Antioxidant and anti-inflammatory properties of a Cucumis melo LC. extract rich in superoxide dismutase activity

Ioannis Vouldoukis a, b, Dominique Lacan c, Caroline Kamate a, Philippe Coste c, Alphonse Calenda a, Dominique Mazier b, Marc Conti b, Bernard Dugas a, b, ∗

a Isocell Nutra SAS, 53 Bld du Général Martial Valin, 75015 Paris, France
b INSERM U511, Immunobiologie Cellulaire et Moléculaire des Infections Parasitaires, CHU Pitié Salpêtrière, Université Paris VI, 75013 Paris, France
c BIONOV sarl, 84911 Avignon, France

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Abstract

The present study was conducted to evaluate in vitro and in vivo the antioxidant and anti-inflammatory properties of a cantaloupe melon (Cucumis melo LC. Cucurbitaceae) extract (CME) selected for its high superoxide dismutase activity. Peritoneal macrophages were pre-activated in vitro with 300 IU of interferon-γ (IFN-γ) and were then challenged in culture with IgG1/anti-IgG1 immune complexes (IgG1 IC) in presence of various CME extracts. The subsequent production of free radicals (superoxide anion, nitric oxide, and peroxynitrite) and of pro-(TNF-α) and anti-(IL-10) inflammatory cytokines was evaluated. The CME inhibited in a dose-dependent manner the production of superoxide anion with a maximal effect at 100 µg/ml. This inhibitory effect of CME appeared to be closely linked to the SOD activity because it was dramatically decreased after heat inactivation of the SOD activity (HI-CME). In addition, the CME inhibited the production of peroxynitrite strengthening the antioxidant properties of this CME rich in SOD activity. The production of the pro- and anti-inflammatory cytokines, namely TNF-α and IL-10, being conditioned by the redox status of macrophages we also evaluated the effect of CME and HI-CME on the IgG1 IC-induced cytokine production. When the SOD activity was present in the CME it promoted the IgG1 IC-induced production of IL-10 instead of TNF-α. These data demonstrated that, in addition to its antioxidant properties, the anti-inflammatory properties of the CME extract were principally related to its capacity to induce the production of IL-10 by peritoneal macrophages. The particular properties of wheat gliadin (Triticum vulgare, Poaceae) for the oral delivery of functional proteins led us to test it in a new nutraceutical formula based on its combination with the CME thus monitoring the SOD activity release during the gastro-intestinal digestive process. In these experiments C57BL/6 mice were supplemented orally everyday during 28 days with: (1) the placebo, (2) the CME extract alone, (3) the gliadin, (4) the CME/gliadin combination, or (5) the HI-CME/gliadin combination (SOD inactivated). At the end of the supplementation period all the animals were injected intra-peritoneal (i.p.) with the pro-inflammatory cytokine IFN-γ (300 IU) and peritoneal macrophages were harvested 24 h after to test their capacities to produce free radicals, TNF-α and IL-10 after triggering with IgG1 IC. We demonstrated that animals supplemented during 28 days with the CME/gliadin combination were protected against the pro-inflammatory properties of IFN-γ while the other products were inefficient. These data did not only indicate that the SOD activity is important for the antioxidant and anti-inflammatory properties of the CME extract, but also demonstrated that when the SOD activity is preserved during the digestive process by its combination with wheat gliadin it is possible to elicit in vivo the pharmacological effects of this antioxidant enzyme.

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Keywords: Superoxide dismutase; Cucumis melo; Antioxidant; Inflammation

1. Introduction

Several classes of antioxidant dietary compounds have been suggested to present health benefits, and there are evidences that consumption of these products leads to a reduction of the expression of various pro-inflammatory and/or oxidative stress biomarkers (Halliwell, 2002; Peng et al., 2000; Jacob et al., 2003). The active principles in these vegetal extracts are principally water soluble or lipophilic antioxidant molecules. Indeed, most of these plant extracts contain various amounts of Vitamin E, Vitamin C, β-carotene, and other flavonoids (Aruoma, 1994; Aruoma,
2003), and were used as potential antioxidant prophylactic agents for both health and disease management (Peng et al., 2000; Clarkson and Thompson, 2000; Ursu and Clarkson, 2003; Diplock et al., 1998; Sardesai, 1995). However, and until now it was not possible to use the antioxidant enzymes (e.g. superoxide dismutase (SOD) etc.) naturally present in various plant extracts (Sandalto et al., 1997; Gardner, 1984) as nutritional supplement. Indeed, these antioxidant enzymes are usually inactivated and digested all along the gastro-intestinal transit thus destroying the antioxidant pharmacological properties of these detoxifying proteins (Giri and Misra, 1984; Zidenberg-Cherr et al., 1983).

The recent development of new vegetal galenic systems allows the oral delivery of functional proteins and peptides (Vasir et al., 2003; Chourasia and Jain, 2003). Among them the biopolymeric wheat gliadin was shown to protect active molecules against the digestive process (Redl et al., 1996; Renard et al., 2002; Mauguet et al., 2002), but also to interact with the intestinal epithelial barrier, thus assuming the mucosal delivery of the active molecules (Fassano et al., 2000; Ezpeleta et al., 1999; Coyler et al., 1987). Indeed, with respect to the differential binding pattern of gliadin in healthy and celiac disease patients (Pittschier et al., 1994) we have considered that the hydrophobic interactions between gliadin and other proteins could define a new class of effective product that promote the oral delivery of functional proteins such as SOD (Calderon de la Barca et al., 1996). The resulting monophasic gliadin/protein combination is then able to target the biological surface of enterocytes (Coyler et al., 1987; Farre-Castany et al., 1995) and thus increases protein absorption to promote the pharmacological effect of the protein. These drug delivery systems open new fields of investigation for functional proteins by the oral route not only because they protect them against the digestive process but also because they target proteins or peptides to a specific body site controlling their release rates and thus displaying an enormous impact on the healthcare system.

During the last decade important efforts were made to develop vegetal antioxidant enzymes (e.g. SOD) as nutraceutical products but the results were disappointing because of the poor bioavailability of these non-protected molecules (Regnault et al., 1996; Giri and Misra, 1984; Zidenberg-Cherr et al., 1983). In the present study we demonstrated that the SOD activity present in a Cucumis melo LC extract (cell line 95LS444, USA Patent, 5,747,043) can be combined to the mucosal delivery system formed by the biodegradable gliadin biopolymer and thus can be delivered efficiently by the oral route. The advantages of delivering proteins such as the antioxidant enzyme SOD, are to develop new classes of nutraceutical supplements that could reduce the biological disorders induced by various pro-inflammatory and/or oxidative stress mechanisms (Germano, 2001; Dugas, 2002; US Patent 6,045,809).

2. Material and methods

2.1. Animals, chemicals, and reagents

C57BL/6 mice (6–8 weeks) were purchased from IFIA-Credo (Orleans, France). Dulbecco’s modified Eagle’s medium (DMEM) containing t-glutamine and 4.5 g/l glucose was obtained from Sigma Chemical Co. (St Louis, MO, USA), as were all reagents not otherwise noted. Recombinant murine interferon-γ (IFN-γ) was purchased from ImmungeneX (Los Angeles, CA). The melon extract isolated from the Cucumis melo LC pulp (US Patent 5,747,043) revealed an SOD activity of 100 IU/NBT/mg of powder which is 5–7 times more than in the classical “melon charantais.” All tissues and culture reagents and Cucumis melo extracts (CME) were assayed for endotoxin contamination by the Limulus lysate test (E-Toxate, Sigma) and were found to be less than 10 pg/ml. Heat-inactivated SOD in the CME (HI-CME) was obtained after warming the extract at 56 °C during 30 min. The content in other antioxidants in HI-CME was verified and remained unaffected after warming (data not shown).

2.2. Peritoneal macrophages preparation

Peritoneal cells were isolated from C57BL/6 mice after washing the peritoneal cavity with 5 ml of physiological water. The cells were washed twice and seeded at densities of 5–6 × 10⁵ cells/cm² on petri dishes (100 × 15 mm) in RPMI-1640 (GIBCO, Grand Island, NY) containing 10% heat-inactivated fetal bovine serum (FBS), penicillin (100 IU/ml), and streptomycin (100 µg/ml). RPMI-FBS. Peritoneal macrophages were allowed to adhere for 2–3 h in 5% CO₂ humidified atmosphere. Then, the non-adherent cells were removed by washing twice with 10 ml pre-warmed medium and dishes were incubated for 10 min at 4 °C. The supernatants were then carefully removed and discarded and the plates were washed once with pre-warmed Dulbecco’s phosphate buffered saline (PBS: GIBCO). Cold PBS (15 ml) containing 1.5% FBS (PBS-FBS) was first added followed by 0.3 ml of 0.1 M EDTA (pH 7.0). The plates were incubated for 15 min at room temperature and the macrophages removed by 10 washes using a 10 ml syringe. The viability of the resulting cells was assessed by Trypan blue exclusion and the rate of macrophages determined after cytoplasmic staining with acridine orange and examination using a fluorescence microscope. Cell preparations were >95% viable and contained >95% macrophages.

2.3. Macrophage culture and activation

Peritoneal macrophages were maintained in DMEM containing 3.7 g/l of sodium bicarbonate supplemented with 10% of fetal calf serum (Bioproducts, France) and 1% of non-essential amino acids, in a humidified 37 °C atmosphere with 10% CO₂. For the in vitro activation, macrophages
were seeded at 10^6 cells/ml, 100 μl/well in 96-well tissue culture plates (Becton Dickinson, Grenoble, France). Plates were incubated for approximately 1–2 h prior to activation to allow adherence of macrophages. Macrophages were then activated during 48 h with IFN-γ (300 IU/ml) and then challenged with IgG1IC complexes (anti-IgG1 20 μg and IgG1 100 μg) as previously described (Vouldoukis et al., 2000) in the presence or in the absence of different doses of CME or HI-CME. The production of TNFα, IL-10, and nitrite were checked in the cell-free supernatant after 24 h while only after 2 h for the cellular production of superoxide anion and peroxynitrites.

2.4. Preparation of the CME/gliadin combination

Considering their hydrophobic nature, wheat gliadin biopolymers (Calderon de la Barca et al., 1996) were evaluated to improve the oral bioavailability of drugs (Regnault et al., 1996; Ezpeleta et al., 1999). In the present study, we used these hydrophobic gliadin biopolymers (Triticum vulgare) to preserve the SOD activity present in the CME during the digestive process (Stella V et al., 1995) and to activate the intestinal mucosal immune system (Fassano set al., 2000).

The CME/gliadin combinations were done as already described for many other drug delivery systems (Stella et al., 1995; Ezpeleta et al., 1999; Mauguet et al., 2002). Briefly, CME (100 IU/mg) preparations were mixed with gliadin in a 40% hydro-alcoholic solution, in such a ratio that the global SOD activity contained in the CME/gliadin combination was checked after dissolution of the product in acetic acid as already described (Stella et al., 1995). Briefly, the SOD activity was measured in the solubilized CME/gliadin combination by the reduction of nitroblue tetrazolium NBT in the presence of 100 nM of dihydrorhodamine 123. The peroxidation of dihydrorhodamine 123 was checked after addition of IC. Cells (2 × 10^5/ml) were pre-incubated for 6 h in the presence of 100 nM of dihydrorhodamine 123. The suspension was pre-warmed at 37 °C before a 50 μl addition of IgG1 immune complexes (IgG1IC) and fluorescence was then measured on a spectrophotometer (LS-50, Perkin-Elmer Corporation, Norwalk, CT) with excitation and emission slit widths of 2.5 and 3.0 nm, respectively, and excitation wavelength of 500 and 536 nm, respectively, and emission wavelength of 500 and 536 nm, respectively, and excitation and emission slit widths of 2.5 and 3.0 nm, respectively, and 30 min after addition of IC. Cells (2 × 10^5/ml) were measured under the different culture conditions.

2.5. Oral supplementation with free CME, gliadin, or the CME/gliadin preparations

C57BL/6 mice of 6–8 weeks old (25–30 g) were divided into five groups (n = 10) receiving, respectively, by oral force feeding during 28 days: (1) the placebo in water, (2) the CME (0.05 mg equivalent of 5 IU/NBT of SOD), (3) the gliadin (1 mg), (4) the gliadin/CME combination (5 mg equivalent to 5 IU/NBT of SOD), and (5) the gliadin/HI-CME combination (5 mg) inactivated for the digestive process (Vouldoukis et al., 1995) and to activate the intestinal mucosal immune system (Fassano et al., 1995). The generation of O$_2$•− was assayed by measuring the reduction of ferricytochrome C at 37 °C by adherent cells (McCord and Fridovich, 1989) pre-incubated in the presence of 150 μM ferricytochrome C. After 150 μM ferricytochrome C. After 2 additional hours of incubation in the presence or in the absence of IgG1IC the absorbance change was assayed at 550 nm in a spectrophotometer.

To measure ONOO$^-$ production was evaluated after oxidation of dihydroethidium 123 to fluorescent rhodamine as previously described (Kony et al., 1994). Briefly, 5 × 10^5 cells in 500 μl RPM1 medium were pre-incubated for 6 h in the presence of 100 nM of dihydroethidium 123. The suspension was pre-warmed at 37 °C, before a 50 μl addition of IgG1 immune complexes (IgG1IC) and fluorescence was then measured on a spectrophotometer (LS-50, Perkin-Elmer Corporation, Norwalk, CT) with excitation and emission wavelength of 500 and 536 nm, respectively, and excitation and emission slit widths of 2.5 and 3.0 nm, respectively, and 30 min after addition of IC. Cells (2 × 10^5/ml) were measured under the different culture conditions.

2.6. TNF-α and IL-10 measurements

At the end of the culture period the cell-free supernatants were kept at −80°C in order to avoid TNF-α degradation. TNF-α and IL-10 levels were measured using ELISA Kits from British Biotechnology (England) and the threshold of detection were 10 pg/ml.

2.7. Assay for O$_2$•−, NO•, H$_2$O$_2$, and ONOO$^-$ production by resting and activated cells

To assess the amount of NO produced, the stable end product of NO, NO$_2$− was measured using the Griess reaction as previously described (Vouldoukis et al., 1995, Dugas et al., 1996). The generation of O$_2$•− was assayed by measuring the reduction of ferricytochrome C at 37 °C by adherent cells (McCord and Fridovich, 1989) pre-incubated in the presence of 150 μM ferricytochrome C. After 2 additional hours of incubation in the presence or in the absence of IgG1IC the absorbance change was assayed at 550 nm in a spectrophotometer.

3. Results

3.1. Effect of the CME extract on the redox status of macrophages

The CME extract is a rich antioxidant nutritional extract that naturally contain a high SOD activity (an average...
Table 1: Antioxidant composition of the 95LS444 Cucumis melo LC extract (CME).

<table>
<thead>
<tr>
<th>Enzymes</th>
<th>Amounts in the extract (IU/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide dismutase</td>
<td>95 ± 8 IU/mg</td>
</tr>
<tr>
<td>Catalase</td>
<td>10 ± 2 IU/mg</td>
</tr>
<tr>
<td>Glutathione peroxidase</td>
<td>1 ± 0.5 IU/mg</td>
</tr>
<tr>
<td>Natural antioxidant</td>
<td></td>
</tr>
<tr>
<td>Co-enzyme Q10</td>
<td>54.0 ± 4 mg/100 g</td>
</tr>
<tr>
<td>Lipase</td>
<td>19.3 ± 1 mg/100 g</td>
</tr>
<tr>
<td>GSX</td>
<td>215 ± 12 µg/100 g</td>
</tr>
<tr>
<td>GSNG</td>
<td>507 ± 53 µg/100 g</td>
</tr>
<tr>
<td>Selenium</td>
<td>2.5 ± 0.2 µg/100 g</td>
</tr>
<tr>
<td>Carotenoids</td>
<td>350 ± 34 µg/100 g</td>
</tr>
<tr>
<td>Vitamin E</td>
<td>240 ± 22 µg/100 g</td>
</tr>
<tr>
<td>Vitamin A</td>
<td>10000 ± 154 µg/100 g</td>
</tr>
<tr>
<td>Vitamin C</td>
<td>5000 ± 523 µg/100 g</td>
</tr>
</tbody>
</table>

Table 2: Effect of CME and heat-inactivated HI-CME on superoxide, nitric oxide, and peroxynitrite production by IFN-γ-activated peritoneal macrophages challenged with IgG1 IC.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Superoxide (µM)</th>
<th>Nitrite (µM)</th>
<th>Peroxynitrite (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>0.1 ± 0.2</td>
<td>0.4 ± 0.1</td>
<td>35 ± 12</td>
</tr>
<tr>
<td>CME</td>
<td>1.2 ± 0.3</td>
<td>0.8 ± 0.2</td>
<td>30 ± 9</td>
</tr>
<tr>
<td>HI-CME</td>
<td>1.0 ± 0.2</td>
<td>0.7 ± 0.1</td>
<td>25 ± 3</td>
</tr>
<tr>
<td>HI-CME</td>
<td>2.6 ± 0.3</td>
<td>0.8 ± 0.3</td>
<td>195 ± 7</td>
</tr>
</tbody>
</table>

Fig. 1. In vitro effects of CME and heat-inactivated CME (HI-CME) on IgG1 IC-induced superoxide anion production by IFN-γ-activated macrophages. Peritoneal macrophages (10^6 cells/ml) were pre-activated for 48 h in the presence of IFN-γ (100 IU/ml) and then challenged during 2 h with IgG1 IC in presence of various doses of CME or HI-CME.

As described in Section 2, peritoneal macrophages (10^6 cells/ml) were pre-activated during 48 h with IFN-γ (500 IU/ml). Cells were then challenged with IgG1 IC in presence or in absence of the CME extracts. After 2 h, the generation of superoxide anion was assayed by reduction of ferricytochrome C and peroxynitrite was measured by the induction of rhodamine fluorescence. Nitric oxide production was evaluated after measurement of NO_2^- production in the cell-free supernatant after 24 h of culture. The data represent the mean ± S.D. of quadruplicates of one representative experiment out of six.

CME could reduce the production of superoxide anion by IgG1 IC-treated macrophages and the NO_2^- production was significantly reduced in the presence of CME. The oxidative metabolism being intimately linked to the pro- and/or anti-inflammatory capacities of macrophages we evaluated the effect of the different CME extracts on the IgG1 IC-induced production of superoxide anion by macrophages. Stimulation of IFN-γ-activated macrophages by IgG1 IC-induced production of superoxide and peroxynitrites by macrophages.

3.2. Effect of the CME extract on the production of TNF-α and IL-10 by macrophages

The oxidative metabolism being intimately linked to the pro- and/or anti-inflammatory capacities of macrophages we evaluated the effect of the different CME extracts on the IgG1 IC-induced production of TNF-α (pro-inflammatory cytokine) and IL-10 (anti-inflammatory cytokine) production by macrophages. Stimulation of IFN-γ-activated macrophages by IgG1 IC-induced production of TNF-α and of IL-10 (Fig. 2). The inverse correlation observed between TNF-α and IL-10 concentrations in these experiments suggests the existence of a physiological equilibrium between the production processes of these two cytokines.

of 100 IU/NBT per mg of dry extract) but also catalase activity (10 IU/mg), residual Gpx activity and number of natural antioxidant quenching molecules (Table 1). Considering that antioxidants are essential for reducing oxidative stress (Halliwell et al., 1995; Yagi et al., 2002; Liu et al., 1998; Lee and Man-Fan Wan, 2000) and that heterologous SOD (antigenic) may have some immunoregulatory properties (He et al., 2002; Mullerad et al., 2002), we evaluated in vitro the effect of the crude CME on resident peritoneal macrophages of C57BL/6 mice. As shown in Fig. 1, the crude CME extract retaining its SOD activity inhibited in a dose-dependent manner the production of superoxide anion by IgG1 IC-stimulated macrophages, the maximal inhibitory effect being reached at 100 µg/ml of CME (equivalent of 10 IU/NBT per mg of dry extract) but also catalase activity (10 IU/mg), residual Gpx activity and number of natural antioxidant quenching molecules (Table 1). Consid-
ering that antioxidants are essential for reducing oxidative stress (Halliwell et al., 1995; Yagi et al., 2002; Liu et al., 1998; Lee and Man-Fan Wan, 2000) and that heterologous SOD (antigenic) may have some immunoregulatory properties (He et al., 2002; Mullerad et al., 2002), we evaluated in vitro the effect of the crude CME on resident peritoneal macrophages of C57BL/6 mice. As shown in Fig. 1, the crude CME extract retaining its SOD activity inhibited in a dose-dependent manner the production of superoxide anion by IgG1 IC-stimulated macrophages, the maximal inhibitory effect being reached at 100 µg/ml of CME (equivalent of 10 IU/NBT per mg of dry extract). With the heat-inactivated CME (HI-CME, lacking its SOD activity) we observed a significant difference in the inhibitory effect. This suggested that even whether the other antioxidant products were able to reduce the production of superoxide anion by IgG1 IC-treated macrophages the SOD activity was essential to reduce oxidative stress.

Considering that the IgG1 IC-stimulation process is known to stimulate the NO-dependent pathway we evaluated the effect of the two different extracts on the production of nitrogen species derived from the iNOS pathway (Dugas et al., 1995) and on the production of the highly pro-oxidant perox-
ynitrates that results from the chemical combination of NO^• with O_2^•- (Ischiropoulos et al., 1992). As demonstrated in Table 2, both extracts were able to reduce the production of peroxynitrates without affecting significantly the NO pro-
duction but this inhibitory effect was more pronounced with CME extract when the SOD activity was maintained. Taken together these data suggested that even whether we cannot totally exclude the inhibitory effects of the different antiox-
idant products (enzyme and/or quencher molecules) present in the CME, the SOD activity is at least responsible for the suppressive effect of CME on IgG1 IC-induced production of superoxide and peroxynitrates by macrophages.
Fig. 2. Correlation between TNF-α and IL-10 production by IFN-γ-activated macrophages stimulated with IgG1 IC. Peritoneal macrophages (10^6 cells/ml) were pre-activated for 48 h in the presence of IFN-γ (300 IU/ml) and then challenged during 24 h with IgG1 IC. Cell-free supernatants were then harvested and frozen prior TNF-α and IL-10 measurements by specific ELISA. Data represent the mean ± S.E.M. of four different experiments, and correlation between IL-10 and TNF-α contents was evaluated using the Spearman rank test.

In the presence of CME the production of TNF-α was significantly reduced (P < 0.01) whereas the IL-10 production was enhanced (P < 0.01) suggesting that the oxidative metabolism is likely involved in the different processes of cytokine productions (Fig. 3). Interestingly, in the same experiments the HI-CME reduced the production of TNF-α (P < 0.05) but did not significantly affect the production of IL-10 suggesting that the SOD activity in the CME extract is an important component to promote its anti-inflammatory properties.

3.3. In vivo anti-inflammatory properties of the CME extract combined or not to a wheat gliadin

The possible use of hydrophobic gliadin biopolymers not only as potent drug delivery system (Stella et al., 1995; Ezpeleta et al., 1999; Mauguet et al., 2002), but also as promoter of mucosal tolerance immunity (Rossi et al., 1999; Husby, 2000), led us to evaluate the anti-inflammatory effect of oral supplementations with the CME extract. Considering the relative activity of the SOD in the CME extract we have designed a 28 days long protocol of oral supplementation which correspond to the minimal period of treatment to obtain an in vivo anti-inflammatory effect on chronic diseases (Baret et al., 1984; Jadot et al., 1995). After this period of supplementation, resident peritoneal macrophages were harvested to evaluate their pro- and anti-inflammatory profiles. As shown in Fig. 4, after the CME/gliadin supplementation period the IgG1 IC-induced TNF-α (Fig. 4A) production in IFN-γ-activated macrophages was significantly reduced (P < 0.01) whereas the production of IL-10 (Fig. 4B) was significantly enhanced (P < 0.01). When compared to the other per os treatment it was found that the non-protected CME extract was unable to affect the IgG1 IC-induced production of TNF-α (Fig. 4A) and of IL-10 (Fig. 4B), whereas the gliadin alone or the gliadin/HI-CME (without SOD activity) slightly (P < 0.05) enhanced the production of...

Fig. 5. Effect of per os mice supplementation by CME, gliadin, CME/gliadin, or HI-CME/gliadin on superoxide (A) nitric oxide (B), and peroxynitrite (C) production after injection i.p. of IFN-γ. Peritoneal macrophages from animals that received i.p. injections of 300 U.E. of IFN-γ as described in Fig. 4, (10^6 cells/ml) were challenged during 24 h with IgG1 IC. After 2 h, the generation of superoxide anion (A) was assayed by reduction of ferricytochrome C and peroxynitrite was measured by the induction of rhodamine fluorescence (C). The nitric oxide production was evaluated after measurement of NO_2− in the cell-free supernatant after 24 h of culture (B). Data represent the mean ± S.E.M. of 10 different mice.

TNF-α (Fig. 4A) but did not affect the production of IL-10 (Fig. 4B).

In a second set of experiments we demonstrated that this protective effect of CME/gliadin supplementation was effective even after intra-peritoneal injection of the pro-inflammatory cytokine IFN-γ (300 U.E.). As demonstrated in Fig. 5, the IFN-γ injection not only induced the production of the pro-inflammatory cytokine TNF-α by IgG1 IC-activated macrophages but also promoted an important oxidative stress as revealed by the production of superoxide anion (Fig. 5A), nitric oxide (Fig. 5B), and peroxynitrite (Fig. 5C). In the same experiments only animals supplemented with CME/gliadin were protected against the pro-inflammatory and pro-oxidative effects of IFN-γ (Murata et al., 2002). Taken together these data confirmed that, even whether we cannot exclude the effect of the other antioxidant components, the SOD activity is essential for the anti-inflammatory effect of the CME. In addition, they also demonstrated that the binding abilities of gliadin polymers with epithelial intestinal cells allowed to obtain a new oral combination able to promote the anti-inflammatory properties of the CME extract thus defining a new class of antioxidant nutraceutical product, GliSODin® (Germano, 2001; Dugas, 2002).
4. Discussion

Intensive research was conducted over the last 10 years to promote the antioxidant nutritional medicine in the nutraceutical field (Lee et al., 2003; Dugas et al., 1999; Introna et al., 1997). Plant extracts with antioxidant activities were traditionally used to strengthen the natural immune defences and to promote host defence mechanisms (Panossian et al., 1999; Xiao et al., 1993). However, even whether these plant extracts are of interest as immune support it is always very difficult to clearly demonstrate in vivo their immune supporting effects. In the present work we demonstrated that Cucumis melo L.C. extracts rich in SOD activity are able to promote concomitantly antioxidant and anti-inflammatory properties likely by inducing the production of IL-10 by macrophages. The SOD activity in these extracts appeared to be important since the production of IL-10 was significantly reduced after heat inactivation of the enzymatic activity. These data fit with previous reports indicating that the anti-inflammatory properties of SOD were not only related to its enzymatic antioxidant property but also to the concomitant stimulation of the production of anti-inflammatory and immuno-regulatory molecules (Vouldoukis et al., 2000; Mullerad et al., 2002; He et al., 2002). Even whether we cannot exclude the effect of the other antioxidant molecules in the CME extracts we demonstrated that the SOD activity present in this extract was able to control the production of the redox-sensible anti-inflammatory cytokine, IL-10 (Haddad and Fahlman, 2002). The antioxidant and anti-inflammatory effects of melon extracts have been already described (Murcia et al., 2001; Campanella et al., 2003) but little was known on the potent pharmacological effect of the SOD activity in these extracts (Palma et al., 1997; Sandaio et al., 1997). In the present report and according to the current literature on SOD (Torrens, 1988), we demonstrated that the SOD activity in the melon extract is essential for its pharmaco-protective effect.

In addition, we demonstrated that the combination of CME with other vegetal extracts such as hydrophobic gliadin biopolymers, led to the development of a 100% vegetal product that promotes the oral pharmacology of SOD (Germano, 2003; Dugas, 2002). Indeed, we demonstrated that the per os supplementation by the CME/gliadin combination (GliSODin®) is essential to promote its delivery in the intestinal mucosa and to trigger the immune system. Without excluding the effects of other components (antioxidant molecules present in the CME and/or gliadin) once absorbed in the intestinal mucosa, the antigenic melon SOD is able to promote locally the activation of the immune system and then to induce in cascade the activation of macrophages in the overall body. In that particular situation the antigenic SOD can be presented as a ying/yang immunoregulatory molecule. Indeed, the intrinsic antioxidant/anti-inflammatory properties of SOD limit the consequence of an uncontrolled activation of the immune system that could induce certain immuno-pathological situations (Lipscomb and Masten, 2002). This dual function of the antigenic SOD defines a new class of super-antioxidant molecules that not only present the classical antioxidant properties of such molecules but also stimulate a protective immune response (Germano, 2001; Erwonwu and Sanders, 2001). At the nutritional level, the development of this novel class of molecule is of importance because in the search of nutritional antioxidant efficacit the CME/gliadin combination (GliSODin®) provides useful tools for the validation of new health nutraceutical biomarkers (Griffiths et al., 2002; Lindsay and Astley, 2002; Potischman, 2003). In conclusion, our results provide strong evidence that in the present Cucumis melo extract, the SOD activity is essential to promote its antioxidant and anti-inflammatory properties. In addition, we demonstrated that, using vegetal hydrophobic biopolymers, it is possible to define a new
class of super-antioxidant nutraceuticals that promote the oral pharmacology of the SOD.

References


