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Research paper

Shared epitopes of avian immunoglobulin light chains

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ABSTRACT

Like all jawed vertebrates, birds (Aves) also produce antibodies i.e. immunoglobulins (Igs) as a defence mechanism against pathogens. Their Igs are composed of two identical heavy (H) and light (L) chains which are of lambda isotype. The L chain consists of variable (VL), joining (JL) and constant (CL) region. Using enzyme immunoassays (EIA) and two monoclonal antibodies (mAbs) (3C10 and CH31) to chicken L chain, we analysed their cross-reactivity with sera from 33 avian species belonging to nine different orders. Among Galliformes tested, mAbs 3C10 and CH31 reacted with L chains of chicken, turkey, four genera of pheasants, tragopan and peafowl, but not with sera of grey partridge, quail and Japanese quail. Immunoglobulins of guinea-fowl reacted only with mAb 3C10. Both mAbs reacted also with the L chain of Eurasian griffon (order Falconiformes) and domestic sparrow (order Passeriformes). Sera from six other orders of Aves did not react with either of the two mAbs. EIA using mAbs 3C10 and CH31 enabled detection of antibodies to major avian pathogens in sera of chickens, turkeys, pheasants, peafowl, Eurasian griffon and guinea-fowl (only with mAb 3C10). The N-terminal amino acid sequence of pheasant L chain (19 residues) was identical to that of chicken. Sequences of genes encoding the L chain constant regions of pheasants, turkey and partridge were determined and deposited in the public database (GenBank accession numbers: FJ 649651, FJ 649652 and FJ 649653, respectively). Among them, amino acid sequence of pheasants is the most similar to that of chicken (97% similarity), whereas those of turkey and partridge have greater similarity to each other (89%) than to any other avian L chain sequence. The characteristic deletion of two amino acids which is present in the L chain constant region in Galliformes has been most likely introduced to their L chain after their divergence from Anseriformes.

1. Introduction

Like other jawed vertebrates, birds (Aves) have an adaptive immune system characterized by the production of antibodies (immunoglobulins, Igs) in response to antigens, including those of pathogens (Ratcliffe, 2006). Birds produce only three classes of Igs: IgA, IgG (or IgY) and IgM (Ratcliffe, 2006; Choi et al., 2010; Huang et al., 2012). Their Igs consist of two identical L chains and two identical H chains. While other vertebrates have two or even three isotypes of L chains, birds have only one, which is similar to the lambda (λ) isotype in other vertebrates (Ratcliffe, 2006; Grant et al., 1971; Magor et al., 1994; Das et al., 2010; Huang et al., 2012; Bao et al., 2012). Their L chain consists of a variable domain (VL) and of a constant domain (VC) which is encoded by a single copy of the light chain gene (Ratcliffe, 2006; Reynaud et al., 1983; Das et al., 2010; Bao et al., 2012). In chicken (*Gallus gallus*), zebra finch (*Taeniopygia guttata*) and turkey

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(*Meleagris gallopavo*) analyses of IgL loci revealed similar features. They have a single functional Vλ gene, about 20 Vλ pseudogenes, and a single functional joining-constant (Jλ-*C*λ) block (Ratcliffe, 2006; Das et al., 2010; Bao et al., 2012). Recombinations (gene conversions) with Vλ pseudogene sequences generate diversification of VL chain sequence in chicken and probably also in other birds, including turkey, duck, zebra finch and ostrich (*Struthio camelus*) (Ratcliffe, 2006; Das et al., 2010; Huang et al., 2012; Bao et al., 2012). This mechanism enables production of diverse repertoire of VL chain sequences and wide repertoire of antibody specificities (Ratcliffe, 2006).

Recently, the L chain sequences of zebra finch, ostrich and turkey have been published (Das et al., 2010; Huang et al., 2012; Bao et al., 2012). However, our knowledge about the L chains in birds remains very limited because they represent an enormously diverse group of vertebrates (~9000 living species).

Monoclonal antibodies (mAbs) are a valuable tool for the study of avian Igs, their shared epitopes, as well as reagents detecting antibodies to avian pathogens (Mockett, 1986; Narat et al., 2004; Kothlow et al., 2008). The mAb 14A3 to duck L chain cross- reacted with L chain of 4 different duck species, two swan species and two goose species (Kothlow et al., 2008). Our mAb 3C10/F6 to chicken L chain cross-reacted with L chains of turkey, pheasant and sparrow (*Passer domesticus*), whereas mAb 1F5, to chicken IgY H chain, cross-reacted with H chain of turkey, pheasant (*Phasianus colchicus*) and peafowl (*Pavo cristatus*) (Narat et al., 2004).

In this study we used two mAbs to chicken L chain (mAbs 3C10 and CH31) to assess their reactivity with Igs of 33 species of birds belonging to nine orders of Aves. We determined the N- terminal amino acid sequences of the L chain of pheasant and, based on DNA sequencing, sequences of coding regions of L chains (joining and constant domains) for pheasant, turkey and grey partridge (*Perdix perdix*) and compared them with sequences from other avian species.

2. Materials and methods

2.1. Samples collected from various avian species

Most serum samples were collected from birds in Slovenia, including sera from our previous studies (Zorman-Rojs et al., 2000; Benčina et al., 2003, 2005; Narat et al., 2004). Serum samples of Eurasian griffon (*Gyps fulvus*), sun conure (*Aratinga solstitialis*), ara (*Ara glaucogularis*) and Major Mitchell's cockatoo (*Cacatua leadbeateri*) were kindly donated by Dr Ursula Heffels Redman (Justus Liebig University, Giessen, Germany). Spleens used to isolate DNA from pheasants, grey partridges and turkeys were from birds reared on farms in Slovenia. They were donated by Dr. Andrej Bidovec and Dr. Rahela Juršič Cizerl (Veterinary Faculty, Ljubljana, Slovenia). Peafowl DNA was from the birds examined in a previous study (Benčina et al., 2003). DNA (spleen samples) from pigeon, greylag goose (*Anser anser var. domestica*), peregrine falcon (*Falco peregrinus*), tawny owl (*Strix aluco*), budgerigar (*Melopsittacus undulatus*), grey parrot (*Psittacus erythacus*) and muscovy duck (*Cairina moschata*) were donated by Dr. Olga Zorman Rojs and Dr. Alenka Dovč (Veterinary Faculty, Ljubljana, Slovenia) and Dr. Tamas Bakonyi (Faculty of Veterinary Science, Szent Istvan University, Budapest, Hungary). Chicken B-cell line (DT 40) was kindly provided by Dr. Jean-Marie Buerstedde (Institute for Molecular Radiobiology Neuherberg, Germany).

2.2. mAbs and other antibodies

The monoclonal Ab CH 31 (C7910, Sigma) reacts according to the producer's specification with L chains of chicken and turkey, but not with sera of duck, dove, goose, ostrich, crow and cattle egret. Monoclonal Ab 3C10/F6 (in short 3C10) to chicken L chain was produced in our previous study and reacted with sera of chicken, pheasant, turkey and sparrow, but not with sera of peafowl, duck, goose, parrot, pigeon and quail (Narat et al., 2004). We also used mAb M1 (to chicken IgM, H chain) and mAb 1F5 (to chicken IgY, H chain) from our previous studies, which also cross-react with Igs of a variety of bird species (Narat et al., 2004; Cizelj et al., 2011).

In enzyme immunoassays (EIA) we used rabbit antisera to IgG (IgY) of goose, pigeon, budgerigar and ostrich to determine relative quantity of Igs in samples. Those antisera were produced as described previously (Benčina and Bradbury, 1991). Monoclonal Ab (to rabbit IgG) conjugated with horseradish peroxidase (HRP) (A1949, Sigma) was used as a secondary antibody.

Rabbit IgG to chicken IgG conjugated with HRP (A9046, Sigma) and goat IgG to mouse IgG conjugated with HRP (A9917, Sigma) were also used in EIA.

2.3. Enzyme immunoassays

2.3.1. Dot-immunobinding assay (DIBA)

DIBA was used to determine which samples react with mAbs 3C10 and CH31 (Narat et al., 2004; Benčina et al., 2005). Serum samples were assayed in serial dilutions ranging from 1:100 to 1:25600, or even higher, when reactions were strong.

DIBA was used in assays investigating whether mAbs 3C10 and CH31 could be useful for the detection of avian antibodies which react with major avian pathogens (Narat et al., 2004; Benčina et al., 2003). Briefly, bacterial or viral antigens were dotted as five separate dots (2–3 μl) on strips cut from the membrane (Immobilon P). The strips were first incubated for 1 h with avian sera diluted 1:100 in this particular test. After washings (3 × 15 min), the strips were incubated with mAb 3C10 or CH31 for 1 h and washed again. Then strips were incubated for 50 min in HRP-conjugated goat antibodies to mouse IgG. The chromogenic substrate TrueBlue (Kirkegaard and Perry Laboratories) was used to develop reactions. Controls included at the first step of DIBA were reference chicken sera containing or lacking specific antibodies.

2.3.2. ELISA

Reactions of mAbs 3C10 and CH31 with avian sera were measured and quantified by ELISA. Serum samples (50 μl) were serially diluted two- or four-fold (from 1:100