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Human γ-interferon expression in the mammary gland of transgenic mice

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Transgenic mice carrying a hybrid gene consisting of ovine β-lactoglobulin gene sequences and human γ-interferon (hIFN-g) cDNA were produced. hIFN-g expression in the mammary gland of two lactating transgenic founder females was found. The concentration of active hIFN-g in the milk was estimated as being ca. 1800 IU/ml. The hIFN-g ability to express in the mammary gland was found in the progeny of transgenic founder male.

γ-Interferon; β-Lactoglobulin; Human; Ovine; Transgenic mouse; Milk

1. INTRODUCTION

The human γ-interferon (hIFN-g) is an immunomodulator displaying antiviral and antiproliferative properties [1]. The recombinant non-glycosylated hIFN-g expressed in *E. coli* cultures can presently be purchased on the market from various sources. But the medical use of the non-glycosylated hIFN-g seems however to be limited, because the recombinant non-glycosylated proteins are known to possess the immunogenic activity (as is, for example, the case in granulocyte/macrophage colony stimulating factor [2]).

One way to obtain large amounts of glycosylated hIFN-g is to develop transgenic forms of domestic mammal species able to synthesize the protein in their mammary glands. Obviously, prior to the commercial application of the mentioned approach, the possibility of the synthesis of biologically active hIFN-g in the mammary gland and of its secretion into the milk is to be proved on laboratory animals.

Here we report the results of the development of a hybrid gene intended for expression of the hIFN-g in the mammary gland of transgenic animals and prove the presence of biologically active interferon in the milk samples taken from transgenic mice carrying such a gene.

2. MATERIALS AND METHODS

2.1. *Construction of the hybrid gene*

A full-length hIFN-g cDNA has been cloned from a human spleen cDNA library as described in [3]. It contained both initiating and terminating codons and was identical in nucleotide sequence to previously published sequence [4]. The GC-tails were removed from the cDNA ends by site-directed mutagenesis [5]. The ovine genomic library in the phage lambda EMBL-3 was produced according to [6], the genomic DNA being isolated from the ovine liver. For the screening of the library and β-lactoglobulin (BLG) gene searching the partially overlapping oligonucleotides ON1 (5′-GTGGCGTCCAGGCCATCATCGTC-3′) and ON2 (5′-TTTCATGGTCTGGGTGACGATGATGG-3′) (Fig. 1A) complementary to the already established cDNA sequence of BLG gene [7] were used as probes. The *SalI–XbaI* fragment of the DNA containing the ovine BLG gene and flanking 5′ and 3′ sequences (Fig. 1A) was finally isolated. The BLG gene fragment containing untranslated region of the penultimate exon, the last intron, and the last exon of BLG gene (including polyadenylation signal) was amplified using polymerase chain reaction (PCR) [8] with two oligonucleotides, ON3 (5′-TACGTAGGTGAGCCCCTGCCGGTGC-3′) and ON4 (5′-AAGCTTCCAGCAAAGACTCAGAAGG-3′) (Fig. 1A). They contained at their 5′-ends recognition sequences for restriction endonucleases *Sna*BI and *Hin*dIII and were complementary to flanks of the BLG gene fragment [9]. The amplified fragment (616 bp) bearing newly created sites *Sna*BI at its 5′-end and *Hin*dIII at its 3′-end, was ligated into the *Pvu*II site that resides within the untranslated region of the BLG gene first exon (Fig. 1A). hIFN-g cDNA was inserted into *Sna*BI site (Fig. 1B). Thus derived BLG-hIFN-g hybrid gene (Fig. 1C) was then used to produce transgenic mice.

2.2. *Production and identification of transgenic mice*

200 to 400 copies of the hybrid gene BLG-hIFN-g had been microinjected into the NMRI mice zygote pronuclei; 1 100 injected zygotes were transplanted into oviducts of pseudopregnant females; genomic DNA was isolated from tails of 90 two-week-old mice, developed from microinjected zygotes [10]. To detect the presence of exogenous DNA in the mouse genome, a PCR [11] was conducted using oligonucleotides ON5 (5′-AATGCAGGTCATTCAGATGTAGCGG-3′) and ON6 (5′-CGAATAATTAGTCAGCTTTTCGAAG-3′) as primers which were complementary to the hIFN-g cDNA (Fig. 1C).

2.3. *RNA isolation and analysis*

RNA from lactating mammary gland and other organs was isolated as described in [12]. The isolated total RNA was analysed by application of reversed transcription followed by PCR (RT-PCR) [9]. RNA was submitted to the reversed transcription reaction in the presence of M-MuLV reverse transcriptase with ON7 (5′-CCCAGAGGAGTCCAAGGCTCCCGGG-3′) (Fig. 1C) as primer. The obtained cDNA

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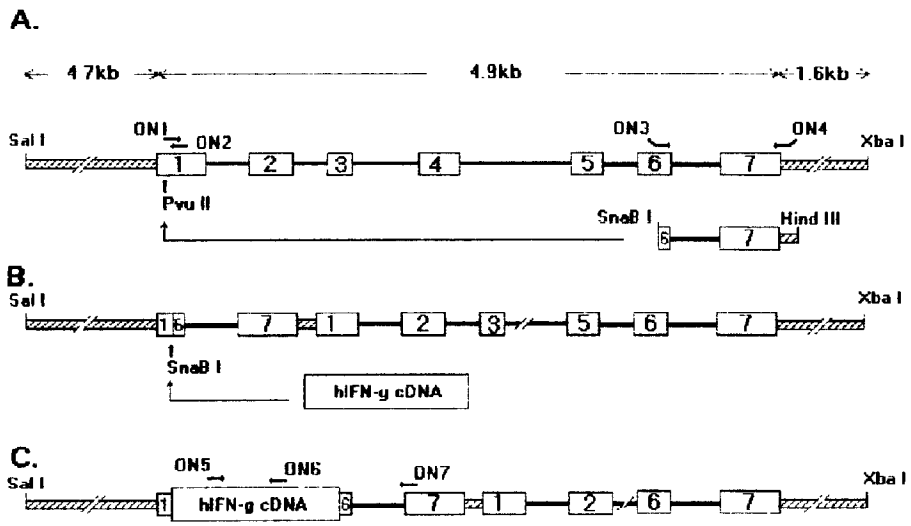

Fig. 1. Sketch of the hybrid BLG-hIFN-g gene construction. (A) Structure of cloned BLG gene and localization of primers ON1, ON2, ON3, ON4. The long angled arrow indicates the insertion site of amplified fragment of the BLG gene within the untranslated region of the first exon of the BLG gene. Numbered boxes are exons of the BLG gene, solid lines: introns of BLG gene; shaded boxes: 5′ and 3′ flanking sequences of the BLG gene; short horizontal arrows: primers; bent-off ends of short arrows: non-complementary fragments of primers ON3 and ON4, containing recognition sites for restriction endonucleases *Sna*BI and *Hin*dIII, respectively. (B) Insertion of the hIFN-g cDNA into the *Sna*BI site under the control of the BLG gene promoter. C. Structure of the hybrid gene BLG-hIFN-g, used for microinjections, and localization of primers ON5, ON6, ON7.

was used in PCR in the presence of oligonucleotides ON5 and ON7 (Fig. 1C) [13].

2.4. *Analysis of the milk samples*

Milk was collected from transgenic lactating mice 12–14 days after parturition. Milk samples were diluted 1:2 in distilled water and defatted by repeated centrifugation (12 000 × *g*). The determination of interferonic activity was based on protection of a human fibroblasts monolayer culture against the cytopathic effects of vesicular stomatitis virus strain Indiana [14]. hIFN-g titers in milk samples were calculated in international units (IU) according to international reference Standard Gg23–901–530.

3. RESULTS AND DISCUSSION

The BLG-hIFN-g hybrid gene was created (Fig. 1). When creating the hybrid gene based on cloned BLG gene and hIFN-g cDNA we aimed to retain all the possible regulatory sequences of the BLG gene which could presumably be present in its introns and exons. Nine transgenic founder mice (four females and five males) having BLG-hIFNg hybrid genes were produced. To identify the transgene within mouse genomic

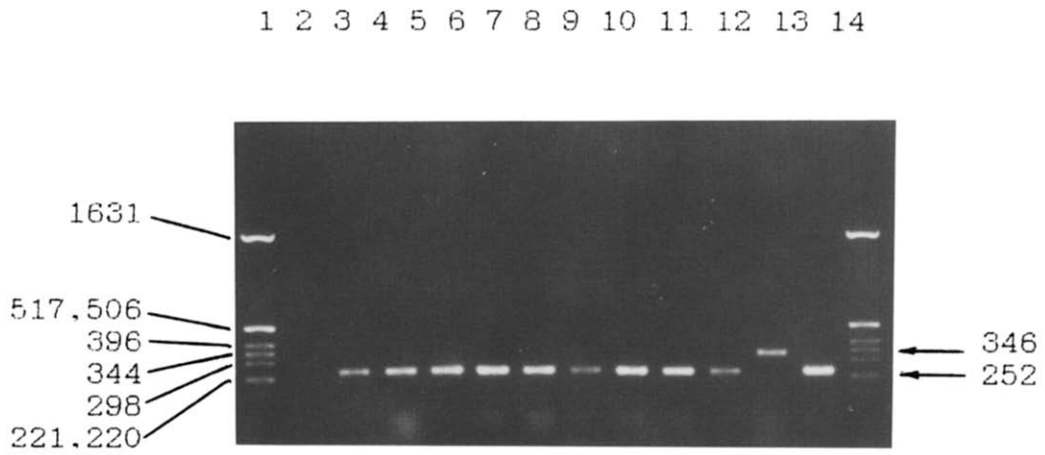

Fig. 2. Identification of transgenic mice by PCR analysis. (1) marker pBR322/*Hin*fI; (2) negative control (DNA of mouse NMRI); (3) DNA of mouse 26; (4) DNA of mouse 28; (5) DNA of mouse 30; (6) DNA of mouse 34; (7) DNA of mouse 40; (8) DNA of mouse 45; (9) DNA of mouse 53; (10) DNA of mouse 65; (11) DNA of mouse 71; (12) DNA from human placenta; (13) positive control (hybrid gene BLG-hIFN-g used for microinjections); (14) marker pBR322/*Hin*fI. Arrows show the amplified DNA fragments, whose lengths of 252 bp and 346 bp are calculated according to nucleotide sequences of hIFN-g cDNA and genomic gene of hIFN-g, respectively.

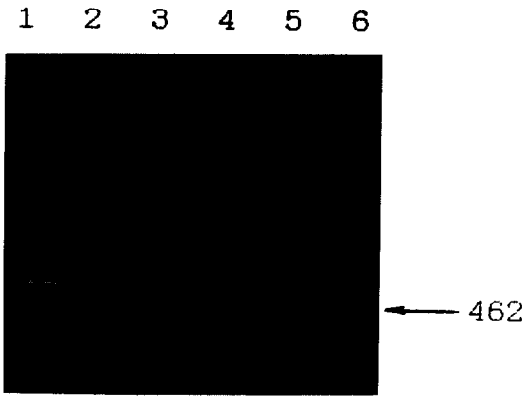

Fig. 3. RT-PCR analysis of RNA isolated from the mammary glands of transgenic females. (1) marker pBR322/*Hin*fI; (2) negative control (RNA of mouse NMRI); (3) RNA of mouse 26; (4) RNA of mouse 28; (5) RNA of mouse 34; (6) RNA of mouse 71. Arrow shows the amplified DNA fragment, whose length (462 bp) is calculated according to nucleotide sequences of hIFN-g cDNA and ovine BLG genomic gene.

DNA, we conducted PCR, which is known to provide a rapid screening method for transgenic mice [7]. The results of transgene identification within the mouse genome are displayed in Fig. 2. Eight out of nine founder transgenic mice have transmitted the transgene to their progeny (Table I). The offspring of founder mouse 65 was also tested for ability to transmit the transgene to their progeny (Table I).

Expression of the transgene was found in the mammary gland of lactating founder transgenic mice 26 and 28: the hybrid BLG-hIFN-g mRNA was detected in the mammary gland tissue (Fig. 3) and an interferonic activity of about 1,800 IU/ml was observed in the milk samples (Table I), which corresponds to the hIFN-g concentration of about 20 ng/ml, assuming an hIFN-g specific activity to be ca. $1 \cdot 10^8$ IU/mg. The interferonic activity was observed also in the mammary gland of lactating transgenic females 65.37, 65.38, and 65.39 descended from transgenic male 65 (Table I).

The transgenic lactating female 65.39 was tested for tissue specificity of transgene expression. The hybrid mRNA of BLG-hIFN-g was found in the mammary gland tissues, but not in lung, liver and kidney (data not shown).

The low observed level of hIFN-g expression (ca. 1,800 IU per ml of milk) seems to be due to the absence of introns of the genomic gene hIFN-g in the hybrid transgene. Indeed, it was reported that in some experiments the genomic gene containing introns and forming a part of a hybrid gene is expressed in transgenic animals more effectively than the cDNA [15]. This is the reason to produce a new hybrid gene containing as structural element the genomic hIFN-g gene.

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Table I

Summary of the hybrid BLG-hIFN-g gene expression in transgenic mice

	Mouse	Sex	Transgene transmission to offspring (transgenic offspring/total offspring)	BLG-hIFN-g expression in the mammary gland	
				mRNA	Interferonic activity of milk (IU/ml)
Founder mice	26	f	3/14	+	1,900
	28	f	5/14	+	1,800
	30	m	1/1	nt	nt
	34	f	3/18	–	240
	40	m	4/11	nt	nt
	45	m	14/20	nt	nt
	53	m	2/23	nt	nt
	65	m	10/16	nt	nt
	71	f	0/5	–	0
	NMRI	f		–	240
Desdendants of mouse 65	65.37	f	nt	nt	750
	65.38	f	2/5	nt	1,500
	65.39	f	2/7	+	750
	NMRI	f		–	64

f, female; m, male; nt, not tested.

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