Non-Polarized Secretion of Mouse Interferon-β from Gene-Transferred Human Intestinal Caco-2 Cells

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Purpose. The intestinal epithelium is considered to be a feasible target for somatic gene therapy. To this end, Caco-2 cells derived from human colon carcinoma were transfected with a mouse interferon-β (IFN-β) expression vector and several stable sublines were established; this hetero-specific cytokine allows unexpected cellular effects to be avoided. Using the highest mouse IFN-β-producing sublines, the mode of IFN secretion was examined.
Methods. The secretion polarity of mouse IFN-β in its gene-transduced Caco-2 sublines was studied in a bicameral culture system in which the chambers were separated by microporous filters.
Results. Mouse IFN-β was secreted to the same extent from both apical and basolateral surfaces of the transduced cells regardless of cell aging.
Conclusions. These results suggest that in the intestinal epithelium exogenous gene products such as IFNs can be delivered to both the luminal and blood sides *in vivo*. Thus, the intestinal epithelium may be suitable for systemic and local delivery of therapeutic proteins by gene transfer.

KEY WORDS: gene transfer; interferon-β; Caco-2 cells; non-polarized secretion; gene therapy.

INTRODUCTION

Recombinant proteins have recently become a novel class of therapeutic agents and efforts have been made to optimize the delivery of these protein drugs *in vivo* (1,2). However, their oral application is highly restricted due to physical and enzymatic barriers presented by the intestinal wall to proteins. One possible alternative method to deliver the proteins within the wall is gene transfer into the intestinal epithelial cells. Expression of exogenous genes encoding secretory proteins in the polarized intestinal epithelium will result in delivery of the gene products to the systemic circulation and/or luminal space. There is, nevertheless, little information in the literature about intestinal gene transfer.

Caco-2 cells, an intestinal epithelial cell line derived from human colorectal carcinoma, display a spontaneous enterocytic differentiation on aging under standard culture conditions (3). The cellular change from the exponentially-dividing phase to the non-dividing stage mimics the differentiation of the small intestine from proliferating crypt cells to non-dividing villi cells (4). In this study, Caco-2 cells were transfected with plasmid-encoding mouse interferon (IFN)-β using Lipofectin. This mouse cytokine was selected as a model of secretory proteins to allow analysis of the results without possible biological side-effects on the cells. We were able to show non-polarized secretion of mouse IFN-β from the transduced Caco-2 cells and discuss the feasibility of the intestinal epithelium as a target for somatic gene therapy.

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MATERIALS AND METHODS

Cell Line

Human colon adenocarcinoma Caco-2 cells (ATCC HTB37), obtained from the American Type Culture Collection (Rockville, MD), were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, 1% non-essential amino acids, 100 U/ml penicillin and 100 μg/ml streptomycin. For subculture, cells were split every week at a ratio of 1:3. The medium was changed every 2–3 days.

Plasmid Construction and Transfection

A mouse IFN-β cDNA (5) was ligated into the unique restriction Xho I site of a mammalian expression vector pcDNA3 (Invitrogen, San Diego, CA) to produce an IFN-expression plasmid, designated pCMV-Muβ.

Caco-2 cells were seeded on 6-well plates at a density of 2×10^5 cells/well, cultured for 24 h and transfected with IFN-expression plasmid mixed with Lipofectin (GIBCO-BRL, Gaithersburg, MD) in serum-free medium. After 6 h, the medium was changed to a serum-complete medium and incubated for a total of 48 h after transfection. The conditioned medium was recovered for measurement of IFN activity and the cells were split into 100 mm dishes followed by selection with medium containing 800 μg/ml G418 (GIBCO-BRL). Two weeks later G418-resistant single colonies were transferred to individual wells of a 24-well plate. Successfully-grown sublines in G418-containing medium were examined for IFN production and the highest IFN-producing clone, designated Caco-2(CMβ)2, was used in this work.

RT-PCR Analysis

Total RNA from Caco-2 and Caco-2 (CMβ) 2 cells was extracted using guanidine thiocyanate (6) and processed in a reverse transcription-polymerase chain reaction (RT-PCR). The oligonucleotide primers used were 5′-CCTGGCTTCCATCAT GAACA-3′ (forward) and 5′-AACTGACAGGTCTTCAG TTT-3′ (reverse) for mouse IFN-β, and 5′-ACCACAGTCCAT GCCATCAC-3′ (forward) and 5′-TCCACCACCCTGTTGCT GTA-3′ (reverse) for human glyceraldehyde 3-phosphate dehydrogenase (GAPDH) which generate 575 bp and 452 bp fragments, respectively.

IFN Impermeation of Caco-2 Cell Monolayer

After Caco-2 cells (2×10^5 cells/cm^2) were cultured on Transwell filters for 15 days, 200 IU/ml mouse IFN-β or -γ was added to the upper or lower compartments. After 24 h

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incubation the IFN activity in the culture fluids in both compartments was measured as described below.

IFN Assay

IFN activity in the supernatants from cell cultures was measured as described previously (7,8). In brief, the activity was determined by the reduction in the cytopathic effect of vesicular stomatitis virus on L cells and expressed in IU, calibrated against reference mouse IFN-α/β (NIH G002-904-511).

Polarity of IFN Secretion

IFN-β-producing Caco-2(CMβ)2 cells were seeded onto Transwell filters (1 cm^2 area) (Costar, Cambridge, MA) at a density of 1×10^5 cells/well and cultured, replacing the medium every 3 days. On the 5th, 10th, 15th, and 20th days, the culture system was thoroughly washed and the cells were incubated in fresh medium. At that time, 60 IU mouse IFN-γ (per culture), prepared from concanavalin A-stimulated mouse T cell line B5 (9), was added to the upper tray as a permeability marker. Twenty-four hours after incubation, supernatants of the apical and basolateral compartments were individually assayed for IFN activity (9) in the presence or absence of neutralizing monoclonal antibodies against mouse IFN-β (7F-D3) (10) or mouse IFN-γ (R4-6A2) (11) to discriminate between mouse IFN-β secreted by Caco-2(CMβ)2 cells and applied mouse IFN-γ.

RESULTS

Caco-2 cells, following transfection with pCMV-Muβ, secreted 190 IU/ml IFN into the culture medium. From the transfectants, several stable sublines were established as G418-resistant cell lines, some of which constitutively produced a detectable amount of IFN in the medium (Table I). The highest producer Caco-2 (CMβ)2 cells were verified for mouse IFN-β mRNA expression by RT-PCR (Fig. 1).

Caco-2 cells form a tight monolayer during long-term culture over about 5 days, through which macromolecules are prevented from being transported. This was proved by the inability of hetero-species specific mouse IFN-β or -γ to permeate when added to the upper or lower compartment of the bicameral culture system (Table II).

After culturing on Transwell filters for 5, 10, 15 or 20 days, Caco-2 (CMβ)2 cells were incubated with fresh medium for a further 24 h, and IFN secretion into the upper and lower compartments was examined (Table III). The tightness of the monolayer during the last incubation was confirmed by showing that mouse IFN-γ applied to the upper compartment did not reach the lower compartment by crossing the monolayer. Discrimination of each type of IFN activity in the supernatants was performed by neutralization with specific monoclonal antibodies against mouse IFN-β and -γ. Table III shows that mouse IFN-β activity was almost equal in both compartments, indicating that the IFN-expressing Caco-2 cell line secretes mouse IFN-β from both apical and basolateral membranes, despite aging.

Table I. Production of Mouse IFN-β by Transduced Caco-2 Sublines

Subline	IFN yield (IU/day/2×10^5 cells)
parental Caco-2	<1
Caco-2 (CMβ)1	<1
Caco-2 (CMβ)2	90
Caco-2 (CMβ)3	33
Caco-2 (CMβ)4	16
Caco-2 (CMβ)5	<1
Caco-2 (CMβ)6	<1

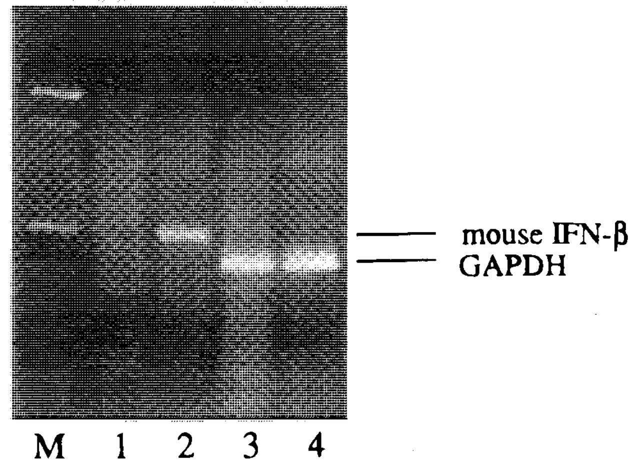

Fig. 1. RT-PCR analysis of MuIFN-β mRNA expression in Caco-2 (CMβ)2 cells. Total RNAs from Caco-2 cells (lanes 1 and 3) and Caco-2 (CMβ)2 cells (lanes 2 and 4) were extracted and RT-PCR was performed with primers for MuIFN-β (lanes 1 and 2) and GAPDH (lanes 3 and 4). Lane M shows the size marker 100 bp ladder.

Table II. IFN Impermeation Through Caco-2 Cell Monolayers[a]

	Partition[a] (%) of IFN activity			
	Apically applied		Basolaterally applied	
	IFN-β	IFN-γ	IFN-β	IFN-γ
upper compartment	71 ± 0.7	62 ± 5.0	<1.0	<0.4
lower compartment	<12	<4.9	86 ± 6.8	94 ± 8.9

[a] Cells were cultured for 15 days in a bicameral system.
[b] Results are the mean ± S.D. of three independent experiments.

DISCUSSION

The intestinal epithelium is an attractive target organ for gene therapy (12–14). Expression of exogenous genes for secretory proteins in the tissue may be effective for treating disorders of the epithelium itself and other sites in the body through systemic delivery. Important issues in intestinal gene transfer to be settled include the choice of vectors and target epithelial cell types (crypt or villi), ways to circumvent physical barriers such as mucus (15,16) and secretion polarity of gene products.

In the present study, using Caco-2 cells as a model of intestinal epithelial cells, we showed that a stable line transduced with mouse IFN-β gene, non-polarized, secreted IFN-β regardless of cell aging or differentiation, suggesting that the intestinal epithelial cells have the potential to deliver gene

Table III. Non-Polarized Secretion of Mouse IFN-β from Caco-2(CMβ)2 Cells

Antibody added on assay	IFN activity[a] (IU per culture) of culture fluids on							
	Day 5		Day 10		Day 15		Day 20	
	Upper compartment[b]	Lower compartment	Upper compartment	Lower compartment	Upper compartment	Lower compartment	Upper compartment	Lower compartment
no antibody	94 ± 5	54 ± 4	110 ± 10	63 ± 8	91 ± 8	48 ± 9	88 ± 7	42 ± 6
anti MuIFN-β	46 ± 2	<3	52 ± 5	<3	33 ± 1	<3	40 ± 3	<4
anti MuIFN-γ	53 ± 3	49 ± 1	57 ± 7	59 ± 8	54 ± 4	45 ± 7	50 ± 5	43 ± 6
anti MuIFN-β + anti MuIFN-γ	<3	<3	<3	<3	<3	<3	<4	<4

[a] IFN activities were measured in the presence or absence of indicated antibodies, each capable of reducing corresponding IFN activity to a fiftieth. The values represent the average and standard deviation of duplicate cultures.
[b] A constant amount of mouse IFN-γ (60 IU per culture) was added to all the upper compartments.

products such as IFN-β to both the luminal and blood sides, after intestinal gene transfer *in vivo*. To date, it has been demonstrated that aged or differentiated Caco-2 cells have a propensity for polarized secretion of various proteins; secretory proteins such as apolipoproteins (17–19), transferrin (17), α-fetoprotein (17), nerve growth factor (20), urokinase-type plasminogen activator (21) and uteroglobin (22) are secreted predominantly through the basolateral membrane of Caco-2 cells, and lysosomal α-glucosidase is secreted preferentially from the apical surface (23), while Caco-2 cells display relatively equal secretion of α_1-antitrypsin via both basolateral and apical pathways (24). Similar polarized basolateral secretion in Caco-2 cells has been seen for exogenous proteins expressed by gene transfection, including growth hormone (17) and bacterial enzyme (25). Although the mechanisms underlying these phenomena are poorly understood, our demonstration of the non-polarized secretion of exogenous IFN-β from both apical and basolateral surfaces in Caco-2 cells shows the potential for gene transfer to the intestinal epithelial cells as a basis for delivering therapeutic proteins to the luminal as well as the blood side *in vivo*.

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