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Adjuvant effect of imiquimod when combined with HSV glycoprotein vaccine in guinea pigs with recurrent HSV-2 genital herpes. C.J. HARRISON*, R.L. MILLER AND D.I. BERNSTEIN. Creighton Univ. and Univ. of Neb. M.D., Omaha, NE, 3M Pharmaceuticals, St. Paul, MN, and J.N. Gamble Inst. Med. Res., Cincinnati, OH.

Therapy for frequently recurrent genital herpes with daily antiviral ceases to be effective when therapy is discontinued. Preliminary data suggested that imiquimod, a cytokine inducer, might provide adjuvant activity for immunotherapy of recurrent disease in addition to its *in vivo* anti-herpes effect. We therefore evaluated the effect of 21d of imiquimod (3 mg/kg/day subQ) alone or in conjunction with HSV glycoprotein immunization on frequently recurrent genital herpes using the guinea pig model. Guinea pigs (n = 45) that had just recovered from primary genital herpes were randomized on d14 after initial HSV genital inoculation. Imiquimod for 21d alone (d14-35) reduced recurrences by 89% during therapy and by 82% for the 4 wks after therapy was discontinued. Adding immunization on d14 and 35 to the 21 days of imiquimod further reduced recurrences by an additional 42% in the first 3 wks and 66% in the next 4 wks. For days 14 through 90, cumulative recurrent disease scores were reduced from 33.8 in placebo to 7.3 ($p < 0.001$) in the imiquimod group and to 2.8 ($p < 0.001$) in the imiquimod + immunization group. Among the immunologic responses assayed, *in vitro* IL-2 response to HSV-2 antigen, MHC-unrestricted cytolysis of HSV targets, and ELISA HSV antibody were enhanced by immunization plus imiquimod. Proliferative responses were reduced in both groups receiving imiquimod and ELISA antibody was reduced in the 21 day imiquimod alone group, despite significant protection from disease in both groups. Imiquimod provided an adjuvant effect for HSV glycoprotein vaccine, effectively reducing recurrences in this animal model.

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DNA Vaccine Protects Mice from Vaginitis and Death due to Herpes Simplex Virus Type 2 (HSV-2). JD Kriesel, JW Ricigliano, SL Spruance, and BA Araneo. University of Utah and Paradigm Biosciences, Inc., Salt Lake City, Utah, USA

Vaccines using viral gene constructs offer promise as a new strategy to induce humoral and cellular immunity. Co-administration of vitamin D3 with an antigen enhanced humoral and mucosal immune responses (*Ann NY Acad Sci* 1994, 730:144). We investigated primary intramuscular vaccination with a construct containing the HSV-2 glycoprotein D (gD2) sequence (p-gD) and a homologous plasmid lacking the gD sequence (p-control) for the prevention of genital herpes in female Balb/C mice. Three groups of 10 animals each were vaccinated 3 times at weekly intervals and 2 weeks later given intravaginal challenge with HSV-2. The vaccination regimens were: 1) 100 µg p-gD, 2) 100 µg p-gD and one 0.1 µg im dose of vitamin D3 (p-gD/D3), and 3) 100 µg p-control. The disease was evaluated by clinical vaginitis score, HSV culture from vaginal washings, and serum antibody. A dramatic effect on mortality was seen in the p-gD/D3 group where 10/10 (100%) animals survived, compared to 4/10 (40%) that received p-gD, and 0/10 (0%) that received p-control ($p<0.001$). The p-gD/D3 and p-gD groups had marked reductions in mean vaginitis scores compared to the p-control group (2.5, 5.2, and 10.7, respectively, $p<0.001$). Lower vaginal viral titers were seen among the p-gD and p-gD/D3 groups compared to the p-control group ($p<0.001$). Serum anti-gD (ELISA) and HSV neutralizing antibodies were detected only among the p-gD and p-gD/D3 groups. We conclude that this gD expression vector is immunogenic and provides protection against clinical disease and death in the murine vaginitis model. Co-administration of vitamin D3 appears to attenuate vaginitis and enhance survival.