

Effect of *in vitro* and *in vivo* administration of dexamethasone on rat macrophage functions: comparison between alveolar and peritoneal macrophages

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Effect of in vitro and in vivo administration of dexamethasone on rat macrophage functions: comparison between alveolar and peritoneal macrophages. Y. Nakamura, T. Murai, Y. Ogawa. ©ERS Journals Ltd 1996.

ABSTRACT: Resident alveolar macrophages (AMs) and peritoneal macrophages (PMs), though they originate from common precursor cells, differ morphologically and functionally. The two types of macrophages residing in different tissues may respond differently to glucocorticoids.

In the present study, we compared the effects of a synthetic glucocorticoid, dexamethasone (Dex), on rat AMs and PMs with regard to their phagocytic activity and tumour necrosis factor- α (TNF- α) releasability.

In vitro exposure of the macrophages to Dex caused the depression of phagocytic activity of AMs but not of PMs. In contrast, TNF- α releasability was depressed in the both types of macrophages, and no difference was found between AMs and PMs in their susceptibility to TNF- α regulation by Dex. When Dex was administered subcutaneously into rats, phagocytic activity was severely depressed in AMs but not in PMs. On the other hand, TNF- α releasability was depressed both in AMs and PMs by the *in vivo* Dex administration. The depression in PMs, however, was transitory and less severe than that in AMs.

These results suggest that alveolar macrophages and peritoneal macrophages differ intrinsically in responses to glucocorticoid, and that the cell location and the cell's microenvironment can also modulate the effects of glucocorticoid on macrophage functions.

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Glucocorticoids have multiple effects on immune and inflammatory responses, and have been extensively used for the treatment of various immunological and inflammatory diseases [1–3]. Resident tissue macrophages, which play a critical role in the immune responses, have been proposed as a target for the immunosuppressive effects of glucocorticoids [4]. It is now recognized that tissue macrophages differ morphologically and functionally between anatomical sites [5–7]. Macrophages differentiate in tissues under the influence of local micro-environmental stimuli, and as a consequence express functions suited to their particular site in the body [7]. It is possible that the functionally different macrophages differ in response to glucocorticoids, though such a comparative approach has rarely been employed in examining the effect of glucocorticoids on tissue macrophages.

In the present study, we compared the susceptibility of rat alveolar macrophages (AMs) and peritoneal macrophages (PMs) to depressive effects of dexamethasone (Dex) on their phagocytic activity and tumour necrosis factor- α (TNF- α) releasability. AMs have been shown to exhibit many functional differences from PMs or macrophages from other sources [8–12]. We now show

that rat AMs, as compared with PMs, are more susceptible to *in vivo* Dex administration.

Materials and methods

Dex administration

Male Wistar rats (Japan SLC Inc., Hamamatsu, Japan) weighing 280–300 g were used throughout this study. The rats were given a single *s.c.* dose of saline or dexamethasone disodium phosphate (Dex, Sigma Chemical Co., St. Louis, MO, USA) of 1.25, 5 or 20 mg·kg⁻¹ body weight (BW) 17 h before collection of macrophages.

Preparation of macrophages

AMs were obtained by bronchoalveolar lavage with phosphate-buffered saline (PBS), and washed with Hanks' balanced salt solution (HBSS, Life Technologies Inc., Grand Island, NY, USA). PMs were obtained by

peritoneal lavage with PBS containing 20 U·mL⁻¹ heparin, and were purified by density-gradient centrifugation. Preparations of AMs and PMs were suspended in Dulbecco's modified Eagle's medium (DMEM, Life Technologies Inc.) and plated into plastic tissue culture plates or dishes. The plates were incubated for 2 h at 37°C in a humidified atmosphere containing 5% CO₂, and then the macrophage monolayers were washed with HBSS to remove nonadherent cells.

Assay of phagocytic activity

The macrophage monolayers (10⁶ cells in 35 mm diameter dish) were incubated with 1 mL of DMEM containing 100 µg·mL⁻¹ serum-opsonized zymosan particles for 45 min at 37°C. The ingestion of the particles was evaluated by direct microscopic counting of at least 500 cells per dish, and the percentage of phagocytosing cells was determined.

Determination of TNF-α releasability

The macrophage monolayers (10⁶ cells in 35 mm diameter dish or 5×10⁵ cells·well⁻¹ in 24 well tissue culture plate) were incubated with DMEM containing 0.1 or 1 µg·mL⁻¹ lipopolysaccharide (LPS) for 17 h at 37°C, and TNF-α was determined in the macrophage culture supernatants by cytotoxicity assay using L929 cells (obtained from Japanese Cancer Research Resources Bank-Cell). In brief, L929 cells were incubated with serial dilutions of the macrophage culture supernatants in the presence of actinomycin D (1 µg·mL⁻¹). After incubation for 17 h, the plates were washed with PBS and the remaining cells were stained with crystal violet. The amount of cell lysis was determined from the absorbance at 570 nm. One TNF-α unit was defined as the amount of TNF-α giving 50% cell lysis. To determine whether the L929 cell lysis was specifically induced by TNF-α, several test samples were also incubated with rabbit anti-murine TNF-α neutralizing antibody (Endogen Inc., Boston, MA, USA), which is cross-reactive to rat TNF-α, prior to addition to L929 cells.

Dex treatment of macrophages in vitro

AMs and PMs were obtained from nontreated rats. The macrophage monolayers were incubated with DMEM containing 10 or 1,000 nM of Dex for 1 h, and then their phagocytic activity and TNF-α releasability were determined as described above.

Statistical analysis

All data were presented as mean±SEM. The statistical comparisons were made using analysis of variance (ANOVA) and Dunnett's multiple comparison. The level of significance chosen was p-value less than 0.05.

Results

Firstly, functional differences between rat AMs and PMs were examined. With regard to phagocytic activity, PMs tended to phagocytose more zymosan particles than did AMs; this difference, however, did not reach statistical significance (data not shown). On the other hand, a marked difference was found in their TNF-α releasability. With a LPS dose of 10 µg·mL⁻¹, the activity

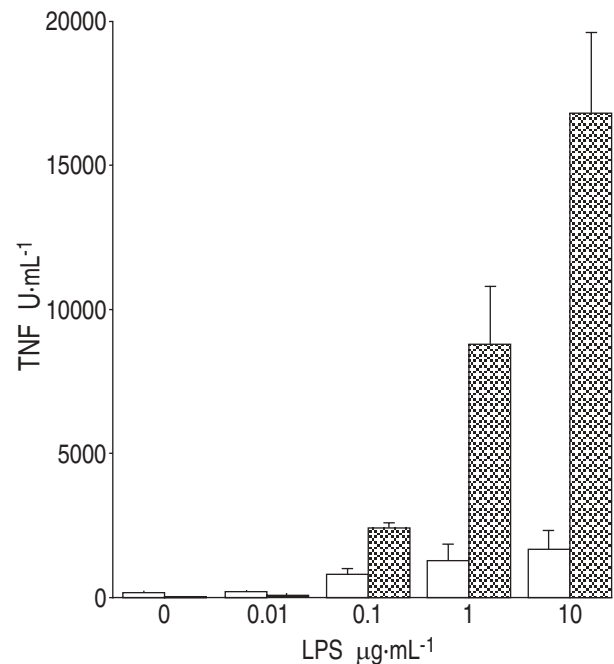


Fig. 1. — Comparison of tumour necrosis factor-α (TNF-α) releasability in AMs and PMs. Macrophages were obtained from nontreated rats. The macrophage monolayers (10⁶ cells in 35 mm diameter dish) were incubated in the medium containing 0, 0.01, 0.1, 1.0 or 10 µg·mL⁻¹ LPS for 17 h, and TNF-α activity was determined in the culture supernatants. Data are presented as mean±SEM of three separate experiments. □: peritoneal macrophages (PMs); ■: alveolar macrophages (AMs). LPS: lipopolysaccharide.

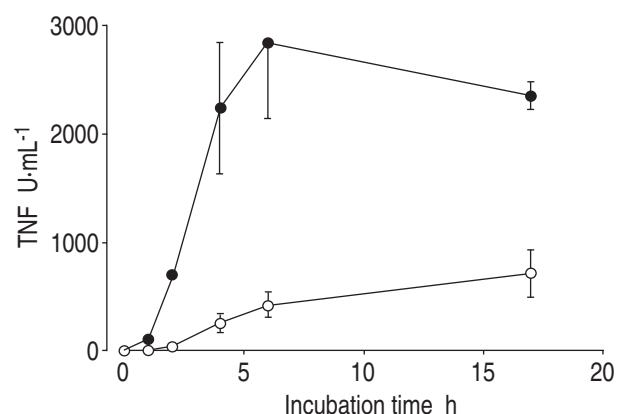


Fig. 2. — Kinetic analysis of tumour necrosis factor-α (TNF-α) releasability in AMs and PMs. Macrophages were obtained from nontreated rats. The macrophage monolayers (10⁶ cells in 35 mm diameter dish) were incubated in the medium containing 1.0 µg·mL⁻¹ LPS for various times, and TNF-α activity was determined in the culture supernatants. Data are presented as mean±SEM of three separate experiments. —●—: alveolar macrophages (AMs); —○—: peritoneal macrophages (PMs). LPS: lipopolysaccharide.

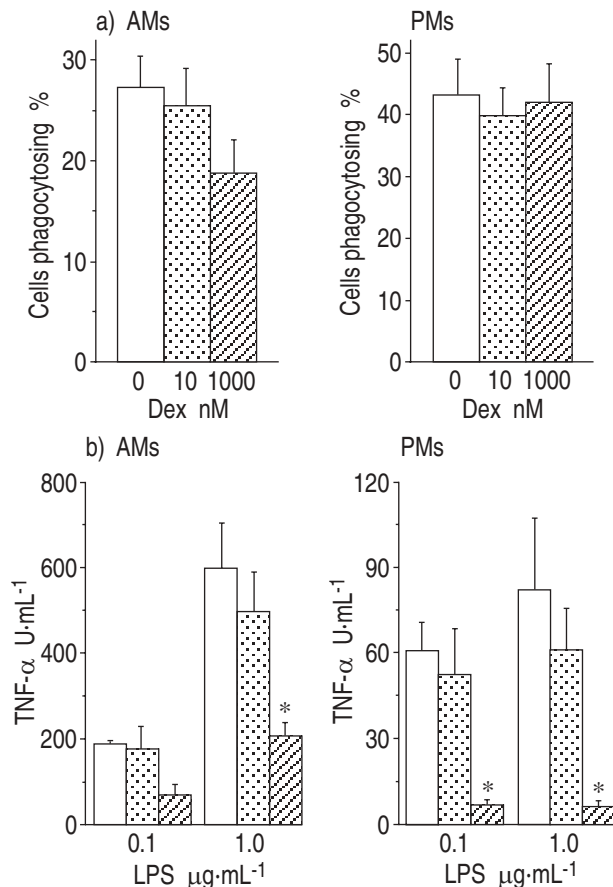


Fig. 3. – Susceptibility of AMs and PMs to dexamethasone (Dex) *in vitro*. Macrophages obtained from nontreated rats were plated into 10^6 cells in 35 mm diameter dish for the assay of phagocytic activity, or 5×10^5 cells-well $^{-1}$ in 24 well plate for the assay of tumour necrosis factor- α (TNF- α) releasability. The macrophage monolayers were incubated in the medium containing 10 or 1,000 nM of Dex for 1 h, and then their phagocytic activity and TNF- α releasability were determined. The percentage of cells phagocytosing zymosan was determined as the phagocytic activity. The macrophage monolayers were incubated in the medium containing 0.1 or 1.0 $\mu\text{g}\cdot\text{mL}^{-1}$ LPS for 17 h, and TNF- α activity in the culture supernatants was determined. Data are presented as mean \pm SEM of four separate experiments. *: significantly different from the 0.0 nM group ($p < 0.05$). a) phagocytic activity; b) TNF- α releasability. AMs: alveolar macrophages; PMs: peritoneal macrophages; LPS: lipopolysaccharide. \square : Dex 0 nM; \square : Dex 10 nM; \square : Dex 1,000 nM.

of TNF- α released from AMs was 10 or more times greater than that from PMs (fig. 1); whilst there was no difference in the time-dependent release of TNF- α between AMs and PMs (fig. 2). The specificity of the measures of TNF- α was confirmed by the neutralization test using anti-murine TNF- α antibody (data not shown).

Next, the susceptibility of the two types of macrophages to Dex was examined by exposing the cells to Dex directly *in vitro*. Macrophages obtained from nontreated rats were incubated with Dex (10 or 1,000 nM) for 1 h and then their functions were determined (fig. 3a and b). The Dex administration *in vitro* depressed phagocytic activity of AMs but not of PMs. On the other hand, TNF- α releasability was depressed significantly not only in AMs but also in PMs, and no difference was found between

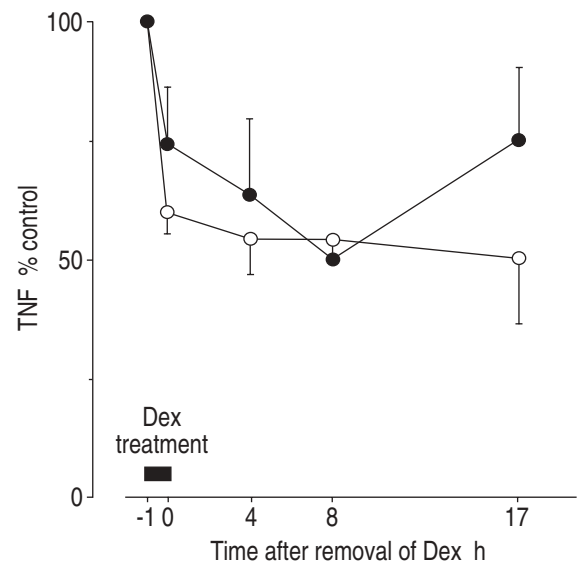


Fig. 4. – Time-course effect of *in vitro* dexamethasone (Dex) administration on tumour necrosis factor- α (TNF- α) releasability of AMs and PMs. Macrophages were obtained from nontreated rats. Macrophage monolayers (5×10^5 cells-well $^{-1}$ in 24 well plate) were incubated with the medium containing 1,000 nM of Dex for 1 h and were then washed. Macrophages were incubated for various times after the removal of Dex, and then incubated in medium containing 1.0 $\mu\text{g}\cdot\text{mL}^{-1}$ LPS for 17 h, and TNF- α activity was determined in the culture supernatants, and expressed as a fall from pre-administration time. Data are presented as mean \pm SEM of three separate experiments. \bullet — \bullet : alveolar macrophages (AMs); \circ — \circ : peritoneal macrophages; LPS: lipopolysaccharide.

the two types of macrophages in their susceptibility to TNF- α regulation by Dex. Time-course effects of Dex administration *in vitro* on TNF- α releasability in AMs and PMs are presented in figure 4. Macrophages were exposed to Dex (1,000 nM) for 1 h, then washed, and their TNF- α releasability was determined 0, 4, 8 and 17 h after the removal of Dex. TNF- α releasability in PMs was depressed from 0–17 h after the removal of Dex. With regard to AMs, almost the same time-dependent depression of TNF- α releasability was observed, though AMs showed a tendency to recover 17 h after the removal of Dex.

The effect of the *in vivo* administration of Dex (1.25, 5, or 20 $\text{mg}\cdot\text{kg}^{-1}$, *s.c.*) was examined. AMs and PMs were harvested from rats 17 h after Dex administration. As shown in table 1, the number of PMs obtained was

Table 1. – Effect of dexamethasone (Dex) pretreatment on cell yields ($\times 10^6$ cells-rat $^{-1}$)

	AMs	PMs
Saline	8.5 \pm 0.61	15.3 \pm 1.48
Dex $\text{mg}\cdot\text{kg}^{-1}$		
1.25	8.7 \pm 0.63	9.3 \pm 1.05*
5.0	7.7 \pm 0.89	9.1 \pm 1.94*
20.0	9.7 \pm 1.06	8.1 \pm 1.41*

Data are presented as mean \pm SEM of six rats. Macrophages were harvested 17 h after Dex administration. *: significantly different from the saline treated group ($p < 0.05$). AMs: alveolar macrophages; PMs: peritoneal macrophages.

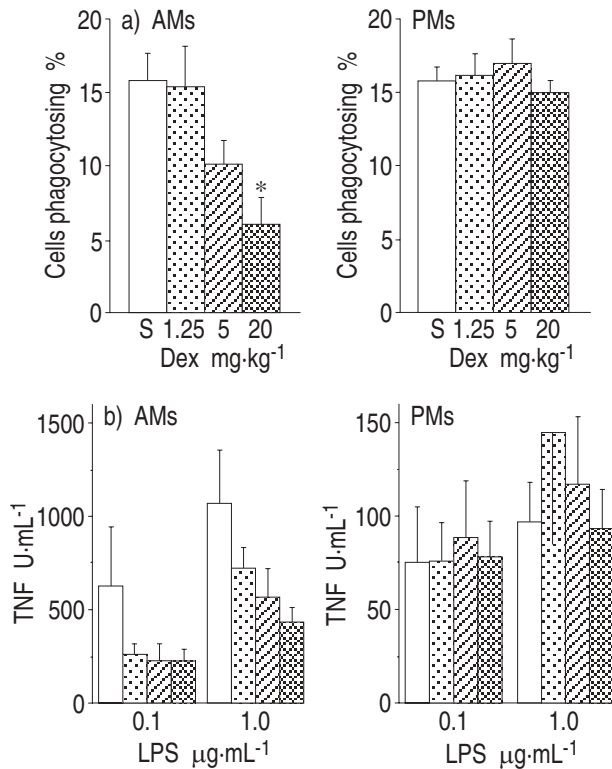


Fig. 5. — Effects of the *in vivo* dexamethasone (Dex) administration on the functions of AMs and PMs. Macrophages were harvested 17 h after either Dex administration (1.25, 5 or 20 mg·kg⁻¹ *s.c.*) or saline (S) control. Macrophages were plated into 10⁶ cells in 35 mm diameter dish for the assay of phagocytic activity, or 5×10⁵ cells·well⁻¹ in 24 well plate for the assay of tumour necrosis factor-α (TNF-α) releasability. The percentage of cells phagocytosing zymosan was determined as the phagocytic activity. The macrophage monolayers were incubated in the medium containing 0.1 or 1.0 µg·mL⁻¹ LPS for 17 h, and TNF-α activity was determined in the culture supernatants. Data are presented as mean±SEM of six rats. *: significantly different from the saline group (*p*<0.05). a) phagocytic activity; b) TNF-α releasability. AMs: alveolar macrophages; PMs: peritoneal macrophages; LPS: lipopolysaccharide. □: saline; ▨: Dex 1.25 mg·kg⁻¹; ▩: Dex 5.0 mg·kg⁻¹; ▪: Dex 20 mg·kg⁻¹.

decreased significantly by *in vivo* Dex administration. In contrast, the number of AMs was not affected. The effects of Dex on AM and PM phagocytic activity and TNF-α releasability are presented in figure 5a and b. Phagocytic activity was significantly depressed in AMs; in the group given 20 mg·kg⁻¹ Dex, the activity of AMs was decreased to 43% of the control group value (fig. 5a). In contrast, phagocytic activity of PMs was not affected by Dex administration. TNF-α releasability was also depressed in AMs but not in PMs (fig. 5b). In order to examine the *in vivo* effects of Dex on the TNF-α releasability more precisely, AMs and PMs were harvested 2, 7, 17 and 40 h after administration of Dex (20 mg·kg⁻¹ *s.c.*). As shown in figure 6, TNF-α releasability in PMs decreased until 7 h after Dex administration, but thereafter showed a tendency to recover, in contrast to a more profound and long-lasting depression in AMs. Thus, TNF-α releasability was depressed *in vivo* in PMs as well as in AMs, however the depression in PMs was transient and less severe than that in AMs.

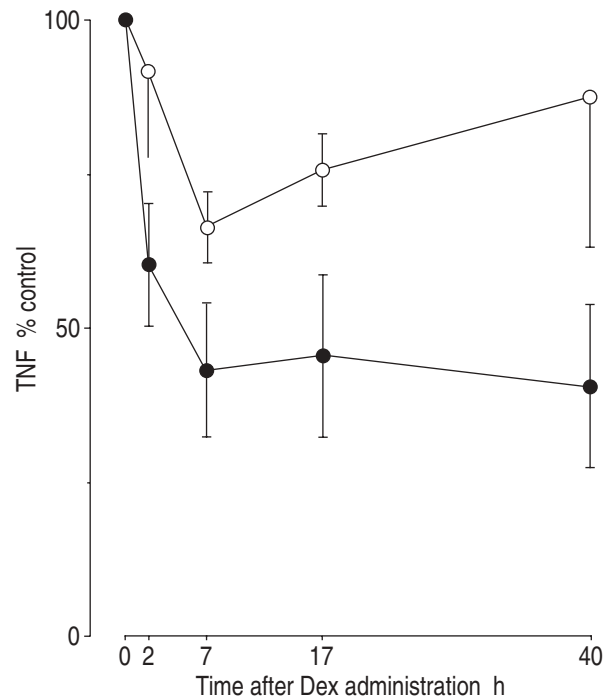


Fig. 6. — Time-course effect of *in vivo* dexamethasone (Dex) administration on tumour necrosis factor-α (TNF-α) releasability. Macrophages were harvested from rats at various times after Dex administration (20 mg·kg⁻¹ *s.c.*). Macrophage monolayers (5×10⁵ cells·well⁻¹ in 24 well plate) were incubated in the medium containing 1.0 µg·mL⁻¹ LPS for 17 h, and TNF-α activity was determined in the culture supernatants. Data are presented as mean±SEM of four rats. —●—: alveolar macrophages (AMs); —○—: peritoneal macrophages (PMs). LPS: lipopolysaccharide.

Discussion

It has been shown that there are considerable structural and functional differences in tissue macrophages between anatomical sites. AMs, in particular, are known to differ in a number of ways from other tissue macrophages. These differences include the bioenergetics [13], expression of surface receptors [14], antigen presentation [15], bacterial killing [16], and cytokine generation [17]. We have also shown that rat AMs have the ability to produce far greater amount of TNF-α in response to LPS when compared with PMs. It would be reasonable to assume that the functionally different macrophages differ in response to glucocorticoids. In the present study, we compared the effects of Dex administration on rat AMs and PMs. The main findings of this study can be summarized as follows: 1) decrease in the recovered cell number of PMs but not of AMs after *in vivo* Dex administration; 2) depression of phagocytic activity in AMs but not in PMs by Dex administration both *in vitro* and *in vivo*; and 3) depression of TNF-α releasability in both types of macrophages *in vitro* and *in vivo*; the *in vivo* depression in PMs, however, was transient and less severe than that in AMs.

The reason for the decrease in the number of PMs recovered from Dex-treated animals is unclear, at present. It is also unclear why the number of AMs was not affected by Dex administration. AMs are the only

macrophages residing in aerobic conditions and this environment may influence the behaviour of these cells at the population level in response to Dex. From these findings on the recovered cell number, we had expected that functional activities of PMs might be more susceptible to Dex *in vivo* than those of AMs. Contrary to expectation, phagocytic activity was depressed in AMs but not in PMs by *in vivo* Dex administration. In the *in vitro* experiments, the phagocytic activity of PMs was not depressed by the direct exposure of the cells to Dex. The findings prove that rat AMs and PMs differ intrinsically in response to Dex. Though the mechanisms by which glucocorticoids depress phagocytic function of macrophages are unknown, it has been postulated that the depression may be due to reduced fluidity of macrophage membranes, since fluid membranes capable of expansion and movement are required for the process of phagocytosis [18]. It seems possible, therefore, that the difference observed between AMs and PMs reflects the difference in properties of the cell membranes. Resident AMs are constantly exposed to infectious agents, allergens and environmental pollutants, and as a consequence they exist in a more activated state relative to resident PMs [19]. Since functional fluid membranes may be required for macrophage activities, the properties of the cell membranes may differ with the state of activation of the macrophages.

It has been shown that glucocorticoids inhibit TNF- α production by macrophages at both the transcriptional and the translational level by blocking gene transcription and messenger ribonucleic acid (mRNA) mobilization [20]. In the present study, we observed that TNF- α releasability of AMs was depressed more severely than that of PMs by *in vivo* Dex administration. On the other hand, no difference was found *in vitro* between AMs and PMs at the cellular level in their susceptibility to Dex with regard to the depression of TNF- α releasability. It is probable, therefore, that the difference observed *in vivo* is due to the cell location and the cell's microenvironment. Although we did not study the tissue distribution of Dex after the systemic administration, it seems possible that Dex had not reached the PM environment in high enough concentrations as compared with the AM environment.

It has been shown that there are many kinds of factors which influence the functions of macrophages; cytokines, immunoglobulins, leukotrienes and others. Some of them activate the functions of macrophages and others depress them. For instance, interferon- γ (IFN- γ), a well-known macrophage-activating factor, enhances TNF- α production by macrophages and interleukin-10 inhibits TNF- α production. It is also possible, therefore, that the microenvironment modified the depressive effect of Dex on TNF- α releasability in macrophages. PMs reside in the peritoneum with other kinds of cells, such as lymphocytes, in contrast to the exclusive residence of AMs in alveoli. It has been reported that activation of antimicrobial activity of macrophages is not suppressed by the addition of Dex to mixed-cell systems composed of proliferating lymphocytes and macrophages, in spite of marked suppression of lymphocyte proliferation [21].

Furthermore, it has been demonstrated that lymphokines, such as IFN- γ , can restore the microbicidal activity of glucocorticoid-suppressed macrophages [22]. Thus, it is conceivable that lymphocytes coexisting with PMs in the peritoneum could counterbalance the suppressive effect of Dex on the macrophages.

It is notable that TNF- α releasability, as well as phagocytic activity, was depressed in AMs to a greater extent than in PMs by systemic Dex administration. PMs, which can be prepared easily, are commonly used as a model of tissue macrophages in immunological study. Our results, however, indicate the importance of studying the role of macrophages in immunity with cells from the appropriate anatomical sites. Human AMs have been shown to be less susceptible to Dex than peripheral blood monocytes *in vitro*, and it has been suggested that the sentinel role of AMs as a critical first line of defence may preclude dramatic swings in regulation [23]. The present study, however, provides evidence that rat AMs, when compared with PMs, are more susceptible to Dex *in vivo*. AMs comprise the first line of defence in the lower respiratory tract, and recurrent infections of the respiratory tract are a frequent and serious side-effect of chronic glucocorticoid treatment [24]. Thus, the relatively high susceptibility of AMs to Dex can lead, in parallel with the Dex-induced suppression of a number of biological processes, to a decrease of lung defences and the subsequent development of an infectious condition.

In summary, the present study showed that the effect of systemic administration of Dex on macrophages varies depending on the specific cell location, and that the functions of AMs, which are critical in lung defence, are more susceptible to Dex than those of PMs.

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