Humoral and cell-mediated immune responses to the glycoproteins of infectious laryngotracheitis herpesvirus

Brief Report

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Summary. The viral glycoproteins of infectious laryngotracheitis virus, an alphaherpesvirus, were the dominant antigens recognised by immune chickens. Glycoproteins with molecular weights of 205, 160, 115, 90, 67, 60, and 52 k reacted strongly in Western blotting studies with a majority of chicken antisera. Viral glycoproteins immunoprecipitated using monoclonal antibodies were also able to elicit a delayed-type hypersensitivity reaction in chickens previously vaccinated with a live vaccine. The 60 k glycoprotein alone and the antigenically related family of higher molecular weight glycoproteins (205, 160, 115, 90, and 85 k) both elicited significant increased in the thickness of the wattles of immune cockerels. Because the glycoproteins induce both antibody and cell-mediated immune responses they may prove to be important protective immunogens in a subunit vaccine.

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Infectious laryngotracheitis (ILT) is an acute upper respiratory tract infection of chickens caused by infectious laryngotracheitis virus (ILTV), family *Herpesviridae*, subfamily *Alphaherpesvirinae* [8]. ILT occurs worldwide, and as outbreaks can result in mortalities of 10–40% and reduced egg production [8], the disease is of considerable economic importance to the intensive poultry industry. At present ILT is controlled by the use of attenuated live vaccines, but a subunit vaccine or a recombinant viral vector-based vaccine expressing the protective immunogen(s) of the virus would be safer and could possibly be used in the eradication of the disease in certain regions of the world, particularly Australia.
Both antibody and cell-mediated immune mechanisms are thought to play a role in immunity to herpesvirus infections; antibody in preventing infection and the establishment of latent infections, and cell-mediated immunity in recovery from an existing infection [22]. The glycoproteins of herpesviruses are important targets of both the humoral and cell-mediated arms of the host immune response (reviewed in [13]). Furthermore vaccination with either purified glycoproteins [2, 10, 11, 12] or recombinant virus vectors expressing herpesvirus glycoproteins [3, 14, 21] protects experimental animals against lethal challenge infection with herpes simplex virus type 1 (HSV-1) or type 2 (HSV-2) or pseudorabies virus.

In ILT, cell-mediated mechanisms are known to be important in immunity following vaccination. Naive chickens can be protected against challenge infection by the transfer of immune lymphoid cells [7], while bursectomised chickens that are unable to synthesise specific antibodies are protected against a challenge infection by vaccination [6, 15].

Development of a subunit or recombinant vaccine for ILT requires knowledge of the protective immunogens of the virus. Two families of ILTV glycoproteins have been described [23], but little is known of other antigens of the virus, or of the significance of the immune response to the viral glycoproteins. This study reports the specificity of the serum antibody response of chickens to protein and glycoprotein antigens of ILTV as determined by Western blotting, and the ability of ILTV glycoproteins to elicit a cell-mediated immune response.

SA-2, the vaccine strain of ILTV used in Australia, was propagated and assayed in monolayer cultures of primary chicken kidney (CK) cells [6].

Detergent extracts of virus-infected cells were prepared at 18–20 h post infection using 1% (v/v) Nonidet P 40 and 1% (w/v) sodium deoxycholate [23]. The glycoprotein fraction of the detergent extract was obtained by affinity chromatography on a lentil lectin Sepharose 4B column [23].

For Western blotting, detergent extracts and glycoprotein fractions were separated by sodium dodecyl sulphate gel electrophoresis (SDS-PAGE) under reducing conditions at an acrylamide concentration of 8%, and transferred to nitrocellulose membrane [1]. The membrane was cut into 5 mm strips and the binding of immune chicken serums was detected using anti-chicken IgG and ¹²⁵I-labelled Protein A [24].

Immune chicken serum was collected 8 to 12 weeks after eyedrop vaccination of 6-week-old specified pathogen-free (SPF) White Leghorn chickens (CSIRO SPF Poultry Unit, Maribyrnong, Vic., Australia) with approximately 10⁵ PFU of SA-2 ILT vaccine (Arthur Webster Pty Ltd, Sydney, N.S.W., Australia). For a time course study of the development of antibodies to ILTV, two 8-week-old chickens were vaccinated with SA-2 by eyedrop. Blood was collected on days 0, 7, 14, 21, and 28 days post vaccination.

ILT antigens obtained by immunoprecipitation of detergent extracts with monoclonal antibodies (Mabs) to ILTV were tested for their ability to elicit a delayed-type hypersensitivity (DTH) reaction in the wattle of cockerels vacci-