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Production of interferon induced by *Streptococcus thermophilus:* role of $CD4^+$ and $CD8^+$ lymphocytes

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The effects of non-pathogenic bacteria on the production of cytokines by circulating mononuclear cells from healthy subjects were studied both in vivo *and* in vitro. *Feeding a diet containing fermented dairy products, induced a significant increase of 2–5A synthetase activity in these cells suggesting a production of interferons. An* in vitro *study showed that mononuclear cells produced interferon-γ in presence of lactic bacteria like* Lactobacillus acidophilus, Streptococcus lactis, *and* Streptococcus thermophilus. S. thermophilus *induced also the production of interleukin-1β. All the studied bacteria, including* Bifidobacterium *and* Lactobacillus casei *induced the production of interleukin-6. In the presence of* S. thermophilus, *76% and 62% reduction of interferon-γ was observed when anti-CD4 co-receptor and anti-CD8 co-receptor antibodies were added in the medium, respectively. Thus, the recognition of* S. thermophilus *involved the two pathways of major histocompatibility complex, and muramyl dipeptide a component of bacterial wall could explain the part recognized by CD4+ lymphocytes.* © Elsevier Science Inc. 1997 (J. Nutr. Biochem. 8:25–31, 1997.)

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Introduction

Eating latic acid bacteria or fermented milks to healthy human or to experimental animals has been reported to stimulate several functions of the immune system such as peritoneal macrophage activity, antibody and cytokines production.[1–4] The property to increase in vitro the production of several cytokines of blood mononuclear cells (BMC) is also shown by non-pathogen bacteria from the human gut flora.[4,5] In conventional mice, BMC show a higher activity of 25A synthetase, a marker of the production of interferons (IFNs), than germ-free animals, suggesting a role of the gut flora in the production of these cytokines.[6] Interestingly, feeding 10^{11} lactic bacteria from yogurt increased the 2-5A synthetase activity of circulating mononuclear cells from healthy subjects.[7] This would indicate that eating non-pathogen bacteria is susceptible to stimulate the production of IFNs and that this production is added to that spontaneously observed in healthy subjects. In vitro, a significant production of IFN-γ, but not IFN-α by blood mononuclear cell was induced in the presence of bacteria from yogurt, this effect being due to their wall, but not to their cytoplasm.[4] The walls of gram-positive bacteria, which include the bactic bacteria, are mainly composed of peptidoglycans and polysaccharides or teichoic acids (or both).[8] Several studies[9–12] have reported data on the effect of surface components of gram-positive bacteria on the cellular release of various cytokines. Muramyl dipeptide (MDP), a common component of peptidoglycans, stimulates macrophage to release IL-1, mainly concerned with activation of T lymphocytes.[13–15] Similarly, MDP induces IFN-γ production by lymphocytes.[11] The mechanism by which surface components activates these cells have not been elucidated yet. Levy et al. have demonstrated that lipoteichoic acid isolated from *Streptococcus faecalis* and *Streptococcus pyrogenes* caused direct activation of the respiratory burst in human

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peripheral blood monocytes.[16] This activity appears to be related to the ability of lipoteichoic acid to bind to the monocyte membrane.

In this work, we have studied the effect of a regular ingestion of yogurt on the production of IFNs in healthy subjects. In in vitro studies, we have compared different species of dairy bacteria on their ability to induce the production of cytokines by human mononuclear cells. Furthermore, we attempted to explain the mechanisms whereby *Streptococcus thermophilus* induces the production of IFN-γ.

Materials and Methods

Subjects

Samples of BMC, for 2-5A synthetase activity determination were prepared from blood of eight healthy subjects [2 females and 6 males (26 ± 6 years)], who were included in a crossover protocol during two periods of 15 days each, during which they were asked not to change any of their dietary practice, except during one period to eat at least one yogurt per day and the other period not to eat any fermented milk. Samples of blood were taken at the end of each period.

The in vitro study was carried out on four healthy volunteers. All gave their informed consent. They were free of disease and took medications during the month before as well as during the study. Procedures on human subjects had been approved by the INSERM Comité National d'Ethique.

2-5A synthetase activity in BMC

Cells extracts were prepared by resuspending the BMC in 100 µL lysis buffer: 10 mM HEPES buffer pH 7.6, 10 mM KCl, 2 mM Mg(OAc)2, 4 H_2O, 7 mM 2-mercaptoethanol, with 0.5% Nonidet P-40. The suspension was incubated at 4°C for 10 min and centrifuged 10 min at 5000 × g. The cytosol was used immediately for the enzyme assay.

Cytoplasmic activity of 2-5A synthetase was determined as described previously.[17] Briefly, poly(rI):(rC)-agarose beads (Pharmacia Fine Chemicals, Uppsala, Sweden) were washed with several volumes of 5 mM Mg(OAc)2, 1 mM dithiothreitol, 25 mM KCl, 10% glycerol, 1 mM EDTA, 20 mM Tris-HCl pH 8, (buffer A), and 30 µL aliquots were placed in microtiter tubes. Aliquots (100 µL) of cytosol were mixed with the beads and incubated for 15 min at room temperature. The beads were washed three times with buffer A and all liquid carefully removed. The beads were then incubated with 10 µL reaction mixture: 25 mM Mg(OAc)2, 0.25 mg/mL bovine serum albumin, 12 µg poly(rI):(rC), 7 mM ^{32}ATP (50 mCi/mL > 15 TBq/mmol, Amersham, UK), 0.25 mg/mL creatin kinase, 0.01 M creatin phosphate, in 20 mM Tris-HCl buffer pH 8 for 2 h at 27°C. The reaction was stopped with 20 µL of 50 mM EDTA and 6 µL samples of incubation mixture were spotted onto PEI cellulose plate. The plates were chromatographed for 16 hr in 2 M Tris-HCl, pH 8.6 to separate 2-5A oligoadenylates from ATP. The radioactivity spots were located using x-ray film, cut out, collected in scintillation liquid and counted in the ^{32}P-channel of a scintillation counter. The percentage conversion of ATP to oligoadenylates was calculated as pmoles ATP incorporation/min per 10^7 cells. Tubes containing cells extracts from BMC stimulated with IFN and tubes containing no cell extract were included in each assay to minimize the variations due to the batch of ^{32}ATP used or other experimental parameters.

Bacteria strains

S. thermophilus (strain 158) was kindly provided by Centre de Recherche International Daniel Carasso (Plessis-Robinson, France). *Bifidobacterium, Lactobacillus, acidophilus, Streptococcus lactis,* and *Streptococcus casei* were obtained from Pilege (Champtoceaux, France). Before use, the bacteria were washed three times with DMEM medium without fetal calf serum (FCS) and antibiotics. *S. thermophilus* was chosen because of its high efficiency to stimulate the production of IFN-γ.[4]

Preparation of cells and cultures

Blood was drawn in heparin (40 U/mL) and the mononuclear cell fraction (BMC) was obtained by density gradient centrifugation on Ficoll-Hypaque (Pharmacia Co. Fine Chemicals, New Jersey, USA).[18] BMC were washed three times with phosphate buffered saline and counted.

In some experiments, BMC were enriched in lymphocytes (EBMC) and used instead of BMC. Preliminary experiments have shown a higher and well standardized production of IFN-γ (mainly produced by lymphocytes T) with EBMC. EBMC were obtained by incubating the BMC with L-leucine methyl ester (Leu-OMe, Sigma, France): Leu-OMe was dissolved in phosphate buffered saline (PBS, Flow Laboratories, Ivrine, UK), and filtered through a 0.45 µm filter immediately before use. Cells were suspended in PBS at concentration of 5 × 10^6 cells/mL. They were incubated in 17 × 100 mm polypropylene culture tubes (Becton Dickinson, Rutherford, New Jersey, USA) with 5 mM Leu-OMe at 22°C for 40 min.[19] When incubation interval was completed, 10% of FCS was added, the cells were washed twice with PBS. The population remaining was found to contain 6% monocytes as shown by flow cytofluorometry analysis.

EBMC were suspended in DMEM medium (Flow Laboratories) containing 10% FCS, 200 mM glutamine, 2 g/L sodium bicarbonate, 100 µg/mL streptomycin, 100 U/mL penicillin. The proportion of viable cells before culture was determined by trypan blue exclusion. Samples containing 2 × 10^6 BMC or EBMC were incubated with 2 × 10^7 bacteria, 10 µg/mL of muramyl dipeptide (MDP) or 10 µg/mL of teichoic acid (TA) from *Streptococcus aureus*, in a total volume of 1 mL in 24-well plates. Incubation was performed for 48 hr at 37°C in a humifified 5% CO_2 incubator. It was previously established that IL-1, TNF-α, and IFN-γ were at their maximal level at 48 h.[4] MDP and TA were purchased from Sigma (Paris, France). BMC incubated with 10 µL of ConA (mitogen) for T lymphocytes) (250 µg/mL) was used as a positive control for IFN-γ. Anti-CD4 and anti-CD8 (Immunothec, Marseille, France) were monoclonal IgG type antibodies for CD4 co-receptor of T helper lymphocytes and CD8 co-receptor of T cytotoxic/suppressor lymphocytes respectively, synthetised in mice. Anti-IL-2 was produced in rabbits, using highly purified recombinant human IL-2 as immunizing antigen (Tebu, Paris, France), IgG type. Ten µg/mL of each antibody were added 1 hr before addition of bacteria and 24 hr after incubation. At the end of the incubation, cell-free supernatants were obtained and stored at −80°C until assayed for cytokines.

Cytokines assay

IL-1β, IL-6, IFN-γ and TNF-α were measured by specific immunoradiometric assays (Medgenix, Fleurus, Belgium); the sensitivities of the assays were: >5 pg/mL, >6 pg/mL, >6 U/mL and >5 pg/mL, respectively. Radioactivity was measured in an automatic gamma counter (LKB-Wallac, Turku, Finland).

Statistics

Statistical significance was calculated using paired *t* test and Mann-Whitney test. The results are reported as means ± SEM.

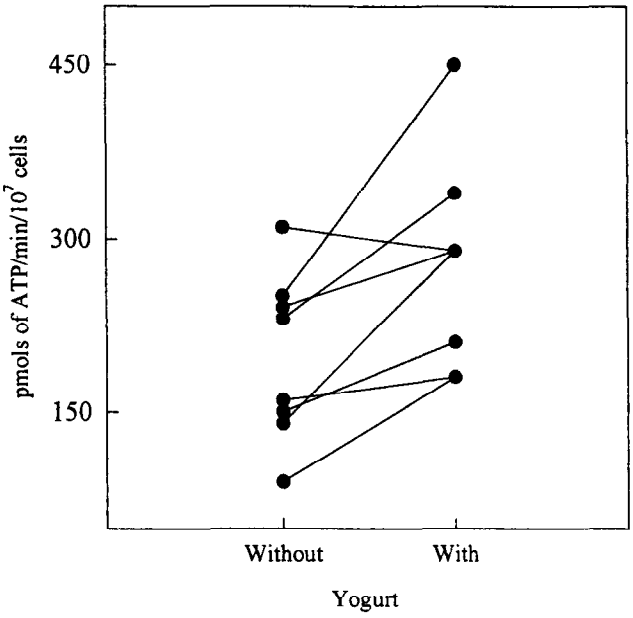

Figure 1 2-5A synthetase activity of blood mononuclear cells from healthy subjects consuming a diet containing or not at least a yogurt per day during two periods of 15 days. $n = 8$ The activity of 2-5A synthetase in the BMC was significantly higher when the diet contained yogurt (265 ± 33) than that of the period without yogurt (192 ± 24) ($P < 0.05$).

Results

2-5A synthetase activity

Eating yogurt for 15 days roughly doubled the levels of 2-5A synthetase found in the circulating BMC of five subjects. The enzyme activity increased modestly in two subjects and declined in one subject (*Figure 1*). However, a significant increase was shown when considering all of them (192 ± 24 vs 265 ± 33, $P < 0.05$).

Cytokines production by different bacteria

A high production of IL-1β was observed when BMC were incubated with *S. thermophilus,* whereas, a small, but significant secretion was shown for others bacteria (*Table 1*). All the bacteria induced a high production of IL-6. Secretion of IFN-γ was minimal with either *L. casei* and *Bifidobacterium,* large quantities of IFN-γ were induced by *S. lactis, L. acidophilus* and to a lesser degree by *S. thermophilus* (*Table 1*). As expected, the production of IFN-γ was induced by ConA mitogen. A significant TNF-α secretion was observed from LPS stimulated cells, this quantity remained small compared to BMC stimulated by *S. thermophilus* (*Table 2*).

IFN-γ production: Effect of antibodies (anti-CD4 co-receptor, anti-CD8 co-receptors)

The production of IFN-γ was 16 fold increased when EBMC were incubated in presence of *S. thermophilus*. The addition of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies reduced this production by 76% and 62%, respectively. However, adding the two antibodies together, did not inhibit completely the production of IFN-γ. Anti-CD4 co-receptor and anti-CD8 co-receptor in the absence of *S. thermophilus* did not change the production of IFN-γ when compared to that observed with cells alone (*Figure 2*). The incubation of ConA with EBMC stimulated the production of IFN-γ, this production was not reduced by the addition of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies (*Figure 3*). To explain the remaining production of IFN-γ, we tested the effect of anti-IL-2 upon this production. This production by EBMC in presence of *S. thermophilus* was significantly decreased when anti-IL-2 was added to the medium (32.2 ± 7.9 U/mL vs 25.4 ± 6.61 U/mL, $p = 0.036$).

The production of IFN-γ by EBMC was also stimulated by both MDP and TA. These stimulations were higher than those induced by *S. thermophilus*. The CD4 blockade was able to reduce IFN-γ stimulated by MDP to 38% compared with that of EBMC (*Figure 4*). Anti-CD4 co-receptor and anti-CD8 co-receptor had no effect on the production of IFN-γ by EBMC induced by teichoic acid (*Figure 5*).

Discussion

The data presented here confirm that the ingestion of yogurt by healthy subjects was able to increase the level of 2-5A synthetase in circulating mononuclear cells which the number and phenotypes are unchanged by consumption of yogurt or fermented milk.[20,21] 2-5A synthetase is an enzyme,

Table 1 IL-1β, IL-6 and IFN-γ production by BMC (2×10^6) in the presence of lactic bacteria (2×10^7) for up 48 hr, $n = 8$

	IL-1β ng/mL	IL-6 ng/mL	IFN-γ U/mL
BMC	0.59 ± 0.02	2.20 ± 0.06	3.46 ± 0.10
BMC + *S. thermophilus*	29.01 ± 1.89**	72.20 ± 1.96**	13.69 ± 0.40**
BMC + *Bifidobacterium*	1.02 ± 0.02*	13.84 ± 0.42**	4.14 ± 0.33
BMC + *L. acidophilus*	0.96 ± 0.05*	46.35 ± 0.80**	40.94 ± 1.38**
BMC + *S. lactis*	1.73 ± 0.07*	56.88 ± 1.34**	48.10 ± 1.31**
BMC + *L. casei*	0.72 ± 0.01*	14.79 ± 0.52**	3.97 ± 0.09*

Comparison between BMC and BMC + bacteria.
*$P < 0.01$
**$P < 0.005$

Table 2 IL-1β, TNF-α and IFN-γ production by BMC (2×10^6) in the presence of *S. thermophilus* (2×10^7), ConA (2.5 μg/mL) or LPS (10 μg/mL) for up to 48 hr, $n = 6$

	IL-1β (ng/mL)	TNF-α (ng/mL)	IFN-γ (U/mL)
BMC	0.5 ± 0.2	0.3 ± 0.1	3.73 ± 0.8
BMC + ConA	0.9 ± 0.4	1.7 ± 0.1**	24.4 ± 2.7**
BMC + LPS	1.9 ± 0.3*	1.9 ± 0.2**	13.8 ± 2.5*
BMC + *S. thermophilus*	24.8 ± 3.7**	9.5 ± 1.1**	17.6 ± 2.6*

As compared to BMC
**$P < 0.001$
*$P < 0.01$

which converts ATP to oligonucleotides with a 2-5A phosphodiester bond, and inhibits protein synthesis by activating a nuclease. The 2-5A synthetase activity is specifically induced by IFNs and remains long after the IFNs has disappeared.[22] Thus, the observed increase level of 2-5A synthetase suggest an augmentation of a spontaneous production of IFNs. However, the change observed here was less than that observed previously in healthy subjects.[7] In this previous work, the subjects ingested once in the lab a controlled yogurt enriched in bacteria (2×10^{11}/g). Here, the subjects were allowed to buy commercial yogurts of their choice and to eat them at home. Thus a precise control of the quality and quantity ingested was not possible. Nor was it possible to be sure that no fermented dairy products were fed during the control period. Interestingly, there was a significant increase in the activity of the 2-5A synthetase, indicating that a regular ingestion of yogurt is compatible with the maintenance of a higher level of the enzyme activity and probably with higher production of interferons. In agreement with this, Muscettola et al. showed in vitro a higher production of IFNs from spleen cells of mice fed live bacteria.[23] These authors suggest that lactic bacteria could interact with M cells, which activate Peyer's patches lymphocytes. These cells might be liberated from the intestine and reach systemic circulation as indicated by a higher level of 2-5A synthetase. A nearby mechanism due to gut flora might be involved to explain the spontaneous level of 2-5A synthetase observed in these subjects when they were not eating fermented dairy products. Other dietary substances, like lectins from peas, have been suggested to explain this level of 2-5A synthetase,[24] but it is more likely that this is the result of a stimulation in the large intestine of the colon flora.[6] Indeed, it has been shown that some of these bacteria are able to induce the production of IFNs in vitro.[4,5] Taken together, these data suggest that the spontaneous —thus physiological— production of IFNs, indirectly detected by the spontaneous activity of the 2-5A synthetase, might be

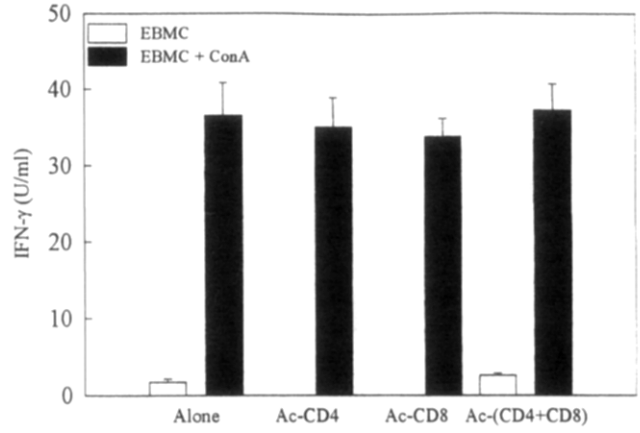

Figure 3 Effect of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies on IFN-γ (U/mL) produced by EBMC stimulated by Concanavalin A (ConA). EBMC (BMC enriched in lymphocytes population) (2×10^6) incubated 48 hr with ConA (2.5 μg/mL) in the presence of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies (10 μg/mL). The results are Mean ± S.E.M. $n = 4$.

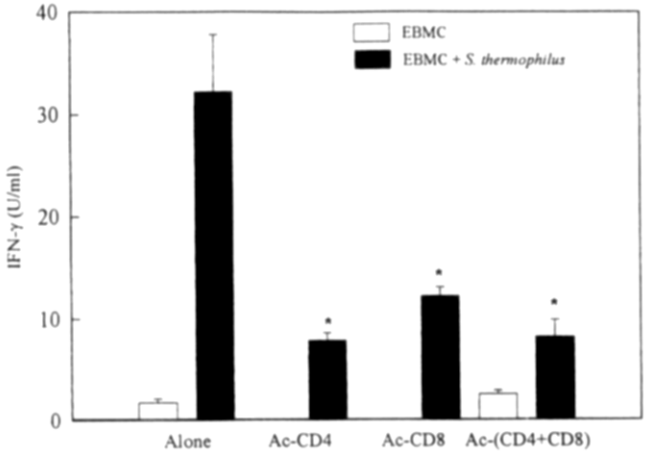

Figure 2 Effect of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies on IFN-γ (U/mL) produced by EBMC stimulated by *S. thermophilus*. EBMC (BMC enriched in lymphocytes population) (2×10^6) incubated 48 hr with *S. thermophilus* (2×10^7) in the presence of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies (10 μg/mL). The results are Mean ± S.E.M. $n = 4$; *: $P < 0.05$ (comparison between EBMC + bacteria and EBMC + bacteria + antibodies).

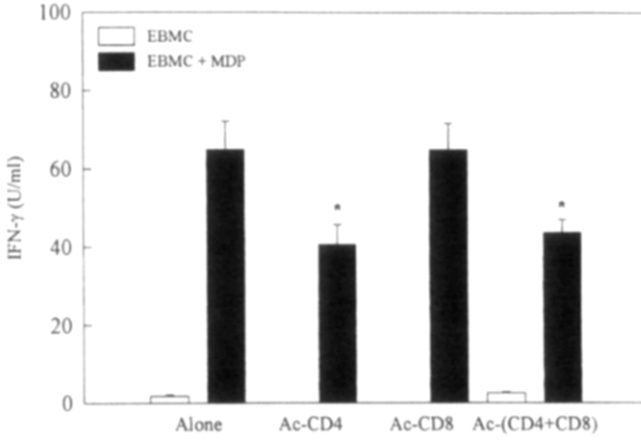

Figure 4 Effect of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies on IFN-γ (U/mL) produced by EBMC stimulated by MDP. EBMC (BMC enriched in lymphocytes population) (2×10^6) incubated 48 hr with MDP (muramyl dipeptide) (10 μg/mL) in the presence of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies (10 μg/mL). The results are Mean ± S.E.M. n = 4, *: $P < 0.05$. (comparison between EBMC + MDP and EBMC + MDP + antibodies).

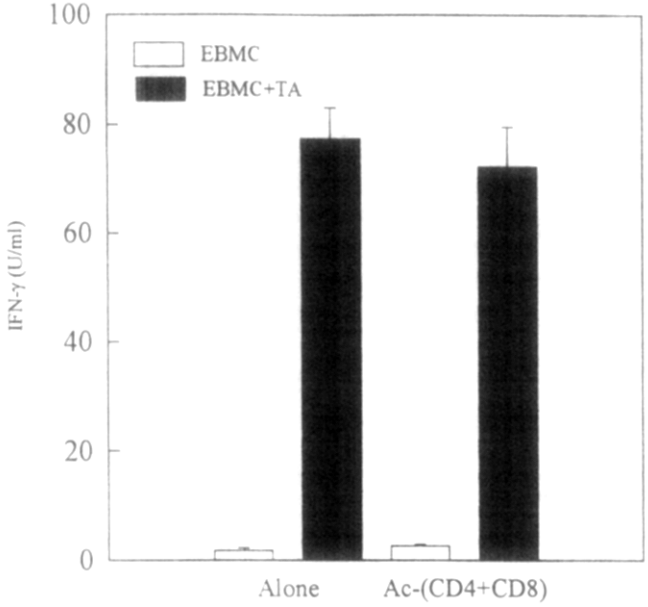

Figure 5 Effect of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies on IFN-γ (U/mL) produced by EBMC stimulated by TA. EBMC (BMC enriched in lymphocytes population) (2×10^6) incubated 48 hr with TA (thecoic acid) (10 μg/mL) in the presence of anti-CD4 co-receptor and anti-CD8 co-receptor antibodies (10 μg/mL). The results are mean ± S.E.M. n = 4.

regulated by dietary non-pathogen bacteria. 2-5A synthetase can be induced by interferons α, β, and γ. Previously, in our laboratory, Solis et al.[4] have shown that bacteria used in yogurt do not induce the production of IFN-α by blood mononuclear cells from healthy subjects. Thus, the increase in the level of 2-5A synthetase activity observed in the in vivo study could be due to the production of IFN-γ and/or IFN-β.

The different bacteria tested were able to induce the production of cytokines in vitro. Their effect vary with species and the kind of cytokine considered. Indeed, *S. thermophilus* was the only bacteria that induced a high production of IL-1β, and large quantities of IFN-γ were induced by *S. lactis, L. acidophilus,* and *S. thermophilus*. All these bacteria induced a high production of IL-6 (*Table 1*). This suggests that there might be different mechanisms involved depending on the composition of the bacteria.

The mechanisms of presentation of non-pathogen bacteria have been poorly studied. Monocytes are known to be necessary to present antigen to lymphocytes.[25,26] We have observed that a decrease in the proportion of monocytes from BMC to 2% of total leucocytes decreased the production of IFN-γ (data not shown). Moreover, pure T lymphocytes, obtained by positive selection using magnetic beads, could not produce IFN-γ (data not shown). In this study, we examined whether anti-CD4 co-receptor or anti-CD8 co-receptor antibodies could inhibit the stimulation of EBMC cells by *S. thermophilus* to produce IFN-γ. This production was reduced by 76% and 62% when anti-CD4 co-receptor and anti-CD8 co-receptor antibodies were added in the medium, respectively. It is known that T cells activation requires the interaction between antigen and products of major histocompatibility complex MHC, HLA (Human Leukocyte Antigen for human) and the antigen-presenting cell (APC). In all likelihood, exogenous antigens from non-pathogen bacteria taken up from the extra cellular environment by macrophages and other APC should be processed in endocytic compartment. Association of processed antigen with class II MHC gene products should be expressed on the cell surface for subsequent recognition by CD4+ T cells.[26–29] Indeed, this pathway seems to be implicated in our study because the anti-CD4 co-receptor antibody blocked this way, which cause a dramatic inhibition of the production of IFN-γ by CD4+ lymphocytes. This is strengthen by the data obtained with ConA. The increase in the production of IFN-γ induced by ConA (known to act by a non-MHC pathway) was not modified by the addition of the two antibodies. This suggests that a possible side effect of this antibodies such as the induction of the production of cytokines like IL-10 known to inhibit the production of IFN-γ was not present in our incubating conditions. Thus, the decrease in IFN-γ production observed in *Figure 2* could be attributable to the implication of the MHC pathway. In contrast, processed antigenic proteins synthesised within APC (as in the case of viral infection) are associated with class I MHC gene products and recognized by CD8+ T cells.[30,31] Interestingly, we showed that here *S. thermophilus* could induce IFN-γ production by CD8+ lymphocytes in vitro via CD8/TCR/HLA class I complex, as this production was markedly inhibited by anti-CD8 co-receptor antibody. Here also, the addition of the CD8 co-receptor antibody had no effect in the presence of ConA indicating that its inhibitory effect on the production of IFN-γ was due to the implication of MHC I. Thus, *S. thermophilus* could be processed not only by class II pathway but also by class I pathway, and could stimulate T lymphocytes (CD4+ and CD8+) to produce the largest part of IFN-γ observed in vitro. The involvement of the two pathways is now accepted for virus[32] such as Influenza virus and very recently for pathogen micro-organisms like *Listeria monocytogenes*.[33] We showed that these mechanisms could also be applied for non-pathogen bacteria.

The walls of gram-negative bacteria contain as major components lipopolysaccharide and relatively little peptidoglycans (10% of the total cell wall), whereas, walls of gram-positive bacteria are composed mainly of peptidoglycans (30 to 70% of the total cell wall), polysaccharides or/and teichoic acid. Little is known about the immunogenic structures of gram-positive bacteria, which includes the lactic bacteria. It has been reported that a molecule such as muramyl dipeptide (MDP) constantly present in the peptidoglycans in this type of bacteria can induce cytokines production in vitro.[9–11] MDP is known to be a minimal effective structure for adjuvant activity peptidoglycans.[34,35] Tufano et al.[11] have shown that MDP stimulates in vitro the production of IL-1, IL-6, and TFN-α by monocytes, and of IL-4 and IFN-γ by lymphocytes. Our results showed that MDP caused the release of IFN-γ and that this production was reduced by anti-CD4 co-receptor (*Figure 4*) and by depletion of monocytes (data not shown). Thus, MDP could stimulate EBMC to produce IFN-γ through CD4/TCR/HLA complex and could be the component of *S. thermophilus* wall who was recognized by monocytes and presented to CD4+ lymphocytes via HLA context. In contrast, MDP was not implicated in the CD8/TCR/HLA pathway because anti-

CD8 co-receptor did not reduce the IFN-γ production stimulated by MDP. Other component(s) of *S. thermophilus* should be involved to explain the observed production of IFN-γ by way of CD8/TCR/HLA complex.

A significant production of IFN-γ was still existing in presence of both anti-CD4 co-receptor and anti-CD8 co-receptor antibodies, suggesting the involvement of (an) other pathway(s) in this production. Interactions between several cytokines have been reported.[36,37] For example, IL-2 is known to play an important role in IFN-γ secretion.[38] To explore this possibility anti-IL-2 was used. Indeed, the addition of an anti-IL-2 antibody reduced significantly the production of IFN-γ induced by *S. thermophilus,* which could explain the remaining production of IFN-γ observed in presence of anti-CD4 co-receptor and anti-CD8 co-receptor. IL-1 can stimulate IFN-γ synthesis. In our experiments, IL-1β, was produced in vitro in presence of *S. thermophilus*. Non proteic components of *S. thermophilus* could also stimulate immune cells, independently of HLA system. Here, we showed an effect of a commercially available teichoic acid from *S. aureus* on the production of IFN-γ by EBMC. Several studies have established that these teichoic acids stimulate more or less the production of IFN-γ by EBMC. Several studies have established that these teichoic acids stimulate more or less the production of IL-1, TFN-α and IL-6 by monocytes in vitro depending in their structure.[9–12,39] Further experiments are necessary to elucidate the structure and composition of the walls of lactic bacteria including those of teicoic acid. Another mechanism has been suggested by De Simone et al.: these authors showed that *Lactobacillus bulgaricus* and *S. thermophilus* bind spontaneously to CD4+ and CD8+ lymphocytes, suggesting the presence of receptor-binding sites for lactic bacteria on human lymphocyte membranes.[40] Moreover, Dziarski demonstrates the presence of specific peptidoglycan-binding protein in lymphocytes and macrophages.[41] This protein could be the site for *S. thermophilus* to stimulate directly the production of IFN-γ. However, when we incubated pure CD8+ lymphocytes in presence of *S. thermophilus,* no production of IFN-γ was observed (data not shown).

In conclusion, non-pathogen (gram-positive) bacteria such as those used in dairy products were able to stimulate in vitro the production of different cytokines from circulating mononuclear cells of healthy subjects. The regular ingestion of a diet rich in some of these bacteria (yogurt) appeared to increase the level of 2-5A synthetase in circulating mononuclear cells, suggesting an in vivo increased production of IFNs. It was also shown that in vitro, *S. thermophilus* could stimulate CD4+ and CD8+ lymphocytes and involved class II and class I histocompatibility system. Muramyl dipeptide, a component of the walls common to these bacteria, could be a possible candidate to explain the part recognized by CD4+ lymphocytes. However, the role of this IFN-γ production is not yet clear. It is known, for example, that eating lactic bacteria renders laboratory animals more resistant to *Salmonella* infection,[42,43] but the underlying mechanism has not been established. Such protection to infection, might be compatible with a role of the induction of the production of cytokines in a system of immune surveillance, but this has not been yet demonstrated.

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