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Controlled release of antibodies for long-term topical passive immunoprotection of female mice against genital herpes

Jill K. Sherwood, Larry Zeitlin[1], Kevin J. Whaley[1], Richard A. Cone[1], and Mark Saltzman*

*Departments of Chemical Engineering and [1]Biophysics, The Johns Hopkins University, Baltimore, MD 21218. *Corresponding author (e-mail: saltzman@jhu.edu).*

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Current methods for sexually transmitted diseases (STD) prophylaxis, which can be disruptive and inconvenient, must be used before each act of sexual intercourse, so a method that provides protection over the course of many acts is desirable. We used a mouse model of vaginally-transmitted herpes simplex virus 2 (HSV-2) infection to test polymeric controlled-release devices for sustained passive immunoprotection. Vaginal disks were prepared by dispersing a monoclonal antibody to HSV-2 (III-174) within a matrix of poly(ethylene-co-vinyl acetate); these disks released 2 to 40 μg/day of antibody into buffered water. When disks were placed in the vagina, large amounts of III-174 (5 to 3,000 ng) were recovered from the vaginal fluid over the next 8 days. Mice were vaginally challenged with 10 ID_{50} of HSV-2 either 3 or 7 days after disk placement; no mice receiving III-174 disks became infected, while 65% of control mice receiving identical disks with nonspecific IgG did. Controlled-release disks with III-174 provided significant protection against HSV-2 infection ($p < 0.005$). This new technology for long-term STD prophylaxis should increase user compliance, a factor limiting the efficacy of current methods.

Keywords: antibody, herpes simplex virus 2 (HSV-2), topical passive immunization, mouse vagina, polymer

According to the Centers for Disease Control and Prevention, the number of people newly infected with herpes simplex virus type 2 (HSV-2) in the United States has been steadily increasing for the past 25 years[1]. Today, 1 out of 6 people in the US may be infected with this virus[2]. Genital herpes is incurable and is characterized by repeated occurrences of genital lesions, which compromise the structural integrity of the genital skin and mucus epithelium increasing susceptibility to other infections. As a result, herpes has been linked with the transmission of human immunodeficiency virus (HIV)[3]. Women are at a higher risk for sexually transmitted diseases (STDs) than men, owing to a larger surface area of vulnerable mucosa and increased periods of contact with semen and urethral fluids[4,5]. The high prevalence of herpes and its association with HIV infection make HSV-2 a serious public health problem; since herpes is incurable, prevention is essential.

Mice pretreated with progesterone are highly susceptible to vaginal transmission of HSV-2, and they exhibit acute visible symptoms when they become infected. The IgG2a monoclonal antibody (MAb) III-174 is a potent neutralizer of HSV-2 *in vitro*[6]; it is believed to block infection by preventing the virion from fusing with its target cell[7]. Vaginal administration of monoclonal III-174 prevents vaginal transmission of HSV-2 in mice challenged with virus immediately after administration in solution[8]. Since the residence half-life of vaginally delivered IgG in solution to the mouse vagina is only 5 hours[9], controlled-release devices may be necessary to achieve long-term protection against vaginally transmitted pathogens. In addition, these devices would reduce problems of user compliance associated with current barrier methods[4,10,11].

We have been investigating the use of polymeric controlled-release devices for sustained delivery of antibodies to the female reproductive tract for passive immunoprotection against vaginally transmitted pathogens. Our device is made of poly(ethylene-co-vinyl acetate) (EVAc); EVAc is biocompatible and has Food and Drug Administration (FDA) approval for use in the human uterus and eye[12,13]. EVAc has been shown to release a variety of drugs, including proteins, over periods ranging from days to years in a reproducible manner[14,15]. Since human cervical mucus does not act as a barrier to antibody diffusion[16], antibodies released from these devices should be able to distribute easily through the vaginal lumen and may provide protection to the entire vaginal epithelium. Previously, we have shown that EVAc devices can provide sustained release of antibodies to the mouse vagina[17]. Here, we demonstrate that these devices can provide long-term protection against HSV-2 infection in mice.

Results

***In vitro* controlled release.** Two different formulations were used to incorporate the same amount of antibody into EVAc matrices. The resulting devices differed in antibody release rates (Fig. 1). In Figure 1, the lower curve represents data from devices that contained unsieved particles. These devices released antibody at a slower rate (2–26 μg/day) than the devices with sieved particles (2–40 μg/day), as indicated in the upper curve.

Intravaginal antibody concentrations. Mice that received high-releasing disks had significant amounts of MAb III-174 in the vaginal lumen from 3 to 7 days after receiving a disk: 3200 ng after 3 days, 1800 ng after 5 days, and 600 ng 7 days after disk insertion (Fig. 2). Figure 2 also shows the amount of antibody in the vaginal lumen predicted by a pharmacokinetic model (Experimental protocol, eqn. 1). The only adjustable parameter in equation 1 was the elimination constant calculated from the half-life of IgG in the mouse vaginal mucus using equation 2, with $t_{1/2} = 5 \pm 2$ hours. The

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optimum k was 0.23 h^{-1}, which corresponded to a half-life of 3 hours. Lavage samples from mice that received low-releasing disks contained an average of 82 ng of MAb 4 days after insertion of the disk and about 4 ng after 8 days (Fig. 2). The low-releasing data were not correlated with the model because these mice were challenged with HSV-2 (in 10 μl of solution) 1 day before lavage samples were collected, which would have rinsed out a significant amount of MAb.

Efficacy of protection against genital herpes. In the first study, mice received low-releasing disks; 3 days after insertion they were challenged with 10 ID_{50} of HSV-2 (Fig. 3A). One group of mice was given devices with MAb III-174, and a control group was given disks that contained nonspecific polyclonal mouse IgG. These two groups of mice were collared when the disks were inserted. After 17 days of observation, none of the 11 mice with III-174 disks showed any sign of infection. In contrast, 7 out of 10 of the mice in the control group were infected by this time ($p < 0.005$).

In the second study, high-releasing disks were inserted in mice (Fig. 3A), and 7 days later the mice were vaginally challenged with 10 ID_{50} of HSV-2. None of the 12 mice with III-174 MAb–loaded devices became infected, although 7 out of 12 control mice became infected. Significant sustained protection was achieved for up to 7 days ($p < 0.005$).

In the third study, mice that received low-releasing devices were challenged 7 days later with 500 ID_{50} of HSV-2 (Fig. 3B). With this superinfectious dose of virus, only 5 out of 12 of the III-174 MAb group became visibly infected but all of the 6 control mice became infected ($p < 0.05$). In addition, the 6 control mice that received a bolus of MAb/ficoll powder in PBS all became infected when challenged with HSV-2 3 days later (Fig. 3B).

Discussion

The controlled–release devices used in these studies were two-phase matrices of solid particles dispersed in an EVAc polymer phase; they have been described in detail elsewhere[13–15]. The dispersed particles create a partially connected tortuous network through the continuous polymer phase. When the device is placed in an aqueous environment, the particles at the surface dissolve and dissolved molecules diffuse out of the matrix, leaving evacuated pores. Water then seeps into these pores and dissolves particles deeper within the matrix. Particle clusters that are completely surrounded by polymer (i.e., not connected to the surface) are trapped and, therefore, not released. An increase in particle size increases the total fraction of releasable protein since larger particles are more likely to touch the surface of the matrix or other particles in the matrix[15,18]. Furthermore, increasing particle size also results in the formation of larger pores in the matrix, which decreases the diffusional path length, thereby increasing the rate of protein release[14,15]. Originally, an unsieved formulation was used because sieving the particles (to 90–180 μm) resulted in the loss of a significant amount of MAb (i.e., many of the particles in the original powder were less than 90 μm). In this particular case, sieving removed smaller particles. When sieved particles were used, the mean particle size of the powder was higher, resulting in the high-releasing formulation. The significant number of small particles (<90 μm) in the low-releasing formulation accounts for its low rate of protein release as well as the apparent low fraction of releasable protein *in vitro*.

The low-releasing formulation also delivered significantly less MAb to the vaginal lumen during the 4 to 8 days after disk insertion than did the high-releasing formulation. It should be noted that the values for the low-releasing disks in Figure 2 are approximately one order of magnitude lower than they would be since 10 μl of liquid was introduced into the vaginal lumen when the mice were challenged with HSV-2, which would have washed out

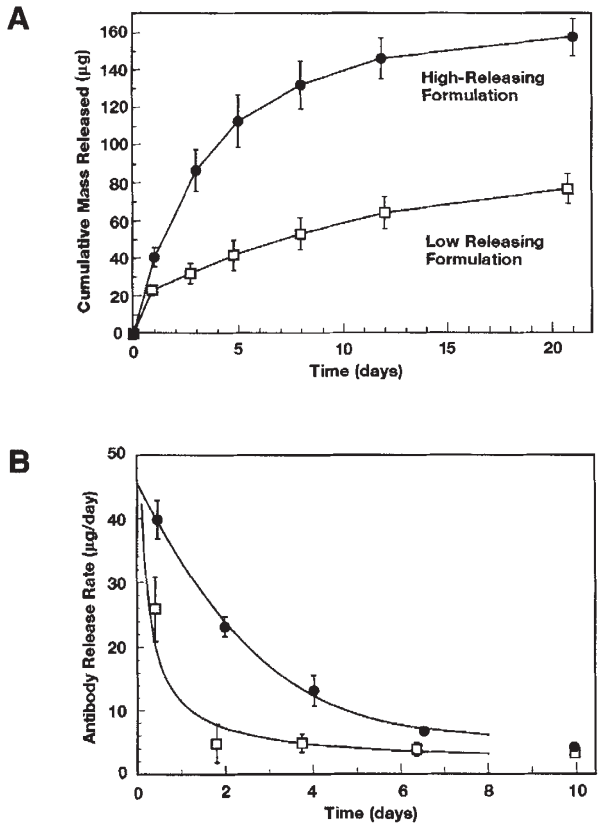

Figure 1. ***In vitro*** **controlled release of mouse IgG into well-stirred PBS. Each device contained 205 μg of antibody. The squares represent low-releasing devices that contained unsieved particles and the solid circles represent high-releasing devices with sieved particles. The error bars indicate the standard deviation of three devices. (A) Cumulative mass released versus time. (B) Rate of antibody release versus time. The lines represent functions that were adjusted to fit the experimentally measured release rates up to 8 days.**

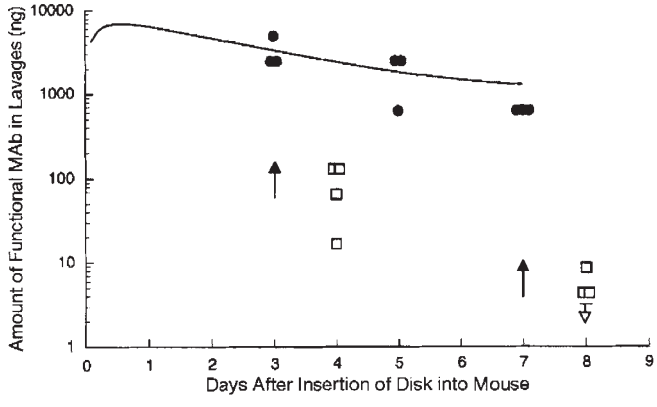

Figure 2. Amount of functional anti-herpes MAb III-174 in the vaginal lavages after receiving a MAb device. Each device contained 205 μg of antibody. The squares represent samples from individual mice with low-releasing devices and solid circles denote samples from mice that received high-releasing devices. The line represents the predicted pharmacokinetics for the high-releasing data. The amount of antibody in the lavage sample from one mouse at day 8 that received a low-releasing device was below the detection limit of the assay, indicated by the hollow arrow at day 8. The two solid arrows indicate the time that the mice with low-releasing disks were challenged with 10 μL of HSV-2, which washed out approximately 90% of the antibody in the vaginal lumen within a few hours.

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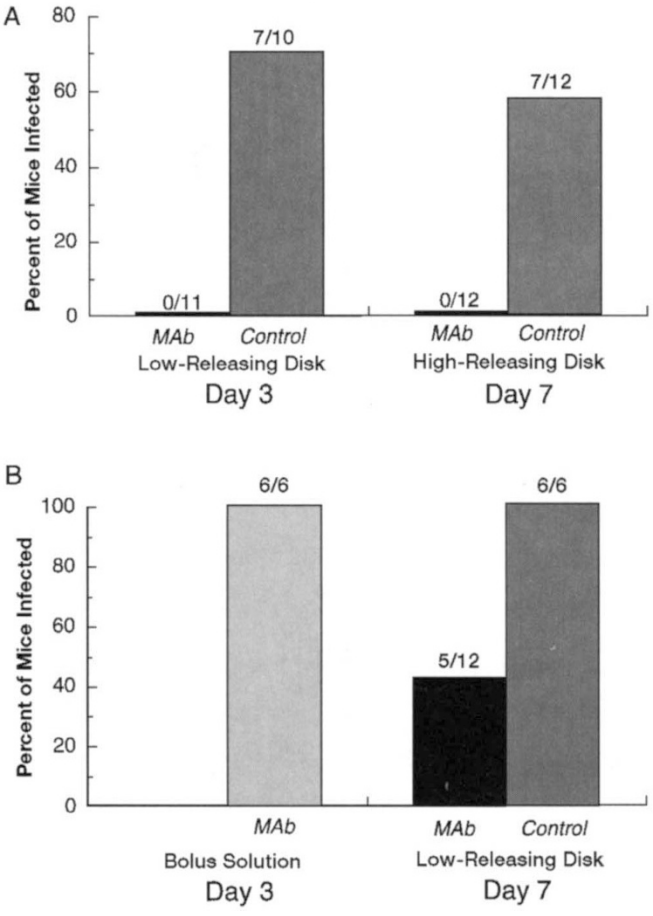

Figure 3. Long-term protection of mice against herpes. The percentage of mice infected after receiving MAb against herpes or control IgG. The mice were vaginally challenged either 3 or 7 days after disk implantation (205 μg/disk) or bolus administration (205 μg in PBS). (A) Mice challenged with 10 ID_{50} of HSV-2. (B) Mice challenged with 500 ID_{50} of HSV-2.

some of the antibody. This 10 μl bolus of solution would have eliminated ~90% of the antibody in the vaginal lumen[9]. The model used to predict *in vivo* MAb concentrations corresponded well to the *in vivo* data for the high-releasing disks. The model was not used for the low-releasing devices because of the washout effect of the viral challenge.

The low-releasing MAb devices completely protected mice when challenged 3 days after insertion with 10 ID_{50}, whereas 70% of the control mice with nonspecific polyclonal IgG disks became infected. The high-releasing devices, which maintained a higher vaginal antibody concentration after 7 days, protected all of the mice when they were challenged 7 days after receiving the disks. Previously, it was demonstrated that 1 μg of III-174 MAb dissolved in buffered saline was needed for protection against a vaginal challenge of HSV-2 when the viral inoculation immediately followed the MAb dose[8]. Our present results suggest that ~1 μg of III-174 MAb may be necessary at the time of challenge for complete protection in mice. The results presented here also suggest that the high-releasing devices may have been able to protect the mice for 2 weeks or more, since antibody concentrations *in vivo* remained above 1 μg for an extended period of time. Previous reports indicate that similar devices, with optimal particle size and loading, can release active antibodies *in vitro* for months or years[14,15], and to the mouse vaginal lumen for 30 days[17]. This suggests that simple changes in the device design will lead to even longer periods of protection. To our knowledge, this is the first study that shows long-term localized immunoprotection against a vaginally transmitted pathogen. To test the bounds of efficacy, some mice received low-releasing devices and were challenged 7 days later with 500 ID_{50} of HSV-2. Even under these rigorous conditions, the controlled–release devices were able to reduce the number of infected animals by half, indicating that the amount of III-174 MAb present in the vagina at 7 days was enough to reduce the infectivity of the viral inoculum by a factor of 500. In contrast, all 6 mice that received a bolus of MAb/ficoll solution became infected when challenged with 500 ID_{50} of HSV-2 only 3 days later.

STDs can have many devastating long-term consequences, including infertility, cervical cancer, pelvic inflammatory disease, and death. Current methods of topical protection, such as detergents used in spermicides, can be irritating to the vaginal epithelium[19]. Since antibodies are natural protective agents in mucosal secretions, the risk of undesired side effects is reduced. Current vaginal barrier devices such as diaphragms could be modified to release antibodies, and newer devices, perhaps in the form of a vaginal ring, could provide continuous long-term protection. It has been estimated that vaginal rings in women would need to deliver <100 μg of antibody a year for protection against an STD pathogen[20]. The devices described here can easily hold 10,000 times this much antibody; therefore, hundreds of different MAbs could be incorporated into the same intravaginal device, for potentially months or years worth of broad spectrum protection. These devices could contain a variety of antibodies against STD pathogens as well as antisperm antibodies for contraception. The primary advantage of these devices is that they would eliminate efficacy problems due to user compliance since they would only need to be replaced every few months. Thus, they would be more desirable than current barrier methods since they do not interfere with sexual behavior. Such devices have the potential to become the basis of a new form of contraception that simultaneously protects against STDs.

Experimental protocol

Materials. The anti-herpes virus mouse monoclonal antibody III-174 used was an IgG2a against a surface epitope of glycoprotein D (cell line a gift from Patricia G. Spear, Northwestern Univ., Evanston, IL). The antibody was partially purified from ascites fluid using a recombinant protein A device (Nygene, Yonkers, NY). Nonspecific polyclonal mouse IgG (Rockland, Gilbertsville, PA) was used for control groups. The controlled release devices were made with poly(ethylene-co-vinyl acetate) (Elvax 40W, 34 wt% vinyl acetate, M_n=70 kD, M_w/M_n=2.4, DuPont, Wilmington, DE), which was washed in a Soxhlet extractor for 48 h with water and then for 48 h with acetone. All procedures used 8–12 week old female C57BL/6 mice (Harlan, Indianapolis, IN or The Jackson Laboratory, Bar Harbor, ME) and were approved by the Johns Hopkins Animal Care and Use Committee.

Fabrication of controlled–release devices. Vaginal controlled–release disks were fabricated by dispersing antibody particles in poly(ethylene-co-vinyl acetate) using a solvent evaporation process described previously[15,17]. To achieve the particle loading necessary to yield the desired release rate, we used the polysucrose Ficoll (Ficoll 400, M_w=426 kD, Pharmacia Biotech, Piscataway, NJ) as a codispersed inert macromolecule. Ficoll was dissolved 150 mg/ml in phosphate buffered saline (PBS, Sigma, St. Louis, MO). Approximately 20 mg of antibody in solution was mixed with 1.3 ml of Ficoll solution and the resulting mixture was lyophilized. This powder was then ground and either sieved to obtain 90–180 μm particles or used unsieved. This powder was dispersed within EVAc by solvent evaporation using methylene chloride[15,17]. The resulting matrix was cut into disks, which were 4 mm in diameter, ~1 mm thick, weighed 10–14 mg, and contained ~205 μg of antibody/disk.

***In vitro* controlled release.** To characterize the release kinetics of antibody from the EVAc devices, the devices were incubated in a well-stirred solution. Three disks were individually incubated in polypropylene vials containing 1 ml of PBS with 0.02% gentamicin (Sigma) as an antimicrobial. The vials

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were kept at 37°C on an orbital shaker. At the desired time, the disk was removed and placed in a new vial and the amount of antibody that was released into solution was determined using a total protein assay (Pierce Coomassie Protein Plus Assay Reagent, Pierce, Rockford, IL) or an enzyme-linked immunosorbent assay (ELISA) for polyclonal mouse IgG. The samples were refrigerated at 4°C until analysis.

Polyclonal mouse IgG ELISA. A 96-well plate was coated overnight at 4°C with 2 μg/ml of horse anti-mouse IgG antibody (Vector, Burlingame, CA) in 0.1 M sodium carbonate buffer. The plate was blocked for 2 h with 3% bovine serum albumin (BSA, Sigma). The mouse IgG samples were diluted in 1% BSA and 50 μl/well was incubated for 1 h. Next, the plate was incubated with 7.5 μg/ml of biotinylated horse anti-mouse IgG (Vector) for 1 h, followed by a 1 h incubation with ABC conjugated to horseradish peroxidase (1:500, Vector). Next, the ABTS substrate (Vector kit) added to the plate was allowed to develop for 20 min in the dark before being analyzed at 405 nm.

Progestin treatment. To increase susceptibility of mice to HSV-2 infection as well as to eliminate differences caused by the estrous cycle, we induced an acyclic diestrus-like state by treating the mice with a long-acting progestin (Depo-Provera, Upjohn, Kalamazoo, MI) 7 days prior to viral challenge[21]. The mice were given 2.5 mg of progestin in 0.1 ml of PBS subcutaneously in the back of the neck with a 26-gauge needle.

Disk implantation. Mice either received disks containing anti-HSV-2 MAb or control disks with nonspecific polyclonal mouse IgG. Disks were inserted using a previously described procedure[17]. At this time, Elizabethan collars (turned down) were placed on the first group of mice to prevent them from grooming themselves; the collars were removed when the mice were challenged. We found that collars resulted in a large mucus plug that deviated from normal conditions and would likely alter the vaginal residence time of the released antibodies; therefore, we did not use collars in later experiments. All mice were housed individually with continual access to food and water at all times. As a second control, 6 mice were administered 3.5 mg of MAb/ficoll powder (containing 205 μg of III-174) in 10 μl of PBS. This was the same powder incorporated into the matrix, and the same amount of powder contained in one disk. Three days later the mice were challenged with 500 ID_{50} of HSV-2.

Collection of mucus samples and neutralization assay. The amount of MAb in the vaginal lumen of mice was determined by lavaging the lumen with 20-μl aliquots of medium (Tissue Culture Refeeding Medium, Baxter, McGaw Park, IL). In the first study, lavage samples were taken from mice 4 or 8 days after receiving a MAb disk, which was 1 day after they had been challenged with virus. Since little or no virus remains in the lumen 24 h after inoculation (unpublished data), there was no concern that the virus would interfere with the neutralization assay used to determine the amount of antibody. In the next study, we were able to collect lavage samples at days 3, 5, and 7 from mice that were not challenged with virus. Initially, mice were lavaged twice, and the two samples were combined and diluted with 60 μl of medium. Later, mice were lavaged four times, and the samples for each mouse were pooled and diluted with 20 μl of medium. In previous experiments, we demonstrated that lavaging 4 times recovered 80±10% of the intravaginal IgG[9]. Each sample was serially diluted, and 75 μl of each dilution was mixed with 25 μl of MAb containing 5000 $TCID_{50}$ (i.e., 5000 times the tissue culture infectious dose needed to infect half of the control wells not containing any antibody) of HSV-2 (Virotech, Rockville, MD) in a 96-well microplate. The samples were incubated with the virus for 1 h at 37°C on an orbit shaker and then placed on target cells (human diploid foreskin fibroblasts, Baxter) and incubated at 37°C in a 5% CO_2 environment for 2 to 3 days. The target cells were then visually inspected for cytopathic effect.

Viral challenge and determination of infection. The mice were vaginally challenged with 10 μl of 10 ID_{50} or 500 ID_{50} (i.e., 10 or 500 times the infectious dose needed to infect half of untreated mice) of HSV-2 7 days after progestin treatment. The virus was administered using a fire-polished 10-μl Wiretrol pipette (Drummond, Broomall, PA). The mice were visually inspected for symptoms of herpes infection (perineal hair loss, swelling, reddishness, and lesions) for 17 days after viral challenge; the mice were sacrificed at the first definitive sign of infection. Statistical significance was determined by Fisher's exact test[22]. In these experiments, viral infection was not determined by isolating virus from lavage samples since the antibodies being released from the devices, as well as naturally occurring antibodies, would have interfered with viral detection. In previous experiments, detection of the virus in vaginal lavages correlated well with observation of visible lesions (R = 0.85; unpublished data).

Data analysis. A simple pharmacokinetic model was used to correlate the *in vitro* release kinetics to the MAb concentrations found in the vaginal lumen. The rate of MAb accumulation in the vaginal lumen was assumed equal to the rate of antibody release from the polymer *in vitro* minus the amount of MAb eliminated from the lumen:

$$V_L \cdot \frac{dC_L}{dt} = \frac{dM_P}{dt} - k \cdot V_L \cdot C_L \qquad (1)$$

where V_L was the volume of the vaginal lumen (0.025 μl), C_L was the concentration of MAb in the lumen, dM_P/dt was the rate of MAb release from the polymer *in vitro*, and k was the *in vivo* antibody elimination constant. The rate of MAb released was determined by using the best curve fit to the *in vitro* controlled release data. Previously, the half-life of IgG in the mouse vaginal mucus, $(t_{1/2})\beta$, was found to be 5 h[9]; k was related to the half-life by:

$$t_{1/2} = \frac{\ln(2)}{k} \qquad (2)$$

where $t_{1/2}$ was the half-life of IgG in the mouse vaginal lumen.

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1. Division of STD/HIV Prevention. Annual Report 1994. U.S. Department of Health and Human Services, Public Health Service. Atlanta: Centers for Disease Control and Prevention. September 1995.
2. Koutsky, L. A., Stevens, C. E., Holmes, K. K., Ashley, R. L., Kiviat, N. B., Critchlow, C. W., and Corey, L. 1992. Underdiagnosis of genital herpes by current clinical and viral-isolation procedures. *N. Engl. J. Med.* **326**:1533–1539.
3. Hook, E. W., Cannon, R. O., Nahmias, A. J., Lee, F. F., Campbell Jr., C. H., Glasser, D., and Quinn, T. C. 1992. Herpes simplex virus infection as a risk factor for human immunodeficiency virus infection in heterosexuals. *J. Infect. Dis.* **165**:251–255.
4. Stone, K. M. 1994. HIV, other STDs, and barriers, pp. 203–212 in *Barrier Contraceptives: Current Status and Future Prospects*. Mauck, C., Cordero, M., Gabelnick, H., Spieler, J., and Rivera, R. (eds.). Wiley-Liss, New York.
5. Cohen, J. 1995. Women: absent term in the AIDS research equation. *Science* **269**:777–780.
6. Para, M. F., Parish, M. L., Noble, A. G., and Spear, P. G. 1985. Potent neutralizing activity associated with anti-glycoprotein D specificity among monoclonal antibodies selected for binding to herpes simplex virions. *J. Virol.* **55**:483–488.
7. Fuller, A. O. and Spear, P. G. 1987. Anti-glycoprotein D antibodies that permit adsorption but block infection by herpes simplex virus 1 prevent virion-cell fusion at the cell surface. *Proc. Natl. Acad. Sci. USA* **84**:5454–5458.
8. 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.
9. Sherwood, J. K., Zeitlin, L., Chen, X., Whaley, K. J., and Cone, R. A. 1996. Residence half-life of IgG administered topically to the mouse vagina. *Biol. Reprod.* **54**:264–269.
10. Elias, C. J. and Heise, L. L. 1994. Challenges for the development of female-controlled vaginal microbicides. *AIDS* **8**:1–9.
11. Feldblum, P. and Joanis, C. 1994. *Modern Barrier Methods: Effective Contraception and Disease Prevention*. Family Health International, Research Triangle Park, NC.
12. Zador, G., Nilsson, B. A., Nilsson, B., Sjoberg, N. D., Westrom, L., and Wiese, J. 1976. Clinical experience with the uterine progesterone (Progestasert). *Contraception* **13**:559–568.
13. Langer, R., Brem, H., and Tapper, D. 1981. Biocompatibility of polymeric delivery systems for macromolecules. *J. Biomed. Mat. Res.* **15**:267–277.
14. Rhine, W. D., Hsieh, D. S. T., and Langer, R. 1980. Polymers for sustained macromolecular release: procedures to fabricate reproducible delivery systems and control release kinetics. *J. Pharm. Sci.* **69**:265–270.
15. Saltzman, W. M. and Langer, R. 1989. Transport rates of proteins in porous materials of known microgeometry. *Biophys. J.* **55**:163–171.
16. 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.
17. 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.
18. Siegel, R. A. and Langer, R. 1984. Controlled release of polypeptides and other macromolecules. *Pharm. Res.* **1**:2–10.
19. Niruthisard, S., Roddy, R. E., and Chutivongse, S. 1991. The effects of frequent nonoxynol-9 use on the vaginal and cervical mucosa. *Sex. Transm. Dis.* **18**:176–179.
20. 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.
21. 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.
22. Snedecor, G. W. and Cochran, W. G. 1980. *Statistical Methods*, 7th ed. The Iowa State University Press, Iowa.