Avian Reovirus Induces an Inhibitory Effect on Lymphoproliferation in Chickens

S. Neelima¹, G.C. Ram¹*, J.M. Kataria² and T.K. Goswami³

¹Immunology Section and ²Division of Avian Diseases, Indian Veterinary Research Institute, Izatnagar 243 122 (UP), India
*Correspondence: E-mail: gcram@ivri.up.nic.in


ABSTRACT

The cellular immune responses of chickens inoculated with the vaccine strain S-1133 and/or a field isolate VA-1 of avian reovirus (ARV) were studied. Both strains of virus caused inhibition of the phytohaemagglutinin (PHA)-induced lymphoproliferative response of peripheral blood mononuclear cells (PBMC) and splenic mononuclear cells (SMC) during the initial stage from day 4 up to day 10 post-inoculation (PI), with a later return to the normal value. The inhibition in the PHA-induced lymphoproliferation of SMC could be partially overcome by depletion of adherent cells. The supernatant of the PHA-stimulated SMC culture was also checked in vitro for the presence of suppressive factor(s) produced in response to ARV infection. The culture supernatant from chickens at day 5 PI caused significant inhibition of the PHA-induced lymphoproliferation of control birds, suggesting the presence of suppressive factor(s). ARV infection also significantly inhibited IL-2 production on day 5. There was a significant increase in nitric oxide production by the splenic mononuclear cells of chickens inoculated with either strain of ARV.

Keywords: avian reovirus, chicken, immunity, interleukin-2, lymphoproliferation, peripheral blood mononuclear cells, splenic mononuclear cells, phytohaemagglutinin, nitric oxide

Abbreviations: ARV, avian reovirus; Con A, concanavalin A; DMSO, dimethyl sulfoxide; IL-2, interleukin-2; LPA, lymphoproliferation assay; MTT, 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide; NO, nitric oxide; PBMC, peripheral blood mononuclear cells; PI, post-inoculation; PPD, purified protein derivative; TGF-β, transforming growth factor-β; SI, stimulation index; SMC, splenic mononuclear cells

INTRODUCTION

Avian reoviruses (ARVs) are ubiquitous in commercial poultry and are frequently isolated from the gastrointestinal and respiratory tracts of chickens with acute or inapparent infections (Rosenberger and Olson, 1991). ARV has been implicated in a variety of disease syndromes, including viral arthritis/tenosynovitis (Daltun and Henry, 1967) and the malabsorption or running-stunting syndrome (Kouwenhoven et al., 1978). In India, the prevalence of arthritis induced by reovirus was first reported by Kataria and colleagues (1982), later serological screening revealing its widespread prevalence in the poultry population (Kataria et al., 1983). The stunting syndrome and high economic wastage due to ARV infection on several broiler farms around Bangalore in India was reported by Joshi and Gowda (1995).
ARV may cause immunosuppression in chickens (Springer et al., 1983; Montgomery et al., 1985) and predispose the host to other infectious agents and stresses present in the environment. Immunosuppression caused by ARV may also influence the success of vaccination against other infectious diseases, such as infectious bursal disease and inclusion body hepatitis (Kudron et al., 1982). Chickens infected with reovirus in the field have an increased incidence of secondary bacterial infections with Staphylococcus aureus (Kibenge et al., 1982a,b).

Although this immunosuppression is well documented, the mechanism underlying it is still poorly defined. ARV is known to replicate in macrophages (von Bulow and Klasen, 1983; Hafer, 1984, Mills and Wilcox, 1993), to induce suppressor macrophages that inhibit the T-cell proliferative response to mitogens and to induce enhanced nitric oxide production in response to T-cell mitogens (Pertle et al., 1995).

The primary objective of this study was to determine the effect of ARV infection on the proliferative ability of lymphocytes and to study the characteristics of the immunosuppression induced by this virus.

MATERIALS AND METHODS

**Chickens**

Unvaccinated day-old broiler chicks of either sex were used. The chicks were reared in isolation and feed and water was provided ad libitum.

**Viruses**

Two isolates of ARV were used: a commercial reovirus vaccine based on the S-1133 isolate was obtained from Intervet (India), Hyderabad, India, and a field isolate (VA-1) of ARV isolated from a case of arthritis (Kataria et al., 1982), was obtained from the Avian Disease Division, IVRI, Izatnagar, India. The stock viruses were titrated on 10- to 11-day-old embryonated chicken eggs and the titre was expressed as the 50% embryo infective dose (EID$_{50}$).

**Media and chemicals**

RPMI-1640 growth medium (RPMI-GM), pH 7.2, contained RPMI-1640 medium (obtained from Sigma, St Louis, MO, USA) with 10% newborn calf serum, 25 mmol/L Hepes, 20 mmol/L sodium bicarbonate, penicillin (100 IU/ml) and streptomycin (100 µg/ml). Heparin, Histopaque 1083, trypan blue, sulphanilamide, phytohaemagglutinin (PHA-P), concanavalin A (Con A) and naphthylene-diamine dihydrochloride were all obtained from Sigma. Dimethyl sulphoxide (DMSO) and 3-[4,5-dimethylthiazole-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were obtained from Sisco Research Laboratories, Mumbai, India.