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Changes in the Superoxide Production and Other Macrophage Functions Could Be Related to the Mortality of Mice with Endotoxin-Induced Oxidative Stress

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Summary

Free radicals and proinflammatory cytokines from phagocytes have been implicated in the pathogenesis of endotoxic shock, a disease with high mortality caused by Gram-negative bacterial endotoxin. In the present study, male BALB/c and Swiss mice received intraperitoneally lipopolysaccharide (LPS) at 100 mg/kg and 150 mg/kg, respectively, that led to a lethal endotoxic shock (100 % of mortality before 30 h). Swiss mice injected with 100 mg/kg, that did not show lethal endotoxic shock, were also studied. Peritoneal macrophages were obtained from animals at 2, 4, 12 or 24 h after injection of LPS or saline (control) solutions. Superoxide anion and tumor necrosis factor (TNFα) production were determined in these cells as well as other functions such as adherence capacity, chemotaxis and phagocytosis. The increase in superoxide anion production after endotoxin injection was higher in cells from mice with lethal shock than in those with non-lethal shock. However, the enhancement of TNFα production was similar in all cases, although in Swiss mice the highest levels of TNFα were observed at 1.5 h after endotoxin injection, while in BALB/c mice they occurred at 2 h after LPS injection. This oxidative stress was also revealed by the other functions analyzed, since adherence to substrate and phagocytosis were stimulated and chemotaxis was decreased after endotoxin injection as compared to controls, the differences being even more significant in animals with lethal shock. These data suggest that these changes, mainly the increased production of free radicals even more than the TNFα release, could be involved in mouse mortality caused by LPS.

Key words

Immune function • Macrophage • Mice • Oxidative Stress • Superoxide anion

Introduction

Septic shock is a systemic response that develops as the result of severe infection. The shock caused by Gram-negative bacteremia is often associated with a high mortality. Lipopolysaccharide (LPS), a major component of the outer membrane of Gram-negative bacteria, is the endotoxin considered to be responsible for initiation of host response to these microorganisms (Yoshikawa *et al.* 1994). The initial symptoms of endotoxic shock are usually associated with acute inflammation due to activation of phagocytic cells (Ayala and Chaudry 1996) and their products (Laskin and Pendino 1995).

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Macrophages are widely recognized as cells that play a central role in the regulation of immune and inflammation activities as well as tissue remodeling, and peritoneal macrophages represent other macrophage population (Unanue 1989). In response to LPS, macrophages secrete proinflammatory cytokines such as TNFα, IL-6, IL-1β or IL-12 (Laskin and Pendino 1995), and free radicals or reactive oxygen species (ROS) such as superoxide anions (Víctor *et al*. 1998, 1999, 2000).

The ROS production is involved in the morbidity and mortality response in infectious processes (Henson and Johnston 1987, Novelli *et al*. 1989, Weiss 1989, Peristeris *et al*. 1992, Yoshikawa *et al*. 1994). TNFα contributes to pathophysiological changes associated with several acute and chronic inflammatory conditions, including septic shock, autoimmune diseases, wasting, rheumatoid arthritis, inflammatory bowel disease, and respiratory distress syndrome (Beutler and Cerami 1989, Van Snick 1990, Vassalli 1992). The release of ROS is stimulated by several cytokines, including TNFα and IL-1β (Meier *et al.* 1989, Jensen *et al.* 1992), whereas the oxygen intermediates, in turn, up-regulate cytokine synthesis by macrophages, creating thus a vicious circle for the production of both types of inflammatory mediators (Jensen *et al*. 1992, DeForge *et al*. 1992).

In previous work, female BALB/c mice with lethal endotoxic shock showed a significant increase of superoxide anion and TNFα production by peritoneal macrophages after LPS injection (Víctor *et al*. 1998, 1999, 2000). Thus, the increase of both TNFα and superoxide anions after endotoxin injection could be involved in the animal mortality. In order to know which of these two oxidative stress mediators plays the most important role in the lethal effect of endotoxin, two strains of mice, BALB/c and Swiss which showed a lethal and non-lethal response to the same LPS dose, were used in the present work.

Moreover, the oxidative stress linked to free radicals and proinflammatory cytokine production can affect other functions of macrophages, namely adherence to tissue, chemotaxis, and ingestion of foreign agents. In fact, female BALB/c mice showed increased adherence and ingestion as well as decreased chemotaxis of peritoneal macrophages after LPS injection (Víctor *et al*. 1998, 1999, 2000). In relation to the above statement, the aim of the present work is to study superoxide anion and TNFα production by peritoneal macrophages from two strains of mice, namely Swiss mice with lethal and non-lethal endotoxic shock and BALB/c mice with lethal endotoxic shock, as well as the effect of the oxidative stress on other macrophage functions.

Methods

Adult male BALB/c and Swiss (OF-1) mice (Harlan Iberica, Barcelona, Spain) aged 24±2 weeks were used, since previous work from our laboratory has shown that at that age the mice do not show any sign of immune function decline (Víctor *et al*. 1998, 1999, 2000). It is the same age that we used in the original study on female BALB/c mice (Víctor *et al*. 1998)**.**

The mice were maintained at a constant temperature (22±2 ^{o}C), in sterile conditions, under a 12-hour light/dark cycle, fed Sander Mus pellets (Panlab L.S. Barcelona, Spain) and water *ad libitum*. The animals did not show any sign of malignancy or other pathological processes. Mice were treated according to the guidelines of the European Community Council Directives 86/6091 EEC.

Five groups of animals were used. The two Swiss (OF-1) experimental groups comprised mice receiving lethal or non-lethal intraperitoneal injection (i.p.) of *Escherichia coli* lipopolysaccharide (LPS) (055:B5, Sigma, St Louis, USA) at a concentration of 150 mg/kg or 100 mg/kg, respectively. The dose of 100 mg/kg of LPS was chosen in agreement with previous studies on other mouse strains by us and other authors (Mansilla-Roselló *et al*. 1997, Víctor *et al*. 1998) showing that it caused a lethal endotoxic shock. On the other hand, after previous assays, the dose of 150 mg/kg was chosen in order to obtain a lethal endotoxic shock in male Swiss mice. The third group, namely the BALB/c group mice, received a lethal injection of *E. coli* at a concentration of 100 mg/kg in agreement with the original study on female BALB/c. The fourth and fifth groups served as the two control groups, one of Swiss and the other of BALB/c mice, which received a phosphate buffer saline solution (PBS) injection. Each animal was injected with LPS or PBS between 9:00 and 10:00 a.m. We analyzed the functions of the peritoneal macrophages from 5 animals (one per group) every day for two weeks.

At 2, 4, 12, and 24 h after injection, peritoneal suspensions were obtained by a procedure described previously (Ferrández and De la Fuente 1999)**.** Briefly, 3 ml of Hank's solution adjusted to pH 7.4 were injected intraperitoneally, then the abdomen was massaged and the peritoneal exudate cells consisting of lymphocytes and macrophages were collected allowing recovery of 90-

95 % of the injected volume. Macrophages, identified by morphology and nonspecific esterase staining, were counted and adjusted in Hank's solution to $5x10^5$ macrophages/ml. Before and after each experiment cellular viability was routinely measured by the trypan-blue exclusion test. In all cases the viability was higher than 95 %. All incubations were performed at 37 °C in a humidified atmosphere of 5 % CO_2.

Superoxide anion production was evaluated following a method described previously (Ferrández and De la Fuente 1999), which assessed the activity of this anion on the basis of nitroblue tetrazolium (NBT, Sigma, St Louis, MO, USA) reduction to a formazan measured by spectrophotometry at 525 nm (Bagasra *et al*. 1988). Briefly, aliquots of 250 µl of peritoneal suspension were mixed with 250 µl of NBT solution (1 mg/ml in Hank's solution) and 50 µl of latex beads (Sigma, St Louis, MO, USA) in the stimulated samples, or 50 µl of Hank's solution in the nonstimulated samples. After 60 min of incubation in a bath at 37 °C, the reaction was stopped, the samples were centrifuged, and the supernatants discarded. The reduced NBT was extracted with dioxan (Merck, Darmstadt, FRG) and the absorbance of the supernatants was determined in a spectrophotometer at 525 nm. The obtained data were expressed as nmoles of NBT reduced per 10^6 macrophages by extrapolating from a standard curve of NBT reduced with 1,4-dithioerythritol (Sigma, St Louis, MO, USA).

Table 1. Mortality of animals after LPS injection

Time (h)	**% of mortality**		
	Swiss		**BALB/c**
	100 mg/kg	**150 mg/kg**	**100 mg/kg**
0-12	0	16	0
13-20	0	32	16
20-24	0	64	50
24-30	0	100	100

Each value represents the cumulative percentage of mortality of animals after injection of LPS. The number of animals used was 10 per group.

Table 2. Effect of LPS in macrophage number ($x10^4$)/ml peritoneal suspension

Time (h) after LPSinjection	**Swiss mice**		**BALB/c mice**
	100 mg/kg	**150 mg/kg**	**100 mg/kg**
Control	73± 5	72±6	60± 7$^{\#}$
2 h	63± 6*	60± 8*	57± 7
4 h	64± 6*	56± 7*^{a}	53± 6^{a}
12 h	66± 5	52± 7*^{a}	50± 6*^{a}
24 h	68± 7	50± 6*^{a}	50± 7*^{a}

Values are means ± S.D. of 8 values corresponding to 8 animals, each value being the mean of duplicate measure. The cells were obtained at 2, 4, 12 and 24 h after LPS injection or after PBS injection (control group). $^{*}p<0.05$ *with respect to the values in the Control group. Calculated by one-way repeated measures ANOVA.* $^{a}p<0.05$ *with respect to the corresponding values in the Swiss 100 group. Calculated by one-way ANOVA on Ranks.* $^{\#}p<0.05$ *with respect to corresponding values in the Swiss 150 group. Calculated by one-way ANOVA on Ranks.*

The concentration of mouse tumor necrosis factor alpha (TNFα) was determined on macrophage culture supernatants. Peritoneal cells were incubated with Hank's solution at a final concentration of $2x10^5$ macrophages/200 µl/well in 96 well plates (Costar, Cambridge, MA, USA) for 1 h to allow macrophages to

form a monolayer. Then, after two washes, RPMI-1640 medium without phenol red and with L-glutamine and 10% heat-inactivated (56 °C, 30 min) calf serum (Gibco, Burlington, Ontario, Canada) was added. After 24 h of incubation, plates were centrifugated and the TNFα production was quantified in the supernatants using a mouse TNFα (Endogen, Woburn, MA, USA) immunoassay with recombinant mouse TNFα. A minimum detectable dose of mouse TNFα was 10 pg/ml and a limit of procedure was up to 1500 pg/ml.

The quantification of substrate adherence capacity was carried out by a method described previously (De la Fuente *et al*. 1991). Aliquots of 200 µl of peritoneal suspension were placed in Eppendorf tubes. At 10 min of incubation, 10 µl from each sample were removed after gently shaking to resuspend the sedimented cells and the number of non-adhered macrophages was determined by counting in Neubauer chambers using an optical microscope (40x magnification lens). The adherence index (AI) was calculated according to the following equation:

$$AI = 100 - [(\text{macrophages/ml supernatant})/(\text{macrophages/ml original sample})] \times 100$$

Chemotaxis was evaluated according to the method of Boyden (1962) in a slight modification (Ferránez and De la Fuente 1999) which consisted basically in the use of chambers with two compartments separated by a filter with a pore diameter of 3 µm (Millipore, Bedford, MA, USA). Aliquots of 300 µl of peritoneal suspension were deposited in the upper compartment and aliquots of 400 µl of a chemoattractant f-met-leu-phe (10^{-8} mol/l) (Sigma, St Louis, MO, USA) were put into the lower compartment. The chambers were incubated for 3 h and then the filters were fixed and stained. The chemotaxis index was determined by counting the total number of macrophages in the lower face of the filter using an optical microscope (100x magnification lens).

Phagocytosis assay of inert particles (latex beads) was carried out by incubating aliquots of 200 µl of the peritoneal suspension in MIF plates (Sterilin, Teddington, UK) for 30 min. The adhered monolayer obtained was washed with prewarmed PBS, and then 200 µl of Hank's medium and 20 µl of latex beads (1.09 µm of diameter; Sigma, St Louis, MO, USA) were added. After 30 min of incubation, the plates were washed, fixed and stained, and the number of particles ingested by 100 macrophages was determined by counting in an optical microscope (100x magnification lens) (Ferrández and De la Fuente 1999). The number of ingesting macrophages per 100 macrophages was also determined.

The data are expressed as the mean ± standard deviation (± SD). The data were analyzed by one-way repeated measures analysis of variance (ANOVA) since data did not show Gaussian distribution in the different groups of mice at 2, 4, 12, and 24 h after LPS injection. One-way ANOVA on Ranks or one-way ANOVA were used to compare the values obtained in the two strains. The Bonferroni and Student-Newman-Keuls test with a level of significance set at $p<0.05$ were used for posthoc comparisons.

Results

Mortality

Table 1 shows the cumulative percentage of mortality of Swiss and BALB/c mice at different times after LPS administration. The results show that in lethal shock groups, Swiss and BALB/c mice receiving 150 mg/kg and 100 mg/kg of LPS, respectively, the percentage of mortality was 100 % at 30 h, while in non-lethal shock group of Swiss mice (100 mg/kg of LPS) there was no mortality at this time, with 100 % survival of the animals.

Since there were no differences at 2, 4, 12, or 24 h after PBS injection in control animals, the values are always shown together. Table 2 shows the total number of macrophages per ml of peritoneal suspension that were obtained at different times after LPS injection**.** The statistical analysis shows that in the Swiss mice lethal shock group the number of macrophages decreased significantly at all times, and in the Swiss non-lethal group at 2 and 4 h after LPS injection. In the BALB/c group, the number of cells decreased at 12 and 24 h. The comparison of the results obtained in the Swiss and BALB/c mice between the non-lethal and the lethal group showed a significant decrease of macrophage in this later group.

Superoxide anion production

In Figure 1, the values of superoxide anion production are shown expressed as nmoles/10^6 cells, measured by NBT reduction in non-stimulated samples (in the absence of ingested material, Fig. 1A), and in stimulated samples (in the presence of ingested material, Fig 1B) from the Swiss mice non-lethal shock group (100 mg/kg), Swiss mice lethal shock group (150 mg/kg), and BALB/c mice lethal shock group (100 mg/kg) at 2, 4,

12, and 24 h after LPS injection. Superoxide anion production increased significantly with respect to the corresponding control group (PBS) at all times after LPS injections. Comparing the results obtained from the non-lethal and the lethal groups of Swiss mice, the superoxide anion production after LPS injection was higher in the lethal group. In relation to the comparison between the two strains, the values of superoxide anion production obtained in the control groups were higher in cells from Swiss mice than in those of BALB/c mice. In animals injected with 100 mg/kg of LPS, superoxide anion production was higher in BALB/c mice than in Swiss mice. In the lethal shock groups, the values obtained in Swiss mice were significantly increased with respect to those in BALB/c animals.

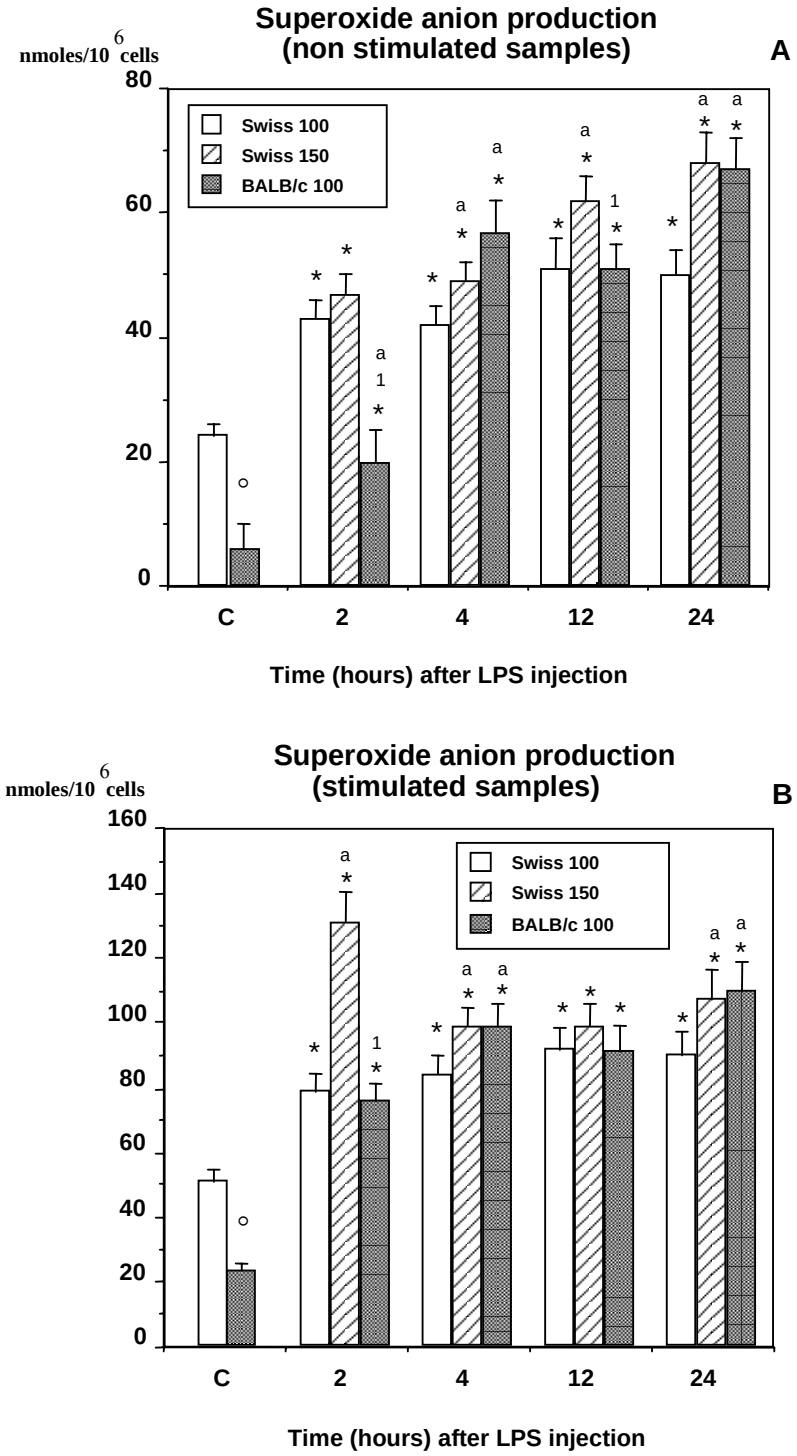

Fig. 1. *A: Superoxide anion production (nmol/10^6 cells) by peritoneal macrophages in nonstimulated samples. B: Superoxide anion production (nmol/10^6 cells) by peritoneal macrophages in stimulated samples. Swiss 100 (non-lethal group): cells from animals injected with 100 mg/kg of LPS; Swiss 150 (lethal group): cells from animals injected with 150 mg/kg of LPS; BALB/c 100 (lethal group): cells from animals injected with 100 mg/kg of LPS. The cells, in all cases were obtained at 2, 4, 12 and 24 h after injection. Each column represents the mean ± S.D. of 8 values corresponding to 8 animals, each value being the mean of duplicate assays. °$p<0.05$ with respect to the corresponding value in the Swiss group. *$p<0.05$ with respect to the values in the C (control) group (time 0). $^a p<0.05$ with respect to the corresponding values in the Swiss 100 group. $^1p<0.05$ with respect to corresponding values in the Swiss 150 group.*

TNFα release

TNFα production by peritoneal macrophages from all groups at 0.5, 1, 1.5, 2, 4, 12 and 24 h after LPS injection is shown in Figure 2. In the Swiss mice groups, the production of TNFα increased with respect to the control value at 0.5, 1 and 1.5 h after LPS injection with the highest value at 1.5 h. In the BALB/c group, TNFα production was increased with respect to the control value, at all times after LPS injection, with the highest value at 2 h. With regards to the comparison of the two strains, the values of TNFα obtained in the Swiss mice (lethal and non-lethal groups) were lower at 2, 4, 12 and 24 h after the LPS injection than those in BALB/c mice.

Adherence capacity

Figure 3 shows the adherence indexes at 10 min of incubation of murine peritoneal macrophages from all groups of mice. In the Swiss and BALB/c mice lethal groups, the adherence index increased at all times after LPS injection in comparison to the PBS group, while in the Swiss non-lethal shock group it was only increased at 4 and 12 h after LPS injection. In the Swiss mice, a significant increase of the adherence index was observed in the lethal group (as compared to the non-lethal one) at all measured times after injection. The comparison between the two strains showed higher adherence values in cells from Swiss mice than in those from BALB/c mice of the control groups. In animals injected with 100 mg/kg of LPS, the adherence indexes were higher in BALB/c mice (lethal group) than in Swiss mice (non-lethal group). In the lethal shock groups, the values obtained in the Swiss mice were significantly increased at 12 h with respect to those of BALB/c animals.

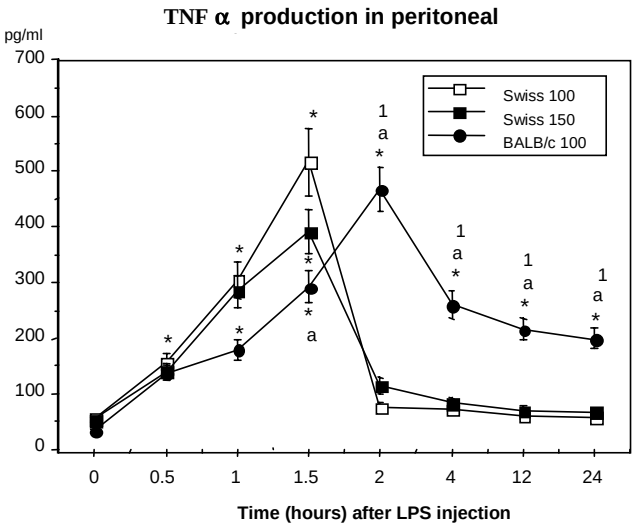

Fig. 2. *TNFα production of peritoneal macrophages. Swiss 100 (non-lethal group): cells from animals injected with 100 mg/kg of LPS; Swiss 150 (lethal group): cells from animals injected with 150 mg/kg of LPS; BALB/c 100 (lethal group): cells from animals injected with 100 mg/kg of LPS. In all cases the cells were obtained at 2, 4, 12 and 24 h after injection. Each column represents the mean ± S.D. of 8 values corresponding to 8 animals, each value being the mean of duplicate assays.* * *p<0.05 with respect to the values in the C (control) group (time 0).* a *p<0.05 with respect to the corresponding values in the Swiss 100 group.*

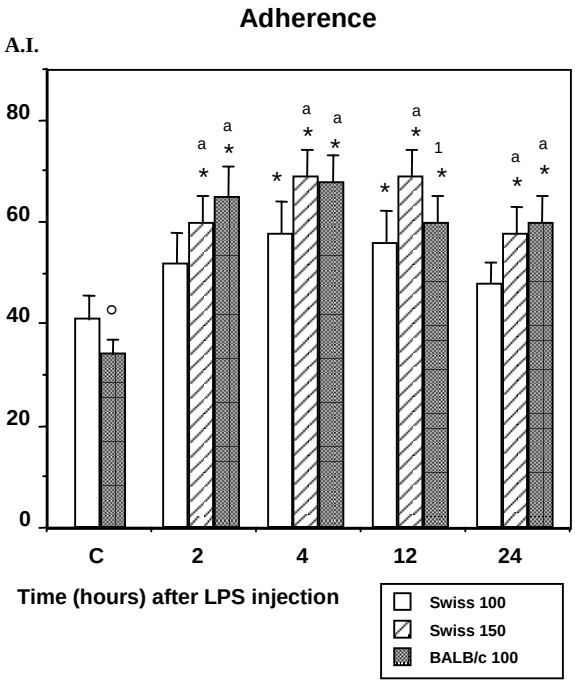

Fig. 3. *Adherence indexes at 10 min of incubation of peritoneal macrophages. Swiss 100 (non-lethal group): cells from animals injected with 100 mg/kg of LPS; Swiss 150 (lethal group): cells from animals injected with 150 mg/kg of LPS; BALB/c 100 (lethal group): cells from animals injected with 100 mg/kg of LPS. In all cases the cells were obtained at 2, 4, 12 and 24 h after injection. Each column represents the mean ± S.D. of 8 values corresponding to 8 animals, each value being the mean of duplicate assays.* ° *p<0.05 with respect to the corresponding value in the Swiss group.* * *p<0.05 with respect to the values in the C (control) group (time 0).* a *p<0.05 with respect to the corresponding values in the Swiss 100 group.* 1 *p<0.05 with respect to corresponding values in the Swiss 150 group.*

Chemotaxis capacity

The chemotaxis indexes of murine peritoneal macrophages from all groups of mice are shown in Figure 4. In the lethal groups, in comparison to the control group, the chemotaxis was decreased at all times after LPS injection, while in the Swiss non-lethal group, this capacity was only decreased at 2 and 4 h after LPS injection. When the results obtained in the non-lethal and the lethal groups of Swiss mice were compared, a smaller chemotaxis was observed in the later group. Comparing the two strains, chemotaxis indexes in cells from BALB/c mice were higher than those in cells from control, lethal and non-lethal groups of Swiss mice.

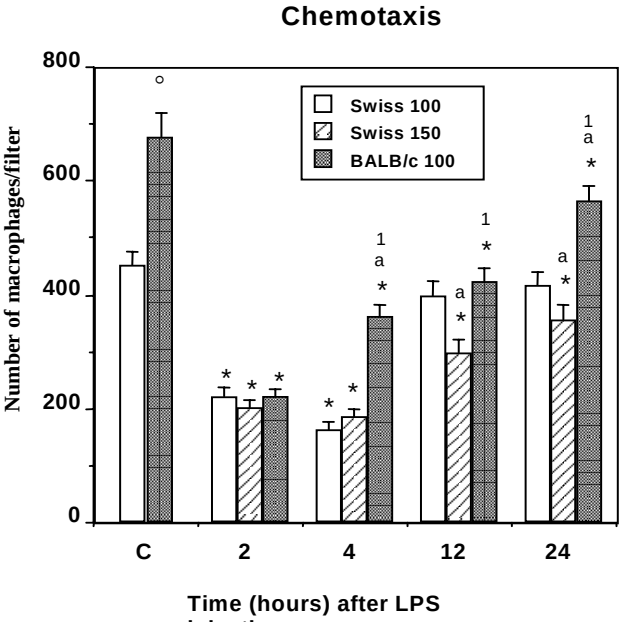

Fig. 4. *Chemotaxis indexes (macrophages/filter) of peritoneal macrophages. Swiss 100 (non-lethal group): cells from animals injected with 100 mg/kg of LPS; Swiss 150 (lethal group): cells from animals injected with 150 mg/kg of LPS; BALB/c 100 (lethal group): cells from animals injected with 100 mg/kg of LPS. In all cases the cells were obtained at 2, 4, 12 and 24 h after injection. Each column represents the mean ± S.D. of 8 values corresponding to 8 animals, each value being the mean of duplicate assays.* ° *$p<0.05$ with respect to the corresponding value in the Swiss group.* * *$p<0.05$ with respect to the values in the C (controls) group (time 0).* ^{a} *$p<0.05$ with respect to corresponding values in the Swiss 100 group.* ^{1}*$p<0.01$ with respect to the corresponding values in the Swiss 150 group.*

Phagocytosis capacity

Figures 5A and 5B show the number of inert particles (latex beads) ingested by macrophages and the number of ingesting cells from the different groups of mice. In all groups injected with LPS, the number of ingested particles and the cells with ingested latex beads increased significantly with respect to the corresponding control group. In the Swiss mice, the ingestion capacity was higher in the lethal than in the non-lethal group. Comparing control groups of the two strains, the values were higher in cells from BALB/c mice than in those from Swiss mice. In animals injected with LPS, the values were higher in BALB/c (lethal group) than in Swiss lethal and non-lethal groups.

Discussion

Oxidative stress has been suggested as the underlying injurious process responsible for the high mortality associated to endotoxic shock, among other mechanisms of LPS-induced damage (McKechnie *et al*. 1986, Víctor *et al*. 1998, 1999, 2000, 2002). Peritoneal macrophages are easily available in relatively great amount in mice and can be obtained without sacrificing the animal (Unanue 1989). Moreover, these cells play an essential role in the immune response of the host to inflammatory and infectious processes such as endotoxic shock (Ayala and Chaudry 1996, Víctor *et al*. 1998). Since these peritoneal macrophages seem to play an important role in the pathogenesis of LPS (Goode and Webster 1993, Víctor *et al*. 1998, 1999, 2000), the study of the toxic effect of these cell products, such as ROS and TNFα, is important for understanding the mechanisms involved in the LPS-induced injury as well as the possible therapies that can reduce this injury. In this study, we have observed that LPS injection producing a lethal endotoxic shock (150 mg/kg in Swiss mice and 100 mg/kg in BALB/c) results in a significant increase of superoxide anion production by peritoneal macrophages. These animals showed 100 % mortality after 24-30 h of LPS injection. However, less superoxide production occurred with 100 mg/kg in the Swiss strain non-lethal endotoxic shock group. The increased ROS are a result of the respiratory burst induced by the activation of LPS in macrophages (Hagenlocker *et al*. 1990, Goode and Webster 1993). Although ROS and TNFα play an important role in the pathogenesis of endotoxic shock, the present work shows that the levels of TNFα are similar in both lethal and non-lethal groups of Swiss mice. This fact suggests that free radicals, such as superoxide anions,

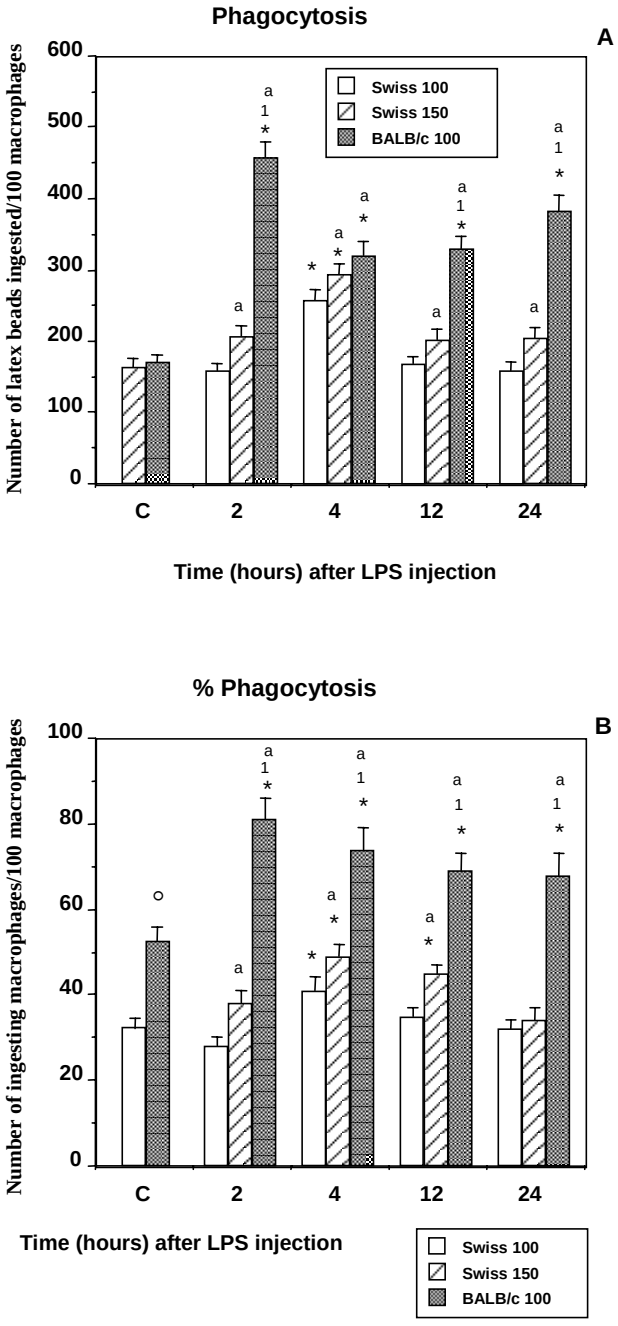

Fig. 5. A: *Number of latex beads ingested per 100 peritoneal macro-phages. B: Number of ingesting cells per 100 peritoneal macrophages. Swiss 100 (non-lethal group): cells from animals injected with 100 mg/kg of LPS; Swiss 150 (lethal group): cells from animals injected with 150 mg/kg of LPS; BALB/c 100 (lethal group): cells from animals injected with 100 mg/kg of LPS. In all cases the cells were obtained at 2, 4, 12 and 24 h after injection. Each column represents the means ± S.D. of 8 values corresponding to 8 animals, each value being the mean of duplicate assays.* ° $p<0.05$ *with respect to the corresponding value in the Swiss group.* * $p<0.05$ *with respect to the values in the C (control) group (time 0).* ^{a} $p<0.05$ *with respect to corresponding values in the Swiss 100 group.* $^{1}p<0.01$ *with respect to the corresponding values in the Swiss 150 group.*

could be more important mediators than TNFα in endotoxic shock for the Swiss strain. The production of ROS, which is necessary in order to eradicate the infection, is useful if their levels are controlled. Conversely, an uncontrolled production of ROS exceeds the antioxidant defense capacity of cells or extracellular fluids and provokes oxidative damage in all organ targets, as happens in endotoxic shock. The involvement of free radicals in tissue damage has been reported in animal models of endotoxic shock and in humans with septic shock (Planas and García 1997)**.** The results of the present study show that the injection of LPS causes an oxidative stress and this stimulates different steps of the phagocytic process in murine peritoneal macrophages, such as adherence and ingestion of inert particles (latex beads), whereas an inhibition of mobility occurs. All these changes were quite evident in Swiss and BALB/c mice with lethal endotoxic shock. The similar results were obtained in our previous work on female BALB/c mice (Víctor *et al*. 1998).

Phagocytic cell adherence to a smooth plastic surface is comparable to that taking place in animal tissues (Noga *et al*. 1984). This activity was stimulated at every time studied in the cells from mice with lethal endotoxic shock, being maximal at 4 and 12 h in Swiss mice, and at 2 and 4 h in BALB/c mice. This stimulation could be due to the increased adhesion molecule expression produced by free radicals (Breviario *et al*. 1988, Cheng *et al*. 1988). The free radical levels increase after LPS injection (Baeuml *et al*. 1997, Mansilla-Roselló *et al*. 1997). Moreover, the total number of macrophages obtained from the peritoneal exudate at different times after LPS injection was significantly decreased in comparison with the control animals. This could explain the adherence stimulation by the oxidative stress state of animals injected with endotoxin. Our results are in agreement with other studies in which striking changes of the amount of monocytes and neutrophils were found after LPS injection in the peritoneal cavity (Sultzer and Castagna 1988)**.** Since adherence activity is the first step of the phagocytic process, the stimulating effect produced by LPS could represent an activation stage of macrophages. However, the next step in phagocytic response to infectious agents, namely chemotaxis, significantly decreased at every time studied after LPS injection, mainly in mice with lethal endotoxic shock. This chemotaxis decline could be a consequence of the production of migration inhibitory factor (MIF) induced by LPS (Calandra and Bucala 1997)**.**

With regards to phagocytosis, an increment of this function both in the number of particles ingested and ingesting macrophages was observed at every time after LPS injection in the lethal shock groups (Swiss with 150 mg/kg and BALB/c with 100 mg/kg), while in Swiss mice with non-lethal shock (100 mg/kg of LPS) the increased phagocytosis was only shown at 4 h. This effect can be explained by the activation of macrophages after LPS injection, which induces an oxidative stress. A raised oxidative state in guinea pigs with deficient vitamin E ingestion showed similar changes (De la Fuente *et al*. 2000).

In summary, the above data suggest that macrophages contribute to the oxidative state produced by LPS and they can also show changes in their functions as consequence of the oxidative stress. Although there are different factors implicated in the pathogenesis of endotoxic shock, free radical production such as superoxide anions seems to be more closely related to the mortality found than TNFα release. These results are important for possible therapies that implicate antioxidants and other scavengers of free radicals compounds.

Acknowledgement

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