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Differential expression of U2AF^{35} in the arthritic joint of avian reovirus-infected chicks

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Abstract

To identify cell types and genes that are differentially expressed during immunopathogenesis of avian reovirus (ARV)-induced viral arthritis (VA), we inoculated arthrotropic strain S1133 of ARV into 1-day-old broilers, and examined tissue histology as well as RNA expression at different days post-inoculation (PI). Using immunohistochemical staining, we detected many CD68 expressing macrophages in and around the blood vessels of the arthritic joints. By RT-PCR, we found that expression of matrix metalloproteinase-2 (MMP-2) and bone morphogenetic protein-2 (BMP-2) was induced earlier in footpads and hock joints of ARV-infected chickens. By employing suppression subtractive hybridization (SSH) technique and RT-PCR, we further identified that small subunit of U2 snRNP auxiliary factor (U2AF^{35} or U2AF1) mRNA was differentially induced in the joint of ARV-infected chickens. By *in situ* hybridization (ISH), mRNA signals of U2AF^{35} and BMP-2 were located in chondrocytes within/near the epiphyseal plate and secondary center of ossification, and in epidermal cells and dermal fibroblast-like cells of arthritic joints. In addition, U2AF^{35} mRNA was expressed in the inflammatory infiltrates of the bone marrow of ARV-infected arthritic joints, while MMP-2 was mainly detected in chondrocytes. Interestingly, among U2AF^{35}, MMP-2, and BMP-2 that were differentially expressed in the joint of ARV-infected chickens, only U2AF^{35} induction correlated well with arthritic manifestation. Because U2AF^{35} may assist in mRNA splicing of proinflammatory chemokines and cytokines, our results indicated that U2AF^{35} induction might play an immunopathological role in ARV-induced arthritis. This study has first associated U2AF^{35} to viral arthritis.

Keywords: Avian reovirus (ARV); Bone morphogenetic protein-2 (BMP-2); Matrix metalloproteinase-2 (MMP-2); Small subunit of U2 snRNP auxiliary factor (U2AF^{35} or U2AF1); Viral arthritis (VA)

1. Introduction

Avian reovirus (ARV) infections have been associated with viral arthritis (VA) (also known as tenosynovitis), enteric diseases and other pathological conditions such as myocarditis, and pericarditis in chickens and turkeys (Clark et al., 1990; Robertson and Wilcox, 1986; van der Heide et al., 1981; Walker et al., 1972). Among these diseases, the causative role of ARV has only been established in VA (Robertson and Wilcox, 1986). Young chicks are especially susceptible to ARV infection, and resistance to infection increases with age (Rosenberger and Olson, 1991).

The apparent lesions of ARV-induced VA include gross swelling and inflammatory infiltration of the

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tibiotarsal and hock joints of infected chickens (Glass et al., 1973; Kerr and Olson, 1969; Pertile et al., 1996). In addition to VA lesions developed in the joints, ARV can lead to a transient reduction of immune responses in chickens (Montgomery et al., 1985; Neelima et al., 2003; Pertile et al., 1995; Springer et al., 1983), which may predispose chickens to secondary infections (Kibenge et al., 1982). Interestingly, arthritic lesions and temporal immunosuppression developed in ARV-infected chickens resemble symptoms that are exhibited in patients with rheumatoid arthritis (RA) (Harris, 1990; Verwilghen et al., 1990). RA is a chronic inflammatory joint disease mediated by activated macrophages, T lymphocytes, and B lymphocytes. Macrophages, in particular, play a pivotal role during RA pathogenesis by expressing major histocompatibility complex (MHC) class II molecules, proinflammatory cytokines, and matrix degrading enzymes (Kinne et al., 2000). In fact, Pertile et al. (1996) have studied in detail the pattern of lymphocyte (CD4- and CD8-positive T lymphocytes, B lymphocytes/plasma cells) infiltration in ARV-induced VA and found it to be similar to that of RA. Apart from the well-characterized lymphocytes, they have also described the infiltration of Ia (class II MHC antigen)-positive non-lymphocyte cell populations during acute phase arthritis (Pertile et al., 1996). However, it remains to be determined whether these non-lymphocyte cells are macrophages.

Studies in RA and other human arthritis have shown that matrix metalloproteinases (MMPs) are major catabolic enzymes participating in the destruction of cartilage and bone matrix during arthritis pathogenesis (Giannelli et al., 2004; Vincenti and Brinckerhoff, 2002). As a counterbalance, anabolic factors such as bone morphogenetic proteins (BMPs) are induced to repair damage in arthritis (Nakase et al., 2003). However, it is still not clear whether MMPs and BMPs are involved in ARV-induced VA as well.

In this study we employed immunohistochemistry, suppression subtractive hybridization (SSH) technique (Diatchenko et al., 1996), and reverse transcription-polymerase chain reaction (RT-PCR) to recognize the identity of the CD68-positive macrophage infiltrates and to identify the gene(s) that may participate in immunopathogenesis of ARV-induced viral arthritis.

2. Materials and methods

2.1. Virus and virus titration

The arthrotropic virulent strain S1133 of ARV (van der Heide and Kalbac, 1975), originally obtained from Vineland Laboratories (Vineland, NJ, USA), is a challenge strain used for the vaccine evaluation. The virus was propagated briefly in chicken embryo fibroblasts (CEF). Virus titration was performed as described (Robertson and Wilcox, 1984), and virus titer was expressed as 50% tissue culture infectious dose ($TCID_{50}$).

2.2. Experimental chickens

Sixty specific pathogen free (SPF) broiler chickens (1-day-old) were used in this study. Each chicken was inoculated either with 10^3 $TCID_{50}$/50 μl of S1133 strain ARV (ARV-infected group) or 50 μl of phosphate buffered saline (PBS) (control group) into each footpad. ARV-infected and PBS-inoculated chickens were housed in separate isolation units with biologically filtered air. At 12 h, 1–4, 6, 8, 10, 12, 14, 16, 18, 21, 28, and 35 days after inoculation, two chickens, respectively from the ARV-infected group and the control group were sacrificed. The experiment was carried out following the guideline of the Institutional Animal Care and Usage Committee, National Chung Hsing University, Taiwan.

2.3. Enzyme-linked immunosorbent assay (ELISA)

Serum was collected from each chicken before sacrifice. A commercial ELISA kit (avian reovirus antibody test kit, Idexx Laboratories, Westbrook, ME, USA) was used to determine the titer of anti-ARV antibody.

2.4. Histology and immunohistochemistry

Lung, heart, liver, spleen, kidney, bursa of Fabricius, proventriculus, duodenum, jejunum, ileum, footpads, and hock joints were dissected from each chicken and cut into half. One half of the tissue was frozen in liquid nitrogen and stored at −70 °C before RNA extraction. The other half of the tissue was fixed with 10% PBS buffered formalin, embedded in paraffin wax, sectioned at 5 μm, and then mounted on glass slides. Two slides for each tissue were stained with hematoxylin and eosin.

For immunohistochemistry, slides were incubated with anti-CD68 monoclonal antibody (KP1) (Dako, Carpenteria, CA, USA) by using the labeled streptavidin–biotin (LSAB) method as described previously (Chow et al., 2004; Peng et al., 1999).

2.5. RNA extraction

Frozen tissues of hock joints and footpads were removed from −70 °C and ground under liquid nitrogen