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# Use of Native Lactococci as Vehicles for Delivery of DNA into Mammalian Epithelial Cells[▽]

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**The use of the food-grade bacterium *Lactococcus lactis* as a DNA delivery vehicle at the mucosal level is an attractive DNA vaccination strategy. Previous experiments showed that recombinant *L. lactis* expressing the *Listeria monocytogenes inlA* gene can deliver a functional gene into mammalian cells. Here, we explored the potential use of noninvasive *L. lactis* strains as a DNA delivery vehicle. We constructed two *Escherichia coli-L. lactis* shuttle plasmids, pLIG:BLG1 and pLIG:BLG2, containing a eukaryotic expression cassette with the cDNA of bovine β-lactoglobulin (BLG). The greatest BLG expression after transfection of Cos-7 cells was obtained with pLIG:BLG1, which was then used to transform *L. lactis* MG1363. The resulting *L. lactis* strain MG1363(pLIG:BLG1) was not able to express BLG. The potential of *L. lactis* as a DNA delivery vehicle was analyzed by detection of BLG in Caco-2 human colon carcinoma cells after 3 h of coincubation with (i) purified pLIG:BLG1, (ii) MG1363(pLIG:BLG1), (iii) a mix of MG1363(pLIG) and purified pLIG:BLG1, and (iv) MG1363. Both BLG cDNA and BLG expression were detected only in Caco-2 cells coincubated with MG1363(pLIG:BLG1). There was a decrease in the BLG cDNA level in Caco-2 cells between 24 and 48 h after coincubation. BLG expression by Caco-2 cells started at 24 h and increased between 24 and 72 h. BLG secretion by Caco-2 cells started 48 h after coincubation with MG1363(pLIG:BLG1). We conclude that lactococci can deliver BLG cDNA into mammalian epithelial cells, demonstrating their potential to deliver in vivo a DNA vaccine.**

Allergy to cow's milk is an important health problem worldwide, affecting 2 to 3% of infants in the first 2 years of life in various countries of northern Europe (21). Bovine β-lactoglobulin (BLG) is the most abundant whey protein of cow's milk and is considered a dominant allergen; it is an 18-kDa lipocalin glycoprotein with a globular structure, containing two intramolecular disulfide bonds (32).

*Lactococcus lactis* is a food-grade gram-positive lactic acid bacterium considered to be noninvasive and noncolonizing. *L. lactis* can deliver antigens and cytokines to the systemic and mucosal immune systems via mucosal routes (42). Therefore, its use as a vaccine delivery system using different antigens and cytokines has been widely studied (5, 6, 7, 17, 28, 33, 34, 35, 44; for a review, see reference 30). We previously showed that administration of *L. lactis* strains producing either BLG or a BLG epitope induces a mucosal immune response and partially protects against sensitization in mice (9, 10, 3).

In contrast to bacterially mediated delivery of protein antigens, bacterially mediated delivery of DNA vaccines could lead to host expression of posttranslationally modified antigens and therefore to the presentation of conformationally restricted epitopes (15). Attenuated strains of pathogenic bacteria, such as *Shigella*, *Listeria*, and *Salmonella*, have been used as live vectors to deliver DNA into mammalian cells (18, 39). Nevertheless, the risk associated with possible reversion to a virulent phenotype of these pathogenic bacteria is a major concern (13).

The use of food-grade lactic acid bacteria as a DNA delivery vehicle is a promising alternative for a DNA vaccine carrier. We previously investigated whether *L. lactis* could deliver DNA vaccines by expressing the *Listeria monocytogenes inlA* gene, which encodes internalin (InlA). The resulting *L. lactis* *inlA*$^+$ strains were able to enter intestinal cells in vivo after oral inoculation of guinea pigs (20). Moreover, a functional eukaryotic *gfp* gene carried by such strains could be delivered into 1% of internalized Caco-2 human colon carcinoma cells (20). The limits of such a strategy were (i) the necessity of working either with transgenic mice expressing human E-cadherin or with guinea pigs and (ii) the need for using recombinant invasive lactococci.

Here, we used native lactococci to deliver BLG cDNA (a eukaryotic expression cassette encoding the BLG antigen) into mammalian cells. Genetic immunization with this cDNA previously induced a preventive and persistent inhibition of specific anti-BLG immunoglobulin E (IgE) responses in mice (2). Caco-2 cells and an *L. lactis* strain harboring BLG cDNA were cocultured to evaluate the ability of lactococci to deliver DNA into mammalian cells. This coincubation led to the expression and secretion of the BLG protein by Caco-2 cells.

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TABLE 1. Strains and plasmids

| Plasmid(s)[a] | Strain(s) | Properties | Reference or source |
|---|---|---|---|
| pIL253* | *L. lactis* MG1363 | High-copy-number lactococcal vector | 40 |
| pLIG1*† and pLIG2*† | *E. coli* Top10, *L. lactis* MG1363 | Cointegrate between pIL253 and pcDNA3 (Clontech) | This work |
| pcDNA3BLG5† | *E. coli* Top10 | pcDNA3 with $P_{cmv}$ promoter fused to *blg* cDNA | 8 |
| pLIG:BLG1*† | *E. coli* Top10, *L. lactis* MG1363 | Cointegrate between pIL253 and pcDNA3BLG5 | This work |
| pLIG:BLG2*† | *E. coli* Top10, *L. lactis* MG1363 | Cointegrate between pIL253 and pcDNA3BLG5 | This work |
| pLEISS-BLG‡ | *L. lactis* NZ9000 | Lactococcal vector containing a nisin induction system with a LEISS fragment and the *blg* gene | 31 |

[a] *, erythromycin; †, ampicillin; ‡, chloramphenicol.

These are the first steps toward the use of lactococci as DNA vaccine delivery vectors. The ease of generating such vehicles for DNA delivery makes the use of *L. lactis* as a carrier for oral DNA vaccination highly attractive for further in vivo research and vaccine development.

## MATERIALS AND METHODS

**Bacterial strains, plasmids, media, and growth conditions.** The strains and plasmids used in this study are listed in Table 1. The plasmids pLIG:BLG1 and pLIG:BLG2 are cointegrates obtained by ligating BglII-linearized pcDNA3BLG5 (8) and BamHI-linearized pIL253 (Fig. 1) (40). To avoid self-ligation, pcDNA3BLG5 was dephosphorylated before ligation. The same constructions were performed with pIL253 and pcDNA3, with pLIG1 and pLIG2 used as a negative control. Products of ligation were transformed in $CaCl_2$ competent cells of *Escherichia coli* strain Top10 (Invitrogen) and plated onto Luria-Bertani agar plates containing 100 μg ampicillin/ml and 150 μg erythromycin/ml. pLIG:BLG1, pLIG:BLG2, pLIG1, and pLIG2 were electrotransformed in *L. lactis* subsp. *cremoris* MG1363 (16), as described elsewhere (25). Enzymatic digestion of both cointegrates with HindIII produced a distinct pattern, allowing selection of clones. These strains were grown in M17 medium (43) containing 0.5% glucose (GM17). Transformants were plated on GM17 agar plates containing 5 μg erythromycin/ml after 24 h of incubation at 30°C.

**DNA manipulations.** DNA manipulations were performed as previously described (37), with the following modifications. For plasmid DNA extraction from *L. lactis*, TES (25% sucrose, 1 mM EDTA, 50 mM Tris-HCl [pH 8]) containing lysozyme (10 mg/ml) was added for 10 min at 37°C to prepare the protoplasts. Enzymes were used as recommended by suppliers.

**BLG expression and extraction in Cos-7 cells.** BLG expression in Cos-7 cells was performed by transfection with LipofectAmine PLUS reagent (Life Technologies, Paisley, United Kingdom). Briefly, 50 to 80% confluent cells cultured in Dulbecco modified Eagle medium, 10% fetal calf serum, 2 mM L-glutamine (BioWhittaker, Cambrex Bio Science, Verviers, Belgium), 100 U penicillin, and 100 μg streptomycin were transfected with pcDNA3BLG5, pLIG:BLG1, and pLIG:BLG2 previously complexed with LipofectAmine. Two days after transfection, the medium was collected and cells were harvested, centrifuged in phosphate-buffered saline (PBS), counted, and sonicated. The cellular extract was centrifuged for 15 min at 10,000 × *g* at 4°C. The supernatant (S) was

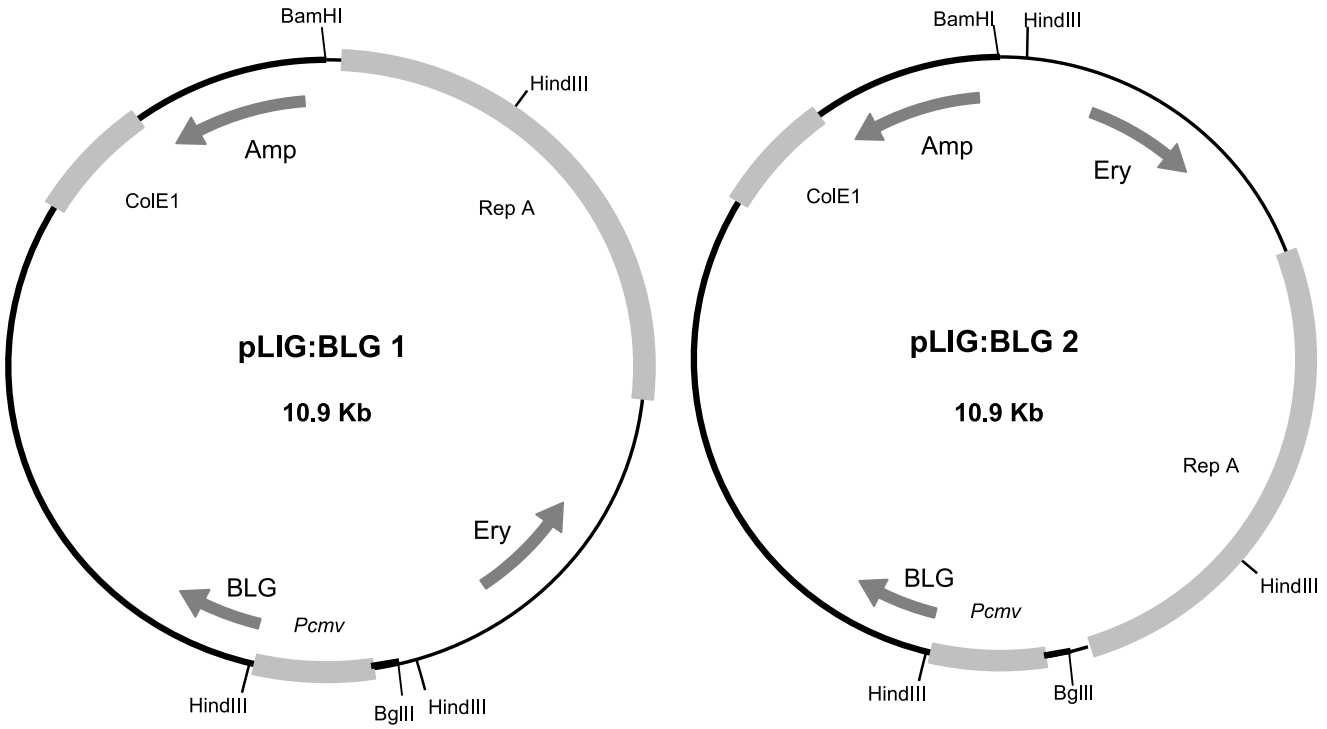


FIG. 1. Structures of pLIG:BLG1 and pLIG:BLG2. Arrows indicate the directions of transcription of BLG cDNA, the ampicillin resistance gene (Amp), and the erythromycin resistance gene (Ery). Boxes indicate $P_{cmv}$, the cytomegalovirus eukaryotic promoter; RepA, the origin of replication of *L. lactis*; and ColE1, the origin of replication of *E. coli*. The thin part of the vector is derived from plasmid pIL253, and the thick part is derived from pcDNA3BLG5. The BamHI and BglII restriction sites used to obtain the two plasmids and the HindIII restriction site are shown.

collected. The pellet was suspended in 6 M GuHCl–100 mM $NaH_2PO_4$–10 mM Tris at pH 8 for 30 min at room temperature and then centrifuged for 10 min at 20,000 × *g* at 4°C. The supernatant (I) was collected. Medium and I extracts were dialyzed against PBS. Native BLG (nBLG) and denatured BLG (RCM-BLG) were assayed in medium and S and I extracts by a specific two-site enzyme immunometric assay (EIA) described below.

**Detection of BLG under native and denatured conformation by two-site EIA.** Two-site EIAs for nBLG and RCM-BLG were performed as previously described (29). Briefly, assays were performed in 96-well microtiter plates coated with a monoclonal antibody (capture antibody) specific for either nBLG or RCM-BLG. Fifty microliters of standard (nBLG or RCM-BLG) or 50 μl of the samples was added; then, 50 μl of tracer was added, consisting of a second monoclonal antibody labeled with acetylcholinesterase (AChE), a conjugate recognizing either nBLG or RCM-BLG. The capture and tracer antibodies were directed against different complementary epitopes. After an 18-h reaction at 4°C, the plates were washed and solid-phase-bound AChE activity was measured by Ellman's method (14). Detection limits of 30 and 200 pg/ml were obtained for nBLG and RCM-BLG, respectively.

**Total RNA extraction and purification from bacteria and Caco-2 cells and detection of specific mRNA by RT-PCR.** Total mRNA was extracted and purified with the RNeasy Fibrous Tissue Mini kit (QIAGEN) as described by the supplier. Reverse transcriptase (RT) PCR was performed on 1 μg of total RNA with the OneStep RT-PCR kit from QIAGEN. In order to detect β-actin mRNA or BLG mRNA, we used oligonucleotides specific for β-actin, ATGGATGACGA TATCGCTGCGCTGGTCGTC (β-actin Fwd) and CTAGAAGCACTTGCGG TGCACGATGGAG (β-actin Rev), and oligonucleotides specific for BLG, CT CATCGTCACCCAGACCATGAAGGGCC (BLG5′) and GATGTGGCACTG CTCCTCCAGCTGGGTTGGG (BLG3′).

**Coculture assays of *L. lactis* strains and Caco-2 human epithelial cells.** Caco-2 epithelial cells were cocultivated with (i) purified pLIG:BLG1, (ii) *L. lactis* MG1363(pLIG:BLG1), (iii) a mix of *L. lactis* MG1363(pLIG1) and purified pLIG:BLG1, and (iv) *L. lactis* MG1363(pLIG1) in order to determine the potential of lactococci to deliver DNA in epithelial host cells. The coculture assays were performed with the human colon carcinoma cell line Caco-2 (ATCC HTB37), as described by Dramsi et al. (12). These cells were cultured in RPMI supplemented with 2 mM L-glutamine (BioWhittaker, Cambrex Bio Science, Verviers, Belgium) and 20% fetal calf serum (complete RPMI). Under these experimental conditions, Caco-2 cells from passages 9 to 12 were used, maintained without antibiotics. The number of cells tested was $5 \times 10^5$ per dish. Four 50-mm petri dishes (Corning Glass Works) were used to analyze each coculture assay. *L. lactis* strains were grown to an optical density at 600 nm of 0.9 to 1.0, washed, and diluted in 1× PBS so that the multiplicity of infection was about $10^3$ bacteria per cell, giving about $5 \times 10^8$ per dish. pLIG:BLG1 is a derivative of pIL253 usually present at a rate of 80 copies per cell (40); consequently, the quantity of pLIG:BLG1 contained in $5 \times 10^8$ bacteria was estimated to be 90 ng. Either the bacterial suspension or the purified pLIG:BLG1 was added to mammalian Caco-2 cells. After 3 h of coculturing, the cells were incubated for 24, 48, or 72 h in complete RPMI medium with gentamicin (20 mg/liter) to kill noninternalized lactococci (12). Bacterial counting was performed after 24, 48, and 72 h of incubation in the presence of gentamicin to estimate bacterial survival. A stable survival level of $\sim 10^2$ lactococci was observed (data not shown).

**Detection of DNA transfer from *L. lactis* to Caco-2 cells by PCR.** At 24, 48, and 72 h, genomic DNA from gentamicin-treated Caco-2 cells was extracted with the DNeasy tissue kit (QIAGEN), as described by the supplier. Plasmid DNA was detected by PCR with the oligonucleotides pcDNA3 start (ATCCCCTATGGT CGACTCTCAGTACAATCT), priming at the N terminus of pcDNA3; pcDNA3 end (GACGTCAGGTGGCACTTTTCGGGGAAATGT), priming at the C terminus of pcDNA3; BLGn2 (CTAATCGTCACCCAGACCATGAAGGGC), priming at the N terminus of BLG cDNA; and BLGc2 (GATGTGGCACTGC TCCTCCAGCTGGGTTGGG), priming at the C terminus of BLG cDNA.

**BLG extraction and detection in Caco-2 cells.** At 24, 48, and 72 h after gentamicin treatment, the medium was collected and Caco-2 cells were harvested. Proteins were extracted as described above for Cos-7 cells, and BLG was assayed in various extracts with both EIAs described above.

**BLG extraction and detection from cultures of bacteria.** Total proteins were extracted from cultures of bacteria, as previously described (9). BLG was then detected in extracts with both EIAs described above.

## RESULTS

**BLG expression in Cos-7 cells is higher with pLIG:BLG1.** Plasmids pLIG:BLG1 and pLIG:BLG2, carrying a eukaryotic expression cassette of the *blg* gene, are depicted in Fig. 1. Cos-7 cells were transfected with the purified plasmids pcDNA3BLG5 (positive control), pLIG1 (negative control), pLIG:BLG1, and pLIG:BLG2. Forty-eight hours after transfection, nBLG and RCM-BLG were assayed in culture supernatants and cell extracts. RCM-BLG was never detected in the culture supernatant. The amount of BLG detected in cell extracts was significantly lower with pLIG:BLG2 than with pcDNA3BLG5 or pLIG:BLG1 (Fig. 2A). In contrast, the amount of BLG detected in culture supernatants was not significantly different between pLIG:BLG1 and pLIG:BLG2 (Fig. 2B). About 10% of the BLG found in the cell extract was denatured. We used pLIG:BLG1 for further experiments.

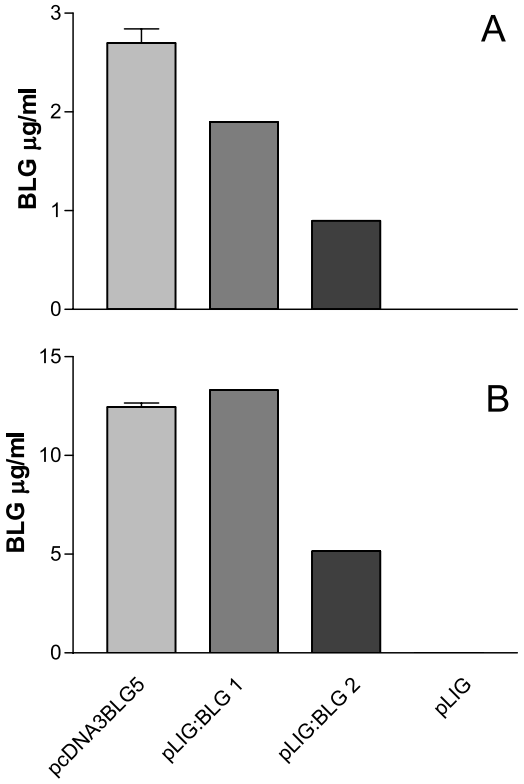


FIG. 2. BLG expression in Cos-7 cells after transfection. Cos-7 cells were transfected with plasmids pcDNA3BLG5, pLIG:BLG1, and pLIG:BLG2. Two days later, the transfection medium was collected and cells were harvested. BLG was assayed in the culture medium and in cell extracts. Results are expressed in micrograms per milliliter of culture medium (A) and of cell extracts (B). The results correspond to the averages of two independent assays. Error bars correspond to standard deviations. Statistical significance was set at a *P* value of $<0.05$.

***Lactococcus lactis* MG1363(pLIG:BLG1) is not able to express BLG.** Total mRNA was extracted from cultures of MG1363(pLIG1) and MG1363(pLIG:BLG1). Figure 3A shows RT-PCR experiments performed with total mRNA from MG1363(pLIG1) and MG1363(pLIG:BLG1), using β-actin-specific oligonucleotides as an RNA positive control (lanes 1 and 2, respectively). No BLG mRNAs were detected in MG1363(pLIG1) or in MG1363(pLIG:BLG1) (Fig. 3A, lanes 3 and 4, respectively). An EIA was performed to confirm the RT-PCR data (Fig. 3B). Total protein was extracted from cultures of wild-type MG1363, MG1363(pLIG1), and MG1363

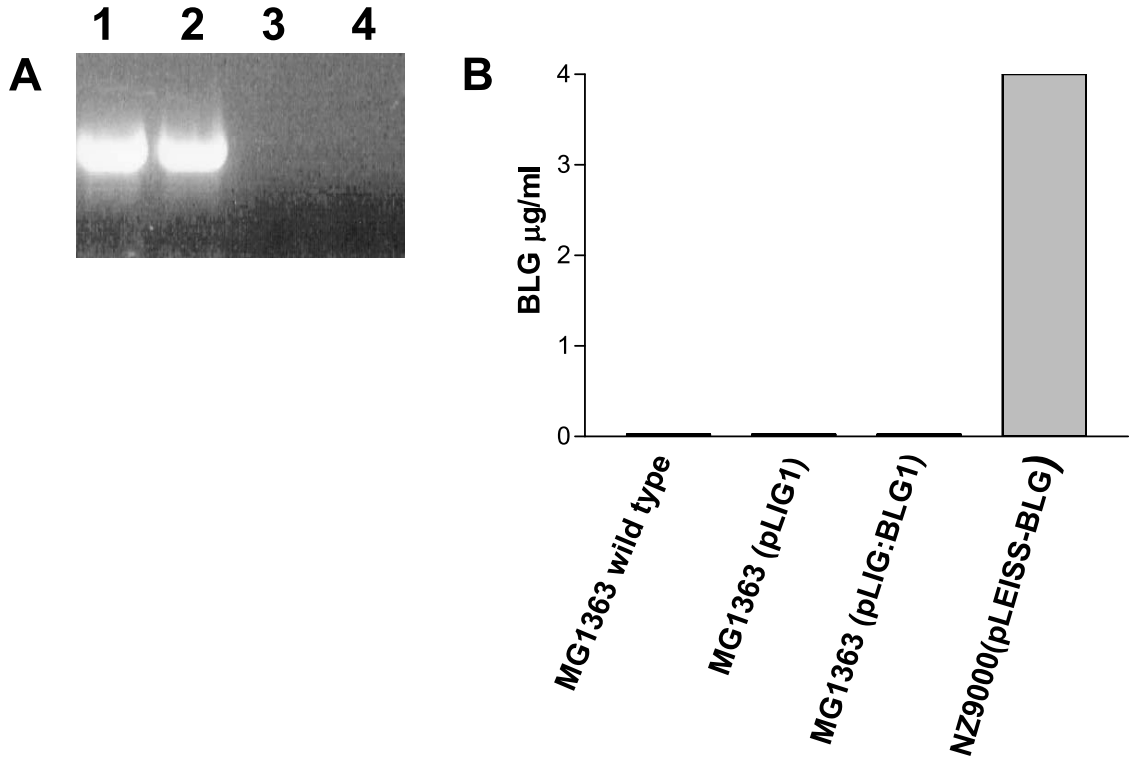


FIG. 3. BLG expression in MG1363(pLIG1) and MG1363(pLIG:BLG1). (A) Detection of β-actin and BLG-specific mRNA by RT-PCR in total RNA from saturated growth of MG1363(pLIG1) and MG1363(pLIG:BLG1). Lanes 1 and 2, β-actin mRNA detection by RT-PCR from MG1363(pLIG1) and MG1363(pLIG:BLG1), respectively; lanes 3 and 4, BLG mRNA detection by RT-PCR from MG1363(pLIG1) and MG1363(pLIG:BLG1), respectively. (B) BLG assay of *L. lactis* strains grown to saturation.

(pLIG:BLG1). BLG was assayed from supernatant and total protein extracts. No BLG was detected in any strain. No trace of BLG mRNA or BLG protein was detected in wild-type *L. lactis* MG1363, MG1363(pLIG1), or MG1363(pLIG:BLG1). We checked that BLG was produced only by our positive control, a nisin-induced culture of *L. lactis* NZ900(pLEISS-BLG) (31).

***Lactococcus lactis* is able to transfer genetic material to Caco-2 cells.** In order to evaluate the feasibility of plasmid delivery from *L. lactis* to eukaryotic cells, MG1363(pLIG1), MG1363(pLIG:BLG1), or purified plasmid pLIG:BLG1 was cocultivated for 3 h with Caco-2 cells, which are routinely used as a model of epithelial cells. DNA was extracted, purified, and quantified from these cells at 24, 48, and 72 h after incubation with gentamicin. Specific amplification by PCR was performed on 1 µg of total DNA with specific oligonucleotides priming at the N terminus and C terminus of pcDNA3 (Fig. 4A) and the N terminus and C terminus of BLG cDNA (Fig. 4B). No amplification was observed with any primer pair when purified pLIG:BLG1 was

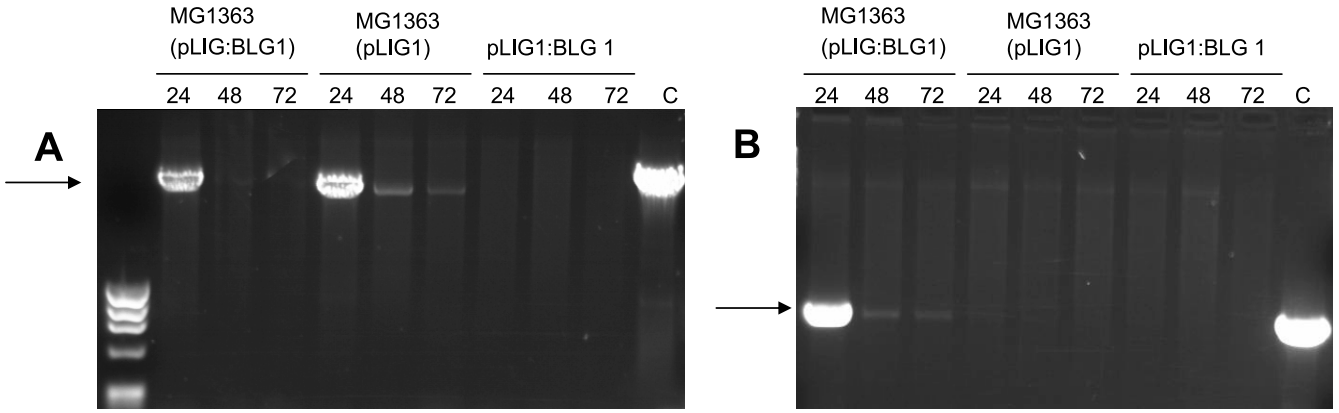


FIG. 4. Plasmid transfer detection in Caco-2 cells determined by amplification by PCR from total DNA purified 24, 48, and 72 h after gentamicin treatment of Caco-2 cells cocultured with MG1363(pLIG:BLG1), MG1363(pLIG1), and purified plasmid pLIG:BLG1. (A) Specific amplification by PCR using oligonucleotides primed at the N terminus and the C terminus of pcDNA3. (B) Specific amplification by PCR using oligonucleotides primed at the N terminus and the C terminus of BLG cDNA. Control (C) amplifications were made with purified plasmid pcDNA3BLG5.

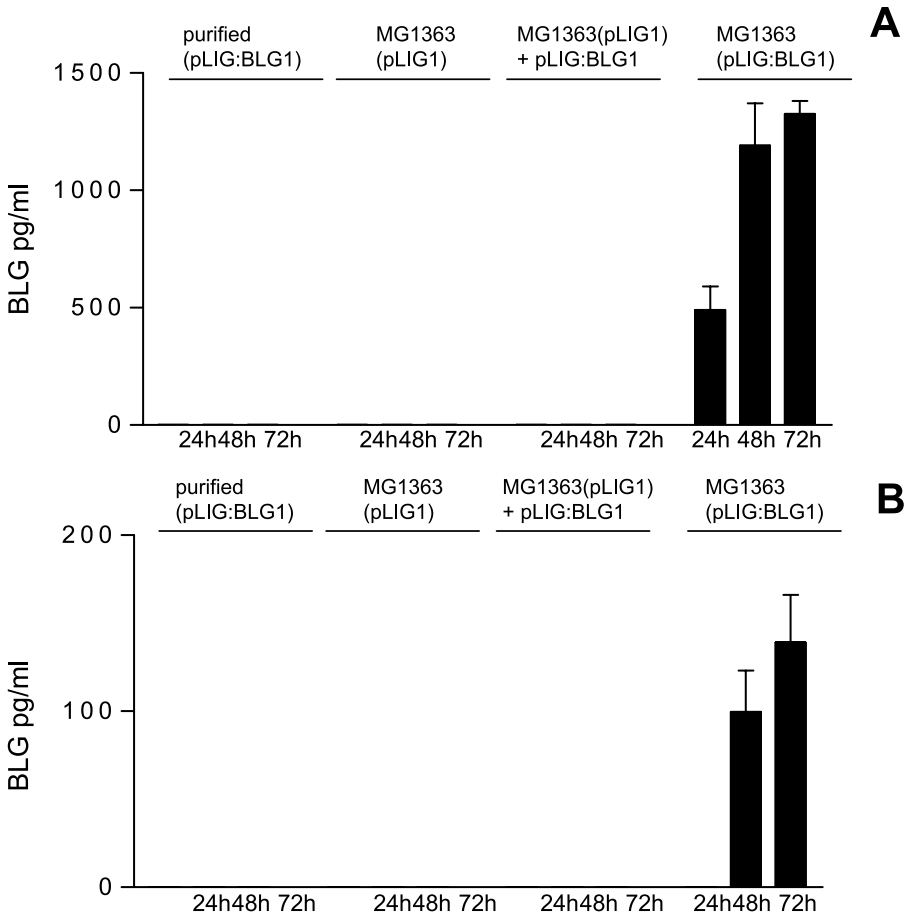


FIG. 5. BLG expression in Caco-2 cells. Caco-2 cells were cocultivated with purified plasmid pLIG:BLG1, MG1363(pLIG1), purified plasmid pLIG:BLG1 mixed with MG1363(pLIG1), and MG1363(pLIG:BLG1). Twenty-four, 48, and 72 h after treatment with gentamicin, the cells were harvested and culture supernatants were collected. (A) BLG assayed in the cellular extract. (B) BLG assayed in the culture supernatant. The results correspond to the averages of three independent assays. Error bars correspond to standard deviations.

added to Caco-2 cells. In cocultures of Caco-2 cells with MG1363(pLIG1), the plasmid was detected at 24, 48, and 72 h with pcDNA3-specific oligonucleotides (Fig. 4A). Nevertheless, a dramatic decrease in pLIG1 concentration was observed after 24 h (Fig. 4A). In cocultures of Caco-2 cells with MG1363(pLIG:BLG1), the plasmid was detected at 24 h (Fig. 4A) and at 24, 48, and 72 h (Fig. 4B), with either the pcDNA3 or BLG primer pairs, respectively. A similarly dramatic decrease in the pLIG:BLG1 concentration after 24 h was observed (Fig. 4B). *L. lactis* was able to transfer genetic material to Caco-2 cells, and this DNA remained detectable even 72 h after coincubation.

**The Caco-2 epithelial cell line is able to express and secrete BLG after coculture with MG1363(pLIG:BLG1).** In order to determine whether *L. lactis* is able to deliver a functional plasmid, we used the coculture assay with Caco-2 cells and either (i) purified pcDNA3:BLG1, (ii) MG1363(pLIG1), (iii) purified pLIG:BLG1 mixed with MG1363(pLIG1), or (iv) MG1363(pLIG:BLG1). The cellular extracts and media from 24-, 48-, and 72-h gentamicin-treated Caco-2 cells were analyzed by a highly specific BLG EIA. We did not detect BLG expression when Caco-2 cells were cocultured with purified pLIG:BLG1, MG1363(pLIG1), or purified pLIG:BLG1 mixed with MG1363(pLIG1) (Fig. 5). BLG expression and secretion were detected only when Caco-2 cells were cocultured with MG1363(pLIG:BLG1), showing that the plasmid should be inside the bacteria to be successfully delivered. In this case, we observed an increase in BLG expression in the cellular extract from 500 pg/ml to about 1,500 pg/ml between 24 and 48 h, reaching a maximum at 72 h (Fig. 5). We also observed an increase in BLG secretion from 100 pg/ml to 150 pg/ml between 48 and 72 h in the cellular supernatant. Seventy hours after coculture, 80% of the BLG was found in the medium. Denatured BLG conformation was never detected. Taken together, these results demonstrate that (i) *L. lactis* was able to deliver fully functional plasmid into Caco-2 cell lines and (ii) these host cells were able to incorporate this DNA, expressing and secreting the BLG protein. Moreover, the presence of BLG mRNA in Caco-2 cells was confirmed by RT-PCR performed with BLG-specific oligonucleotides (Fig. 6).

## DISCUSSION

We evaluated the potential of native noninvasive lactococci as DNA delivery vectors by coculture of Caco-2 epithelial cells and *L. lactis* strains carrying an expression cassette encoding BLG cDNA under the transcriptional control of the human

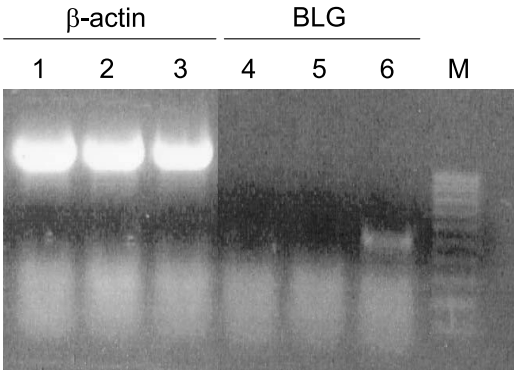


FIG. 6. Detection of BLG mRNA in Caco-2 cells. Caco-2 cells were cocultivated with purified plasmid pLIG:BLG1 (lanes 1 and 4), MG1363(pLIG1) (lanes 2 and 5), and MG1363(pLIG:BLG1) (lanes 3 and 6). Forty-eight hours after treatment with gentamicin, the cells were harvested and total RNA was collected and used for RT-PCR experiments with β-actin- and BLG-specific oligonucleotides. M, nucleic acid marker.

cytomegalovirus eukaryotic promoter ($P_{cmv}$). We showed that *L. lactis* was able to deliver the *blg* gene with subsequent expression of the BLG protein by the eukaryotic host cell. It was previously shown that native lactococcus strains are able to adhere to the human cell line Caco-2 (23). Moreover, a similar adhesion background was also observed after coincubation of recombinant invasive *L. lactis* strains and epithelial cells (4, 20, 27, 36, 41). Our hypothesis is that after coculture of *L. lactis* and Caco-2 epithelial cells, some lactococcal strains are internalized and probably lysed within the phagolysosome, releasing BLG cDNA into the cytosol via leakage from host cell phagosomes. This type of leakage between these two compartments has already been proposed for the transfer of certain protein antigens from the phagosome to the cytosol in primary macrophages and in dendritic cells (1, 19, 22, 24).

After 24 h, the BLG-specific DNA concentration significantly decreased inside Caco-2 cells, suggesting that it progressively disappeared by degradation by cytoplasmic nucleases and/or by entering the nucleus and being stable inside it (26). The turnover of DNA delivered by microinjection into the cytosol has been shown to be rapid, with an apparent half-life of 50 to 90 min in HeLa and COS-1 cells (26). Bacterial DNA delivery to eukaryotic cells was previously achieved at a low rate with noninvasive strains of *E. coli* (38). The low rate of BLG expression by Caco-2 cells could be due to the low efficiency of nuclear DNA importation into the nucleus (11). No production of BLG was observed when Caco-2 cells were coincubated with purified plasmid mixed or not mixed with the bacterium, suggesting that the plasmid must be inside the bacterium to achieve transfer and consequent BLG production.

Some advantages of using lactococci as a DNA delivery vehicle can be considered. (i) Oral administration of such a DNA vaccine vector could induce specific protection against food allergies by inducing a local IgA immune response (9). (ii) In contrast to immunization with naked plasmid DNA, no further plasmid amplification and purification steps are needed, considerably reducing cost and labor. Finally, (iii) an improved safety profile can be attained due to the fact that lactococci are food-grade bacteria that deliver genes encoding only the desired antigens, in contrast to similar delivery systems with pathogens. This strategy could be improved by increasing the quantities of delivered DNA, which could be performed by analyzing the incidence of plasmid size and copy number. This could optimize DNA nuclear importation and consequently BLG expression by the host cell. The results presented here are highly encouraging; the next step is to evaluate the immunological response after oral administration in mice of this *Lactococcus* strain as a bacterial carrier to determine whether it can be used as a DNA vaccine against BLG in vivo.

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