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# ADAPTIVE IMMUNITY IN CONJUNCTIVA ASSOCIATED LYMPHOID TISSUE AFTER OCULAR IMMUNIZATION

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Conjunctiva-associated lymphoid tissues (CALT) constitute along with the Harderian glands the chickens main para-ocular lymphoid system. These tissues are the first to be exposed to pathogens entering by the ocular or nasal routes and to provide adaptive immunity and, consequently, influence avian disease resistance. Although considerable knowledge has accumulated regarding the role of Harderian glands in ocular immunity, the role of CALT in generating antigen-specific ocular immune responses has not been well defined. It is assumed that CALT, like Harderian glands, have a sentinel role protecting mucosal surfaces of the eye. To test whether CALT plays an important role in generating protective ocular immunity, chickens were ocularly immunized with a human, replication-deficient adenovirus vector of serotype 5 (Ad5). Initially, the T cell composition of CALT was analyzed by fluorescence-activated cell sorting (FACS). Approximately 37% of the lymphocytes were CD3<sup>+</sup> of these CD3<sup>+</sup> lymphocytes about half of them were CD4 positive and ~20% CD8 positive. Thus, both T helper and cytotoxic T cells are present in CALT, which can drive humoral and cell mediated immune responses after ocular Ad5 immunization. The induction by Ad5-specific ocular immunity was supported by elevated levels of IFN-gamma in tears. In order for B cell responses to be effective at mucosal surfaces, transport of IgA across the epithelium by the polymeric immunoglobulin receptor (pIgR) would be required. Our RT-PCR and immunohistochemistry results indicated, that the pIgR is expressed by epithelial cells of the eyelid. Analyses by RT-PCR and ELISPOT demonstrated that ocular immunization with Ad5 resulted in increased production of IFN-gamma in both CD4<sup>+</sup> and CD8<sup>+</sup> lymphocytes as well as induction of Ad5-specific antibody secreting B cells in CALT. Thus, CALT are important mucosal inductive sites producing antigen-specific antibodies and activated cytotoxic T cells and T helper type 1 cells after ocular Ad5 immunization. The epithelium of the eyelid expressed the pIgR allowing active transport of IgA. The prevalence of IgA producing cells in CALT combined with the presence of pIgR demonstrates that CALT contributes to mucosal immune protection of the eye.