Probiotics and Antimicrobial Proteins
https://doi.org/10.1007/s12602-023-10041-1

RESEARCH

Lactococcus lactis as an Interleukin Delivery System for Prophylaxis and Treatment of Inflammatory and Autoimmune Diseases

Gabriela Munis Campos[1] · Monique Ferrary Américo[1] · Andria dos Santos Freitas[1] · Fernanda Alvarenga Lima Barroso[1] · Joyce da Cruz Ferraz Dutra[1,2] · Ludmila Silva Quaresma[1] · Bárbara Fernandes Cordeiro[1] · Juliana Guimarães Laguna[1] · Luís Cláudio Lima de Jesus[1] · Aparecida Maria Fontes[3] · Alexander Birbrair[4,5] · Tulio Marcos Santos[1,6] · Vasco Azevedo[1]

Accepted: 9 January 2023

Abstract

Target delivery of therapeutic agents with anti-inflammatory properties using probiotics as delivery and recombinant protein expression vehicles is a promising approach for the prevention and treatment of many diseases, such as cancer and intestinal immune disorders. *Lactococcus lactis*, a Lactic Acid Bacteria (LAB) widely used in the dairy industry, is one of the most important microorganisms with GRAS status for human consumption, for which biotechnological tools have already been developed to express and deliver recombinant biomolecules with anti-inflammatory properties. Cytokines, for example, are immune system communication molecules present at virtually all levels of the immune response. They are essential in cellular and humoral processes, such as hampering inflammation or adjuvating in the adaptive immune response, making them good candidates for therapeutic approaches. This review discusses the advances in the development of new therapies and prophylactic approaches using LAB to deliver/express cytokines for the treatment of inflammatory and autoimmune diseases in the future.

Keywords Lactic Acid Bacteria · Cytokines · Inflammation, Heterologous protein expression · Immune signalization

Introduction

Lactic Acid Bacteria (LAB) are a heterogeneous group of Gram-positive, mesophilic, non-LPS-producing bacteria, and with the ability to produce lactic acid from sugar fermentation [1]. This group consists of bacteria of the genus *Lactobacillus* (which has been reclassified into 25 new genera), *Lactococcus*, *Streptococcus*, *Enterococcus*, *Leuconostoc*, *Carnobacterium*, *Oenococcus*, *Pediococcus*, *Tetragenococcus*, *Vagococcus*, and *Weissella* [1–4].

The absence of endotoxins makes LAB an important group for industrial applications [5]. In this context, the long track record in fermentative processes of cheese, yogurt, and other dairy products, as well as their ability to produce some food-preserving acids, led to LABs receiving GRAS (generally recognized as safe) status from the Food and Drug Administration (FDA) [2, 6–8].

In addition to their widespread use in the food industry, some LAB strains are used as probiotics, defined as "live microorganisms that, when administered in adequate amounts, confer a health benefit to the host" [9–11]. The

Tulio Marcos Santos, and Vasco Azevedo contributed equally to this work.

✉ Vasco Azevedo
vascoariston@gmail.com

1 Department of Genetics, Ecology, and Evolution, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

2 Department of Microbiology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

3 Genetics Department, Ribeirão Preto School of Medicine, University of São Paulo, Ribeirão Preto, São Paulo, Brazil

4 Department of Dermatology, School of Medicine and Public Health, University of Wisconsin-Madison, Madison, WI, USA

5 Department of Pathology, Federal University of Minas Gerais, Belo Horizonte, MG, Brazil

6 Uniclon Biotecnologia, Belo Horizonte, MG, Brazil

Published online: 07 February 2023

efficacy of these microorganisms as probiotics has been observed in the treatment and prevention of several inflammatory diseases, including inflammatory bowel diseases (IBD), rheumatoid arthritis [12], diabetes [13], allergy [14], and cancer [15].

Besides their use as probiotics, microorganisms of the LAB group, mainly *Lactococcus lactis,* have also been used as bioreactors for heterologous expression of biomolecules with therapeutic effects, and as biosystems for delivery of DNA vectors used in gene therapy and immunization [1, 16]. *Lactococcus lactis* has become the most widely used model for developing of genetic engineering tools because it is easy to grow and genetically manipulate, survives gastrointestinal tract (GIT) stress, and produces no LSP, reducing the risk of endotoxin shock [6, 9, 17], among others. These properties make LAB promising for production or delivery of interesting biomolecules (e.g., anti-inflammatory proteins, vaccine antigens, cytokines, and antimicrobial molecules) [6, 9, 17] using various routes of administration, including intranasal, genital, and oral [18–20].

Bioengineered *Lactococcus lactis:* Expression Systems

Several expression systems have been developed for use in *L. lactis*, including the XIES, NICE, and SICE systems, used for heterologous protein production, and pVALAC and pExu for expression of biomolecules/antigens by host's cells [1]. The main expression/delivery systems are described hereafter (Fig. 1).

NICE System

In 1995 an expression system was developed using genes involved in the regulation of nisin, an antimicrobial peptide widely used as protective in the food industry. The nisin controlled expression system (NICE) is based on the expression of genes related to the regulation of the NIS operon (*nis*A, *nis*F, and *nis*R), regulated by the pNisA promoter. In this system, the nisin inducer binds to the histidine kinase *Nis*K transmembrane protein, activating *Nis*R through the phosphorylation pathway. Consequently, this process activates

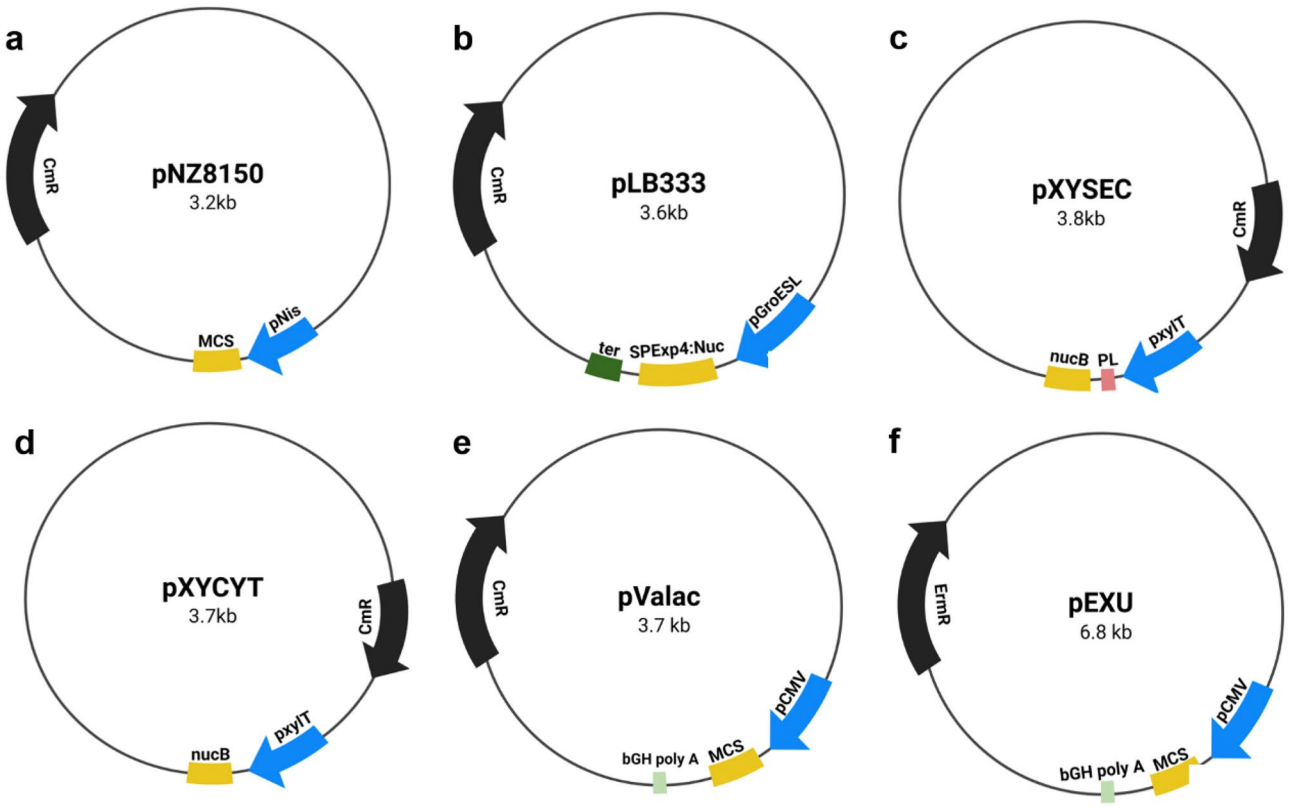

Fig. 1 Representation of the main plasmid vectors of prokaryotic expression (**a**–**d**) and eukaryotic expression (**e**, **f**) used in *Lactococcus lactis*. Blue: promoters; yellow: multiple cloning sites/nuclease gene; black: antibiotic resistance (CmR, chloramphenicol resistance; EmR, erythromycin resistance). Green: other transcriptional elements (poly A tail; transcription termination factor); pink: signal peptide

the PnisA/PnisF promoters, thus, inducing the expression of genes regulated by them (Fig. 1a) [16, 21].

SICE System

The stress-induced controlled expression system (SICE) is a vector that carries an expression cassette under the transcriptional control of a stress-inducible promoter. This system is based on the groESL operon, which after induction with different stressors agents, including those related to gastrointestinal tract (GIT) (pH, heat-shock, or bile salts), can induce protein synthesis of cloned open reading frames (ORFs) (Fig. 1b) [22, 23].

XIES System

Another important heterologous expression system is the xylose-inducible expression system, developed by Miyoshi et al. [24]. This system uses the promoter p*xyLT* from *Lactococcus lactis* NCDO2118 to control the expression of genes of interest. With the addition of xylose in the bacterial growth medium, the promoter p*xyLT* is activated, producing the protein of interest. It is essential to highlight that glucose in the medium shuts down the system.

The XIES system has two versions: the secretion version (pXIES:SEC), where the bacteria produce and secrets the protein (Fig. 1c), and the cytoplasmic version (pXIES:CYT), where the protein produced remains inside the bacteria (Fig. 1d) [24].

pVALAC Vector

Other expression systems have also been explored for protein expression directly by host cells using *L. lactis* as a delivery vector. One of these new approaches is the pVALAC (vaccination using lactic acid bacteria) vector, which was constructed to deliver antigens via *Lactococcus* spp. as a vaccinal and gene therapy approach. The construction of this plasmid was idealized using the fusion of a eukaryotic region containing the promoter pCMV originated from cytomegalovirus, allowing the cloning of antigens of interest for expression in eukaryotic host cells, and a prokaryotic region, where replication rolling-circle type, and also selection in bacteria (chloramphenicol gene marker) can be performed (Fig. 1e) [25].

pExu Vector

The pExu (extra chromosomal unit) vector, containing the pCMV promoter and erythromycin as a selection marker, was constructed as a vaccinal/gene therapy vector to be expressed only by eukaryotic cells. Unlike pVALAC, delivered only by *Lactococcus* sp., pExu can replicate in Gram-positive and Gram-negative bacteria, including *Lactococcus lactis, Lactobacillus sp.,* and *E. coli* strains. In addition, this vector has a theta-type replication. This makes it more stable (Fig. 1f) [26, 27].

Immune System and Cytokines

Immune system cells require a communication network in order to act specifically, locally, or systemically. To achieve this, the immune system has a chain of molecules necessary for its functioning, in which cytokines are among the best characterized [28–30].

Cytokines are a large group of non-enzymatic proteins involved in all immunity levels, including innate and adaptive responses, antigen presentation, cell recruitment, and expression of adhesion molecules. These cytokine networks are complex, and their production is usually transient due to the rapid elimination of the inducing stimulus, feedback mechanisms, and negative receptor regulation. In this context, it is essential to highlight that the prolonged action of cytokines in the immune system can cause damage to the healthy organism, such as the development of chronic inflammation [31].

Restoration of cytokine balance benefits the body in immune and inflammatory responses, making them a source for developing new therapeutic targets [32, 33]. In general, cytokines are used in therapeutic/vaccination strategies only to act as adjuvants-substances capable of enhancing the immune response [34] when co-administered with the molecule of interest [35, 36], allowing both delivery to antigen-presenting cells (APCs) and to modulate the specificity, duration, and type of response (cellular or humoral) [37]. Based on these properties, some cytokines have been evaluated for their biotherapeutic properties.

IL-10

The IL-10 family of cytokines can be divided into three subgroups based on their functions. The first group, represented only by IL-10, is present in innate and adaptive immune responses and performs immunosuppressive functions by reducing tissue damage caused by excessive inflammation [38, 39]. The second group includes cytokines of the IL-20 subfamily, which act mainly on epithelial cells and induce innate defense mechanisms. Finally, the third group consists of the IL-28 subfamily, generally classified as type III interferons (IFNs) with a preference for tissue epithelial cells [38].

Interleukin-10 (IL-10) has anti-inflammatory properties and plays a critical role in the prevention of inflammatory processes [40]. Mature human IL-10 has 160 amino acids and can be produced by various leukocytes and tissue

epithelial cells, such as innate immune system cells, including dendritic cells (DC), macrophages, mast cells, natural killer (NK) cells, eosinophils, neutrophils, $CD4^+$, $CD8^+$, and B cells [29, 30, 40]. IL-10 is not a cell-specific cytokine, but is widely expressed by several immune cells and exerts its anti-inflammatory effects through multiples induction pathways, making it an important molecule to test for delivery or local expression using *L. lactis* as a delivery vehicle.

For this purpose, Schotte et al. [41] investigated the ability of *Lactococcus lactis* MG1363 to synthesize and secrete murine IL-10 fused to a secretion signal peptide of the Usp45 protein of lactococcal origin. The authors constructed a plasmid (pTREX1) based on the pVAX plasmid and the mIL-10 sequence. Bacteria carrying the mIL-10 sequence were able to produce and secrete the protein even at low concentrations [41].

Martin et al. [42] and Benbouzine et al. [23] used the vector pSICE containing the IL-10 sequence carried by *L. lactis* to treat IBD induced by dinitrobenzene sulfonic acid (DNBS). The recombinant *Lactococcus lactis* producing IL-10 decreased intestinal permeability by partially improving and modulating the expression of the tight junctions *F11r* and *Zo-1*. Administration of the recombinant bacteria also resulted in protective effects on immune activation and intestinal function parameters [23, 42].

Del Carmo [33] tested the effects of IL-10 cytokine delivered/expressed by two plasmids (pValac and pGroesESL) in a 2,4,6-trinitrobenzene sulfonic acid (TNBS)-induced chronic colitis model. Administration of both plasmids decreased the severity of inflammation, improved the intestinal damage score and modulated the IL-10/IL-6 expression ratio [33].

Strategies have also been developed using pValac carrying IL-10 in models of intestinal inflammation. For example, Del Carmen et al. [43] constructed a *Lactococcus lactis*-producing fibronectin binding protein A ($FnBPA^+$) of *Staphylococcus aureus* (*L. lactis* MG1363 $FnBPA^+$) capable of harboring the plasmid pValac carrying the mIL-10 gene. This strain could efficiently internalize and induce the expression of $FnBPA^+$ from human epithelial cells [43] and was also able to reduce the severity of TNBS-induced colitis. Zurita-Turk et al. [44] also tested this invasive strain in an IL-10 knockout (IL-10 −/−) model that spontaneously develops intestinal inflammation. Oral administration of *L. lactis* MG1363 FnBPA + (pValac:il-10) modulated the organism to produce IL-10 and reduced the intestinal inflammation severity, presenting lower scores and histological damage and tended to reduce the levels of the pro-inflammatory cytokine IL-6. Based on previous results, the authors carried out a new strategy to evaluate the therapeutic effect of an invasive strain of *L. lactis* MG1363 $FnBPA^+$ (pValac:IL-10) compared to a wild-type of strain of *L. lactis* MG1363 (pValac:IL-10) in preventing inflammation in a sodium dextran sulfate (DSS)-induced colitis model. The results showed that both strains were able to deliver the vector to the inflammation sites, reducing the severity of inflammation and avoiding side effects [32, 43, 44].

Based on the previous effects of treatment with IL-10-expressing *Lactococcus lactis*, Bermúdez-Humaran et al. [45] showed that oral administration of these strains exhibited moderate anti-inflammatory effects in mice compared to the administration of serine and protease inhibitors, which showed more promising results at the mucosal level for the treatment of IBD [45]. There is limited efficacy for the use of IL-10 secreted *Lactococcus lactis* as a primary source of treatment for IBD, and this can be explained by the choice of the molecule, which may not be the best option, as well as due to the amount of IL-10 produced and released by the bacteria is not being sufficient to generate an efficient response of the intestinal environment [45, 46].

The cytokine hIL-10 has also been tested in models, such as autoimmune diabetes, where it was used as an immunomodulatory cytokine in a biological system that secretes human insulin autoantigen. Administration of IL-10 in combination with a low dose of systemic anti-CD3 was well tolerated and induced a long-term autoantigenicity, allowing stable reversal of newly diagnosed autoimmune diabetes in mice. Furthermore, Takiishi et al. [47] and Robert et al. [48] used hIL-10 combined with a T1D GAD65 autoantigen in *Lactococcus lactis,* and administered in the intestine in a mouse model of diabetes. The combination with anti-CD3 and the treatment stabilized insulitis, preserved β cells, and restored normoglycemia in mice [47, 48].

Oral administration of *L. lactis* carrying IL-10 was tested to treat food tolerance by preventing sensitization in a mouse model of food allergy. Sensitization was performed with β-lactoglobulin in the presence of cholera toxin. Pretreatment contributed to the reduction of anaphylaxis and inhibited the production of antigen-specific IgE and IgG and increased the production of antigen-specific IgA in the intestine [49]. Marinho et al. [50] used *L. lactis* NCDO2118 to support the XIES expression system producing LL-CYT and LL-SEC IL-10 expression cassettes from *Ratus novergicus*. They were administered orally and both treatments with the recombinant strains resulted in decreased cytokines observed during an inflammatory response in allergic disorders (IL-4, IL-5, CCL3), EPO activity, IgE, IgG1 anti-OVA levels, pulmonary inflammation, and mucus hypersecretion [50].

IL-22

IL-22 is a member of the IL-10 cytokine family and a critical regulator of epithelial homeostasis. The primary function of IL-22 is to provide a protective response against pathogens at barrier surfaces. It has been implicated in multiple aspects

of epithelial barrier function, including regulation of epithelial cell growth and permeability, mucus and antimicrobial protein (AMP) production, and complement activation [38, 51, 52]. IL-22 acts exclusively on epithelial cells to promote cell regeneration and tissue repair. The IL-22 receptor is widely expressed on epithelial cells in boundary tissues, such as the gut, lung, liver, and skin [53].

This cytokine is constitutively expressed in the small intestine of humans and mice to maintain the integrity of the epithelial barrier against enteric microorganisms, which plays an essential role in the pathogenesis of IBD, and the level of expression depends on the type of inflammation. In healthy humans and mice, the expression level of IL-22 is rarely detectable [54, 55]. Many studies have shown that IL-22 is essential in various cardiovascular diseases, inhibiting inflammation in myocarditis, atherosclerosis, and myocardial infection [56].

In the Loera-Arias et al. [57] work, a safe vector was constructed to produce hIL-22 *in vivo* in *L. lactis* NZ9000 using a nisin-inducible system. Western blotting was performed to confirm the expression, and an ELISA assay was performed to quantify the secreted protein. Recombinant *L. lactis* was added to a culture of Colo-205 cells, a cell line that secretes IL-10 upon IL-22 stimulation, to evaluate the bioactivity of the recombinant IL-22. The recombinant strain induced IL-10 production by Colo-205 cells, and the amount of IL-10 secreted was proportional to the number of recombinant bacteria used, demonstrating that the secreted hIL-22 was biologically active [57].

To evaluate *L. lactis* carrying the plasmid ProbiH1-IL-22 on symptoms of post-infectious irritable bowel syndrome (PI-IBS) symptoms, Maëva et al. [58] used a model of *Citrobacter rodentium* infection in C57BL6/J mice since symptoms resemble enterobacterial gastroenteritis. The infected mice exhibited persistent colonic hypersensitivity, cognitive impairment, and anxiety-like behaviors associated with low-grade inflammation, and increased intestinal permeability. This study presented an alternative treatment using recombinant *L. lactis* as a therapeutic approach, enabling the production of IL-22 in epithelial cells, after which the treatment with this recombinant strain improved intestinal permeability, normalized colonic sensitivity, restored cognitive performance, and also reduced anxiety-like behaviors [58].

IL-4

IL-4 is a member of the T helper 2 (Th2) cytokine family. These cytokines are key mediators of allergic inflammation, are potent B-cell growth and survival factors, promote immunoglobulin isotope conversion to IgE and IgG, and play a critical role in macrophage regulation [59, 60]. Several innate immune cell types are known to be IL-4 producers: epithelial cells, lymphocytes, eosinophils, basophils, and mast cells. They have a wide range of overlapping, but also distinct biological functions, particularly in inflammatory and allergic diseases [61].

Cytokines are part of a chain of events that contribute to immunomodulation, and stimulate risk factors that may be present in the development of type 1 diabetes (DM1). They can induce the destruction of T cells present in pancreatic islets. In this context, studies have been developed to evaluate immunomodulation by administration of IL-4 and IL-10 [62–64].

Preisser et al. [62] used a synergistic strategy with the invasive strain *Lactococcus lactis* MG1363 FnBPA$^+$ by using plasmids encoding IL-4 and IL-10 in a multidose streptozotocin (STZ) diabetes model and non-obese diabetic (NOD) mice. In the STZ model, the treatments used (IL-4; IL-10; IL-4/IL-10) did not alter glycemic levels after the induction of type 1 diabetes (T1D) and showed similar levels of IL-4 and IL-10 measured in serum and pancreas. The groups presented blood glucose levels close to the saline group, with a progressive increase in the incidence of diabetes in all groups tested, even those receiving oral treatment. Although all groups presented diabetic animals, the groups in which IL-4 and IL-10 were administered presented a higher number of prevention of hyperglycemia and reduction of pancreatic islets destruction [62].

NOD mice were also treated orally with *Lactococcus lactis* MG1363 FnBPA$^+$ carrying pValac:IL-4 and pValac:IL-10. The groups receiving *L. lactis* carrying IL-4 and IL-10 exhibited a normoglycemic pattern, consisting of glycemic levels below 200 mg/dL. The synergistic treatment also protected the mice from developing T1D throughout the experimental period and induced significantly higher levels of IL-4 and IL-10 compared to the saline group. At the end of the experimental period, only mice in the saline group developed diabetes [62].

IL-4 and IL-13-producing Th2 cells mediate exacerbated inflammation in colitis, resulting in a deregulated immune response in the intestinal mucosa [65]. IL-4 also plays a critical role in the pathogenesis of ulcerative colitis. Souza et al. [66] used *L. lactis* and pValac::dts:: *IL-4* as a therapeutic molecule against TNBS-induced colitis. Intragastric administration of the recombinant strain effectively reduced the severity of colitis by decreasing IL-12, IL-6, and myeloperoxidase (MPO) activity and increasing IL-4 and IL-10 levels, thereby contributing to the restoration of intestinal homeostasis [66].

IL-2

Interleukin IL-2 is an autocrine T-cell growth factor that plays a role in the stimulation and proliferation of these cells, the generation of effector memory T cells, and also has the potential to induce self-tolerance. In addition to its originally

recognized role in T cell proliferation, IL-2 increased the cytolytic activity of NK cells and lymphokine-activated killer cells [67–69]. It also produced primarily $CD4^+$T cells, but high-affinity IL-2 receptors are expressed by $CD4^+$ and $CD8^+$ T cells and other cell populations [68].

IL-2 was the first cytokine to be administered to humans with cancer and HIV/AIDS, demonstrating success as an immunotherapy. Its limitations were demonstrated by the administration of high doses administration resulting in toxic effects and vascular leakage syndrome [67]. One of the most studied cytokine receptors is the common gamma chain family (γc). It is present in IL-2, IL-4, IL-7, IL-9, IL-15, and IL-21, and is named after the use of the γc subunit for its receptors [70].

The use of *L. lactis* carrying IL-2 for various therapeutic protocols may become a key to immunomodulation without causing toxic effects to the body. For example, Steidler et al. [71] constructed a vector capable of expressing mIL-2 in *Lactococcus lactis* under the controll phage promoter T7, where the cytokine expression cassette was fused to the *usp*45 gene [71]. Fernández et al. [72] showed that *Lactococcus lactis* has the potential to secrete mIL-2 under the control of the nisin promoter *pnisA* without the need for a signal peptide, and the expression was confirmed by Western blot technique [72].

Steidler et al. [73] constructed two strains of *L. lactis* carrying tetanus toxin fragment C (TTFC) in the cytoplasmic compartment, which could also secrete IL-2 or IL-6. By intranasal immunization of mice with the constructed strains, anti-TTFC antibodies could be visualized. Anti-TTFC antibody titers were also measured in groups treated with recombinant bacteria that also secreted IL-2 or IL-6. When the recombinant bacteria were killed with mitomycin C, the adjuvant effect was lost, and it was shown that live bacteria are necessary for the secretion of these interleukins by *L. lactis* [73].

To evaluate *Lactococcus lactis* as a delivery vector for an oral brucellosis vaccine, Rezaei et al. [74] engineered an Omp16-IL-2 fusion gene and evaluated the delivery and expression of this gene in *L. lactis* MG1363 [74]. The researchers successfully described the secretion of a sequence of porcine IL-2 in *Lactococcus lactis* NZ9000. Expression was confirmed in a cell proliferation assay in an IL-2-dependent CTLL-2 cell line [75].

IL-6

IL-6 is a prototypical homeostatic cytokine with a pleiotropic effect on the immune response, particularly inflammation, hematopoiesis, bone metabolism, embryonic development, and other fundamental processes, such as neural and cardiovascular development [76–79]. When infection or tissue injury disrupts homeostasis, IL-6 is immediately produced and contributes to host defense against emerging stress by activating the acute phase of inflammation [76].

The IL-6 family includes many cytokine members: IL-6, IL-11, IL-27, IL-39, oncostatin M (OSM), leukemia inhibitory factor (LIF), ciliary neurotrophic factor (CNTF), cardiotrophin 1 (CT-1), and cardiotrophin-like cytokine factor 1 (CLCF1) [78]. Members of this family play prominent roles in chronic inflammation, autoimmunity, infectious diseases, and cancer. As a result, the IL-6 family of cytokines is now considered a primary therapeutic target for clinical intervention [79]. During inflammation, IL-6 cytokines regulate innate immunity through direct effects on innate immune cells and indirectly through activation of stromal tissue cells resident at the site of inflammation [79]. IL-6 promotes antibody production by acting directly on plasma cells and indirectly by promoting Bcl6-dependent follicular differentiation of $CD4^+$ T cells in the presence of IL-21 and T cell receptor (TCR) stimulation [78]. In disease, IL-6 is an important growth factor in myeloma cells. Its concentration is elevated in patients with Crohn's disease and is overproduced in the germinal centers of hyperplastic lymph nodes in patients with Castleman's disease [80]. Thus, IL-6 is being investigated as a potential immunomodulatory cytokine.

Li et al. [81] developed a new mucosal adjuvant consisting of a cytokine generated by conjugating the C-terminal portion of mIL-6 with an M-cell targeting peptide (CKS9) to enhance a mucosal immune response. The researchers used the *L. lactis* IL1403 strain as a host to express and secrete the recombinant IL-6-CKS9 protein, and this potential adjuvant was tested with the *Brachyspira sp.* membrane B protein (M-BmpB) antigen, administered orally. Analyses performed by the authors showed an increase in anti-M-BmpB antibody levels at mucosal and systemic levels. There was a successful production and secretion of mIL-6, demostrating that the use of recombinant *L. lactis* has the potential to be used as a vaccine adjuvant [81].

IL-12

IL-12 is a member of the IL-12 cytokine family, which is part of the IL-6 superfamily. The IL-12 family consists of four heterodimeric cytokines that share sequence homology, including IL-12, IL-23, IL-35, an anti-inflammatory cytokine produced by regulatory T cells (T_{reg} cells), and IL-27 [82]. IL-12 has biological functions such as the differentiation of naïve $CD4^+$ T cells into IFNγ-producing T_H1 cells. The ability of these cytokines to modulate immune responses in cancer has been of greatt interest. IL-12 cytokines family are typically secreted by innate immune cells, but can also be secreted by adaptive immune cells depending on the disease and immune context [82, 83]. Their importance in the context of cancerl and the major clinical trials conducted are reviewed in the work of Nguyen et al. [84].

To test the production of IL-12 by *L. lactis*, Bermúdez-Humarán et al. [35] constructed an NZ9000 strain with a singlestrand of the ssIL-12 gene coupled to a nisin promoter. The biological activity of IL-12 was confirmed *in vitro* by its ability to induce IFN-γ production in mouse splenocytes and *in vivo* in C57BL/6 mice administered intranasally, where IL-12 induced production of IFN-γ on the mucosal surface [35]. Cortez-Perez et al. [85] administered a strain of *L. lactis* carrying bovine β-Lactoglobulin (BLG) and IL-12 as an adjuvant to evaluate the modulating effect on allergen sensitization and allergic response. The prophylactic effect of administration in an allergy model was evaluated by challenging BALB/c mice with intranasal administration of BLG. The authors observed an increase in IFN-γ levels in the treated mice, suggesting that IL-12 promotes the induction of Th1 response thought the induction of BLG-specific IgG2a [85].

Another study investigated the administration of recombinant *L. lactis* for the prevention of allergic diseases. It evaluated the immunomodulatory effects of IL-12 secreting *L. lactis* for intranasal administration in a mouse model of ovalbumin (OVA)-induced asthma. Mice that received the treatment showed less epithelial damage and mononuclear cell infiltration, reduced airway hyperresponsiveness, and lung inflammation [37].

Fernandez et al. [86] tested a novel signal peptide for *L. lactis,* the SLPmod, which was used to secrete mIL-12 in C3H/HeJ mice. This signal peptide was more effective in producing IL-12 than the Usp45-derived signal secretion [86]; this may be of great utility as the use of *L. lactis* secreting mIL-12 may be beneficial in reducing the toxic side effects associated with systemic delivery of heterologous proteins.

IL-27

Interleukin (IL)-27 is a cytokine that plays a pleiotropic role in the immune system and can directly modify the effector functions of $CD4^+$ and $CD8^+$ T cells. These changes lead to the induction of the cytokine IL-10 and the promotion of specialized regulatory T-cell responses (Treg) [87]. IL-27 is mainly produced by antigen-presenting cells, such as dendritic cells, monocytes, neutrophils, and macrophages. IL-27 production is mainly induced by Toll-like receptors (TLRs) and IFN-γ [88, 89].

IL-27 can be used in therapies against diseases associated with Th1-type inflammation [88]. The use of anti-IL-27 to treat mice exposed to cigarette smoke resulted in a decrease in IFN-γ and attenuated inflammation [90]. In ovalbumin-induced asthma models, prophylactic administration of IL-27 attenuated airway inflammation and hyperresponsivess [91].

In this context, Hanson et al. [92] used *L. lactis*-IL27 by gavage in a model of colitis in $Rag^{-}/^{-}$ mice. This administration protected the mice from enterocolitis by reducing disease scores, intestinal pathology, and inflammatory cytokine levels and increased IL-10 production [92]. These results demonstrate that the use of *L. lactis* has the potential to be an effective and safe treatment for IBD.

All experimental studies with *Lactococcus lactis* expressing or delivering cytokines are listed in Table 1.

IL-35

IL-35 is an anti-inflammatory cytokine, a member of the IL-12 family with a unique expression pattern within the family, and is predominantly secreted by Treg cells and regulatory B cells (Breg) [99]. One of the most prominent functions of IL-35 is the ability to propagate infectious tolerance and to generate a potent population of IL-35-expressing regulatory cells, which have been shown to exhibit immunosuppressive activity [99, 100]. Functional analysis studies suggested that IL-35 plays a critical role in many autoimmune diseases such as multiple sclerosis (MS), systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), and inflammatory bowel disease (IBD) [101–104].

Using oral therapeutic administration of *Lactococcus lactis* carrying mIL-35, this cytokine has been shown to have immunosuppressive functions in several models of autoimmune disease. For example, Maddaloni et al. [98] tested the ability of this cytokine to improve collagen-induced arthritis (CIA). Oral administration of *L. lactis*-IL35 effectively reduced the incidence and severity of CIA disease, reducing IFN-γ and IL-17, and increasing the production of IL-10. This study showed the visibility and benefits of probiotics in immune diseases [98].

IL-35 is an anti-inflammatory cytokine that has been shown to be produced preferentially by Treg cells. It can also facilitate Treg cell generation and limit inflammatory Th17 cells [105]. Hence, Wang et al. [97] investigated its preventive potential. A dairy *L. lactis* NZ9000 strain was engineered to express murine IL-35 and used to prevent the development of DSS-induced mouse colitis. The results showed that oral administration of NZ9000/IL-35 induced the accumulation of IL-35 in the intestinal lumen of normal mice. When administrated prophylactically, the recombinant strain suppressed the DSS-induced colitis progression and decreased the IL-17, IL-6, IFN-γ, and TNF expression [97].

IFN

Interferons (IFNs) are a class of cytokines thar are elicited upon challenge to the host defense and are essential for

Table 1 Cytokines secreted or delivered by *L. lactis* tested in disease models

Cytokine	BAL	Model studied	Organism tested	Main results	References
IL-10	*Lactococcus lactis MG1364 (LL-IL10; LL-OVA + IL10)*	Diabetes	NOD mice	Increased levels of $CD4^{+}Foxp3^{+}CD25^{+}$ regulatory T cells	[48]
IL-10	*Lactococcus lactis*	Diabetes	NOD mice	Combination of oral proinsulin and IL-10 via oral with low-dose aCD3 therapy restored beta-cell tolerance	[93]
IL-10/ IL-4	*Lactococcus lactis MG1363*	Diabetes	Mouse model C57BL/6/NOD	Lower incidence of diabetes and more preserved pancreatic islets	[62]
IL-10	*Lactococcus lactis MG1363 (LL-Thy12)*	Crohn's disease	Crohn's disease patients	A decrease in disease activity	[94]
IL-10	*Lactococcus lactis MG1363 (LL-mIL10)*	Food allergy	Mouse model of food allergy	Induction of IL-10 secretion by Peyer's patches cells	[49]
IL-10	*Lactococcus lactis NCDO2118*	OVA-induced acute airway inflammation	Mouse model BALB/c	Decrease in eosinophil count, EPO activity, anti-OVA IgE and IgG1 levels, IL-4 and CCL3 production, lung inflammation, and mucus hypersecretion	[50]
IL-10	*Lactococcus lactis MG1363*	TNBS-induced chronic colitis	Mouse model BALB/c	Reduced weight loss, lower disease scores, and immune activation	[95]
IL-10 / TGF-β1	*Lactococcus lactis MG1363 / L. lactis NZ9000*	DSS-induced colitis	Mouse model C57BL/6	Moderate anti-inflammatory effects	[45]
IL-10	*Lactococcus lactis MG1363*	DSS-induced colitis	Mouse model C57BL/6	Reduce inflammation	[32]
IL-4	*Lactococcus lactis MG1363; L. lactis MG1363 FnBPA +*	Crohn's disease	Mouse model BALB/c	Decreased the severity of colitis; decreased levels of IL-12, IL-6, and MPO activity; and increased levels of IL-4 and IL-10	[66]
IL-22	*Lactococcus lactis*	Enterobacteria gastroenteritis	Mouse model C57BL/6	Alleviates colon hypersensitivity, cognitive impairment, and anxiety-like behaviors by acting on intestinal mucosal integrity	[58]
IL-27	*Lactococcus lactis*	Enterocolitis	Mouse model Rag-/-	Reduce the severity of colitis by increasing the production of IL-10	[92]
IL-17	*Lactococcus lactis MG1363*	Human Papillomavirus (HPV)-induced cancer	Mouse model pathogen-free C57BL/6	Partial protection against TC-1-induced tumors	[96]
IL-12	*Lactococcus lactis*	ovalbumin (OVA)-induced asthma	Mouse model BALB/c	A shift from Th2 to Th1 with elevated IFN-γ and decreased IL-4 levels	[37]
IL-35	*Lactococcus lactis* NZ9000	DSS-induced colitis	Mouse model C57BL/6	Suppression of DSS-induced colitis progression	[97]
IL-35	*lactococcus lactis* IL1403	Collagen-Induced arthritis (CIA)	Mouse model C57BL/7	Reduced IFN-γ, IL-17, and increased IL-10 production	[98]

mobilizing immune responses against pathogens. All three classes of IFNs are named for their common property to interfere with viral replication in the host [106].

The type I IFN classes are represented by the two best characterized and most widely expressed genes of this subtype: IFN-α, encoded by more than a dozen genes, and IFN-β, a single gene family. Within this family, there are other types known as IFN-ε, IFN-κ, IFN δ, IFN-ζ, IFN-ω, and IFN-τ [106, 107].

IFN-α has emerged as a important factor in several autoimmune and rheumatic diseases. It is produced by several cell types, and plasmacytoid dendritic cells (pDCs) are the major contributors to IFN-α production following pathogen infection [108]. It is also considered a potential therapeutic strategy to treat COVID-19 disease because the innate immune system rapidly produces IFN-α as a first line of defense to combat viral infections [109].

Due to its important role in host defense, many researchers are trying to make use of new strategies to manipulate IFN-α as a therapy for various diseases. In a study performed by Bayar et al. [110], *Lactococcus lacti*s MG1363 and NZ9000 strains carrying the sequence encoding IFN-α were able to express it [110]. Zhang et al. [111] fused a synthetic LEISSTCDA (LEISS) to the N-terminus of hIFN-α, increasing its secretion by *L. lactis* and its yield, representing a new therapeutic delivery strategy [111]. These studies open doors for the use of *L. lactis* in several new therapeutic approaches for the treatment of diseases, where INF-α production is deficient or where higher expression of this cytokine is required.

IFN-β, an important cytokine, plays a critical role in stimulating innate and adaptive immune responses and has been reported to have pro-bacterial activity and antiviral immunity [112]. The interferon family has been strongly associated with T1D pathogenesis [113]. IFN-β reduces the inflammatory response that is mediated by immune cell infiltration into the brain and is used to treat patients with multiple sclerosis [114]. In their study, Zhuang et al. [115] cloned the huIFN-β sequence in an expression system (NICE) for expression in *L. lactis*. The recombinant strains were able to secrete huIFN-β [115]. The ability of *L. lactis* to express IFN-β opens new doors for its use in the treatment of diabetes and multiple sclerosis.

Type II IFN or IFN-γ is best known as a critical cytokine secreted during activated NK- and T-cell responses. IFN-γ is a protein encoded by the IFNG gene [106]. It is a pleiotropic cytokine with antiviral, antitumor, and immunomodulatory functions, such as enhancing antigen presentation for improved recognition via T-cell interaction, increasing the production of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNIs) [116, 117]. The presence of IFN-γ is essential in combating mycobacterial infections through its ability to regulate various protective functions and maintain $CD4^+$ and $CD8^+$ cell activity [118].

Using a coding sequence of IFN-γ, Rupa et al. [119] provided a tool to analyze the predisposition of pigs to food allergy. They used *L. lactis* as a delivery system for a cloned sequence of porcine IFN-γ conjugated to the *usp45* secretion signal. The authors evaluated the biological activity of rpIFN-γ through bioassays that determine the positive regulatory capacity of MHC II expression increased in 3D4/31 cells. These data suggest that recombinant *L. lactis* may enhance the type 1 immune response , which in turn may reduce susceptibility to allergy [119].

IL-1Ra

The interleukin-1 (IL-1) cytokine and receptor family is unique because of its shared similarity to the toll-like receptor (TLR) families, including similar functions. More than any other cytokine family, the IL-1 family has emerged as a key cytokine involved in innate and adaptive immunity [120]. There are 11 members of the IL-1 family of cytokines (IL-1α, IL-1β, IL-18, IL-33, IL-36α, IL-36β, and IL-36γ) and 10 members of the IL-1 family of receptors [121, 122]. IL-1R1 is the major receptor for IL-1a and IL-1b ligands, being expressed by various cell types, including innate and adaptive immune cell types, epithelial cells, endothelial cells, adipocytes, chondrocytes, and fibroblasts [123].

Namai et al. [124] used a nisin-controlled expression vector (NICE) with the sequence encoding mIL-1RA, an IL-1 receptor antagonist (pNZ8148#2:SEC-IL1Ra), to form a recombinant *L. lactis* strain (gmLAB). The authors tested the expression of IL-1RA in a DSS-induced colitis model by oral administration. gmLAB suppressed weight loss and exacerbation of the disease index. In mice with acute colitis, oral administration of the recombinant strain reduced the expression of IL-17. In EL4 cells, NOB-1 rmIL-1Ra produced by gmLAB played an antagonist role by suppressing the expression of IL-1[124].

IL-18

IL-18 is a pro-inflammatory cytokine of the IL-1 family produced by immune cells, such as macrophages, Langerhans cells, DCs, and many non-immune cells, such as osteoblasts, chondrocytes, endothelial cells, keratinocytes, and intestinal epithelial cells [125, 126]. IL-18 is involved in the activation and differentiation of several T-cell populations and joins with IL-12 in the Th1 lymphocyte paradigm characterized by a predominant IFN-γ production by T and B cells and NK cells [127, 128]. The post-translational regulation of IL-18 is similar to that IL-1β. Both cytokines are produced as inactive pro-forms require procssing to become biologically active [129].

Based in the ability of *L. lactis* to secrete biologically active cytokines, Feizollahzadeh et al. [130] cloned

a sequence of mIL-18 to enhance the immune response, coupled to a nisin and *lacF* promoter in the strain NZ3900, which is unable to utilize lactose. For the detection of secreted mIL-18, Western blotting was performed to evaluate the biological activity of IL-18, and the authors used mouse splenic T cells and determined the increased secretion of IFN- γ. The results indicated the generation of a novel recombinant strain of *L. lactis* expressing biologically active mIL-18 [130]. This IL-18-producing strain can be tested in models of inflammatory bowel disease and microbial infections to enhance host innate immunity and potentiate treatment strategies.

IL-17

IL-17 is a versatile pro-inflammatory cytokine that is critical for a variety of processes, including host defense, tissue repair, the pathogenesis of the inflammatory disease, psoriasis, and cancer progression [131, 132]. The IL-17 family consists of six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F [133].

The IL-17 family shows broad heterogeneity of function in different inflammatory contexts. IL-17B is upregulated during intestinal inflammation and promotes neutrophil migration after intraperitoneal administration, suggesting a pro-inflammatory role. However, the same cytokine has anti-inflammatory functions by blocking IL-25 signaling during mucosal inflammation [134, 135]. IL-17A-producing TH17 cells protect the integrity of the intestinal mucosa and stimulate the local maturation of IgA-producing plasma cells, but IL-17A has a relevant pathogenic role in several diseases where there is an alteration of the microbiota (dysbiosis), such as obesity, type 2 diabetes, Crohn's disease, rheumatoid arthritis, multiple sclerosis, and systemic lupus erythematosus [136, 137].

IL-17A has been implicated in the immunopathology of several inflammatory diseases. IL-17A signaling in target cells bearing IL-17 receptors, including fibroblasts, epithelial cells, and synovitis, results in the transcription of pro-inflammatory cytokines (IL-6, TNF, and IL-1). In addition, IL-17A increases the production and secretion of granulocyte colony-stimulating factor (G-CSF) and granulocyte–macrophage colony-stimulating factor (GM-CSF), macrophages, and T cells [136, 138].

In addition to its antiviral responses, IL-17 also has a proeminet role in promoting viral infection. Lahiri et al. [139] investigated chicken IL-17A-mediated antiviral immune effects on avian influenza virus (AIV) infection in primary chicken embryo fibroblast cells (CEFs). The authors used a strain of *L. lactis* secreting bioactive recombinant chicken IL-17A. This activity was confirmed by transcriptional upregulation of several genes associated with antiviral host responses, demonstrating that pretreatment of primary CEFs cells with sChIL-17A prior to influenza virus infection induces a pro-inflammatory state and protects the cells from viral infection [139].

To understand IL-17A in cancer disease, it was constructed a recombinant *L. lactis* producing this cytokine in a stress-inducible vector (pSICE) and determined its biological activity in a bioassay test in a murine fibroblasts 3T3 L1 cell line and a murine model of human papilloma virus (HPV)-induced cancer. This recombinant *L. lactis* was able to expresss a biologically active IL-17A under stress. On average, 26% of treated mice did not develop the tumors. These results demonstrate that administration of a genetically engineered strain of *L. lactis* secreting IL-17 results in partial protection against tumors in mice [96].

Production of Cytokines Using Food-Grade Systems

The broad applications of cytokine production and delivery are only possible because of the genetic modifications and molecular tools available. However, only a few recombinant *L. lactis* have been approved for human clinical trials [140]. Genetically modified bacteria are considered under the safety regulations for genetically modified organisms (GMOs) by regulatory agencies to prevent adverse effects on human health and the environment [141].

Many of the expression systems developed have antibiotic resistance genes (ARGs) as selective markers and are among the major concerns because the possibility of transfer ARGs to microbial communities of the host microbiota or the environment. To overcome these concerns, the use of food-grade systems is an alternative strategy that can be based on auxotrophy, complementary markers, or the development of biocontainment strategies [8, 142]. In a pioneering study, the sequence of murine *Il10* was integrated into the bacterial genome, replacing the *thya* gene of *Lactococcus lactis* which is essential for bacterial growth. This promoted a biocontainment system in which the recombinant strain *L. lactis* $thyA^{-}hIL10^{+}$ is depends on thymine for its growth, preventing this GMO from spreading into the environment. This recombinant strain was tested in a DSS-induced colitis model, causing a 50% reduction in colitis with less inflammation and also preventing the onset of colitis in $IL10^{-/-}$ mice [143]. Following these results, the authors integrated the human *Il10* sequence into the bacterial genome, replacing the *thya* gene of *Lactococcus lactis* and tested this strain in patients with Crohn's disease. The results of this first phase I clinical trial showed positive effects on the use of modified strains in terms of containment, tolerance, and also safety [94, 144]. In addition, the company ActoGenix, which is specializedcialized in the development of LAB strains genetically engineered to secret therapeutic

molecules, conducted tests in people with ulcerative colitis. The results of this study confirmed that the system is adequate for biological containment applied in humans, but there were no significant clinical effects.

Conclusions and Future Prospects

Lactic Acid Bacteria, including *Lactococcus lactis*, *Lactococcus lactis* have been widely used in the dairy industry. Currently, the biotechnological tools developed to the harbor and express therapeutic biomolecules by *Lactococcus lactis* represent a major advance in bioengineering, providing efficient expression systems that allow therapeutic applications with promising results in several disease models. The use of *L. lactis* for delivery of cytokines is an interesting approach as it can provide a more straightforward therapeuticl target, focusing on pathways of the immune system that could be disease-specific, seeking immunomodulation towards healing or protection of the organism. As we have described in this review, the construction of recombinant LAB strains that secrete or deliver recombinant cytokines has been widely reported in the literature; however, few disease models have been tested and even fewer have reached the clinical trial phase, demonstrating that this field of researchis only beginning to be explored. In addition, the use of recombinant LAB in future clinical trials requires a risk assessment that takes safety guidelines for the use of genetically modified organisms provided by regulatory agencies around the world, which are essential for understanding and establishing new therapies. Therefore, future works should focus on ensuring the safety of LAB-producing therapeutic molecules, the long-term effect of these recombinant systems on different diseases, and expanding the applications while deepening the understanding of signaling molecules in each disease.

Author Contribution Conceptualization: Vasco Azevedo, Gabriela Munis Campos; writing—original draft preparation: Gabriela Munis Campos; writing—review and editing: Monique Ferrary Americo; Andria dos Santos Freitas, Fernanda Alvarenga Lima Barroso; Joyce da Cruz Ferraz Dutra, Ludmila Silva Quaresma; Bárbara Fernandes Cordeiro; Juliana Guimarães Laguna; Luís Cláudio Lima de Jesus; Aparecida Maria Fontes; Alexander Birbrair; Tulio Marcos Santos and Vasco Azevedo; supervision: Vasco Azevedo; funding acquisition: Vasco Azevedo. All authors read and approved the final manuscript.

Funding Financial support and fellowships were provided by the Pró-Reitoria de Pesquisa — Universidade Federal de Minas Gerais, Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq), Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES), and Fundação de Amparo à Pesquisa do Estado de Minas Gerais (FAPEMIG).

Declarations

Consent for Publication All authors consent to the publication of this article.

Conflicts of Interest The authors declare no competing interests.

References

1. Tavares LM, de Jesus LCL, da Silva TF et al (2020) Novel strategies for efficient production and delivery of live biotherapeutics and biotechnological uses of *Lactococcus lactis*: the lactic acid bacterium model. Front Bioeng Biotechnol 8:1269. https://doi.org/10.3389/fbioe.2020.517166
2. Cano-Garrido O, Seras-Franzoso J, Garcia-Fruitós E (2015) Lactic acid bacteria: reviewing the potential of a promising delivery live vector for biomedical purposes. Microb Cell Factories 14:1–12. https://doi.org/10.1186/S12934-015-0313-6
3. Stiles ME, Holzapfel WH (1997) Lactic acid bacteria of foods and their current taxonomy. Int J Food Microbiol 36:1–29. https://doi.org/10.1016/S0168-1605(96)01233-0
4. Zheng J, Wittouck S, Salvetti E et al (2020) A taxonomic note on the genus *Lactobacillus*: Description of 23 novel genera, emended description of the genus Lactobacillus beijerinck 1901, and union of Lactobacillaceae and Leuconostocaceae. Int J Syst Evol Microbiol 70:2782–2858. https://doi.org/10.1099/IJSEM.0.004107
5. Khelissa S, Chihib NE, Gharsallaoui A (2020) Conditions of nisin production by *Lactococcus lactis* subsp. *lactis* and its main uses as a food preservative. Arch Microbiol 203:465–480. https://doi.org/10.1007/S00203-020-02054-Z
6. Song AAL, In LLA, Lim SHE, Rahim RA (2017) A review on *Lactococcus lactis*: from food to factory. Microb Cell Fact 16. https://doi.org/10.1186/S12934-017-0669-X
7. Kleerebezem M, Bachmann H, van Pelt-KleinJan E et al (2020) Lifestyle, metabolism and environmental adaptation in *Lactococcus lactis*. FEMS Microbiol Rev 44:804–820. https://doi.org/10.1093/femsre/fuaa033
8. Plavec TV, Berlec A (2020) Safety aspects of genetically modified lactic acid bacteria. Microorganisms 8:297. https://doi.org/10.3390/microorganisms8020297
9. Leblanc De Moreno De, A, Del Carmen S, Chatel JM et al (2015) Current review of genetically modified lactic acid bacteria for the prevention and treatment of colitis using murine models. Gastroenterol Res Pract
10. Cortes-Perez NG, de LeBlanc A, de M, Gomez-Gutierrez JG et al (2021) Probiotics and trained immunity. Biomolecules 11. https://doi.org/10.3390/BIOM11101402
11. Hill C, Guarner F, Reid G et al (2014) The International Scientific Association for Probiotics and Prebiotics consensus statement on the scope and appropriate use of the term probiotic. Nat Rev Gastroenterol Hepatol 11:506–514. https://doi.org/10.1038/nrgastro.2014.66
12. Bungau SG, Behl T, Singh A et al (2021) Targeting probiotics in rheumatoid arthritis. Nutrients 13. https://doi.org/10.3390/nu13103376
13. Gomes AC, Bueno AA, De Souza RGMH, Mota JF (2014) Gut microbiota, probiotics and diabetes. Nutr J 13:1–13. https://doi.org/10.1186/1475-2891-13-60
14. Suzuki T, Nishiyama K, Kawata K et al (2020) Effect of the *Lactococcus Lactis* 11/19-B1 Strain on atopic dermatitis in a clinical test and mouse model. Nutrients 12. https://doi.org/10.3390/nu12030763

15. Kim S, Kim Y, Lee S et al (2022) Live biotherapeutic *Lactococcus lactis* GEN3013 enhances antitumor efficacy of cancer treatment via modulation of cancer progression and immune system. Cancers (Basel) 14:4083. https://doi.org/10.3390/cancers14174083
16. de Castro CP, Drumond MM, Batista VL et al (2018) Vector development timeline for mucosal vaccination and treatment of disease using *Lactococcus lactis* and design approaches of next generation food grade plasmids. Front Microbiol 9:1805. https://doi.org/10.3389/fmicb.2018.01805
17. Berlec A, Škrlec K, Kocjan J et al (2018) Single plasmid systems for inducible dual protein expression and for CRISPR-Cas9/CRISPRi gene regulation in lactic acid bacterium *Lactococcus lactis*. Sci Rep 8:1009. https://doi.org/10.1038/s41598-018-19402-1
18. Cook DP, Gysemans C, Mathieu C (2018) *Lactococcus lactis* as a versatile vehicle for tolerogenic immunotherapy. Front Immunol 8. https://doi.org/10.3389/fimmu.2017.01961
19. Shende P, Basarkar V (2019) Recent trends and advances in microbe-based drug delivery systems. Daru 27:799–809. https://doi.org/10.1007/S40199-019-00291-2
20. Azizpour M, Hosseini SD, Jafari P, Akbary N (2017) *Lactococcus lactis* : a new strategy for vaccination. Avicenna J Med Biotechnol 9:163
21. Kuipers OP, Beerthuyzen MM, De Ruyter PGGA et al (1995) Autoregulation of nisin biosynthesis in *Lactococcus lactis* by signal transduction. J Biol Chem 270:27299–27304. https://doi.org/10.1074/jbc.270.45.27299
22. Desmond C, Fitzgerald GF, Stanton C, Ross RP (2004) Improved stress tolerance of GroESL-overproducing *Lactococcus lactis* and probiotic *Lactobacillus paracasei* NFBC 338. Appl Environ Microbiol 70:5929–5936. https://doi.org/10.1128/AEM.70.10.5929-5936.2004
23. Benbouziane B, Ribelles P, Aubry C et al (2013) Development of a stress-inducible controlled expression (SICE) system in *Lactococcus lactis* for the production and delivery of therapeutic molecules at mucosal surfaces. J Biotechnol 168:120–129. https://doi.org/10.1016/j.jbiotec.2013.04.019
24. Miyoshi A, Jamet E, Commissaire J et al (2004) A xylose-inducible expression system for *Lactococcus lactis*. FEMS Microbiol Lett 239:205–212. https://doi.org/10.1016/j.femsle.2004.08.018
25. Guimarães V, Innocentin S, Chatel JM et al (2009) A new plasmid vector for DNA delivery using lactococci. Genet Vaccines Ther 7:1–7. https://doi.org/10.1186/1479-0556-7-4
26. Mancha-Agresti P, Drumond MM, Carmo FLR, do, et al (2017) A new broad range plasmid for DNA delivery in eukaryotic cells using lactic acid bacteria: in vitro and in vivo assays. Mol Ther - Methods Clin Dev 4:83–91. https://doi.org/10.1016/j.omtm.2016.12.005
27. Barroso FAL, de Jesus LCL, de Castro CP et al (2021) Intake of *Lactobacillus delbrueckii* (pExu: hsp65) prevents the inflammation and the disorganization of the intestinal mucosa in a mouse model of mucositis. Microorganisms 9:1–27. https://doi.org/10.3390/microorganisms9010107
28. Saraiva M, O'Garra A (2010) The regulation of IL-10 production by immune cells. Nat Rev Immunol 10:170–181. https://doi.org/10.1038/nri2711
29. Rutz S, Ouyang W (2016) Regulation of interleukin-10 expression. Adv Exp Med Biol 941:89–116. https://doi.org/10.1007/978-94-024-0921-5_5
30. Wang X, Wong K, Ouyang W, Rutz S (2019) Targeting IL-10 family cytokines for the treatment of human diseases. Cold Spring Harb Perspect Biol 11. https://doi.org/10.1101/cshperspect.a028548
31. Ouyang W, Rutz S, Crellin NK et al (2011) Regulation and functions of the IL-10 family of cytokines in inflammation and disease. Annu Rev Immunol 29:71–109. https://doi.org/10.1146/annurev-immunol-031210-101312
32. Zurita-Turk M, del Carmen S, Santos ACG et al (2014) *Lactococcus lactis* carrying the pValac DNA expression vector coding for IL-10 reduces inflammation in a murine model of experimental colitis. BMC Biotechnol 14:1–11. https://doi.org/10.1186/1472-6750-14-73
33. Del Carmen S, Rosique RM, Saraiva T et al (2014) Protective effects of lactococci strains delivering either IL-10 protein or cDNA in a TNBS-induced chronic colitis model. J Clin Gastroenterol 48:S12–S17. https://doi.org/10.1097/mcg.0000000000000235
34. Nouaille S, Ribeiro LA, Miyoshi A et al (2003) Heterologous protein production and delivery systems for *Lactococcus lactis*. Genet Mol Res 2:102–111
35. Bermúdez-Humarán LG, Langella P, Cortes-Perez NG et al (2003) Intranasal immunization with recombinant *Lactococcus lactis* secreting murine interleukin-12 enhances antigen-specific Th1 cytokine production. Infect Immun 71:1887–1896. https://doi.org/10.1128/iai.71.4.1887-1896.2003
36. Alizadeh P, Ahmadpour E, Daryani A et al (2019) IL-17 and IL-22 elicited by a DNA vaccine encoding ROP13 associated with protection against *Toxoplasma gondii* in BALB/c mice. J Cell Physiol 234:10782–10788. https://doi.org/10.1002/jcp.27747
37. Wu C, Yang G, Bermúdez-Humarán LG et al (2006) Immunomodulatory effects of IL-12 secreted by *Lactococcus lactis* on Th1/Th2 balance in ovalbumin (OVA)-induced asthma model mice. Int Immunopharmacol 6:610–615. https://doi.org/10.1016/j.intimp.2005.09.010
38. Ouyang W, O'Garra A (2019) IL-10 family cytokines IL-10 and IL-22: from basic science to clinical translation. Immunity 50:871–891. https://doi.org/10.1016/j.immuni.2019.03.020
39. Bedke T, Muscate F, Soukou S, et al (2019) Title: IL-10-producing T cells and their dual functions. Semin Immunol 44. https://doi.org/10.1016/j.smim.2019.101335
40. Neumann C, Scheffold A, Rutz S (2019) Functions and regulation of T cell-derived interleukin-10. Semin Immunol 44. https://doi.org/10.1016/j.smim.2019.101344
41. Schotte L, Steidler L, Vandekerckhove J, Remaut E (2000) Secretion of biologically active murine interleukin-10 by *Lactococcus lactis*. Enzyme Microb Technol 27:761–765. https://doi.org/10.1016/S0141-0229(00)00297-0
42. Martín R, Martín R, Chain F et al (2014) Effects in the use of a genetically engineered strain of *Lactococcus lactis* delivering in situ IL-10 as a therapy to treat low-grade colon inflammation. Hum Vaccin Immunother 10:1611–1621. https://doi.org/10.4161/hv.28549
43. Del Carmen S, Zurita-turk M, Alvarenga Lima F et al (2013) A novel interleukin-io dna mucosal delivery system attenuates intestinal inflammation in a mouse model. Eur J Inflamm 11(3):641–654. https://doi.org/10.1177/1721727X1301100308
44. Zurita-Turk M, Mendes Souza B, Prósperi De Castro C et al (2020) Attenuation of intestinal inflammation in IL-10 deficient mice by a plasmid carrying *Lactococcus lactis* strain. BMC Biotechnol 20:1–12. https://doi.org/10.1186/s12896-020-00631-0
45. Bermúdez-Humarán LG, Motta JP, Aubry C et al (2015) Serine protease inhibitors protect better than IL-10 and TGF-β anti-inflammatory cytokines against mouse colitis when delivered by recombinant lactococci. Microb Cell Fact 14:1–11. https://doi.org/10.1186/s12934-015-0198-4
46. Fedorak RN, Gangl A, Elson CO et al (2000) Recombinant human interleukin 10 in the treatment of patients with mild to moderately active Crohn's disease. Gastroenterology 119:1473–1482. https://doi.org/10.1053/gast.2000.20229
47. Takiishi T, Korf H, Van Belle TL et al (2013) Reversal of autoimmune diabetes by restoration of antigen-specific tolerance using genetically modified *Lactococcus lactis* in mice. Diabetes Technol Ther 15:1717–1725. https://doi.org/10.1089/dia.2013.1510

48. Robert S, Gysemans C, Takiishi T et al (2014) Oral delivery of glutamic acid decarboxylase (GAD)-65 and IL10 by *Lactococcus lactis* reverses diabetes in recent-onset NOD mice. Diabetes 63:2876–2887. https://doi.org/10.2337/db13-1236
49. Frossard CP, Steidler L, Eigenmann PA (2007) Oral administration of an IL-10-secreting *Lactococcus lactis* strain prevents food-induced IgE sensitization. J Allergy Clin Immunol 119:952–959. https://doi.org/10.1016/j.jaci.2006.12.615
50. Marinho FAVV, Pacífico LGGG, Miyoshi A et al (2010) An intranasal administration of *Lactococcus lactis* strains expressing recombinant interleukin-10 modulates acute allergic airway inflammation in a murine model. Clin Exp Allergy 40:1541–1551. https://doi.org/10.1111/j.1365-2222.2010.03502.x
51. Keir ME, Yi T, Lu TT, Ghilardi N (2020) The role of IL-22 in intestinal health and disease. J Exp Med 217. https://doi.org/10.1084/jem.20192195
52. Sabihi M, Böttcher M, Pelczar P, Huber S (2020) Microbiota-dependent effects of IL-22. Cells 9. https://doi.org/10.3390/cells9102205
53. Wei HX, Wang B, Li B (2020) IL-10 and IL-22 in mucosal immunity: driving protection and pathology. Front Immunol 11:1315. https://doi.org/10.3389/fimmu.2020.01315
54. Mizoguchi A, Yano A, Himuro H et al (2018) Clinical importance of IL-22 cascade in IBD. J Gastroenterol 53:465–474. https://doi.org/10.1007/s00535-017-1401-7
55. Kidess E, Kleerebezem M, Brugman S (2021) Colonizing microbes, IL-10 and IL-22: keeping the peace at the mucosal surface. Front Microbiol 12. https://doi.org/10.3389/fmicb.2021.729053
56. Che Y, Su Z, Xia L (2020) Effects of IL-22 on cardiovascular diseases. Int Immunopharmacol 81. https://doi.org/10.1016/j.intimp.2020.106277
57. Loera-Arias MJ, Villatoro-Hernández J, Parga-Castillo MA et al (2014) Secretion of biologically active human interleukin 22 (IL-22) by *Lactococcus lactis*. Biotechnol Lett 36:2489–2494. https://doi.org/10.1007/s10529-014-1626-y
58. Maëva M, Elodie B, Nathalie R, et al (2022) AhR/IL-22 pathway as new target for the treatment of post-infectious irritable bowel syndrome symptoms. Gut Microbes 14. https://doi.org/10.1080/19490976.2021.2022997
59. Iwaszko M, Biały S, Bogunia-Kubik K (2021) Significance of interleukin (IL)-4 and IL-13 in inflammatory arthritis. Cells 10. https://doi.org/10.3390/CELLS10113000
60. Ho IC, Miaw SC (2016) Regulation of IL-4 expression in immunity and diseases. Adv Exp Med Biol 941:31–77. https://doi.org/10.1007/978-94-024-0921-5_3
61. Shi J, Song X, Traub B et al (2021) Involvement of IL-4, IL-13 and their receptors in pancreatic cancer. Int J Mol Sci 22:1–16. https://doi.org/10.3390/ijms22062998
62. Preisser TM, Da Cunha VP, Santana MP, et al (2021) Recombinant *Lactococcus lactis* carrying IL-4 and IL-10 coding vectors protects against type 1 diabetes in NOD mice and attenuates insulitis in the STZ-induced model. J Diabetes Res. https://doi.org/10.1155/2021/6697319
63. Papaccio G, Pisanti FA, Di MR et al (2002) Th1 and Th2 cytokines exert regulatory effects upon islet microvascular areas in the NOD mouse. J Cell Biochem 86:651–664. https://doi.org/10.1002/jcb.10250
64. Ukah TK, Cattin-Roy AN, Chen W et al (2017) On the role IL-4/IL-13 heteroreceptor plays in regulation of type 1 diabetes. J Immunol 199:894–902. https://doi.org/10.4049/jimmunol.1700410
65. Hoving JC, Keeton R, Höft MA et al (2020) IL-4 receptor-alpha signalling of intestinal epithelial cells, smooth muscle cells, and macrophages plays a redundant role in oxazolone colitis. Mediators Inflamm. https://doi.org/10.1155/2020/4361043
66. Souza BM, Preisser TM, Pereira VB et al (2016) *Lactococcus lactis* carrying the pValac eukaryotic expression vector coding for IL-4 reduces chemically-induced intestinal inflammation by increasing the levels of IL-10-producing regulatory cells. Microb Cell Fact 15:1–18. https://doi.org/10.1186/S12934-016-0548-x
67. Abbas AK, Trotta E, Simeonov DR et al (2018) Revisiting IL-2: biology and therapeutic prospects. Sci Immunol 3. https://doi.org/10.1126/sciimmunol.aat1482
68. Spolski R, Li P, Leonard WJ (2018) Biology and regulation of IL-2: from molecular mechanisms to human therapy. Nat Rev Immunol 18:648–659. https://doi.org/10.1038/s41577-018-0046-y
69. Wrangle JM, Patterson A, Johnson CB et al (2018) IL-2 and Beyond in Cancer Immunotherapy 38. https://doi.org/10.1089/jir.2017.0101
70. Yang Y, Lundqvist A (2020) Immunomodulatory effects of IL-2 and IL-15; implications for cancer immunotherapy. Cancers (Basel) 12:1–20. https://doi.org/10.3390/cancers12123586
71. Steidler L, Wells JM, Raeymaekers A et al (1995) Secretion of biologically active murine interleukin-2 by *Lactococcus lactis* subsp. *lactis*. Appl Environ Microbiol 61:1627–1629. https://doi.org/10.1128/AEM.61.4.1627-1629.1995
72. Fernández A, Rodríguez JM, Bongaerts RJ et al (2007) Nisin-controlled extracellular production of interleukin-2 in *Lactococcus lactis* strains, without the requirement for a signal peptide sequence. Appl Environ Microbiol 73:7781–7784. https://doi.org/10.1128/aem.01247-07
73. Steidler L, Robinson K, Chamberlain L et al (1998) Mucosal delivery of murine interleukin-2 (IL-2) and IL-6 by recombinant of *Lactococcus lactis* coexpressing antigen and cytokine. Infect Immun 66:3183–3189. https://doi.org/10.1128/iai.66.7.3183-3189.1998
74. Rezaei M, Khorasgani MR, Esfahani SHZ et al (2019) Production of Brucella melitensis Omp16 protein fused to the human interleukin 2 in Lactococcus lactis MG1363 toward developing a Lactococcus-based vaccine against brucellosis. Can J Microbiol 66(1):39–45. https://doi.org/10.1139/cjm-2019-0261
75. Åvall-Jääskeläinen S, Palva A (2006) Secretion of biologically active porcine interleukin-2 by *Lactococcus lactis*. Vet Microbiol 115:278–283. https://doi.org/10.1016/j.vetmic.2006.02.007
76. Tanaka T, Narazaki M, Kishimoto T (2018) Interleukin (IL-6) Immunotherapy. Cold Spring Harb Perspect Biol 10:a028456. https://doi.org/10.1101/cshperspect.a028456
77. Hirano T (2021) IL-6 in inflammation, autoimmunity and cancer. Int Immunol 33:127–148. https://doi.org/10.1093/intimm/dxaa078
78. Murakami M, Kamimura D, Hirano T (2019) Pleiotropy and specificity: insights from the interleukin 6 family of cytokines. Immunity 50:812–831. https://doi.org/10.1016/j.immuni.2019.03.027
79. Jones SA, Jenkins BJ (2018) Recent insights into targeting the IL-6 cytokine family in inflammatory diseases and cancer. Nat Rev Immunol 18:773–789. https://doi.org/10.1038/S41577-018-0066-7
80. Choy EH, De Benedetti F, Takeuchi T et al (2020) Translating IL-6 biology into effective treatments. Nat Rev Rheumatol 16:335–345. https://doi.org/10.1038/s41584-020-0419-z
81. Li HS, Piao DC, Jiang T et al (2015) Recombinant interleukin 6 with M cell-targeting moiety produced in *Lactococcus lactis* IL1403 as a potent mucosal adjuvant for peroral immunization. Vaccine 33:1959–1967. https://doi.org/10.1016/j.vaccine.2015.02.061
82. Moschen AR, Tilg H, Raine T (2018) IL-12, IL-23 and IL-17 in IBD: immunobiology and therapeutic targeting. Nat Rev Gastroenterol Hepatol 16:185–196. https://doi.org/10.1038/s41575-018-0084-8

83. Mirlekar B, Pylayeva-Gupta Y (2021) IL-12 Family cytokines in cancer and immunotherapy. Cancers 13:167. https://doi.org/10.3390/cancers13020167
84. Nguyen KG, Vrabel MR, Mantooth SM et al (2020) Localized interleukin-12 for cancer immunotherapy. Front Immunol 11. https://doi.org/10.3389/fimmu.2020.575597
85. Cortes-Perez NG, Ah-Leung S, Bermúdez-Humarán LG et al (2007) Intranasal coadministration of live lactococci producing interleukin-12 and a major cow's milk allergen inhibits allergic reaction in mice. Clin Vaccine Immunol 14:226–233. https://doi.org/10.1128/CVI.00299-06
86. Fernandez A, Horn N, Wegmann U et al (2009) Enhanced secretion of biologically active murine interleukin-12 by *Lactococcus lactis*. Appl Environ Microbiol 75:869–871. https://doi.org/10.1128/aem.01728-08
87. Shahi A, Afzali S, Salehi S et al (2020) IL-27 and autoimmune rheumatologic diseases: the good, the bad, and the ugly. Int Immunopharmacol 84:106538. https://doi.org/10.1016/j.intimp.2020.106538
88. Mei Y, Lv Z, Xiong L et al (2021) The dual role of IL-27 in CD4+T cells. Mol Immunol 138:172–180. https://doi.org/10.1016/j.molimm.2021.08.001
89. Povroznik JM, Robinson CM (2020) IL-27 regulation of innate immunity and control of microbial growth. Futur Sci OA 6. https://doi.org/10.2144/fsoa-2020-0032
90. Qiu SL, Duan MC, Liang Y et al (2016) Cigarette smoke induction of interleukin-27/WSX-1 regulates the differentiation of Th1 and Th17 cells in a smoking mouse model of emphysema. Front Immunol 7:553. https://doi.org/10.3389/fimmu.2016.00553
91. Liu X, Li S, Jin J et al (2019) Preventative tracheal administration of interleukin-27 attenuates allergic asthma by improving the lung Th1 microenvironment. J Cell Physiol 234:6642–6653. https://doi.org/10.1002/jcp.27422
92. Hanson ML, Hixon JA, Li W et al (2014) Oral delivery of IL-27 recombinant bacteria attenuates immune colitis in mice. Gastroenterology 146. https://doi.org/10.1053/j.gastro.2013.09.060
93. Cook DP, Cunha JPMCM, Martens PJ et al (2020) Intestinal delivery of proinsulin and IL-10 via *Lactococcus lactis* combined with low-dose anti-CD3 restores tolerance outside the window of acute type 1 diabetes diagnosis. Front Immunol 11:1103. https://doi.org/10.3389/fimmu.2020.01103
94. Braat H, Rottiers P, Hommes DW et al (2006) A phase I trial with transgenic bacteria expressing interleukin-10 in Crohn's disease. Clin Gastroenterol Hepatol 4:754–759. https://doi.org/10.1016/j.cgh.2006.03.028
95. del Casado Muñoz M, C, Benomar N, Lerma LL, et al (2014) Antibiotic resistance of *Lactobacillus pentosus* and *Leuconostoc pseudomesenteroides* isolated from naturally-fermented Aloreña table olives throughout fermentation process. Int J Food Microbiol 172:110–118. https://doi.org/10.1016/j.ijfoodmicro.2013.11.025
96. Jacouton E, Maravilla ET, Boucard AS et al (2019) Anti-tumoral effects of recombinant *Lactococcus lactis* strain secreting IL-17A cytokine. Front Microbiol 10:1–7. https://doi.org/10.3389/fmicb.2018.03355
97. Wang J, Tian M, Li W, Hao F (2019) Preventative delivery of IL-35 by *Lactococcus lactis* ameliorates DSS-induced colitis in mice. Appl Microbiol Biotechnol 103:7931–7941. https://doi.org/10.1007/s00253-019-10094-9
98. Maddaloni M, Kochetkova I, Hoffman C, Pascual DW (2018) Delivery of IL-35 by *Lactococcus lactis* ameliorates collagen-induced arthritis in mice. Front Immunol 9:2691. https://doi.org/10.3389/fimmu.2018.02691
99. Sakkas LI, Mavropoulos A, Perricone C, Bogdanos DP (2018) IL-35: a new immunomodulator in autoimmune rheumatic diseases. Immunol Res 66:305–312. https://doi.org/10.1007/S12026-018-8998-3
100. Liu K, Huang A, Nie J et al (2021) IL-35 regulates the function of immune cells in tumor microenvironment. Front Immunol 12:2004. https://doi.org/10.3389/FIMMU.2021.683332
101. Li Y, Yao L, Liu S et al (2019) Correlation between Serum IL-35 Levels and Bone Loss in Postmenopausal Women with Rheumatoid Arthritis. Mediators Inflamm 2019:9139145. https://doi.org/10.1155/2019/9139145
102. Badihian S, Shaygannejad V, Soleimani P et al (2018) Decreased serum levels of interleukin-35 among multiple sclerosis patients may be related to disease progression. J Biol Regul Homeost Agents 32:1249–1253
103. Ye Z, Jiang Y, Sun D et al (2019) The Plasma Interleukin (IL)-35 Level and frequency of circulating IL-35+ regulatory B cells are decreased in a cohort of chinese patients with new-onset systemic lupus erythematosus. Sci Reports 9:1–12. https://doi.org/10.1038/s41598-019-49748-z
104. Su F, Berry K, Ioannou GN (2021) No difference in hepatocellular carcinoma risk between chronic hepatitis B patients treated with entecavir versus tenofovir. Gut 70:370–378. https://doi.org/10.1136/GUTJNL-2019-319867
105. Wang Y, Mao Y, Zhang J et al (2018) IL-35 recombinant protein reverses inflammatory bowel disease and psoriasis through regulation of inflammatory cytokines and immune cells. J Cell Mol Med 22:1014–1025. https://doi.org/10.1111/JCMM.13428
106. Negishi H, Taniguchi T, Yanai H (2018) The interferon (IFN) class of cytokines and the IFN regulatory factor (IRF) transcription factor family. Cold Spring Harb Perspect Biol 10. https://doi.org/10.1101/cshperspect.a028423
107. Li G, Fan Y, Lai Y et al (2020) Coronavirus infections and immune responses. J Med Virol 92:424–432. https://doi.org/10.1002/JMV.25685
108. De Ceuninck F, Duguet F, Aussy A et al (2021) IFN-α: a key therapeutic target for multiple autoimmune rheumatic diseases. Drug Discov Today 26:2465–2473. https://doi.org/10.1016/j.drudis.2021.06.010
109. Hoffmann HH, Schneider WM, Rice CM (2015) Interferons and viruses: an evolutionary arms race of molecular interactions. Trends Immunol 36:124–138. https://doi.org/10.1016/J.IT.2015.01.004
110. Bayat O, Baradaran A, Ariff A et al (2014) Intracellular production of IFN-alpha 2b in *Lactococcus lactis*. Biotechnol Lett 36:581–585. https://doi.org/10.1007/s10529-013-1390-4
111. Zhang Q, Zhong J, Liang X et al (2010) Improvement of human interferon alpha secretion by *Lactococcus lactis*. Biotechnol Lett 32:1271–1277. https://doi.org/10.1007/s10529-010-0285-x
112. Sabir N, Hussain T, Shah SZA et al (2017) IFN-β: A contentious player in host–pathogen interaction in tuberculosis. Int J Mol Sci 18:2725. https://doi.org/10.3390/IJMS18122725
113. Li Y, Sun F, Yue TT et al (2021) Revisiting the antigen-presenting function of β cells in T1D pathogenesis. Front Immunol 12:2848. https://doi.org/10.3389/FIMMU.2021.690783
114. Tresse E, Riera-Ponsati L, Jaberi E, et al (2021) IFN-β rescues neurodegeneration by regulating mitochondrial fission via STAT5, PGAM5, and Drp1. EMBO J 40:e106868. https://doi.org/10.15252/EMBJ.2020106868
115. Zhuang Z, Wu ZG, Chen M, Wang PG (2008) Secretion of human interferon-β 1b by recombinant *Lactococcus lactis*. Biotechnol Lett 30:1819–1823. https://doi.org/10.1007/s10529-008-9761-y
116. Jorgovanovic D, Song M, Wang L, Zhang Y (2020) Roles of IFN-γ in tumor progression and regression: a review. Biomark Res 8:1–16. https://doi.org/10.1186/S40364-020-00228-X

117. Burke JD, Young HA (2019) IFN-γ: A cytokine at the right time, is in the right place. Semin Immunol 43:101280. https://doi.org/10.1016/J.SMIM.2019.05.002
118. Kak G, Raza M, Tiwari BK (2018) Interferon-gamma (IFN-γ): exploring its implications in infectious diseases. Biomol Concepts 9:64–79. https://doi.org/10.1515/BMC-2018-0007
119. Rupa P, Monedero V, Wilkie BN (2008) Expression of bioactive porcine interferon-gamma by recombinant *Lactococcus lactis*. Vet Microbiol 129:197–202. https://doi.org/10.1016/j.vetmic.2007.11.010
120. Mantovani A, Barajon I, Garlanda C (2018) IL-1 and IL-1 regulatory pathways in cancer progression and therapy. Immunol Rev 281:57–61. https://doi.org/10.1111/IMR.12614
121. Dinarello CA (2018) Overview of the IL-1 family in innate inflammation and acquired immunity. Immunol Rev 281:8–27. https://doi.org/10.1111/IMR.12621
122. Dinarello CA (2019) The IL-1 family of cytokines and receptors in rheumatic diseases. Nat Rev Rheumatol 15:612–632. https://doi.org/10.1038/s41584-019-0277-8
123. Zhang W, Borcherding N, Kolb R (2020) IL-1 signaling in tumor microenvironment. Adv Exp Med Biol 1240:1–23. https://doi.org/10.1007/978-3-030-38315-2_1
124. Namai F, Shigemori S, Ogita T et al (2020) Microbial therapeutics for acute colitis based on genetically modified *Lactococcus lactis* hypersecreting IL-1Ra in mice. Exp Mol Med 52:1627–1636. https://doi.org/10.1038/s12276-020-00507-5
125. Abbate A, Toldo S, Marchetti C et al (2020) Interleukin-1 and the inflammasome as therapeutic targets in cardiovascular disease. Circ Res 1260–1280. https://doi.org/10.1161/circresaha.120.315937
126. Nanda JD, Ho TS, Satria RD, et al (2021) IL-18: The forgotten cytokine in dengue immunopathogenesis. J Immunol Res 2021. https://doi.org/10.1155/2021/8214656
127. Vecchié A, Bonaventura A, Toldo S et al (2021) IL-18 and infections: Is there a role for targeted therapies? J Cell Physiol 236:1638–1657. https://doi.org/10.1002/JCP.30008
128. Yasuda K, Nakanishi K, Tsutsui H (2019) Interleukin-18 in health and disease. Int J Mol Sci 20. https://doi.org/10.3390/ijms20030649
129. Mühl H, Bachmann M (2019) IL-18/IL-18BP and IL-22/IL-22BP: Two interrelated couples with therapeutic potential. Cell Signal 63. https://doi.org/10.1016/j.cellsig.2019.109388
130. Feizollahzadeh S, Khanahmad H, Rahimmanesh I et al (2016) Expression of biologically active murine interleukin-18 in *Lactococcus lactis*. FEMS Microbiol Lett 363:1–6. https://doi.org/10.1093/femsle/fnw234
131. Li X, Bechara R, Zhao J et al (2019) IL-17 receptor-based signaling and implications for disease. Nat Immunol 20:1594–1602. https://doi.org/10.1038/S41590-019-0514-Y
132. Bunte K, Beikler T (2019) Th17 cells and the IL-23/IL-17 axis in the pathogenesis of periodontitis and immune-mediated inflammatory diseases. Int J Mol Sci 20. https://doi.org/10.3390/IJMS20143394
133. Gorczynski RM (2020) IL-17 Signaling in the tumor microenvironment. Adv Exp Med Biol 1240:47–58. https://doi.org/10.1007/978-3-030-38315-2_4
134. McGeachy MJ, Cua DJ, Gaffen SL (2019) The IL-17 family of cytokines in health and disease. Immunity 50:892–906. https://doi.org/10.1016/j.immuni.2019.03.021
135. Bie Q, Jin C, Zhang B, Dong H (2017) IL-17B: a new area of study in the IL-17 family. Mol Immunol 90:50–56. https://doi.org/10.1016/j.molimm.2017.07.004
136. Brevi A, Cogrossi LL, Grazia G, et al (2020) Much more than IL-17A: cytokines of the IL-17 family between microbiota and cancer. Front Immunol 11. https://doi.org/10.3389/FIMMU.2020.565470
137. Zwicky P, Unger S, Becher B (2020) Targeting interleukin-17 in chronic inflammatory disease: a clinical perspective. J Exp Med 217. https://doi.org/10.1084/JEM.20191123
138. Taams LS, Steel KJA, Srenathan U et al (2018) IL-17 in the immunopathogenesis of spondyloarthritis. Nat Rev Rheumatol 14:453–466. https://doi.org/10.1038/s41584-018-0044-2
139. Lahiri A, Bhowmick S, Sharif S, Mallick AI (2021) Pre-treatment with chicken IL-17A secreted by bioengineered LAB vector protects chicken embryo fibroblasts against Influenza Type A Virus (IAV) infection. Mol Immunol 140:106–119. https://doi.org/10.1016/j.molimm.2021.10.003
140. Berlec A, Ravnikar M, Strukelj B (2012) Lactic acid bacteria as oral delivery systems for biomolecules. Pharmazie 67:891–898. https://doi.org/10.1691/ph.2012.1705
141. Ferrary AM, Azevedo V, de Carvalho RDO (2022) Genetically modified lactic acid bacteria in food and beverages: Safety concerns for industry and clinical use. In: Lactic Acid Bacteria in Food Biotechnology. Elsevier Inc., pp 349–363. https://doi.org/10.1016/B978-0-323-89875-1.00003-1
142. Mignon C, Sodoyer R, Werle B (2015) Antibiotic-free selection in biotherapeutics: now and forever. Pathog 4:157–181. https://doi.org/10.3390/pathogens4020157
143. Steidler L, Hans W, Schotte L et al (2000) Treatment of murine colitis by *Lactococcus lactis* secreting IL-10. Science 289:1352–1355
144. Steidler L, Neirynck S, Huyghebaert N et al (2003) Biological containment of genetically modified *Lactococcus lactis* for intestinal delivery of human interleukin 10. Nat Biotechnol 21:785–789. https://doi.org/10.1038/nbt840