Available online at www.sciencedirect.com
SCIENCE DIRECT®
Veterinary Microbiology 105 (2005) 163–167
veterinary microbiology
www.elsevier.com/locate/vetmic

Th1/Th2 polarization by viral and helminth infection in birds

Winfried G.J. Degen[a], Nancy van Daal[a], Lisa Rothwell[b], Pete Kaiser[b], Virgil E.J.C. Schijns[a,*]

[a]*Department of Vaccine Technology and Immunology, Intervet International BV, P.O. Box 31, 5830 AA Boxmeer, The Netherlands*
[b]*Institute for Animal Health, Compton, Berkshire RG20 7NN, UK*

Received 30 August 2004; received in revised form 18 November 2004; accepted 1 December 2004

Abstract

Mammals developed an immune system able to functionally polarize into so-called type 1 or type 2 immune pathways, to resolve infections with intracellular and extracellular pathogens, respectively. In the well-studied avian immune system of the chicken, however, no evidence for polarized immunity could be found, as yet. To investigate whether these two major arms of mammalian immunity, regulated by a T helper (Th)1/Th2 cytokine balance, evolved similarly in birds, chickens were exposed to a prevalent intracellular (viral) or extracellular (helminth) infection. By using semi-quantitative RT-PCR analysis we provide evidence that polarization of Th1/Th2 type immunity extends beyond mammalian species, and, therefore, has been evolutionary conserved for more than 300 million years, when the lineages of mammalian and avian vertebrates are assumed to have segregated.

Keywords: Th1/Th2 polarization; Chicken; Mammalian; Semi-quantitative RT-PCR; ChIL-13; ChIFN-γ

1. Introduction

Mammals developed an immune system able to functionally polarize into so-called type 1 or type 2 immune pathways (Mosmann et al., 1986), to resolve infections with intracellular and extracellular pathogens, respectively (Janeway, 1992). The polarization of acquired immune reactions is largely regulated by antigen-specific Th cells (Abbas et al., 1996). Naïve Th cells can differentiate into either Th1 or Th2 type cells, driving counterbalanced cell-mediated and humoral immune reactions, respectively (Mosmann and Sad, 1996). Th1 type cells typically produce IFN-γ, crucially driven by early IL-12 and IL-18, and are associated with inflammatory cytolytic responses; generally necessary for destroying cells infected by viruses and other intracellular microbes. By contrast, synthesis of IL-4, IL-5 and IL-13 form hallmarks of Th2 cells, which are facilitated by IL-4 or IL-13 and are associated with antibody production, anti-helminth reactions and IgE synthesis. Polarization of cytokine production and

* Corresponding author. Tel.: +31 485 587688; fax: +31 485 585313.
E-mail address: Virgil.Schijns@Intervet.com (Virgil E.J.C. Schijns).

0378-1135/$ – see front matter © 2004 Elsevier B.V. All rights reserved.
doi:10.1016/j.vetmic.2004.12.001

cellular responses by T helper cells was later extended to CD8 + α/β T cell receptor (TCR) cells, γ/δ TCR cells (Mosmann and Sad, 1996) and to dendritic cells (Liu et al., 2001; Kapsenberg, 2003). The ultimate source of Th1/Th2 phenotype bias remains largely unknown. Th1 responses likely involve recognition of conserved microbial motifs by innate immune cell receptors (Kapsenberg, 2003), while Th2-dominated reactions may result form default responses and non-microbial adjuvants (Schnare et al., 2001).

Whether the two major arms of immunity, regulated by a T helper (Th)1/Th2 cytokine balance, evolved similarly in birds is unknown. In the well-studied chicken (*Gallus gallus*) immune system immunoglobulin (Ig) E or IgG subtypes are missing. Also, Th2-associated allergies are unknown for birds. Chickens possess few eosinophils, basophils and mast cells—all hallmarks of mammalian Th2 responses. As yet, only Th1 type cytokines, including chicken (Ch) interferon (IFN)-γ (Digby and Lowenthal, 1995) and Ch interleukin (IL)-18 (Schneider et al., 2000), have been identified, with amino acid identities of only 30–35% relative to their mammalian orthologues (Secombes and Kaiser, 2003). The functional homologue of the long-searched-for ChIL-12, driving ChIFN-γ synthesis, was identified only very recently (Degen et al., 2004). Birds evolved delayed-type hypersensitivity reactions and produce a single IgG equivalent, known as IgY, possibly guided by a regulatory pathway of Th1 cytokines only. Recently, however, the first non-mammalian (chicken) Th2 cytokine gene sequences, with ChIL-5 identified as a pseudogene, were identified (Avery et al., 2004). This allowed us to examine whether avian Th2 type cytokine expression has been conserved over 300 million years, since the great radiation of birds and mammals (Kumar and Hedges, 1998). In the present study, we provide evidence that chickens are able to mount a typical Th1- or Th2-biased cytokine response to experimental viral and helminth parasite infections, respectively.

2. Materials and methods

2.1. Chickens

Three weeks old Normal White Leghorn SPF chickens were derived from the Intervet Animal Facilities and housed under SPF conditions. The animals received water and food ad libitum. All experiments were carried out according to protocols approved by the Intervet Animal Welfare Committee.

2.2. Microbial infection

Groups of three-week-old SPF White leghorn layer chickens ($n = 5$ per group) were infected with paramyxoviral NDV strain Herts 33/56 (1×10^4 ELD50/0.2 ml, intramuscularly) or embryonated *Ascaridia galli* (strain Liederbach) worm eggs (1000 per dose, orally). At several time points post-infection chickens were sacrificed, spleen and ileal lymphoid tissues extracted, and total RNA isolated from these tissues.

2.3. RNA isolation

Total RNA was isolated using Trizol reagent (Invitrogen Life Technologies, Grand Island, NY) as described by the manufacturer. RNA quality was evaluated on 1.2% agarose gels. RNA samples were used subsequently for semi-quantitative RT-PCR (semi-Q-RT-PCR) or stored at −70 °C.

2.4. Semi-quantitative RT-PCR

Two micrograms of total RNA was reverse-transcribed into cDNA using the Superscript II RT protocol (Invitrogen Life Technologies) in a 20 μl reaction. One microliter of cDNA was mixed with 0.5 μl of 1 unit/μl Supertaq (HT Biotechnology, Cambridge, U.K.), 1 μl of 10 ng/μl of each primer, 1.6 μl of 2 mM dNTPs, and 2 μl of 10× ST PCR buffer (HT Biotechnology) in a final volume of 20 μl. The reaction cycling conditions were: 5 min 94 °C, variable cycles (30 s 94 °C, 1 min 55 °C, 1 min 72 °C), and 5 min 72 °C for a final extension. To determine the optimum number of PCR cycles required for a near linear relationship between the amount of RNA and amplified DNA band intensity, a variable number of cycles was performed for each marker. Internal primers used are described in Table 1. PCR products were separated on a 1.5% agarose gel and analysed. ChGAPDH was used for RT-PCR control and semi-quantitative comparison for ChIFN-γ, ChIL-4 and ChIL-13 mRNA expression.