Culture Refeeding Medium. The inoculum used was 10 times the dose required to infect 50% of mice (10 ID_{50}) when infection was assessed by visible lesions, and was 30 ID_{50} when infection was assessed by culture of vaginal lavage[30]. After delivery of the inoculum the pipette was moved in and out four times to simulate the stirring action of coitus. Mice were assessed for infection by surveillance for visible lesions and by assaying for viral shedding from the vagina by culturing of vaginal lavages taken 3 days after inoculation (infected animals shed peak amounts of virus on day 3; data not shown). Mice were examined for visible lesions (perineal hair loss, reddening, and swelling) daily for 17 days after viral inoculation; mice displaying lesions were killed immediately to prevent undue suffering. Vaginal lavages were obtained by pipetting 20 µl of medium (Bartels Tissue Culture Refeeding Medium) in and out of the vagina 10 times; lavages were diluted to 0.1 ml, and placed on target cells (human newborn foreskin diploid fibroblast cells; Bartels). This assay does not evaluate the quantity of virus shed vaginally, but rather detects whether or not virus is present in the vaginal lavage. CPE was evaluated 2 days later. Statistical significance was determined by Fisher's exact test[47]. Test and control groups contained eight animals each. All protocols were approved by the Johns Hopkins University Animal Care and Use Committee.

1. Eng, T. and Butler, W. 1997. *The hidden epidemic*. National Academy Press, Washington, D.C.
2. Cone, R.A. and Whaley, K.J. 1994. Monoclonal antibodies for reproductive health: part I. Preventing sexual transmission of disease and pregnancy with topically applied antibodies. *Am. J. Reprod. Immunol*. **32:**114–131.
3. Davidson, G.P., Whyte, P.B., Daniels, E., Franklin, K., Nunan, H., McCloud, P.I. et al. 1989. Passive immunisation of children with bovine colostrum containing antibodies to human rotavirus. *Lancet* **2:**709–712.
4. Tamura, S., Funato, H., Hirabayashi, Y., Suzuki, Y., Nagamine, T., Aizawa, C. et al. 1991. Cross-protection against influenza A virus infection by passively transferred respiratory tract IgA antibodies to different hemagglutinin molecules. *Eur. J. Immunol*. **21:**1337–1344.
5. Whaley, K.J., Zeitlin, L., Barratt, R.A., Hoen, T.E., and Cone, R.A. 1994. Passive immunization of the vagina protects mice against vaginal transmission of genital herpes infections. *J. Infect. Dis.* **169:**647–649.
6. Zeitlin, L., Whaley, K.J., Sanna, P.P., Moench, T.R., Bastidas, R., De Logu, A. et al 1996. Topically applied human recombinant monoclonal IgG1 antibody and its Fab and F(ab')2 fragments protect mice from vaginal transmission of HSV-2. *Virology* **225:**213–215.
7. Zeitlin, L., Doctoral dissertation, Topical methods for preventing genital herpes infection in the mouse. The Johns Hopkins University, Baltimore, MD, 1996.
8. Co, M.S. and Queen, C. 1991. Humanized antibodies for therapy. *Nature* **351:**501–502.
9. Burton, D. and Barbas, C. 1994. Human antibodies from combinatorial libraries. *Adv. Immunol.* **57:**191–280.
10. Winter, G., Griffiths, A., Hawkins, R., and Hoogenboom, H. 1994. Making antibodies by phage display technology. *Annu. Rev. Immunol*. **12:**433–455.
11. Williamson, R., Burioni, R., Sanna, P., Partridge, L., Barbas, C., and Burton, D. 1993. Human monoclonal antibodies against a plethora of viral pathogens from single combinatorial libraries. *Proc. Natl. Acad. Sci. USA* **90:**4141–4145.
12. Hiatt, A., Caffertey, R., and Bowdish, K. 1989. Production of antibodies in transgenic plants. *Nature* **342:**76–78.
13. Ma, J., Hiatt, A., Hein, M., Vine, N., Wang, F., Stabila, P. et al. 1995. Generation and assembly of secretory antibodies in plants. *Science* **268:**716–719.
14. Hiatt, A. 1990. Antibodies produced in plants. *Nature* **344:**469–470.
15. Wright, A. and Morrison, S. 1997. Effect of glycosylation on antibody function: implications for genetic engineering. *Trends Biotechnol.* **15:**26–32.
16. Co, M.S., Deschamps, M., Whitley, R.J., and Queen, C. 1991. Humanized antibodies for antiviral therapy. *Proc. Natl. Acad. Sci. USA* **88:**2869–2873.
17. Fleming, D., McQuillian, G., Johnson, R., Nahmias, A., Aral, S., Lee, F. et al. 1997. Herpes simplex virus type 2 in the United States, 1976 to 1994. *N. Engl. J. Med.* **337:**1105–1111.
18. Hook, E.W., Cannon, R.O., Nahmias, A.J., Lee, F.F., Campbell, C.H., Glasser, D. et al. 1992. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. *J. Infect. Dis.* **165:**251–255.
19. Gwanzura, L., McFarland, W., Alexander, D., Burke, R.L., and Katzenstein, D. 1998. Association between human immunodeficiency virus and herpes simplex virus type 2 seropositivity among male factory workers in Zimbabwe. *J. Infect. Dis.* **177:**481–484.
20. Saltzman, W.M., Radomsky, M.L., Whaley, K.J., and Cone, R.A. 1994. Antibody diffusion in human cervical mucus. *Biophys. J.* **66:**508–515.
21. Cone, R. 1999. Mucus, pp. 43–64 in *Handbook of mucosal immunology,* 2nd ed. Ogra, P.L., Mestecky, J., Lamm, M.E., Strober, W., McGhee, J.R., Bienenstock, J. (eds.). Academic Press, Washington, D.C. In press.
22. Whaley, K.J., Barratt, R.A., Zeitlin, L., Hoen, T.E., and Cone, R.A. 1993. Nonoxynol-9 protects mice against vaginal transmission of genital herpes infections. *J. Infect. Dis.* **168:**1009–1011.
23. Cai, W., Gu, B., and Person, S. 1988. Role of glycoprotein B of herpes simplex virus type 1 in viral entry and cell fusion. *J. Virol.* **62:**2596–2604.
24. Hiatt, A. and Ma, J.-C. 1992. Monoclonal antibody engineering in plants. *FEBS Lett.* **307:**71–75.
25. Fiedler, U. and Conrad, U. 1995. High-level production and long-term storage of engineered antibodies in transgenic tobacco seeds. *Bio/Technology* **13:**1090–1093.
26. Hiatt, A. and Ma, J.-C. 1993. Characterization and applications of antibodies produced in plants. *Int. Rev. Immunol.* **10:**139–152.
27. Friberg, J. 1974. Sperm-agglutinating antibodies and immunoglobulins G and A in stored human seminal fluid. *Acta Obstet. Gyn. Scand. Suppl.* **36:**59–63.
28. Tjokronegoro, A. and Sirisinha, S. 1974. Degradation of immunoglobulins by secretions of human reproductive tracts. *J. Reprod. Fertil.* **38:**221–224.
29. Castle, P. 1995. Contraceptive effect of sperm-agglutinating monoclonal antibodies in rabbit. (Doctoral dissertation). Johns Hopkins University, Baltimore, MD.
30. Zeitlin, L., Whaley, K.J., Hegarty, T., Moench, T.R., and Cone, R.A. 1997. Tests of vaginal microbicides in the mouse genital herpes model. *Contraception* **56:**329–335.
31. Elias, C. and Heise, L. 1994. Challenges for the development of female-controlled vaginal microbicides. *AIDS* **8:**1–9.
32. Stone, A. and Hitchcock, P. 1994. Vaginal microbicides for preventing the sexual transmission of AIDS. *AIDS* (suppl 1) **8:**S285–S293.
33. Stein, Z. 1990. HIV prevention: the need for methods women can use. *Am. J. Public Health* **80:**460–462.
34. Sherwood, J.K., Zeitlin, L., Chen, X., Whaley, K.J., Cone, R.A., and Saltzman, W.M. 1996. Residence half-life of IgG administered topically to the mouse vagina. *Biol. Reprod.* **54:**264–269.
35. Sherwood, J.K., Zeitlin, L., Whaley, K.J., Cone, R.A., and Saltzman, W.M. 1996. Controlled release of antibodies for long-term topical passive immunoprotection of female mice against genital herpes. *Nat. Biotechnol.* **14:**468–471.
36. Radomsky, M.L., Whaley, K.J., Cone, R.A., and Saltzman, W.M. 1992. Controlled vaginal delivery of antibodies in the mouse. *Biol. Reprod.* **47:**133–140.
37. Ma, J.K. and Hein, M.B. 1995. Immunotherapeutic potential of antibodies produced in plants. *Trends Biotechnol.* **13:**522–527.
38. Miele, L. 1997. Plants as bioreactors for biopharmaceuticals: regulatory considerations. *Trends Biotechnol.* **15:**45–50.
39. Ma, J. K-C., Hikmat, B.Y., Wycoff, K., Vine, N.D., Chargelegue, D., Yu, L. et al. 1998. Characterization of a recombinant plant monoclonal secretory antibody and preventive immunotherapy in humans. *Nat. Med.* **4:**601–606.
40. Sherman-Gold, R. August 1997. Monoclonal antibodies: the evolution from '80s magic bullets to mature, mainstream applications as clinical therapeutics. *Genetic Engineering News*. August, p. 4.
41. Wong, J. 1998. Monoclonals to hit stride in '98. *Genetic Engineering News*. January, p. 21.
42. Zeitlin, L., Cone, R.A., and Whaley, K.J. Monoclonal antibodies for preventing mucosal transmission of epidemic infectious diseases. *Emerg. Infect. Dis*. In press.
43. Francisco, J.A., Gawlak, S.A., Miller, M., Bathe, J., Russell, D., Chance, D. et al. 1997. Expression and characterization of bryodin 1 and a bryodin 1-based single-chain immunotoxin from tobacco cell culture. *Bioconjugate Chem.* **8:**708–713.
44. McCabe, D.E., Swain, W.F., Martinell, B.J., and Christou, P. 1988. Stable transformation of soybean (Glycine max) by particle acceleration. *Bio/Technology* **6:**923–926.
45. Wolf, D. 1989. Designing, building, and using a fluorescence recovery after photobleaching instrument. pp 271-306 in *Fluorescence microscopy of living cells in culture. Part B.* Taylor, D. and Wang, Y. (eds.). Academic Press, San Diego, CA.
46. Barisas, B. and Leuther, M. 1977. Fluorescence photobleaching recovery measurement of protein absolute diffusion constants. *Biophys. Chem.* **10:**221–229.
47. Matthews, D. and Farewell, V. 1985. Using and understanding medical statistics. Karger, Basel, Switzerland.