EFFECT OF IMMUPLUS (A HERBAL IMMUNOMODULATOR) ON PARASPECIFIC IMMUNE RESPONSES IN CHICKS

Balwinder Singh Dhote, G.K. Singh and *R.S. Chauhan

Department of Anatomy, College of Veterinary and Animal Sciences,
G.B. Pant University of Agriculture and Technology,
Pantnagar - 263145 (Uttaranchal)
** Joint Director, CADRAD, Indian Veterinary Research Institute, Izatnagar (U.P)*

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Introduction

The immune system of birds and animals is highly susceptible to the hostile environment and invading pathogens. The conditions that cause stress either physical or psychological may lead to immune dysfunction. In ancient Indian literature, several herbal preparations have been described, which can be given to animals to augment the immune response (Bhargava and Singh, 1981; Chauhan, 1999). Kumar *et al*. (2003) concluded that Immuplus up regulates immune response to FMD vaccine in calves.

Materials and methods

To assess the effect of Immuplus on paraspecific immunity in developing stages of chicks, 104 chicks were procured on the day of hatch and randomly divided into two groups of 52 each. The chicks were raised up to 60 days of age under ideal and uniform husbandry conditions. One group of chicks was given herbal preparation Immuplus 25 mg/kg body weight in drinking water and the other group was kept as control. Blood samples were collected, in heparin, at 5 days interval i.e. day 0, 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, and 60 from 4 randomly selected experimental chicks from each group by cardiac puncture. All the serum samples were stored at -20°C till use.

Immunization programme

All the chicks were vaccinated against New Castle disease (NCD) and Infectious bursal disease (IBD) with primary (for both) and booster dose (for NCD) at 30 days of age.

Paraspecific Immune response

A. Total leucocyte count (TLC)

TLC (µl) was estimated as per the method of Natt and Herrick (1952) using a diluent containing 0.1 M NaCl, 0.008 M $Na_2HPO_4.12\ H_2O$, 0.002 M KH_2PO_4, 7.5 ml formaldehyde and 0.1 g methyl violet 2B.

B. Absolute lymphocyte count (ALC)

The ALC values in each group of birds were calculated by using the formula: ALC/µl of blood = (% Lymphocyte X Total leucocyte count)/100.

C. Macrophage function test-NBT reduction assay

To measure the metabolic activity of phagocyte cells, the nitroblue tetrazolium (NBT) reduction assay was conducted at every 5 days interval using NBT kits (Sigma, USA) as described by Chauhan (1998) and Chauhan and Tripathi (2002).

D. Interleukin-1 and 2 (IL- 1 and 2)

Test procedure

Triplicate cultures were made using 100 µl of cell suspension with 50 µl of medium alone in one set and medium containing LPS 4 µg/ml in second set of wells in flat bottom sterile microtitre plates (Corning, Germany). Plates were sealed with cello tape and incubated for 3 days at 37°C in CO_2 chamber,50µl of the supernatant from each well was pipetted out to parallel wells of separate round bottom 96 well microtitre plates for estimation of interleukins-1 and 2 and kept overnight at 4°C. Washing and tapping steps were performed thrice. The nonspecific binding sites were blocked by adding 100 µl of 5% skimmed milk powder in each well and plates were incubated for 2h at 37°C. After usual washing and tapping, 100 µl of antibodies against IL-1 and 2 (raised in goat, dilution 1:10,000 for both) were pipetted separately into wells containing mitogens-ConA and LPS. The plates were incubated for 2h at 37°C followed by routine washing and tapping. Then 100 µl of antigoat IgG peroxidase conjugate (1:10,000) was added to each well and plates were incubated at 37°C for 2h. The plates were washed and tapped 3 times, and then 100 µl of OPD (Sigma) in citrate phosphate buffer (pH 5.0) was added to each well. Plates were incubated at 37°C for 30 min. Colour development was stopped by adding 100 µl of 1N sulphuric acid to all the wells and the absorbance was read at 492 nm using ELISA reader (ECIL, India). Duplicate positive and negative sera were also included as positive and negative controls in each plate. The ELISA values were calculated by dividing the absorbance value of various test samples by absorbance value of negative control.

Results and discussion

Lymphocytes constitute the key component of the immune system. A rise or fall in the concentration of these cells affects the health/immune constitution of the body as they are known to recognize the foreign antigens and mount an immune response. In the present study

the TLC ($x10^3/\mu l$) values evinced a continuous increasing trend in the treated group of birds (Table.1). The values for days 50, 55 and 60 were significant ($P<0.05$) between the two groups. The values for treated group were 27.26, 27.78 and 28.07 and the corresponding values for control group were 25.73, 26.21 and 26.88, respectively. The absolute lymphocyte count (ALC) also revealed an increasing trend (Table.1). The values were significant ($P<0.05$) between the two groups at 50, 55 and 60 days of age. A rise in cell concentration implies strengthening and consolidation of the body defense system. The total leucocytic counts and absolute lymphocytic counts were found to be increased by 5.95, 5.99 and 4.43% and 10.38, 10.52 and 9.12%, respectively in Immuplus treated birds in comparison to the control group on 50, 55 and 60 day of observation. Chauhan (2001) also reported that with increasing age the concentration of lymphocytes increased. Borkar *et al*. (2002) also observed that 'Growell' an herbal immunomodulator caused a significant increase in the haematological parameters (TEC, TLC, Hb and PCV) in IBD vaccinated chicks.

The macrophages process and present the antigens in association with MHC molecules to the T- or B-cells. The number of NBT positive cells in the treated group was higher than those of the control group indicating increased activity of macrophages in the birds treated with Immuplus (Fig.1). Upadhyay *et al*. (1992) and Rao *et al*. (1995), reported enhancing effects of various immunomodulators on the macrophage functioning. The increase in the number of NBT positive cells was observed from 5^{th} day onwards which is in agreement with the observations of Janse and Jeurissen (1991) that macrophages start to absorb antigens few days after hatching. Qureshi *et al*. (1994) stated that macrophages owing to their phagocytic action, form the first line of defense against infectious organisms and they do so by several effector functions such as chemotaxis, bacterial uptake, killing and phagocytosis, biosynthesis of nitric oxide, oxygen metabolites and proteolytic enzymes, and monokines like IL-1. The mean ELISA values for IL-1 were almost equal in both the groups during most part of the experiment but it showed an increase after 50 days of age. A significant increase ($P\leq0.05$) of 8.49% and 8.14% was observed at 55 and 60 days of age (Fig.2).

The IL-2 values of the treated group were more than those of the control group of birds at all the ages (Fig.3). The increase in IL-2 values of treated birds over control was 7.80, 7.69 and 8.52% at 50, 55 and 60 days of age, respectively. This increase was significant at ($P\leq0.05$). According to Sharma and Sharma (2001) IL-1 is secreted by macrophages and lymphocytes, which target the T-helper cells (Th-cells) and B-cells, while IL-2 is liberated from Th-cells and NK-cells that target the T- and B-cells. However, many more cells secrete

more cytokines to bring about multiple biological activities (pleotropy), which may overlap with those of others (redundancy). Kumar *et al*. (2003) observed significant increase in IL-1, IL-2 values in FMD vaccinated calves fed with Immuplus.

Withania somnifera is known to positively moderate the immune system of man and animals (Kuttan, 1996). Other ingredients of Immuplus *Tinospora cordifolia* (Karande *et al*., 1991), *Ocimum sanctum* (Godhwani *et al*., 1988) and *Emblica officinalis* (Vasudevan, 1994) are also reported to have immunomodulatory properties.

Conclusion

The findings of the present investigation indicate that Immuplus has potentiating effect on the paraspecific responses against ND and IBD antigens as detected by TLC, ALC NBT and for IL-1 and IL-2 ELISA test in chicks.

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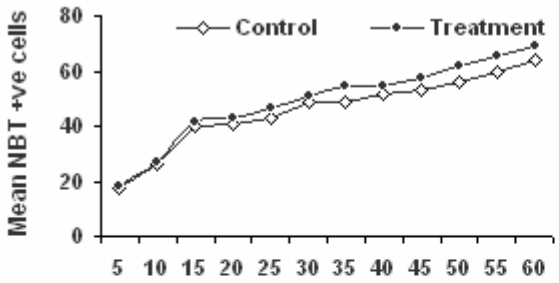

Fig. 1. Effect of Immuplus on Macrophage Function of chicks

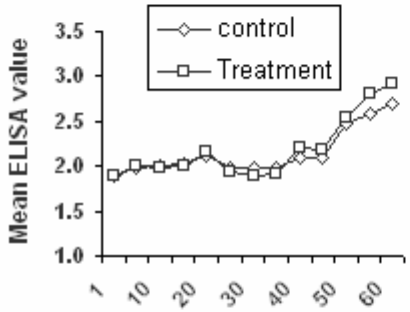

Fig. 2. Effect of Immuplus on Interleukin-1 level of chicks

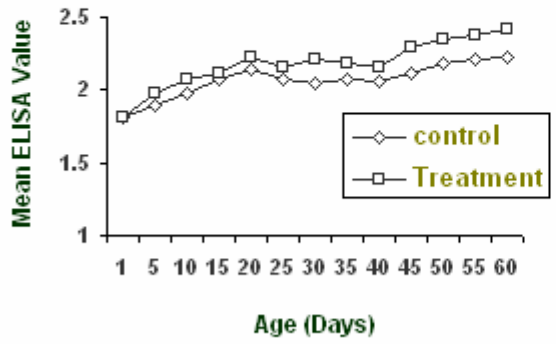

Fig. 3. Effect of Immuplus on Interleukin-2 level of chicks

Age (days)	TLC: $10^3 \mu l^{-1}$		ALC: $10^3 \mu l^{-1}$	
	C	T	C	T
1	20.06 ±0.26	20.09 ±0.22	14.24 ±0.19	14.01 ±0.08
5	20.51 ±0.29	20.61 ±0.27	14.62 ±0.22	14.53 ±0.22
10	21.25 ±0.35	21.47 ±0.31	15.30 ±0.19	15.67 ±0.25
15	21.86 ±0.29	22.17 ±0.38	16.23 ±0.25	16.46 ±0.31
20	22.45 ±0.41	22.65 ±0.31	16.61 ±0.36	17.34 ±0.42
25	23.19 ±0.21	23.74 ±0.21	17.50 ±0.32	18.35 ±0.46
30	23.79 ±0.24	24.28 ±0.23	17.84 ±0.36	18.94 ±0.36
35	24.30 ±0.26	24.81 ±0.26	18.66 ±0.41	19.48 ±0.42
40	24.86 ±0.26	25.74 ±0.36	18.78 ±0.65	20.40 ±0.46
45	25.17 ±0.37	26.23 ±0.37	19.19 ±0.57	20.49 ±0.43
50	25.73 ±0.36	27.26 ±0.45*	19.75 ±0.39	21.80 ±0.43*
55	26.21 ±0.37	27.78 ±0.48*	20.24 ±0.37	22.37 ±0.51*
60	26.88 ±0.29	28.07 ±0.30*	20.84 ±0.38	22.74 ±0.20*

C: Control; T: Treatment

*Significant difference from control within column ($P \leq 0.05$)

Table-1 Total leucocyte count (TLC) and Absolute lymphocyte count (ALC) values in chicks