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Expression of a bioactive recombinant human interleukin-11 in chicken HD11 cell line

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Abstract

To direct the synthesis and secretion of recombinant human interleukin-11 (rhIL-11) in chicken HD11 cells, a plasmid targeting the c-lysozyme gene has been constructed which contains the mature cytokine cDNA in frame with the lysozyme leader sequence. The upregulation of rhIL-11 mediated by LPS proves the knock-in of hIL-11 cDNA in the lysozyme gene. The bioactivity of the expressed protein is demonstrated and quantified with the hIL-11 dependent 7TD1 and B9 cell lines. The electrophoretic mobility, receptor binding properties and growth promoting effect of the chicken-derived cytokine are identical to those of a rhIL-11 expressed in *Escherichia coli*. These results describe the secretion of a biologically active rhIL-11 expressed by an avian cellular machinery.

Keywords: Human interleukin-11; Chicken HD11; c-Lysozyme locus

1. Introduction

The interleukin-11 (IL-11), a member of the IL-6 sub-family of cytokine [1–4], exhibits multiple biological activities in both hematopoietic and non-hematopoietic systems [5]. Its predominant activity is to stimulate the differentiation, maturation and proliferation of megakaryocytes, resulting in increased thrombopoiesis [6–9]. Preclinical and clinical trials have demonstrated the efficiency and safety of this cytokine when used to prevent severe chemotherapy-induced thrombocytopenia [10–13]. A functional modified form of hIL-11, purified from *Escherichia coli*, is given to patients with nonmyeloid malignancies to reduce the need of platelet transfusions [12].

Several cellular models like bacteria [14], yeast [15], silkworm [16] and monkey cells [17] have been used to express, purify and characterize native or truncated rhIL-11. In many respects, chick is considered as a very promising system to produce eukaryotic proteins, including the large glycosylation potentialities which pattern, in the case of IgGs, is closer to human than other mammalian species [18]. Exogenous bioactive proteins have been expressed in the oviduct [19–21] and the chicken egg white [19,20], and germline transmission of transgenes has been described [19,22,23]. However, many efforts have to be made to circumvent low expression level [24] and silencing due to the random

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integration of the replication-defective retroviral vectors used in these studies. An alternative method would be to introduce the transgene into defined genomic loci by a homologous recombination ("knock-in") approach [25]. The transgene localization and its spatio-temporal expression would then be controlled.

The c-lysozyme gene is expressed in the mature oviduct and the myeloid cell lineage [26] and is considered as a locus of choice for foreign protein expression strategies [27]. When used as a transgene, the chicken lysozyme gene resists to chromosomal position effect-mediated silencing [26]. Furthermore, several studies have demonstrated the efficiency of its signal peptide for the secretion of heterologous proteins [27–30]. Lipopolysaccharide (LPS) activated HD11 cell line has been widely used for functional and structural studies of c-lysozyme expression in myelomonocytic cells [31–34]. In the present study, we use the chicken cell line to evaluate in vitro rhIL-11 expression by a bird cellular machinery, using a homologous recombination vector constructed with c-lysozyme genomic sequences and human IL-11 cDNA. We report the expression of a biologically active rhIL-11 fused with the lysozyme leader sequence, under the control of the lysozyme regulatory elements.

2. Results

2.1. Expression of rhIL-11 in HD11 cells transfected with pLyso1-18/IL-11

Previous northern blot analysis has shown a constitutive expression of the lysozyme gene in the macrophage cell line HD11 as well as an upregulation mediated by LPS [44]. To evaluate rhIL-11 expression, the supernatant of HD11 cells stably transformed with pLyso1-18/IL-11 was collected after 2 days of culture and subjected to an ELISA quantification. Fig. 1 shows specific expression of rhIL-11 in supernatants of transfected cells with a 2 fold upregulation of rhIL-11 following a LPS treatment. The main regulatory sequences involved in the control of tissue specific expression and differentiation state activation of the lysozyme gene in chicken myeloid cells have been localized at −6.1 kb, −3.9 kb, −2.7 kb, −2.4 kb, −1.0 kb, −0.25 kb and −0.2 kb (for a review see Ref. [45]). The 5′ homologous arm of our construct which is 2.7 kb length contains 5 of these elements. Since the enhancer that mediates LPS activation of the lysozyme gene in HD11 cells is located 6.1 kb upstream of the transcription start site [44], the LPS upregulation of rhIL-11 expression is possible only if the construct inserts by homologous recombination in the lysozyme locus. PCR amplification on transfected HD11 genomic

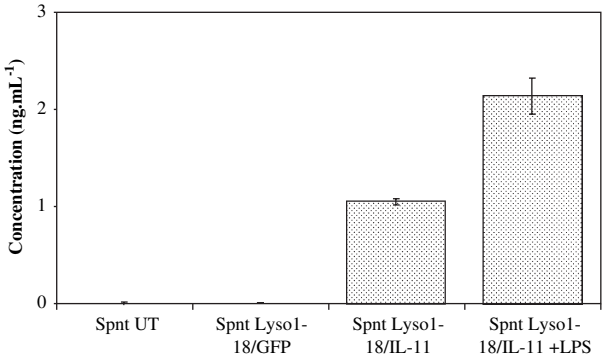

Fig. 1. Expression of rhIL-11 detected by ELISA quantification. HD11 cells were transfected or not (UT) with pLyso1-18 vector encoding GFP or hIL-11. After 48 h of culture, supernatant (Spnt) of transfected neomycin resistant and UT cells was collected and analysed for rhIL-11 detection. Cells transfected with plyso1-18/IL-11 were either treated with 5 μg mL^{-1} LPS or not. Each sample was tested in duplicate.

DNA confirmed that our targeting vector is able to recombine in the lysozyme locus (Fig. 2).

We determined the optimal culture conditions regarding serum and LPS concentrations to get the best rhIL-11 expression. The results shown in Fig. 3A and B confirmed the LPS effect previously observed and showed that upregulation of rhIL-11 expression was almost maximal at 1% serum and 2 μg mL^{-1} of LPS. As a consequence, these culture conditions were applied to produce HD11 supernatant.

2.2. Recombinant hIL-11 expressed in HD11 cells is biologically active

Cell proliferation assays were carried out using the IL-11 dependent murine hybridoma cell line 7TD1 to evaluate the bioactivity of rhIL-11 secreted by transfected HD11 cells (Fig. 4A). Supernatants from pLyso1-18/IL-11 transfected cells had a much stronger effect than those from non-transfected cells in the 7TD1 proliferation assay (Fig. 4A). When comparing the dilutions giving half-maximal proliferation, a 100 fold ratio was measured, indicating that about 99% of the growth promoting activity contained in the supernatant of transfected cells was due to the expression of the human transgene. Neutralization experiments proved that this activity was due to secreted rhIL-11 (Fig. 4B). The H56 monoclonal antibody has been previously shown to interact with an epitope located within site II of human IL-11 (C. Blanc, unpublished data), a site that is involved in the recruitment of the gp130 transducing receptor chain [46], and to block IL-11 action [42]. This antibody completely inhibited the growth promoting activity of both *E. coli*-derived rhIL-11 and transfected HD11 supernatants. As a negative control, proliferation induced by IL-6 was not affected by H56. Proliferation