An exopolysaccharide produced by Geobacillus thermodenitrificans strain B3-72: Antiviral activity on immunocompetent cells

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Abstract

The immunomodulatory and antiviral effects of an extracellular polysaccharide (EPS-2), produced by a strain of Geobacillus thermodenitrificans isolated from a shallow marine vent of Vulcano Island (Italy), were evaluated. In the present study, we show for the first time that EPS-2 treatment hinder HSV-2 replication in human peripheral blood mononuclear cells (PBMC) but not in WISH cells. In fact, high levels of IFN-γ, IL-12, IFN-α, IL-18 were detected in supernatants of EPS-2 treated PBMC. Moreover, this effect was dose-dependent. Taken together, our results confirm that the immunological disorders determined by HSV-2 could be partially restored by treatment with EPS-2.

1. Introduction

Microorganisms in natural systems offer a rich source of useful biomolecules as complex polysaccharides, pigments and polyesters that have useful function and can be used in various industrial processes. The marine environment shows an extraordinary diversity but at the moment marine organisms represent an almost unexploited resource of biomolecules. Many marine bacteria produce exopolysaccharides as a strategy for growth, adhering to solid surfaces, and to survive to adverse conditions [1,2]. There is growing interest in isolating new exopolysaccharides producing bacteria from marine environments, particularly from extreme marine environments characterised by extreme pressure and temperature, high concentrations of H2S and heavy metals [3]. Bacterial exopolysaccharides produced in these habitats with novel chemical compositions, properties and structures have been found to have potential applications in different industrial fields [4]. From deep-sea hydrothermal vents some bacteria, such as Alteromonas macleodii subsp. fijensis [5], Vibrio diabolicus [6], Alteromonas infernus [7,8] and strain HYD721 [9] produced EPS with original structures to survive in their extreme environment. More recently the hyperthermophilic Thermotoga maritima and Thermococcus litoralis have been reported as producers of extra polysaccharides [10].

A growing attention in the isolation and identification of new marine microbial polysaccharides that have applied in many sectors of industry has been focused [11]. Pharmaceutical applications, as antiviral, antitumor and immunostimulant activities of polysaccharides produced by marine bacteria have been yet demonstrated [12–14].

New thermostolerant and thermophilic EPS producing bacteria from marine Mediterranean shallow vents have been described [15–20].

In a previous study we reported the purification and characterization of two different exopolysaccharides produced by a thermophilic strain Geobacillus denitrificans isolated from a shallow marine hot spring of Vulcano Island (Italy) [18]. The exocellular polysaccharide (EPS-2) produced by this bacterium, possesses a trisaccharide repeating unit essentially constituted by sugars with a manno-pyranosidic configuration.

Recently we have reported the antiviral and the immunoregulatory effect of a new exopolysaccharide from a thermostolerant Bacillus licheniformis isolated from a shallow vent of Vulcano Island (Italy) [21]. Its EPS appeared to improve immune surveillance of PBMC toward HSV-2 infection by triggering the production of Th1-type cytokines.

It is well known that the interaction between different immune cells, in order to counteract the development of infectious diseases, is mediated by a complex network of cytokines and chemokines with pleiotropic effects which orchestrate immune response. Defined effector mechanisms include the release of...
cytokines such as IFN-γ, tumor necrosis factor-α (TNF-α) and other types of interferons as IFN-α, with wide-ranging antiviral effects. In addition, interleukin 12 (IL-12), produced by monocytes and antigen-presenting cells, promotes the differentiation of naive CD4+ T cells to the Th1 phenotype and decreases the synthesis of the type 2 cytokines IL-4 and IL-10 by CD4+ T cells [22].

Furthermore, IL-18 plays a critical role in the host defense against viral infection, promoting cell-mediated immunity via activation of NK and Th1-type cells [23]. In addition to its potent action in inducing IFN-γ, IL-18 alone activates CD8+ T cells, which play an important role in viral clearance, suggesting that this cytokine could influence the outcome of viral infection. Indeed, the importance of IL-18 has been demonstrated in various viral diseases. In particular, a protective effect of IL-18 against infection with herpes simplex virus (HSV) was shown in a mouse model [24]. It is noteworthy that IL-18 can also promote Th2 differentiation by promptly enhancing IL-4 production by NK T lymphocytes [25,26].

It is well known that IL-4 is a prototypical anti-inflammatory cytokine that modulates macrophage activity through global suppression of proinflammatory cytokines [27,28], in addition to pleiotropic effects on the development of immune cells and the immune response [29].

IL-10 was originally identified as a cytokine synthesis inhibitory factor because of its ability to inhibit the production of IFN-γ by Th1 cell clones [30]. It is now known to be an important suppressor of both T-lymphocyte and antigen-presenting cell effector function. It also acts on macrophages to down-regulate MHC class II expression, resulting in the inhibition of cytokine synthesis by activated T cells and NK cells. Thus, the induction, perpetuation, and collapse of a particular cytokine network and of the cellular events that it controls are strongly influenced by dynamic relationships between pro- and anti-inflammatory cytokines, as well as their rates of production.

In the present paper, we studied the effects of EPS-2 on the replication of HSV-2 in human PBMC and WISH cells. Furthermore, we investigated whether the anti-HSV-2 activity observed only in PBMC could be related to its modulatory activity on cytokine production by the same cells.

2. Materials and Methods

2.1. Production and physicochemical characterization of the exopolysaccharide

The B3-72 strain of Geobacillus thermodenitrificans, isolated from a shallow, marine vent of Vulcano Island (Eolian Islands, Italy) at Porto Levante was grown in liquid mineral medium plus sucrose 0.6% (w/v) at 65 °C and pH 7.0 [18].

Production and characterization of EPS-2 have been performed as previously reported [18]. Briefly, cells at stationary phase of growth were harvested by centrifugation (9800 × g, 20 min at +4 °C). The liquid phase was treated with 1 volume of cold ethanol added drop by drop under stirring. Alcoholic solution was kept at −20 °C overnight and then centrifuged at 15,000 × g for 30 min. The pellet was washed two times with ethanol and dissolved in hot water, dialysed against distilled water and lyophilised.

The rheological properties were analysed by studying the specific viscosity (η) and the degradation temperature by means a thermogravimetical analysis of EPS-2.

Measurements of specific viscosity as a function of concentration of aqueous solutions of the polysaccharide were carried out using Cannon-Ubbelohde 75 suspended level viscometers at 30 °C.

Thermogravimetical analysis was performed by using a Mettler Toledo Star System apparatus. Sample (5 mg) was heated from 30 to 400 °C at a heating rate of 20 °C/min under nitrogen.

2.2. Isolation of human peripheral blood mononuclear cells (PBMC) and EPS-2 treatment

PBMC were isolated from freshly collecteduffy coats of healthy blood donors (Centro Trasfusionale, Policlinico Universitario “G. Martino”, Messina, Italy), after centrifugation over Ficoll-Hypaque gradient. PBMC were then washed three times in RPMI 1640 medium (Sigma) and cultured in 24-well plates at a concentration of 2 × 10⁶ cells/ml per well in RPMI 1640 medium. PBMC were cultured at 37 °C in 5% CO₂ atmosphere, in RPMI 1640 supplemented with 50 μg/ml gentamycin and 5% fetal calf serum (FCS, Sigma).

PBMC were then treated with the exopolysaccharide EPS-2 at different concentrations (300, 200, 100 and 50 μg/ml) and incubated for 24 h at 37 °C in 5% CO₂.

Lipopolysaccharide (LPS) from Escherichia coli strain 055:B5 was used as positive control.

Twenty-four hours post-treatment, the supernatants were harvested, and suitable aliquots were stored at −80 °C until cytokine analysis.

2.3. Treatment and HSV-2 infection

In a second series of experiments, PBMC and WISH cells were seeded onto 24-well culture plates at a density of 2 × 10⁶ cells per well. Treatments were performed with different concentrations (300, 200, 100 and 50 μg/ml) of EPS-2 and after 24 h infected with HSV-2 at a multiplicity of infection (MOI) 0.1 and incubated for further 24 h at 37 °C in 5% CO₂. Then the plates were frozen and thawed three times in order to release the intracellular virus. Cell lysates and supernatants were kept at −80 °C until virus titration. The virus titer was expressed as plaque forming unit (PFU) per ml.

2.4. Virus

HSV-type 2 strain G was used throughout the study. HSV-2 infection was propagated on WISH cell lines. Viral stocks were prepared by pelleting infected cells exhibiting cytopathic effect, and freezing aliquots at −80 °C. The virus titer was assessed on WISH cells and expressed as plaque forming unit (PFU) per ml.

2.5. Cytotoxicity test

Cytotoxicity test was performed on PBMC as well as on WISH cells. EPS-2 was diluted in apyrogenic sterile water at a concentration of 1 mg/ml.

To determine the effect of different concentrations of EPS-2 on cells viability a colorimetric assay was used as described by Mossmann [31]. The assay is based on the tetrazolium salt 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), a pale yellow substrate that is cleaved by active mitochondria to produce a dark blue formazan product. Briefly, cells were seeded onto 96-well culture plates at a number of 10⁴ per well. After 4 h of incubation to allow seeding of the cells, various concentrations of compounds (1000, 800, 600, 500, 400 and 300 μg/ml) were added into each well. The plate was then incubated at 37 °C with atmosphere of 5% of CO₂ for 24 h. Later, the medium was discarded and the MTT reagent was added. The plate was re-incubated at 37 °C for an additional 3 h to allow the development of formazan. The plates were read with a microtiter reader using a wavelength of 570 nm. Cytotoxicity percentage was calculated as follows:

\[
\text{Cytotoxicity percentage} = \left( \frac{\text{experiment OD} - \text{lysis control OD}}{\text{cell control OD} - \text{lysis control OD}} \right) \times 100
\]
2.6. Limulus test

Culture media and reagents tested for the presence of endotoxin by E-Toxate kit (Sigma, Milan) were found to contain ≤10 pg of endotoxin per ml.

2.7. Cytokine evaluations

 Supernatants from PBMC, in different experimental conditions, were harvested, centrifuged and kept at –80°C until titration for the presence of IFN-α, TNF-α, IL-12, IL-18, IFN-γ, IL-4 and IL-10 by an immunoenzymatic method (ELISA) all from Bender MedSystems (Milan, Italy). The limits of detection were of 3.1, 0.13, 2.1, 9.2, 0.06, 0.1 and 0.05 pg/ml, respectively.

2.8. Cytokine and monoclonal antibodies

Rh-IL-18 was used at a concentration of 20 and 40 ng/ml; Rh-IL-18 was added to human PBMC at the time of HSV-2 infection. Rh-TNF-α was used at a concentration of 5 and 10 ng/ml. Monoclonal anti-human TNF-α (ND50 was 0.015–0.06 pg/ml in the presence of 0.25 ng/ml recombinant human TNF-α) and monoclonal anti-human IL-18 (ND50 was 0.1 µg/ml in the presence of 40 ng/ml of recombinant human IL-18) were added to human PBMC at the time of EPS-2 treatment. All reagents were purchased from R&D System (ND50 was 0.015–0.06 pg/ml, respectively).

2.9. Statistical evaluation

Results were expressed as the means of three experiments ± standard deviation (S.D.). Data were analysed by one-way analysis of variance (ANOVA) and the Student–Newman–Keuls test. Differences were considered statistically significant for p value of <0.05.

3. Results

3.1. Physicochemical properties of EPS-2

The molecular weight of EPS-2 was on average 400,000 Da. The optical rotation was [θ]D20 14. The infrared spectrum showed strong signal at 3420 cm−1 attributable to OH stretching, CH stretching signal at 2929 cm−1; signal at 1420 cm−1 an intense signal attributable to CH deformation and at 1055 cm−1 attributable to OH deformation. The ultraviolet spectrum showed no strong absorption peaks in the range of 350–210 nm. Hydrolysis with 2 M trifluoroacetic acid yielded mannose and glucose, as principal constituents, in a relative ratio of 0.3:1 and 1:0.2, respectively.

The measurement of the viscosity was 0.33η at a concentration of 0.5% in aqueous solution. Thermogravimetical analysis showed a weight loss due to water presence, centred at 60–80°C. From this temperature to 220°C the biopolymer was very stable and started to decompose at about 240°C indicating a behaviour similar to that of other polysaccharides.

3.2. Cytotoxicity test

As the purpose was to determine the EPS-2 concentrations that did not affect cell viability and to be used for subsequent assays, the cytotoxic effect of EPS-2 toward human PBMC and WISH cells was evaluated using the MTT assay.

Results showed in Table 1 demonstrate that EPS-2 displayed different, dose-dependent cytotoxicity in both cell culture systems employed. The EPS-2, however, was found to be non-toxic toward PBMC as well as WISH cells at concentrations of 300 µg/ml or below.

<table>
<thead>
<tr>
<th>EPS-2</th>
<th>PBMC</th>
<th>WISH</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1000 µg/ml</td>
<td>51 ± 10.8</td>
<td>60 ± 13.1</td>
</tr>
<tr>
<td>800 µg/ml</td>
<td>40 ± 7.3</td>
<td>56 ± 10.2</td>
</tr>
<tr>
<td>600 µg/ml</td>
<td>29 ± 6.8</td>
<td>33 ± 7.4</td>
</tr>
<tr>
<td>500 µg/ml</td>
<td>12 ± 2.2</td>
<td>18 ± 3.1</td>
</tr>
<tr>
<td>400 µg/ml</td>
<td>4 ± 0.6</td>
<td>7 ± 0.8</td>
</tr>
<tr>
<td>300 µg/ml</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Therefore, only concentrations of 300 µg/ml or below were selected for the subsequent studies.

3.3. Anti-HSV-2 activities of EPS-2

The inhibitory effect of EPS-2 on HSV-2 infection was investigated by plaque reduction assay. Results in Fig. 1 demonstrated that treatment of PBMC with EPS-2 inhibited HSV-2 replication in a dose-dependent manner. In particular, PBMC in the presence of 300 and 200 µg/ml of EPS-2 produced 7.5 × 104 (±0.3) PFU/ml, 9.8 × 104 (±0.5) PFU/ml respectively vs. 2.3 × 104 (±0.3) PFU/ml of untreated PBMC (p < 0.05) whereas at the concentration of 100 and 50 µg/ml it did not show any significant antiviral effect. Moreover, EPS-2 treatment on WISH cells did not show any significant inhibition of HSV-2 replication at all tested concentrations.

The antiviral activity observed on PBMC and not on WISH cells prompted us to investigate if the activity of exopolysaccharide could be related to an immunomodulatory mechanism. Since, the production of different cytokines, such as IFN-α, IL-12, IFN-γ, TNF-α, IL-18, was evaluated on EPS-2 treated PBMC infected or not with HSV-2. The results are shown in Table 2. The effect of EPS-2 was dose-dependent. High levels of all these cytokines were detected in supernatants of EPS-2 treated PBMC. It is noteworthy that, at a concentration of 300 µg/ml EPS-2 induced a significantly higher amount of IL-18 compared to that induced by 1 µg/ml LPS treatment (p < 0.05). Furthermore, EPS-2 at the concentration of 100 µg/ml triggered PBMC in releasing appreciable levels of IFN-γ, TNF-α and IL-18, whereas very low amounts of IFN-α and IL-12 productions were found. Moreover, at the concentration of 50 µg/ml only modest amounts of TNF-α was detected.

On the other hand, when PBMC were treated with EPS-2 and then infected with HSV-2, cytokine productions was downregulated. In particular, the amounts of all cytokines assayed was significantly lower (p < 0.05) with respect to those induced from the...
same concentrations (300 and 200 µg/ml) of EPS-2 by uninfected PBMC. Furthermore, the down-regulating effect of HSV-2 infection was particularly evident on cells treated with 100 µg/ml of EPS-2. In fact, the release of IFN-γ, IFN-α, and IL-12 was completely inhibited, whereas appreciable amounts of TNF-α and IL-18 were still produced. At the concentration of 50 µg/ml no cytokines were found. The significant reduction of Th1-type cytokines production by infected cells was directly related to a significant increase of viral replication (Fig. 1). In order to understand if this down-regulation could be due to the presence in supernatants of Th2 cytokines, we analysed the release of IFN-4 and IL-10.

In Table 3 are reported the results of IL-4 and IL-10 production after treatment with different concentrations of EPS-2 by PBMC infected or not with HSV-2.

### Table 3

<table>
<thead>
<tr>
<th>Inducer</th>
<th>IL-4</th>
<th>IL-10</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPS-2 (1 µg/ml)</td>
<td>82 ± 13</td>
<td>193 ± 25</td>
</tr>
<tr>
<td>None</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (300 µg/ml)</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (200 µg/ml)</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (100 µg/ml)</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (50 µg/ml)</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (300 µg/ml) + HSV-2</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (200 µg/ml) + HSV-2</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (100 µg/ml) + HSV-2</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>EPS-2 (50 µg/ml) + HSV-2</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
</tr>
<tr>
<td>HSV-2</td>
<td>&lt;0.06</td>
<td>&lt;0.06</td>
</tr>
</tbody>
</table>

| Significant difference (p < 0.05) compared with EPS-2 treated and HSV-2 infected. |
| Significant difference (p < 0.05) compared with untreated controls. |

EPS-2 treatment did not trigger uninfected-PBMC to release IL-4 and IL-10 that are strong hallmarks of Th2 responses, at any concentration tested. Whereas, the HSV-2 infection of untreated PBMC induced an appreciable amounts of IL-4 and IL-10. Furthermore, the HSV-2 infection of PBMC treated with 300 and 200 µg/ml of EPS-2 did not produce any amount of IL-4 and IL-10, whereas, at the concentration of 100 and 50 µg/ml, unable to hinder virus replication, we found unexpected amounts of IL-4 and IL-10.

In order to verify if amounts of IL-4 and IL-10, detected at the concentrations of 100 and 50 µg/ml of EPS-2, could be due to presence in supernatants of IL-18 or TNF-α, we added monoclonal antibodies vs. IL-18 or vs. TNF-α to PBMC treated with 100 µg/ml of EPS-2 and infected with HSV-2. The results (Table 4) demonstrated that the neutralization of IL-18 determined a significant down-regulation of production of IL-4 and TNF-α, whereas no effect was observed on IL-10 production. Conversely, the neutralization or addition of TNF-α did not produce any effect on production of cytokines (data not shown). Furthermore, the addition of Rh-IL-18 (20 and 40 ng/ml) triggered PBMC infected with HSV-2 to release marked amounts of IL-4 and TNF-α in a dose-dependent manner, whereas did not exert any effect on IL-10 production. These results indicated that the addition of exogenous Rh-IL-18 had the capacity to modulate the production of IL-4 as well as TNF-α.

### Table 4

<table>
<thead>
<tr>
<th>Inducer</th>
<th>IL-4</th>
<th>IL-10</th>
<th>TNF-α</th>
<th>IL-18</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPS-2 (100 µg/ml)</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
<td>508 ± 41</td>
<td>51 ± 6.7</td>
</tr>
<tr>
<td>EPS-2 (50 µg/ml)</td>
<td>&lt;0.1</td>
<td>&lt;0.05</td>
<td>121 ± 20</td>
<td>&lt;9.2</td>
</tr>
<tr>
<td>EPS-2 (100 µg/ml) + HSV-2</td>
<td>48 ± 4.8*</td>
<td>22 ± 3.1</td>
<td>106 ± 24*</td>
<td>43 ± 2.9</td>
</tr>
<tr>
<td>EPS-2 (50 µg/ml) + HSV-2</td>
<td>30 ± 4.2*</td>
<td>20 ± 4.6</td>
<td>&lt;0.13</td>
<td>&lt;9.2</td>
</tr>
<tr>
<td>HSV-2</td>
<td>34 ± 3.1</td>
<td>29 ± 5.8</td>
<td>&lt;0.13</td>
<td>&lt;9.2</td>
</tr>
<tr>
<td>Rh-IL-18 + HSV-2</td>
<td>18 ± 2.3</td>
<td>31 ± 6.2</td>
<td>28 ± 8.6</td>
<td>–</td>
</tr>
<tr>
<td>Rh-IL-18 + HSV-2</td>
<td>95 ± 11§</td>
<td>19 ± 6.8</td>
<td>344 ± 29§</td>
<td>–</td>
</tr>
</tbody>
</table>

| Significantly different (p < 0.05) compared with amounts induced by neutralization of IL-18. |

### Discussion

The efficient elimination of infectious agents such as viruses requires a proinflammatory host response and development of type 1 immunity [32]. This type of response is characterised by activation of mononuclear cells and production of proinflammatory cytokines such as interferons (IFN), tumor necrosis factor (TNF) and...
interleukins (IL-12 and IL-18). In fact, these cytokines, regulating the inflammatory and immune responses, may induce an antiviral state in the cells (i.e. via IFNγ) or destroy virus-infected cells (i.e. via TNF-α), may stimulate cytotoxicity and cytokine production by T-cells and NK cells, and may initiate development of Th1 cells (i.e. via IFNγ, IL-12 and IL-18) [33].

On the other hand, viruses have devised multiple mechanisms to manipulate cytokine production, in order to survive as successful pathogens [34,35].

Polysaccharides with high molecular weight, >90,000 Da, are good immunogens [7]. In vitro studies demonstrated that sulphated polysaccharides, such as dextran sulphated, have antiviral effects against enveloped viruses (herpes simplex virus, human cytomegalovirus, vesicular stomatitis virus, Sindbis virus and human immunodeficiency virus) [36]. The sulphated EPS are known to interfere with the absorption and penetration of viruses into host cell and to inhibit various retroviral reverse transcriptases [37,38].

Our in vitro results indicate that the HSV-2 infection of PBMC do not induce any amounts of type 1 cytokines; whereas an appreciable production of IL-4 and IL-10 was found. On the basis of this datum, it can be hypothesized that the induction of IFN-γ and TNF-α, IL-12 and IL-18 is not equally pronounced. In particular, the EPS-2 (100 μg/ml) played a protective role during HSV-2 infection increasing, in a dose-dependent way, the inflammatory response supported by Th-1 cytokines such as IFN-γ, IFN-α, TNF-α, IL-12 and IL-18. It is noteworthy that, at a concentration of 300 μg/ml EPS-2 induced a significantly higher amount of IL-18 compared to that induced by 1 μg/ml LPS treatment.

The treatment of PBMC with 100 μg/ml of EPS-2 did not hinder HSV-2 replication, but was still able to trigger infected PBMC to release some type 1 cytokine such as TNF-α and IL-18, as well as appreciable amounts of IL-4 and IL-10. In particular, the level of IL-4 was higher than that induced by virus alone. In order to verify whether the production of IL-4 was supported by the virus and/or by TNF-α and IL-18 induced by EPS-2, in further experiments we neutralized IFN-γ or TNF-α with monoclonal antibodies. Conversely, in other experiments the same recombinant cytokines were added to PBMC. Our results demonstrate that TNF-α could not modulate IL-4 production whereas, surprisingly, the levels of the same cytokine were markedly increased, in a dose-dependent way, by addition of exogenous Rh-IL-18.

Data obtained by neutralization of IL-18 induced by EPS-2, as well as by addition of Rh-IL-8, suggest that different amounts of EPS-2 (100 μg/ml and even more evidently 50 μg/ml), are unable to restrict the replication of HSV-2. Moreover, the virus in turn induces type 2 cytokines such as IL-4 and IL-10 supporting its own replication. In particular, the EPS-2 (100 μg/ml) induced IL-18 seems to enhance the viral strategy of immune evasion by upregulating the production of IL-4.

On the contrary, EPS-2 at higher concentrations (300 and 200 μg/ml) induces the release of a greater amount of Th1 cytokines. As a consequence the development of a cytokine network with a net proinflammatory effect, related to increased resistance to viral replication, can be hypothesized.

These data support the notion that, in contrast with the exclusive IFN-γ inducer IL-12, IL-18 can behave in a bivalent way as a costimulatory factor in both pro-Th1 and pro-Th2 responses depending on the cytokine profile present in the microenvironment.

Therefore, the effects of this innovative compound may contribute to improve immune surveillance of PBMC toward virus infection, by triggering polarization in favor of the Th1 immune response. Thus, our results could represent the basis to support the potential strategy of EPS-2 for therapeutic manipulation direct toward equilibrating the immune response in viral diseases. Taken together, our results seems to indicate the potential role of EPS-2 toward equilibrating the immune response during viral infection.

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References


