Relationship between virus replication and apoptosis events in IgM + cells from chicken spleen and bursa of Fabricius infected with Malaysia strain of very virulent infectious bursal disease virus

ABSTRACT

Background: Infection of IBDV was reported to be endemic in worldwide including Malaysia and can be spread orally thru polluted fodder and water source, thus causing economic losses especially in commercial poultry industry. The infection resulted in depletion of B lymphocytes and subsequently destruction of the bursa which leaded to immunosuppression of the bird and it was postulated that the depletion of cells in the bursa was due to induction of apoptosis. In the current study, the infection of Malaysia isolated very virulent IBDV UPM0081 on IgM bearing B lymphocytes (IgM+ cells) from chicken spleen and bursa was compared.

Materials, Methods & Results: A total of sixty eggs were obtained and raised until the age of 3 weeks old. The birds were divided into two groups (n = 30), which one of them served as control while IBDV strain UPM0081 was used to infect another group of birds at the concentration of 103 ELD50. The birds were observed and sacrificed at day 2, 4 and 5 post infections. Spleen and bursa of Fabricius were harvested and subjected to IgM+ cell enrichment using microbeads. The cell viability of enriched cells was assayed using MTT and cell cycle was analyzed using propidium iodide. Annexin V FITC and acridine orange/propidium iodide double stain assays were used to determine the event of apoptosis in the enriched IgM+ cells. Also, the IBDV viral load was also quantified by using real time PCR to evaluate the relationship between virus replication and apoptosis events in the infected chickens. Current results showed that the apoptotic events were observed to be significantly higher in IgM+ cells isolated from chicken bursa as compared to the cells isolated from spleen. The bursal B lymphocytes cell viability was observed to be decreasing following the infection of very virulent IBDV. The cells were then investigated of their apoptotic rate and data showed that increasing apoptotic cells (early and late apoptosis) were observed in AO/PI double stain as well as increment of SubG0/G1 population in the cell cycle analysis and also increment of Annexin V FITC bound cells in the apoptosis study. As for B lymphocytes from chicken spleen, the magnitude of damage caused by very virulent IBDV was not as severe as what being observed in the chicken bursa, with the cell viability drastically decreased on day 4 following IBDV infection.

Discussion: IBDV caused severe destruction in bursa of Fabricius compared to spleen, in which cell death events in the former was reported to be directly caused by the virus. Apoptotic event in chicken spleen following IBDV infection was observed to be caused by oxidative stress. Thus, viral replication played a role in inducing bursal IgM+ cells death while such phenomenon was not observed in spleen isolated IgM+ cells. In summary, the cell death events of IgM+ cells in chicken spleen and bursa of Fabricius may be accounted by different factors upon infection with Malaysia strain of IBDV UPM0081. It is obvious that

IgM+ cells from chicken bursa suffered from apoptotic cell death in an increasing manner considerably with time of infection and RNA load detected in the cells, which supported by previous literature that IBDV induces host cells apoptosis, with both VP2 and VP5 playing a role in binding and apoptosis. Meanwhile, the cell death events of B lymphocytes in chicken spleen was observed to be more relevant to other factors such as the oxidative stress or proinflammatory cytokines that caused by the virus infection rather than the viral RNA load.

Keyword: Infectious bursal disease virus; Apoptosis; Avian; Spleen; Bursa of Fabricius; IgM+ cells