PROTECTION ACTIVITY OF T CELL LYMPHOKINES AGAINST INFECTIOUS BURSAL DISEASE IN LAYER PULLETS

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ABSTRACT

The present study was aimed to administering hyperimmunized avian salmonella-immune lymphokines (S-ILK) to 200 one-day-old layer pullets to improve the immunological response against Gumboro (IBDV) divided into four groups. On the first day, the following was applied to all groups: G1: intraperitoneal injection of 0.50 mL S-ILK followed by a 30-minute challenge with 0.1 mL IBDV (ELD_{50} $10^{3.2}$); G2: intraperitoneal injection of 0.5 mL of S-NILK followed by a 30-minute challenge by 0.1 mL IBDV. G3: challenged with 0.1 mL of IBDV only, G4: uninfected, unchallenged group consider as a negative control. The results of the findings indicated the greatest a statistically significant rise ($p \leq 0.05$) in IgG and IFN-γ titres in that group and the viral load test revealed that at 7 and 14 days after infection, The largest number of IBDV RNA copies were found in G2 and G3. in the bursa of Fabricius. The first group had the lowest mortality rate compared to the other groups. Early S-ILK administration improves maternal resistance to IBDV infection and inhibits viral replication in the fabricius bursa following IBDV challenge. Thus, we may reduce the amount of time, effort, and money spent on immunization procedures that do not completely protect against diseases.

Key words: Gumboro, IFN-γ, ELISA, viral load.

الفعالية الوقائية للمفوكينات الخلايا التائية ضد مرض جراب فابريشيا المعدي في افراخ البياض

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المستخلص

هدفت الدراسة الحالية إعطاء اللمفوكينات المناعية للطيور الممنعة ضد السالمونيلا (S–ILK) إلى 200 فرخة بياضة التي يبلغ بعمر يوم واحد لتعزيز المناعة ضد الإصابة بمرض كمبورا مقسمة إلى أربع مجاميع. في اليوم الأول حقنت المجاميع على النحو الاتي G1: الحقن داخل الصفاق 0.5 مل من S–ILK متبوعًا بعد 30 دقيقة بتحدي مع كمبوا 0.1 مل (ELD_{50} $10^{3.2}$)؛ G2: الحقن داخل الصفاق بمقدار 0.5 مل من S–NILK متبوعًا بتحد بعد 30 دقيقة بتحدي مع كمبورا. G3: تم اختباره بـ 0.1 مل من كمبورا فقط. G4: تعتبر مجموعة سيطرة سالبة. أظهرت النتائج أكبر زيادة معنوية ($p \leq 0.05$) في معيار IgG and IFN–γ للمجموعة الاولى وكذلك اختبار الحمل الفيروسي أنه بعد 7 و14 يومًا من الإصابة، كان لدى المجموعة الثانية والمجموعة الثالثة أعلى مستوى من نسخ حمض الرايبي للكمبورا في جراب فابريشيا. المجموعة الأولى سجلت أقل معدل هلاكات مقارنة بالمجموعات الأخرى، نستنتج من الدراسة الحالية ان إعطاء S–ILK يحسن المناعة الاموية المبكرة لمقاومة اصابة الكمبورا وتمنع تكاثر الفيروس في جراب فابريشيا بعد التحدي بفيروس الكمبورا. وبالتالي، نقلل مقدار الوقت والجهد والمال الذي يتم إنفاقه على إجراءات التحصين التي لا تحمي من الإصابة بشكل مطلق.

الكلمات المفتاحية: كمبورا, IFN–γ, الاليزا, الحمل الفيروسي.

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INTRODUCTION

Infectious bursal disease is among the costliest ailments that plague hens raised for commercial purposes. (IBD or Gumboro disease) (26). The RNA virus with a double-stranded structure IBD-virus (IBDV), which is the cause of this immunosuppressive condition. IBDV damages the bursa of Fabricius by destroying B cells that are still growing. In the field, a variety of clinical IBD manifestations are seen, to high mortality rates of up to 90–100% in flocks, from mild immunosuppression that have not received vaccinations. additional lymphoid tissues, including the Fabricius bursa may be destroyed temporarily or permanently by IBDV infection, based on the severity of the IBDV strain, age of infection, IBDV antibody status, and genetic make-up of the chicken were evaluated (4, 7). induced immunosuppression by IBDV is a result of the B cells' and macrophages' functions being destroyed (17). The standard sanitary procedures used on chicken farms are insufficient to prevent this illness IBDV is extremely environment-resistant. As a result, immunization is a crucial technique for IBD prevention (2). It was demonstrated in both experimental settings and a field trial show the appearance of bursal lesions, IBDV multiplication, and the emergence of humoral immunity after administration of a commercially available intermediate IBDV vaccine are all directly related (9, 21). If birds were immunized at the ideal time, when MDA were below the vaccine's breakthrough level, when they were below the ideal period, all immunized flocks developed IBDV antibodies and bursal lesions up to 14 days after immunization. Using MLVs, however, in the presence of high MDA titres may reduce their effectiveness against IBDV field problems. Inflammatory reactions are mostly carried by small molecules known as cytokines, consisting of proteins and glycoproteins. A variety of resistance mechanisms interact with these physiologically active proteins (20). It is more effective to stop outbreaks of major IBDV infections when lymphokines are used to boost immunological resistance (22, 26). The objective of the present investigation was to show that (S-ILK) can dramatically lessen the harmful consequences of infected bursal illness in layer pullets.

MATERIALS AND METHODS

Lymphokines production: The Department of Poultry Diseases at the University of Baghdad's College of Veterinary Medicine submitted a specimen of *S. enteritidis* that had not yet been reported. The isolate was raised in peptone water and nutrition broth. After 24 hours, the colonies and white sediments emerged. When certain colonies were taken out and allowed to grow on medium that are selective the MacConkey and SS agar tests for salmonella, these colonies displayed black colonies. The API20 test was utilized to locate the isolate biochemically. Finally, the sample was infused with 1×10^8 colony-forming units/ml of bacteria (3). 20-layer pullets that were one day old were split into two groups. In a different breeding facility, the second group—which served as a control—received PBS treatment. At ages 7, 14, and 21 days, the first group received three oral doses of a bacterial solution (1×10^8 CFU). At 30 days, both groups were slaughtered. After being sliced and crushed, in the spleen put PBS-filled petri dishes. To separate the mixture, a centrifuge was employed. T lymphocyte cells from the first group T lymphocytes implanted in tissue culture from the first group were stimulated to produce lymphokines using con-A, and T cells in the first group's spleen were used to obtain S-ILK. In the T cells of the second group's spleen, (S-NILK) were discovered.

The viral inoculum was prepared

An unpublished isolate of the IBDV was found on CAM at 10–11 days in the pathology and poultry diseases branch of the University of Baghdad's College of Veterinary Medicine (26). An embryonated chicken egg hemagglutination test yielded an ELD_{50} of $10^{5.2}$, which was used to challenge the ELD_{50} of $10^{3.2}$ according to Reed and Muench's method (23). Until use, in a deep freezer (-80°C), the sample was kept.

Experimental strategy

200- one day old layer pullets are separated into five groups. On the first day, each group received the following treatment: G1: intraperitoneal injection of 0.5 mL of S-ILK followed by a 30-minute challenge by 0.1 mL

IBDV (ELD_{50} $10^{3.2}$); G2: intraperitoneal injection of 0.50 mL S-NILK followed by a 30-minute challenge with 0.1 mL IBDV. G3 group to be challenged with 0.1 mL of IBDV only, G4 is the uninfected, unchallenged group that performs the role of a negative control. Following the challenge, we recorded all clinical indicators and mortality rates.

Sampling

From the right jugular vein, five blood samples were taken to determine IgG and IFN-γ titres. Using anticoagulant-free glass tubes and a centrifuge set. For 15 minutes, 1000 rpm was used to separate the serum from the blood samples. Each group received five samples from the Bursa of Fabricius at days 7, and 14 following the challenge, they were kept at -80 degrees Celsius in a deep freezer. RT-PCR was used to determine the number of IBDV RNA copies.

Mortalities and morbidities rates

Throughout the course of the trial, clinical signs in the birds that survived the initial infection were noted. Depression, fatigue, drooping wings, and diarrhea with water the thigh and pectoral muscles both have hemorrhages. Along with renal alterations, there is an increase in gastrointestinal mucous. The surface of the bursa's serosal cavity is covered in a gelatinous, yellowish transudate in cases of severe dehydration, edema, and hyperemia (11, 15).

Actual time (RT-PCR)

In order to ascertain the quantity of RNA copies of the IBDV present in bursa tissues, Hazare (10) evaluated the test data. RNA was extracted from the infected birds' tissues using the TRIzol reagent. bursa of fabricius tissues (Invitrogen, Carlsbad, CA, USA). Specialized primers are used to detect IBD viruses. Forword: 5′-ACTGTCCTCAGCTTACCCACAT-3′. Reverse: 5′-TCTGTGACCAGGTTCTTTGCTA-3′. AIV RNA was discovered using 22 probes. Real-time PCR was performed using a single-step Prime Script RT-PCR kit (8). the LightCyler® 480 real-time PCR system from Roche Diagnostic Germany GMBH, Mannheim, Germany (16).

An ELISA tests: The ELISA kit used in the test, which was used to find chicken serum from afflicted birds contains IgG and IFN-γ against IBDV., was manufactured by the ProFlock AIV company (SunLong Biotech Co.,LT).

Analytic statistically

To analyze the dataset, the Statistical Analysis System was employed. Using the least significant difference (LSD) test, the means were separated. To evaluate statistical significance, a P value of 0.05 was used (24).

RESULTS AND DISCUSSION

IgG and (IFN-γ) immunity: At the age of one days old, 10 hens were randomly selected and divided into groups to assess humoral and cellular immunity (IFN-γ and IgG) in the serum against IBDV. 20.2±3.4 and 12657.7±342 are the respective numbers. The study provided evidence for how SILK improves the immune response to the IBDV challenge. As shown in Tab. 1 and 2, IgG and IFN-γ titers against IBDV are consistently significantly different at the level of ($P \leq 0.05$). At 21 days, G2 and G3 had the highest mean (IgG) and (IFN-γ) titres, but G1 had the least mean (IgG) and (IFN-γ) titres without significant mortality. In comparison to the other groups, the control group's (IgG) and (IFN-γ) antibodies against IBDV were significantly lower.

Table 1. IgG antibody titers are present at different periods in layer pullets

Periods	7 days	14 days	21 days	35 days
Groups	IgG Means ± Stander error			
G1	3567.1±213.8 B	6876.2±352.3 C	8213.2±654.7 C	10654±564.3 B
G2	2103.4±241.3 D	7895.8±545.1 AB	18765.8±1654 AB	22876.5±1342 AB
G3	2765.2±767.3 C	8435±678 A	19453±2017 A	24536.3±2140 A
G4	7436.4±266.3 A	2176.8±144.1 D	1109.8±122 D	786.4±78.3 C
LSD	233.28	724.49	1694.36	2703.3

A significant difference (P≤0.05) is denoted by capital letters in column

Table 2. IFN-γ titre in layer pullets at different periods against IBDV

Periods	7 days	14 days	21 days	35 days
Groups	IFN-γ titre Means ± Stander error			
G1	21.1±7.8 A	34±9.3 B	56±12.7 B	77.3±21 B
G2	25.4±5.3 A	44.8±10.1 A	67.8±16 A	103.7±32 AB
G3	23.2±8.3 A	49±14.2 A	70±22.1 A	120±33.2 A
G4	6.6±2.3 B	8±1.2 C	7.8±2.1 C	12.8±3 C
LSD	5.4	11.3	13.8	18.3

A significant difference (P≤0.05) is denoted by capital letters in column

These findings are consistent with those of Petkov *et al.* (19), who discovered a decrease with relation to the IgA and IgG B-cell populations in the IgM B-cell population after IBDV infection. Subsequently, there are two different subpopulations of IgM B cells. More recently, it was discovered that the highest titer of IgG against IBDV without mortality in the first group returned to the protection activity of T cell lymphokines that activated the macrophage (1). Although it has been proposed that IBDV enters cells by membrane perforation, it is still entirely unknown what cellular mechanism is being exploited to allow it to do so. Recent evidence reveals that the entire V-ATPase is supplied with an IBDV particle. positive vesicles for uncoating and implicates an important function for clathrin-independent endocytosis during the viral entrance (22). T cells and IFN- play a crucial part in the pathogenesis of IBD. Indeed, between 1 and 10 dpi, CD4+ and CD8+ lymphocytes influx and infiltrate into the BF, likely exacerbating cellular injury (5). The participation of T cells is also essential. Throughout an infection, a sophisticated network of cytokines regulates both inflammatory and targeted immune responses. Cytokines, which control the onset and upkeep of host defenses, eventually decide on the type of response and the effector mechanisms produced to mediate resistance. To regulate the intensity and duration of the immunity, cytokines function as effector molecules that are produced momentarily and locally. Consequently, cytokines are crucial in the control of both inflammation and immunity. Shown to contribute to the etiology of IBD, there is a considerable infiltration of CD4 cells, CD8+ cells, and macrophages, particularly in the BF, as early as one day after infection (dpi) (5). Bursal T cells are stimulated and show increased gene transcription for pro-inflammatory cytokines like CXCi2, ChIL-1b, and ChIL-6 (6). It was also discovered that high ChIFN-γ and ChIL-6 were present at the acute phase after vvIBDV challenge, implying the importance of an aggravated innate immune response that produced a "cytokine storm" in the early stages of the infection (22). The IL12a and IL18 mRNA levels in splenic macrophages, as well as pro-inflammatory factors like Inducible nitric oxide, ChIL-1b, and ChIL-6 synthase (iNOS), were all linked with the up-regulation of ChIFN-γ (18).

Viral loads

The results of the RT-PCR used to quantify viral load after the IBDV challenge are shown in Table 3. The number of IBDV RNA copies within Fabricius' bursal tissues at 7 days PI increased marginally significantly (P≤0.05) more in G3 and G2 than in G1, which had the lowest number. But by day 14, G3 and G2 had increased their IBDV RNA copies the most noticeably (P≤0.05), while G1 had decreased the most**.**

Table 3. IBDV RNA copies in bursa of fabricius tissues were analyzed using RT-PCR

Groups	7 days PI	14 days PI
G1	576±12.2 C	1428±432 C
G2	2569±321 B	5646.7±789.6 B
G3	3215.9±366.6 A	7645±933 A
G4	0±0 D	0±0 D
LSD	422	899.7

A significant difference (P≤0.05) is denoted by capital letters in column

These findings concur with those of Shihab (25), who noted that monocyte-macrophage lineages can also be infected in a persistent both the spread of the virus and the development of disease in a vitally important and efficient manner. The lowest IBDV particles in the first group that return to the efficacy of T cells' lymphokines to reduce virus multiplication in Fabricius' bursa (14). Between 1 and 7 dpi, RT-PCR and immunochemistry were used to detect viral RNA in bursal macrophages and viral proteins (12). Confocal microscopic analysis of the cells indicated that they were positive for both KUL01 (a macrophage surface marker) and R63 (an IBDV-VP2 marker), proving that the virus was present in the macrophages (18). The IBDV infection therefore alters the macrophage functions, particularly the phagocytic activity, and upregulates the production of cytokine genes, which affects the infected birds' normal immunological reactivity (5). Finally, even if they are immune to infection, releasing large quantities of mediators including nitric oxide (NO), chemokines like IL-8 and MIPa, and proinflammatory cytokines like IL-6 and IL-1 (12, 14, 20, 26). Additionally, the signal transduction mechanisms related to macrophage activation have been investigated (13). These findings indicate that IBDV utilizes cellular signal transduction mechanisms. Macrophages producing more NO, IL-8, and COX-2 may be a factor in the bursal inflammatory responses that are frequently observed during the acute phase of IBDV infection (12).

Morbidities and mortality

Birds from groups G3 and G2 had the highest prevalence of clinical symptoms and the highest rates of morbidity (both 100%), whereas birds from group G1 had the lowest incidence of clinical signs and the lowest rates of death (both 40 and 8%, respectively).

Table 4. Mortality and morbidity rates following the IBDV challenge

Groups	Morbidity	Mortality
G1	40% (10/25) B	8% (2/25) B
G2	100% (25/25) A	80% (20/25) AB
G3	100% (25/25) A	88% (22/25) A
G4	0% (0/25) C	0% (0/25) C

Capital letters signify a difference that is significant at the level of (P≤0.05)

The first group showed lower rates of compare the rates of morbidity and mortality to G2 and G3, which is consistent with Jumaa *et al.* (11) who recorded that finding of cell-mediated immunity, though also linked to the immunopathogenesis of the virus, is crucial for the removal of IBDV and recovery. Rauf *et al.* (20) recently published a study that looked at the molecular basis of cytotoxic T cell responses in the emergence of IBD in chickens. Chickens with IBDV contamination additionally infiltrated by CD4+ and CD8+ T cells. Importantly, immunohistochemistry also detected CD4+ and CD8+ T cells that produce PFN in the bursa of IBDV-infected hens. Strongly elevated Th1 cytokine production, including that of IL-2 and IFN-c, also points to T cell activation. The outcomes of this study showed how cytotoxic T cells can remove virus-infected cells.

CONCLUSION

Early S-ILK administration enhance Maternal immunity protects against IBDV infection and prevents viral multiplication in bursal tissue post challenge with IBDV. Thus, we may reduce the time, effort, and money spent on vaccinations procedures.

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