Virology 522 (2018) 56–64

Contents lists available at ScienceDirect

Virology

journal homepage: www.elsevier.com/locate/virology

Characterizaton of gamma delta T cells in Marek's disease virus (Gallid herpesvirus 2) infection of chickens

Adrianna M.S. Laursen[a], Raveendra R. Kulkarni[a], Khaled Taha-Abdelaziz[a,b], Brandon L. Plattner[a], Leah R. Read[a], Shayan Sharif[a,*]

[a] Department of Pathobiology, Ontario Veterinary College, University of Guelph, Guelph, Ontario, Canada N1G 2W1
[b] Pathology Department, Faculty of Veterinary Medicine, Beni-Suef University, Al Shamalah, 62511 Beni-Suef, Egypt

ARTICLE INFO

Keywords:
Marek's disease
Chickens
γδ T cells
Immune response
Innate lymphocytes
Cytokines

ABSTRACT

Immunity against Marek's disease (MD), caused by Gallid herpesvirus 2 (GaHV-2), in chickens is mediated by both innate and adaptive responses. The present study evaluated the effects of GaHV-2 infection on distribution and frequency of γδ T cells in tissues, as well as their expression of cytokines. We found that the infected chickens had significantly higher number of γδ T cells in their spleens by 10 and 21 days post-infection (d.p.i.) and nearly 100% of these γδ T cells were $CD8^+$ at 21 d.p.i. Conversely, the number of γδ T cells in the cecal tonsils of GaHV-2-infected birds decreased compared to uninfected birds. Splenic γδ T cells had up-regulated expression of interferon-γ early in infection followed by simultaneous gene expression of interleukin-10 during the later phases. In conclusion, these results suggest a potential role for γδ T cells in host response to GaHV-2 and further elucidate the underlying immunological mechanisms of interactions between this virus and its host.

1. Introduction

Marek's disease (MD) is a highly infectious lymphoproliferative disease caused by caused by Gallid herpesvirus 2 (GaHV-2), an avian herpesvirus of the sub-family *Alphaherpesvirinae*. The virus causes immunosuppression in susceptible birds and may ultimately result in the formation of T cell lymphomas. GaHV-2 is a cell-associated virus that gains entry into its host through the respiratory system where it is taken up by phagocytic cells such as macrophages, and spread to lymphoid organs (as reviewed by Haq et al., 2013). In the early cytolytic phase of infection, mainly B cells become infected with the virus (Shek et al., 1983). However, prior to entering the latent phase, activated $CD4^+$ T cells become infected with GaHV-2 (Schat and Xing, 2000). During the neoplastic phase of infection, productive infection occurs in $CD4^+$ T cells leading to the release of cell-free virus particles from the feather follicle epithelium (FFE) and skin epithelium (Heidari et al., 2016). As there are still many gaps in the literature pertaining to GaHV-2, it is important to further our understanding of this virus and its interaction with the chicken's immune system.

The host response to GaHV-2 infection first involves the innate response followed by the adaptive immune response. GaHV-2 is a cell-associated virus during infection in the host; therefore, understanding the cell-mediated immune response is critical. $CD4^+$ T cells serve as targets of transformation; however, they are also able to influence the micro-environment by creating different cytokine milieus depending on the stage of GaHV-2 pathogenesis (Parvizi et al., 2009). Meanwhile $CD8^+$ T cells have important cytotoxic capabilities as previously described against certain GaHV-2 antigens (Schat et al., 1992). When $CD8^+$ T cells are depleted, the result is a higher GaHV-2 load in $CD4^+$ T cells (Morimura et al., 1998); therefore, these cells can be characterized as having a unique and central role in the avian immune response. Other cell subsets that remain to be studied in response to GaHV-2 infection include γδ T cells.

Despite the high frequency of γδ T cells in chickens, little is known in regard to the nature and function of these cells. γδ T cells comprise about 10% of thymocytes, 20% of circulating T cells, and 30% of splenocytes in adult chickens (Bucy et al., 1991). In comparison, most research on γδ T cells has been focused on mice and humans; however, these species have a much lower frequency of γδ T cells relative to chickens (Haas et al., 1993). Given the number and location of γδ T cells and the range of immune system molecules they produce, these cells are thought to be involved in immunity against pathogens, including viruses.

Studies in mice and humans have revealed that the number and function of these cells are increased during viral infections, including herpesviruses infections (Nishimura et al., 2004; Sciammas et al.,

* Corresponding author.
E-mail address: shayan@uoguelph.ca (S. Sharif).

https://doi.org/10.1016/j.virol.2018.06.014
Received 26 March 2018; Received in revised form 24 June 2018; Accepted 26 June 2018

1997). For example, humans infected with herpes simplex virus (HSV) exhibit a 24-fold increase in the number of γδ T cells by 10 d.p.i. (Bukowski et al., 1994). Another study in cattle showed that bovine herpesvirus type I infection is associated with a substantial increase in peripheral blood γδ T cells (Amadori et al., 1995). In the context of infection with influenza virus, Carding et al. (1990) found that mice infected intranasally with influenza A virus had an inflammatory response in the lungs predominated by γδ T cells.

Although both T helper (Th)1-like and Th2-like cytokine gene expression has been shown to be up-regulated in the spleen of chickens at different phases of GaHV-2 infection (Parvizi et al., 2009; Sarson et al., 2006; Abdul-Careem et al., 2007), the range of cytokines produced by chicken γδ T cells following infection with GaHV-2 have not yet been studied. It has been demonstrated that murine γδ T cells exhibit plasticity in their response depending on the nature of the infecting pathogen (Ferrick et al., 1995). In this study, mice infected with an intracellular bacterium had increased expression of interferon (IFN)-γ by γδ T cells whereas those infected with an extracellular parasite had increased expression of interleukin (IL)-4. Furthermore, research on avian $CD8^+$ γδ T cells has shown mRNA expression of IFN-γ but not IL-4 in response to *Salmonella* infection, consistent with Th1 polarization during this type of infection (Pieper et al., 2008). This plasticity in their role during host immune response to pathogens demonstrates that γδ T cells are able to discriminate between pathogens posing a risk to the host and respond in the appropriate fashion. Previous work has also shown that chicken γδ T cells are able to express Toll-like receptor (TLR) 3 (Iqbal et al., 2005). With the ability to express pattern recognition receptors (PRRs) such as TLRs, and produce cytokines, chicken γδ T cells seem to be capable of detecting and responding to viral invasion as a 'first line of defense'. The cytokines produced by γδ T cells in response to infection may also aid in creating a cytokine milieu, and thus influencing the differentiation of $CD4^+$ T cells into a Th1 or Th2 subset (Parvizi et al., 2009).

The goal of the present study was to elucidate the role of γδ T cells in immunity against MD in order to further our understanding of this virus and its interaction with the chicken immune system. The objective was to evaluate changes in the frequency and absolute numbers of γδ T cells during the course of GaHV-2 infection. Furthermore, we set out to elucidate the role of γδ T cells in response to GaHV-2 by examining the expression of cytokine and TLR genes in these cells. GaHV-2 is a constantly evolving virus; therefore, it is of interest to understand the underlying immunological mechanisms that mediate immunity to MD with the ultimate goal of creating novel vaccines.

2. Results

2.1. GaHV-2 meq genome copy number in feather tips of GaHV-2-infected chickens

As determined by conventional PCR, all the samples from uninfected control groups were *meq* negative. Additionally, samples from GaHV-2-infected chickens at 4 d.p.i. were *meq* negative in both trials (Fig. 1a, trial 1 and Fig. 1b, trial 2). As shown in Fig. 1, GaHV-2-infected chickens from both trials had high *meq* copy numbers in their feather follicles with a mean of $2.98X10^6$ (trial 1) and 4.4×10^4 (trial 2), respectively by 10 d.p.i. Furthermore, the *meq* copy numbers were significantly higher at 21 d.p.i., 1.94×10^7 (trial 1) and 9.1×10^5 (trial 2) respectively, indicating increased virus replication and shedding during the late cytolytic stage of GaHV-2 infection.

2.2. Analysis of splenic γδ T cells in GaHV-2-infected chickens

To determine changes in the splenic γδ T cell population in GaHV-2-infected chickens, splenocytes were isolated at 4, 10, and 21 d.p.i and stained with anti-TCRγδ specific monoclonal antibody for flow cytometry analysis. As shown in Fig. 2a, the splenic γδ T cell frequency in

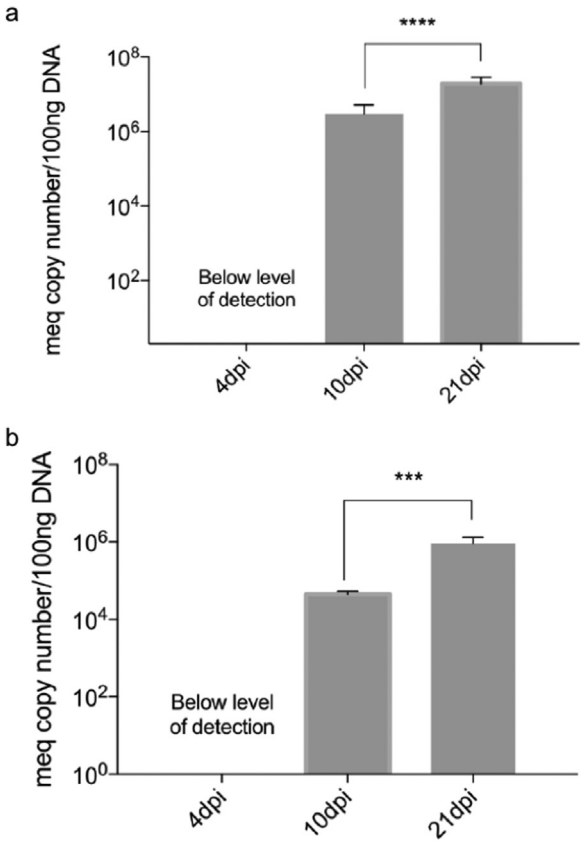

Fig. 1. GaHV-2 copy number in feather follicles at 4, 10, and 21 days post-infection (dpi) for (1a) trial 1 and (1b) trial 2. *Meq* gene was used to calculate virus copy number from 100 ng of DNA. The groups included in this representation were GaHV-2-infected birds only as control birds lacked the presence of *meq*. Data presented are the mean ± standard error of the mean (SEM) for ten (a) and six (b) biological replicates at each time point. Asterisks denote a significant difference between groups ($p \leq 0.0001$ (****), or ($p < 0.001$ (***)).

GaHV-2-infected chickens was significantly reduced at all time points when compared to control birds. In contrast, we found that the GaHV-2-infected chickens had significantly higher numbers (absolute counts) of γδ T cells in spleens by 21 d.p.i. compared to uninfected control chickens (Fig. 2b). However, no significant changes in the number of splenic γδ T cells at 4 and 10 d.p.i. were observed between infected and control chickens. We also performed an intra-group temporal analysis and found that the absolute γδT cell numbers were significantly higher at 21 d.p.i. when compared to either 4 or 10 d.p.i. within both the infected and uninfected groups. This was possibly due to an age-dependent increase / splenic organ development leading to an increase in cell numbers.

Because the absolute cell counts represent an unbiased analysis of changes in cell populations, the results presented here and conclusions are based on absolute numbers rather than cell frequencies. This was done because the infection-induced changes in tissues such as the spleen can lead to changes in cell frequency but not necessarily the population of a specific cell-type. Hence, in the present study, we investigated the absolute γδ T cell counts in the tissues.

2.3. Evaluation of splenic γδ T cell subsets in GaHV-2-infected chickens

From previous studies, it has been determined that there are three distinct γδ T cells subsets in chickens including $CD8^+$, $CD4^+$, or $CD4^-CD8^-$ double negative γδ T cells (Bucy et al., 1991). Subsequent to analyzing the total γδ T cell population in the spleens of GaHV-2-