Infection of human B lymphoma cells by *Mycoplasma fermentans* induces interaction of its elongation factor with the intracytoplasmic domain of Epstein-Barr virus receptor (gp140, EBV/C3dR, CR2, CD21)

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Abstract

Human cell lines are often infected by mycoplasma strains. We have demonstrated that when infected by *Mycoplasma fermentans*, human B lymphoma cell proliferation increased strongly. These infected B cells expressed a p45 kDa protein which interacted with the intracellular domain of CD21, the EBV/C3d receptor. p45 analysis demonstrated that this is a new gene which encodes an elongation factor originating from *Mycoplasma fermentans*. p45 interaction with CD21 was specific, there being no interaction with CD19. This is the first demonstration that *Mycoplasma fermentans*, in infecting human B cells, generates a p45 *Mycoplasma* component that interacts with CD21, which is involved in B cell proliferation.

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**Keywords:** Mycoplasma infection; B lymphocyte proliferation; CD21

1. Introduction

It is well known that human cell cultures can be infected by *Mycoplasmas*, more often carried by humans. Indeed, *Mycoplasmas* (and especially of the *fermentans* strain) have been found in mucosal tissues, in saliva and oropharyngeal of 45% of healthy adults [1]. In addition, evidence suggests that certain species may invade the host cells and can be found intracellularly [2]. Specific binding of *Mycoplasma fermentans* predominantly directed towards B cells has been detected by flow cytometry [3]. *Mycoplasma fermentans* was also able to fuse with lymphocytes, allowing numerous *Mycoplasma* components to be expressed in the host cells, affecting normal cell functions and changing the immunological characteristics of human cells [2] The signals generated in human host cells by invasive *Mycoplasmas* are not yet understood [2]. Thus, identification of the mechanisms by which *Mycoplasmas* may modulate the functions of human B cells would allow us to understand their impact on human biology.

We observed that when infected by *Mycoplasma*, human B lymphoma cell lines in culture very often
present a higher level of proliferation than non-infected cells. These observations led us to analyze whether *Mycoplasma* infection could interfere with CD21, the receptor for the C3d fragment of complement [4] and for the Epstein-Barr virus (EBV) [5] (gp140, EBV/C3dR, CR2). Indeed, CD21 plays a pivotal role in the regulation of human B cell proliferation [6,7] and transformation [5]. CD21 crosslinking on the cell surface by its extracellular ligands, which is the first step in these biological events, induces B cell proliferation [6,7]. In addition, it was demonstrated that these regulations are mediated by the intracytoplasmic carboxy-terminal domain of CD21, despite its short length of just 34 amino acids. Indeed, cells transfected with CD21 deleted of this intracytoplasmic domain did not allow cell transformation by EBV [8]. Furthermore, B lymphocyte proliferation induced by CD21 activation on the cell surface was specifically inhibited by intracellular expression of pep34, a synthetic peptide whose sequence corresponded to the CD21 intracytoplasmic domain [9]. CD21 activation on the cell surface specifically triggers, in the absence of CD19 and sIgG, PI 3-kinase activation through tyrosine phosphorylation of the p95 nucleolin [10,11].

2. Materials and methods

2.1. Cells

Two human cells, both expressing CD21, were used: Raji, an EBV positive Burkitt B lymphoma cell line, and Ramos, an EBV negative Burkitt B lymphoma cells line [4–7]. Cells were grown in RPMI 1640 supplemented with 10% FCS, 2 mM L-glutamine, 1000 U ml⁻¹ penicillin, and 100 μg ml⁻¹ streptomycin at 37 °C in 5% CO₂.

2.2. Antibodies and Mycoplasma strain

BL13 and OKB-7, two monoclonal anti-CD21 antibodies (moAbs) were purchased from Immunotech (Westbrook, ME) and Ortho (Raritan, NJ, USA), respectively. Anti-CD19 moAb (HD37) was from Cymbus Biotechnology (Hants, England). Polyclonal anti-glutathione–S-transferase (GST) Ab was from Oncogene (France) and anti-CD19 Ab (C-20) from Santa Cruz (USA). Polyclonal anti-p45 Ab was prepared by immunizing rabbits, as previously described [10], with pep22, a 22 amino acid synthetic peptide whose sequence MAKQDFRNKDHVNGTIGHVD corresponds to the N-terminal domain sequence of p45 highly purified from human B lymphoma cells (see Section 3). *Mycoplasma fermentans* strain PG-18 was obtained from ATCC-LGC (France).

2.3. Detection of Mycoplasmas in B lymphoma cells

*Mycoplasma* infection of B lymphoma cells was detected using a PCR ELISA kit (Roche, Meylan, France). This kit detects all *Mycoplasma*, *acholyplasma* and *ureaplasma* species that have been identified so far, using a ready-to-use mixture of *Mycoplasma* -specific primers. The assay was performed following the manufacturer’s instructions.

2.4. Infection of human B lymphoma cells by Mycoplasma fermentans

10⁶ human B lymphoma cells were contaminated with 10⁶ colony forming units per ml of *Mycoplasma fermentans* strain PG-18 as described [2]. Detection of *Mycoplasma* infection in human B lymphoma cells was controlled as described above.

2.5. Immunoprecipitation, affinity chromatography and immunoblotting

Solubilization of total proteins, immunoprecipitations, proteins analysis by SDS and immunoblotting and cell activation by anti-CD21 and anti-CD19 moAbs were performed as previously described [6,7,9–11]. For protein translation analysis, 10 μl rabbit reticulocyte lysate, obtained using the in vitro TNT Rabbit Reticulocyte Translation System (Promega) and which contains 35S methionine, was used in immunoprecipitation assays. Then, proteins were immunoprecipitated on indicated antibody and analyzed as previously described [9–11].

2.6. Genomic DNA extraction, RNA extraction, cDNA synthesis and PCR

Genomic DNA was purified from B lymphoma cells with the Turbogen kit (Invitrogen). Total RNA was prepared with RNAzole (Bioprobe). Then, a cDNA library was constructed using the ZAP-cDNA synthesis kit (Stratagene) as previously described [12] and Marathon cDNA amplification kit (Clontech). PCR were performed with the different specific primers described below, as previously described [12].

Intracytoplasmic domain (p34 or p34/Mut) and external (CD21/Ext) domains of CD21 were constructed from PCR samples which contained 500 ng PBS-CD21M1 coding for CD21 (kindly provided by M. Holers). These samples were heated for 5 min at 94 °C, then submitted to 30 cycles at 94 °C for 1 min, 55 °C for 1 min, 72 °C for 1.5 min, with final extension at 72 °C for 7 min. The p34 amplicon was obtained using 5’ primer 5’-CGGGATCCATTACCTTATACGTGATA-3’ (nt 3139–3156 with a BamHI site) and 3’ primer
5'-TAGAATTCGCTGGCTGGGTGTAGG-3' (nt 3244–3261 with an EcoRI site); the p34/Mut amplicon was obtained using the specific 5' primer 5'-CGGGACTCATTACCTATAGCTGATAGCCCAAG-3' and 3' primer 5'-TAGAATTCGCTGGCTGGGTGTAGG-3' (nt 3244–3261 with an EcoRI site), and the CD21/Ext was obtained using 5' primer 5'-ATGGATCCATTTTCTTGGGGTCCTCTCC (nt 61–80 with a BamHI site) and 3' primer 5'-CGAATTCTAGACTTTTTTCTTCAATG-3' (nt 170–190 with an EcoRI site). The corresponding amino acid sequence of these amplicons is described in Section 3. For p45 cloning, PCR mixtures with 15 μl of human B lymphocyte cDNA library were submitted to 94°C for 10 min, then to 30 cycles at 94°C for 2 min, 45°C for 3 min, 72°C for 1 min, with final extension at 72°C for 10 min. The different degenerate primers used were 5' primer 45Ext1D1: 5'-ATG GCA GCN AAR CGR AAR TAR AAY TGN-3', 45Ext2D1: 5'-GGA ACN ATT GGA CAC GTA GAT-3' and 3' primer 45Int2D1 5'-ATT GTC CCC NGC YTG NAC TTC-3' or 45Int3D1: 5'-GTN CGR AAR TAR AAY TGN GG-3'.

2.7. Construction of recombinant proteins

Recombinant proteins corresponding to wild-type or mutated intracytoplasmic domains of CD21 (p34 or p34/Mut) and external (CD21/Ext) domain of CD21, were prepared using the PCR fragments obtained above and purified, after agarose gel electrophoresis, using the Sephaglass Band Prep Kit (Pharcol). The modified 3' end coding for tryptophan by PCR performed on 200 ng pcDNA3-45M. The modified 3' end of the cDNA codon was modified to a TGG codon coding for the CD21/Ext was obtained using 5' primer 5'-ATGGATCCATTTTCTTGGGGTCCTCTCC (nt 61–80 with a BamHI site) and 3' primer 5'-CGAATTCTAGACTTTTTTCTTCAATG-3' (nt 170–190 with an EcoRI site). The corresponding amino acid sequence of these amplicons is described in Section 3. For p45 cloning, PCR mixtures with 15 μl of human B lymphocyte cDNA library were submitted to 94°C for 10 min, then to 30 cycles at 94°C for 2 min, 45°C for 3 min, 72°C for 1 min, with final extension at 72°C for 10 min. The different degenerate primers used were 5' primer 45Ext1D1: 5'-ATG GCA GCN AAR CGR AAR TAR AAY TGN-3', 45Ext2D1: 5'-GGA ACN ATT GGA CAC GTA GAT-3' and 3' primer 45Int2D1 5'-ATT GTC CCC NGC YTG NAC TTC-3' or 45Int3D1: 5'-GTN CGR AAR TAR AAY TGN GG-3'.

2.8. Site-directed mutagenesis of p45 cDNA

The p45 cDNA obtained by PCR was subcloned first into the pCR-TOP01 vector (Invitrogen), then into pcDNA3 (Invitrogen). The cDNA sequence was performed by Genome Express (Meylan, France). The TGA stop codon was modified to a TGG codon coding for tryptophan by PCR performed on 200 ng pcDNA3-45M using specific primers described below. The modified 5' fragment was obtained with 5' primer 45GN1 (5'-GGGATCCATGGCTCAACAAGATTTCAAT-3', localized on nt 1–21 of p45 cDNA, with a BamHI site) and 3' primer 45R3 (5'-GAGTTTGAATCCTGAGCTTTCCTTCT-3' located on nt 610–588 of p45 cDNA). The modified 3' fragment was obtained with 5' primer 45R4 (5'-GTGATCCATGGCTTTCCTTCTTCT-3' located on nt 3244–3261 with an EcoRI site) and 3' primer 45Ext1D1 5'-ATGGATCCATTTTCTTGGGGTCCTCTCC (nt 61–80 with a BamHI site) and 3' primer 5'-CGAATTCTAGACTTTTTTCTTCAATG-3' (nt 170–190 with an EcoRI site). The corresponding amino acid sequence of these amplicons is described in Section 3. For p45 cloning, PCR mixtures with 15 μl of human B lymphocyte cDNA library were submitted to 94°C for 10 min, then to 30 cycles at 94°C for 2 min, 45°C for 3 min, 72°C for 1 min, with final extension at 72°C for 10 min. The different degenerate primers used were 5' primer 45Ext1D1: 5'-ATG GCA GCN AAR CGR AAR TAR AAY TGN-3', 45Ext2D1: 5'-GGA ACN ATT GGA CAC GTA GAT-3' and 3' primer 45Int2D1 5'-ATT GTC CCC NGC YTG NAC TTC-3' or 45Int3D1: 5'-GTN CGR AAR TAR AAY TGN GG-3'.

2.9. Purification of p45 protein from Mycoplasma infected B lymphocytes

10⁶ human B lymphoma cells infected by Mycoplasma fermentans were solubilized as described [4–6]. In parallel, the same amounts of non-infected B lymphocytes were used as control. Then, cell extracts were incubated with 10 mg of GST-p34 bound on 2 ml glutathione–sepharose beads. After extensive washes, proteins bound on GST-p34 fusion protein were eluted in sample buffer, submitted to SDS-PAGE under reducing conditions and electrotransferred on polyvinylidene difluoride (PVDF) membranes (Amersham). Proteins present on PVDF membranes were stained with Ponceau Red. The band containing the p45 kDa protein, purified only from Mycoplasma-infected B lymphoma cells, was excised and submitted to amino acid analysis.

2.10. Cell proliferation assay

Human B lymphoma cell proliferation assay was performed by measuring the cellular conversion of tetrazolium dyes into a formazan product by the action of NADH-generating dehydrogenases present in metabolically active cells [13].

3. Results and discussion

3.1. Proliferation of human B lymphoma cells infected by Mycoplasmas

Infection of human B lymphoma cells by Mycoplasmas was detected using a PCR ELISA kit (Table 1, Columns A and B) in five cultures (cultures no. 1, 2, 4, 6 and 7). In cultures no. 3 and 5, human cells were not infected. As shown Fig. 1, proliferation of non-infected cells (from culture no. 3) used as control was very low when CD21 was not activated (lane 3), but was strongly increased when CD21 was activated on the cell surface (lane 8), in accordance with our previ-
ous data [6,7]. However, among Mycoplasma-infected B lymphoma cells, four of them (lanes 1, 2, 6 and 7) already presented a high level of proliferation, similar to CD21 activated cells (lane 8) and CD21 activation on their surface did not modify their proliferation (lane 9). Furthermore, B lymphoma cells from culture no. 4 (lane 4), although infected by Mycoplasmas, presented the same low level of proliferation as non-infected or CD21-non activated cells (lane 3).

3.2. CD21 interacts through its intracytoplasmic domain with a p45 protein expressed only in human B lymphoma cells infected by Mycoplasmas

The above data suggested to us a possible relationship between CD21 activation and Mycoplasma infection of human B lymphoma cells. Thus, we constructed different GST recombinant fusion proteins: (1) GST-p34, the whole 34 amino acids of the CD21

Table 1
Correlation between infection by Mycoplasma fermentans of human B lymphoma cells and expression of p45

<table>
<thead>
<tr>
<th>Culture of human B lymphoma cells no.</th>
<th>Detection of Mycoplasma absorbance unit ((A_{450, \text{nm}})) obtained by PCR ELISA(^a)</th>
<th>Detection of Mycoplasma fermentans by PCR(^b)</th>
<th>Expression of p45(^c)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1.83 ± 0.08</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>1.56 ± 0.04</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>0.12 ± 0.03</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>1.4 ± 0.05</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>5</td>
<td>0.16 ± 0.02</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>6</td>
<td>1.73 ± 0.08</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>7</td>
<td>1.79 ± 0.09</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>Negative control</td>
<td>0.1 ± 0.02</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Infected with PG-18 strain</td>
<td>1.8 ± 0.09</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>

\(^a\) The presence of Mycoplasmas was detected using an PCR ELISA kit, which uses a ready-to-use mixture of primers specific to the different strains of Mycoplasma. Values are means of absorbance values at 450 nm ± standard deviations (four samples for each preparation of human B lymphoma cells), with absorbance value < 0.2 considered as negative.

\(^b\) Mycoplasma fermentans was detected by PCR using specific primers [19].

\(^c\) Expression of p45 was analyzed by SDS–PAGE and immunoblotting.

Fig. 1. Proliferation of Mycoplasma-infected and non-infected human B lymphoma cells. Cultures of human B lymphoma cells (lanes 1–7), described in Table 1, Column A, were tested for their level of proliferation. Proliferation of cells from culture no. 3 (lane 3), not infected by Mycoplasmas, was increased under CD21 activation (lane 8). Proliferation of cells from culture no. 7 (lane 7), infected by Mycoplasmas, was not increased under CD21 activation (lane 9). Proliferation of these cells was analyzed with the CellTiter 96 Aqueous One Solution by measuring absorbance at 490 nm in a microtiter reader plate. There is a linear relationship between cell number present in the assays and the amount of formazan product produced and measured by absorbance. Each assay was performed in triplicate. These data represent the mean and standard deviation obtained from four independent experiments.
intracytoplasmic domain fused to GST, i.e., amino acids 1047–1087 (IVITLYVISKHRNRYTDTSQKEAFHLEAREVYSVDYPNPA); (2) GST-p34/Mut, mutant p34 form deleted in the first ten amino acids of the N-terminal region of CD21 intracytoplasmic domain, i.e., amino acids 1053–1064 (ITLYVISQKEAFHLEAREVYSVDYPNPA); and (3) as another control, GST-CD21/Ext, a fusion protein whose sequence corresponded to a region of the external domain of CD21, i.e., amino acids 21–63 (ISCGSPPILNQRISYYSTPIAVGTFTVIRYSCSGTFRLIGEKS). Equal amounts of these GST recombinant proteins were incubated with proteins solubilized from human B lymphoma cells infected by *Mycoplasmas* (cultures no. 1, 2, 6 or 7). SDS–PAGE and Coomassie blue staining allowed detection (Fig. 2A) of a protein of an apparent molecular weight of 45 kDa on GST-p34 (lane 2). In the control, this p45 was not detected on GST (lane 1), GST-p34/Mut (lane 3) or GST-CD21/Ext (lane 4). In addition, this p45 protein was not detected on GST-34 when solubilized proteins were prepared from non-infected human B lymphoma cells of culture 3 (lane 5). Then, this p45 protein was purified using solubilized proteins prepared from 10^8 human B lymphoma cells infected by *Mycoplasmas*, from culture 1, and incubated with recombinant GST-p34 bound on sepharose beads. After washing and elution by sample buffer, SDS–PAGE, transfer onto PVDF membrane and Ponceau Red staining, the purified p45 kDa protein was isolated and submitted to amino-acid analysis. Its N terminal sequence was determined as MAKQDFNRNKDHV-NIGHTIGHVD. Data bank analysis demonstrated that these 22 amino acids presented 81% sequence identity with a region of elongation factor Tu of *Mycoplasma hominis* (EFTU-MYCHO). A polyclonal anti-p45 Ab was prepared against a synthetic peptide whose sequence corresponded to the above 22 amino acid sequence. This anti-p45 Ab (Fig. 2B) specifically recognized the p45 protein, which specifically interacted with GST-p34 and was purified from *Mycoplasma*-infected human B lymphoma cells (B1, lane 1). In the control, the anti-p45 Ab did not recognize any component interacting with GST-p34 in total cell extracts prepared from human B lymphoma cells not infected by Mycoplasmas (B1, lane 2), while the same amount of GST-p34 was used in both assays (Fig. 2B). These data demonstrated that a p45 protein, only expressed in human B lymphoma cells infected by *Mycoplasmas* and absent in non-infected cells, specifically interacted with the 34 amino acid intracytoplasmic domain of CD21.

### 3.3. Cloning of p45 present in human B lymphocytes infected by *Mycoplasmas*

The p45 cDNA was cloned from a cDNA library prepared from human B lymphoma cells infected by *Mycoplasmas* (culture 1). PCR reactions were performed with degenerate primers. The sequences of these primers were derived from the sequences of the 5′ terminal peptide and of the three internal peptides of the p45 protein, whose sequences were obtained after its purification and its amino acid analysis. A 1183 bp cDNA, corresponding to an estimated 43.5 kDa protein, was obtained (Fig. 3). This sequence is registered in GenBank under Accession No. AY838558. Analysis of this sequence using data banks demonstrated the presence in its N-terminal domain of an ATP/GTP-binding site motif A (P-loop) (a.a. 19–26), and a GTP-binding elongation factor signature (a.a. 51–66). When this 1183 bp cDNA was in vitro translated (Fig. 4A) and then immunoprecipitated on the anti-p45 Ab prepared above, only a truncated form of 22 kDa was detected (lane 2). In the control, this 22 kDa component was not recognized by an irrelevant Ab (lane 1). This difference in molecular weight suggested the presence of a stop codon in the middle of the nucleotide sequence of p45. Indeed, a TGA stop codon was present (nucleotides 598–600) in the sequence corresponding to one of the three purified peptides used to clone p45 cDNA. However, the amino acid sequence...
of this peptide, obtained from p45, showed that this TGA stop codon was translated and used as a tryptophan in human B lymphoma cells. The fact that this CD21-binding p45 protein was purified from Mycoplasma-infected human B lymphoma cells as a 45 kDa and not as a 22 kDa protein suggested that Mycoplasmas infecting these human B lymphoma cells were using their own genetic code to translate their own proteins inside the cells. The only partial identity of the p45 amino acid sequence with regions of the elongation factor of Mycoplasma hominis strain suggested that this p45 protein originated from another Mycoplasma strain. Thus, we performed PCR on genomic DNA from human B lymphoma cells infected by Mycoplasma (cultures 1, 2, 4, 6...
and 7) with specific primers from different Mycoplasma strains [13,14]. Data demonstrated (Table 1, Columns C and D) that Mycoplasma fermentans was the strain present in the infected human B lymphoma cells which expressed the p45 component (cultures 1, 2, 6 and 7). In the control, B cells from culture no. 4, while found to be positive for Mycoplasma infection by PCR ELISA, were not infected by Mycoplasma fermentans and did not express the p45 protein. In addition, when human B cells were infected by the Mycoplasma fermentans PG-18 strain, this p45 protein was also expressed. To obtain the cDNA corresponding to the full-length p45 protein, site-directed mutagenesis of the TGA stop codon contained in the p45 cDNA was performed to transform this TGA stop codon into a TGG tryptophan codon. Mutation of the TGA codon into a TGG codon was verified by sequencing (Fig. 4B). The integrity of the full-length p45m was confirmed by immunoprecipitation using anti-p45 Ab. The corresponding proteins obtained after in vitro translation were analyzed by SDS-PAGE (A) after immunoprecipitation either on non-immune serum (lanes 1 and 3) or on anti-p45 Ab (lanes 2 or 4).

3.4. In vivo interaction of p45 elongation factor with CD21

We then analyzed the in vivo interaction of p45 Mycoplasma elongation factor with CD21 in human B lymphoma cells infected by Mycoplasma fermentans (Fig. 5). Total cell extracts, prepared from these lymphoma cells, which expressed CD21 (A, lane 1) and CD19 (B, lane 1), were submitted to immunoprecipitation on anti-p45 Ab. Immunoblotting analysis, using anti-CD21 moAb or anti-CD19 Ab, demonstrated that in these infected B lymphoma cells, p45 elongation factor specifically interacted with CD21 (A, lane 2) but not with CD19 (B, lane 2).

All the above data obtained using Raji cells, an EBV positive Burkitt B lymphoma cell line, were also obtained using Ramos cells, an EBV negative Burkitt B lymphoma cell line, both cells expressing CD21. Thus, the presence of the EBV genome in human cells did not modify cell infection by Mycoplasma fermentans, the expression of this p45 elongation factor, nor its interaction with the intracytoplasmic domain of CD21.

Fig. 4. Mutation of the codon TGA into TGG leads to a 45 kDa protein. pcDNA3-p45 and pcDNA3-p45M were sequenced (B). The A → G mutation is indicated (*). The corresponding proteins obtained after in vitro translation were analyzed by SDS-PAGE (A) after immunoprecipitation either on non-immune serum (lanes 1 and 3) or on anti-p45 Ab (lanes 2 or 4).

Fig. 5. Interaction of p45 with CD21 in human B lymphoma cells. Proteins solubilized from human B lymphoma cells infected by Mycoplasma fermentans (A and B) were incubated with anti-p45 Ab (lanes 2) bound to sepharose beads. After extensive washes, bound proteins were eluted and submitted to SDS-PAGE. In the control, total solubilized proteins were submitted directly to SDS–PAGE (lanes 1). Then, proteins were analyzed by immunoblotting using either anti-CD21 moAb diluted 1/1000 (A, lanes 1 and 2) or anti-CD19 Ab diluted 1/1000 (B, lanes 1 and 2).
Certainly, further studies are needed to analyze whether infection by *Mycoplasma fermentans* of human B lymphocytes of patients may also induce the expression of this p45 elongation and its interaction with CD21. Indeed, the involvement of *Mycoplasmas* in a broad range of immunoregulatory events has been described, mediated by cytokine production and direct effects on macrophages, B and T lymphocytes [15]. Furthermore, *Mycoplasma fermentans* promotes immortalization of human peripheral mononuclear cells [16]. Linking of *Mycoplasma fermentans* and *Mycoplasma penetrans* to AIDS pathogenesis and to malignant transformation [16] has been also implicated as a cause of systemic infections in AIDS patients [17]. Furthermore, the effect of p45-CD21 interaction on the signal transduction pathway specifically triggered by CD21 activation [10,11] also needs to be determined.

In conclusion, our data strongly support that the specific interaction between p45 elongation factor and CD21 may contribute to the activated state of cell proliferation always “turned on”, which characterises human B lymphoma cells infected by *Mycoplasma fermentans*.

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