Research Article

Effects of Fruit and Vegetable Low Molecular Mass Fractions on Gene Expression in Gingival Cells Challenged with *Prevotella intermedia* and *Actinomyces naeslundii*

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Low molecular mass (LMM) fractions obtained from extracts of raspberry, red chicory, and Shiitake mushrooms have been shown to be an useful source of specific antibacterial, antiadhesion/coaggregation, and antibiofilm agent(s) that might be used for protection towards caries and gingivitis. In this paper, the effects of such LMM fractions on human gingival KB cells exposed to the periodontal pathogens *Prevotella intermedia* and *Actinomyces naeslundii* were evaluated. Expression of cytokeratin 18 (CK18) and $\beta4$ integrin ($\beta4$INT) genes, that are involved in cell proliferation/differentiation and adhesion, and of the antimicrobial peptide $\beta2$ defensin (HβD2) in KB cells was increased upon exposure to either live or heat-killed bacteria. All LMM fractions tested prevented or reduced the induction of gene expression by *P. intermedia* and *A. naeslundii* depending on the experimental conditions. Overall, the results suggested that LMM fractions could modulate the effects of bacteria associated with periodontal disease in gingival cells.

1. Introduction

Periodontal diseases are a heterogeneous group of inflammatory conditions that involve the supporting tissues of the teeth and include gingivitis, in which only the gingivae are involved, and the various forms of periodontitis, chronic inflammatory conditions initiated by a polymicrobial infection that leads to gingival tissue destruction and alveolar bone resorption [1]. Gingivitis is the most prevalent form of periodontal disease that can be defined as "a nonspecific inflammatory process of the gingivae (gums) without destruction of the supporting tissues". This is a reversible condition as a return to meticulous dental hygiene practices will restore gingival health [2]. Several bacterial species have been implicated as aetiological agents of this disease: these include *Actinomyces israelii*, *Actinomyces naeslundii*, *Actinomyces odontolyticus*, *Lactobacillus* spp., *Prevotella intermedia*, *Treponema* spp., and *Fusobacterium nucleatum* [3].

Bacteria and their products can directly damage periodontal tissues and/or initiate inflammation locally. The clinical outcomes of these events are determined by the host response to the infections [1]. Different experimental systems can be utilized to evaluate cellular responses to different bacteria or antibacterial agents, from fibroblasts

derived from human periodontal ligaments to epithelial cells and fibroblasts derived from human gingivae [4].

Foodstuffs as a source to obtain agents/fractions that can improve oral health have been the focus of intensive research because such natural agents are likely to be nontoxic and edible; for example, they can be used to supplement various oral hygiene products. In studies described elsewhere in this issue, it has been shown that low molecular mass (LMM) fractions obtained from extracts of raspberry, chicory, and mushrooms inhibit coaggregation, biofilm formation, and adhesion to hydroxyapatite and/or cultured gingival cells of oral bacteria involved in caries and/or gingivitis [5]. To further evaluate the beneficiary effect on oral health of these dietary fractions, the present study was designed to determine the effect of the raspberry, chicory, and mushroom LMM fractions on the ability of gingivitis-associated bacteria to induce deleterious gene expression in the gingival KB cell line.

2. Materials and Methods

2.1. Bacterial Cultures. *A. naeslundii* ATCC 19039 and *P. intermedia* ATCC 25611 were employed. Bacteria were grown in Brain Heart Infusion Broth (BHIB, Difco Laboratories, Detroit, Mich.) supplemented with haemin (final concentration, $5 \, \text{mg} \, \text{mL}^{-1}$) and vitamin K (final concentration, $1 \, \text{mg} \, \text{mL}^{-1}$) and incubated at $37°\text{C}$ under anaerobic conditions. Cells were harvested at stationary phase by centrifugation ($5,000 \times g$ for 10 min at $4°\text{C}$) and washed twice with 10 mM phosphate buffered saline, pH 7.0. Bacterial suspensions (final concentration, $2 - -5 \times 10^8 \, \text{cfu} \, \text{mL}^{-1}$) were prepared in PBS ($0.1 \, \text{M} \, \text{Na}_2\text{HPO}_4$, $0.1 \, \text{M} \, \text{KH}_2\text{PO}_4$, $0.15 \, \text{M} \, \text{NaCl}$, pH 7.2 to 7.4) alone or suspensions containing different concentrations of test LMM fractions (pH adjusted to 7). Aliquots ($10–100 \, \mu\text{L}$) of the bacterial suspensions were suitably added to KB cell monolayers in order to reach a nominal bacteria: KB cell ratio of 50 and incubated at $37°\text{C}$ for different periods of time in 5% CO_2 atmosphere with gentle shaking. For each strain, untreated controls were included. In experiments with killed bacteria, bacteria were heat inactivated at $70°\text{C}$ for 15 min, centrifuged at $10,000 \times g$, and washed twice in PBS, before being adjusted to the appropriate density in the same buffer.

2.2. LMM Fraction Preparation. Food and vegetable extracts and fractions were prepared as described elsewhere in this issue [6]. Briefly, aliquots of frozen mushroom (*Lentinus edodes*) (400 g) and fresh chicory (*Cichorium intybus* var. *silvestre*) (500 g) and fresh raspberry fruit (*Rubus idaeus* L. var. *tulameen*) (200 g) were homogenized and centrifuged (10 min, 8000 rpm). The juice, after separation from the solid part, was filtered on paper filter. Mushroom and chicory extracts were then fractionated into low and high molecular weight (LMM and HMM) fractions using an ultradiafiltration system. The obtained LMM and HMM fractions contained all the compounds with molecular mass lower and higher than 5000 Da, respectively. Raspberry extract was fractionated by dialysis with cellulose ester

membrane (Spectrum Europe B.V.) with a 3500 Da MWCO. The obtained LMM and HMM fractions contained all the compounds with molecular mass lower and higher than 3500 Da, respectively. All LMM fractions were subjected to sterile ultrafiltration and freeze dried.

2.3. Gingival Cell Culture and Treatments. The gingival fibroblast KB cell line (accession number ICLC HTL96014) obtained from Cell bank Interlab Cell Line Collection (ICLC) of IST-Istituto dei Tumori di Genova (Genoa, Italy) was cultured in a complete medium consisting of Dulbecco's Modified Eagle's Medium (DMEM)-high glucose, with $4.5 \, \text{g} \, \text{L}^{-1}$ glucose and sodium bicarbonate supplemented with 10% fetal calf serum, penicillin ($100 \, \text{U} \, \text{mL}^{-1}$), streptomycin ($100 \, \mu\text{g} \, \text{mL}^{-1}$), and 2 mM L-glutamine. Cells were incubated at $37°\text{C}$ in a 5% CO_2 atmosphere to about 90% confluence and used after 5–10 passages.

For MTT assay, cells were seeded in flat-bottom 96-well plates, at a density of 2×10^4 cells per well, in 0.2 mL of complete medium without antibiotics in triplicate and incubated for 24 h at $37°\text{C}$.

For RT-Q-PCR analysis cells were plated (3×10^5 cells) in to 25 cm^2 flasks in 5 mL of complete medium and incubated for 48 hrs at $37°\text{C}$.

After 24 hrs of starvation in DMEM without serum, cell monolayers were washed twice with PBS and then exposed to bacterial suspensions alone or suspensions containing LMM test fractions at the indicated concentrations. A parallel set of untreated cells in triplicate was utilised as a control. All experiments were performed in quadruplicate.

2.4. Viability Assay. KB cell viability was evaluated by the MTT assay [7]. After each treatment MTT [3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide)] solution in phosphate buffered saline (PBS, pH 7.4) (final concentration of 1 mg/mL) was added to each well and incubated for 3 h at $37°\text{C}$. Following incubation, $100 \, \mu\text{L}$ of 0.04 N HCl isopropanol was added to each sample and the plates incubated for 10 minutes at room temperature. The absorbance at 550 nm was determined by a Varian Cary 50-bio UV-visible spectrophotometer. Data are expressed as percent of control values (mean ± SD).

2.5. RT-Q-PCR. The bacteria-induced changes in mRNA expression of keratinocyte growth factor receptor (KFGR), cytokeratin 18 (CK18), $\beta 4$ integrin (B4ITG), and β defensin 2 (HβD2) were evaluated by quantitative RT-PCR analysis. After harvesting, total cellular RNA was isolated by the procedure of Chomczynski and Sacchi [8] using Tri-Reagent (SIGMA, Milan, Italy). RNA purity was evaluated by measuring the 260/280 nm absorbance ratio, and only samples with $\text{OD}_{260/280} > 1.8$ were processed, resolved on a 1.5% agarose gel, and stained with ethidium bromide, to check for purity. cDNA synthesis was performed from 1.5 μg of total DNaseI (Fermentas, M-Medical, Italy) treated RNA using 200 units RevertAid H Minus M-MuLV Reverse Transcriptase (Fermentas Italy, M-Medical, Milan) in presence of 201 pmoles oligo (dT)18 (TIB MOLBIOL,

TABLE 1: Oligonucleotide primers used for quantitative RT-PCR analysis.

Gene	Primer forward (5′–3′)	Primer reverse (5′–3′)	GenBank
KGFR	GATTACAGCTTCCCCAGACTACC	GAAGAGAGGCGTGTTGTTATCC	M80634
CK18	TCAGAGACTGGAGCCATTACTTC	CAGTCGTGTGATATTGGTGTCAT	NM199187
ITGB 4	GCCGCTACGAGGGTCAGTT	TCCATTACAGATGCCCCCATT	NM000213
HβD2	GGGTCTTGTATCTCCTCTTCTCG	ACAGGTGCCAATTTGTTTATACC	NM004942
GAPDH	GTCAGTGGTGGACCTGACCT	AGGGGTCTACATGGCAACTG	M17851

Genova, Italy), 40 units RiboLock (ribonuclease inhibitor), and 1 mM dNTPs (Fermentas Italy, M-Medical, Milan) at 42°C for 60 min in a reaction volume of 20 μL according to the manufacturer's protocol. 5 μL of cDNA was then used to amplify the genes of interest using a Chromo 4 System real-time PCR apparatus (Biorad Italy, Segrate, Milan) in a final volume of 20 μL containing 1x iTaq SYBR Green Supermix with Rox (Biorad, Milan, Italy) and 0.25 μM of each primer (TIB MolBiol, Genoa, Italy). The primer pairs were used, and their accession numbers are shown in Table 1. The primers for KFGR, CK18, HβD2, and GAPDH were designed with the *Primer3* (v.0.4.0) software [9], those for B4ITG were from Wang et al. [10].

The thermal protocol consisted of 3 min initial denaturation at 95°C, followed by 40 cycles: 15 s at 95°C; 30 s at 54°C; 20 s at 72°C. A melting curve of PCR products 55–94°C) was also performed to ensure the absence of artefacts. Gene expression was determined relative to the expression of the gene Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) by the comparative C_T threshold method [11] using the Biorad software tool Genex-Gene Expression Macro [12]. The normalized expression obtained was expressed as relative quantity of mRNA with respect to control samples.

2.6. Data Analysis. Data representing the mean ± SD of at least 4 experiments in triplicate were analysed by the Mann-Whitney U test ($P \leq 0.05$).

3. Results

3.1. Effects of Live Bacteria and LMM Fractions, Alone and in Combination, on KB Cell Viability and Gene Expression. The effects of the gingivitis-associated *P. intermedia* and *A. naeslundii* and LMM fractions (Raspberry, Chicory, Mushroom) alone on KB cell viability were first evaluated by the MTT assay. LMMs were tested at different concentrations (0.2x, 0.5x, 1x) and times of incubation with monolayers (4 and 6 h).

The viability of KB cells treated with live bacteria alone (at the nominal bacteria: KB cell ratio of 50) was not affected at 4 h; the number of viable bacteria remained the same during the assay as evaluated by cfu counting. KB cell incubation for longer than 4 h or in the presence of 0.5x and 1x concentrations of LMM fractions alone decreased by 20–30% (data not shown). Therefore, all subsequent experiments with live bacteria were performed by treating KB cells for 4 h with bacteria and 0.2x LMM fractions, both
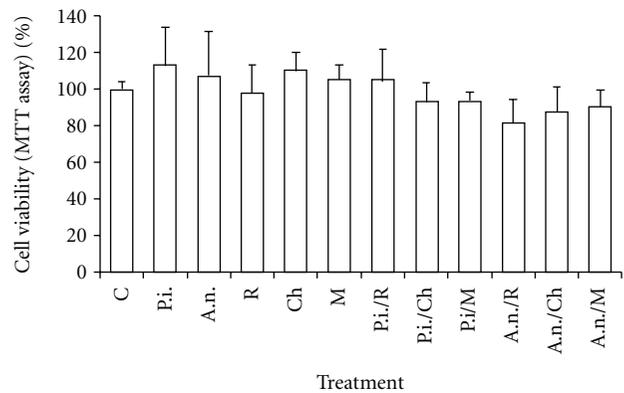

FIGURE 1: Effects of live *P. intermedia* and *A. naeslundii,* and of Raspberry, Chicory, and Mushroom LMM fractions on KB cell viability. Cells were exposed to bacteria (at the nominal bacteria: KB cell ratio of 50) and fractions (0.2x), alone and in combination, for 4 h. Cell viability was evaluated by the MTT assay as described in Methods. Data, expressed as percent cell viability with respect to controls, represent the mean ± SD of four experiments in triplicate. P.i.: *P. intermedia*; A.n.: *A. naeslundii*; R: Raspberry; Ch: Chicory; M: Mushroom; P.i./R: *P. intermedia*/R; P.i./Ch: *P. intermedia*/Ch; P.i./M: *P. intermedia*/M; A.n./R: *A. naeslundii*/R; A.n./Ch: *A. naeslundii*/Ch; A.n./M: *A. naeslundii*/M.

alone and in combination. In these conditions, no effects on KB cell viability were observed (Figure 1).

The effects of KB cell treatment for 4 h with *P. intermedia* and *A. naeslundii* and LMM fractions, alone and in combination, on the expression of different genes (KGFR, CK18, β4INT and HβD2) were evaluated. No appreciable changes could be detected in the expression of KGFR by KB cells in response to different treatments (not shown). In contrast, *P. intermedia* and *A. naeslundii* alone decreased significantly the transcription of CK18 by 40% and 18%, respectively ($P \leq 0.05$) (Figure 2(a)). No significant changes in CK 18 expression were noted in KB cells exposed to LMM fractions. Nevertheless, raspberry and chicory LMM fractions prevented the downregulation of CK18 induced by both bacteria, whereas the LMM mushroom fraction did not.

All bacteria and LMM fractions, when tested alone, induced a decrease in transcription of β4ITG with respect to controls (Figure 2(b)). The effect was significant for all treatments, with decreases in β4ITG mRNA level ranging from about −60% *(P. intermedia)* to −40% (mushroom fraction) ($P \leq 0.05$). However, when KB cells were exposed to a mixture of bacteria and LMM fractions, raspberry,
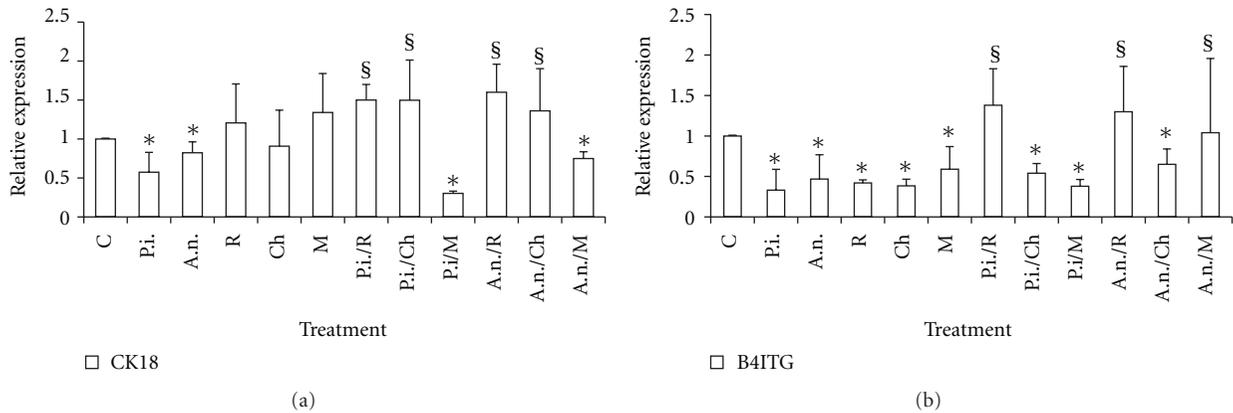
(a)

(b)

FIGURE 2: Effects of live *P. intermedia* and *A. naeslundii,* and of Raspberry, Chicory, and Mushroom LMM fractions on expression of Cyt18 (a) and 4βINT (b) in KB cells. Cells were exposed to bacteria (at the nominal bacteria: KB cell ratio of 50) and fractions (0.2x), alone and in combination, for 4 h. Gene expression was evaluated by RT-Q-PCR as described in Methods. Data, expressed as relative expression with respect to controls, represent the mean ± SD of four experiments in triplicate. Abbreviations as in legend to Figure 1. *= $P \leq 0.05$: all treatment versus controls; § = $P \leq 0.05$: bacteria/LMM fractions versus bacteria alone. Mann-Whitney U test.

the decrease in β4ITG expression was attenuated; moreover, mushroom LMM fraction prevented the effect induced by *A. naeslundii*. On the other hand, no effect was observed with chicory fractions.

3.2. Effects of Heat-Killed Bacteria and LMM Fractions, Alone and in Combination, on KB Cell Viability and Gene Expression.

The same experiments as above were carried out with heat-killed *P. intermedia* and *A. naeslundii* alone and in combination with LMM extracts. Longer times of incubation and lower concentrations of LMM extracts (0.1x) were tested: however, from 8 to 20 hr incubation, decreases in cell viability were observed in different experimental conditions with both bacteria and test substances, with raspberry LMM extracts in particular. At 6 hr, stable cell viability was observed in all experimental conditions, and therefore this time of exposure was chosen for subsequent experiments; however, since raspberry LMM still induced significant loss in cell viability, it was no longer utilized for subsequent experiments. In Figure 3, data on viability of KB cells exposed for 6 h to *P. intermedia* and *A. naeslundii*, alone and in combination with chicory and mushroom LMM fractions, are reported.

Possible changes in gene expression induced by KB cell exposure for 6 h to inactivated bacteria and LMM fractions, alone and in combination, were evaluated, and the results are reported in Figure 4. Heat-killed *P. intermedia* and *A. naeslundii* alone did not affect CK18 expression. On the other hand, in these experimental conditions, chicory fraction caused a significant decrease (-27%) and shiitake mushroom fraction an increase ($+43\%$) in CK18 expression ($P \leq 0.05$) (Figure 4(a)). In cells incubated with bacteria and LMM fractions, upregulations of CK18 were observed: in the presence of *P. intermedia*, both chicory and mushroom fractions induced an increase in CK18 mRNA levels with respect to control cells ($+58\%$ and $+133\%$, resp.; $P \leq 0.05$); a larger increase was observed in the presence of *A. naeslundii*
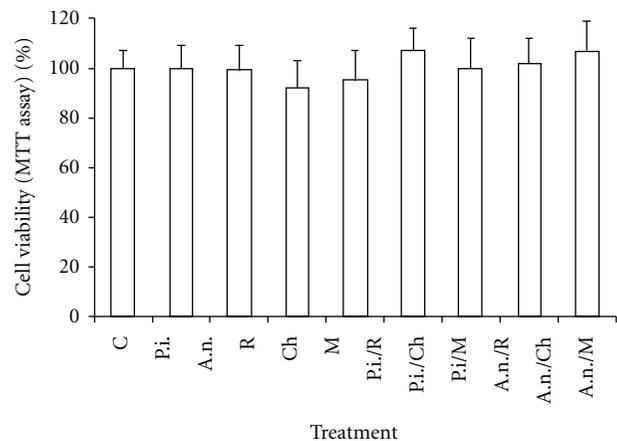

FIGURE 3: Effects of heat killed *P. intermedia* and *A. naeslundii*, and of Chicory and Mushroom LMM fractions on KB cell viability. Cells were exposed to bacteria (at the nominal bacteria: KB cell ratio of 50) and fractions (0.1x), alone and in combination, for 6 h. Cell viability was evaluated by the MTT assay as described in Methods. Data, expressed as percent cell viability with respect to controls, represent the mean ± SD of four experiments in triplicate. P.i.: *P. intermedia*; A.n.: *A. naeslundii*; Ch: Chicory; M: Mushroom; P.i./Ch: *P. intermedia*/Ch; P.i./M: *P. intermedia*/M; A.n./Ch: *A. naeslundii*/Ch; A.n./M: *A. naeslundii*/M.

which was induced by both chicory and mushroom ($+269\%$ and $+247\%$, resp., $P \leq 0.05$) (Figure 4(a)).

Changes in β4ITG expression were observed with heat-killed bacteria alone, with *P. intermedia* inducing an increase ($+119\%$; $P \leq 0.05$), and *A. naeslundii* a decrease (-30%; $P \leq 0.05$) in the level of β4ITG mRNA (Figure 4(b)). Both chicory and mushroom LMM fractions alone caused a decrease in β4ITG expression (-39% and -54%, resp.; $P \leq 0.05$) (Figure 4(b)). However, both fractions prevented the upregulation of β4ITG induced by *P. intermedia* and the downregulation of β4ITG induced by *A. naeslundii*.
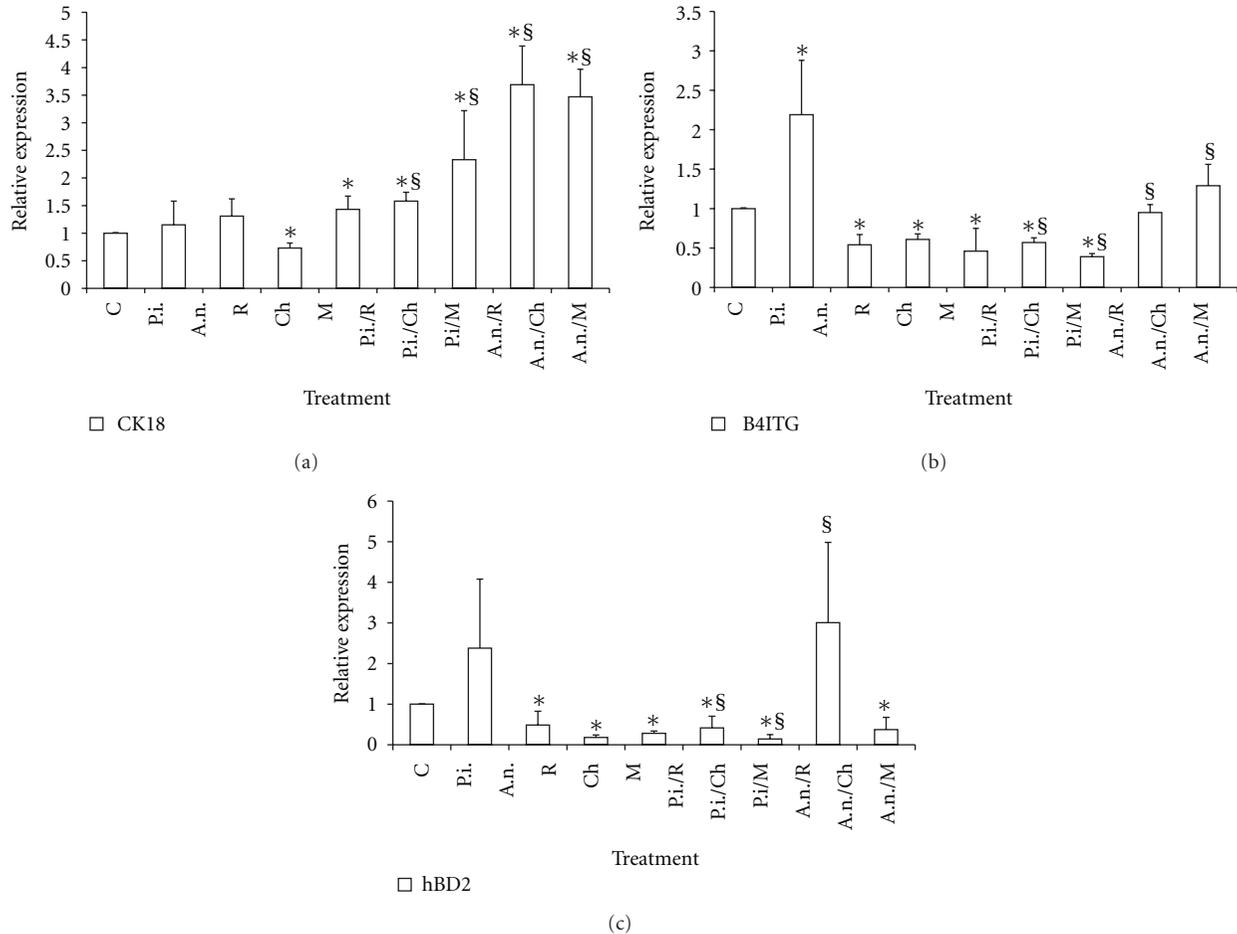
(a)

(b)

(c)

FIGURE 4: Effects of heat killed *P. intermedia* and *A. naeslundii,* and of Chicory and Mushroom LMM fractions on expression of Cyt18 (a), 4βINT (b), and HβD2 (c) in KB cells. Cells were exposed to bacteria (at the nominal bacteria: KB cell ratio of 50) and fractions (0.2x), alone and in combination, for 4 h. Gene expression was evaluated by RT-Q-PCR as described in Methods. Data, expressed as relative expression with respect to controls, represent the mean ± SD of four experiments in triplicate. Abbreviations as in legend to Figure 4. $*= P \leq 0.05$: all treatment versus controls; $§ = P \leq 0.05$: bacteria/LMM fractions versus bacteria alone. Mann-Whitney U test.

In these experimental conditions, changes in expression of the defensin gene HβD2 were also observed (Figure 4(c)). *P. intermedia* alone induced a 2-fold increase in the level of HβD2 mRNA; however, the effect was not significant due to large sample variability. In contrast, *A. naeslundii* alone induced a decrease in transcription of HβD2 (−52% with respect to controls; $P \leq 0.05$). Even larger decreases were observed with chicory and shiitake mushroom LMM fractions alone (−80% and −70%; $P \leq 0.05$). When tested in combination with *P. intermedia*, both fractions prevented the increase in HβD2 expression induced by bacteria. Moreover, chicory fractions prevented the decrease in HβD2 expression induced by *A. naeslundii*, whereas mushroom was ineffective.

4. Discussion

In this work, the effects of different fruit and vegetable LMM fractions, alone or in combination with the gingivitis-associated bacteria *P. intermedia* and *A. naeslundii*, on cell viability and gene expression by human KB gingival

cells were evaluated. In preliminary experiments, different concentrations of LMM fractions and times of exposure with bacteria and fractions were tested; however, since loss in cell viability was observed in different experimental conditions, assays with live bacteria were carried out at 4 h of exposure and at a concentration of LMM fractions of 0.2x, whereas assays with heat-killed bacteria were carried out for longer times (6 h) in the presence of lower concentrations of LMM fractions (0.1x). In these conditions, no effects on cell viability were observed, as evaluated by the MTT assays, that measures the number of live and metabolically active cells.

The possible effects of bacteria and LMM fractions, alone and in combination, on the expression of selected genes associated with cell differentiation, proliferation, and adhesion, and the production of antimicrobial peptides were evaluated. Significant changes in expression of Cyt18, β4ITG, and HβD2 were observed in different experimental conditions. When expression of other genes such as KGFR (keratinocyte growth factor receptor), β1 integrin, or β3 defensin (HβD3) was evaluated, no changes were induced by different bacteria and LMM fractions, alone and in

combination (data not shown). The lack of effect could be probably due to the short duration of the experiments (4–6 h) that did not allow appreciable changes in the level of mRNAs for these genes to be observed.

Cytokeratins belong to intermediate filament proteins and are characterized by remarkable biochemical diversity, represented in epithelial tissues by at least 20 different polypeptides. Their expression varies with proliferation, differentiation, and the states of cell development and transformation [13]; Cyt 18 in particular has been utilized as a marker for healthy gingival cells in culture [4, 14]. Cytokeratins are connected through transmembrane cell-matrix junctional complexes, the hemidesmosomes, to the ECM [15]. Integrins, a component of hemidesmosomes, are heterodimeric transmembrane glycoproteins formed by the noncovalent association of α and β subunits [16]. $\beta 1$ and $\beta 4$ ($\beta 4$ITG) are also involved in apical migration of junctional epithelium during the periodontal attachment loss [17]. $\beta 4$ITG is expressed in the basal side of cells cultured from rat oral epithelium [18]. Cytokeratins and integrins are important proteins in regenerating oral epithelium [19].

Distinct changes in gene expression were observed in response to live and heat-killed bacteria, alone and in combination with LMM fractions. At 4 h incubation with live bacteria and fractions, when no changes in cell viability were observed, only changes in Cyt18 e 4βINT could be detected in different experimental conditions. Both live *P. intermedia* and *A. naeslundii* induced downregulation of Cyt18. The effect was reduced by both raspberry and chicory LMM fractions, whereas Mushroom was ineffective.

All live bacteria and fractions alone induced a decrease in expression of 4βINT. Interestingly, the effect of *P. intermedia* was prevented by Raspberry fractions and that of *A. naeslundii* by raspberry and mushroom, whereas chicory was ineffective. This indicates that simultaneous exposure to bacteria and LMM fractions in certain combinations has a synergistic protective effect on the expression of 4βINT.

Overall, these data indicate a protective effect of LMM fractions (raspberry > chicory > mushroom) on the decrease in the expression of genes involved in gingival cell proliferation and differentiation, as well as in cell attachment and signalling, induced by live *P. intermedia* and *A. naeslundii.*

Distinct effects were observed in experiments carried out with heat-killed bacteria at longer times of incubation and lower concentrations of LMM chicory and mushroom fractions. Heat-killed bacteria alone did not affect Cyt18 expression at 6 h incubation, whereas small decreases and increases were observed with chicory and mushroom fractions, respectively. Interestingly, combined exposure to bacteria and LMM fractions induced small upregulation of Cyt18 expression. This synergistic action indicates a beneficial effect of chicory and mushroom fractions on cells exposed to heat killed bacteria. *P. intermedia* alone induced an increase in expression of 4βINT, whereas all the other individual treatments resulted in downregulation. Both chicory and mushroom prevented the upregulation of 4βINT induced by *P. intermedia*. Moreover, combined exposure to *A. naeslundii* and LMM fractions abolished the downregulation induced by individual treatments, again

indicating a synergistic effect between LMM fractions and heat-killed bacteria.

In these experimental conditions, changes in expression of the antimicrobial peptide HβD2 were also observed. Antimicrobial peptides (AMPs) are components of the host innate immune defence system, exerting broad-spectrum antimicrobial activity via the binding and perforation of cell membranes [20, 21], as well as exerting neutralising effects on the LPS activity of Gram negative bacteria, including periodontopathogens such as *P. intermedia* [22]. Human β-defensins are small, cationic AMPs: β-defensin 1 (HβD1) is expressed constitutively in epithelial tissues, whereas HβD2 and HβD3 are expressed in response to bacterial stimuli or inflammation [23–26]. Defensins, including HβD2, may also participate in epithelial differentiation and tissue damage repair in periodontal disease and contribute to the host defense by recruiting neutrophils to the site of inflammation and modulate the expression of cytokines, thus playing an important role in the control of oral health [22, 26–31]. Gingival epithelial cells and tissue express HβD2 mRNA and peptide in response to inflammatory mediators and challenge from commensal bacteria naturally present in the oral cavity [32]. In gingival epithelial cells, HβD2 mRNA was induced in response to the supernatant from *Porphyromonas gingivalis*, and this expression might be associated with periodontal health and disease [33]. Cell wall components of periodontal pathogens induce HβD2 expression through activation MAP kinases [34]; such effect can be mediated by activation of fibronectin-integrin components [35].

The results here obtained with heat-killed *P. intermedia* and *A. naeslundii* and with chicory and mushroom LMM fractions show that individual treatments induced changes in the level of HβD2 mRNA similar to those observed for that 4βINT, with upregulation by *P. intermedia* and downregulation by *A. naeslundii* and LMM fractions. Both chicory and mushroom prevented the upregulation of HβD2 induced by *P. intermedia* and combined exposure to *A. naeslundii* and LMM fractions abolished the downregulation induced by individual treatments. Again, the results indicate a comparable effect of Chicory and Mushroom LMM fractions on the overall changes in gene expression induced by heat-killed gingivitis-associated bacteria.

5. Conclusions

On the basis of the results of various assays, reported elsewhere in this issue, raspberry, chicory, and mushroom LMM fractions were chosen as the most active test materials towards the gingivitis-associated species *P. intermedia* and *A. naeslundii* [5]. With respect to studies of coaggregation, biofilm formation, and adhesion to hydroxyapatite and cultured gingival cells [5], in this work LMM fractions were utilised at lower concentrations and longer times of exposure in order to maintain cell viability and allow for detection in changes in mRNA transcription, respectively. Our data indicate that both chicory and mushroom LMM fractions, at concentrations that do not affect viability of KB gingival cells, can modulate the expression of different genes induced by both live and heat-killed *P. intermedia* and

A. naeslundii. This supports the hypothesis that chicory and mushroom LMM fractions can modulate the responses of gingival cells to periodontopathogens and can be used as source for obtaining agents to be included in toothpaste, mouthwashes, and other oral health care products.

Acknowledgments

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