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Rajesh Singathia
Department of Veterinary Microbiology, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Ravindra Sharma
Department of Veterinary Microbiology, College of Veterinary Sciences, Hisar, Haryana, India

Satish Kumar Batra
Department of Veterinary Microbiology, College of Veterinary Sciences, Hisar, Haryana, India

Corresponding Author
Rajesh Singathia
Department of Veterinary Microbiology, College of Veterinary and Animal Science, Navania, Vallabhnagar, Udaipur, Rajasthan, India

Cell-mediated immune response against F-1 and LaSota strains of new castle disease virus in chicken

Rajesh Singathia, Ravindra Sharma and Satish Kumar Batra

Abstract
F-1 and LaSota strains of New castle disease were propagated in the laboratory using embryonated hen eggs via the allantoic cavity route. One group of thirty chicks were infected with F-1 strain and the other with LaSota strain with virus via oral and ocular route. Sequential lymphocyte from blood samples at days 0, 3,7,14, 21, and 28 post infection were collected and cell mediated immune response against F-1 and LaSota strains of New castle disease virus (NDV) at different days post inoculation was analyzed by performing lymphocyte proliferation assays and by fluorescence-activated cell sorting analysis of phenotypic markers of T lymphocytes. In both the group of chicks immunized with F-1 and LaSota strains of NDV, lymphocyte proliferation responses at different days post infection were significantly higher ($P<0.05$) compared to that of the control group on *in vitro* stimulation with antigen. On *in vitro* stimulation with Concanavalin A the mean stimulation index values in the group of birds immunized with F-1 or with LaSota strain of NDV were found to be significantly higher than that of control at most of the days. A statistically lower ($P<0.05$) proportion of CD_4^+ T lymphocyte was observed on days 3 & 7 post infection in group of chickens inoculated with either F-1 or LaSota strain of NDV as compared to that of control birds. A lower proportion of CD_8^+ T cells were observed on days 7 & 14 post infection in birds inoculated with either F-1 or with LaSota strain of NDV. The Cellular immune response is generated in the form of first suppression (day 3) followed by an increase in CD_4^+ T lymphocytes and CD_8^+ T lymphocytes.

Keywords: Newcastle disease virus, Cell mediated immune response, F-1, LaSota, Chicks

Introduction
Newcastle disease **(**ND) is one of the important contagious, lethal, zoonotic disease causing very much economic losses in the poultry industry and is caused by an avian paramyxovirus serotype-1 (APMV-1) classified under genus *Avulavirus* of family *Paramyxoviridae* (Singathia *et al*., 2019) [30]. Inactivated as well as live virus vaccines are being used for the immunization of birds. The live vaccines are reported to induce both humoral immune response (HIR) and cell-mediated immune response (CMIR). A bird to be protected from infection with the New castle disease virus (NDV) should have both humoral and cell-mediated immune response against the virus. The author had already studied systemic and local humoral immune response against F-1 and LaSota strains of New castle disease virus (NDV) in chicken (Singathia *et al*., 2019) [30]. So in continuation, the author planned to analyze cell-mediated immune response elicited by the F-1 and LaSota strains of NDV in chicken.

Materials and Methods
Virus and experimental chickens
A seed stock of F-1 and LaSota strain of NDV were procured in lyophilized form and propagated in 10-day old embryonated eggs via allantoic cavity route by the standard method (Singathia *et al.*, 2019) [30]. For use as antigen in lymphocyte proliferation assay, the purified virus was inactivated by exposing to ultraviolet light for 40 minutes (Reynolds and Maraqa, 2000b) [23] and virus titration was performed by calculating the egg infective dose 50 (EID_{50}) of both F-1 and LaSota strain in 10-day old embryonated chicken eggs by the standard method (Reed and Muench,1938) [21].
Ninety broiler chicks were randomly divided into three groups of 30 birds each. Of these, one batch was vaccinated with F-1 strain and the second with LaSota strain via an oral and ocular route with 100 µl of virus (10^5 egg infective doses). Birds in the third group *i.e.* control group were mock-infected similarly with 100 µl of PBS each.

Collection of blood samples and separation of lymphocytes from blood

Samples of blood (3 ml) were collected from immunized and control birds directly from the heart in sterile plastic tubes containing heparin (10 I.U. /ml of blood). Blood samples were mixed with an equal volume of RPMI -1640 medium. Diluted blood was then carefully layered onto histopaque-1077 (Sigma Chemical Co.) column in sterile centrifuge tubes. The tubes were centrifuged at 750xg for 30 min at 4^0C in a cooling centrifuge (Remi Rev. tabletop centrifuge C-23). After centrifugation, the white interface layer was harvested carefully into another sterile 15-ml tube. These cells were then resuspended in RPMI-1640 medium and washed twice at 450xg for 10 min a cooling centrifuge. The viability of mononuclear cells was estimated by the trypan blue dye exclusion method after transferring the suspension to a hemocytometer. The cells were finally suspended in RPMI complete medium at a concentration of 10^7 live cells/ml.

Lymphocyte Proliferation Assay

Lymphocyte proliferation assay was performed in 96 well flat bottom microtitre plates (Nunc.). One hundred microliters of cell suspension ($1x10^6$ cells) were dispensed in each of the 3 wells of a 96 well flat bottom microtitre plate. These cells were then stimulated by adding 50 µl of optimally diluted (1:32) virus antigen (U.V. inactivated NDV) or mitogen [Concanavalin A (Sigma Chemical Co.) @ 10 µg/ml] or optimally diluted (1:32) Mock Ag. Controls wells received RPMI 1640 medium only. The plates were then incubated at 37^0C in a CO_2 incubator for 72 h. After the incubation, 20 µl of working solution (5mg/ml) of 3-(4, 5-dimethylthiazo-2-lyl)-2, 5-diphenyl tetrazolium bromide; Thiazolyl–Blue (MTT) dye (Sigma Chemical Co.) was added in each well, and plates were further incubated at 37^0C in CO_2 incubator for 4 h. Following incubation, the supernatant of each well was discarded carefully and 100 µl of dimethyl sulfoxide (DMSO) was added into each well to solubilize the formazon crystals. Finally, optical density was recorded using Multiskan Ex (Labsystems) microplate reader at 540 nm wavelength and the stimulation index (SI) was calculated by the following formula (Mosmann, 1983; Denizot and Lang, 1986) [20, 7].

$$\text{SI of antigen driven proliferation} = \frac{\text{Absorbance of antigen stimulated cells}}{\text{Absorbance of antigen unstimulated cells}}$$

$$\text{SI of Mitogen driven proliferation} = \frac{\text{Absorbance of Mitogen stimulated cells}}{\text{Absorbance of Mitogen unstimulated cells}}$$

Cytofluorometric analysis

The population of lymphocytes having different T-cell markers *i.e.* CD_4^+, CD_8^+ was analyzed using monoclonal antibodies (mab) against these T-cell markers by flow cytometry as per the method of Sharma (1990) [29].

Approximately 10^6 live mononuclear blood cells from each bird after washing were incubated with optimally diluted [(CD_4^+ 1:50),(CD_8^+ 1:200)] monoclonal antibodies in blocking buffer to label CD_4^+, CD_8^+cells for 1 h at 4^0C. After incubation three washings were given with washing buffer (PBS containing 0.2% sodium azide) by centrifugation at 450xg for 5 min. Labeled cells were incubated with optimally diluted (1:200) goat anti-mouse IgG-FITC conjugate in blocking buffer for one h at 4^0C. Following incubation, three washings were given with washing buffer and cells were resuspended in PBS containing paraformaldehyde (3% v/v) and glucose (2% w/v). Fixed cells were analyzed by flow cytometry using the fluorescence-activated cell sorting (FACS) Calibur machine (Becton Dickson, Franklin Lakes, NJ). Data were processed using Cell Quest software (Becton Dickson). The percentage of positive cells out of 10,000 was calculated by subtracting background staining of cells with fluorescent conjugate alone.

Result

The CMIR against F-1 and LaSota strains of NDV at different days post-inoculation (DPI) was analyzed by performing lymphocyte proliferation assays and by FACS analysis of phenotypic markers of T lymphocytes.

Lymphocyte proliferation assay

Proliferation responses of blood lymphocytes obtained from the three groups of broiler chicks in response to *in vitro* stimulation with viral antigen or mitogen were studied on 0, 3, 7, 14, 21 and 28 days post-immunization using MTT assay.

Lymphocyte proliferation response on *in vitro* stimulation with NDV strain F-1

Proliferation responses in the form of stimulation indices of blood lymphocyte cultures on *in vitro* stimulation with NDV strain F-1 in different groups of broiler chicks on different DPI are depicted in table 1. In the group of chicks immunized with F-1 NDV strain, lymphocyte proliferation responses at different days post-infection were significantly higher ($P<0.05$) compared to the control group, with the peak response (S.I.= 6.10) observed on 14 DPI. In the group of chicks immunized with LaSota strain, lymphocyte proliferation responses were significantly higher ($P<0.05$) compared to the control group. The stimulation index value showed a gradual increase on 3rd and 7th DPI and remained at the same level up to 21 days and was maximum on 28th DPI.

Lymphocyte proliferation response on *in vitro* stimulation with Concanavalin A

Table 2 depicts the proliferation responses as stimulation indices of lymphocyte cultures on *in vitro* stimulation with Concanavalin A in different groups of broiler chicks on different DPI. The mean SI values in the group of birds immunized with F-1 NDV strain were found to increase on day 7 post-immunization and were significantly higher than control ($P<0.05$) on day 14th through 28th when a peak S.I. of 7.38 was observed. The SI values in the group of birds infected with LaSota strain showed a similar increase pattern at different intervals post-infection and peaked on day 28. However, the extent of stimulation (peak S.I. value =6.20) was slightly lower than that of F-1.

Phenotypic characterization of T lymphocytes

Different T- Cell subpopulations in the blood of broiler chicks immunized with different strains of NDV were analyzed by FACS analysis of blood T lymphocyte after staining with marker-specific anti-chicken monoclonal antibodies. The sequential alterations in the CD_4^+ T lymphocytes subpopulation at different DPI are shown in the form of percentage values in table 3 and figure 1 and 2. A statistically lower ($P<0.05$) proportion of CD_4^+ T lymphocytes was observed on day 3 & 7 post-infection in a group of chickens inoculated with either F-1 or LaSota strains of NDV as compared to that of control birds. The proportion of CD_4^+ T lymphocyte on days 14 through 21 was not different from that

of the control group. A statistically lower proportion of CD_8^+ T lymphocyte was recorded in chicks immunized with either F-1 or LaSota strains of NDV as compared to control chick at day 7 and 14 post-inoculation but at day 3 and 21 the proportions of CD_8^+ T cells in immunized chicks was found to be equal to that of control chick (Table 4 and figure 3).

The ratio of CD_4^+/CD_8^+ T cells in immunized chicks were comparable to that of control chick at day 3,7,21 post-infection. The ratio of CD_4^+/CD_8^+ T cells in immunized chicks peaked on day 14 post-infection (Table 5).

Table 1: Lymphocyte proliferation responses on *in vitro* stimulation with NDV strain F-1

Mean S.I. ± S.E. of cultures stimulated with NDV strain F-1						
Groups	**Days post immunization**					
	0	**3**	**7**	**14**	**21**	**28**
Control	$1.05^{A} \pm 0.24$	$1.44^{B} \pm 0.20$	$1.22^{B} \pm 0.15$	$0.77^{C} \pm 0.05$	$1.20^{C} \pm 0.05$	$1.61^{B} \pm 0.26$
F-1	$0.97^{A} \pm 0.27$	$1.16^{B} \pm 0.05$	$2.53^{A} \pm 0.23$	$6.10^{A} \pm 0.84$	$4.60^{A} \pm 0.46$	$4.17^{A} \pm 0.58$
LaSota	$1.27^{A} \pm 0.29$	$2.44^{A} \pm 0.24$	$2.85^{A} \pm 0.28$	$2.95^{B} \pm 0.17$	$3.42^{B} \pm 0.09$	$3.79^{A} \pm 0.80$

Means with the same letter are not significantly different ($P<0.05$)

Table 2: Lymphocyte proliferation responses on *in vitro* stimulation with Concanavalin A

Mean S.I. ± S.E. of cultures stimulated with Concanavalin A						
Groups	**Days post immunization**					
	0	**3**	**7**	**14**	**21**	**28**
Control	$1.56^{A} \pm 0.19$	$2.42^{A} \pm 0.52$	$2.52^{A} \pm 0.09$	$1.68^{B} \pm 0.06$	$2.17^{B} \pm 0.27$	$6.98^{A} \pm 0.41$
F-1	$1.56^{A} \pm 0.19$	$1.50^{A} \pm 0.23$	$3.05^{A} \pm 0.28$	$5.21^{A} \pm 0.83$	$3.80^{A} \pm 0.64$	$7.38^{A} \pm 1.38$
LaSota	$1.56^{A} \pm 0.19$	$2.57^{A} \pm 0.23$	$2.97^{A} \pm 0.70$	$2.52^{B} \pm 0.14$	$4.45^{A} \pm 0.57$	$6.20^{A} \pm 1.23$

Means with the same letter are not significantly different ($P<0.05$)

Table 3: Sequential alterations in the CD_4^+ T lymphocyte population in the blood of chicks

% CD_4^+ T lymphocyte				
Groups	**Days post immunization**			
	3	**7**	**14**	**21**
Control	$7.67^{A} \pm 0.45$	$5.64^{A} \pm 0.52$	$6.16^{A} \pm 0.76$	$9.38^{A} \pm 0.96$
F-1	$5.62^{AB} \pm 1.24$	$2.32^{B} \pm 0.59$	$15.16^{A} \pm 5.03$	$13.84^{A} \pm 2.21$
LaSota	$4.05^{B} \pm 0.45$	$2.28^{B} \pm 0.46$	$11.76^{A} \pm 2.51$	$11.50^{A} \pm 4.23$

Means with the same letter are not significantly different ($P<0.05$)

Table 4: Sequential alterations in the CD_8^+ T lymphocyte population in the blood of chicks

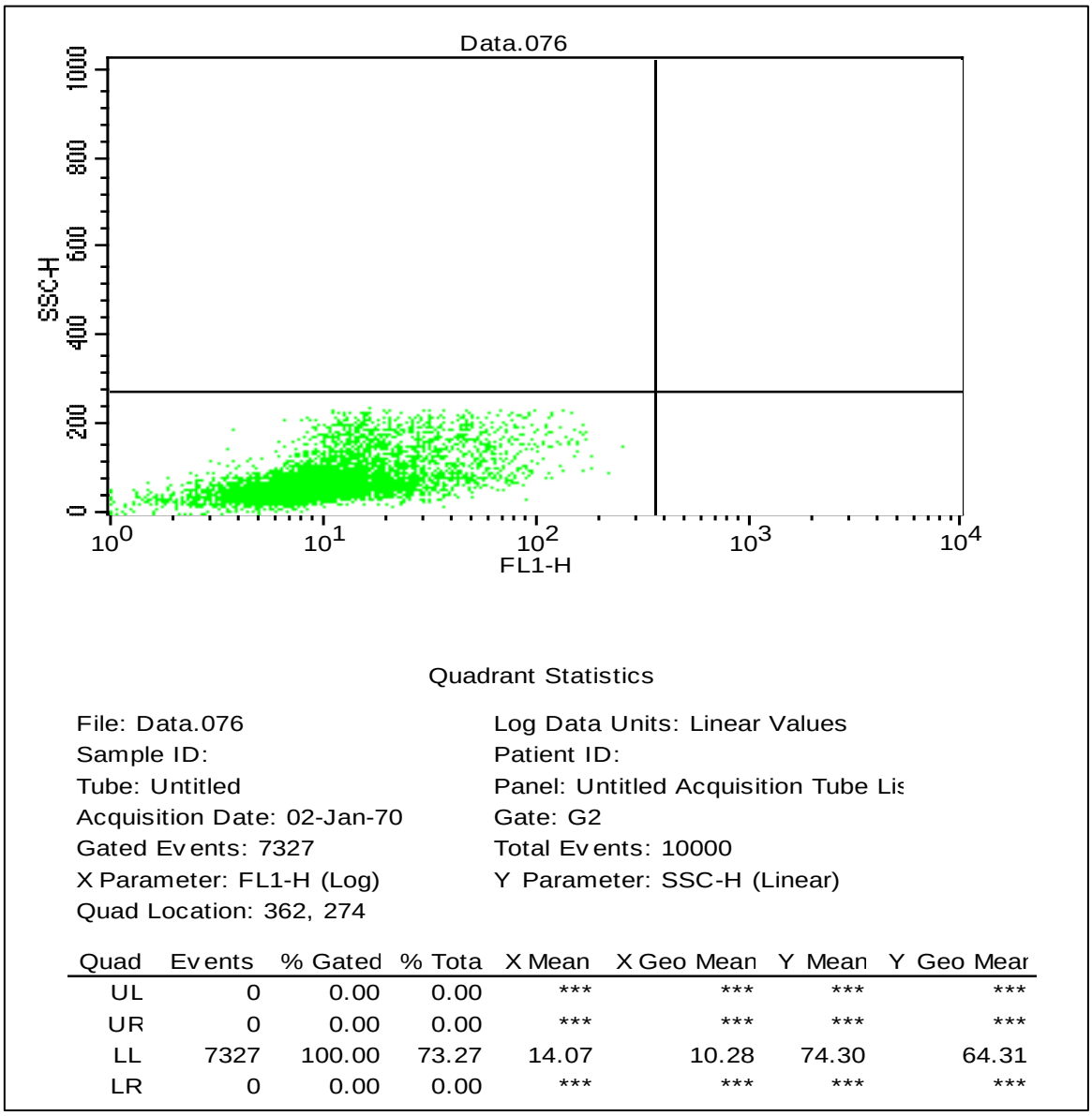
% CD_8^+ T lymphocyte				
Groups	**Days post immunization**			
	3	**7**	**14**	**21**
Control	$18.25^{A} \pm 1.60$	$12.06^{A} \pm 0.56$	$8.51^{A} \pm 1.55$	$12.06^{A} \pm 1.11$
F-1	$22.01^{A} \pm 2.98$	$3.68^{B} \pm 0.40$	$3.18^{B} \pm 0.37$	$11.50^{A} \pm 1.65$
LaSota	$14.35^{A} \pm 2.41$	$4.50^{B} \pm 1.00$	$4.60^{B} \pm 0.47$	$21.15^{A} \pm 6.22$

Means with the same letter are not significantly different ($P<0.05$)

Table 5: Sequential alterations in the ratio of CD_4^+/CD_8^+ T lymphocyte population in the blood of chicks

CD_4^+/CD_8^+ T lymphocyte				
Groups	**Days post immunization**			
	3	**7**	**14**	**21**
Control	$0.44^{A} \pm 0.05$	$0.47^{A} \pm 0.05$	$0.95^{B} \pm 0.35$	$0.80^{A} \pm 0.10$
F-1	$0.33^{A} \pm 0.14$	$0.65^{A} \pm 0.16$	$4.99^{A} \pm 1.91$	$1.30^{A} \pm 0.32$
LaSota	$0.33^{A} \pm 0.09$	$0.60^{A} \pm 0.15$	$2.81^{AB} \pm 0.74$	$1.03^{A} \pm 0.68$

Means with the same letter are not significantly different ($P<0.05$)



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Acquisition Date: 02-Jan-70
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Log Data Units: Linear Values
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Gate: G2
Total Events: 10000
Y Parameter: SSC-H (Linear)

Quad	Events	% Gated	% Tota	X Mean	X Geo Mean	Y Mean	Y Geo Mear
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UR	0	0.00	0.00	***	***	***	***
LL	7327	100.00	73.27	14.07	10.28	74.30	64.31
LR	0	0.00	0.00	***	***	***	***

Fig 1: FACS profile of avian mononuclear cells labelled with FITC conjugate alone

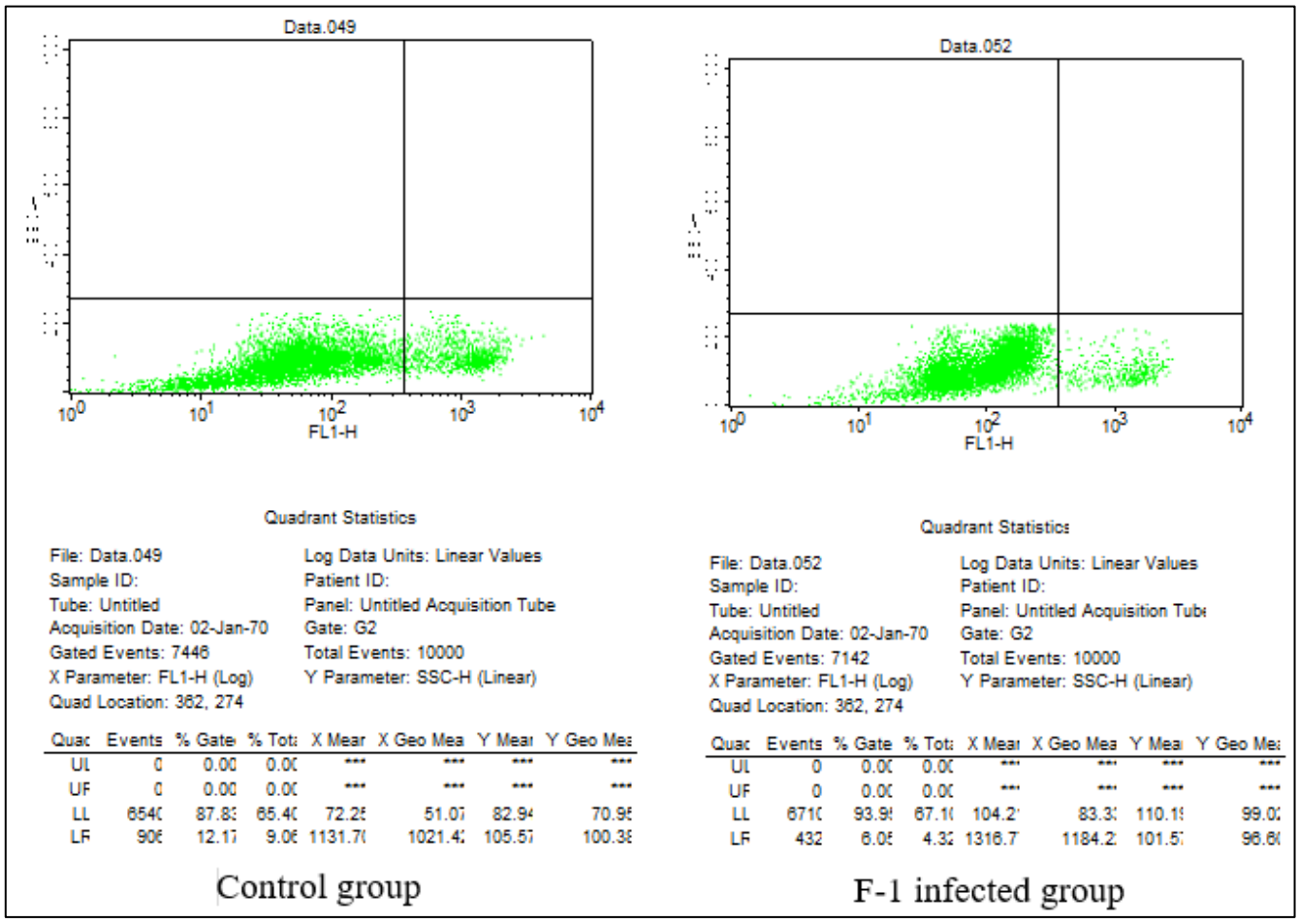

Fig 2: FACS profile of avian CD_4^+ T CELLS

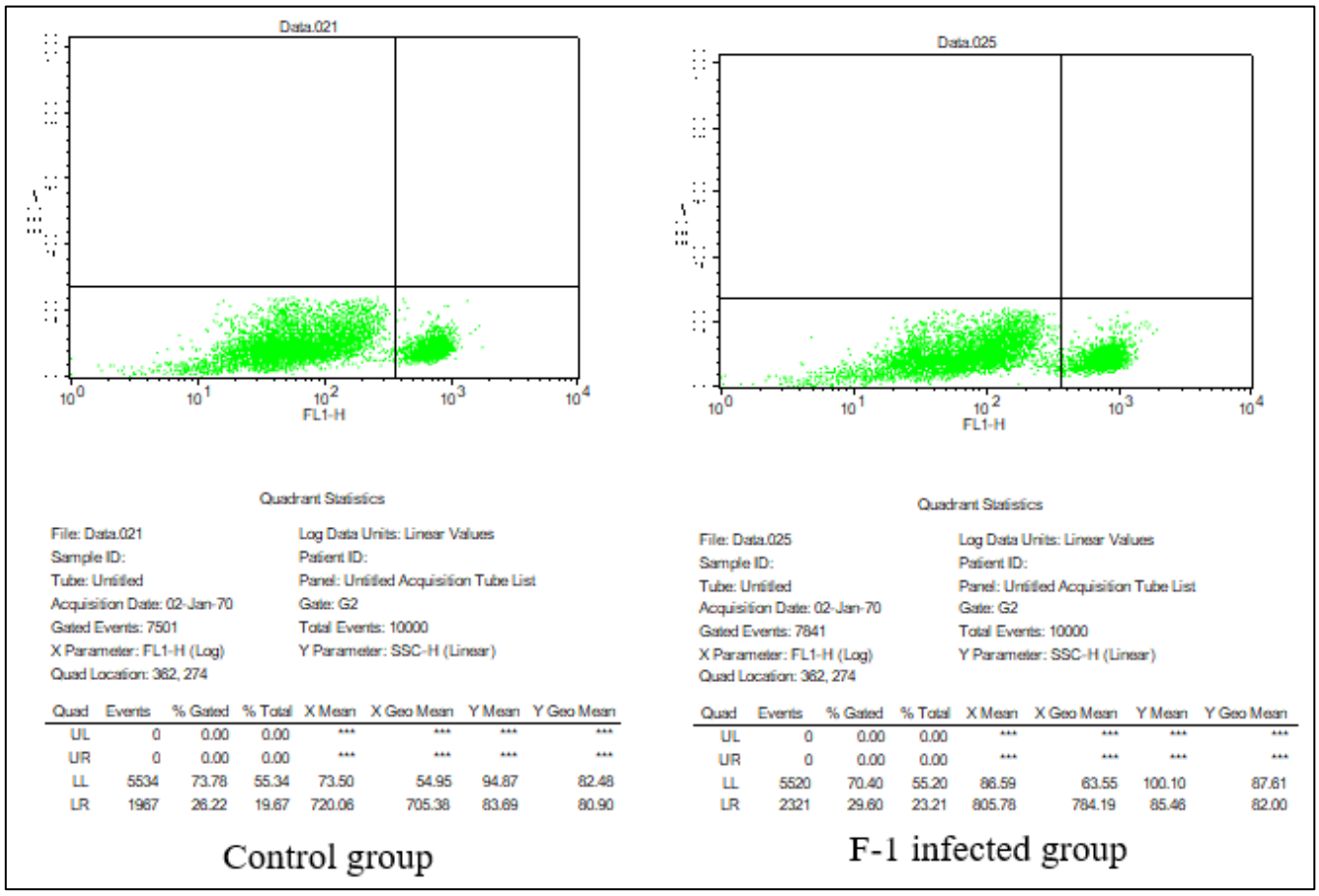

Fig 3: FACS profile of avian CD_8^+ T Cells

Discussion

In addition to antibodies, cell mediated immunity also plays an important role in immunity against NDV (Dandapat *et al.*, 2005) [5]. To examine the functional significance of T-cell, experiment was planned to study the CMIR. The cellular immune response was measured using MTT based lymphoproliferation assay and by cytofluorometric analysis.

Chicks immunized with either F-1 or with LaSota strain of NDV exhibited significantly higher lymphocyte proliferation responses than control group at different days post infection. On *in vitro* stimulation of lymphocytes from chicks immunized with either F-1 or LaSota strain of virus, the SI values showed gradual increase on 7th and 3rd DPI, respectively while peak stimulation index response was observed on 14th and 28th DPI, respectively. These results of present study are supported by the findings of Dandapat *et al.* (2005) [5] who reported that the peak SI (1.395 ± 0.046) of lymphocytes from birds immunized using NDV conventional vaccine was observed at the 2nd week PI. Lambrecht *et al.* (2004) [15] while using the lymphocyte proliferation assay, observed a peak S.I. on 3rd week after vaccination with live NDV vaccine in the chicks. Our result are also on agreement with the findings of Ghumman and Bankowski (1976) [11], who reported detection of CMIR as early as 2-3 days post vaccination with the live NDV. Our results are also similar with the findings of Mishra *et al.* (1984) [19] who reported CMIR by leucocyte migration inhibition test (LMI) and skin reactive factor test and found that a maximum of migration inhibition and production of skin reactive factor was observed on day 21st post vaccination with R_2B strain of NDV by subcutaneous route. The difference in peak may be due to use of different strain of the virus and a different route of inoculation. Similarly, Kumar *et al.* (1988) [14] have reported CMIR measured by leucocyte migration inhibition test (LMI) was significantly higher from 2nd week to 8th week after primary vaccination. Similarly, Timms and Alexander (1977) [32] also reported CMIR by LMI, highest Cell mediated immunity (CMI) levels were demonstrated in 3 or 7-week old birds which were vaccinated with live vaccine by aerosol method. Al-Shahery *et al.* (2008) [1] also showed CMIR employing LMI test. Reynolds and Maraqa (2000a) [22]; Linghua *et al.*, (2007) [16] also reported that the CMI generated by NDV. In the present study, on stimulation with mitogen, we recorded high SI in the control group also. This finding may be ascribed to the non-specific viral mitogenesis induced in the PBMCs culture by the paramyxo-viruses (Russell, 1988) [25].

CMI is considered a major protective mechanism against viral diseases (Erf, 2004). One of the conventional methods of *in vitro* CMI evaluation is by measuring the antigen specific blastogenic response (Breed *et al.*, 1997) [3]. The antigenic specific *in vitro* proliferation assay also exhibits most of the immunological phenomena taking place *in vivo* including expression of innate markers. This offers an excellent opportunity to appraise the CMI response by measuring the production of the markers. An advantage of this approach is that a broader picture of CMIR could emerge, by evaluating these parameters, which isn't possible with the conventional proliferation assay. In the present study, phenotypic characterization of T lymphocytes was done by cytofluorometric analysis. Immuno-phenotype study was conducted to evaluate the percentage of CD_4^+ and CD_8^+ positive cells in the blood of chicks immunized with different strains of NDV. Cytotoxic T cells typically express CD_8^+ molecules, while the T helper cell expresses CD_4^+ molecules (Chan *et al.*, 1988) [4].

The CD_4^+ and CD_8^+ T cell number was found to be less than that of control birds during 3rd and 7th day post infection indicating leukopenia. Lymphopenia appears to be a normal feature during the acute phase of NDV infection (Beard and Hanson, 1984; Dhir *et al.*, 1986; Rombout *et al.*, 1992) [2, 6, 24] and can possibly be explained by cytolysis of peripheral blood lymphocytes directly by virus contact or indirectly by the elimination of NDV infected cells (Hao and Lam, 1986) [13]. Rinderpest virus another member of family *Paramyxoviridae* has been reported to cause lymphopenia as the virus replicates in lymphocytes resulting in lymphopenia (Scott, 1990; Yamanouchi *et al.*, 1993) [34]. A significant increase in the number of CD_4^+ cells in the peripheral blood was observed on day 14 post infection onwards in birds immunized with either F-1 or LaSota strain of NDV. An increase in CD_4^+ T cell response has been observed in cattle following vaccination with a paramyxovirus (Rinderpest virus) and a specific response has been reported to haemagglutinin, fusion, nucleocapsid and matrix proteins of virus (Lund *et al.*, 2000) [17]. Antigen specific CD_4^+ T cells have been reported to modulate the immune response to respiratory virus infections such as parainfluenza virus through regulation of antibody production (Doherty *et al.*, 1997) [8] and are also involved in clearance of virus infection in association with CD_8^+ T cells (Graham and Braciale, 1997; Zhong *et al.*, 2000) [12, 36].

A less than normal CD_8^+ T cell count in the peripheral blood lymphocytes was observed during the present study up to 14 DPI followed by an increase to the normal values. This finding is in consistence with that of Rombout *et al.* (1992) [24]. This finding is, however, in contrast to that of Russell *et al.* (1997) [26] who reported a six fold increase in the CD_8^+ T cells in immunoperoxidase stained sections of Harderian gland of chickens vaccinated with Hitchner B1 strain of NDV. The initial suppression of CD_8^+ T cell may be due to leukopenia as observed during the present study and reported by several workers (Beard and Hanson, 1984; Dhir *et al.*, 1986; Rombout *et al.*, 1992) [2, 6, 24]. In several other paramyxoviruses like respiratory syncytial virus infection in calves, it has been reported that depletion of CD_8^+ T cells prolongs clearance of virus from body and this plays a dominant role in recovery of animals from virus infection (Taylor *et al.*, 1995) [31]. CD_8^+ cells have been reported to clear paramyxovirus virus infections in mice and domestic animals (Young *et al.*, 1990; Taylor *et al.*, 1995; Seal *et al.*, 2000) [35, 31, 28] and measles virus infection in humans (Weidinger *et al.*, 2000) [33].

The CD_4^+ and CD_8^+ T cell ratio is generally regarded as a good estimate of helper T cell and cytotoxic T cell balance and was calculated in chicken peripheral blood lymphocytes. CD_4^+ and CD_8^+ T lymphocyte ratio in experimental group of birds were statistically indifferent from that of control birds on 3rd, 7th and 21st DPI. It showed an increase on 14th DPI. The small number of samples used in this study however limits us in concluding much from phenotypic studies. This is compounded by the fact that samples are acquired on different dates. The $CD_4^{+:}$ CD_8^+ cell ratio observed in the present study is well within the range of previous studies (Erf *et al.*, 1998) [10].

The results of the present study indicate that cellular components are stimulated by the vaccine virus. Cellular arm of the immune response following NDV infection responds by initial leukopenia followed by proliferation of virus specific T

lymphocytes of both CD_4^+ and CD_8^+ T series. These studies are in consistent with the findings of Reynolds and Maraqa (2000a, 2000b) [22, 23]. It would be interesting to investigate further the exact role of cytotoxic T cells in recovery/clearance of NDV infection from body.

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References

1. Al-Shahery MN, Al-Zubeady AZ, Al-Baroodi SY. Evaluation of cell-mediated immune response in chickens vaccinated with Newcastle disease virus. Iraqi J. Vet. Sci 2008;**22**(1):21-24.
2. Beard CW, Hanson RP. Newcastle disease. In: *Diseases of Poultry*. 8th ed. Hofstad, M.S., Barnes, H.J., Calnek, B.W., Reid, W.M. and Yoder, H.W. (eds.). Ames, IA: Iowa State University Press 1984, 452-470.
3. Breed DG, Schetters TP, Verhoeven, NA, Vermeulen, AN. Characterization of phenotype related responsiveness of peripheral blood lymphocytes from *Eimeria tenella* infected chickens. Parasite Immunol 1997;19(12):563-569.
4. Chan MM, Chen CLH, Ager LL, Cooper MD. Identification of avian homologue of mammalian CD_4 and CD_8 antigens. J. Immunol 1988;140:2133-2138.
5. Dandapat S, Nagarajan K, Kataria JM, Bash BB, Yadav MP. Humoral and cell mediated immune response to Ranikhet disease vaccine delivered through PLG microspheres in broiler chickens. Indian J. Poult. Sci 2005;40(1):47-51.
6. Dhir RC, Gary UK, Chhabra IS, Data IC. Studies on haematological and biochemical parameters in chickens after infection with Newcastle disease virus of varying virulence. Ind. J. Poult. Sci 1986;21:322-325.
7. Denizot F, Lang R. Rapid colorimetric assay for cell growth and survival. Modification to the tetrazolium dye procedure giving improved sensitivity and reliability. J. Immunol. Methods 1986;89:271-277.
8. Dohert PC, Topham DJ, Tripp RA, Cardin RD, Brooks JW, Stevenson PG. Effector CD_4^+ and CD_8^+ T cells mechanism in the control of respiratory virus infections. Immunol 1997;159:105-117.
9. Erf GF. Cell-mediated immunity in poultry. Poult. Sci 2004;83(1-6):580-590.
10. Erf GF, Botte WG, Bersi TK. CD_4, CD_8 and TCR defined T-cell subsets in thymus and spleen of 2- and 7-week old commercial broiler chickens. Vet. Immunol. Immunopathol 1998;62(4):339-348.
11. Ghumman JS, Bankowski F. *In vitro* DNA synthesis in lymphocytes from turkey vaccinated with LaSota, TC, and inactivated Newcastle disease vaccines. Avian Dis, 1976;20:18-31.
12. Graham MB, Braciale TJ. Resistance to and recovery from lethal influenza virus infection in B lymphocyte deficient mice. J. Exp. Med 1997;186:2063-2068.
13. Hao Q, Lam KM. Interaction between chicken lymphocytes and Newcastle disease virus. Avian dis 1986;31:649-653.
14. Kumar KU, Swamy SK, Reddy TV. Humoral and cell-mediated immune response in chicks vaccinated against Newcastle disease. Kerala J. Vet. Sci 1988;19(2):116-121.
15. Lambrecht B, Gonze M, Meulemans G, Van de berg TP. Assesment of the cell-mediated immune response in chickens by detection of chickens interferon-γ in response to mitogen and recall Newcastle disease viral antigen stimulation. Avian Pathol 2004;33(3):343-350.
16. Linghua Z, Xingshan T, Fengzhen Z. Vaccination with Newcastle disease vaccine and CpG oligodeoxynucleotides induces specific immunity and protection against Newcastle disease virus in SPF chicken. Vet. Immunol. Immunopathol 2007;115:216-222.
17. Lund BT, Tiwari A, Galbraith S, Baron MD, Morrison WI, Barrett T. Vaccination of cattle with attenuated rinderpest virus stimulates CD_4^+ T responses with broad viral antigen specificity. J. Gen. Virol 2000;81:2137-2146.
18. Mayo MA. A summary of the taxonomic changes recently approved by ICTV. Arch. Virol 2002;147:1655-1663.
19. Mishra SC, Rai A, Jaiswal TN. Assay of cell mediated immune response in chickens vaccinated with Ranikhet disease vaccine. Indian J. Comp. Microbiol. Immunol. Infect. Dis 1984;5(4):157-161.
20. Mosmann T. Rapid colorimetric assay for cellular growth and survival: application to proliferation and cytotoxicity assays. J. Immunol. Meth 1983;65:55-63.
21. Reed LV, Muench H. A simple method of estimating fifty per cent end points. Am. J. Hyg 1938;**27**:493-497.
22. Reynolds DL, Maraqa AD. Protective immunity against Newcastle disease: the role of cell-mediated immunity. Avian Dis 2000a;44:145-154.
23. Reynolds DL, Maraqa AD. Protective immunity against Newcastle disease: the role of antibodies specific to Newcastle disease virus polypeptides. Avian Dis 2000b;44:138-144.
24. Rombout JHWM, Van Rens BTTM, Sijtsma SR, Van der Weide MC, West CE. Effect of vitamin A deficiency and Newcastle disease virus infection on lymphocyte subpopulations in chicken blood. Vet. Immunol. Immunopathol 1992;31:155-166.
25. Russell PH. The non-specific stimulation of avian peripheral blood lymphocyte from uninfected chickens by paramyxoviruses and influenza viruses. Vet. Microbiol 1988;16:181-188.
26. Russell PH, Dwivedi PN, Davison TF. The effects of cyclosporin A and cyclophosphamide on the population of B and T cells and virus in the Harderian gland of chickens vaccinated with the Hitchner B1 strain of Newcastle disease virus. Vet. Immunol. Immunopathol 1997;60:171-185.
27. Scott GR. Rinderpest virus. In: *Viral infections of Ruminants*. Dinter, Z. and Morein, B. (ed.). Amsterdam: Elsevier Science Publishers 1990, 341-354.
28. Seal BS, King JK, Sellers HS. The avian response to Newcastle disease virus. Dev.Comp. Immunol 2000;24:257-268.
29. Sharma R. Immunopathogenesis of respiratory syncytical virus in experimentally infected lambs. Ph.D. Thesis submitted to University of Liverpool, U.K 1990.
30. Singathia R, Sharma R, Batra SK. Systemic and Local Humoral Immune Response against F-1 and LaSota Strains of New Castle Disease Virus in Chicken. International Journal of Current Microbiology and

Applied Sciences 2019;8(9):194-203.
31. Taylor G, Thomas LH, Wyld SG, Furze J, Sopp P, Howard CJ. Role of T- lymphocytes subsets in recovery from respiratory syncytial virus infection in calves. J. Virol 1995;69:6658-6664.
32. Timms L, Alexander DJ. Cell-mediated immune response of chickens to Newcastle disease vaccines. Avian Pathol 1977;6:51-59.
33. Weidinger G, Czub S, Neumeister C, Harriott P, Meulen VT, Niewiesk S. Role of CD_4^+ and CD_8^+ T cells in the prevention of measles virus-induced encephalitis in mice. J. Gen. Virol 2000;81:2707-2713.
34. Yamanouchi K, Inui K, Sugimoto M, Asano F, Nishimaki F, Kitching RP *et al*. Immunisation of cattle with a recombinant vaccinia vector expressing the haemagglutinin gene of rinderpest virus. Vet. Rec 1993;132:152-156.
35. Young DF, Randall RE, Hoyle JA, Souberbielle BE. Clearance of persistent paramyxovirus infection is mediated by cellular immune response but not by serum-neutralizing antibody. J. Virol 1990;64(11):5403-5411.
36. Zhong W, Marshall D, Coleclough C, Woodland DL. CD_4^+ T cell priming accelerates the clearance of Sendai virus in mice, but has a negative effect on CD_8^+ T cell memory. J. Immunol 2000;164:3274-3282.