Assessment of Humoral Immune Response in Mink (Mustela Vison): Antibody Production and Detection

Authors: Smits, Judit E. G., and Godson, Dale L.

Source: Journal of Wildlife Diseases, 32(2) : 358-361

Published By: Wildlife Disease Association

URL: https://doi.org/10.7589/0090-3558-32.2.358

Journal of Wildlife Diseases, 32(2), 1996, pp. 358–361

Assessment of Humoral Immune Response in Mink (*Mustela Vison*): Antibody Production and Detection

Judit E. G. Smits[1] **and Dale L. Godson,**[2] [1]Department of Veterinary Pathology, University of Saskatchewan, 52 Campus Drive, Saskatoon, Saskatchewan, S7N 5B4, Canada; [2]Veterinary Infectious Diseases Organization, 120 Veterinary Road, Saskatoon, Saskatchewan, S7N 5E3, Canada

ABSTRACT: A method for investigating the humoral immune response in mink (*Mustela vison*) was developed between October 1993 and March 1994. Protein A, 1:8000 dilution, had a high affinity for mink immunoglobulin, while anti-ferret (*Mustela putorius*) antibody, 1:200 dilution, had a weaker affinity. Four adult mink were immunized with a hapten, dinitrophenol (DNP), conjugated to a large carrier protein, keyhole limpet hemocyanin (KLH), and received two boosters at 3-week intervals. This provoked a strong T-lymphocyte dependent humoral immune response. An indirect enzyme linked immunosorbent assay (ELISA) was used to quantify the antibody produced. All mink had undetectable anti-DNP-KLH antibody in the pre-immune sera, with antibody levels increasing post-immunization, and peaking after the first or second booster.

Key words: Humoral immunity, mink, *Mustela vison*, antibody response, DNP-KLH, protein A.

Modulation of the immune response of animals may occur due to exposure to environmental contaminants. Mink (*Mustela vison*), semi-aquatic mammals at the top of the aquatic and terrestrial food web, have a wide natural distribution throughout temperate North America, making them a potential sentinel for monitoring environmental health. They are also amenable to being raised in captivity. This makes them a useful mammalian model for the study of immune function, which may be affected by environmental xenobiotics.

Previous immune function studies in mink were initiated because an important disease of captive mink, Aleutian Disease, appeared to be immune mediated. The antibody response of mink against goat red blood cells (RBC) was assessed by determining the number of plaque-forming cells (antibody-producing lymphocytes) present in lymphoid tissues of mink inoculated with goat RBC (Lodmell et al., 1971). In mink vaccinated with a keyhole limpet hemocyanin (KLH) preparation, specific immunoglobulins against the KLH antigen were identified as 19S β globulin likely immunoglobulin M (IgM) (Lodmell et al., 1970). Later, three specific classes of mink immunoglobulins, IgG, IgA and IgM, were identified using KLH as antigen (Coe and Hadlow, 1972). An enzyme-linked immunosorbent assay (ELISA) for detecting *Pseudomonas aeruginosa* antibodies in mink has been described in which a staphylococcal cell wall peptide, protein A, was shown to be a good indicator for mink immunoglobulin (Rivera et al., 1994).

In this study a method was developed to assess and measure antibody production in mink. This entailed the identification of a conjugate with a high affinity for mink immunoglobulin, the formulation of a vaccine which would stimulate a T-cell dependent antibody response in mink, and the development of an ELISA to measure the antibody produced.

Four healthy young adult (<1 yr) mink, (two males, two females) demibuff cohorts from a mink ranch at Pike Lake, Saskatchewan, Canada, were housed indoors in the Animal Care Unit, University of Saskatchewan, in standard stainless steel wire cages (20 cm × 20 cm × 25 cm), under a 12 hr light/dark cycle. They were fed commercial maintenance fox and mink ration (Feedrite Ltd. Humboldt, Saskatchewan) at 150 g per day for females and 180 g per day for males); tap water was given ad libitum. Conditions for the mink met requirements of the Canadian Council of Animal Care.

Detection of antibodies in the sera from

a healthy mink and ferret were tested using three possible conjugates: horseradish peroxidase conjugated goat anti-ferret antibody (Kirkegaard Perry Laboratories, Canadian Life Technologies, Burlington, Ontario, Canada); recombinant protein G-horseradish peroxidase (Dimension Laboratories Zymed, Mississauga, Ontario, Canada), and developing reagent protein A-horseradish peroxidase (Dimension Laboratories, Zymed). In a 96-well Immulon II plate (Dynatech Laboratories Inc., Alexandria, Virginia, USA), two-fold serial dilutions of mink and ferret sera in phosphate buffered saline (PBS) pH 7.3 were prepared, beginning with a 1:10 dilution. The plate was incubated at 4C for 15 hr, then emptied and washed four times with distilled water. One hundred ml of 2% chicken ovalbumin (Sigma Chemical Company, St. Louis Missouri, USA) in PBS, was added to all wells to block any unbound sites, and the plate was incubated at 20 C for 1 hr. The plate was emptied and washed as described. The test conjugates were added as follows: anti-ferret serum 1:200, anti-ferret serum 1:2000, protein G 1:4000, and protein A 1:8000. The plate was incubated in the dark for 40 min at 37 C and washed. We added 2,2'azinobis (3-ethylbanzo-thiazoline-6-sulfonic acid) (ABTS) peroxidase substrate (Mandel Scientific, Guelph, Ontario) to all wells. After 15 min, absorbance was read on a BioRad 3550 microplate reader, with a 405 nm filter (BioRad Laboratories, Mississauga, Ontario).

A vaccine was formulated using a single epitope antigen, dinitrophenol (DNP), conjugated to a carrier molecule, KLH, as follows. A stock solution of 1 mg/ml DNP-KLH (Calbiochem, Terochem Laboratories Ltd., Edmonton, Alberta, Canada) in 10 mM 3-[N-morpholino] propane-sulfonic acid buffer pH 7.2 was prepared. Two ml of this DNP-KLH, 1400 µl carbonate buffer pH 9.5, 1200 µl Rehydragel HPA aluminum hydroxide gel (Reheis, Inc. Berkeley Heights, New Jersey, USA), 600 µl Tween 80 (Sigma Chemical Co.), 240 µl Span 80 (Sigma Chemical Co.) and 1000 µl Freund's Incomplete Adjuvant (Sigma Chemical Co.) were emulsified. The mink were vaccinated in the paralumbar region with 200 µl of vaccine at each of two sites, one subcutaneous, one intramuscular. Mink received two booster vaccinations with 200 µl of vaccine, on day 25 and day 47 after the initial immunization. Sera were collected in serum vacutainers by anterior vena caval puncture, on mink anesthetized with an intramuscular injection of 18 to 20 mg/kg ketamine HCl (Rogar/STB Inc. Montréal, Québèc, Canada), and 1 mg/kg xylazine HCl (Bayvet, Etobicoke, Ontario) on days 0 (pre-immune serum) 10, 32, and 59 after the initial immunization.

To quantify mink anti-DNP-KLH antibodies, an indirect ELISA was developed. One hundred µl of 0.5 µg/ml DNP-KLH in carbonate coating buffer 0.05 M pH 9.5 was added to all wells of a 96-well Immunlon II plate and incubated at 4 C for 15 hr. The plate was washed four times using 0.2% Tween 20 in deionized, distilled water and tapped dry. Two-fold dilutions of pre- and post-immunization sera beginning with a dilution of 1:10, were incubated at 20 C for 2 hr, then washed as described. One hundred µl of 0.25% bovine serum albumin (BSA) (bovine albumin fraction V, Sigma Chemical Co.) in phosphate buffered saline with 0.05% Tween 20 (PBST) (Aldrich Chemical Company, Inc., Milwaukee, Wisconsin, USA) was added to each well for 30 min at 20 C then washed as described. One hundred µl of 1:8000 protein A-HPO in 0.05% PBST was added to each well and the plate was incubated at 20 C in the dark for 1 hr, then washed. One hundred µl ABTS substrate was added to each well. Absorbance was read after 15 min on a microplate reader at 405 nm.

As anti-mink immunoglobulin reagents were not commercially available at the time of this study, the first objective was to find a reagent which would bind mink antibodies. *Staphylococcus aureus* cell wall

peptide Protein A, at a 1:8000 dilution, had the strongest affinity for mink immunoglobulin with a titer of 1:5120. Unexpectedly, commercially available anti-ferret (*Mustela putorius*) immunoglobulin had less affinity for mink immunoglobulin than did protein A. The 1:200 goat anti-ferret antibody had distinct, but weaker recognition of mink antibodies, with a titer of 1:640, while the 1:2000 dilution did not result in a detectable titer. Thus, the avidity of protein A is strong for the fragment, crystalline (Fc) portion of mink antibodies, as it is for numerous other mammalian species including human, rabbit and guinea pig (Langone, 1982). Although protein G, a 30 to 35 kilodalton (kD) cell wall peptide from β hemolytic *Streptococcus* spp., does have strong affinity for bovine, equine and porcine antibodies (Harlow and Lane, 1988), at the 1:4000 dilution used here, it had no detectable binding with mink immunoglobulin.

Using the protein A conjugate, we were able to develop an indirect ELISA for the detection of antibody after vaccination with the hapten-carrier conjugate DNP-KLH. Although high absorbance readings were seen at sera dilutions between 1:10 and 1:160 in both pre-immune and post-immunization sera, with more dilute sera, conjugate binding only occurred in the post-immunization sera; thus it was specific for anti-DNP-KLH immunoglobulins. Given this limitation on the sensitivity of the assay, no pre-immune sera had detectable levels of anti-DNP-KLH antibodies. After primary immunization, all mink sera had detectable antibody. One male mink had the highest antibody level after booster 2, while the others had peak levels after booster 1 (Fig. 1).

The affinity with which specific immunoglobulins bind to protein A or protein G varies with class and subclass of immunoglobulins and with species (Åkerström et al., 1985), which must be considered during assay development. Gershon and Paul (1971) demonstrated the affinity of anti-DNP antibody to be dependent upon

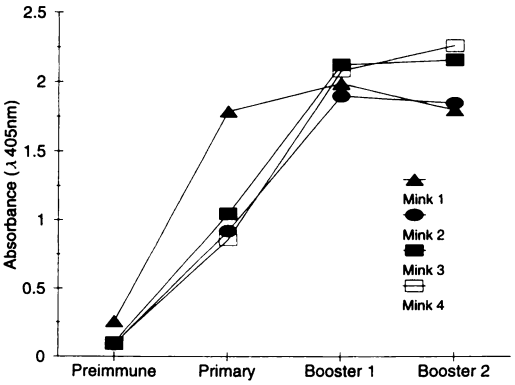

FIGURE 1. Relative antibody levels in 1:1280 dilution of sera from DNP-KLH vaccinated mink. Serum samples collected before immunization, 10 days after primary immunization (primary), and 7 and 9 days after boosters 1 and 2, respectively, were analyzed for DNP-KLH specific antibody levels using an indirect ELISA. Mink 1 and 2 are females, mink 3 and 4 are males.

the nature of the carrier molecule. Keyhole limpet hemocyanin conjugated to DNP produced strong, high affinity, anti-hapten antibodies. The DNP antigen alone is not immunogenic because of its small size, and thus must be conjugated to a large carrier molecule (KLH 8-9 × 10^6 MW) (Mälekä and Seppälä, 1986) which is recognized by Th lymphocytes following antigen processing. Cytokine release from these helper T cells direct antigen stimulated B-lymphocytes to mature and differentiate into antibody producing plasma cells (Abbas et al. 1991). Thus, the production of antibodies reflects the operational integrity of many aspects of the immune system.

A primary immune response was evident by day 10 post-immunization, at which time absorbance levels reached approximately half of the peak values seen in the secondary response between days 25 and 47 post-immunization. These results were similar to those seen by Lodmell et al. (1971) using sheep-RBC vaccinated mink, in which hemagglutination titers peaked at 42 days post-immunization, and a strong post-booster anamnestic response was seen.

An assay was required that would not cause disease or in any way compromise the health status of the test animals, yet would provoke a strong humoral immune response. Using the method described here, we have shown that nonpathogenic DNP-KLH is an effective immunogen. This system provides a practical method for assessing the function of the immune system.

LITERATURE CITED

ABBAS, A. K., A. H. LICHTMAN, AND J. S. POBER. 1991. Cellular and molecular immunology. W. B. Saunders Co. Philadelphia, Pennsylvania, pp. 186–204.

ÅKERSTRÖM, B. T. BRODIN, K. REIS, AND L. BJÖRCK. 1985. Protein G: A powerful tool for binding and detection of monoclonal and polyclonal antibodies. Journal of Immunology 135: 2589–2592.

COE, J. E., AND W. J. HADLOW. 1972. Studies on immunoglobulins of mink: Definition of IgG, IgA and IgM. Journal of Immunology 108: 530–537.

GERSHON, R. K., AND W. E. PAUL. 1971. Effect of thymus-derived lymphocytes on amount and affinity of anti-hapten antibody. Journal of Immunology 106: 872–874.

HARLOW, E., AND D. LANE. 1988. Antibodies: A laboratory manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York, pp. 615–622.

LANGONE, J. J. 1982. Protein A of *Staphylococcus aureus* and related immunoglobulin receptors produced by streptococci and pneumococci. Advances in Immunology 32: 157–25.

LODMELL, D. L., W. J. HADLOW, J. J. MUNOZ, AND H. W. WHITFORD. 1970. Hemagglutinin antibody response of normal and Aleutian Disease-affected mink to keyhole limpet hemocyanin. Journal of Immunology 104: 878–887.

———, R. K. BERGMAN, W. J. HADLOW, AND J. J. MUNOZ. 1971. Cellular and humoral antibody response of normal pastel and sapphire mink to goat erythrocytes. Infection and Immunity 3: 221–227.

MÄLEKÄ, O., AND I. J. T. SEPPÄLÄ. 1986. Haptens and carriers. In Handbook of experimental immunology, Vol. 1. Immunochemistry, D. M. Weir (ed.). Blackwell Scientific Publications, Alden Press, Oxford, United Kingdom pp. 3.1–3.13.

RIVERA, E., M. JACKERT-JERNBERGER, T. MEJERLAND, AND K. A. KARLSSON. 1994. Evaluation of protein A and protein G as indicator system in an ELISA for detecting antibodies in mink to *Pseudomonas aeruginosa*. Veterinary Microbiology 42: 265–271.

Received for publication 12 June 1995.