Vaccine xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

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

# Vaccination against histomonosis limits pronounced changes of B cells and T-cell subsets in turkeys and chickens

Taniya Mitra [a], Wilhelm Gerner [b], Fana Alem Kidane [a], Patricia Wernsdorf [a], Michael Hess [a,c], Armin Saalmüller [b], Dieter Liebhart [a,*]

[a] Clinic for Poultry and Fish Medicine, Department for Farm Animals and Veterinary Public Health, University of Veterinary Medicine Vienna, Austria
[b] Institute of Immunology, Department of Pathobiology, University of Veterinary Medicine Vienna, Austria
[c] Christian Doppler Laboratory for Innovative Poultry Vaccines (IPOV), University of Veterinary Medicine Vienna, Austria

ARTICLE INFO

*Article history:*
Received 10 May 2017
Received in revised form 30 May 2017
Accepted 12 June 2017
Available online xxxx

*Keywords:*
*Histomonas meleagridis*
Histomonosis
Vaccination
Chicken
Turkey
Immune response

ABSTRACT

The protozoan parasite *Histomonas meleagridis* is the causative agent of histomonosis in gallinaceous birds. In turkeys, the disease can result in high mortality due to severe inflammation and necrosis in caecum and liver, whereas in chickens the disease is less severe. Recently, experimental vaccination was shown to protect chickens and turkeys against histomonosis but dynamics in the cellular immune response are not yet demonstrated. In the present work, different groups of birds of both species were vaccinated with attenuated, and/or infected with virulent histomonads. Flow cytometry was applied at different days post inoculation to analyse the absolute number of T-cell subsets and B cells in caecum, liver, spleen and blood, in order to monitor changes in these major lymphocyte subsets. In addition, in chicken samples total white blood cells were investigated.

Infected turkeys showed a significant decrease of T cells in the caecum within one week post infection compared to control birds, whereas vaccination showed delayed changes. The challenge of vaccinated turkeys led to a significant increase of all investigated lymphocytes in the blood already at 4 DPI, indicating an effective and fast recall response of the primed immune system.

In the caecum of chickens, changes of B cells, $CD4^+$ and $CD8\alpha^+$ T cells were much less pronounced than in turkeys, however, mostly caused by virulent histomonads. Analyses of whole blood in non-vaccinated but infected chickens revealed increasing numbers of monocytes/macrophages on all sampling days, whereas a decrease of heterophils was observed directly after challenge, suggesting recruitment of this cell population to the local site of infection.

Our results showed that virulent histomonads caused more severe changes in the distribution of lymphocyte subsets in turkeys compared to chickens. Moreover, vaccination with attenuated histomonads resulted in less pronounced alterations in both species, even after challenge.
© 2017 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

## 1. Introduction

The flagellated protozoan parasite *Histomonas meleagridis* is the aetiological agent of histomonosis (synonyms: enterohepatitis or blackhead disease) of poultry [1]. The pathogenesis can vary between species of gallinaceous birds: in turkeys (*Meleagris gallopavo*) the disease can cause high mortality whereas in chickens (*Gallus gallus*) histomonosis is generally less fatal. The pathogen primarily targets the caecum before it reaches the liver through the hepatic portal vein. The lesions are characterized by severe fibrinous inflammation of the caecum and multifocal areas of inflammation and necrosis in the liver [2]. Effective prophylactic and therapeutic options are not available for food producing birds in most industrial countries due to consumer safety regulations resulting in re-emergence of the disease and economic losses in the poultry industry [3,4].

Previous investigations on vaccination to prevent histomonosis showed that the transfer of antibodies or the use of inactivated

*Abbreviations:* DPI, day post inoculation; FCM, flow cytometry; VT, vaccinated turkeys; VC, vaccinated chickens; IT, infected turkeys; IC, infected chickens; VIT, vaccinated and infected turkeys; VIC, vaccinated and infected chickens; CT, control turkeys; CC, control chickens; LS, lesion score; PBS, phosphate buffered saline; FCS, fetal calf serum; PBMCs, peripheral blood mononuclear cells; IELs, intraepithelial lymphocytes; aa, amino acid; mAb, monoclonal antibody.

* Corresponding author.
*E-mail address:* dieter.liebhart@vetmeduni.ac.at (D. Liebhart).

http://dx.doi.org/10.1016/j.vaccine.2017.06.035
0264-410X/© 2017 The Authors. Published by Elsevier Ltd.
This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

Please cite this article in press as: Mitra T et al. Vaccination against histomonosis limits pronounced changes of B cells and T-cell subsets in turkeys and chickens. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.06.035

*H. meleagridis* was not effective to protect birds from the disease [5–7]. In contrast, the application of attenuated histomonads to prevent histomonosis was earlier demonstrated [2] and recently performed experimental studies showed that clonal *in vitro* attenuated *H. meleagridis* are effective and safe in protecting turkeys and chickens [7–10]. However, data on the immune response against histomonads are limited. Varying cytokine expression profiles in caecum and liver between chickens and turkeys indicated an innate immune response of chickens against histomonosis [11]. In the same work, the occurrence of different populations of lymphocytes in liver and spleen by immunohistochemistry was demonstrated. Moreover, co-infection of *Heterakis gallinarum* and *H. meleagridis* of chickens showed the involvement of T cells in the caecum with induction of Th1 and Th2 type cytokines [12]. The activation of the local humoral immune response was demonstrated by detecting specific antibodies in different parts of the intestine of chickens infected with histomonads [13]. Anyhow, there are no data available about detailed changes in lymphocyte distribution following *H. meleagridis* infection or vaccination.

Therefore, the aim of the present work was to investigate changes in the kinetics of lymphocytes during inoculation of turkeys and chickens with attenuated and virulent *H. meleagridis*. $CD4^+$ and $CD8\alpha^+$ T cells together with B cells in different organs and blood were investigated by flow cytometry (FCM) for the first time to obtain insights into the local and systemic cellular immune response after vaccination and/or infection. In addition, data on the cell dynamics of B cells, total T cells, macrophages/monocytes together with heterophils in whole blood of chickens were generated by FCM analysis following inoculation with *H. meleagridis* in chickens.

## 2. Materials and methods

### 2.1. Birds

A total of sixty turkeys (B.U.T. 6™; Aviagen Turkeys Ltd, Tattenhall, UK) and the same number of specific pathogen free (SPF) layer type chickens (VALO, BioMedia, GmBH, Osterholz-Scharmbeck, Germany) were included in the present study. At the first day of life every bird was marked with subcutaneously fixed tags for identification.

### 2.2. Preparations of parasites for inoculation

The clonal culture *H. meleagridis*/Turkey/Austria/2922-C6/04 [14] was co-cultivated with intestinal flora of the host bird before used for vaccination and infection of the birds: attenuated histomonads, established by long-term cultivation for 295 passages, were used for vaccination whereas for infection the virulent cultured histomonads (21 passages) were administered as previously described [7]. Both cultures were stored at −150 °C prior to inoculation. $6 \times 10^5$ cells of *H. meleagridis* in 600 μl culture medium consisting of Medium 199 with Earle's salts, L-glutamine, 25 mM HEPES and L-amino acids (Gibco™ Invitrogen, Lofer, Austria), 15% foetal calf serum (FCS) (Gibco™ Invitrogen) and 0.66 mg rice starch (Sigma-Aldrich, Vienna, Austria) were administered per bird, split between the oral and cloacal route using a syringe together with a crop tube, respectively a pipette. Birds of the control groups were sham infected with the equal volume of pure culture medium.

### 2.3. Setup of the in vivo trial

Water and feed (unmedicated turkey, respectively chicken starter feed) were provided *ad libitum*, except for 5 h of feed restriction after inoculation. The different groups consisted of 15 birds of each species and were kept separated in four rooms depending on the inoculation scheme: vaccinated turkeys (VT), vaccinated chickens (VC), infected turkeys (IT), infected chickens (IC), vaccinated and infected turkeys (VIT), vaccinated and infected chickens (VIC), control turkeys (CT) and control chickens (CC) (Table 1). Vaccination of groups VIT and VIC was applied on the first day of life. The challenge infection of the same birds was performed 28 days later together with the inoculation of the IT and IC groups. On the same day (28th day of life) birds from the only vaccinated group were inoculated with the attenuated strain and control birds were inoculated with culture medium only. From this day onwards, 3 previously determined birds (ascending order of tag numbers) per group were sacrificed 4, 7, 10, 14 and 21 days post inoculation (DPI).

### 2.4. Clinical examination, post-mortem and sampling

Behaviour, plumage, faeces, feed and water intake together with body weight were examined throughout the experiment for any clinical signs indicative for histomonosis. Re-isolation of viable parasites from cloacal swabs of every bird was performed in intervals of 2–3 days following vaccination and/or infection to confirm the successful inoculation. For that, after sampling each swab was placed into a 2 ml Eppendorf tube containing culture medium as described above and incubated at 40 °C. Following a propagation period of 2–3 days the re-isolations were microscopically examined. Blood samples of every bird were collected directly before the birds were killed. Birds that were killed or had to be euthanized were anaesthetized by intravenous application of thiopental (Sandoz, Kundl, Austria) before bleeding to death. Dead birds were necropsied and pathological changes in caecum and liver were evaluated using a previously established lesion score (LS) system: LS 0 was applied for normal organs whereas LS 1–4 classified mild to severe changes [13]. Caecum, liver and spleen were weighed and collected in cold phosphate buffered saline (PBS) (Gibco™ Invitrogen) containing 2% FCS (Gibco™ Invitrogen) (PBS + FCS) as soon as possible after death and further processed as described below.

### 2.5. Cell isolation

Single cell suspensions were prepared according to standard procedures from caecum, liver, spleen and blood PBMCs together with total white blood cells. Brief descriptions are given below for each type of sample.

#### 2.5.1. Caecum

Intraepithelial lymphocytes (IELs) were isolated from both caeca of each bird as described previously [15] with some modification. In detail, faeces were removed and the organ samples were rinsed with cold PBS + FCS. After cleaning, the caeca were cut longitudinally in pieces of approximately 1 cm before isolation was performed in a solution of 50 ml PBS (Gibco™ Invitrogen) containing 500 μl of 1 M DTT (Sigma-Aldrich) and 10 μl of 0.5 M EDTA at 37 °C for 30 min during continuous stirring. The sediment consisting of sloughed tissue was left behind and the supernatant was transferred into two 50 ml tubes in equal amounts. The tubes were filled up with cold PBS + FCS and centrifuged at 4 °C, 220*g* for 10 min. The supernatant was collected passed through 40 μm nylon cell strainer (BD Falcon©), whereas the pellet was resuspended in 50 ml cold PBS + FCS to obtain more single cells by further centrifugation steps at 4 °C, 350*g* for 10 min. By that, single cells of the supernatants from the centrifugation steps were obtained before passed through the cell strainer. Following centrifugation the pellet was resuspended in 10 ml PBS + FCS.

The prepared suspension was then slowly layered above a double volume of Histopaque®-1077 (Sigma-Aldrich, Vienna, Austria) for density gradient centrifugation. The cells from interphase layer

Please cite this article in press as: Mitra T et al. Vaccination against histomonosis limits pronounced changes of B cells and T-cell subsets in turkeys and chickens. Vaccine (2017), http://dx.doi.org/10.1016/j.vaccine.2017.06.035

Download English Version:

https://daneshyari.com/en/article/5536440

Download Persian Version:

https://daneshyari.com/article/5536440

Daneshyari.com