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Research paper

Cytokine gene expression in splenic CD4$^+$ and CD8$^+$ T cell subsets of genetically resistant and susceptible chickens infected with Marek's disease virus

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ABSTRACT

T cells from the spleens of B^{19}/B^{19} and B^{21}/B^{21} chickens infected with MDV JM-16 strain were fractionated by flow cytometry at 4, 10, 21 days post infection (d.p.i.). The expression of cytokine and viral genes (meq and glycoprotein B (gB)) was measured by real-time RT-PCR. It was determined that CD4$^+$ and CD8$^+$ T cells had both become infected with Marek's disease virus (MDV) in both chicken lines. There was significantly higher expression of meq in CD4$^+$ T cells compared to CD8$^+$ T cells at 10 and 21 d.p.i. Furthermore, at 10 and 21 d.p.i., there was a tendency for higher expression of meq in both T cell subsets of B^{19} chickens compared to those of B^{21} chickens. There were temporal changes in the expression of cytokines, interferon (IFN)-γ, interleukin (IL)-18, IL-6, and IL-10, in various T cell subsets. Among these changes, there was an increase in IL-10 expression in both T cell subsets at different time points, especially in the susceptible line at 10 and 21 d.p.i. Our results indicate that cytokines could be differentially induced by MDV in CD4$^+$ and CD8$^+$ T cell subsets and that IL-10 may play a role in the modulation of immune response to MDV. However, an association between cytokine gene expression in T cell subsets and resistance or susceptibility to MD was not established.

1. Introduction

Marek's disease is caused by a herpesvirus which, based on its genomic structure, is a member of the subfamily *Alphaherpesvirinae* (Davison, 2002). Several pathotypes of Marek's disease virus (MDV) have so far been characterized, ranging from mild to very virulent plus strains. Among these pathotypes, the JM-16 strain is considered a virulent one (Izumiya et al., 2001). MDV infection occurs through inhalation of cell-free virus particles, which are produced in feather follicle epithelium and are present in feather dander, by susceptible chickens (Abdul-Careem et al., 2008). The virus is then taken to lymphoid organs by macrophages (Lessard et al., 1996). Based on the model proposed by Calnek (1986), MDV infects B cells in the early cytolytic phase of the infection (2–7 days post infection, d.p.i.). The cytolytic phase is followed by a latent phase, which is marked by the infection of CD4$^+$CD8$^-$ T cells through infected B cells (Schat et al., 1982). This phase begins around 7 d.p.i. and continues until about 14 d.p.i (Schat et al., 1991). In addition to CD4$^+$ T cells, CD4$^-$CD8$^+$ and CD4$^-$CD8$^-$ T cell subpopulations have also been shown to be latently infected between 7 and 10 d.p.i. (Lee et al., 1999). From approximately 18 d.p.i. onward, in

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susceptible birds, there is a second phase of cytolytic infection as well as a transformation phase which mainly occurs in $CD4^+$ $CD8^-$ T cells and is manifested by the presence of tumours in various organs (Calnek, 2001). Clinical signs of MD, most notably paralysis, are usually associated with infiltration of transformed T cells into various tissues, including the nervous system (Calnek, 2001). Therefore, based on this model, 4, 10, and 21 d.p.i. were chosen in our experiments to represent various phases of MDV pathogenesis.

The are several factors that determine the development of MD in chickens, including the serotype and pathotype of the virus, age of the host, the presence of relevant innate defence mechanisms as well as adaptive immune responses, immunity conferred by vaccines, and importantly genetic resistance of the host (Cole, 1967; Calnek, 2001). Several genetic loci are associated with resistance or susceptibility to MD, among which major histocompatibility complex (MHC or B-complex) plays an important role (Pazderka et al., 1975; Longenecker et al., 1976). It has been shown that different B haplotypes confer various degrees of resistance or susceptibility to MD. For example, chickens that are homozygous for B^{19} haplotype display susceptibility to disease, whereas B^{21} homozygote chickens are resistant (Bacon et al., 2001). The differential susceptibility to MD among chickens may be attributed to the quantity and quality of host responses to MDV antigens. For instance, cytotoxic T lymphocytes (CTL) responses against some of MDV antigens such as ICP4, were detected *in vitro* in MDV-stimulated splenocytes from B^{21}/B^{21} chickens, but these responses were absent in B^{19}/B^{19} splenocytes. This may partly explain genetically regulated susceptibility of B^{19}/B^{19} chickens (Markowski-Grimsrud and Schat, 2002).

Cytokines have a pivotal role in steering the immune response and possibly in genetic resistance and susceptibility to MD. Similar to mammals, T helper (Th)1 and Th2-like responses have also been described in chickens (Degen et al., 2005). In addition to the cytokines associated with Th1/Th2-like responses, type I interferons and chemokines have been studied for their role in immunity against MD. It has been reported that the amount of the transcripts for interferon (IFN)-α and IFN-γ in the blood of susceptible chickens is lower than that in resistant birds in early days of infection (Quere et al., 2005). Similarly, IFN-γ transcripts are elevated in splenocytes of infected $B^{21}B^{21}$ chickens in the early cytolytic phase (Xing and Schat, 2000b). On the contrary, interleukin (IL)-6 and IL-18 are expressed more in susceptible lines (7_2 and P) than in resistant lines (6_1 and N) in the early cytolytic phase of the infection (Kaiser et al., 2003). It has also been shown that CC chemokines such as macrophage inflammatory protein 1 β (MIP1β) and K203 are up-regulated in resistant chickens which may in turn induce the production of IFN-γ via natural killer (NK) cells (Djeraba et al., 2002).

In general, there is little known about the repertoire of cytokines produced by $CD4^+$ and $CD8^+$ T cell subsets in chickens and in the case of MDV infection in particular, no information is currently available. The objectives of the present study were, therefore, to characterize the cytokines, including IFN-γ, IL-18, IL-6 as indicators of a proinflammatory/Th1 responses, IL-4 as an indicator of a Th2 response, and IL-10 as an indicator of regulatory T cell phenotype, produced by $CD4^+$ and $CD8^+$ T cell subsets in the course of MDV infection. These cytokine profiles were then correlated with resistance or susceptibility of B^{19}/B^{19} and B^{21}/B^{21} chickens to MD. Here, we show that MDV is transcriptionally active in both $CD4^+$ and $CD8^+$ T cell subsets of infected chickens. Furthermore, we provide evidence that cytokine gene expression is temporally regulated in these T cell subsets in the course of MDV infection.

2. Materials and methods

2.1. Virus

The virulent MDV strain JM-16 was provided by Dr. K.A. Schat (Cornell University, NY, USA). Virus was passaged 14 times on chicken kidney cell cultures (CKC) based on the method previously described (Calnek and Madin, 1969). Briefly, CKC were prepared using specific pathogen free (SPF) chickens which were 10–20 days old. CKC cultures were then inoculated with 20 plaque-forming units (PFU) of the JM-16 viral stock propagated *in vivo*. The seeded cultures were kept under the same conditions for 48 h and transferred to a fresh CKC culture by trypsinisation with 1:10 dilution of trypsin (Invitrogen Life Technologies, Carlsbad, CA, USA) for 5–8 min. This procedure was repeated 14 times and finally CKC cultures with the propagated virus were trypsinised and frozen in the freezing medium (DMEM (Dulbecco's modified Eagle's medium nutrient) 65%, 10% TPB (Tryptose phosphate buffer), 10%DMSO (Dimethyl sulfoxide), 15% FBS (fetal bovine serum) in cryogenic vials (Corning Inc., NY, USA). The *in vitro* propagated virus was used to infect the chickens (Jarosinski et al., 2002). The final titer of the propagated virus was 30,000 PFU/ml.

2.2. Experimental animals

SPF eggs from genetically defined lines N2a (B^{21}/B^{21} haplotype) and P2a (B^{19}/B^{19} haplotype) (Briles and Briles, 1982) were received from Cornell Veterinary College (Cornell University, New York, USA). Eggs were hatched in the Arkell Poultry Research Unit, University of Guelph and chicks were placed at the Isolation Unit of Ontario Veterinary College (University of Guelph, Guelph, Ontario, Canada).

2.3. Experimental design

Forty two chicks from each line were randomly divided into two groups. Twenty-one chicks were infected intraperitoneally on day 5-post hatch with 10,000 PFU of JM-16 (Passage#14) propagated *in vitro* as previously described. The rest of chickens were kept in a separate room as uninfected controls. In a pilot experiment, we determined that inoculation of B^{19} chickens with this dose of the virus resulted in formation of typical MD tumors in all of the infected birds by day 21 post-infection.