A common variant associated with asthma, interleukin 13 R130Q, promotes the production of IgE

Y. Chu, L. Hua, Q. Liu & Y. Bao

Summary
Interleukin (IL)-13 plays an important role in the pathogenesis of asthma. A polymorphic variant of human IL-13 R130Q, results in substitution of an arginine with a glutamine was shown to be associated with asthma in Chinese Han nationality. We examined the functional consequences of this variant in vitro to investigate whether this variant enhanced functional activity compared with wild type IL-13. The wild-type and mutant IL-13 genes were amplified from the plasmid of pET22b-hIL-13 by PCR and site-directed mutagenesis PCR. Both the PCR product and the vector pET28a(+) were digested by the NdeI and BamHI. Then the PCR product was cloned in the prokaryotic expression vector of pET28a(+). The plasmids were constructed and transformed into E. coli BL21(DE3). The positive clones were selected, and tested by sequencing. Peripheral blood mononuclear cells (PBMCs) from healthy participants were isolated and cultured with increasing concentrations of recombinant WT IL-13 and IL-13 R130Q. IgE was detected with ELISA kit in the supernatants. Recombinant WT IL-13 and IL-13 R130Q were successfully expressed into the prokaryotic expression system and their biological activity was consistent with standard protein. Our results show that IL-13 R130Q is more active than WT IL-13 in inducing hydrocortisone-dependent IgE synthesis. There were statistical significances between them. IgE induction by physiologic concentrations was obviously increased. IL-13 R130Q has increased activity compared with wild type IL-13 in vitro. And IL-13 R130Q may be used for new target of asthma for diagnosis and therapy in the future.

Introduction
Asthma is one of the most common chronic inflammatory lung diseases worldwide. It is well known that asthma is an immune-mediated disease and associated with an excessive T-helper type 2 (Th2) immune response (Busse & Lemanske, 2001; Umetsu et al., 2002).

Interleukin (IL)-13 is produced by Th2 cells in response to antigen receptor engagement (Fattouh & Jordana, 2008) and can induce IgE synthesis in cultured B cells (Punnonen et al., 1993). The IL-13 gene is located in the chromosome 5q31-q33 region, and its role in the genetics of allergic diseases, such as asthma, has already been widely investigated (Punnonen et al., 1993). Furthermore, some animal models of allergic lung inflammation have provided compelling evidence that IL-13 plays a pivotal role in the development of cardinal features of allergic asthma including airway hyper-responsiveness, remodelling and eosinophilic inflammation (Wills-Karp, 2004).

The IL-13 gene contains a block of common single-nucleotide polymorphisms (SNPs) in virtually complete linkage disequilibrium (LD), which span the third intron (+1923CT), the fourth exon (+2044GA) and the 3’ untranslated region of the gene (+2525GA, +2580CA, 2749CT) (Graves et al., 2000). IL-13 A2044G is expected to result in the nonconservative replacement of arginine (130R) with glutamine (130Q) and is associated with bronchial asthma (Heinzmann et al., 2000), atopic dermatitis (He et al., 2003) and increased IgE levels (Graves et al., 2000).

Previously, we have shown that a polymorphic variant of human IL-13, R130Q, increased activity compared to wild-type (WT) IL-13 using statistical analysis in children of Chinese Han nationality (Li et al., 2009). The aim of this study was to investigate whether this variant has enhanced functional activity in vitro. Thus, we examined the effects of WT IL-13 and IL-13 R130Q priming on IgE synthesis.

Materials and methods
Expression of recombinant IL-13

The WT and mutant IL-13 genes were amplified from the plasmid pET22b-hIL-13 by PCR and site-directed mutagenesis PCR. The primers were designed as followings: IL-13 forward-primer: 5’-TATACTCGGATATGG GCCCTGTGCTCCTCCCTCTA-3’; IL-13 reverse-primer: 5’-TATGGATCCITTATCGTGGAAACCGTCCCTGC
-3’-IL-13 mutant reverse-primer: 5’-TATGGATCTCTTA TCAGTTGAACCTGGCCTGGG-3’. The primers contain an NdeI and a BamHI site (underlined) at their 5’ and 3’ ends.

Amplification of IL-13 was performed in a 50-μL total volume reaction containing 50 pmol μL⁻¹ of each primer, 10 × PCR buffer, 25 mM dNTPs, plasmid pET22b-hIL-13 and Pfu DNA polymerase. Samples were denatured for 5 min at 95°C and then cycled 23 times through the following steps: 30 s at 95°C, 45 s at 68°C and 1 min at 72°C. The PCR products were subjected to electrophoresis on 2% agarose gel and visualized by ethidium bromide staining. The PCR product was then digested at 37°C for 12 h using NdeI and BamHI restriction enzymes (MBI Fermentas, Glen Burnie, ML, USA). The digests were cloned into the pET28a(+) expression vector to construct the expression plasmids that were transformed into E. coli BL21(DE3). To identify positive clones, we used specific primers and site-directed mutagenesis primer to amplify gene fragment. The plasmid that contained the target gene was purified and sequenced.

 expression of recombinant protein was induced by isopropylthio-β-D-galactoside (IPTG), and the expressed product was purified through a Ni column (Ni-NTA). TF-1 erythroleukemia cells proliferate in response to IL-13, so we analysed the bioactivity of expressed WT IL-13 and IL-13 R130Q using an 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay to measure the proliferative response of TF-1 cells to WT IL-13 and the R130Q variant.

MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay

TF-1 cells exhibited a strong proliferate response to granulocyte–macrophage colony-stimulatory factor (GM-CSF), so we used rhGM-CSF as standard protein in this study. Cells were cultured with various concentrations of WT IL-13, the R130Q variant and rhGM-CSF for 24 h, and then 2 × 10³ cells/well were seeded into 96-well plates in triplicate. Briefly, 10 μL of MTT [5 μg mL⁻¹ in phosphate buffered saline (PBS)] was added to each well, and the cells were incubated at 37°C for 4 h. Cell culture medium was then removed, and 100 μL DMSO was added to the wells. Plates were briefly shaken at 60 rpm for 5 min to dissolve the precipitate and remove the bubbles and then read at 490 nm using a microplate reader. The cellular proliferation curve was delineated by the mean absorbency at different concentrations. Cells that were cultured with PBS were used as the negative control.

Peripheral blood mononuclear cells (PBMCs) culture and IgE determination

The participants (n = 6) were normal nonatopic students from the Medical School of Shanghai Jiaotong University. The participants ranged from 22 to 26 years of age (50% men and 50% women). Approval for human studies protocol was obtained from Xinhua Hospital affiliated to Shanghai Jiaotong University, and informed consent was obtained from all participants. PBMCs isolated through Ficoll-Hypaque density centrifugation were washed and responded (3.0 × 10⁶ cells per mL) in complete RPMI-1640 medium (GIBCO, Grand Island, NY, USA) containing 100U mL⁻¹ penicillin and 100U mL⁻¹ streptomycin (GIBCO) supplemented with 10% foetal bovine serum (HyClone, Glen Burnie, ML, USA) and maintained at 37°C in 5% CO₂. Cells were resuspended and stimulated with increasing concentrations of WT IL-13 or IL-13 R130Q in the presence of hydrocortisone (HC, 1 μM). Culture supernatants were harvested after 7 days and assessed for IgE concentration by ELISA kit (CUSABIO BIOTECH).

Statistical analysis

All statistical analyses were performed using SPSS version 13.0, and all results were expressed as means ± SEs obtained from more than three replicates. A 2-tailed paired Student’s t-test was used to compare bioactivity of recombinant IL-13 as well as response to WT IL-13 and IL-13 R130Q. P values for significance were set at 0.05.

Results

Expression of WT IL-13 and IL-13R130Q

The WT and mutant IL-13 genes were amplified from the plasmid of pET22b-hIL-13 by PCR and site-directed mutagenesis PCR. The products are shown in Fig. 1. This shows that the genes were inserted and there was no mutation or the reading frame shift when we sequenced the recombinant plasmid using universal primer. The sequences were in accordance with the IL-13 target gene.
cDNA, which were published by Genbank (Genbank accession number AC000137.1). The recombinant IL-13 and IL-13R130Q were successfully expressed in the form of inclusion bodies; the polypeptide showed a relative molecular mass of about 14.4 kDa by SDS-PAGE (Fig. 2) in accordance with the design.

The recombinant proteins of WT IL-13 and IL-13 R130Q have biological activity and are consistent with standard protein

TF-1 cells were cultured in the presence of various concentrations of IL-13, R130Q variant or standard rhGM-CSF. Subsequent cell proliferation was detected by MTT assay. The results demonstrated that TF-1 cell proliferation in response to the recombinant proteins WT IL-13 or R130Q variant was comparable to standard rhGM-CSF (Fig. 3). The proliferation of TF-1 cells was promoted after cultured with recombinant WT IL-13, IL-13 R130Q and rhGM-CSF. The biological activity of WT IL-13 or IL-13 R130Q was consistent with rhGM-CSF. There was no significant difference of biological activity between the recombinant WT IL-13 and IL-13 R130Q [OD = (0.17 ± 0.01) vs. OD = (0.18 ± 0.02), P > 0.05] at the concentration of 0.01 ng mL⁻¹. The values of OD of WT IL-13 at the concentration of 0.02, 0.04, 0.1, 0.2 and 0.4 were 0.24 ± 0.03, 0.30 ± 0.02, 0.34 ± 0.02, 0.40 ± 0.02 and 0.54 ± 0.03, respectively. The values of OD of IL-13 R130Q at the concentration of 0.02, 0.04, 0.1, 0.2 and 0.4 were 0.26 ± 0.02, 0.32 ± 0.01, 0.36 ± 0.02, 0.42 ± 0.01 and 0.56 ± 0.01, respectively. There was also no significant difference between two recombinant proteins at various concentrations (P > 0.05).

IL-13 R130Q is more active than WT IL-13 in inducing hydrocortisone-dependent IgE synthesis

PBMCs from nonallergic donors (n = 6) were isolated through Ficoll-Hypaque density centrifugation and incubated with increasing concentrations of WT IL-13 or IL-13 R130Q in the presence of hydrocortisone (HC). Figure 4 shows that IgE synthesis was significantly increased in response to IL-13 stimulation, particularly with IL-13 R130Q. Substantial IgE synthesis, in response to IL-13 R130Q [(0.16 ± 0.02)µg mL⁻¹ vs. (0.49 ± 0.07)µg mL⁻¹, P < 0.001], was detected in cultures simulated with 90 pg mL⁻¹ of variant. The difference in the response to a higher IL-13 concentration (500 pg mL⁻¹) approached statistical significance [(0.16 ± 0.03) µg mL⁻¹ vs. (0.47 ± 0.05) µg mL⁻¹].
in vivo in various ways; IL-13R et al. one another (Kioi et al., 2003). Numerous studies have revealed that IL-13 R130Q, a common variant encoded by the IL-13 A2044G polymorphism, markedly increases the risk of developing asthma (Kim et al., 2006; Hosseini-Farahabadi et al., 2007; Llanes et al., 2009; Park et al., 2009). However, such data provide evidence for the significance of the WT IL-13 and IL-13 R130Q variant by way of statistical analysis, while our experimental results show that IL-13 R130Q is significantly more active in elevating serum IgE in vitro. As mentioned earlier, elevated IgE has been proved to be associated with a higher risk of allergic disease (Sears et al., 1991; Douglass & O’Hehir, 2006). IL-13-induced IgG4 and IgE synthesis reflects immunoglobulin isotype switching and is not because of a selective outgrowth of new B cells committed to IgG4 or IgE production. In the current study, PBMCs from nonallergic donors were incubated with increasing concentrations of WT IL-13 or IL-13 R130Q in the presence of HC, and IgE synthesis was significantly increased. IgE induction by physiologic concentrations was obviously increased, but when we used supraphysiologic concentrations to stimulate PBMCs, the IgE induction was decreased. In contrast, (Tollerud et al., 1991) have found a strong association between elevated IL-13 levels and IgE production in vivo in a group of children. So whether this association between IL-13 and IgE production did occur still needed to be proved in vitro.

The mechanism accounting for the observed increased activity of IL-13 R130Q variant is not yet clear. As we know, the biological activity of IL-13 is regulated via type 1 and type 2 IL-13 receptors. The type 1 receptor is a heterodimer of the IL-4R alpha chain (α) and IL-13Rα1; the type 2 receptor is composed of the IL-13Rα1 and IL-13Rα2 chains (Ly et al., 2003; Finkelman et al., 2010). The IL-13Rα2 chain binds IL-13 with high affinity and internalizes after binding to ligand without involvement of other chains (Donaldson et al., 1998). In addition, IL-13 appears to regulate the expression of IL-13Rα2, suggesting that ligand and receptor may cross-regulate one another (Kioi et al., 2008). However, Kioi et al. (2008) have shown that IL-13Rα2 may act to attenuate IL-13 activity by increasing the strength of IL-13 signalling or negative signalling or simply as a molecular decoy. Currently, many studies have suggested that IL-13Rα2 was a key negative regulator of IL-13 in vivo (Wood et al., 2003). A computer modelling study suggested that Arg130 was directly involved in the interaction with IL-13 receptor and that charge-changing variants were likely to display different biological properties. Alanine scanning mutagenesis revealed 130R to be important for IL-13 binding to IL-13Rα2 (Graves et al., 2000). (Mitchell et al., 2010) suggested that the variant showed a lower affinity with the IL-13Rα2 and an enhanced stability in both human and mouse plasma. Substitution of 130R with the negatively charged aspartic acid created an artificial agonist which bound the IL-13 receptor with 5- to 10-fold improved affinity, so the 130Q changes the activity of the molecular and mutant IL-13 enhanced signal transmission. It was also found that among asthmatic children, subjects homozygous for Gln130 (130Q) had higher levels of serum IL-13 than those homozygous for Arg130 (Arima et al., 2002). The structural IL-13 change led to functional change, so IL-13 R130Q as a functional genetic factor could elevate synthesis of IgE as shown in our results.

It has been shown that hydrocortisone (HC) and its synthetic derivatives are able to potentiate in vitro IL-4-induced IgE production by PBMCs (Nüsslein et al., 1994; Noguchi et al., 2001), but the interaction between HC and IL-13 has not been investigated. There is a positive crosstalk between IL-13 and HC in earlier research, and this crosstalk may reflect unexpected functional differences in the B-cell signalling pathways that lead to IgE synthesis (Nüsslein et al., 1992). Our results confirmed this view by using WT IL-13 and IL-13 R130Q to stimulate PBMCs in the presence of HC. As one of the agents of pharmacotherapy of asthma, HC could control acute asthma (Vladich et al., 2005). Cho et al., (2002) suggested that HC could enhance allergen-specific IgE production by PBMCs from atopic patients. On the other hand, Klebl et al., (1994) suggested that glucocorticoid treatment does not give rise to a substantially enhanced risk for increased IgE synthesis and the development of sensitizations in nonallergic persons receiving steroids. So further studies should be performed in vivo or in vitro.

IL-13 has been proposed as a therapeutic target for bronchial asthma because it plays crucial roles in the pathogenesis of the disease. Choi et al., (2009) have developed an in vitro test system measuring transcriptional downregulatory activities on IL-13 as a primary screening method to select drug candidates from natural products.

In conclusion, our results reported here confirmed that IL-13 R130Q increased activity compared with WT IL-13. The SNP identified in this study may be used to develop markers to assess the risk of asthma. IL-13 R130Q may be used for new target of asthma for diagnosis and therapy in the future.
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Conflicts of interest
The authors declare that they have no competing interests.

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