Title: ANALYSIS OF THE CELL IMMUNE RESPONSE OF AVIAN INFECTIOUS BRONCHITIS VARIANTS BY FLOW CYTOMETRY

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Introduction: Avian infectious bronchitis virus (IBV) is primarily replicates in epithelial cells of the upper respiratory tract. Indeed the great number of IBV strains and variants are routinely pointed out as a cause of outbreaks in vaccinated flocks. However, the association between the local immune responses induced by IBV and the viral immune evasion mechanisms has not yet been completely elucidated. This study investigated and compared the early immune responses induced by the infection with different Brazilian field variants of IBV, in chickens previously immunized or not with the attenuated H120 vaccine. Methods/Results: White Leghorn SPF chicks were housed in separate positive pressure isolators: CN (control group), NP (respiratory non-pathogenic strain (M41-like attenuated strain)), NF (nephrogenic strain (IBV-variant 448/1998) and RP (pathogenic respiratory strain (strain F3735-Brazilian reference M41 field isolate)). All groups were vaccinated with attenuated strain H120 at day 1. At 28 days of age, groups were experimentally infected with strains. Peripheral blood mononuclear cells were obtained at day 1 post-infection (d.p.i) and stained (CD4, CD8?, MHCII, TCR??/V?1, CD28, Kul-01) to characterize phagocytes cells, helper and cytotoxic T cells by flow cytometry. The quantity of phagocytic monocytes in the control group was higher than in the challenged groups (NP: p<0.05; NF: p<0.01; and RP: p<0.001). NF and RP groups presented a higher quantity of APC (p<0.001) than the control group. When we looked to the T cells, a higher number of non-activated T cells in the challenged groups (NP: p<0.05; NF: p<0.001; and RP: p<0.001). However, the non-activated CD8T cells a reduced amount was observed in the challenged groups NP(p<0.001), NF(p<0.001) and RP(p<0.001) when compared to the control group. There was no significant difference in the amount of activated circulating CD4T and CD8T between the groups. Conclusion: As expected, the challenged groups showed a decreased frequency of non-activated CD8T cells, as well as monocytes. Because it is an acute phase of the viral challenge the decrease in the absolute number of lymphocytes may be due to the homing effect, when naïve lymphocytes (CD8?+CD28+) leave the circulation and enter the lymphoid tissues. Our results suggest similar patterns of innate and adaptive immune response at 1 d.p.i elicited by H120 vaccine in the challenged animals demonstrated a protective action against these Brazilian strains.