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Evaluation of the antibody mediated immune response in nestling American kestrels (*Falco sparverius*)

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Abstract

Avian biologists and toxicologists use tests of immune function to evaluate health or quality in birds. Nestlings are widely studied members of the population because of the logistical ease of working with them, and because of their vulnerability to environmental contaminants. Current immunological techniques are designed for domestic poultry and are far from ideal, since poultry are precocial (developmentally mature at hatching), while many wild species are altricial (developmentally immature, i.e. blind, naked and totally dependent at hatching). The purpose of this study was to identify a sensitive means of evaluating in vivo antibody responsiveness in nestling American kestrels. Two antigens, sheep red blood cells (SRBC), and dinitrophenol–keyhole limpet hemocyanin (DNP–KLH), were used to stimulate a B cell mediated response. Antibody production was measured using a hemagglutination assay (SRBC), or an enzyme-linked immunosorbent assay (DNP–KLH). Two formulations of the antigen DNP–KLH were compared. DNP–KLH stimulated a stronger and more consistent antibody response in nestling kestrels than did SRBCs.

Keywords: Antibody response; American kestrel; Antibody mediated immunity; Ontogeny of antibody response; Comparative immune response; DNP–KLH; Sheep red blood cells; Altricial nestlings

1. Introduction

What we know of avian immunology has largely been derived from studies in domestic fowl [1–4]. Testing the immune response is now recognized as a valuable tool in toxicology, ecology and even behavioral studies of wild avian species [5–12]. In studies of wild birds, in which one or more aspects of immune function are examined, immunocompetence is now being used as a surrogate measure of fitness,

Abbreviations: DDA, dimethyldioctadecyl ammonium bromide; DNP–KLH, dinitrophenol–keyhole limpet hemocyanin; DTH, delayed type hypersensitivity; ELISA, enzyme-linked immunosorbent assay; HA, hemagglutination assay; M, molar; μl, microlitre; PBS, phosphate-buffered saline; PHA, phytohemagglutinin; RBC, red blood cells; SE, standard error; SRBC, sheep red blood cells.

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survivability, and quality of bird [11–14]. Tests of immunotoxicity are seen as a means of detecting subclinical changes in health status of wild birds that may become significant if animals are subjected to additive environmental stressors such as contamination, inclement weather, deteriorating habitats, exposure to infectious agents, and disturbed food supply.

Variables related to immunity such as total and differential white blood cell counts, antibody production against specific antigens, and the skin response to the T lymphocyte mitogen phytohemagglutinin (PHA), are the measures of immunological responsiveness most commonly used [6,7,12,15,16]. Not all these measures provide equally meaningful information about the nature of the impact on the immune system from exposure to pathogens, contaminants, or from the ecological perturbation being studied. In mature animals, the immune system is temporally dynamic and highly redundant with numerous interactive elements. Prediciting and interpreting the magnitude and direction of a response can be challenging. Antibody, or B cell mediated immunity responds variably to environmental stressors. Svensson et al. [7] demonstrated that cold temperatures suppress antibody production in blue tits (*Parus caeruleus*). In mallard ducks (*Anas platyrhynchos*), antibody titers against foreign red blood cells (RBC) were unaffected from exposure to selenium [14], whereas lead ingestion suppressed [17], or had no effect [18] on antibody responses in quail (*Coturnix coturnix*). To complicate the interpretation further, antibody production in American kestrels (*Falco sparverius*) exposed to PCBs was increased in adult females and in nestlings of both sexes, while it was suppressed in males [19].

A common means of assessing the humoral, or antibody response in wild birds is through the use of foreign RBC based assays [11,12,20–22]. RBC from one species, when injected into any other species, will generally induce an antibody-mediated immune response. Among other antigens that have been used to promote a humoral immune response in wild birds are the combination of dinitrophenol (DNP), a simple, chemically reactive hapten (having only one antigenic determinant), conjugated to keyhole limpet hemocyanin (KLH), a larger, but still simple respiratory pigment of the keyhole limpet [19]. Other antigens that have been successfully used in birds are KLH alone [23], diphtheria-tetanus vaccine [7,24] and Newcastle disease virus (NDV) [18]. In these studies factors influencing the antibody response were investigated in adult birds. In research on wild birds in which sheep red blood cells (SRBC) have been used to evaluate immunocompetence in adults and young, nestlings fail to produce detectable antibodies [11,20–22]. Smits and Bortolotti found nestling American kestrels to produce a markedly weaker response to DNP–KLH than did the adult birds. Nestling birds are widely studied members of the population because of the relative logistical ease of working with them, and because of their vulnerability to stressors (physical, social, toxicological, infectious). Altricial species hatch young that are developmentally immature (blind, naked and totally dependent on parental support) and their immune systems are likely to be similarly immature, which possibly explains the poor responses seen, relative to those of adult birds. Before researchers can interpret immunological responses, we must know the animals' capacity to respond.

One objective of this study is to identify a means of consistently provoking and detecting an antibody response in altricial, nestling birds, that will be useful for ecological and immunotoxicological studies in the wild. A second objective is to determine whether antibody production in the nestlings can be enhanced through different vaccine formulations. Adjuvants are substances, which when administered together with protein antigens, elicit strong innate immune reactions and inflammation at the site of antigen entry, thus promoting T cell dependent antibody production by mature B lymphocytes [25,26].

Characteristics of the antigen influence the nature of the antibody response, and two different antigens may not be equally effective in stimulating a detectable antibody response. Analytical methods may not be equally sensitive in detecting subsequent antibody levels. In this paper, we examine different means of stimulating and evaluating the B cell immune response in wild birds. American kestrels, small North American falcons, are common over an extensive geographical range, making them popular for studies in toxicology [27–29]. Because of their attributes of being tolerant of human disturbance, their relatively long nestling period (approximately 27 days) and the logistical ease with which they can be