Nigerian Veterinary Journal 24(3): 4-8; 2003.

TOTAL SERUM COMPLEMENT IN CHICKENS EXPERIMENTALLY INFECTED WITH INFECTIOUS BURSAL DISEASE VIRUS WITH OR WITHOUT PREVIOUS VACCINATION

FAGBOHUN, O.A*[1]; TAIWO, V.O[1]; OWOADE, A.A[1]; ODAIBO, G.N[2]; OLUWAYELU, D.O[1]; AIKI-RAJI, C.O.[1] AND OLALEYE, D.O[2]

[1]*Faculty of Veterinary Medicine; and* [2]*Department of Virology, College of Medicine, University of Ibadan, Ibadan.*

* *Author for correspondence.*

Key words: Infectious bursal disease virus, complement.

SUMMARY

Mean titres of serum complement (C′) in 100 broiler chicks from day one to week 13 were determined using the complement fixation test. The chicks were assigned to four groups of 25 each namely, unvaccinated unchallenged (UvUc) which served as the controls, vaccinated unchallenged (VUc), unvaccinated challenged (UvC) and vaccinated challenged (VC). The chicks in groups VUc and VC were vaccinated at week 3 with locally produced Nigerian Fibrogomboyac 10 infectious bursal disease virus (IBDV) vaccine. The chicks in groups UvC and VC were challenged at week 6 by intraocular inoculation with virulent IBDV. Complement was first detected at 2 weeks of age in chickens while the highest mean titre was observed at 8 weeks. There was a 4-fold increase in the mean titre of C′ in the UvC group and a 3-fold increase in the VC group. The mean titre of C′ was lower in the VUc than those of the control (UvUc). Thus, challenge with IBDV significantly increased serum C′ levels in chickens while vaccination caused a decrease. The role of C′ in the pathogenesis of IBD is discussed.

INTRODUCTION

Infectious bursal disease virus (IBDV) induces an acute highly contagious immunosuppressive disease in young chickens which causes significant losses to the poultry industry worldwide (Rautenschlein *et al*, 2002). In the bursa of infected chickens, productive viral replication is often associated with necrosis, apoptosis of lymphoid cells, inflammatory changes, atrophy and haemorrhages (Kim *et al*, 2000). Haemorrhages may also be present in the muscles of the thigh and breast, intestine and proventriculus. Nephritis and tubular nephrosis also occur in infected chickens (Okoye and Uzoukwu, 1990).

The pathogenesis of IBD is not fully understood yet. However, Ivanyi and Morris (1976) hypothesized that immune complexes play a role in the pathogenesis of IBD infections. Skelees, *et al* (1979) described some of the bursal lesions as resembling Arthurs reaction, a type of the immunologic injury induced by antigen-antibody complexes and complement (C′)

and also observed depletion in serum C′ level in 8 weeks old chickens at 3, 5 and 7 days post-infection compared with the uninfected control chickens, which is indicative of serum C′ consumption.

This study was designed to investigate the effect of IBDV infection on the level of serum C′ in chickens and the role of C′ in the pathogenesis of IBD.

MATERIALS AND METHODS

Chicks

One hundred broiler chicks were collected at day old from infectious bursal disease (IBD) vaccinated breeders for this study. The chicks were assigned into four groups of 25 each; unvaccinated unchallenged (UvUc) which acts as the controls, vaccinated unchallenged (VUc), unvaccinated challenged (UvC) and vaccinated challenged (VC).

Vaccination

Groups VUc and VC were vaccinated at 3 weeks of age with the locally produced Nigerian Fibrogombovac 10 vaccine (Vom, Nigeria). The contents of the 200-dose vaccine with the titre 7.26 $\log_{10}$ $TCID_{50}$ per ml were reconstituted in two litres of distilled water. One litre of the reconstituted vaccine was put in a clean drinker in each group and left for the chicks to drink such that each chick would consume 10mls. The chicks were deprived of water overnight to ensure that they drink the whole vaccine within 30 minutes of administration. The chicks in the other two groups (UvUc and UvC) were given the same volume of distilled water.

Preparation of viral inoculum and inoculation

Bursa of Fabricius from naturally IBD infected flock were homogenised in a tissue blender with phosphate buffered saline. The homogenate was centrifuged at 3000g for 10 minutes and the supernatant filtered. The filtrate was confirmed to be IBD positive by agar gel immunodiffusion (AGID) (Okoye, 1984).

At week 6, 0.05ml of the inoculum was carefully applied on the conjunctival of each of the chickens in groups UvC and VC. These were housed separately. Chickens in all the groups were thereafter observed daily for clinical signs of IBD.

Sera collection

At day-one, 10 chicks were selected randomly and about 1ml of blood was collected from each through jugular venapuncture into plain tubes. Thereafter, blood was collected weekly for 8 weeks and lastly at week 13. The blood samples were allowed to clot and kept overnight at 4°C, after which the sera were separated by centrifugation and stored at -70°C until tested.

Preparation of sensitized sheep erythrocytes

Sheep erythrocytes were collected by bleeding sheep from the jugular vein into an equal volume of Alsevier's solution. Erythrocytes were washed and the concentration of cells for sensitization was determined according to Palmer (1980).

Rabbit anti-sheep haemolysin (Difco, UK) was used to sensitize the sheep erythrocytes as described by Gewurz and Suyehira (1980). A dilution of 1:300 was used based on result of a checkerboard titration.

Complement fixation test

This was carried out as described by Palmer (1980). Briefly, two-fold serial dilutions of each serum sample was made in Veronal buffer and 20ml dispensed into each well of 96 - well microtitre plate. The positive control consisting of guinea pig serum known to be rich in C′ was set up in the same manner in a row of wells. The negative control was set up by dispensing 20ml of veronal buffer each to a rows of wells but without serum. Thereafter, 20ml of sensitized sheep erythrocyte was added to each of the wells and incubated at 37°C for 30 minutes. Haemolysis in the various wells was visually assessed and recorded against the various concentrations of serum C′.

RESULTS

Chickens infected with the virus (groups UvC and VC) showed clinical signs of ruffled feathers. Dynamics of C′ levels in the different groups of chicken are shown in Table 1. No detectable level of complement was found in the sera of all the chickens until week 2 and there was no significant variation in complement levels in all the groups up till week 6 (Table 1). At week 7, there was a sharp increase in complement levels in vaccinated unchallenged (VUc) and vaccinated challenged (VC) groups which were vaccinated at week 3. Complement level

TABLE 1: Mean C titres of IBD challenged and unchallenged chickens with or without IBDV vaccination.

Period post challenge or vaccination	Mean C′ titres per group			
	UvUc	VUc	UvC	VC
Day 1	0	0	0	0
Wk 1	0	0	0	0
Wk 2	2.4	2.4	2.4	2.4
Wk 3*	7.0	9.6	7.0	9.6
Wk 4	4.4	4.0	4.4	4.0
Wk 5	8.4	1.3	8.4	1.3
Wk 6**	1.8	1.0	1.8	1.0
Wk 7	2.2	47.1	0.86	34.3
Wk 8	38.4	6.5	167.4	116.6
Wk 13	33.4	9.2	8.6	37.3

UvUc = Unvaccinated Unchallenged, VUc= Vaccinated Unchallenged, UvC= Unvaccinated Challenged, VC = Vaccinated Challenged.
* Period of vaccination, ** Period of challenge with IBDV.

decreased to baseline value from week 8 onwards in the VUc group while it rose remarkably in the VC group (about 300% of the UvUc group) at week 8 and was maintained through a lower level (37.3) till week 13. On the other hand, the UvC group had the highest level of complement (167.4) at week 8, which dropped to near zero level at week 13.

DISCUSSION

It was observed that mean titre of serum C′ in the control, that is, UvUc chickens did not show any appreciable rise until week 8. It could thus be deduced that the peak level of biosynthesis of C′ was at week 8 age of chicken. This observation is consistent with the report of Skeeles *et al.* (1979) in which the pre-infection mean titre of was <1:8 in the 2 week-old chickens.

This is also in line with the works of Polk *et al* (1938) and Gabrielsen *et al* (1973) who reported that C′ titre increased with the age of chickens. The undetectable and low levels of C′ observed at the early ages (day 1 to week 2) might be responsible for the lack of immunologic injury in chickens infected with IBDV at this age range reported by Ivanyi and Morris (1976) unlike in adult chickens 5 to 6 weeks of age. Localized immunologic injury is caused by antigen/antibody complexes which activate C′ resulting in neutrophil chemotaxis and accumulation.

Resident tissue macrophages that encounter immune complexes are stimulated to secrete tumour necrosis factor-α (TNF-α), interleukin 1 (IL-1), platelet activating factor, nitric oxide and oxygen radicals. The TNF-α and IL-1 upregulate the expression of leucocyte adhension molecules especially E-selectin and intercellular adhesion molecules-I (ICAM-I) which facilitates neutrophil adherence (Roitt *et al*, 2002). The neutrophils attracted by C3a, C5a and C567 emigrate from the blood vessel, adhere to immune complexes and promptly phagocytose them (Tizard, 1996). Eventually, immune complex are eliminated. During this process, phagocytes release a variety of other molecules such as toxic oxygen radicals, peroxides and lipid mediators of inflammation such as prostaglandins and leucotrienes -particularly leucotriene B4 (Tizard, 1996). As a result, destruction (especially of blood vessels) causes vasculitis, haemorrhages and development of oedema characteristic of local immunologic injury (Arthus type) similar to the lesions observed especially in the bursa of Fabricius of chickens infected with IBDV (Ivanyi and Morris, 1976).

The production of C′ by chickens in groups VUe and VC was observed at week 7. However, C′ levels dropped significantly to near zero levels from week 8 onward in VUe. This may be due to C′ consumption by vaccine antigen-antibody produced in response to the vaccination. It should be noted that serum C′ levels in VC chicken was slightly lower than those of VUe at week 7. These chickens (VC) were exposed to a field strain of IBDV a week earlier. Hence, some of the C′ produced in response to vaccination may have been consumed by field IBDV antigen/antibody complexes. It should however, be noted that challenge of chickens in UvC and VC on week 6 provoked significant increase in serum C′ levels in these chickens at week 8. The serum C′ level in UvC chickens was almost 150% higher than those of VC group implying that vaccination of chickens in the VC group significantly reduced the serum C′ level post-challenge. This may be related to the degree of complement-mediated tissue destruction in IBDV infected chicken, in which case tissue damage will be less in vaccinated than in unvaccinated chickens. It also should be noted that rapid C′ consumption took place in UvC chickens, while this was not so in VC chickens on week 13. This implied that tissue destruction in UvC chicken was more severe than in the VC. Also, the finding of remarkably high levels of C′ in VC chickens is an indication that the level of field IBDV antigens in these chickens was low

at week 13, most of these have been destroyed by the antibodies produced in response to vaccination at week 3.

It can thus be concluded that vaccination of chickens with IBDV at an early age led to antibody production and protection due to production of lower levels of C′ and therefore the development of milder complement-mediated tissue lesions after field challenge with the virus at a later stage.

It is hereby suggested that chickens should be vaccinated with IBDV at an age not later than 3 weeks so that minimal C′ activation will be provoked in the event of natural infection later in life.

REFERENCES

GABRIELSEN, R.E; PICKERING, R.J; LINNA, T.J. and GOOD, R.A. (1973): Haemolysis in chicken serum II. Ontogenic development. *Immunol.* 25: 179-184.

GEWURZ, H. and SUYEHIRA, L.A. (1980): In: Manual of clinical immunology 2nd Ed.Amer. Soc.Microbiol., 163-174.

IVANYI, J. and MORRIS, R. (1976): Immunodeficiency in the chicken (IV): An immunological study of infectious bursal disease. *Clin. Exp. Immunol.* 23: 154-156.

KIM, I.J; YOU, S.K; KIM, H; YEH, H.Y; and SHARMA, J.M. (2000): Characteristics of bursal T lymphocytes induced by infectious bursal disease virus. *J. Virol.* 74: 84-92.

OKOYE, J.O.A. (1984): Persistence of infectious bursal disease virus and the appearance of precipitins in infected chickens. *Trop. Vet.* 2: 97-102.

OKOYE, J.O.A.and UZOUKWU, M. (1990): Pathogenesis of infectious bursal disease in embryonally bursectiomised chickens. *Avian Pathol.* 19: 555-569.

PALMER, D.F. (1980): Complement fixation test In : Manual of clinical immunology 2nd Ed. Amer. Soc.Microbiol., 35-47.

POLK, A; BUILDING, G.J. and GOODPASTURE, E.W. (1938): An experimental study of complement and haemolytic amboceptor introduced into chick embryos. *Am. J. Pathol.* 14, 71-86.

RAUTENSCHLEIN S; YEH, H.Y; NJENGA, M.K; and SHARMA, J.M. (2002): Role of intrabursal T cells in infectious bursal disease virus infection. T cells promote viral clearance but delay follicular recovery. *Arch. Virol.* 147(2): 285-304.

ROITT, I; BROSTOFF, J. and MALE, D. (2002): In: Immunology. Elsevier Science Limited, 47-64.

SKELLES, J.K; LUKERT, E.V; DE BUYSSECHER, C.J; FLETCHERS, O.J. and BROWN, J. (1979): Infectious bursal disease virus infections II: The relationship of age, complement levels, virus neutralizing antibody, clotting and lesions. *Avian Dis.* 23: 107-117.

TIZARD, I.R. (1996): Veterinary Immunology: An introduction. 5th Ed. W.B. Saunders Company, 35-51.