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Eve 378 FASE NACIONAL
Act 444 [illegible] Folios 40

Re <u>P1.-**GLAXO GROUP LIMITED.**-Solicitud de Patente de Invención en Fase Nacional PCT para **"VACUNA ÚTIL PARA LA INMUNIZACIÓN CONTRA AUTO-PROTEINAS, PARTICULARMENTE CITOQUINAS".**-Clase **C12N 15/24.**-Expediente número **03-075.597.-**</u>

1 Me refiero al auto dictado por ese Despacho notificado mediante fijación en lista de 12 de Enero de 2007

2 De acuerdo con el Concepto Técnico No **2031** se tiene que

"Como resultado de la búsqueda de anterioridades se encontró que la solicitud en estudio corresponde con la solicitud europea y publicada con el número WO 02/070711 El análisis del informe de búsqueda internacional de la solicitud extranjera establece como documentos que afecta la novedad del objeto de la solicitud Colombiana los siguientes·

- **MCKENZIE A N J ET AL. "Structural comparison and chromosomal localization of the human and mouse IL-13 genes" JOURNAL F IMMUNOLOGY, THE WILLIAMS AND WILKINS CO. BALTIM RE, US, Vol 150, No. 12, 15 June 1993 (1993-06-15), pages 5436-5444**

- **BUITKAMP JOHANNES ET AL · "The cattle interleukin-13 gene Genomic organization, chromosomal location, and evolution of the promoter" IMMUNOGENETICS, vol 49, no. 10, 1999, pages 872-878, XP002198871 ISSN:0093-7711**

- **HEINZMANN A ET AL· "Genetic variants of IL-13 signalling and human asthma and atopy" HUMAN M LECULAR GENETICS, vol 9, no 4, 1 March 2000 (2000-03-01), pages 549-559.**

"Dado que el artículo mencionado es el resultado del examen de patentabilidad efectuado a la solicitud extranjera que corresponde con la solicitud Colombiana y habiendo sido imposible la consecución de dicho documento, esta oficina requiere que el solicitante proporcione en un plazo que no excederá de 3 meses copia del artículo mencionados anteriormente Nos permitimos recordarle que si el solicitante no presentara los documentos requeridos dentro del plazo señalado, la oficina nacional competente denegará la patente"

3 Para responder a los requerimientos del señor Examinador de conformidad con el Artículo 46 de la Decisión 486, anexo me permito presentar a ese Despacho copia de los siguientes documentos solicitados

- MCKENZIE A N J ET AL "Structural comparison and chromosomal localization of the human and mouse IL-13 genes" JOURNAL OF IMMUNOLOGY, THE WILLIAMS AND WILKINS CO BALTIMORE, US, Vol 150, No 12, 15 June 1993 (1993-06-15), pages 5436-5444

- BUITKAMP JOHANNES ET AL "The cattle interleukin-13 gene Genomic organization, chromosomal location, and evolution of the promoter" IMMUNOGENETICS, vol 49, no 10, 1999, pages 872-878, XP002198871 ISSN 0093-7711

- HEINZMANN A ET AL "Genetic variants of IL-13 signalling and human asthma and atopy" HUMAN MOLECULAR GENETICS, vol 9, no 4, 1 March 2000 (2000-03-01), pages 549-559

4 En vista de todo lo anterior, ruego a usted proceder de conformidad y conceder el privilegio de patente solicitado

Señor Superintendente,

ALICIA LLOREDA RICAURTE
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0022 1767/93/1501 2-5436$02.00/0
The Journal of Immunology

Vol 150, 5436–5444 No. 12 June 15 1993
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Structural Comparison and Chromosomal Localization of the Human and Mouse IL-13 Genes[1]

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ABSTRACT The genomic structure of the recently described cytokine IL 13 has been determined for both human and mouse genes The nucleotide sequence of a 4 6-kb DNA segment of the human gene is described The human IL 13 gene (*IL13*) occurs as a single copy in the haploid genome and maps to human chromosome 5 A 4 3-kb DNA fragment of the mouse IL 13 gene (*Il13*) has been sequenced and found to occur as a single copy mapping to mouse chromosome 11 Intrachromosomal mapping studies revealed that both genes contain four exons and three introns and show a high degree of sequence identity throughout their length Potential recognition sequences for transcription factors that are present in the 5′ flanking region and are conserved between both genes include IFN-responsive elements, binding sites for AP-1, AP 2 and AP-3, an NF IL 6 site, and a TATA like sequence Both genes map to chromosomal locations adjacent to genes encoding other cytokines, including IL-3, GM-CSF, IL-5 and IL-4 suggesting that IL-13 is another member of this cytokine gene family that may have arisen by gene duplication *Journal of Immunology*, 1993, 150 5436

Human IL-13[4] and mouse IL-13 are recently described cytokines produced by activated Th2 cells in human and mouse (1–3) IL-13 is secreted as a mainly unglycosylated protein with a M_r of 10,000 (3) Initial efforts to characterize its biologic role have revealed interaction with monocytes and B cells Human IL-13 causes human monocytes to differentiate The cells become dendritic and up-regulate their surface expression of MHC class II Ag and the low-affinity receptor for IgE (CD23 or FcεRII) (3) In addition, IL-13 elicits human B cell proliferation similar to that induced by IL-4 IL-13 also regulates Ig secretion by human B cells requiring costimulatory signals provided by the presence of T cells This regulation results in an increase in the secretion of IgG accompanied by a reduction in IgA secretion (3) Significantly IL-13 can also induce IgE secretion by human B cells a process previously attributable only to IL-4 (4)

In this paper we report the complete DNA sequences of the human and mouse genes for IL-13 as well as their chromosomal locations Both the genes contained four exons which account for their entire cDNA sequences, and are located on human chromosome 5 and mouse chromosome 11 respectively They were further localized to re-

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[4] Abbreviations used in this paper *IL13*, human IL-13 gene *Il13*, mouse IL-13 gene, *Il3*, mouse IL-3 gene, *Il5*, mouse IL-5 gene; *Il4*, mouse IL-4 gene, *IL4*, human IL-4 gene; *IL5*, human IL-5 gene *GMCSF* human granulocyte macrophage-colony stimulating factor gene GM-CSF granulocyte macrophage-colony stimulating factor PCR, polymerase chain reaction.

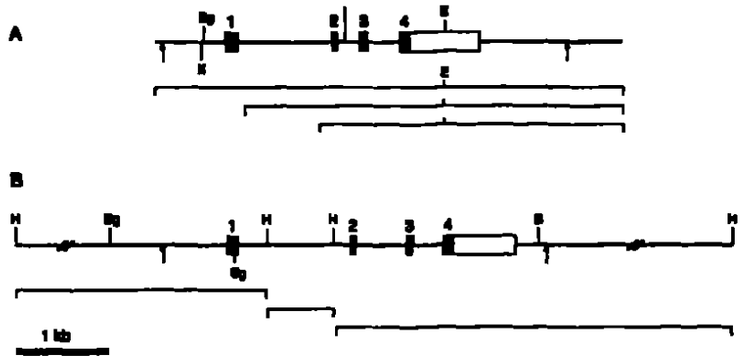

FIGURE 1 Schematic representation and partial restriction map of the human (*A*) and mouse (*B*) *IL13* (*Il13*) genes ■ represent exons 1 to 4 □ indicate untranslated sequences. Restriction fragments that were subcloned are indicated by *bars* below the diagram Regions between the *arrows* were sequenced completely *E*, *EcoRI* *B* *BamHI* *Bg* *BglI* *H* *HindIII* *X*, *XhoI*

gions of these chromosomes where a cluster of genes encoding other immune and hemopoietic factors, including IL-3, GM-CSF IL-5 and IL-4, have previously been mapped We also identified certain shared sequences in the 5′ noncoding regions of the *IL13* and *Il13* genes which may contribute to the control of their expression

Materials and Methods

Isolation and DNA sequence analysis of IL 13 genomic clones

The human *IL13* gene was isolated by plaque hybridization from a human genomic library prepared in the EMBL SP6/T7 λ vector (Clontech, Palo Alto, CA). Hybridization was performed with a ^{32}P labeled 380-bp fragment of the human cDNA (3) produced by PCR using the primers 5′-ACCCAGAACCAGAAGGCTCCG and 5′ TCAGTTGAACCGTCCCTCGCG (oligonucleotides were synthesized using ABI reagents on a Model 394 DNA/RNA synthesizer Applied Biosystems Inc , Foster City CA) Hybridization was overnight at 42°C in 6 × SSPE (1 × SSPE = 150 mM NaCl 10 mM NaH_2PO_4, and 1 mM EDTA pH 7.4) 20% formamide, and 0 1% SDS with 0 1 mg/ml tRNA The filters were then washed three times at room temperature with 0 1× SSPE and 0 05% SDS Positive plaques were reanalyzed by restriction enzyme digestion and Southern blot analysis using the *IL13* cDNA fragment and labeled oligonucleotides corresponding to 5′, middle and 3′ regions of the human cDNA as probes (5) Positively hybridizing *Bam*HI fragments were subcloned into pUC21 (Boehringer Mannheim, Indianapolis, IN) and M13 mp18 and -19, and both strands were sequenced using internal primers and the M13 universal primers (Sequenase 2 0 kit, United States Biologicals, Cleveland, OH) Figure 1*A*

The mouse *Il13* gene was isolated by colony hybridization from a mouse genomic (BALB/c) library prepared in the cosmid vector pWE15 (Clontech, Palo Alto CA) Colony hybridization was performed with a ^{32}P-labeled 400-bp *PstI*/*PvuII* DNA fragment, derived from the mouse cDNA (1) Positive clones were verified by restriction enzyme digestion followed by Southern blot analysis, using labeled oligonucleotides corresponding to the 5′ middle and 3′ regions of the mouse cDNA as probes, by standard procedures (5) Genomic DNA fragments produced by restriction enzyme digestion with *HindIII* or *BglII*, and identified as hybridizing with the mouse cDNA and the oligonucleotides were subcloned into pUC21 and sequenced on both strands using internal primers and the M13 universal primers (Fig 1) The 707-bp *HindIII* fragment was isolated by PCR from the parent cosmid, using flanking primers This fragment was then subcloned into pUC21 and sequenced on both strands (Fig 1*B*)

The sequences were assembled and analyzed using Intelligenetics (Intelligenetics, Mountain View CA) and GCG (Genetics Computer Group Inc. Madison, WI) software

Localization of the human *IL13* gene

A mapping panel consisting of 14 Chinese hamster mouse or rat × human hybrid cell lines was used to assign the *IL13* gene to human chromosomes (6). PCR was performed with template DNA from the panel using primers (5′-CTGGGCTCGCTGAAGGGAAGCTGGCTG and 5′ CAAAGAGGTCAGCACGTGAGTAGAACGC) that directly amplified a 285-bp DNA fragment of the sequence between exons 3 and 4 A 0 2 μg quantity of genomic DNA was amplified by PCR using the following conditions. a preliminary incubation at 95°C for 5 min followed by 28 cycles of 94°C 1 min, 58°C 1 min 72°C 1 min and a final reaction at 72°C for 7 min

The chromosomal location of the *IL13* gene was further defined by fluorescence in situ hybridization as described (7). Briefly a 7 5 kb-fragment of human *IL13* genomic DNA was labeled with biotin-11-dUTP by nick translation (Boehringer Mannheim kit) The labeled probe DNA, together with human placental and salmon sperm DNA as competitor was then hybridized to pretreated and denatured human lymphocyte metaphase chromosomes After washing the slides were treated with avidin/FITC (Vector Laboratories, Burlingame, CA) and the signal amplified using biotinylated goat anti-avidin D antibody and a second incubation with avidin/FITC (Vector Laboratories) The

chromosomes were counterstained with propidium iodide. Twenty metaphase chromosomes were analyzed using fluorescence microscopy with a filter combination specific for FITC and propidium iodide. Signals were counted as specific only when the fluorescence was seen lying in parallel on both chromatids of a chromosome

Localization of the mouse *Il13* gene

PCR primers (5'-GCCGGGATGGGCATTCCACGTGTG and 5'-GGACGCCAAGGTCAAGAACAGTTG) that directly amplified a 265 bp DNA fragment of the sequences between mouse *Il13* exons 3 and 4 were used to screen DNA from a panel consisting of 14 Chinese hamster or rat × mouse hybrid cell lines. A 0.2 μg quantity of genomic DNA was amplified by PCR under the same conditions as in the human gene mapping experiment

Interspecific backcross mapping

Interspecific backcross progeny were generated by mating (C57BL/6J × *Mus spretus*)F_1 females and C57BL/6J males as described (8). A total of 205 N2 mice were used to map the *Il13* locus. DNA isolation, restriction enzyme digestion, agarose gel electrophoresis, Southern blot transfer and hybridization were performed essentially as described (9). All blots were prepared with Zetabind membrane (AMF-Cuno, Meriden, CT). The probe was a ^{32}P-labeled 1.4-kb *Bam*HI fragment of the mouse *Il13* cDNA (1). Washing was performed to a final stringency of 0.1 to 0.5 × SSCP, 0.1% SDS, 65°C. *Bam*HI digestion of C57BL/6J DNA produced fragments of 6.6 and 5.0 kb. *M. spretus* DNA yielded a 10.5-kb fragment. *Sca*I digestion of C57BL/6J DNA produced a 5.2-kb fragment. *M. spretus* DNA yielded a 12-kb fragment. *Eco*RV digestion of C57BL/6J DNA produced a 6.4-kb fragment. *M. spretus* DNA yielded a 7.8 kb fragment. The presence or absence of the *M. spretus*-specific fragments was followed in backcross mice, data from the three mapping experiments were combined. A description of the probes and RFLP for *Il3*, myosin H chain, skeletal muscle (*Myhs*), and transformation-related protein 53 (*Trp53*) have been reported previously (10). The adrenergic receptor α_1 (*Adra1*) locus has not been reported previously for this interspecific backcross. The probe was a ~2-kb fragment of the hamster adrenergic receptor α_1 cDNA (11) and detected 6.8 kb and 5.7-kb fragments in *Bam*HI digested C57BL/6J DNA and 14.0 and 5.7 kb fragments in *Bam*HI digested *M. spretus* DNA. In addition, *Hin*cII digestion produced 8.2-, 3.3-, and 2.2 kb C57BL/6J DNA fragments and fragments of 8.2 and 4.9 kb from *M. spretus* DNA. The *M. spretus*-specific RFLP were followed and the data from the two different enzymes combined. Recombination distances were calculated as described using the computer program SPRETUS MADNESS (12). Gene order was determined by minimizing the number of recombination events required to explain the allele distribution patterns.

Results

Human *IL13* gene structure

A diagrammatic representation of the *IL13* gene and restriction fragments that were isolated for sequence analysis are shown in Figure 1*A*. The complete nucleotide sequence of a 4.6-kb region containing all the coding sequences is shown in Figure 2. The human *IL13* gene consists of four exons and three introns. Although the transcription initiation site has not been determined, our longest cDNA extends from position 732 (Fig. 2) into a region containing at least two potential cap sites (Table I). Exon 1 (extends to position 903) encodes 44 amino acid residues and contains the 5'-untranslated region. Exon 2 (position 1960–2013) and exon 3 (position 2266–2370) encode 18 and 35 amino acid residues, respectively. Exon 4 (position 2717–3651) encodes the C terminal 35 residues of human IL-13 and contains the 3' untranslated region. The length of introns 1, 2, and 3 are 1055 bp, 251 bp, and 345 bp, respectively. In common with other cytokine genes, the intron-exon junctions do not interrupt amino acid codons (13). The existence of the two different *IL13* cDNAs that have been isolated (3) is explained by the presence of an alternative 3'-splice/acceptor site present at the 5'-end of exon 4 that results in the presence or absence of the nucleotides encoding a glutamine residue at this position (Fig. 2).

Assignment of the human *IL13* gene to chromosome 5

Genomic DNA from a mapping panel of rodent × human hybrid cell lines was analyzed by PCR using primers that specifically amplified human *IL13* sequences. The expected 285-bp PCR product was obtained only from a genomic *IL13* clone and from total human DNA, or DNA from hybrid cell lines that had retained human chromosome 5. No specific amplification was seen from the DNA of Chinese hamster, mouse 3T3, or rat 7777 cells under our PCR conditions. As shown in Table II, all human chromosomes were excluded by this panel except chromosome 5, which showed no discordance. The DNA from one hybrid cell line with deletion of the chromosome 5q13-ter region failed to be amplified by PCR, indicating that the *IL13* gene is located on the long arm of human chromosome 5. Fluorescence in situ hybridization using *IL13* genomic DNA as a probe verified this conclusion. A specific fluorescent signal on both chromatids of at least one chromosome 5 was seen in 15 of 20 analyzed metaphase cells. Both chromosome 5 homologues carried specific label in 11 metaphase cells. The *IL13* locus was assigned to band q31 on the long arm of chromosome 5 based on an R banding pattern produced by the incorporation of BrDu after synchronization and counterstaining with propidium iodide (Fig. 3).

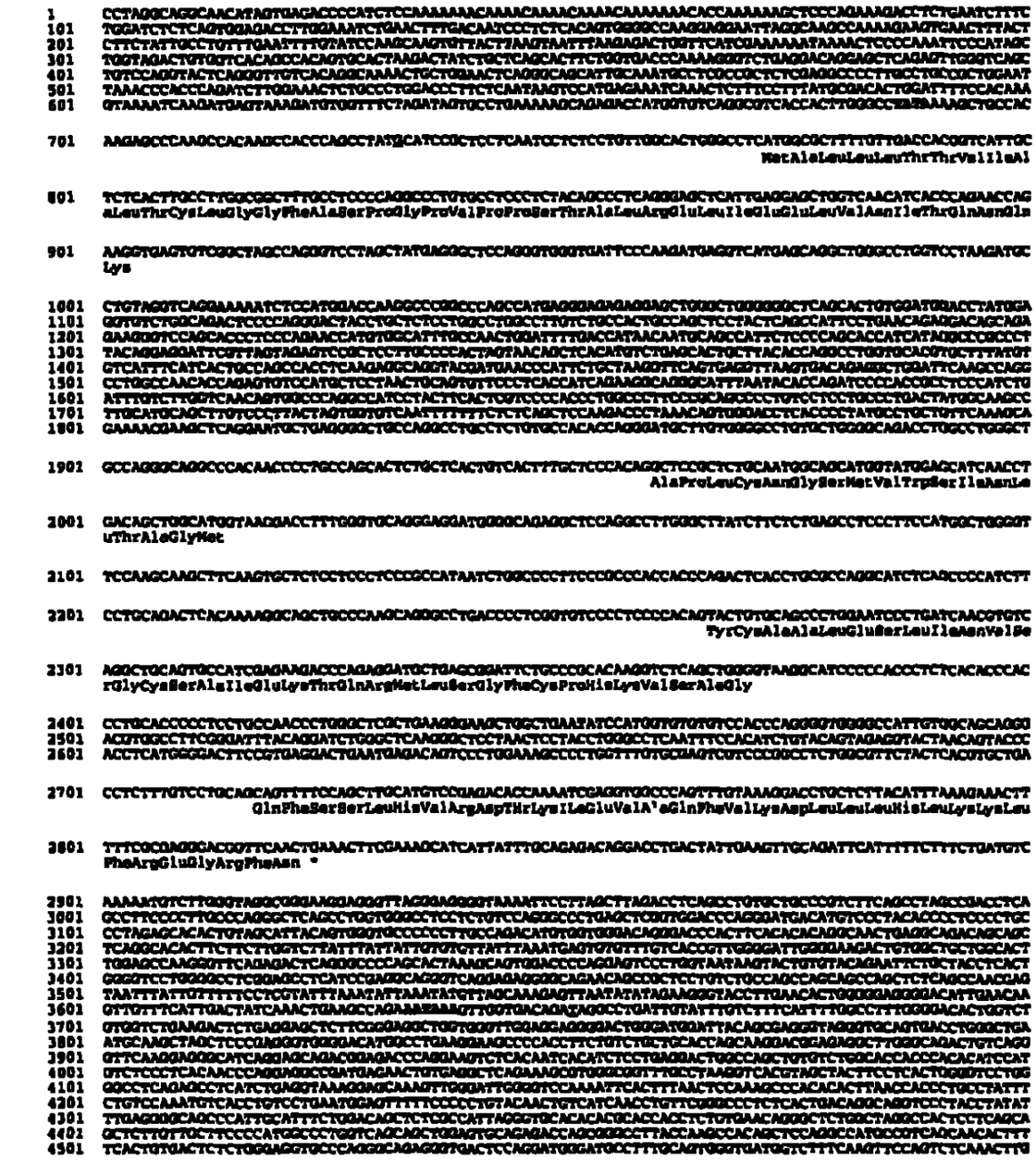

FIGURE 2 DNA sequence of the human *IL13* gene The amino acid sequences encoded by the gene are indicated and the putative TATA box and polyadenylation signal (AATAAA) are shown in *bold*. The nucleotides at the beginning and end of the 5 and 3' untranslated sequences are *underlined* This sequence has been deposited in the GenBank database under accession number L13029

Murine *Il13* gene structure

The mouse *Il13* gene also consists of four exons and three introns. Although the cap site(s) for the *Il13* RNA has not been determined our longest cDNA extends from position 725 (Fig 4) in a region containing several potential cap sites (Table I) The first 47 N terminal amino acid residues and the 5' untranslated region are contained in exon 1 (extends to position 934). Exon 2 (position 2193–2245) and exon 3 (position 2823–2926) encode 18 and 35 amino acid residues respectively Exon 4 (position 3240–4073) encodes the C-terminal 31 amino acid residues of mouse IL-13 and contains the 3'-untranslated region The length

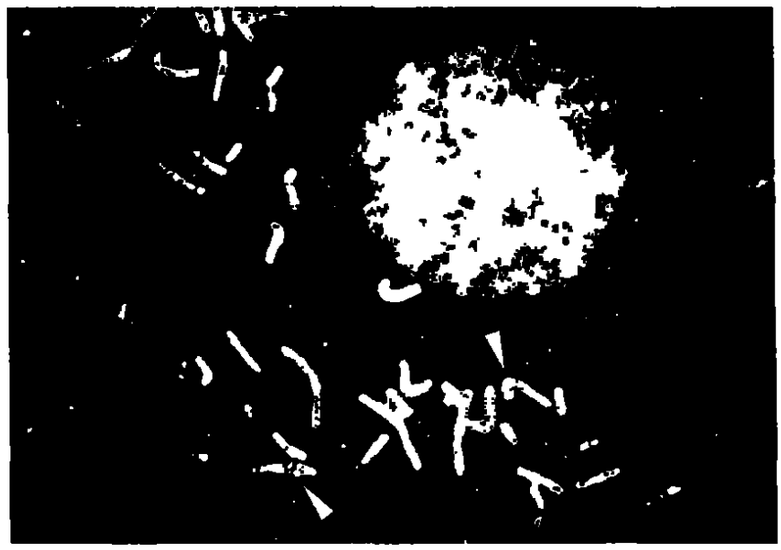

FIGURE 3 Chromosomal mapping of human *IL13* by fluorescence in situ hybridization. A human metaphase cell shown in the figure was stained with biotin/avidin/FITC using an *IL13* genomic fragment as a probe. Double chromatid signals (*yellow dots*) are present on both chromosome 5 homologues (*arrows*).

of introns 1, 2, and 3 are 1258 bp, 576 bp, and 311 bp, respectively. The intron-exon junctions do not interrupt amino acid codons (Fig. 4).

Assignment of the *Il13* gene to mouse chromosome 11

Genomic DNA from a panel of Chinese hamster or rat × mouse cell lines was analyzed by PCR using primers that specifically amplified *Il13* sequences but did not amplify hamster or rat genomic DNA. The results are depicted in Table III. The presence of an *Il13* signal from cell hybrids containing mouse chromosome 11 was in complete concordance. All other mouse chromosomes were excluded by at least 23% of discordant hybrids.

The chromosomal location of the *Il13* locus was further determined by interspecific backcross analysis using progeny derived from matings of [(C57BL/6J × *M. spretus*)F_1 × C57BL/6J] mice. This interspecific backcross mapping panel has been typed for over 1100 loci that are well distributed among all the autosomes as well as the X chromosome (8). DNA from C57BL/6J and *M. spretus* mice was digested with several enzymes and analyzed by Southern blot hybridization for informative RFLP using the mouse IL-13 cDNA probe. The 10.5 kb *Bam*HI, 12.0 kb *Sca*I, and 7.8 kb *Eco*RV *M. spretus*-specific RFLP (Materials and Methods) were used to follow the segregation of the *Il13* locus backcross mice. The mapping results indicated that *Il13* is located in the middle of mouse chromosome 11, linked to *Adra1*, *Il3*, *Myhs*, and *Trp53*. Although 95 mice were analyzed for every marker as shown in the segregation analysis (Fig. 4), up to 144 mice were typed for some pairs of markers. Each locus was analyzed in pairwise combinations for recombination frequencies using the additional data. The ratios of the total number of mice exhibiting recombinant chromosomes to the total number of mice analyzed for each pair of loci and the most likely gene order are: centromere-*Adra1*, 8/123; *Il3*-0/106-*Il13*-5/107-*Myhs*, 7/144-*Trp53*. The recombination frequencies expressed in cM ± SE are *Adra1*, 6.5 ± 2.2; [*Il3*, *Il13*]-4.7 ± 2.0-Myhs, 4.9 ± 1.8-*Trp53*. The lack of recombination between *Il3* and *Il13* in 106 mice suggests that the two loci are within 2.8 cM of each other (95% confidence limit).

Possible transcriptional regulatory sequence motifs in the human and mouse *IL-13* genes

TATAA sequences (TATA box) are located at positions 697–701 and 786–790 in the *IL13* and *Il13* genes, respectively (Figs. 2 and 4). The polyadenylation sequence of the human IL-13 gene is located between positions 4055–4060 and between positions 3633–3638 in the *Il13* gene. Other recognizable sequence motifs that are conserved in the 5′ flanking regions of both the *IL13* and *Il13* genes include putative binding sites for AP-1, AP-2, and AP-3, NF-IL-6, IFN-responsive elements, and PU.1 [Table I (14)]. In addition, the *Il13* gene contains repeated PEA3 binding sites [Table I (14)].

Homology between the *IL13* gene and the *Il13* gene

Although the *IL13* and *Il13* genes display a high degree of sequence identity throughout their entire length, they also contain more defined regions of homology that may prove important for the transcriptional control of these genes. A region of 76% homology starting at positions 330 and 270 in mouse and human, respectively, and extending to 774 (*Il13*) and 733 (*IL13*) exists in the region 5′ of the initiation ATG of each gene. Comparison of the *Il13* and *IL13* intron sequences also identified a high degree of sequence identity. Intron 1 contains a 1716-bp region of 67% sequence

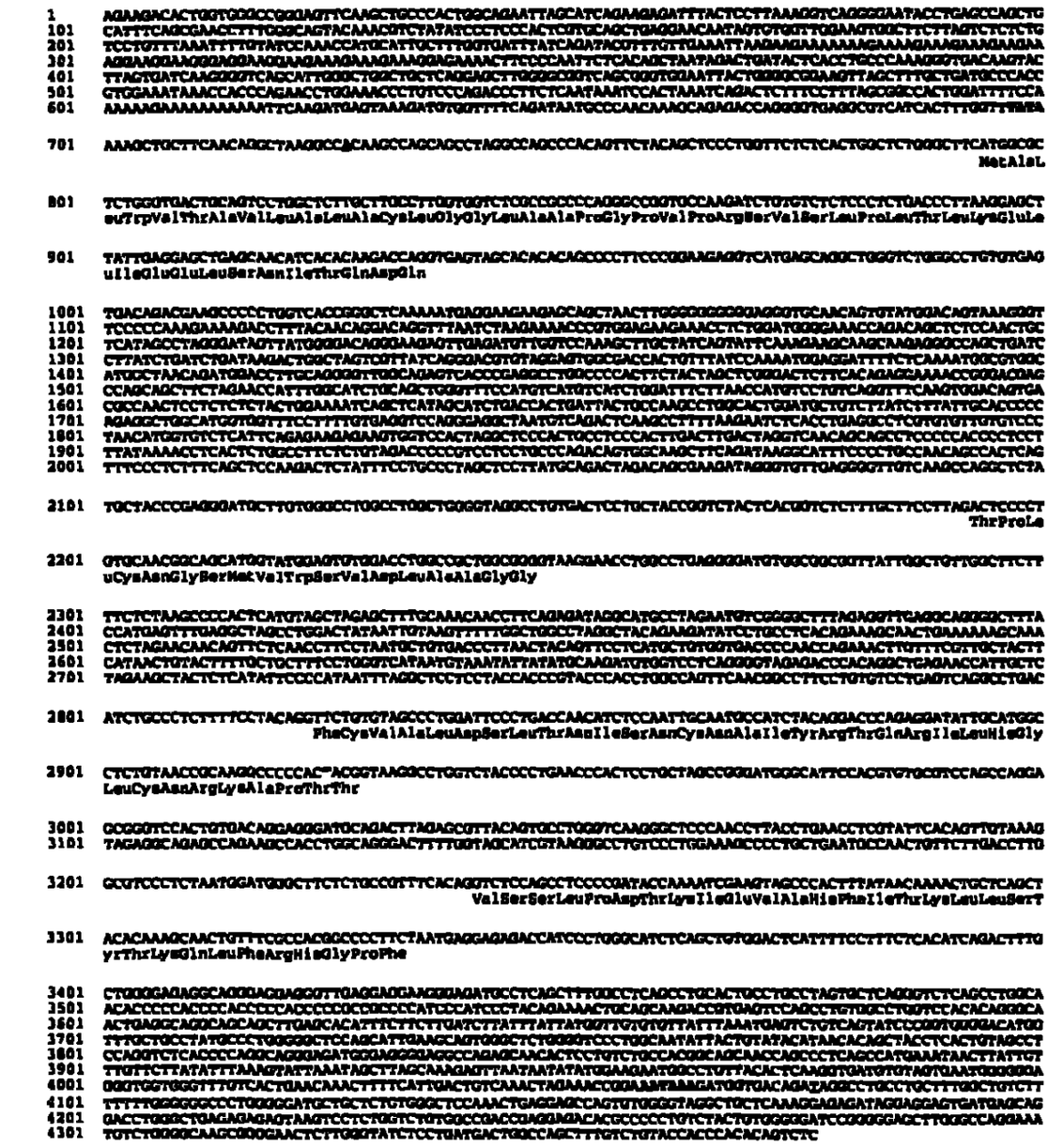

FIGURE 4. DNA sequence of the mouse *Il13* gene The amino acid sequences encoded by the gene are indicated and the *putative TATA box and polyadenylation signal (AATAAA) are shown in bold. The nucleotides at the beginning and end of the* 5′ and 3 -untranslated sequences are *underlined* This sequence has been deposited in the GenBank database under accession number L13028

identity between *Il13* (position 1476–2192) and *IL13* (positions 1182–1959) A 100-bp region of 67% sequence identity was also found in intron 2 between *Il13* positions 2247–2347 and *IL13* positions 2014–2115 Furthermore, intron 3 contained a 288-bp region of high sequence identity (67%) between *Il13* (positions 2944–3232) and *IL13* (positions 2400–2712) Comparison of the *Il13* and *IL13* sequences following the translation stop codon showed an overall identity of 70%

Discussion

We have determined the sequences of both the *IL13* and *Il13* genes. Both loci contain 4 exons which comprise the

Table I
Potential transcriptional control sequences located in the 5′-flanking regions of IL13 and Il13 genes

Recognition Sequence	Position		Possible Significance
	IL13	*Il13*	
TATAA	686–691	697–702	TATA box
CTGGATCT	100–107		IFN-γ inducibility
CTTTACTC	194–201		
CAGGAGCT	378–385	436–443	
CTGGAACT	436–443		
CTGGAAAC		523–530	
CTGGAATT	494–501		
CTGGACCC	531–538		
CTTTATGC	573–580		
CTGGATTT	585–592	589–596	
AAGTGA	187–192		IFN α inducibility
AAAGGA		299–304	
		331–336	
AAGGGA		308–313	
TGAGTAA	613–619	625–631	AP-1 binding site
CCCAAGCC	706–713		
GCCAGCCC		743–750	AP-2 binding site
TGTGGTTT	623–630	635–642	AP-3 binding site
CAGCCT	724–729	735–740	Cap sites
CAATCC	744–749		
CAGCCC		745–750	
CAGTTC		752–757	
CAGCTC		760–765	
TTAGGCAAG	171–179		NF-IL6 recognition site
TTAAGTAAT	243–251		
TGTTGAAAT		259–267	
AGGAAG		301–306	PEA3 binding protein recognition
		305–310	
		313–318	
		3–7–322	
GAGGAA	165–170	158–163	PU box
		312–317	

Table II
Correlation of human IL13 sequences with human chromosomes in rodent × human somatic cell hybrids

Human Chromosome	Signal/Chromosome				
	+/+ᵃ	−/−	+/−	−/+	% Discordance
1	2	5	4	3	50
2	2	5	3	2	42
3	4	2	1	6	53
4	4	5	2	2	31
5	6	8	0	0	0
6	3	3	2	4	50
7	2	3	4	3	58
8	6	5	0	3	21
9	1	5	3	3	50
10	2	6	4	2	43
11	5	3	0	4	33
12	3	4	3	4	50
13	5	4	1	3	31
14	5	1	1	7	57
15	5	2	1	6	50
16	4	5	2	2	31
17	4	6	2	2	29
18	4	4	2	4	43
19	4	3	1	4	42
20	4	6	1	1	17
21	5	1	1	5	50
22	7	1	0	5	39
X	2	1	2	3	63

ᵃ Symbols indicate the presence (+) or absence (−) of the hIL-13 PCR fragment as related to the presence (+) or absence (−) of a particular human chromosome. The number of discordant observations is the sum of the +/− and −/+ observations.

entire coding region of each gene The spatial distribution of the exons is similar in both genes and the intron-exon boundaries do not disrupt amino acid codons in either locus Two forms of the human IL-13 protein have been identified, which although differing at a single amino acid residue (glutamine 98) display identical biologic activity (3) In this study we have found that the codon encoding Gln 98 is at the 5 -end of exon 4 and that its incorporation or absence is probably the result of alternative splicing No alternative splicing has been identified in the mouse gene The *IL13* and *Il13* cDNA sequences have 66% sequence identity and alignment of the two genomic sequences shows that extensive identity also exists in the 5′- and 3′ flanking regions as well as throughout the introns

The *IL13* gene has been mapped to chromosome 5q31 and the *Il13* gene has been localized to a region on mouse chromosome 11 that has known conserved synteny with human 5q and 17p (Fig 5) It is noteworthy that the *IL13* and *Il13* genes localize to regions of these chromosomes where genes for other immunoregulatory and hemopoietic growth factors have been mapped This gene cluster includes genes for IL-3 GM-CSF IL-4 and IL-5 in both human and mouse (15 16) These proteins have a common four α-helix bundle structure (17) and, with the exception of *Il3* the genes encoding them also share a common intron-exon structure with the *IL13* and *Il13* genes consisting of four exons and three introns (13) Studies using pulsed-field gel electrophoresis have estimated that the *Il4* and *Il5* genes are ~110–180 kb apart (15) Physical linkage analysis has also shown that the *IL5* and *IL4* genes are a minimum of 600 kb from the closely linked *IL3* and *GMCSF* genes (16) During the course of this present study others have described the isolation of cDNA by hybridization to a yeast artificial chromosome containing a 425-kb human genomic sequence containing the *IL4* and *IL5* genes One of the cDNAs isolated was that of *IL13*, which mapped upstream and probably within 50 kb of the *IL4* locus (18) The close proximity and similar structure of these genes has led to the suggestion that they have evolved by ancient gene duplication It has also been suggested that this linkage may provide a mechanism for controlling the coordinate expression of these genes (19)

The *IL13* and *Il13* genes show a relatively high degree of nucleotide identity in their 5′-flanking regions and contain a number of conserved transcription factor consensus sequences that may be important for regulating the expression of these genes. Many of these putative cis-acting motifs are associated with transcription factors that are rapidly induced in inflammatory responses, a feature in keeping

Table III
Correlation of mouse Il13 sequences with mouse chromosomes in rodent x mouse somatic cell hybrids

Mouse Chromosome	Signal/Chromosome				
	+/+	−/−	+/−	−/+	% Discordance
1	1	4	1	7	62
2	0	2	2	10	86
3	0	6	2	6	57
4	0	5	2	6	54
5	0	6	2	3	46
6	1	5	1	5	50
7	0	3	2	9	79
8	0	6	2	6	57
9	1	7	1	4	46
10	1	7	1	4	39
11	2	12	0	0	0
12	1	5	1	7	57
13	2	8	0	3	23
14	0	8	2	4	43
15	0	3	2	9	79
16	0	5	2	6	62
17	1	3	1	9	71
18	0	7	2	4	46
19	0	3	2	8	77
X	0	3	2	8	77

Symbols indicate the presence (+) or absence (−) of the mIL-13 PCR fragment as related to the presence (+) or absence (−) of a particular mouse chromosome. The number of discordant observations is the sum of the +/− and −/+ observations.

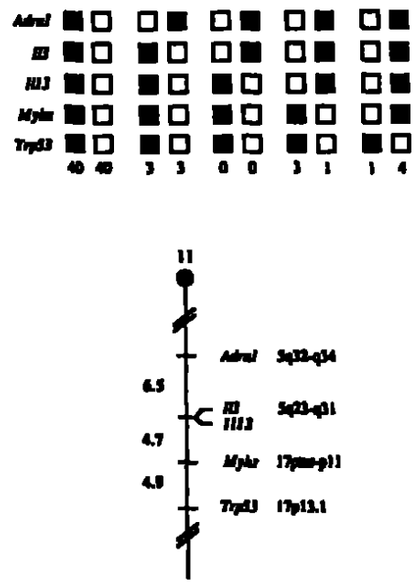

FIGURE 5 *Il13* maps in the central region of mouse chromosome 11. The segregation patterns of *Il13* and flanking genes in 95 backcrossed animals that were typed for all loci are shown at the *top*. For individual pairs of loci up to 144 animals were typed. Each column represents the chromosome identified in the backcrossed progeny that was inherited from the (C57BL/6J × *M. spretus*)F_1 parent. The ■ represent the presence of a C57Bl/6J allele and □ represent the presence of a *M. spretus* allele. The number of offspring inheriting each type of chromosome is listed at the bottom each column. A partial chromosome 11 linkage map showing the location of *Il13* in relation to linked genes is shown at the *bottom*. Recombination distances between loci in cM are shown to the left of the chromosome and the positions of loci in human chromosomes, where known, are shown to the right.

with the induction of IL-13 in activated T cells. Although regions of significant nucleotide sequence homology have been identified between the *IL4* and *IL2* genes (19) and between the *IL3* and *GMCSF* genes (20), alignment of the *IL13* and *Il13* genes with other cytokine genes showed no significant nucleotide sequence homology. Furthermore, despite the fact that IL-13 is also produced by Th2 cells, the sequences presented here do not contain the 11-bp motif found to be important for the regulation of *IL4* transcription (21).

When the amino acid sequences of the IL-4 and IL-13 proteins are aligned they display ~30% homology throughout their lengths and are able to cross-compete for receptor binding on certain cell types, suggesting that IL-13 and IL-4 have shared receptor components (S. Zurawski and G. Zurawski, manuscript in preparation). In addition, the alignment of the IL-4 and IL-13 proteins showed a coincidence of their intron/exon boundaries, a feature that has also been noted in other members of this gene family (13). The relatedness of IL-13 and IL-4 is also reflected in their biologic properties, which include the regulation of IgE synthesis (22), the induction of human B cell proliferation (23), and the induction of macrophage-like dendritic cell morphology (24) with associated CD23 expression on human monocytes (25). Studies are currently in progress to determine the levels of IL-4 and IL-13 mRNA produced upon T cell activation, and preliminary data indicate that IL-13 is produced for more extended periods than IL-4 after activation (R. de Waal Malefyt, manuscript in preparation). We are also in the process of determining the levels of IL-13 message produced by the T cells of mice in which the *Il4* gene has been disrupted by homologous recombination (26). These *Il4* −/− do not produce detectable levels of circulatory IgE after nematode infection, and therefore may prove useful in determining the in vivo function of IL-13. Although it has not yet been ascertained that IL-13 induces IgE secretion by mouse B cells, it is possible that disruption of the *Il4* locus may also influence the expression of receptors for IL-13 or the production of IL-13. The cloning and sequencing of the *Il13* locus should enable manipulation of the *Il13* gene in vivo, facilitating the establishment of in vivo models for the study of IL-13 function.

The clustering of the genes encoding IL-3, GM-CSF, IL-5, IL-4, and IL-13 to the same region of chromosomes 5 and 11 in human and mouse, respectively, suggests that the *IL13* gene is another member of a cytokine gene family that has probably arisen by gene duplication. These shared origins would explain certain of the conserved functional

characteristics that exist between members of this gene cluster This region of human chromosome 5 is known to be deleted in patients with 5q syndrome, a genetic disorder that results in the development of myeloid leukemias (18) The relative importance of the genes within this gene family to the appearance of leukemias remains unknown, but IL-13 must now also be taken into consideration It is also interesting to speculate what other cytokine genes remain to be found in this region of the genome

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The cattle interleukin-13 gene: genomic organization, chromosomal location, and evolution of the promoter

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Abstract Interleukin (IL)-13 plays a key role in the T-helper 2 type immune response In domestic animal species no sequence information is available for the *IL13* gene Here we report the isolation and sequencing of the cattle *IL13* gene Since a heterologous cDNA probe failed to hybridize to cattle genomic DNA the gene was identified by a positional sequencing approach In human and mouse both cytokines are located within a region of about 30 kilobases (kb) Therefore the region upstream of the cattle *IL4* gene has been sequenced by a shotgun approach The cattle *IL13* gene was found to be located about 20 kb upstream of *IL4* in a head-to-tail orientation The exon–intron structure and the promoter region is well conserved across species The alignment of the *IL13* sequences from different species allowed the identification of highly conserved amino acid positions and transcriptional elements Two conserved amino acid positions (glu_{31} and pro_{34}) are most probably homologous to the interleukin-4 signalling residues One of the potential binding sites for transcription factors is located at position -197 to -189 and is closely related to the P elements of the *IL4* promoter Nevertheless, the overall identity of the promoter sequences of *IL13* and *IL4* is low Therefore we conclude that both cytokines use diverse regulatory elements There is evidence that IL-13 but not IL-14 is the key mediator in allergic asthma Furthermore IL-13 is involved in resistance to some infections Therefore IL-13 is of interest for disease association studies in domestic animals

Key words Interleukin-13 Cattle Chromosomal location Cytokine Promoter

The nucleotide sequence data reported in this paper have been submitted to the EMBL nucleotide sequence database and have been assigned the accession number AJ132441

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Introduction

Interleukin (IL)-13 was initially reported by Brown and co-workers (1989) as a transcript preferentially expressed by mouse T-cell clones The cDNA, originally called p600, is abundantly expressed by the cells of the T-helper 2 (T_H2) subset, but was not expressed by the T_H1 subset Other *IL13* cDNA sequences are also available including those from human and rat (Lakkis and Cruet 1993, McKenzie et al 1993a Minty et al 1993, Morgan et al 1992) IL 13 exerts multiple effects on mouse and human cells *in vitro* It induces the isotype switch towards the production of IgG_4 and IgE in human B cells (Punnonen et al 1993) the proliferation of B cells (Cocks et al 1993), and promotes human macrophage fusion *in vitro* (DeFife et al 1997) IL-13 shares many biological properties with IL-4 Both cytokines have the ability to induce and amplify T_H2-type immune responses and to promote IgE formation even though IL-4 seems to be the dominant IgE synthesis inducing cytokine (e g , Punnonen et al 1993 1997) Nevertheless, although the effects overlap significantly some functions within the immune response seem to be unique to each cytokine For example in contrast to IL-4, IL-13 leads to an increase of interferon-γ production by human natural killer cells (Minty et al 1993) The overlap in biological properties is reflected in the sharing of a receptor component (Hilton et al 1996, Lefort et al 1995, Zurawski et al 1995) Both receptors use the Janus kinase/signal transducer and activator of transcription (JAK / STAT) pathway and activate STAT6 (Lin et al 1995) IL-13 seems to play an important role in triggering an effective immune response expelling parasitic worms in mice Furthermore it has been demonstrated in two recent publications that IL-13, but not IL-4, is the key mediator in the expression of mouse asthma (Grünig et al 1998, Wills-Karp et al

1998) Due to their biological properties and functions IL-13 and IL-4 are of therapeutic interest, since both cytokines may be aetiologically involved in atopic diseases For example, many antiallergic drugs inhibit the release of IL-13 and IL-4 from basophil leucocytes that are responsible for the propagation of some allergic diseases (Gibbs et al 1998)

The human *IL13* gene is located on human Chromosome 5 (HSA5q23–31) together with other growth factors such as *IL4, IL5, IL13* and the granulocyte/macrophage-stimulating factor (GM-CSF) The human *IL13* gene is located 12 5 kilobases (kb) upstream of the *IL4* gene (Smirnov et al 1995) Due to the important function within the immune response, the genomic region containing *IL4* and *IL13* is a candidate for the study of the genetic component of autoimmune diseases and of resistance to certain infections Linkage analysis has mapped asthma related phenotypes to the cytokine gene cluster on HSA5q23 31 For example the IgE level and the bronchial hyper-responsiveness (BHR) are associated with microsatellites from HSA5 in asthma patients (CSGA 1997 Marsh et al 1994, Meyers et al 1994 Postma et al 1995) In the mouse an increase in the T_H2-type immune response was shown to be associated with resistance to certain parasitic infections (e g , Else et al 1994)

To our knowledge no sequence information for *IL13* is available in domestic animals The aim of this study was to clone and sequence the genomic region containing the cattle *IL13* gene This sequence information will be the basis for the development of polymorphic markers for disease association studies as well as in–depth characterization of the regulatory elements and the deduced amino acid sequence

Material and methods

Screening of a cattle cosmid library and restriction mapping

A cattle Super Cos 1 cosmid library was screened using a mouse *IL13* and a cattle *IL4* cDNA (Brown et al 1989) as described (Buitkamp et al 1995) A restriction map of the isolated cosmid clone designated 16B was obtained by single and double digests as well as partial digestions of the cosmid insert using the enzymes*Bam* HI *Eco* RI *Hin* dIII *Pst* I and *Xba* I (New England Biolabs, GmbH Schwalbach Germany) Single fragments were identified by subsequent hybridisation with subclone probes as well as oligonucleotide probes hybridizing to exon 4 of *IL13* various simple repetitive sequences, and T3 and T7 bacteriophage promoters as described (Buitkamp et al 1995)

Subcloning, plasmid preparation, and sequencing

Random subclones were generated by cloning *Sau* 3A fragments from the cosmid into the *Bam* HI cloning site either of a phagemid vector (Bluescript II KS$^+$ Stratagene GmbH Heidelberg, Germany) or of pZEr0-1 (Zero Background Cloning Kit Version A Invitrogen Corporation Leek The Netherlands) Subcloning of unique *Bam* HI *Eco* RI *Hin* dIII or *Pst* I DNA fragments, or the preparation of deletion clones was done by the DEAE cellulose method (Sambrook et al 1989) Eluted fragments were ligated to pZEr0-1 digested with the appropriate enzyme according to the recommendations of the manufacturer Fragments were sequenced using double-stranded plasmid DNA either with [α-^{35}S]dCTP using the chain termination method (random *Sau* 3A subclones Sanger et al 1977) or on an automated fluorescence-based system (Applied Biosystems 377) using cycle sequencing with standard FS DyeDeoxy Terminator chemistry (Perkin Elmer Applied Biosystems Foster City Calif) For sequencing of inserts propagated in pZEr0-1 two primers were specifically designed to obtain optimal sequence reactions (M13RevZ-5 GAC GTT GTA AAA CGA CGG CCA GTG 3 Sp6Z-5 AAG CTA TTT AGG TGA CAC TAT AG 3) The following primers were used for completion of the sequence. IL7-5 -CCC AGT TCT TAA AAG ACC TGC-3' TUM83-5 CTT TCC TTT ATG CGA CAC TGG 3 TUM84-5 TTC AGA TCT AGT TGG CAA ATG-3 TUM85-5 TGT CAG CCA GTG TCA CCT GC-3 TUM86-5 -CCA GAT ATC AAG ACA CAG GGC-3 TUM87-5 GGG CAT TAA GAA ATC CCT GGC-3' TUM98-5 -CCT GTT TGA ATT TTG TAT CCA GAG C-3 TUM99-5' AAT AAG AGC GCC ATG GAG CC-3

Sequence data analysis

The sequence data were assembled and analyzed using the Genetics Computer Group (GCG) package (Wisconsin Package Version 9 1 Madison Wis) Alignment of sequences and calculation of similarities were carried out with the VOSTORG package (V5 0 Zharkikh et al 1991)

Results

Cloning and sequencing of the cattle IL13 gene

To isolate the cattle *IL13* gene, a mouse *IL13* cDNA was hybridized to a cattle cosmid library This hybridization revealed no clear signal (nor did a Southern blot of cattle genomic DNA, data not shown) Therefore an alternative approach (i e random sequencing of the '*IL13* candidate' region), was used to identify the cattle *IL13* gene In mouse and human, *IL13* is located less than 20 kb upstream of the gene encoding *IL4* The corresponding region was isolated from a cosmid clone known to contain the cattle *IL4* gene (Buitkamp et al 1995) Thirteen random *Sau* 3A subclones from this cosmid were sequenced from one end One insert showed 77 5% similarity to 140 base pairs (bp) of exon 4 of the human *IL13* gene (accession number L13029) Using the cattle sequence as a starting point, *IL13*-containing fragments were subcloned and sequenced from both ends The whole *IL13* is covered by an 18 kb *Bam* HI subclone To obtain overlapping sequence information for all exons of the cattle *IL13* gene the *Bam* HI plasmid was sequenced with additional primers (IL7 TUM83, TUM84, TUM85 TUM86 TUM87 TUM98 TUM99)

Cattle IL13 gene structure and localization

An overview of the exon intron structure of the cattle *IL13* gene and the complete sequence of a 3.5 kb region containing all of the coding sequence is shown in

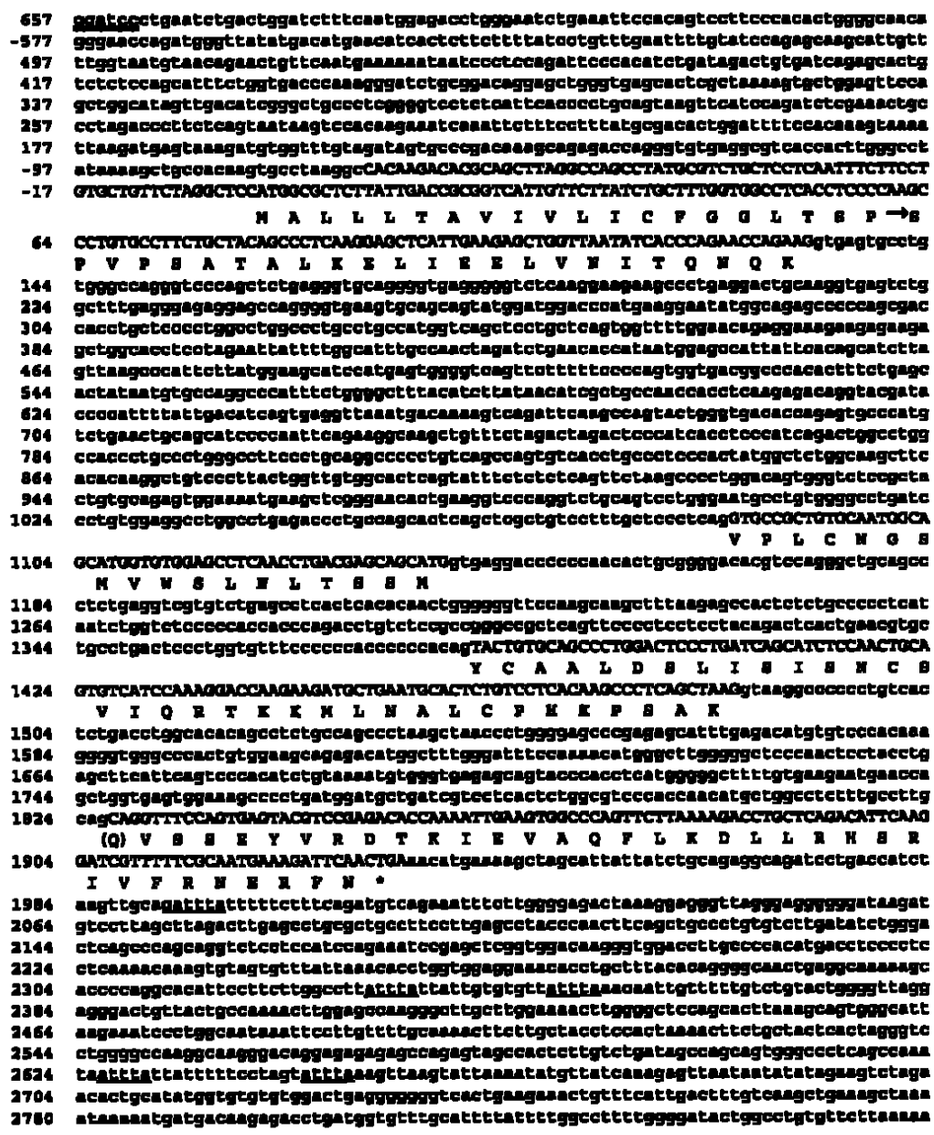

Fig. 1 Nucleotide and derived amino acid sequence of the cattle *IL13* gene The TATA box is indicated in ***bold*** the transcription start site (indicated by *capital letters*) is presumed to be at the position analogous to the mouse *IL13* sequence (McKenzie et al 1993b) the N terminus (serine at amino acid position 21) is indicated by an *arrow* at the position analogous to the first amino acid of the mature human IL-13 (hsIL-13 expressed in Chinese hamster ovary cells Minty et al 1993) The Q at amino acid position 102 which could be encoded by alternative usage of the splice site is given in *parentheses* The *Bam* HI site at the beginning of the sequences (indicated by *double underlining*) corresponds to the cosmid vector insert border The 5 AUUUA motifs in the 3 end are *underlined*

Fig 1 The transcription start site as well as the potential polyadenylation signal were inferred by comparison with the human and mouse data Cattle *IL13* is located about 20 kb upstream of *IL4* in a head-to-tail orientation The orientation of the gene was deduced from the restriction map Cattle *IL13* consists of 4 exons, 201, 51, 105, and 959 bp in length with exon 1 carrying the 5' untranslated region (about 70 nucleotides) and encoding the first 47 amino acids As in other lymphokine genes, all splice sites are located between codons At the 5 end of exon 4, two potential splice sites (at positions 1893–94 and 1897–98 respectively, see Figs 1 and 2) exist Therefore as in human, cattle IL-13 (btIL-13) possibly occurs in two forms Alternative splicing would lead to an insertion of glutamine at amino acid position 98 The *btIL13* gene, together with *IL4*, was mapped to BTA4q23–31 by fluorescence *in situ* hybridization of cosmid 16B to cattle chromosomes as described previously (Buitkamp et al 1995)

The putative cattle IL13 protein

The putative IL-13 amino acid sequence was aligned to the sequences of other species (Fig 2) The atomic structure of IL-13 has not been defined but two models have been proposed using the structural information of other members of the family of helical cytokines [e g., IL-4 or growth hormone (Bamborough et al 1994)] The predicted helices A B C, and D are indicated in Fig 2 (according to PDB entry 3itr Abola et al 1997) The four predicted α-helical regions are shorter than those of IL-4 Whereas helices A and B of IL-13 appear to be highly conserved between the species, helices C

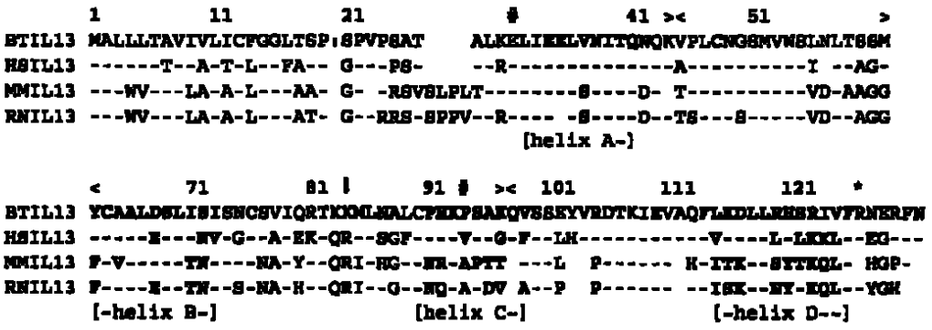

Fig. 2 Alignment of the deduced IL13 aa sequences of *Bos taurus (bt) Homo sapiens (hs) Mus musculus (mm)* and *Rattus norvegicus (rn)* Amino acid positions are numbered according to the *hsIL13* sequence Amino acid sequences were extracted from the EMBL database [accession numbers P35225 (*hs*) P20109 (*mm*) P42203 (*rn*)] – leader peptide cleavage site >< – exon boundaries # – binding residues, * – signalling residues ‡ – position varied in a mutein of the human IL-13 as described by Zurawski and co-workers (1994)

and D show a lower degree of similarity However, amino acids at some of the positions of all helices are identical to those of other helical cytokines For example the leu_{118} and the arg_{127} of IL-13 correspond to the leu_{117} and the arg_{121} of human IL-4 respectively

Putative transcription regulatory sequence elements

The alignment of the 5' region of the cattle human, and mouse sequences is shown in Fig 3 The cattle IL-13 promoter contains the classical TATAA sequence located about 24 nucleotides upstream of the putative transcriptional initiation (the Cap site) at position 70 (see also Fig. 1) but no CCAAT box SRE or HSE elements Several upstream promoter elements were identified by searching the Transfac database (Heinemeyer et al 1998) Some of these are conserved between cattle, mouse and human One of the potential binding sites for factors known to be involved in interleukin gene transcription is located at position -197 to 189 and is closely related to the P elements of the IL-4 promoter The P elements are functionally most important for the expression of IL-4 in the mouse (Abe et al 1992) and highly conserved across different species (Buitkamp et al 1995) Two CREB sites are found close to the TATAA sequence (one highly conserved at -100 to -85, the other at 117 to -106) Highly conserved IFN IE, AP-3 and GAGA 3 sites are indicated in Fig 3 Within the 3 untranslated region, several AUUUA motifs occur From other interleukins these motifs are known to enhance expression by stabilizing the RNA (Richter et al 1990)

Comparison of the cattle IL13 gene with the IL13 genes of other species

The *IL13-IL4* genes are highly conserved with respect to chromosomal location, genomic organization and exon-intron structure As in mouse and human, both cattle cytokine genes are arranged in a head to-tail fashion within a region of about 35 kb Similarities to other transcribed *IL13* sequences are summarized in Table 1 The cattle sequence is most similar to the human sequence, whereas the mouse and rat sequences are more divergent All introns are very similar in length in all species (intron 1 is 0 9–1 2 kb, intron 2 is 0 25–0.55 kb intron 3 is 0 31–0 35 kb in length), but the mouse introns tend to be the longest The intronic sequences of cattle and human *IL13* can be easily aligned and show similarities of 70 4, 66 5, and 69 9% for introns 1, 2 and 3 respectively (data not shown) In contrast the alignment to mouse introns 1 and 2 is not possible The third intron of the mouse and cattle *IL13* can be aligned showing 62 1% identity The identity within the first 700 bp of the 5' region is 78 5% for the cattle and the human and 64 7% for the cattle and the mouse sequence (see also Fig 3)

Discussion

Previous work on IL-13 has focused on the human, mouse and rat systems IL-13 raises a lot of interest since it is involved in atopy Together with IL-4 this cytokine plays an important role in the T_H2 (humoral) immune response and IgE up-regulation These mechanisms are especially important in the immune response to the infection with some macroparasites Infections with macroparasites are widespread in the ruminants and cause severe losses (including sub clinical economic losses) to farmers and breeders In this work we determined the sequence of the cattle *IL13* gene as a starting point for the development of polymorphic markers and functional studies of this cytokine in the ruminants

The cattle *IL13* gene was identified and sequenced directly by its comparative physical location (positional sequencing) This was successful due to the highly conserved genomic organization of the *IL4* family gene

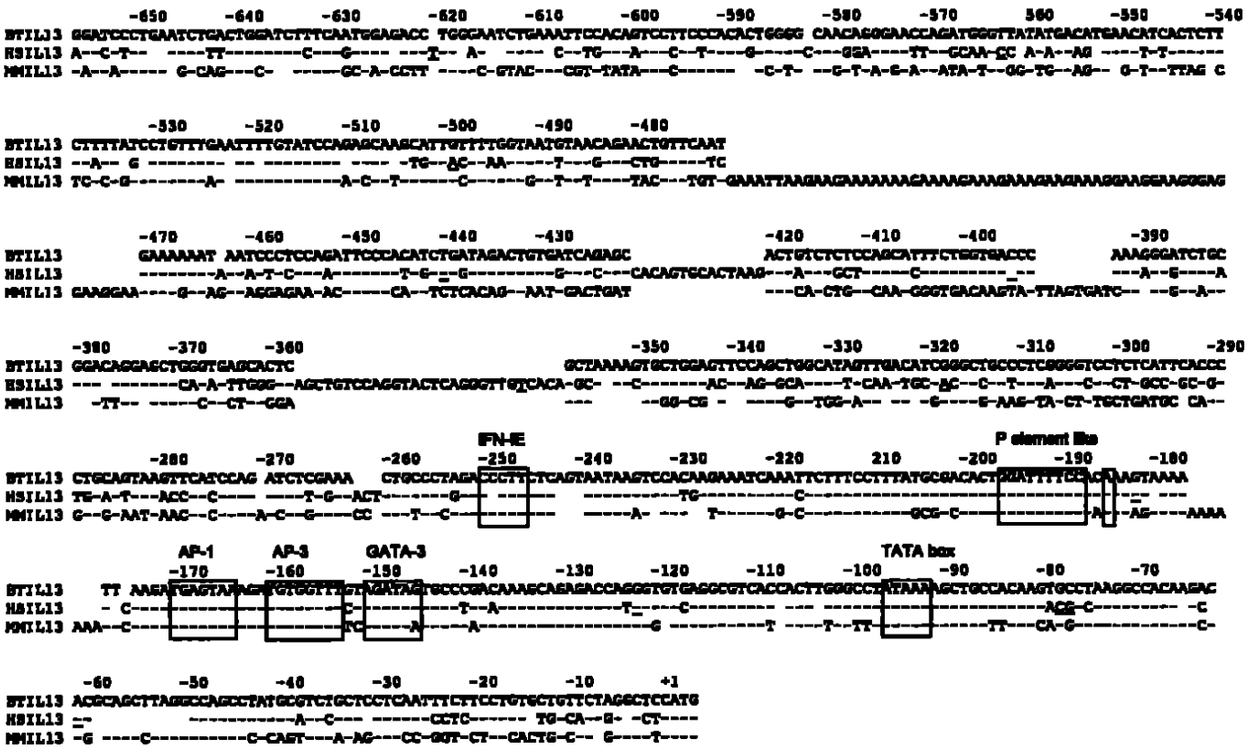

Fig. 3 Sequence alignment of the cattle *(bt)* human *(hs)* and mouse *(mm) IL13* 5′ untranslated and flanking region The numbering is according to the cattle sequence (see Fig. 1) The human and mouse sequences were extracted from the EMBL database [accession numbers U10307 (*hs*) L13028 (*mm*)] residues showing sequence differences from other human sequences (U31120 L13029) are *underlined*

cluster Furthermore the *IL13* gene is relatively compact Under these circumstances this approach could be applied to the identification of other genes when heterologous probes are not available or fail to give a hybridisation signal

The alignment of the *IL13* sequences to different species show a number of highly conserved regions The receptor binding or signalling sites of IL13 have not yet been determined but can be presumed by comparison with the well-investigated IL-4/IL-4-receptor system (Kruse et al 1993) According to the alignment by Bamborough and co-workers (1994) the human IL-4 (*hs*IL-4) binding residues glu_9 and arg_{88} correspond to *bt*IL-13 residues glu_{31} and pro_{94} (indicated in Fig 2) The glutamic acid at (*bt*IL-13) position 31 is conserved between IL-3 and IL-4 across all species The proline at position 94 is replaced by alanine in mouse and rat IL-13 and a valine in human IL-13 These amino acids can be substituted with 95% confidence without changing the properties of the protein (Bordo and Argos 1991) The *hs*IL-4 signalling residues are arg_{121}, tyr_{124}, and ser_{125} The arg_{121} corresponds to *bt*IL-13 arg_{127} and is conserved in IL-13 but is replaced by a lysine in some of the IL-4 proteins This is also a conservative amino acid exchange The tyrosine at position 124 (signalling site, Fig 2) seems to be crucial in the signalling process since a substitution with aspartic acid at that position leads to an IL-4/IL-13 antagonist (Aversa et al 1993) Two positions have been shown to be important by generating mutant proteins (cytokine muteins) of human IL-13 (Zurawski and Zurawski 1994) The first is position 31 (glutamic acid) corresponding to the first binding residue in IL-4 (see above) The second is position 84 (Fig. 2), corresponding to arg_{87} in the mature *hs*IL-13 At that position a lysine occurs in the cattle IL-13 sequence Since an exchange of lysine to an arginine should not change the properties of the protein (Bordo and Argos 1991), both positions are highly conserved across the species The availability of homologous sequences allows the identification of highly conserved amino acid positions This information could be used to identify candidate residues for the design of muteins Cytokine-based diagnostic and therapeutic ap-

Table 1 Inter-species comparison of transcribed *IL13* gene sequences The percentage identity of nucleotide sequences are given to the right of the diagonal and the deduced amino acid sequences are given to the left of the diagonal

		nt identity			
		Cattle	Human	Mouse	Rat
aa identity	Cattle	–	80 95%	70.54%	70.57%
	Human	70 99%	–	72 60%	75 52%
	Mouse	57 93%	65 35%	–	86.61%
	Rat	57 14%	62 20%	79 23	–

proaches are the subject of intensive investigation and are covered by an increasing number of patents Since the production and testing of muteins with respect to antagonistic or agonistic activity is a time-consuming procedure such information could be valuable for future pharmacological research

There are very few functional studies concerning the IL-13 transcriptional elements The existence of a P-element like sequence has been proposed by Dolganov and co workers (1996) They showed that this element binds the transcription initiator protein NF-ATp *in vitro* in human T cells As shown in Fig 3, a conserved AP-1 site probably constituting a NF-ATp/AP 1 composite binding site exists This composite site would be required for the formation of a complex of NF-ATp and proteins of the Fos and Jun-family (components of the AP-1 transcription factor) proteins (Miner and Yamamoto 1991) A number of further potential binding sites had been identified However, the importance of these binding sites remains to be shown Elements known to be functionally important are highly conserved upstream from IL-4 in cattle, human, mouse, and rat (Buitkamp et al 1995) When comparing the IL-13 5' region across species the degree of identity is quite high but not as high as for the IL-4 gene which has 90% identity between cattle and human There are a number of short highly conserved sequence stretches These blocks are prime candidates in the search for additional regulatory factors A striking feature is the expansion of a purine-rich stretch in the mouse IL-13 and IL-4 5 regions at position -472 and position 211 respectively (Fig. 3) Intron 3 is conserved between all three species In this intron a number of potential transcriptional elements are identified For example several conserved binding sites for the Ikaros gene 2 (*IK 2*) product exist. This gene encodes a zinc finger DNA-binding protein that is expressed in a lymphocyte restricted fashion and can strongly stimulate transcription Therefore intron 3 similar to intron 1 in the mouse IL-4 (Henkel et al 1992) could contribute to the regulation of IL-13

The major regulating elements are usually expected to lie within the first few 100 bp of the upstream region For example, in the *IL4* promoter the first 97 bp are sufficient for transcription after *in vitro* stimulation of the mouse T_H2 clone D 10, even though about 3 kb have to be present for the entire *in vivo* tissue specificity and inducible expression (Todd et al 1993) Furthermore the first 300 bp are sufficient to mediate the transcriptional specificity of IL-4 in T_H2 (vs T_H1) cells (Bruhn et al 1993) It has been speculated that the tandem arrangement could be functionally related to the co-expression of these cytokines (Dolganov et al 1996 Smirnov et al 1995) However the regulatory sequences for IL-13 look quite different from those for IL-4 For example, no CS1 or CS2 sites, suspected to mediate T_H2 transcriptional specificity of IL-4 (Bruhn et al 1993), are present in the IL-13 region This finding is inconsistent with functional co-expression but supports clonally diverse (i e, functionally independent) expression of both cytokines as shown by Essayan and co-workers (1996) IL-4 and IL-13 are closely related by function, localization, and genomic structure Both have multiple overlapping functions but their regulatory elements seem to be quite distinct

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Genetic variants of IL-13 signalling and human asthma and atopy

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Asthma and atopy show epidemiological association and are biologically linked by T-helper type 2 (T_h2) cytokine-driven inflammatory mechanisms. IL-4 operates through the IL-4 receptor (IL-4R, a heterodimer of IL-4Rα and either γc or IL-13Rα1) and IL-13 operates through IL-13R (a heterodimer of IL-4Rα and IL-13Rα1) to promote IgE synthesis and IgE-based mucosal inflammation which typify atopy Recent animal model data suggest that IL-13 is a central cytokine in promoting asthma, through the stimulation of bronchial epithelial mucus secretion and smooth muscle hyper-reactivity We investigated the role of common genetic variants of IL-13 and IL-13Rα1 in human asthma, considering IgE levels. A novel variant of human IL-13, Gln110Arg, on chromosome 5q31, associated with asthma rather than IgE levels in case–control populations from Britain and Japan [peak odds ratio (OR) = 2 31, 95% CI 1.33–4.00], the variant also predicted asthma and higher serum IL-13 levels in a general, Japanese paediatric population. Immunohistochemistry demonstrated that both subunits of IL-13R are prominently expressed in bronchial epithelium and smooth muscle from asthmatic subjects Detailed molecular modelling analyses indicate that residue 110 of IL-13, the site of the charge-modifying variants Arg and Gln, is important in the internal constitution of the ligand and crucial in ligand–receptor interaction A non-coding variant of IL-13Rα1, A1398G, on chromosome Xq13, associated primarily with high IgE levels (OR = 3.38 in males, 1 10 in females) rather than asthma Thus, certain variants of IL-13 signalling are likely to be important promoters of human asthma, detailed functional analysis of their actions is needed

INTRODUCTION

Atopy is a common immune disorder characterized by raised IgE levels It is a key predisposition to bronchial asthma between 3 and 20 years of age (1,2). Bronchial hyper-responsiveness (BHR) an exaggerated bronchospastic response to specific and non specific substances, represents a physiological hallmark of asthma induced by T-helper type 2 (T_h2) cytokines such as IL-4, 5 -9 10 and 13 (1,2). The studies on T_h2 cytokines in relation to asthma, however have focused on IL-4 and IL-5 This is due to the crucial role of these two cytokines in generation of T_h2 responses in a variety of animal models IL-4 is essential for the maturation of naïve T cells towards T_h2 cells, and the production of IgE (2,3), whereas IL-5 regulates activation and tissue recruitment of eosinophils (4) However a variety of studies have

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shown that IL-4 and IL-5 alone and in combination cannot fully account for the development of asthma (5,6) in BALB/c mice, BHR to methacholine is mediated by T cells, but is independent of IL-4 and IL-5. Other molecules must be involved in the development of BHR, and hence asthma.

IL-13 is a 12 kDa protein product and shares several biological profiles with IL-4 (1,2), including IgE production, CD23 and MHC class II expression, inhibition of antibody-dependent cell-mediated cytotoxicity with downregulation of IgG type I receptor (FcγRI), and suppression of type I interferon. Although IL-4 and IL-13 possess many similar biological activities (1,2), IL-13 shows some unique activities. Unlike IL-4-deficient mice, IL-13 null mice fail to generate goblet cells, responsible for mucus overproduction in asthma, fail to recover basic IgE levels after stimulation with IL-4 and fail to expel helminths (7). IL-13 operates through IL-13R, a heterodimer of IL-4Rα and IL-13Rα1 chains (1–3). Transgenic mice with the promoter of the Clara cell 10 kDa protein (CC10) driving IL-13 expression selectively in the lungs exhibit BHR to methacholine in addition to bronchial eosinophil prominence, epithelial cell hyperplasia, mucus cell metaplasia, hyperproduction of mucus, deposition of Charcot–Leyden-like crystals and subepithelial airway fibrosis (8); these features are typical of T_H2 inflammation-induced asthmatic airways. These findings suggest that IL-13 is crucial for allergen-induced BHR in experimental animals, and may be relevant to human asthma (9,10). Significantly higher IL-13 levels have been found in asthmatic patients with and without atopy (11,12). One report has related a polymorphism within the promoter region of IL-13 with allergic asthma in a Dutch population (13).

Here we report a variant of the human IL-13 gene, Gln110Arg, which specifically associates with both allergic and non-allergic asthma in case–control studies in both British and Japanese subjects. Our computer modelling suggests that residue 110 is relevant to the internal constitution of the ligand, and is likely to play a crucial role in ligand–receptor interaction. Using immunohistochemical techniques we show that functional IL-13R is specifically expressed on bronchial smooth muscle and epithelium in human asthmatic airways. These findings point to the importance of IL-13 in human asthma. In contrast, we show that a variant of IL-13Rα1 A1398G on chromosome Xq13 (14) associates primarily with high IgE levels in the British subjects, a result that parallels our previous findings for variants of IL-4Rα in Japanese (15,16) and German (17) subjects. Thus, certain variants of IL-13 may promote the development of atopy with high IgE levels, and asthma of diverse types across different ethnic groups.

RESULTS

Genetic association study of an IL-13 variant in case–control populations

A single-strand conformation polymorphism (SSCP) analysis among >200 atopic subjects identified different electrophoretic patterns in exon 4 of IL-13 (Fig. 1A); subsequent direct sequencing identified an A4464G variant (18) (Fig. 1B) indicating replacement of Arg by Glu at position 110 of the mature protein. Despite intensive screening we could not find any further common variants. We went on to test for a genetic association between Gln110Arg and clinical asthma and IgE levels in two populations (Table 1). The genotype frequencies were concordant with Hardy–Weinberg equilibrium. No significant differences in genotype frequencies were seen between two control populations: $P_{Arg} = 0.92$, $P_{Gln} = 0.08$ in a British population, and $P_{Arg} = 0.90$, $P_{Gln} = 0.10$ in a Japanese population. In a case–control study of British subjects, the Gln110 significantly associated with asthma, especially chronic unremitting asthma. In a second case–control study of Japanese subjects, Gln110 associated with atopic [odds ratio (OR) = 1.85, 95% CI 1.05–3.24, $P = 0.033$] and also non-

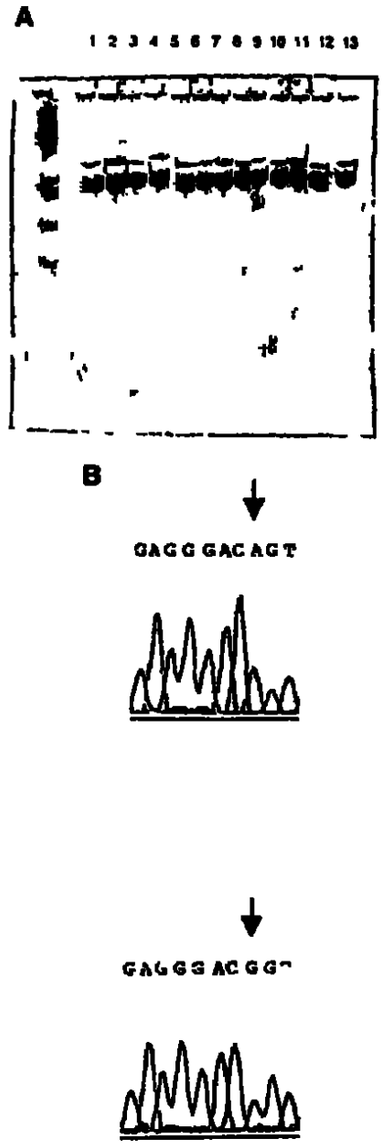

Figure 1. Detection of the Gln110Arg variant of IL-13. (A) SSCP gel for IL-13 exon 4. Lanes 1, 3, 5, 6, 10 and 12, wild-type; lanes 4, 9 and 13, mutant; lanes 2, 7 and 11, heterozygous. DNA markers were run in the left-hand lane. (B) Sequence of the wild-type (top) and mutant (bottom) alleles of IL-13 exon 4. The arrow indicates the G to A change.

Table 1 Odds ratio (95% CI) for IL-13 and its receptor genes, IL-4R and IL-13RA1 by asthma, total serum IgE, ASE and atopy in the Japanese and the British populations

	IL-13 Gln110Arg			IL-4Rα Ile50Val			Arg551Gln			IL-13Rα1 A1398G		
	%Gln	OR (95% CI)	*P* value	%Ile/Ile	OR (95% CI)	*P* value	%Arg (+)	OR (95% CI)	*P* value	%A/A	OR (95% CI)	*P* value
Japanese												
Controls	43			18.0			22.0			40.0		
Atopic asthma	63	1.85 (1.05–3.24)	0.033	59.0	4.42 (1.57–6.69)	<0.0001	32.0	1.66 (0.88–3.13)	0.111	33.0	1.35 (0.76–2.41)	0.304
Non-atopic asthma	62	1.77 (1.01–3.10)	0.047	18.0	1.00	1.00	24.0	1.12 (0.58–2.17)	0.737	36.0	1.19 (0.67–2.10)	0.56
Asthmatics	62.5	1.81 (1.11–2.93)	0.013	38.5	1.52 (0.68–2.98)	<0.151	28.0	1.38 (0.77–2.78)	0.264	34.5	1.27 (0.77–2.08)	0.350
Low serum IgE	56.3			17.6			23.5			38.6		
High serum IgE	60.2	1.18 (0.72–1.90)	0.508	53.5	3.09 (1.98–4.91)	<0.0001	28.5	1.28 (0.81–2.02)	0.288	32.4	1.18 (0.72–1.93)	0.516
ASE (–)	52.7			18.2			23.6			38.9		
ASE (+)	63.9	1.59 (1.00–2.53)	0.051	52.3	3.17 (1.98–5.07)	<0.0001	28.0	1.26 (0.63–2.52)	0.319	33.1	1.29 (0.80–2.08)	0.296
Non-atopic	52.3			17.0			23.3			40.1		
Atopic	64.6	1.66 (1.01–2.57)	0.047	62.4	7.04 (2.08–9.88)	<0.0001	28.8	1.59 (0.91–3.03)	0.093	31.9	1.28 (0.80–2.08)	0.305
British												
Controls	26.7			34.6			41.3			4.0		
Atopic asthma	40.0	1.83 (1.13–2.99)	0.014	32.0	1.12 (0.81–2.34)	0.624	35.5	0.78 (0.49–1.24)	0.342	12.0	3.22 (1.26–8.33)	0.018
Non-asthmatics	23.5			31.3			35.2			4.2		
Asthmatics	39.8	2.14 (1.28–3.60)	0.003	34.3	1.11 (0.68–1.82)	0.770	32.4	0.73 (0.45–1.17)	0.236	10.5	2.70 (0.98–7.14)	0.053
Severe asthmatics	41.5	2.31 (1.33–4.00)	0.003	36.3	1.39 (0.74–2.13)	0.488	33.8	0.78 (0.47–1.30)	0.409	10.0	2.50 (0.87–7.35)	0.090
Low serum IgE	29.3			31.3			37.7			3.6		
High serum IgE	38.3	1.49 (0.92–2.42)	0.100	36.3	1.25 (0.30–1.47)	0.366	39.1	1.06 (0.66–1.69)	0.902	13.5	2.88 (1.11–7.86)	0.021
ASE (–)	30.0			31.2			39.2			4.5		
ASE (+)	38.3	1.27 (0.77–2.10)	0.351	34.9	1.05 (0.29–1.28)	0.856	37.5	0.62 (0.39–1.01)	0.071	10.0	2.32 (0.85–6.45)	0.122
Non-atopic	27.2			30.2			35.2			4.0		
Atopic	36.3	1.52 (0.90–2.58)	0.118	37.3	1.23 (0.73–2.04)	0.515	36.5	0.82 (0.50–1.34)	0.520	10.0	2.63 (0.87–7.69)	0.111

[a]OR in females = 1.10 (95% CI:0.81–2.81 Fisher's exact test $P = 0.765$), in males = 3.39 (95% CI 1.04–9.35, Fisher's exact test, $P = 0.015$ (see text)

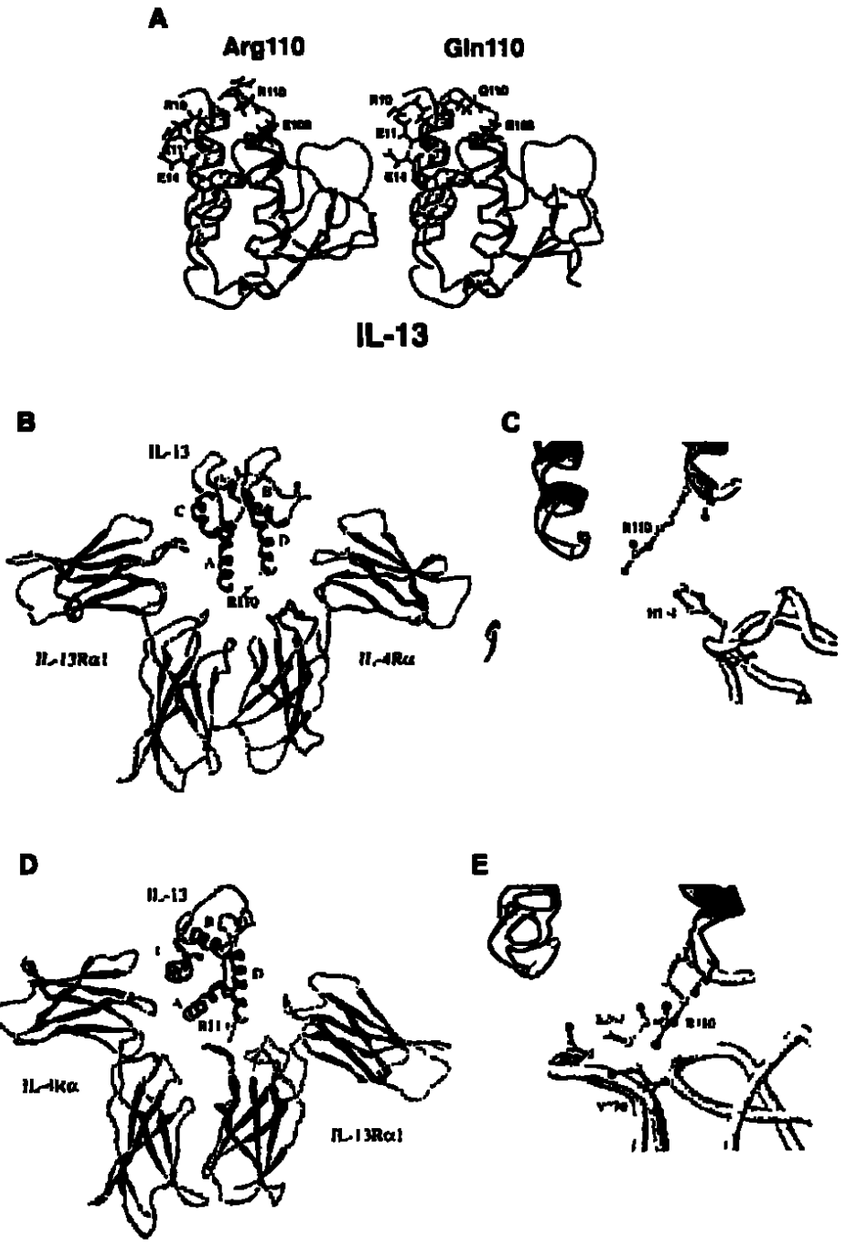

Figure 2. Ribbon diagrams of the IL-13 computer model (A) The effect of replacement at position 110 on the internal constitution of the IL-13 ligand. (Left) Wild-type, Arg110, (right) mutant, Gln110. The blue and pink portions represent α-helices or β-sheets, respectively. Numbering is based on the mature peptide. (B–E) Modelling of IL-13 in relation to its receptors, IL-4Rα and IL-13Rα1 chains. Two models (1 or 2) are shown; the differences between the two depend on whether the IL-13 helix AC interfaces with IL-4Rα [model 1 (B and C)] or IL-13Rα1 [model 2 (D and E)] β-sheets are shown in blue, and α-helices in red. (B) R110 of the IL-13 is predicted to interact with loop BC of the IL-4Rα (magenta). (C) Close-up of the interaction: R110 faces H131 of IL-4Rα. (D) R110 is predicted to interact with loop C'E of the IL-13Rα1 (magenta). (E) Close-up of the interaction: R110 faces E267 of IL-13Rα1. Note that backbone atoms of V270 are also shown

atopic (intrinsic) asthma (OR = 1.77, 95% CI 1.01–3.10, $P = 0.047$); the overall OR for asthma was 1.81 (95% CI 1.11–2.93, $P = 0.017$). There was no association between Gln110 and serum IgE levels in either population.

Table 2. Simple factorial ANOVA analysis for genetic variants in the development of asthma and atopy in the British and the Japanese populations

Phenotype	Genetic factor	Variant	F value (df = 2)			
			British	P value	Japanese	P value
Asthma	IL-4	-590C/T	0.511	0.600	0.490	0.623
	IL-13	Gln110Arg	4.133	0.017	4.283	0.026
	IL-4Rα	Ile50Val	2.511	0.083	2.614	0.076
		Arg551Gln	1.121	0.328	0.998	0.344
	IL-13Rα1	A1398G	2.704	0.069	1.134	0.313
Atopy (IgE levels)	IL-4	-590C/T	0.229	0.795	0.748	0.475
	IL-13	Gln110Arg	0.935	0.394	1.494	0.206
	IL-4Rα	Ile50Val	0.362	0.697	5.272	0.006
		Arg551Gln	1.242	0.290	0.206	0.814
	IL-13Rα1	A1398G	4.288	0.015	0.829	0.479

We found no linkage disequilibrium between the *IL-4* (-590C/T) and the *IL-13* (Gln110Arg) variants in either the British (t = Δ/SE = −0.43) or the Japanese (t = Δ/SE = −1.29) population.

Genetic association study of the IL-13 variant in a general population in relation to serum IL-13 levels

To test the relationship between the Gln110Arg of IL-13 and serum IL-13 levels we conducted a population-based survey of genotype frequencies among Japanese schoolchildren aged between 12 and 13 years (19). A significantly higher frequency of the Gln110 allele was seen among asthmatic children than that among non-asthmatic subjects. Those homozygous for Gln110 had significantly higher levels of serum IL-13 than those homozygous for Arg110.

Genetic association study of an IL-13Rα1 variant in case–control populations

We have searched for single nucleotide polymorphisms (SNPs) by SSCP in the coding region of *IL-13RA1* on the X chromosome (14). all three SNPs discovered were silent and only one of them, A1398G was relatively common (20). This variant showed marginal association with high IgE levels (OR = 2.88, 95% CI 1.11–7.86, P = 0.021 Pc = 0.06) but not with asthma in the British population no association was seen with either high IgE levels or asthma in the Japanese population (Table 1). Since male subjects are hemizygous at *IL-13RA1* we calculated adjusted OR by sex. In the Japanese population, there was no significant difference in OR (1.66 versus 1.21) between male and female subjects for atopy. In the British population OR was 3.39 (Fisher's exact test, P = 0.015) in males and 1.10 in female (Fisher's exact test, P = 0.680) for atopy.

Genetic interaction among variants of IL-4 and IL-13 signalling

To test whether variants of *IL-4* and *IL-13* and of their receptor genes, *IL-4R* or *IL-13RA1* might interact in the development of asthma and atopy we conducted simple factorial analysis of variance (ANOVA). there was no significant genetic interaction between these variants of ligand and receptors in the development of asthma or atopy in either the British or the Japanese populations (Table 2). In the development of asthma, the Gln110 variant of *IL-13* is a significant factor in both populations. atopy associates with either an *IL-13RA1* variant in the British population or an *IL-4R* variant in the Japanese population (15,16) in our German population, atopy is also mainly related to *IL-4R* variants (OR = 0.36, P = 0.0042 for the combination with Pro478 and Arg551) (17).

Computer modelling of IL-13 and its receptor

To address the biological activity of the Gln110 variant of IL-13 molecular modelling was conducted on the basis of sequence alignment between IL-4 and IL-13 (20). Firstly the modelling suggested that the replacement of Arg with Gln at position 110 may allow Arg10 to become closer to Gln110 with electrostatic interaction within IL-13 itself (Fig. 2A) this may result in subsequent change of electrostatic potential around glutamic acids at positions 11 and 14, and the former is believed to be important for IL-13 binding to the receptor on the basis of IL-4 homology (20).

Further molecular modelling focused on the interaction of IL-13 with IL-4Rα and IL-13Rα1 and was conducted on the basis of multiple alignment of cytokine receptors (Fig. 2B–E). Two alternative models (model 1. Fig. 2B. model 2, Fig. 2D) showed that Arg110 is likely to have direct interaction with one or other of the component receptor chains. Closer inspection of Arg110 in model 1 showed that this residue was located in proximity to His131 of IL-4Rα (Fig. 2C). As both residues are positively charged, this predicted a repulsive interaction. A Gln110 mutant would abolish this expulsion and thus enhance receptor binding of IL-13 with consequent upregulation of IL-13 signalling. In model 2, Arg110 lies close to Glu267 and Val270 of IL-13Rα1 implying an attractive interaction (Fig. 2E). A Gln110 mutant would lead to reduced binding. In the light of the association of Gln110 with asthma, model 1 may be more plausible. However model 2 remains interesting since such a mutation might lead to an alternative binding mode or induce reversal of model 2 binding mode to a model 1 binding mode. In either case computer modelling strongly suggests that Arg110 is directly involved in interactions with its receptor and that charge changing variants (Arg, Gln) are likely to display different biological properties.

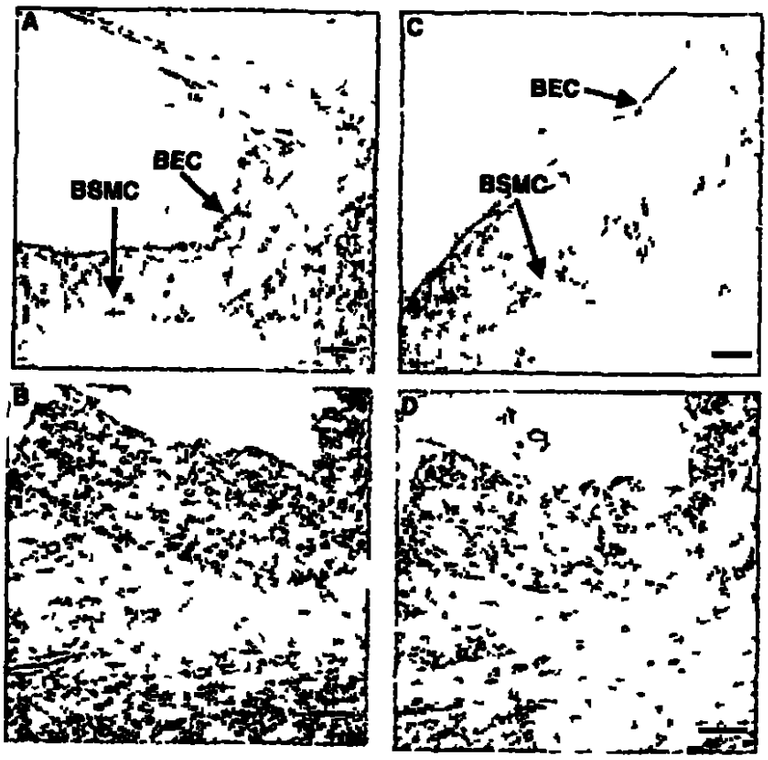

Figure 3. Alkaline phosphatase staining of a human lung probed with anti-IL-13Rα1 antibody (A and B) or anti IL-4Rα antibody (C and D) (A and C) Staining in normal bronchiolus Bar, 220 μm in (A), 100 μm in (C) (B and D) Staining in asthmatic bronchiolus. Bars, 25μm.

Immunohistochemical assay with bronchial specimens

Only in murine studies has the existence of IL-13R been demonstrated in airways (1,2). Therefore, we conducted immunohistochemistry on pulmonary specimens from normal controls and asthmatic subjects, using monoclonal antibodies to both IL-13Rα1 and IL-4Rα (Fig. 3A–G). Both components were present prominently in smooth muscle cells and epithelial cells of the bronchus, but not in fibroblasts (Fig. 3A–D) Within alveolar tissue neither epithelial cells nor pulmonary macrophages showed either component (Fig. 3A–E) Specificity of signal was confirmed by demonstration that the signal was not elicited by the treatment without the primary antibody (Fig 3) Thus the data suggest that functional IL-13R is specifically expressed in epithelial and muscle cells of the human bronchus.

DISCUSSION

Our data suggest an important role for genetic variants of IL-13 in the development of asthma, independently of IL-4, in humans. Many groups have identified linkage of both asthma and IgE levels to chromosome 5q31 (21,22) where *IL-4 IL-13* and *IL-5 cluster is localized. Although IL-4 is crucial for the development* of T_H2 cells (1–3) and hence high IgE levels (23–26), IL-4 may not be a sufficient inducer for asthma itself No functional polymorphism in *IL-5* has been identified in relation to either asthma (27) or eosinophilia (28).

We have identified a novel coding variant of the human *IL-13* Gln110Arg that associates with asthma in both British and Japanese populations. Our computer modelling suggests that Gln at position 110 impacts on ligand–receptor interaction through enhanced charge attraction to IL-13R Thus in turn may enhance signalling, but detailed functional studies are now needed to test this The genetic association of *IL-13* with asthma across different ethnic populations, which is independent of *IL-4* supports the candidacy of *IL-13* as a major locus for asthma on chromosome 5q31 Moreover we found a genetic association of the Gln110Arg variant of *IL-13* with both atopic and non-atopic (intrinsic) asthma—a finding concordant with clinical reports on the significant elevation of IL-13 levels in both types of asthmatic subject (11 12) These findings extend the observation of association between a promoter polymorphism of IL-13 and allergic asthma in a Dutch population (13) and support the contention that IL-13 may be a key promoter of bronchial asthma in humans

There is strong evidence that IL-13 is crucial for the induction of an asthma-like phenotype in animal models, independent of IL-4 (9 10) but which is dependent on IL-4Rα, a common component of IL-4R and IL-13R T cell-deficient mice are capable of inducing an asthma-like phenotype on administration

Figure 3. (E–G) Without (left) and with (right) anti-IL-13Rα1 monoclonal antibody staining. (E) Bronchial epithelium (F) BSMC (G) alveolar space. AC, alveolar cells; BEC, bronchial epithelial cells; BSMC bronchial smooth muscle cells

of IL-13 (9), suggesting that IL-13 may operate through mechanisms other than those that are classically implicated in T_H2 cell-induced immune reactions (10) One possible explanation is that IL-13R is predominantly expressed in bronchial tissues, and that higher production of IL-13 in high risk genotypes induces hypertrophic change of the bronchial smooth muscle, subepithelial fibrosis and goblet cell hyperplasia through IL-13R To investigate this possibility we stained airway specimens from normal controls and asthmatic subjects with anti IL-13Rα1 and anti IL-4Rα monoclonal antibodies. In asthmatic airways in contrast to alveolar tissue, both components of IL-13R are significantly expressed. To date, two types of specific IL-13

receptor unit have been identified IL-13Rα1 (29) and IL-13Rα2 (30) IL-13Rα2 is considered to be a decoy receptor (31,32) whereas the heterodimer consisting of IL-13Rα1 and IL-4Rα acts as the functional receptor for human IL-13 (1,2) Human IL-4Rα is constitutively expressed in airway epithelial cells (33,34) and is essential for mucus production (7–9) Although human IL-4 induces goblet cell hyperplasia *in vivo* (35), T_h2 cells derived from IL-4-null mice cannot activate goblet cells even after transfection into IL-4Rα-null mice, suggesting that, in the absence of IL-4, IL-13 may be critical for goblet cell activity through IL-13R in airways (7–9) Also, IL-13 transgenic mice show BHR to methacholine and subepithelial fibrosis, whereas IL-4 transgenic mice do not (8) The immunohistochemical findings in human lung specimens suggest that functional IL-13R is expressed in bronchial tissues in asthmatic subjects, and support the idea that IL-13 may play a key role in the development of asthma.

IL-13 and IL-4 have overlapping effector profiles (1–3). This overlap is probably due to shared use of IL-4Rα, and the IL-13Rα1 chains in the multimeric receptor complex (1–3) Chromosome 16p11 2/12, where the IL-4Rα gene is encoded, has also been shown to be linked to atopy (36) We have previously identified a common extracellular variant, Ile50Val of the IL-4Rα, which associates strongly with atopic asthma in the Japanese but not in the British population (Table 1) (15 16) Furthermore, we showed that Ile50 strongly and specifically associated with atopic rather than intrinsic or non-atopic asthma. it upregulated cellular IgE synthesis when tested by transfection into B cell lines. Another cytoplasmic variant of IL-4Rα, Arg551Gln associates with atopy (17) in our German population this variant was in tight linkage disequilibrium with Pro478Ser and the latter may change the structure of the receptor leading to altered phosphorylation patterns of signal molecules, and hence lower IgE levels (17)

We now show that a variant of *IL-13RA1* on the X chromosome (14) showed association with IgE levels, but not with asthma, in British male subjects significantly the OR for high IgE levels was 3 38 in males, but 1 10 in females This is the first indication of X linked inheritance of an atopic immune disorder with high IgE levels, and further illustrates the heterogeneity of its genetic basis It may also, in part, explain the previously noted transmission of atopy through non-affected mothers (37 38) The functional role of this variant remains unknown it may be in linkage disequilibrium with so far unidentified polymorphisms in the regulating or coding parts of *IL-13RA1* or variants of *IL-13RA2* (30) close by on the X chromosome (14) Further studies are needed to clarify these points.

In conclusion, we have identified a novel coding variant of the human *IL-13* Gln110Arg, on chromosome 5q31 where genome-wide searches have identified linkage to asthma (23,24) Gln110Arg shows association with clinical asthma across different ethnic populations, including atopic (high IgE levels) and non-atopic asthma (Table 1) it associates with higher serum IL-13 levels Immunohistochemical techniques revealed that both components of IL-13R are present in epithelial and smooth muscle cells of the human bronchus in asthma. Molecular modelling points to modification of ligand–receptor interaction by Gln110Arg substitution with increased ligand–receptor attraction We therefore propose that Gln110Arg IL-13 the product of effector cells in asthmatics promotes smooth muscle hyper reactivity and mucus hyper-secretion through IL-13R in human bronchi and thus promotes clinical asthma. Our observations and conclusions are consistant with the observed central role of IL-13 in experimental asthma in animal models (9 10) detailed functional analyses on the variant are now required. In contrast, we have found that high IgE levels associate with variants of IL-4Rα or IL-13Rα1 with restriction amongst different ethnic groups (Table 1) A multivariate analysis shows that the associations are independent among the variants of IL-4, IL-13 IL-4Rα and IL-13Rα1 (Table 2) The implication of variants of IL-4 and IL-13 signalling in the development of asthma and atopy in humans provides a focus for considering novel therapeutic and preventive strategies

MATERIALS AND METHODS

Populations studied and phenotypes

Three distinct populations were studied, comprising a total of 890 subjects to whom uniform criteria were applied for assignment as clinical asthma or atopy (i) the British case–control group included 150 young adult subjects with clinical asthma and atopy and 150 healthy controls, all from the Oxford region (39) (ii) the Japanese case–control group included 100 young adults with clinical asthma and atopy 100 adults with clinical asthma but no atopy (non-atopic or intrinsic asthma) and 100 young adults attending a well man clinic as controls all drawn from the Osaka region (40) (iii) a general childhood Japanese population of 290 schoolchildren from Wakayama Prefecture who showed negative tuberculin responses at 6 and 12 years old of whom 46.8% showed atopy and 13.4% clinical asthma (19) All the asthmatic subjects had specialist physician-diagnosed asthma with (i) recurrent breathlessness and chest tightness requiring on-going treatment (ii) physician-documented wheeze; and (iii) documented labile airflow obstruction with variability in serial peak expiratory flow rates >30% There were no heavy smokers (>20 cigarettes/day) among the subjects. Allergen specific IgE (ASE) was detected by the CAP ELISA system (Pharmacia, Uppsala, Sweden) and the criteria for a positive titre were as used previously (39,40) A high IgE titre (CAP system) was taken as >2 SD above published normal values (39,40) Atopy was defined as high IgE levels by the presence of a high concentration of total serum IgE, or a positive ASE against one or more highly purified aero-allergens

SSCP analysis and direct sequencing

SSCP analysis for human IL-13 and IL-13Rα1 was done as follows. searching for polymorphisms in the four exons of IL-13 we used the following primers. 5′ AAG CTG CCA CAA GAC GCC AA 3′ and 5′ GCC TGC TCA TGA CCT CAT CT-3 for exon 1 5′-GCA CTC TGC TCA CTG TCA CT 3′ and 5′ AAG ATG GGG CTG AGA TGC CT 3 for exon 2, 5 -CAC AAA AGG CAG CTG CCC AA 3′ and 5′-GGT GGA CAC ACA CCA TGG AT 3 for exon 3 and 5′ TGG CGT TCT ACT CAC GTG CT 3′ and 5′-CAG CAC AGG CTG AGG TCT AA 3′ for exon 4. The annealing temperature was 60°C in all cases. Primers for the IL-13 promoter region were 5′-GCA ACA TAG TGA GAC CCC AT 3 and 5′-GCT ATG GGA ATT TGG GGA GT 3 ,5′ TAA GAG ACT GGT TCA TCG AA 3 and 5′ TTA AT T CCA GCG GCA GGC AA-3 and 5′-GGG CAG CAT TGC AAA TGC CA 3 and 5′-GAT TGA GGA GCG GAT GCA TA-3′ These combinations of primers covered 739 bp of sequence upstream from the ATG codon The annealing temperature for the

first set was 63°C, whereas that for the latter two was 55°C. When searching for polymorphisms in the IL-13Rα1 seven sets of primers were used for SSCP: 5′ TCC GAG GCG AGA GGC TGC AT-3′ and 5′-CAC TGG GAC CCC ACT TGC AG-3′ (60°C) 5′-GTA TTT TAG TCA TTT TGG CG-3′ and 5′-AGT TAG TGT CGG GAC TGG TA-3′ (60°C) 5′-GCA CAA CTT GAG CTA CAT GA-3′ and 5′-TT CAC AGC CGA AGT TAA AGG-3′ (60°C) 5′-AAA ATT AAA CCA TCC TTC AA-3′ and 5′-GGA CCA TGA AAC AAG ATG TA-3′ (50°C) 5′-TGA GAA TCC AGA ATT TGA GA-3′ and 5′-TAA TCT TGA GCC TTT TTA GG-3′ (45°C) 5′-TCA TCG TCG CAG GTG CAA TC-3′ and 5′-AAT GGA GAA TGG GAA GAA TC-3′ (50°C) and 5′-TCA GTG ATG GAG ATA ATT TA-3′ and 5′-ATA AGA TTA ACT CCA CCA CT-3′ (55°C). The annealing temperature is shown in parentheses. The amplified products were resolved on non-denaturing polyacrylamide gels under four different conditions: 10% polyacrylamide gel containing 0 or 10% glycerol at 10 or 20°C for 2 h. The gels were visualized by silver staining. Sequencing was conducted with the big-dye system (Applied Biosystems, Warrington, UK) using downstream primers for IL-13 and IL-13Rα1 and the image was visualized in the commercial POP-6 gel using an automated sequencer (ABI Prism 310 Genetic Analyser).

Serum IL-13 assay

Serum IL-13 was immunoassayed in the Mitsubishi Kagaku BCL laboratories by means of a commercial kit (19). To limit circadian variation in cytokine production, blood samples were obtained between 09:00 and 10:00 h. The minimal detectable level was 3.1 pg/ml.

Genotyping

DNA samples were extracted using the IsoQuick kit (Microprobe, Garden Grove, IL). For genotyping Gln110Arg of IL-13 PCR primers were: 5′-TGG CGT TCT ACT CAC GTG CT-3′ and 5′-TTT CGA AGT TTC AGT AGT AC-3′ (underlined bases were mutated to incorporate a restriction site). Amplified products were digested with *ScaI* at 25°C. For genotyping IL-13Rα1 primers were: 5′-TCA GTG ATG GAG ATA ATT TA-3′ and 5′-TGA GCT GCC TGT TTA TAA AT-3′. Amplification products were digested with *MseI*. Genotyping Ile50Val and Arg551Gln of the IL-4Rα (16) –590C/T promoter of the IL-4 (41) was as described elsewhere.

Computer modelling

To assess the effect of replacement at position 110 on the internal constitution of IL-13 ligand, the modelling was conducted using the Homology module of the graphic program Insight 98 (Biosym, 1998) on a Silicon Graphic OCTANE workstation. The co-ordinate of 2.25 Å crystal structure of IL-4 (Protein Data Bank accession no. 1rcb) was used as a template for homology modelling of IL-13. The Gln110Arg variant of the IL-13 was built up using the Biopolymer module on the basis of IL-13 model structure. The two complete structures were further minimized by heating to 300 K equilibration for 1 ps, 200 steps of steepest gradient minimization and 10 000 steps of conjugate gradient minimization to optimize the hydrogen bonds, ion pairs and hydrophobic interactions. The minimization was performed by the Discover 3 program with the CVFF forcefield.

To investigate interaction between ligand and receptors, the three-dimensional structures of IL-4Rα and IL-13Rα1 were generated by application of restraint- and structure-based homology modelling techniques (42). The extracellular part of IL-4R consists of two cytokine receptor domains. In contrast, IL-13Rα1 has an additional fibronectin type III domain at its N terminus. The shorter extracellular sequence of IL-4Rα and comparison with the structures of the human growth hormone (hGH) and the EPOR (erythropoietin receptor) complexes indicate that, in the complex including IL-4Rα1, IL-13 must bind to domains 2 and 3 of IL-13Rα1. These two domains also show the cytokine receptor-specific cysteine/tryptophan/proline pattern. The structures of hGHR (43), EPOR (44) and gp130 (45) served as templates for the modelling. The crystal structure of IL-4 bound to IL-4Rα has been published recently (46), but the co-ordinates have not been released yet. However the information given about loop conformation, residue exposure and inter-protein orientation was taken into account for modelling of IL-4Rα and the complex. For IL-13 an earlier prediction was considered (47). The models were refined by initial minimization (5000 steps), short molecular dynamics (40 ps) and final minimization using the Amber forcefield. Functional IL-13R is composed of IL-4Rα and IL-13Rα1 (1–3). Current knowledge about the standard structures of Class I cytokine receptor complexes suggests that there are two faces on a cytokine interacting with its two receptor chains. One face is made up of residues predominantly located on helices A and C of the cytokine and interacts with one of the receptor chains. The other face consists of amino acids in the two long loops (loops AB and CD) and helix D of the cytokine. As it was not known whether the AC face of IL-13 interacts in the complex with IL-13Rα1 or IL-4Rα, two models were built. In model 1 the AC face interacts with IL-4Rα (Fig. 2B and C), while interacting with IL-13Rα1 in model 2 (Fig. 2D and E).

Immunohistochemical assay

Monoclonal antibodies to human IL-13Rα1 were established using the extracellular domain of IL-13Rα1 as antigen. One was designated as UU15 and is an IgG2a isotype. We immunostained human B cells, DND39 cells and human IL-13Rα1-transfected DND39 cells with UU15. It has been confirmed that DND39cells do not express mRNA of human IL-13Rα1. The transfectants showed strong staining, whereas parental cells did not give rise to any signal. These results confirmed that UU15 recognizes human IL-13Rα1 specifically in immunohistochemistry. Monoclonal antibody to human IL-4Rα was purchased from Genzyme (Cambridge, MA). Fresh human lung tissues were obtained and embedded in paraffin from patients undergoing surgery informed consent was obtained. Asthmatic specimens were obtained from autopsy lungs. The sections were probed with UU15 by the alkaline phosphatase method. Specificity of signal was confirmed by demonstration that: (i) the signal was not elicited by the treatment without the primary antibody and (ii) adding excess IL-13Rα1 or IL-4Rα to the reaction diminished the signal.

Statistics

Contingency table analysis, ORs, 95% CIs and significance values were calculated by computerized methods (SPSS program v8). If the number in the column was <10, Fisher's exact method was used. Probability values were corrected for multiple comparison by multiplying the P values by the number of loci

compared (Bonferroni correction) ANOVA and multivariate analyses were also performed using this program two- three-, four and five-way step interactions were tested. Linkage disequilibrium was calculated as described (48)

Sequence database

Sequences used here for modelling were derived from the database at DDBJ/EMBL/GenBank IL-13 (accession nos U10307 L06801 L13029), IL-4 (M23442), IL-4Rα (X52425), IL-13Rα1 (U62858), IL-13Rα2 (X95302), EPOR (M34986, M60459) OHR (M28466), gp130 (M57230). Note that numberings in this text is on the basis of mature peptides Protein numbering is based on the cytokine-web (http://www.psymx.co.uk/cytweb/targets/index.html).

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SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO

RADICACIÓN 3-75597 **EDICTO No 8393**

LA SECRETARÍA GENERAL AD-HOC
HACE SABER

Que esta Superintendencia profirió Resolución No 12322 de 30-ABR-2007 Que el contenido de la parte resolutiva es como sigue El Superintendente de Industria y Comercio

RESUELVE

ARTICULO PRIMERO Negar el privilegio de patente de invención para la solicitud correspondiente a "VACUNA que entró en fase nacional en virtud del Tratado de Cooperación en Materia de Patentes (PCT).

ARTICULO SEGUNDO Notificar personalmente el contenido de la presente resolución a la doctora Alicia Llorede Ricaurte o a quien haga sus veces, en su calidad de representante legal de Glaxo Group Limited entregándole copia de la misma, advirtiéndole que contra ella procede el recurso de reposición interpuesto ante el Superintendente de Industria y Comercio al momento de la notificación o dentro de los 5 días hábiles siguientes a ella

NOTIFÍQUESE Y CUMPLASE
Dada en Bogotá D.C. a los 30 ABR. 2007

EL SUPERINTENDENTE DE INDUSTRIA Y COMERCIO

JAIRO BUBIO ESCOBAR

Para notificar a los interesados se fija el presente EDICTO en lugar visible al público en la SUPERINTENDENCIA DE INDUSTRIA Y COMERCIO por el término de (10) diez días hábiles contados a partir de las 8 00 a.m de hoy

Secretaría General Ad-Hoc 15-MAY-2007 **Este edicto se desfija hoy 29-MAY-2007 a las 4 30 p m**
Secretaría General Ad-Hoc
EDICTO No. 8393