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AA AMYLOID FORMATION BY PRIMARY CHICKEN FIBROBLAST-LIKE SYNOVIOCYTES

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Introduction

Some strains of commercial layer chickens are prone to develop AA-amyloid arthropathy in response to chronic arthritis, whereas others are resistant. In previous studies on experimental brown layers during amyloidogenesis in the joint the possibility of extrahepatic production of serum amyloid A (SAA) has been suggested. Isolated fibroblast-like synoviocytes (FLS) appeared to form SAA protein after stimulation with LPS. Recent findings of *in vitro* studies by various authors reveal that small β-pleated sheet structure proteins accelerate amyloid fibrillogenesis from precursor proteins. The so called 'amyloid enhancing factor" which can be obtained from β-pleated proteins such as amyloid fibrils or silk, may act as nidus for the accelerated fibrillogenesis. The aim of the present study was to study extrahepatic AA amyloid formation by synoviocytes, and to investigate the mechanism of AA-amyloidogenesis.

Materials and Methods

Primary chicken FLS originating from brown layer were isolated and cultured *in vitro*. Recombinant chicken SAA (rchSAA) was generated using the pGEX bacterial expression system and fibril-derived amyloid enhancing factor (FAEF) solution was prepared. Fibril formation of rchSAA was assessed by Congo red staining and by electron microscopy. The capability of FLS to degrade rchSAA was examined by gel electrophoresis and immunoblotting. Finally, AA-amyloid fibril formation in cultured FLS was assessed by Congo red staining and immunohistochemistry.

Results

After incubating rchSAA in acidic buffer without cells, small fibril fragments formed which on Congo red staining revealed green birefringence. When FLS were incubated with rchSAA under neutral conditions, degradation products of rchSAA were detected in culture medium within 48 hours. However, prolonged culture time (15 days) did not result formation of amyloid fibrils. Addition of FAEF to this culture system under a neutral environment, evident amyloid fibrils were formed after 48 h and more abundantly after 5 days.

Conclusions

The present findings suggest that chicken FLS which earlier were found to act as a source of SAA in the joint during infection and inflammation, can favour amyloid formation from the formed SAA. This process may be mediated by fragments of degraded amyloid or other β-pleated units which may be derived from inflammatory cells. The amyloidogenesis process itself appeared to be independent of macrophages.