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ORIGINAL ARTICLE

Study of the *APC* gene function in the mouse *APC+/APC*^{1638N} model

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Abstract

OBJECTIVES: Familial adenomatous polyposis (FAP) is an autosomal dominant disease characterized by the presence of hundreds to thousands of benign polyps in the colon. If not removed prophylactically they represent a risk of developing malignant cancer with an almost 100% penentrance. FAP is induced by germline mutation in the *APC* gene. Tumorigenesis launched a second somatic mutation of *APC* gene allele, leading to synthesis of non-functional APC protein. One of the possibilities of cancer prevention could be an alternative gene therapy using bacteria as vectors for delivery of therapeutic protein molecules.
MATERIAL AND METHODS: For this purpose mice model *APC+/APC*^{1638N} with mutation in one allele murine homolog of the APC gene were used. Mice were fed orally commercial nutrition enriched with 0.5 ml PBS buffer with 5% milk containing 5×10^8 recombinant bacterial cells DE3plys6 bearing plasmid with cloned *APC* gene twice a week during 42 weeks. Afterwords mice were killed by thiopental, gastrointestinal tracts were removed, microscopically, macroscopically inspected for polyps/neoplastic lesions and immunohistochemically investigated with polyclonal rabbit antibody against APC protein.
RESULTS: We have cloned full-lenght *APC* gene into vector for expression in bacterial cells *Escherichia coli* BL21(DE3) and BL21(DE3) pLysS. Expression of the APC protein, induced by IPTG, was detected in protein extracts of three bacterial clones: DE3104-11, DE3pLys5, DE3pLys6. APC protein was identified by Western blot analysis using monoclonal and polyclonal antibodies against the APC protein. Bacteria of clone DE3pLys6 were orally administered to *APC+/APC*^{1638N} mice with mutations in the *APC* gene. All transgenic mice without therapy developed adenomatous polyps in the gastrointestinal tract. Transgenic mice treated by oral administration of bacteria expressing functional APC protein developed polyps in 33.3%. The remaining four mice 66.7% were without polyps development.
CONCLUSION: Administration of *APC* gene expressing by bacteria to transgenic mice with mutation in *APC* gene leads to reduction in the number of mice developing polyps in the gastrointestinal tract. The effect of bacterially expressed APC

protein in elimination of intestinal polyps or tumors has been monitored. These are our preliminary results and for possible confirmation of our hypotheses still more research is needed.

Abbreviations:

AGT	- alternative gene therapy
APC	- the adenomatous polyposis coli gene
ECL	- enhanced chemiluminescence
FAP	- familial adenomatous polyposis
CHRPE	- congenital hypertrophy of the retinal pigment epithelium
IL	- Interleukine
IPTG	- isopropyl β-D-1-thiogalactopyranoside
MCR	- the Mutation Cluster Region
PMSF	- phenylmethanesulfonylfluoride
rpm	- rotational speed per minutes
SD	- standard deviation
TBST	- Tris-Buffered Saline, Tween 20
WNT	- signaling pathway

INTRODUCTION

Familial adenomatous polyposis (FAP) is an autosomal dominant disease induced by germline mutations in the adenomatous polyposis coli (*APC*) gene. Predisposition is manifested by the developement of hundreds to thousands benign adenomatous polyps in the colon and rectum. The polyps are pre-malignants, so if the colon is left intact the majority of patients with FAP eventually develop colon cancer by the age of 30-45 years (Eccles *et al.* 1996; Half *et al.* 2009). The incidence is 1:5 000 in the Central Europian population with an almost 100% penetrance, which means that each carrier of mutated allele of the *APC* gene is affected. The *APC* tumor suppressor gene is localized on chromosome 5q21. The coding region consists of 8535 bp (Kinzler *et al.* 1991) and is divided into 15 exons. The majority of germline and somatic *APC* mutations occur in exon 15 and more than 50% occur between codons 1286-1513 known as the Mutation Cluster Region (MCR) (Beroud and Soussi 1996; Matelova *et al.* 2008; Matelova *et al.* 2009). Frameshift, nonsence mutations and polymorphisms are the most common in the FAP families (Wachsmannova-Matelova *et al.* 2009; Fearnhead *et al.* 2001).

The large APC protein (312 kDa) plays a central role in tumor suppression by antagonizing the WNT signaling pathway (Groden *et al.* 1991). The role of *APC* gene/protein in colon carcinogenesis has been further corroborated by the development of mouse models of FAP neoplasia (Shibata *et al.* 1997).These models, such as the *APC*$^{+}$/*APC*^{1638N} mouse, have mutations in one allele of the murine homolog of the *APC* gene and exhibit an autosomal dominantly inherited predisposition to intestinal neoplasia. The mouse *APC* gene is localized on locus 18q53, whereas human homolog is on 5q21. The *APC*^{1638N} mouse model was created by inserting neomycin expression cassette into codon 1638 upstream of *APC* transcription. These mutations lead to the formation of an unstable 182 kDa protein (Kucherlapati *et al.* 2001; Boivin *et al.* 2003). Polyps were developed in 10-to 15-week-old mice and 30-week-old mice already presented intestinal tumors. In contrast to *APC*/Min mice, *APC*^{1638N} mutants survive longer and exhibit rich extracolonic manifestations, as subcutaneous cysts, desmoid tumors and CHRPE similar findings (Yang *et al.* 1997; Smiths *et al.* 1999).

Recent advances in gene therapy can be attributed to improvements of gene delivery vectors. Useful viral and nonviral transport vehicles that considerably increase the efficiency of transfection have been prepared. However, these vectors still have many disadvantages that are difficult to overcome, thus a new approach is needed. One of the prospective approaches of bacterial gene delivery in animal experiments and clinical studies is the alternative gene therapy (AGT) (Celec *et al.* 2005). AGT resembles bacteria-mediated protein delivery, as the therapeutic proteins are produced not by host cells but by the bacteria *in situ* in the cells or in the intercellular space. The expression can be regulated exogenously (Palffy *et al.* 2006).

Intestinal microflora is involved in maintaining a healthy mucosa, modulating the immune system, controlling epithelial proliferation and differentiation, producing anti-tumorigenic or anti-mutagenic compounds, binding and degrading potential carcinogens, and is thus capable to defend the body against pathogens (Tappenden & Deutsch 2007; Fotiadis *et al.* 2008; O‘Keefe 2008). On the other hand, potentially pathogenic microflora may represent a high risk of developing colorectal cancer. A number of studies have shown that these predisposing factors are altered favorably by consumption of probiotics or prebiotics. These components play an important role in maintaining healthy intestinal mucosa and restoring physiological microflora existentially important for the organism. The key to strengthening physiological function is regular consumption of probiotic bacteria, mainly lactic acid bacteria, bifidobacteria and lactobacilli, *Saccharomyces* spp. and *Enterococcus* spp. (Rolfe 2000; Limdi 2006; Mego & Zajac 2008). Another way of using probiotic bacteria could be their application in AGT as potential vectors for production of therapeutic protein. This system eliminates unwanted side effects related to host-bacteria interactions.

The aim of this study was to investigate the *APC* gene/protein function in prevention of colorectal cancer on the mouse *APC*$^{+}$/*APC*^{1638N} model using orally applied bacteria expressing APC protein.

MATERIAL AND METHODS

Preparation of recombinant plasmids for APC gene expression

The *APC* gene from vector pEGFP – C3 (Groden *et al.* 1991) was re-cloning for expression in bacterial cells into pET-24a(+) plasmid. Plasmid pEGFP – C3 was digested by BamHI a NotI and after electrophoresis in

LMP agarose, fragment corresponding to the *APC* gene was cut out and isolated by a commercial kit manual – QIAquick Gel Extraction Kit. The isolated *APC* fragment (0.15 μg) was consequently ligated into pET-24a (+) vector (0.2 μg) open with BamHI a NotI. The ligation mixture was incubated overnight at 16 °C. NovaBlue competent cells (25 μl) were defrosted on ice and transformed by 4 μl of ligation mixture. Recombinants selected on LB agar plates with kanamycine after overnight incubation at 37 °C were analyzed by BamHI and NotI restriction. From plasmids bearing *APC* fragment after amplification, DNA was isolated and four of them were used for transformation of BL21 (DE3) and BL21 (DE3) pLysS cells for *APC* gene expression.

Analysis of APC gene expression

From bacterial clones bearing suitable plasmids, checked by complete sequencing of *APC* gene, 3 ml of overnight culture were transferred into 100 ml LB medium with kanamycin and the cultures were let to grow to optimal optical density. The cultures were divided into two parts – one with IPTG to final concentration of 1 mM IPTG/ml. Both cultures were grown overnight at 37 °C and afterwards were centrifuged at 3 000 rpm for 15 minutes. Pellets were sonicated, 10 μl of 100 mM PMSF was added and centrifuged at 13 000 rpm for 15 min. Prepared cell extracts were electrophorezed (Mini-PROTEAN Tetra System, BioRad) in 10% SDS-PAGE.

Western blotting

Proteins after electrophoresis were transferred from acrylamide gel to nitrocellulose (NC Hybon membrane) overnight at 50 mA. The membrane was incubated in TBS-T buffer and blocked using 5% milk for 1 hr at room temperature. The blocking buffer was removed and the membrane was washed with TBS-T buffer. Appropriately diluted mouse monoclonal APC antibody ALi 12-28 and polyclonal ab15270 (Abcam) in TBS-T buffer with 5% milk was added to the membrane and incubated overnight at 4 °C on a shaker with a rocking motion. The membrane was washed with TBS-T buffer and incubated for 1 hour at room temperature in appropriately diluted goat anti-mouse antibody sc-2005 (Santa Cruz Biotech.) in buffer containing 5% milk. Consequently the membrane was washed with TBS-T buffer and for visualization ECL solutions were used.

Animals in experiment

C57Bl mice with mutation in *APC* gene and C57BL with wild type *APC* gene were used. The mice aged 3.5–4 months, initial weight 18–25 g were tagged using the Ear Tagging-Marking System for animal identification. The mice were housed under standard experimental conditions (temperature 21 ± 2 °C, relative humidity 55 ± 10%, 12/12 hr light-dark cycle, food and water provided *ad libitum*).

Mouse model APC^{+}/APC^{1638N}

Mice (n=19) were divided into: control group of 7 mice, 4 of them were $APC^{+/-}$ and the rest were $APC^{+/+}$; experimental group containing 12 mice of which 6 were $APC^{+/-}$ and 6 were $APC^{+/+}$ mice. The genotype APC^{+}/APC^{1638N}, hereafter referred to as $APC^{+/-}$ (n=10) and the genotype APC^{+}/APC^{+} referred to as $APC^{+/+}$ (n=9). The experiment was approved by the State Veterinary and Food Administration of the Slovak Republic No. 743/09-221/3. Experimental procedures were performed in compliance with the Principles of Laboratory Animal Care issued by the Ethical Committee of the Cancer Research Institute, Slovak Academy of Sciences.

Genotypes were detected from genomic mice DNA by standard techniques from tail snips. Three oligonucleotide primers (primers A–B; A–C which recognize specific sequences in mice DNA) were used in PCR under standard conditions (94 °C for 5 min, 40 cycles of 94 °C for 1 min, 58 °C for 45 sec, 72 °C for 45 sec and 10 min at 72 °C).

Primer A: 5´ TGCCAGCACAGAATAGGCG 3´
Primer B: 5´ TGGAAGGATTGGAGCTACGG 3´
Primer C: 5´ GTTGTCATCCAGGTCTGGTG 3´

Two types of bands were detected: by primers A–B, product size 400 bp; by primers A–C, product size 300 bp. According to product size, we investigated heterozyous $APC^{+/-}$ mice with (400 bp product) or homozygous $APC^{+/+}$ mice (300 bp product) in *APC* gene (Fodde & Smiths 2001) (Figure 1). Mice of the experimental group were fed orally commercial nutrition enriched with 0.5 ml PBS buffer with 5% milk containing $5{\times}10^{8}$ DE3plys6 bacterial cells twice a week during 42 weeks. Mice of the control group were fed commercial nutrition. All mice were in good health condition, put on weight 30–38 g and no mice died during the experiment.

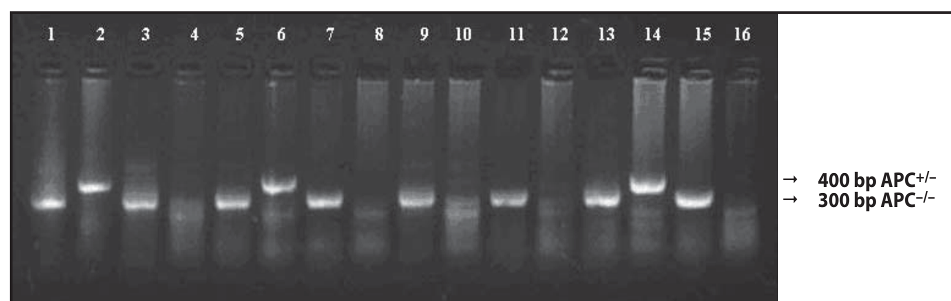

Fig. 1. PCR analysis of genomic DNA: homozygous $APC^{+/+}$ and heterozygous $APC^{+/-}$ mice. Lines 1,2 represent positive control for mutated APC^{1638N} allele and 3, 4 represent positive control for wild type allele. Lines 5,6- mouse 49; line 7,8- mouse 34; line 9,10- mouse 27; line 11,12- mouse 46; line 13,14- mouse 28; line 15,16- mouse 21.

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At the end of the experiment the mice were killed by barbiturate general anesthetic sodium thiopental and the entire gastrointestinal tract was removed. All intestines were opened longitudinally, rinsed with physiological saline and macroscopically inspected for polyps and neoplastic lesions (Hlubinova *et al.* 2004). Then were investigated microscopically and immunohistochemically with polyclonal rabbit antibody against APC protein.

Immunohistochemistry

Formalin-fixed and paraffin-embedded biopsy samples were cut into 5 mm thick slices. After deparafinisation standard procedure of cuts and blocking out of the endogenous peroxidase activity, sections were revitalized in citrate buffer (250 ml, pH 6.0) with microwave irradiation power 750 W during the period of 5 minutes. We repeated the revitalization of 10 minutes for 1 minute. After revitalization, serving as visualize antigenic epitopes, slides were washed in phosphate-buffered saline (50 mM PBS, 150 mM NaCl, 0.005% Tween, pH 7.4) 3×5 minutes. Subsequently, the sections were incubated with primary antibody diluted 1:200 for 1 hour at room temperature. For the detection of APC protein was used polyclonal rabbit anti-APC (Abcam). After incubation, the samples were washed again in buffered solution 3×5 minutes. To detect the primary antibody was used against universal rabbit/mouse anti-polymer conjugated with peroxidase Histofine (Nichirei Biosciences Inc., Japan). Slices were incubated with secondary antibody for 30 minutes at room temperature, then were washed in PBS 3×5 minutes. To visualize the reaction was used 3,3-diaminobenzidine (Dako Cytomation, Glostrup, Denmark). Finally slices were stained with hematoxylin, embedded in acrylic medium and covered with a cover slip. Expression of APC was evaluated by semi-quantitative optical microscopy. For semi-quantitative evaluation, we focused on the histological characteristics of the finding – the presence, respectively absence of positivity and localization of positivity (Figure 2.).

Cytokine detection assay

We detected cytokines (IL-1b; IL-2; IL-4; IL-5; IL-10; IFN-γ; TNF-α) in mouse serum using Bio-Plex Pro™ Assay (Bio-Rad). The principle of this assay is similar to a capture sandwich immunoassay. An antibody directed against the desired cytokine is covalently coupled to internally dyed beads. The coupled beads are allowed to react with sample containing the target biomolecules. Than is added a biotinylated detection antibody specific to an epitope different from that of the capture antibody. This complex is visualised by streptavidine-phycoerythrin reporter.

Statistical analysis

Fisher's exact test was used, to compare differences in proportions in subgroups of mice. Analysis of differences in serum cytokine levels between two groups of mice was performed using the Mann-Whitney *U*. All reported *p*-values were two sided. For all statistical analyses, a *p*-value <0.05 was considered as significant. Statistical analyses were performed using NCSS 2007 software.

RESULTS

The *APC* gene was re-cloned from vector pEGFP – C3 into pET-24a (+) plasmid for expression in bacterial cells. Plasmid pEGFP – C3 was opened with BamHI a NotI and after electrophoresis in LMP agarose the bands were isolated. The *APC* fragment was cut out and ligated into pET-24a (+) vector opened with the same restrictase enzymes. NovaBlue competent cells were transformed by ligation mixture and recombinants were selected on LB agar plates with kanamycine. Isolated plasmids DNA of recombinats were analyzed. After amplification of clones bearing *APC* gene, plasmid DNA was isolated and restricted by BamHI and NotI for detail analysis.

Plasmid DNA of four suitable bacterial clones: L4-26, L4-31, L4-59, L4-104 was completely sequenced to check correctness of the inserted *APC* gene. Plasmid DNA of clones L4-26 and L4-104 were used for trans-

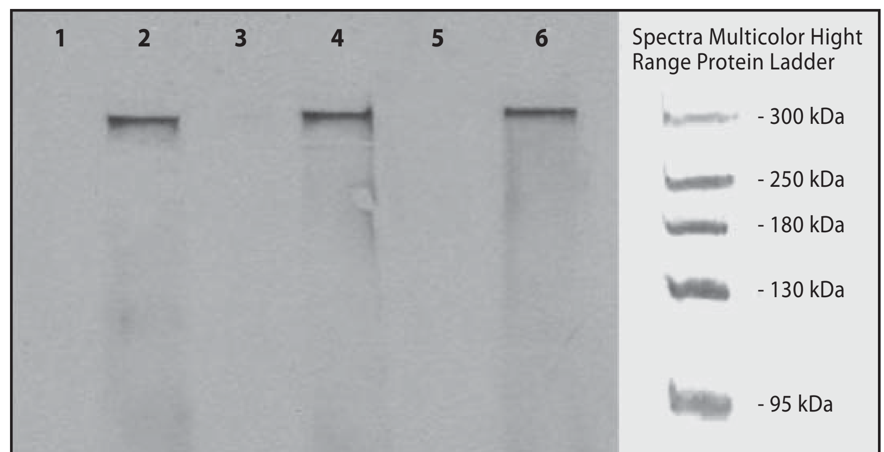

Fig. 2. Immunodetection of bacterially expressed APC protein. Lines: 1, 3, 5 bacterial protein extracts of recombinant clones DE3-104-11, DE3-pLys-5, DE3-pLys-6 without IPTG induction; lines: 2, 4, 6 expression of APC protein in protein extract of bacterial clones DE3-104-11, DE3-pLys-5, DE3-pLys-6 after IPTG induction.

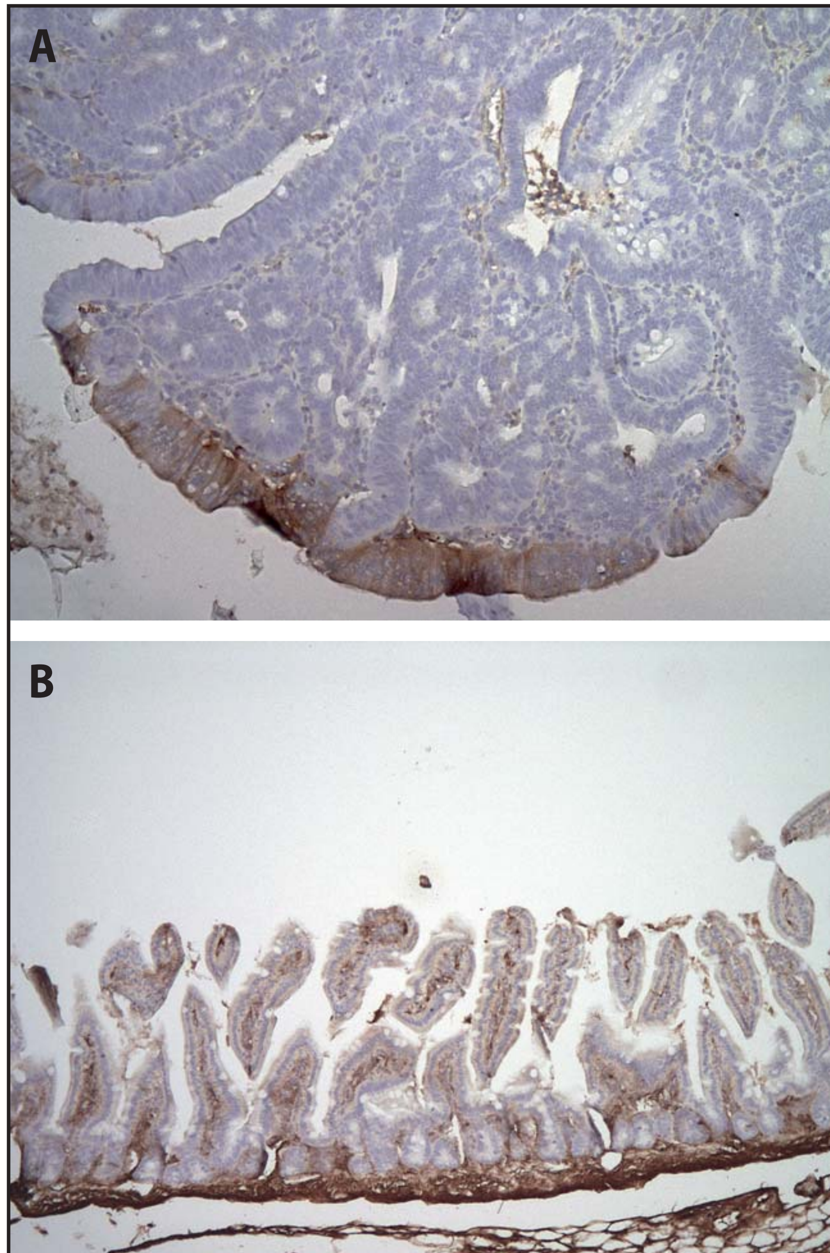

Fig. 3. Immunohistochemistry of mouse small intestine.
A) Heterozygous non-feeded mouse, high positivity of APC protein expression on the polyp surface, original magnification 40×. **B**) High positivity of APC protein expression in duodenal mucose of heterozygous feeded mouse, original magnification 20×.

formation of BL21 (DE3) and BL21 (DE3) pLysS cells for *APC* gene expression. Plasmid DNA of clones DE3-104-11, DE3-pLys-5, DE3-pLys-6 was again sequenced to check correctness of the inserted *APC* gene. Induction of expression of three inspected valid clones was induced with IPTG. Cell extracts prepared from overnight cultures were electrophorezed in 10% SDS-PAGE and proteins were transferred from acrylamide gel to the NC Hybon membrane. The membrane was incubated in diluted mouse monoclonal APC antibody ALi 12-28 and polyclonal ab15270 and the following day in diluted goat anti-mouse antibody sc-2005. ECL solutions were used for final visualization (Figure 2).

Expression of the APC protein was detected in protein extracts of three bacterial clones: DE3-104-11, DE3-pLys-5, DE3-pLys-6. The molecular weight of expressed protein was about 300 kDa, which is in coincidence with the molecular weight of complete human APC protein. Bacteria of clone DE3-pLys-6 were orally administered to APC^{+}/APC^{1638N} mice with mutations in one allele of the murine homolog of the *APC* gene and exhibited colorectal cancer.

The group of heterozygous $APC^{+/-}$ mice had in 2 cases their small intestine attacked with polyps. In the remaining 4 cases no polyps were developed as the result of administration of probiotic bacteria expressing

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functional APC protein, we suppose. Polyps were found in all 4 mice *APC*+/– of the control group. In the experimental and control group of homozygous *APC*+/+ mice, no polyps were detected (Table 1).

In our study 8 cytokines in mice serum were determined. The mice fed with recombinant bacteria had higher level of serum IL1b compared to control group (mean ± SD of IL1b = 198.06 ± 521.92 vs. 174.31 C 469.53; p=0.04). We observed as well, that increased level of IL-1b was associated with absence of polyps compared to lower levels of IL1b (mean ± SD of IL1b = 528.26 ± 429.95 vs. 121.09 ± 84.99; p=0.04). There was no association between other serum cytokine and administration of recombinant bacteria or polyp formation, respectively (Tables 2 and 3).

Immunohistochemistry demonstrated that expression of APC protein was detected. In mentioned four experimental mice which were administrated by *APC* gene expressing bacteria, gastrointestinal tract showed high positivity of APC protein expression (Figure 3).

DISCUSSION

The *APC* gene plays an essential role in the etiology of colorectal cancer. *APC* gene mutation was detected in almost all FAP patients, but also in 70% of patients with sporadic intestinal tumors were found. These facts suggest the importance of the *APC* gene/protein function. In this study a very effective approach was used; exploitation of animal models, as transgenic mice *APC*+/*APC*1638N with mutated murine analog of human *APC* gene, producing incomplete 182 kD protein. For delivery of complete APC protein to transgenic mice a new way in gene therapy – alternative gene therapy (AGT) – was used (Celec *et al.* 2005). Restoring normal APC function to the colonic epithelium of FAP patients with germline *APC* mutation is a feasible therapeutic strategy (Goss and Groden 2000). The advantage of this method of gene transfer is simplicity, specificity and efficiency of DNA transfer. Bacteria as vectors for gene therapy have been contemplated recently.

The effect of bacterially expressed APC protein in elimination of intestinal polyps or tumors has been monitored. After oral feeding of mice with commercial nutrition containing bacterial cells with expressed APC protein, the group of heterozygous *APC*+/– mice had its small intestine attacked with polyps in 2 cases. In the other 4 cases no polyps were developed and we suppose that is the result of probiotic bacteria expressing functional APC protein administration. Percentually, just 33.3% mice fed with recombinant bacteria had polyps, while 100% of mice without this approach developed polyps. Polyps were detected in all 4 mice of the control group. So, the effect of applied bacteria with expressed protein was found highly arguable in statistical evaluation using Fisher's exact test (p=0.076). For statistically significant results, the lowest number of animals is 6 per group. We followed the principles of the "3Rs", i.e. Reduction of numbers, Refinement of techniques aimed at avoiding or diminishing pain and suffering, and Replacement of animal use.

Six mice fed with commercial nutrition with recombinant bacteria in *APC*+/+ genotype group did not develop any polyps. Similarly three mice from the

Tab. 1. Prevention of polyp formation by recombinant bacteria.

Genotype	experimental group* N (%)	control group N (%)	*p*-value **
APC+/–			
polyps present	2 (33.3)	4 (100.0)	0.076
polyps absent	4 (66.7)	0 (0.0)	
APC+/+			
polyps present	0 (0.0)	0 (0.0)	1.00
polyps absent	6 (100.0)	3 (100.0)	
All genotypes			
polyps present	2 (16.7)	4 (57.1)	0.129
polyps absent	10 (83.3)	3 (42.9)	

* mice fed by recombinant bacteria
** Fisher`s exact test

Tab. 2. Effect of recombinant bacteria on serum cytokines.

Cytokines	control group		experimental group		*p*-value*
	mean	± SD	mean	± SD	
IL-1b	174.31	469.53	198.06	521.92	0.04
IL-2	9.51	6.29	9.20	8.57	0.64
IL-4	2.34	1.51	2.65	2.58	0.73
IL-5	19.23	10.71	30.40	29.30	0.79
IL-10	19.66	27.50	19.64	14.91	0.50
GM-CSF	41.18	29.43	55.43	34.25	0.44
IFN-g	60.66	102.26	78.52	78.37	0.62
TNF-a	189.11	208.20	376.87	435.35	0.32

* Mann-Whitney U test

Tab. 3. Associacion between polyp formation and serum cytokines in *APC*+/– mice.

Cytokines	Polyps absent (n=4)		Polyps present (n=6)		*p*-value*
	mean	± SD	mean	± SD	
IL-1b	528.26	429.95	121.09	84.99	0.04
IL-2	14.34	13.25	9.07	6.31	0.91
IL-4	2.62	2.86	1.27	0.85	0.91
IL-5	36.08	30.64	11.08	4.87	0.26
IL-10	13.79	13.68	7.93	2.82	0.83
GM-CSF	54.78	20.78	27.77	33.17	0.53
IFN-g	68.28	74.01	13.32	13.31	0.39
TNF-a	313.16	216.95	118.57	68.45	0.17

* Mann-Whitney U test

control group had no changes in the small intestine. The difference in polyp formation between experimental and control groups was insignificant, 0.0% of experimental mice developed polyps and 100.0% had no polyps. In Fisher's exact test p=1.0. In combined analysis of all mice with genotype $APC^{+/-}$ and $APC^{+/+}$, 16.7% of mice fed with recombinant bacteria developed polyps, but on the other hand, polyps were found in 51.7% mice not fed with recombinant bacteria. Fisher exact test (p=0.129).

There are two explanations of the results reached; 1) by administration of bacteria bearing expressed external APC protein substituting internal one, this tumor suppressor protein stopped the process of polyps development. Therefore, the *APC* and its gene product are attractive targets for the design of therapeutic and chemopreventive strategies for colorectal cancer patients (Goss & Groden 2000); 2) by administration of bacteria bearing *APC* gene the ratio between therapeutic and potentially pathogenic bacteria was changed in the intestinal tract of experimental mice. This approach probably resulted in decreasing the amount of pathogenic bacteria, which were not able afterwards to participate in induction of polyp formation. In our previous study, we confirmed association between intraepithelial bacteria and colorectal tumors. We showed, that, probiotic bacteria could displace these intraepithelial bacteria in patients with colon polyps (Mego *et al.* 2005) and this could be another mechanism, by which recombinant bacteria could influence polyp formation as well.

We performed an analysis of the expression of the APC protein in the mice tissue. It is necessary to emphasize that immunohistochemistry detects the presence of the full-length APC protein. It is a current assumption that almost all of the mutations of the *APC* gene result in the production of a truncated protein that has lost its tumour-suppressor function (Bobe *et al.* 2010). We have detected full-length APC protein using immunohistochemistry in $APC^{+/-}$ mice fed with recombinant bacteria, so we can predict the expression of wild-type APC protein in these knock-out mice. This hypothesis is confirmed of our preliminary results in which we have detected APC protein expression in bacteria isolated from mice feces.

Interleukin 1 (IL-1) is a pluripotent cytokine that promotes angiogenesis, tumor growth and metastasis in experimental models (Elaraj *et al.* 2007). In recently published, Polyp Prevention Trial, investigators observed, that low level of serum IL1b was associated with prevention of colorectal neoplasm (Mego *et al.* 2005). Paradoxically, we observed that mice fed with recombinant bacteria had higher level of serum IL1b compared to control group and that increased serum level of IL1b was associated with absence of polyps compared to lower levels of IL1b. Role of IL1b was not specifically studied in mice with $APC^{+/-}$ genotype, and thus we can´t exclude different role of IL1b on polyp formation in these genetically modified mice. Our study was limited to determination of restricted number of serum cytokines and thus we are unable determine effect of these bacteria on other cytokines

Bacteria-mediated gene transfer might be able to target tissue layer that are inaccessible to non-replicating vectors. The almost unlimited capacity of bacterial plasmid should allow the transfer of large genomic fragments and by using appropriate recombinant bacterial carriers homologous recombination might also be possible. Thus the development of bacteria as efficient transfer vehicle for the therapy of monogenic defects appears to be a desirable goal (Weiss & Chakraborty 2001).

Genetically modified bacteria have been experimentally used as recombinant probiotics also in gastrointestinal disorders, but clinical usage is currently not the case. But in the future a greater use of this kind of approach is expected. One of the possible used strains in gene therapy should be *E. Coli* Nissle 1917. The expression of APC protein in this probiotic strain may be suitable for clinical use as a potential drug.

CONCLUSION

Administration of *APC* gene expressing by bacteria to transgenic mice with mutation in *APC* gene leads to reduction in the number of mice developing polyps in the gastrointestinal tract. The effect of bacterially expressed APC protein in elimination of intestinal polyps or tumors has been monitored. This work presents our preliminary results and for possible confirmation of our hypotheses still more research is needed.

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