CHICKEN MACROPHAGE ACTIVATION THROUGH INTERACTION BETWEEN CD80 AND CD86 RECEPTORS WITH RECOMBINANT PROTEIN CD28

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It is known that double signaling (MHC/CD3 and CD28/CD80-86) act synergically on T cells activation. Both functionally and structural studies on CD28-CD80/86 interaction are mainly focused on lymphocyte pathway activation, while APCs are typically interpreted as supporting cells. The aim of our project is to show that APCs, particularly macrophages, can be engaged in CD28-dependent T cell activation (reverse signaling) in avian immune system. To test this hypothesis, we analyzed cytokine production by chicken macrophages stimulated by recombinant CD28, as a T cell mimetic. Therefore, the ectodomain of wild-type CD28 was cloned in a His-tagged protein vector (pET-28a) and expressed in E. coli system (BL21(DE3)STAR). In addition, a deletion mutant (CD28Δ) devoid of the binding site for CD80-86 (MYPPPY) was generated as control. Since both recombinant proteins were expressed as inclusion bodies, soluble protein was initially obtained only in 8M urea-solubilizing conditions. Using a stock CD28 solution (in 8M urea) we established that the final CD28 concentration desirable to carry out our biological experiments produces only 4mM urea concentration in the macrophage culture media. In these conditions, urea seems to be innocuous to macrophage cultures and remarkable CD28 remains soluble. Meanwhile, qPCR conditions for chicken IL2, IL4, IL6, IL13, TNFα and IFNγ quantification were also established. Preliminary results suggest that IL-6 mRNA expression is increased when HD11, a chicken macrophage established cell line, was challenged with recombinant CD28. This suggests, for the first time, that chicken macrophage could induce reverse signaling mediated by CD28 receptor engagement.

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