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J Vet Res 64, 239-245, 2020
DOI: 10.2478/jvetres-2020-0040

IFNγ production profile in turkeys of different immunological status after TRT vaccination

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Received: March 3, 2020 *Accepted: May 18, 2020*

Abstract

Introduction: Despite vaccination against avian metapneumoviruses (aMPV), cases of turkey rhinotracheitis (TRT) caused by aMPV field strains are frequently reported. Differences have been shown in the level of immune system stimulation after aMPV vaccination between turkeys that do and do not possess specific anti-aMPV maternally derived antibodies (MDA). The article describes the influence of MDA on the production of IFNγ in the spleen of aMPV-vaccinated turkeys. **Material and Methods:** MDA+ or MDA− turkeys were vaccinated against TRT after hatching or on the 14th day of life. Spleen samples were collected 3, 7, and 14 days post vaccination for mononuclear cell isolation. Real-time PCR, flow cytometry, and the enzyme-linked immunospot assay were used to evaluate the levels of IFNγ gene expression, production, and secretion by cells within the spleen samples. **Results:** Increased IFNγ gene expression was noticed after vaccination only in birds that did not possess MDA or possessed MDA at relatively low level (MDA+ birds vaccinated at 14 DOL). In all birds, an increased percentage of T lymphocytes producing IFNγ was recorded. The proportion of anti-aMPV IFNγ-secreting cells was increased only in MDA− birds. **Conclusion:** Besides having a protective role, MDA are known to interfere with vaccination efficacy. The analysis of our results confirms that MDA can decrease the level of immune system stimulation after aMPV vaccination of turkeys.

Keywords: turkeys, avian metapneumovirus, maternally derived antibodies, vaccination.

Introduction

Avian metapneumovirus (aMPV) is a highly infectious RNA virus that induces turkey rhinotracheitis (TRT) in turkey flocks. This virus is a representative of the *Pneumoviridae* family and the *Metapneumovirus* genus and is currently divided into four subtypes (A–D) (4, 6, 12). Avian metapneumovirus infections cause significant losses in the poultry industry due to poorer body weight gains, directly attributable deaths, a decrease in laying performance, and immunosuppression which increases birds' sensitivity to secondary infections (15). The range of aMPV covers the whole globe except for Australia and Canada.

Vaccination against TRT has been found effective when live attenuated and inactivated vaccines are used. Unfortunately, despite the commonness of vaccination, cases of TRT are frequently reported in poultry given the prophylactic because field strains can sometimes defeat post vaccination immunity (15). A large part of the turkey population in Poland comprises poults imported from Canada, which engenders a lack of specific anti-aMPV maternally derived antibodies (MDA) in that part of the domestic poult flock.

As demonstrated earlier, there is no explicit correlation between the level of anti-aMPV specific IgY in blood serum and the upper respiratory tract (URT) and the degree of immunity against TRT, even though these antibodies slightly alleviate the clinical course of the disease (2, 3, 5, 9). This precipitates vaccination of chicks against TRT on the first day of life irrespective of the level of maternal antibodies.

Few studies have been performed so far to determine the effect of MDA on the efficacy of vaccination against TRT. Śmiałek *et al.* (13, 14) demonstrated that MDA-possessing (MDA+) turkeys did not produce specific IgY or IgA after the

vaccination against TRT. Additionally, the authors found abnormalities in the specificity of IgA^+ B lymphocyte response in MDA+ turkeys after vaccination using aMPV subtype A (aMPV/A). They also demonstrated limited replication of vaccine aMPV in the URT of the MDA+ turkeys.

Due to the not fully understood role of humoral immunity, the mechanisms of cell-mediated immunity are increasingly often considered a decisive factor in the protection against TRT. Liman and Rautenschlein (11) demonstrated a significant increase in the $CD4^+$ subpopulation of splenic T lymphocytes as a proportion, and the increase was accompanied by the upregulation of IFNγ gene expression and synthesis in splenocytes after vaccination using aMPV/B of birds past 30 days of life and without anti-aMPV antibodies. In contrast, Cha (2) reported an increased percentage of $CD8^+$ rather than $CD4^+$ cells in URT structures, and no increase in their percentage in the spleen after aMPV/C infection in 14-day-old MDA-lacking (MDA−) birds. In the same study, this author demonstrated greater expression of the IFNγ gene in the URT of the infected birds. These results indicate that cell-mediated immunity, including IFNγ production, is involved in the protection against aMPV and that its mechanisms can be affected by the age of birds and the subtype of aMPV. In addition, Śmiałek *et al.* (13) demonstrated that the stimulation of the local cellular immunity in the URT against TRT may be dependent on MDA level. They also showed differences in the extent of URT structure infiltration by immunocompetent cells ($CD4^+$ and $CD8^+$ subpopulations of T lymphocytes), that were more significant in the MDA− than in MDA+ groups.

Therefore, it seems that by inhibiting the replication of vaccine aMPV the specific MDA impair its immunogenicity, which decrease the level of immune system stimulation after vaccination. For this reason, the scientific goal of the project was to determine the influence of specific anti-aMPV MDA on the stimulation of splenic T lymphocytes and splenocytes for IFNγ production after vaccination of turkeys against TRT.

Material and Methods

Turkeys and vaccination. A total of 180 commercial Hybrid Converter turkeys were used in the experiments. Ninety MDA+ turkeys (50%) (provided by the Grelavi S.A. hatchery, Kętrzyn, Poland) originated from breeder turkeys vaccinated against TRT (three times with live aMPV/A and twice with aMPV/B inactivated vaccines). The other half (90 MDA− turkeys provided by the same hatchery) originated from a Canadian breeder flock. The turkeys for all experiments were provided by the hatchery at the same time.

Turkeys were housed in isolated units maintained at a physical containment level 3 facility. Turkeys of vaccinated groups were vaccinated occulonasally, individually with 10^4 of 50% tissue infectious dose 50% of aMPV/A strain BUT1 #8544 commercial attenuated vaccine (Zoetis, USA). Non-vaccinated birds received vaccine diluent. Water and feed were given to birds *ad libitum*. IgY MDA status was confirmed with ELISA (IDEXX, Westbrook, MN, USA).

Experimental design. Experiments I and II. Experiments were carried out on 45 MDA+ (experiment I) or MDA− (experiment II) day-old turkeys. After their arrival, blood samples (n = 15) were taken from MDA+ and MDA− turkeys for MDA status evaluation. Afterwards, the birds were randomly divided (n = 15) into MDA+ and MDA− vaccinated (0/V) and not vaccinated (0/NV) groups. Turkeys of the former groups were vaccinated against TRT on the day of arrival (0 day of life – DOL). The birds were raised to 14 days of life, and spleen samples (n = 5 per group) were taken at 3, 7, and 14 days post vaccination (DPV) for further analysis.

Experiments III and IV. The experimental design was identical, but older birds were used in these experiments. Experiments were carried out on 45 MDA+ (experiment III) or MDA− (experiment IV) 14-day-old turkeys. Before birds were divided into MDA+ and MDA− vaccinated (14/V) and non-vaccinated (14/NV) groups, blood samples (n = 15) were taken for MDA status evaluation. Birds of vaccinated groups were vaccinated against TRT on 14 DOL. Turkeys were raised to 28 DOL, and spleen samples (n = 5 per group) were taken at 3 (17 DOL), 7 (21 DOL), and 14 DPV (28 DOL) for further analysis.

Serological evaluation. ELISA evaluation was performed with the use of a commercial APV ELISA kit (IDEXX) according to the manufacturer's recommendations. Sample-to-positive (S/P) ratios were used to express the mean MDA level on the day of vaccination.

Isolation and determination of mononuclear cell counts. Isolation of splenic mononuclear cells were performed as described previously (13). Briefly, spleen samples were homogenised in a manual tissue grinder. Cell pellets obtained after centrifugation at 450 g for 10 min at 20°C were resuspended in 40% Percoll density gradient and layered on 60% Percoll. After centrifugation at 1,900 g for 20 min at 20ºC with the breakes off, mononuclear cells were collected from the interphase. The cells were washed twice, and finally, they were resuspended in 1 mL of PBS. Lymphocyte counts were calculated with a Vi-cell XR cell counter (Beckman Coulter Life Sciences, Indianapolis, MN, USA).

Molecular biology. Isolation of the RNA from the splenocytes was carried out with the use of NucleoSpin RNA kit (Macherey-Nagel, Düren, Germany) according to the manufacturer's recommendations. A total of 5×10^6 of mononuclear cells were used for the RNA isolation protocol. The concentration and quality of isolated RNA were evaluated with a NanoDrop 2000 spectrophotometer

Table 1. Primers used for real time PCR

Primer	Sequence 5”–3”	Fragment size (bp)	GenBank accession no.
INFγ F	CTGACAAGTCAAAGCCGCAC	137	XM_003202048.3
INFγ R	AGTCATTCATCTGAAGCTTGGC		
GAPDH F	CCCTGAGCTCAATGGGAAGC	125	NM_001303179.1
GAPDH R	TCAGCAGCAGCCTTCACTAC		

(Thermo Fisher Scientific, Waltham, MA, USA) and a Bioanalyzer 2100 system (Agilent, Santa Clara, CA, USA), respectively.

Reverse transcription was performed with the use of a High-Capacity cDNA Reverse Transcription Kit (Life Technologies, Waltham, MA, USA) following the manufacturer’s protocol. A 0.5 µg mass of standardised RNA per sample was used for cDNA synthesis. In order to determine the IFNγ gene expression, the real-time PCR technique was implemented. The reaction mixture for qPCR was composed of: 10 µL of Power SYBR Green PCR Master Mix (Life Technologies), 1.8 µL of each 10 µM primer, 2 µL of cDNA, and 4.4 µL of ribonuclease-free water. The primers sequences are given in Table 1. The quantitative PCR was conducted under the following conditions: activation of the polymerase was at 95°C for 10 min, 40 two-stage cycles of: denaturation at 95°C for 30 s were run, and primer annealing and chain elongation were at 60°C for 60 s. The relative expression of the IFNγ gene was calculated using the $2^{-\Delta\Delta Ct}$ method normalised to efficiency corrections, the expression levels of the reference gene coding glyceraldehyde 3-phosphate dehydrogenase (GAPDH), and adequate control groups. The analysis was carried out with GenEx 6.1.0.757 data analysis software (MultiD Analyses, Gothenburg, Sweden).

Flow cytometry. Flow cytometry analysis was performed as described previously (16) with minor modifications. Briefly, lymphocytes from individual spleen samples in a 2×10^6 quantity were transferred in triplicate to 24-well plates (Corning, Tewksbury, MA, USA) containing 2 mL of complete culture medium (RPMI-1640, 20 mM 4-(2-hydroxyethyl)-1-piperazineethanesulphonic acid (HEPES), 10% FBS, Antibiotic Antimycotic Solution (Sigma Aldrich, Hamburg, Germany), 4 µL of Leukocyte Activation Cocktail with BD GolgiPlug (BD Pharmingen, Franklin Lakes, NJ, USA), and 0.3 µg/mL of Mouse Anti-Chicken CD28 monoclonal antibody (MCA5760; clone AV7, Bio-Rad, Watford, UK) as a co-stimulatory. After incubation at 40°C in 5% CO_2 for 6 h, 20 µL of 20 mM EDTA solution (Sigma-Aldrich) in PBS was added. Cells were washed twice in PBS with 1% FBS (Sigma-Aldrich). Mouse Anti-Chicken CD4-FITC monoclonal antibody (MCA2164F; Bio-Rad) or Mouse Anti-Chicken CD8 α-FITC monoclonal antibody (MCA2166F; Bio-Rad) was used to stain the cells. After 30 min of incubation, the cells were washed in PBS, and fixed with Leucoperm Reagent A (Bio-Rad). Afterwards, cell samples were suspended in 100 µL of permeabilisation medium (Leucoperm Reagent B; Bio-Rad), and 5 µL of Rabbit Anti-Chicken IFNγ antibody was added (AHP945Z; Bio-Rad). After another incubation, the cells were once again washed with PBS and Sheep Anti-Rabbit IgG:PE (STAR35A; Bio-Rad) secondary antibody was added. After final incubation, the cells were washed. Samples were resuspended in PBS and analysed with a FACSCanto II flow cytometer (BD, San Jose, CA, USA). A fluorescence minus one control (a samples without the primary anti-chicken IFNγ antibody) was prepared for each analysed sample.

ELISpot assay. An enzyme-linked immunospot (ELISpot) assay was performed as described previously (14, 18) with modifications. After membrane activation in 70% ethanol at 50 µL/well for 45 s, MultiScreen ELISPOT plates (Millipore, Burlington, MA, USA) were coated with Rabbit Anti-Chicken IFNγ antibody (as above; Bio-Rad) and incubated at 4°C for 24 h. Splenic lymphocytes in a 1.5×10^4 quantity were added in triplicate directly to the wells of previously prepared ELISpot plates with BD FACSAria II (BD) and prior to incubation at 39.5°C in 5% CO_2 for 24 h, 100 µL of Iscove’s modified Dulbecco’s medium (Sigma Aldrich) with previously titrated vaccine aMPV/A suspension was added to each well. After incubation, the plates were washed four times with PBS-Tween 20 and once with PBS and incubated overnight at 4°C with Anti-Chicken IFNγ Biotinylated Polyclonal Antibody (PBB0448C-050; Kingfisher Biotech, St Paul. MN, USA). The plates were then washed three times with PBS, and after loosening the bottoms of the plates, the membranes were washed with PBS on the reverse. Afterwards, Streptavidin Alkaline Phosphatase (SA-5100; Vector Laboratories, Burlingame, CA, USA) was added to the wells of the plate. After 1 h incubation at room temperature and execution of a washing protocol, the enzymatic reaction was performed with a BCIP/NBT Substrate, Alkaline Phosphatase Kit (SK-5400; Vector Laboratories) for 15–25 min. The enzyme reaction was stopped with water. Counting of IFNγ spot-forming units (SFU) was performed with the use of an Eli.Scan plate scanner and Eli.Analyse software (A.EL.VIS, Hannover, Germany).

Data were expressed as x-fold change of mean IFNγ SFU in vaccinated groups relative to non-vaccinated groups in the same experiment at each specified DPV sampling occasion.

Statistical analysis. The results were processed by Student's *t*-test for independent samples in GraphPad Prism 6 software (GraphPad, San Diego, CA, USA). Differences were considered statistically significant at $P \leq 0.05$.

Results

The results of the serological evaluation of anti-aMPV maternally derived antibody levels are summarised in Table 2.

Experiment I. The results of IFNγ gene expression in splenocytes isolated from the birds of experiment I are summarised in Fig. 1A. In experiment I, the expression level of IFNγ in the vaccinated group was below the level of expression recorded for the control group. No statistical differences in the level of IFNγ gene expression were recorded.

As set out in Tables 3 and 4, a statistically significant increase in the percentage of $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma$ T cells was recorded in the MDA+0/V group in comparison to the control group at 7 ($CD4^+$) and 14 ($CD4^+$ and $CD8^+$) DPV.

The results of ELISPOT IFNγ spot-forming unit determination for experiment I are shown in Fig. 2A. No statistical differences in the mean IFNγ spot-forming unit number were recorded between the MDA+0/V and MDA+0/NV groups.

Table 2. Serum maternally derived anti-aMPV IgY antibody levels on the days of aMPV/A vaccination of turkeys in experiments I–IV

Bird MDA status	Mean MDA S/P ratio ± SD at the time of vaccination	
	0 DOL	14 DOL
MDA+	6.63 ± 2.53 (experiment I)	2.30 ± 1.22 (experiment III)
MDA−	0.00 ± 0.00 (experiment II)	0.00 ± 0.00 (experiment IV)

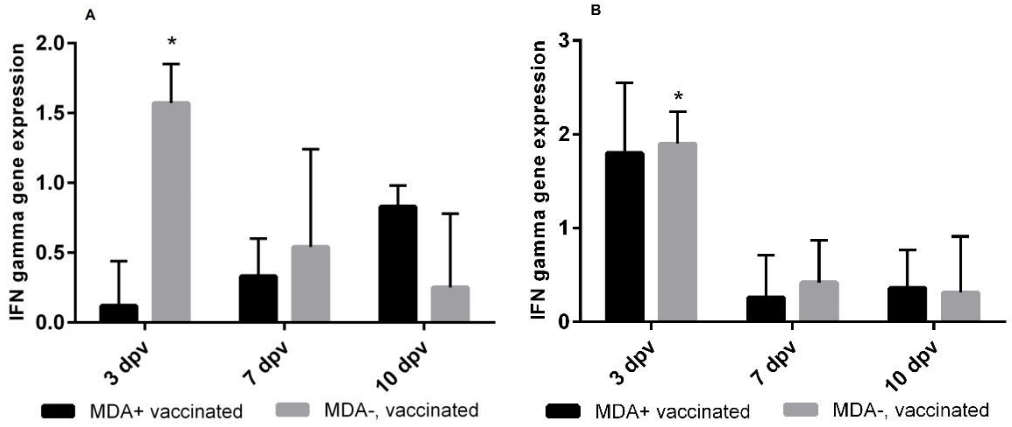

Fig. 1. Summary of mean relative IFNγ gene expression in splenic mononuclear cells on different days post aMPV/A vaccination in experiments I (MDA+ vaccinated) and II (MDA− vaccinated) (A) and III (MDA+ vaccinated) and IV (MDA− vaccinated) (B). The relative expression of the IFNγ gene was calculated using the $2^{-\Delta\Delta Ct}$ method normalised to efficiency corrections, expression levels of the reference gene coding GAPDH and adequate control groups. Bars represent mean IFN gamma expression level against its expression in the adequate control group
* Significant differences at different DPV (*t*-test, $P < 0.05$)

Table 3. Mean percentage of $CD4^+$ $IFN\gamma^+$ cells ± SD within splenic mononuclear cells of turkeys of vaccinated (V) and not vaccinated (NV) groups of experiments I–IV at different DPV

Experiment	Group	Mean percentage of $CD4^+IFN\gamma^+$ cells ± SD		
		3 DPV	7 DPV	10 DPV
I	MDA+0/V	1.11 ± 0.33	2.66 ± 0.97*	3.34 ± 1.55*
	MDA+0/NV	1.26 ± 0.67	0.91 ± 0.32	0.97 ± 0.18
II	MDA−0/V	2.27 ± 0.72*	1.65 ± 1.08	1.01 ± 0.39
	MDA−0/NV	0.94 ± 0.32	0.39 ± 0.11	0.79 ± 0.40
III	MDA+14/V	5.21 ± 2.56*	4.69 ± 3.69	4.21 ± 1.89*
	MDA+14/NV	1.50 ± 0.01	1.32 ± 0.59	1.16 ± 0.91
IV	MDA−14/V	1.68 ± 0.20*	2.71 ± 1.90	1.60 ± 0.52
	MDA−14/NV	0.46 ± 0.28	1.28 ± 0.30	0.50 ± 0.33

* Significant differences at different DPV in the experimental groups in comparison to the adequate control groups (*t*-test, $P < 0.05$)

Table 4. Mean percentage of $CD8^+$ $IFN\gamma^+$ cells ± SD within splenic mononuclear cells of turkeys of vaccinated (V) and not vaccinated (NV) groups of experiments I–IV at different DPV

Experiment	Group	Mean percentage of $CD8^+IFN\gamma^+$ cells ± SD		
		3 DPV	7 DPV	10 DPV
I	MDA+0/V	1.14 ± 0.56	1.4 ± 0.36	5.45 ± 2.21*
	MDA+0/NV	0.64 ± 0.11	1.48 ± 0.8	1.21 ± 0.46
II	MDA−0/V	2.82 ± 1.67*	1.37 ± 0.83	1.17 ± 0.15
	MDA−0/NV	0.5 ± 0.35	0.39 ± 0.02	1.08 ± 0.18
III	MDA+14/V	4.26 ± 1.96	3.57 ± 0.42	3.69 ± 1.46*
	MDA+14/NV	1.55 ± 0.73	2.81 ± 0.83	1.39 ± 0.42
IV	MDA−14/V	1.65 ± 0.11	2.79 ± 1.23	1.55 ± 0.89
	MDA−14/NV	0.91 ± 0.16	2.01 ± 0.63	1.32 ± 0.78

* Significant differences at different DPV in the experimental groups in comparison to the adequate control groups (*t*-test, $P < 0.05$)

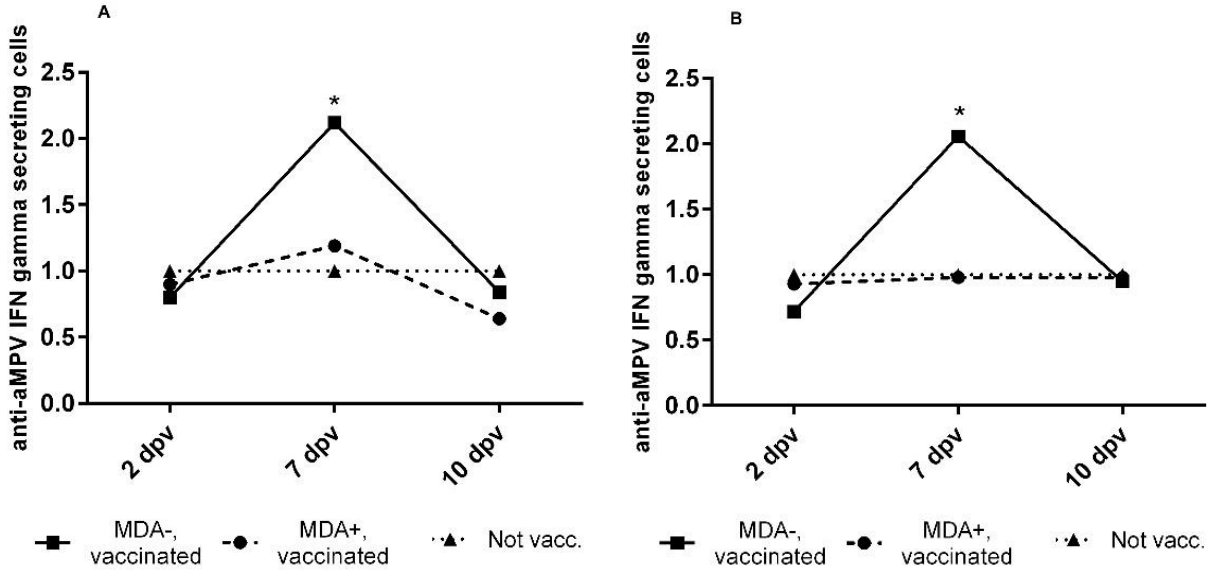

Fig. 2. Summary of mean contribution of anti-aMPV IFNγ-secreting cells within splenic mononuclear cells at different days post aMPV/A vaccination in experiments I (MDA+ vaccinated) and II (MDA− vaccinated) (A) and III (MDA+ vaccinated) and IV (MDA− vaccinated) (B) in relation to the mean contribution of anti-aMPV IFNγ-secreting cells in the spleen of adequate control birds (not vaccinated)
* Significant differences in vaccinated groups of birds in comparison to adequate control groups, at different DPV (*t*-test, $P < 0.05$)

Experiment II. Fig. 1A shows that in experiment II, the expression level of IFNγ in the vaccinated group significantly increased at 3 DPV in comparison to the control group.

As shown in Tables 3 and 4, a statistically significant increase in the percentage of both $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma$ T cells was recorded in the MDA+0/V group in comparison to the control group at 3 DPV.

The results of ELISPOT IFNγ spot forming unit determination for experiment II are presented in Fig. 2A. A statistically significant increase in the mean IFNγ spot-forming unit number was recorded in MDA−0/V group at 7 DPV, in comparison to the MDA−0/NV group.

Experiment III. The results of IFNγ gene expression for experiment III are presented in Fig. 1B. The expression level of IFNγ in the vaccinated group was increased at 3 DPV in comparison to the control group. This difference was not statistically significant.

As Tables 3 and 4 show, a statistically significant increase in the percentage of $CD4^+IFN\gamma^+$ and $CD8^+IFN\gamma$ T cells was recorded in the MDA+14/V group at 3 ($CD4^+$) and 14 ($CD4^+$ and $CD8^+$) DPV, in comparison to the control group.

No statistical differences in the mean IFNγ spot-forming unit number was recorded between the MDA+14/V and MDA+14/NV groups (Fig. 2B).

Experiment IV. In experiment IV, the expression level of IFNγ in the vaccinated group was significantly increased at 3 DPV in comparison to the control group (Fig. 1B).

It can be seen in Table 3 that a statistically significant increase in the percentage of $CD4^+IFN\gamma^+$ T cells was recorded in the MDA−14/V group at 3 DPV in comparison to the control group.

As demonstrated in Fig. 2B, a statistically significant increase in the mean IFNγ spot-forming unit number was recorded in MDA−14/V group at 7 DPV, in comparison to the MDA−14/NV group.

Discussion

In the first weeks of a chick's life, MDA play a significant protective role against common viral and bacterial pathogens. They can prevent or alleviate consequences of infections with these microorganisms at this time. Breeder flocks are vaccinated several times (with live and/or inactivated vaccines) in order to induce high titres of pathogen-specific antibodies which are then transferred to the progeny (10). In turn, the presence of these antibodies in the first weeks of life can influence the efficacy of protective vaccinations performed in this period.

The results of investigations conducted so far support the conclusion that cellular immunity is involved in the protection against aMPV in turkeys, regardless of metapneumovirus subtype. Unfortunately, only few experiments have addressed this subject until now. Liman and Rautenschlein (11) demonstrated a significant increase in the $CD4^+$ subpopulation of splenic T lymphocytes as a proportion a concurrent upregulation of IFNγ gene expression in splenocytes after the vaccination using aMPV/B. In contrast, Cha (2) reported an increased percentage of $CD8^+$ cells in URT and no increase in their percentage in the spleen after aMPV/C infection. Both these experiments were performed on birds which at the moment of vaccination or infection were free of specific antibodies against aMPV.

As demonstrated in our previous studies, the presence of MDA has a very strong impact on the stimulation of both humoral and cellular immunity after bird vaccination against TRT with the aMPV/A subtype (13, 14). In the present experiment, we evaluated the effect of MDA on the stimulation of T lymphocytes and splenocytes for IFNγ gene production after vaccination against TRT.

In experiments II, III, and IV, stronger IFNγ gene expression was demonstrated at 3 DPV in the vaccinated birds, while such an increase was not observed in the birds from experiment I, at any stage of the analyses. Considering the splenic lymphocytes of the vaccinated birds from all experiments, the analyses demonstrated a significant rise in the percentage of both subpopulations of T lymphocytes ($CD4^+$ and $CD8^+$) capable of producing IFNγ in every group except the MDA−14/V turkeys, in which the increase was observed only for $CD4^+$ cells. Differences between the groups of vaccinated birds in particular experiments concerned the time needed for T lymphocyte stimulation because in the MDA+0/V group, the expansion of the subpopulations tested occurred the latest (from 7 DPV) and mainly applied to the percentage of $CD4^+$ lymphocytes, whereas in both MDA− groups (0/V and 14/V) and in the MDA+14/V group this growth was recorded as soon as at 3 DPV. In addition, in the MDA+14/V group, an increased level of both subpopulations of $IFN\gamma^+$ T lymphocytes was demonstrated over the entire experimental period (a statistically significant increase was observed in this group at 3 and 14 DPV). The results of cytometric analyses are somehow consistent with the results of the molecular analysis, as the intensification of IFNγ gene expression coincided with the moment of detecting

an increased level of the $IFN\gamma^+$ T cells. Enhancement of this gene expression was not observed only in experiment I in the MDA+0/V birds, which corresponds with a delayed growth of the $IFN\gamma^+$ T cell population.

In both groups of MDA- birds (experiments II and IV) vaccinated against TRT, we noticed an increment in the percentage of cells specifically producing IFNγ as a result of the repeated contact with vaccine aMPV. In both groups, an increase in the percentage of these cells occurred at 7 DPV, which is consistent with the results of the molecular and cytometric analyses, these demonstrating significant upregulation of gene expression and IFNγ-producing cells at 3 DPV. Similar molecular and cytometric analysis results were obtained for birds from the vaccinated group in experiment III. However, in their case and also in that of vaccinated birds from experiment I, there was no rise in the number of cells specifically producing IFNγ among the splenic cells. We observed similar phenomena in our previous study with B cells isolated from the upper respiratory tract of birds after vaccination against TRT. In the case of vaccinated MDA+ birds, the population of IgA^+ B cells expanded, while no change was noted in the population of cells capable of producing specific IgA in confrontation with vaccine aMPV nor in the level of specific IgA in the URT (13, 14).

Besides having a protective role for birds in the first weeks of their life, MDA are known to interfere with vaccination efficacy. This is a common phenomenon and adheres to both the inactivated and live vaccines, also including vector vaccines. When birds are highly MDA+ with homologous antibodies to the antigen the birds are vaccinated against, a decreased level of maternal antibodies results from the vaccination because they are used to neutralise the antigen, as also do a lower level of humoral immunity and consequently, a more severe clinical course of the disease after experimental infection than in MDA− birds at the moment of vaccination (1, 7, 8, 17).

The analysis of results concerning the immunology of turkeys vaccinated against TRT confirms that differences undoubtedly exist in the extent of immune system stimulation that depend on the MDA level in turkey poults on the day of vaccination. The MDA can influence the efficacy of vaccination against TRT, which may result in more severe clinical symptoms after experimental infection of the vaccinated birds with aMPV (Śmiałek – unpublished data).

Conflict of Interests Statement: The authors declare that there is no conflict of interests regarding the publication of this article.

Financial Disclosure Statement: The authors declare that part of this study was funded by the KNOW (Leading National Research Centre) Scientific Consortium "Healthy Animal – Safe Food", Ministry of Science and Higher Education resolution no. 05-1/ KNOW2/2015. The other part of the study was supported with funds from the National Science Centre, project no. 2016/23/D/NZ6/00099.

Animal Rights Statement: All procedures and animal handling were performed with the approval of the Local Ethical Commission for Experiments with Animals in Olsztyn (resolution no. 71/2015).

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