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MACROPHAGE DERIVED SIGNALLING REGULATES NEGATIVELY THE MEGAKARYOCYTE COMPARTMENT

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Abstract - Megakaryocytopoiesis is the process by which stem cells go through a process of commitment, proliferation and differentiation leading to the production of platelets. In the mouse, this process is accomplished within the bone marrow (BM) and spleen microenvironment and is carried out by regulatory molecules and accessory cells including macrophages, fibroblasts and endothelial-like cells. Previously, we have reported that macrophage depletion following administration of liposomal clodronate (LIP-CLOD) provokes enhancement of both, megakaryocytopoiesis and thrombocytopoiesis. In this report, we investigated the changes in the compartment of megakaryocyte progenitor cells (MK-CFU), their correlation with plasmatic thrombopoietin (TPO) and TPO transcription levels after macrophage depletion. LIP-CLOD-treated mice showed an increase of the MK-CFU in BM and spleen. Concerning TPO plasma levels, kinetic studies revealed a 1.5- and 1.3-fold increase in the TPO concentration at 12 and 24 hr of treatment. We also show evidence of regulation of TPO transcription in the liver and spleen. Although empty liposomes also enhanced TPO gene regulation in these organs, transcriptional TPO up regulation correlated with an increase of protein synthesis only in those animals where macrophages were effectively removed. Taken together, these results suggest that BM and spleen macrophages derived signalling regulates negatively the megakaryocyte compartment.

Key words: Clodronate, macrophages, thrombopoiesis, megakaryocytopoiesis, platelets

INTRODUCTION

Megakaryocytopoiesis is the cellular process by which committed megakaryocyte (MK) progenitor cells proliferate and differentiate into immature MK. These cells undergo a process of cytoplasmic maturation and nuclear endoreplication to generate mature, polyploid-platelet generating MK (6,36).

In the mouse, this event occurs primary in bone marrow (BM) and spleen (35,39). These organs provide an environment that supports the initial steps in MK differentiation (50). The stroma of BM contains a mixed population of cell types including macrophages, endothelial-like cells, myofibroblasts and other cellular types that, even present in lower number, are functionally important (17,18). These cells are possible sources of growth factors that modulate stem cell mitotic activity and differentiation among lineage-committed cells (46).

Megakaryocytopoiesis is modulated by a network of cytokines. For example stem cell factor (SCF) (54) and interleukin-3 (IL-3) (32) that influence the proliferation of committed cells; while IL-11 (8,44), IL-6 (29,31), leukaemia inhibitory factor (8,41) and oncostatin M (57) are mainly involved in MK maturation. In addition, thrombopoietin (TPO), also known as Mpl ligand is a key regulator of megakaryocytopoiesis (6,15,36,58). TPO affects the proliferation of MK committed cells, the maturation of MK and the blood platelets levels by activation of its receptors (the c-mpl) (4).

TPO is produced constitutively in several organs throughout the body, althought its expression predominates in liver and kidney (15) and the endogenous levels in plasma are inversely correlated with the platelet counts (34). The regulation of TPO gene is complex, and two

Abbreviations: **BM**: bone marrow; **CFU**: colony forming units; **CFU-GM**: colony forming units-granulocyte macrophages; **CMP**: common myeloid progenitor; **CFU-MK:** colony forming units-megakaryocytes; **CSF-GM**: granulocyte-macrophage colony stimulating factor; **GMP(s)**: granulocyte/monocyte restricted progenitors; **IL**: interleukins; **LIP-CLOD**: liposome encapsulated clodronate; **LIP-PBS**: liposome phosphate buffer saline; **MEPs**: megakaryocyte/erythrocyte-restricted progenitors; **MK**: megakaryocytes**; RES**: reticulo endothelial system; **SCF**: stem cell factor; **TPO**: thrombopoietin

mechanisms have been proposed. First, it has been demonstrated that TPO production is constant and its plasma concentration is regulated by the binding to platelets (42,52) and to MK (49). Conversely, other lines of evidence demonstrated that the TPO gene is upregulated in response to platelet demand (25,40).

In a previous report we demonstrated that selective depletion of splenic and hepatic macrophages following administration of liposome-encapsulated clodronate (LIP-CLOD) in mice induced an increase of MK mass in bone marrow and spleen, correlating with an increase of the thrombopoietic activity (3).

The aim of the present work was to analyze the effect of macrophage depletion on the amount of spleen and bone marrow megakaryocyte progenitor cells (CFU-MK) and their correlation with plasma levels of TPO. We also investigate whether TPO transcription could be regulated in hematopoietic organs following macrophage depletion.

MATERIALS AND METHODS

Mice

BALB/c male and female mice aged 14 to 16 weeks were bred in the animal facility of the Academia Nacional de Medicina, Buenos Aires. The experiments performed here were conducted according to principles set forth in the Guide for the Care and Use of Laboratory Animals (43).

Liposome-encapsulated clodronate (LIP-CLOD)

Dichloromethylene bisphosphonate (CLOD) was provided by Roche Diagnostics (Mannheim, Germany). CLOD and PBS-containing liposomes were prepared as previously described (55). The i.v. injection of 0.1 ml/10 g body weight of liposomes containing 380 µg/ml CLOD (76 µg CLOD /mouse, respectively) induces the depletion of splenic and hepatic macrophages within 48 hr (2). Control animals were intravenously injected with 0,1 ml NaCl (saline) or 0,1 ml of phosphate buffer saline (PBS) containing liposomes (LIP-PBS).

Preparation of cell suspensions

Spleen single-cell suspensions were prepared by gentle disruption through a stainless steel 100-mesh. A Ficoll discontinuous density gradient was used to enrich leukocytes of spleen. BM cells that were flushed from the tibia and femur with medium. After washing, the cells were resuspended in Iscove's modified Dulbecco's medium (IMDM), counted and adjusted to final concentration.

Progenitor cells assays

Colony-forming unit-megakaryocytes (CFU-MK) and colony-forming unit-granulocyte-macrophages (CFU-GM) were assayed in duplicate according to the technique of Worton (62) and Shimoda (48), respectively, with slight modifications. For culture of CFU-MK, cells were stimulated with 10 ng/ml recombinant murine interleukin-3 (rmIL-3), 20 ng/ml recombinant human IL-11 (rhIL-11) and 50 ng/ml recombinant human thrombopoietin (rhTPO). CFU-GM cultures were stimulated with 20% colony-stimulating factor (CSF-GM) prepared according to Horiuchi *et al.* (26). After a culture period of 7 days for CFU-GM and 12 days for CFU-MK at 37°C, 5% CO_2, colonies (>50) were provisionally counted using an Olympus dissection microscope (Olympus; Tokyo, Japan). For this purpose, cells were fixed with 2,5% glutaraldehyde and stained for acetylcholinesterase activity (megakaryocytes) and hematoxylin-eosin (granulocytes, macrophages and erythroid cells).

Thrombopoietin enzyme-linked immunosorbent assay

Murine TPO was detected by a commercially available enzyme-linked immunosorbent assay (ELISA) system (R&D Systems, Minneapolis, MN) according to manufacturer's instructions. The lower concentration limit of the assay was 20 pg/ml.

Platelet count

Platelets were counted in a hemocytometer using a buffer containing ammonium oxalate 1% for erythrocyte lysis. The range for platelet count in our BALB/c mice was from 620 to 1300 x 10^3/µl, n = 60. This is in agreement with previously reported data (11,45).

Reverse transcription polymerase chain reaction (RT-PCR) for TPO gene expression

A reverse transcriptase-PCR procedure was performed to determine relative quantities of mRNA TPO. Total cellular RNA was extracted from liver, kidney, spleen and bone marrow of mice treated with saline, LIP-PBS or LIP-CLOD for 24 hr using the SV Total RNA isolation system kit (Promega). cDNA was obtained from 2 µg of total RNA. First strand cDNA was synthesized using oligo-dT_{15} primers and M-MLV reverse transcriptase (Promega, Madison, WI). For the semiquantitative assay 1/8 and 1/16 dilutions of the RT product were used as PCR template.

Semiquantitative Nested PCR was performed to check TPO expression and its isoforms. The primer set for the first PCR was S1 5' TTG CAG TGG CAA GAC TAA CT 3' and A1 5' TGG GGA CCT GGA GGT TTG ATT 3' and for the second PCR was S2 5' TGC AGG GCC TCC TAG GAA CCC 3' and A2 5' CTG AAT CCC TGA AGC CTG CT 3'. The housekeeping murine hypoxanthine phosphorybosyl-transferse (HPRT) mRNA was amplified as a loading control (22) using the following primers S 5' GAT TCA CTT GCG CTC ATC TTA GGC 3´ AS 5' AS1 5' GTT GGA TAC AGG CCA GAC TTT GTT G 3'. The primer sets were from Annovis (Aston, PA), the GoTaq DNA polymerase and the others PCR reagents were from Promega.

The amplification conditions were the following: cycles of 94ºC for 30 sec; 53ºC (primers S1 and A1), 55ºC (primers S2 and A2) or 60ºC (HPRT) for 30 sec; and 72ºC for 45 sec, followed by 10 min at 72ºC. HPRT and TPO primer set S1 and A1 were amplified for 30 cycles, and TPO primer set S2 and A2 was amplified for 35 cycles.

Following amplification, products were separated by electrophoresis on 10% polyacrylamide gels and visualized with ethidium bromide under UV light. The TPO products of nested PCR were 304 bp for TPO-6, 292 pb for TPO-1+2+5+7, 225 bp for TPO-3 or 188 bp for TPO-4+8 and 150 pb for HPRT. Images were recorded digitally and computer analyzed (Master 1D Prime, Pharmacia Biotech, NovoDynamics, MI, USA). The results were expressed as the percentage of control HPRT [(amplified target/amplified HPRT ratio) x 100].

Statistical analysis

Intergroup contrasts of dimensional variables were compared with one way analysis of variance (ANOVA) and p values ≤ 0.05 were considered for further analysis by Bonferroni´s test to define the intergroup comparisons. All statistical tests were interpreted in a two-tailed fashion to estimate p values.

Quantification, direction and magnitude of the correlation between two independent variables were calculated by Pearson test.

RESULTS

Effect of macrophage depletion upon megakaryocyte precursors induced by IL-3/IL-11/TPO

In a previous report (3), we showed that the depletion of splenic and hepatic macrophages in mice increases the number of MK followed by a raise in the reticulated

platelet count 48 hr later. To study whether macrophage depletion was also able to modulate the frequency of MK progenitors in bone marrow and spleen, mice were injected intravenously (i.v.) with LIP-CLOD (76 µg CLOD/ mouse), empty liposomes (LIP-PBS) or saline. After 48 hr of treatment, the animals were killed and the number of megakaryocyte precursors (CFU-MK) was assayed by stimulating bone marrow and spleen cell suspensions with hTPO, rmIL-3 and rhIL-11.

Results depicted in Fig 1A indicate that LIP-CLOD-treated mice showed a significant increase in the frequency of bone marrow CFU-MK compared to LIP-PBS and saline-treated mice. This colony expansion was inversely related to the macrophage count detected in this organ (Pearson r = -0.8484, $p<0.001$, two tailed, n =11). The spleen of LIP-CLOD-treated mice (Fig. 1B) also showed a significant increase of MK precursors compared to the control groups. The raise in CFU-MK was also inversely related to the number of macrophages reported in this organ (Pearson r = -0.8624, $p<0.0001$, two tailed, n =13).

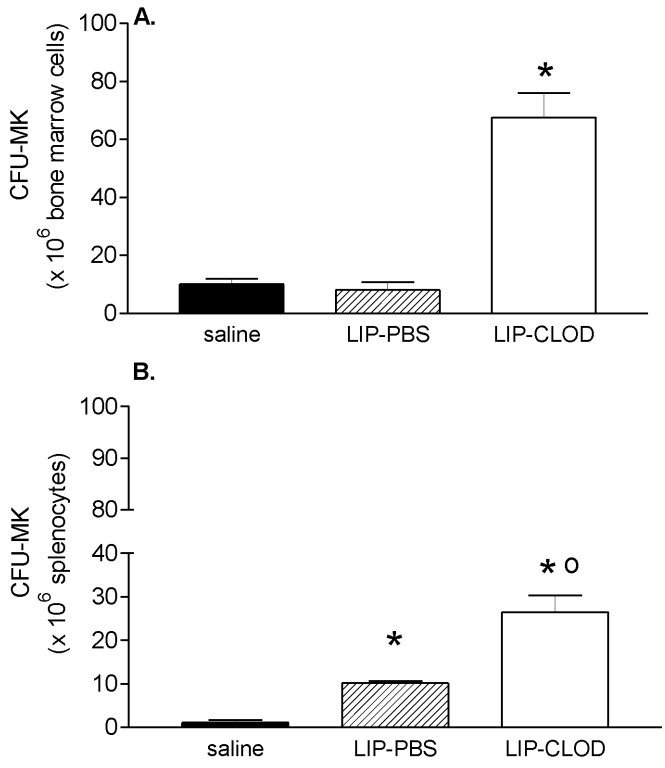

Fig. 1 Effect of LIP-CLOD treatment on bone marrow and spleen UFC-MK. Three groups of mice were injected with saline, LIP-PBS or LIP-CLOD. After 48 hr of treatment the animals were sacrificed and bone marrow (**A**) and spleen (**B**) cell suspensions were assayed for total CFU-MK as described in Materials and Methods. *$p<0.001$ significantly different from saline-treated mice. °$p<0.001$ significantly different from LIP-PBS-treated mice. Bonferroni test (two-tailed), n = 5 mice/ group.

Effect of macrophage depletion upon megakaryocyte and macrophage precursors induced by CSF-GM

Since LIP-CLOD treatment exerts a specific depletion of splenic and hepatic macrophages, we further evaluate the effect of this treatment on the number of bone marrow and spleen macrophage precursors (CFU-GM), using CSF-GM. Since this concentration of CSF-GM could also stimulate MK precursors, the CFU-MK count was evaluated.

For this purpose, mice were injected i.v. with LIP-CLOD, LIP-PBS or saline, and 48 hr later they were sacrificed and the number of CFU-GM and CFU-MK were assayed by stimulating both bone marrow and spleen cells with CSF-GM (see Materials and Methods). The results from total bone marrow CFU showed no statistical

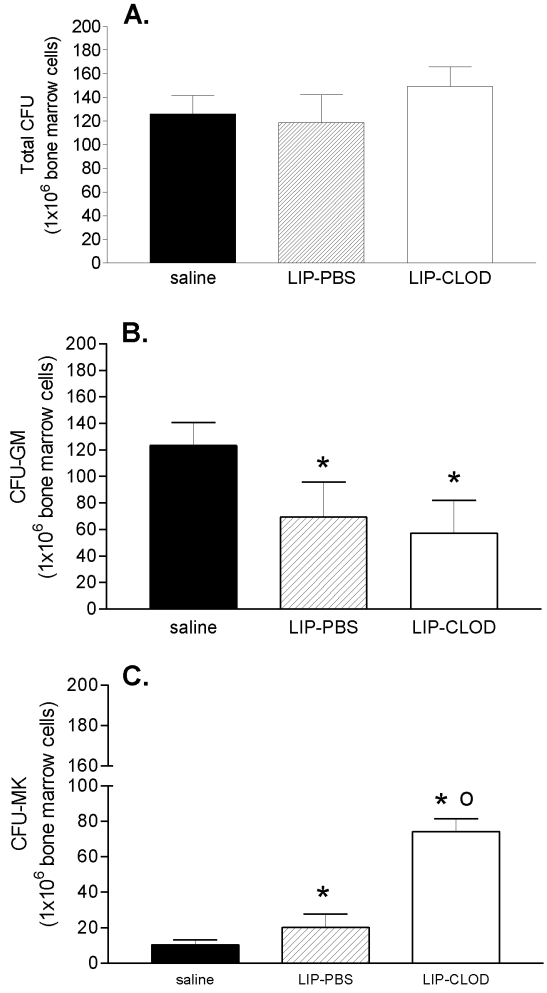

Fig. 2 Effect of LIP-CLOD treatment on bone marrow CFU-GM. Three groups of mice were injected with saline, LIP-PBS or LIP-CLOD. After 48 hr of treatment the animals were sacrificed and bone marrow cell suspensions were assayed for total CFU (**A**), CFU-GM (**B**) and CFU-MK (**C**) using CSF-GM, as described in Materials and Methods. *$p<0.001$ significantly different from saline-treated mice. °$p<0.001$ significantly different from LIP-PBS-treated mice. Bonferroni test (two-tailed), n= 4 mice/ group.

differences between groups (Fig. 2A). However, after colony examination, the LIP-CLOD-treated mice showed a decrease in the CFU-GM count (Fig. 2B), although a lesser effect was observed in LIP-PBS-treated mice. This reduction seemed to be compensated by a significant augmentation of MK precursors in bone marrow (Fig 2C).

Similar to bone marrow cells, in the total spleen colony production, control and experimental groups did not show any changes in the total CFU counts (Fig. 3A). Nevertheless, after colony observation, LIP-CLOD- and LIP-PBS-treated mice exhibited a reduction in the proliferation of GM precursors (Fig. 3B). On the other hand, these groups displayed a significant CFU-MK expansion (Fig. 3C). However, in both cases LIP-PBS exerted a significant but lesser effect than LIP-CLOD.

TPO plasma levels following macrophage depletion

Among humoral growth factors, TPO, is the most potent cytokine that physiologically regulates the megakaryocytopoiesis (15,36,58). Given the increase of megakaryocytic precursors following macrophage depletion, a time kinetic study was performed to evaluate TPO plasma levels in mice injected with saline, LIP-PBS or LIP-CLOD. In addition, since circulating levels of TPO depend mainly on circulating levels of platelets (7,16,34,51), the platelet count was determined at all time points.

Fig. 4 illustrates a significant increase in TPO plasma levels in mice treated with LIP-CLOD for 12 hr and 24 hr, while no changes in saline and empty liposome-treated groups were detected. Platelet count remained unchanged during the time course of the experiment in all experimental groups (not shown).

Transcription of TPO following macrophage depletion

To investigate whether TPO transcription is regulated following macrophage depletion, we determined the mRNA levels of TPO in hematopoietic organs (BM and spleen), and in the main TPO sources (kidney and liver) (15,37,38,53) following saline, LIP-PBS or LIP-CLOD treatment. Then, we performed a semi quantitative RT-PCR on total RNA obtained after 24 hr of treatment. All of the nine TPO mRNA isoforms (Iso) described (63) were detected in the assessed organs.

The kidney (Fig. 5A) and the bone marrow (Fig. 5B) showed no changes in the TPO mRNA expression following macrophage depletion. The Iso 1+2+5+7+9 and 6 were found to be the most abundant in the kidney, while the 4+8 splicing variants were the most profuse in the bone marrow.

In the liver (Fig. 5C), the most abundant TPO mRNA species were 1+2+5+7+9, 4+8 and 6. The liposome-treated mice showed increased levels of all isoforms, and no significant differences were detected between both groups.

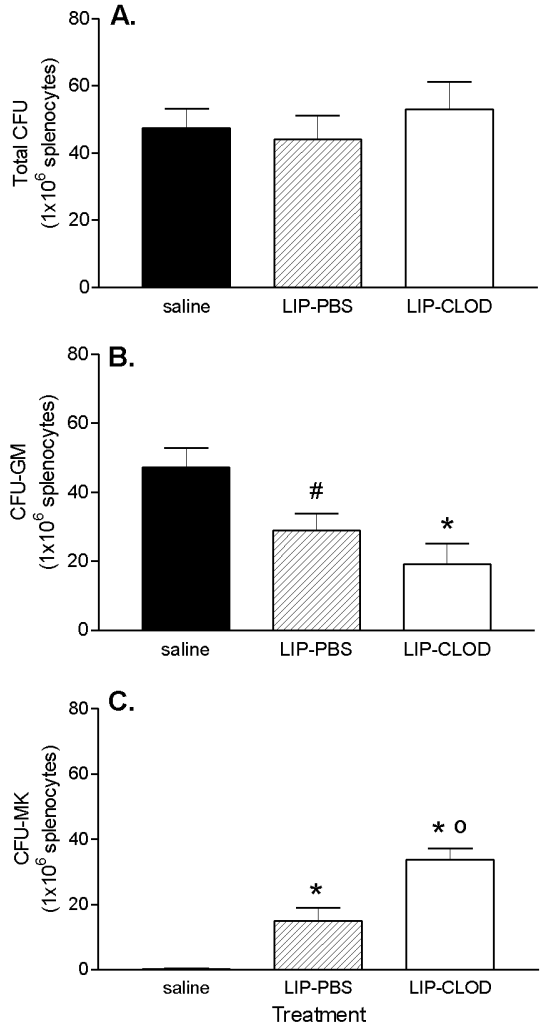

Fig. 3 Effect of LIP-CLOD treatment on spleen CFU-GM. Three groups of mice were injected with saline, LIP-PBS or LIP-CLOD. After 48 hr of treatment the animals were sacrificed and spleen cell suspensions were assayed for total CFU (**A**), CFU-GM (**B**) and CFU-MK (**C**) as described in Materials and Methods. *$p<0.001$ significantly different from saline-treated mice. $^o p<0.001$ significantly different from LIP-PBS-treated mice. Bonferroni test (two-tailed), n= 4 mice/ group.

Concerning the spleen (Fig. 5D), no signs of Iso 6 expression were observed in the saline-treated group, while both LIP-CLOD and LIP-PBS-injected mice showed detectable levels of this splicing variant, and enhanced expression of 4+8. In contrast, a significant reduction of the Iso 1+2+5+7+9 expression was found in the LIP-CLOD- and LIP-PBS-treated mice.

DISCUSSION

We have previously reported that macrophage depletion following administration of LIP-CLOD

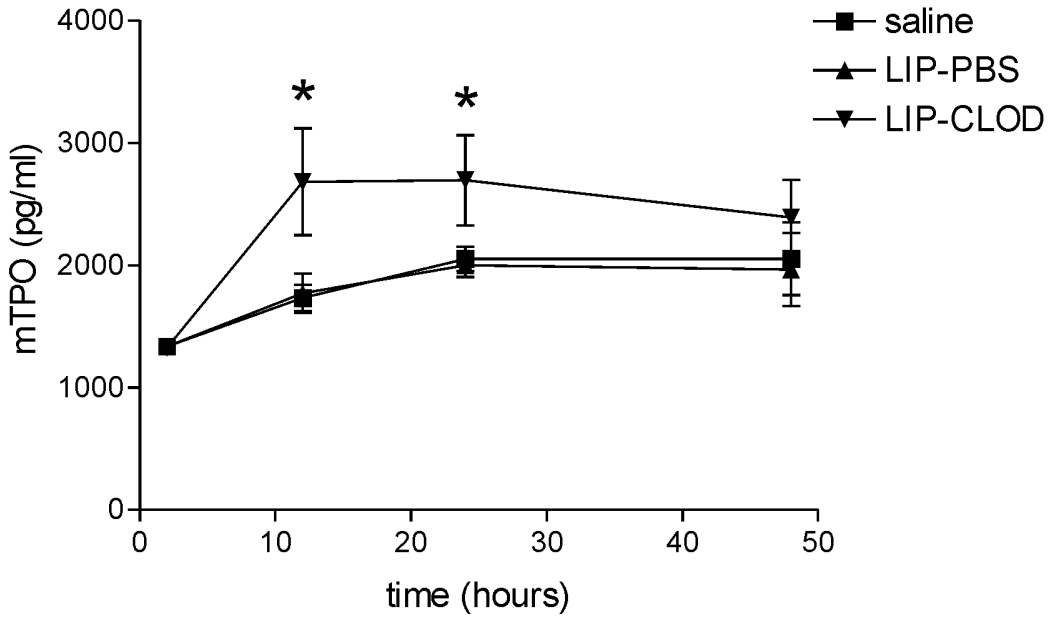

Fig. 4 TPO plasma levels following macrophage depletion. Three groups of mice were injected with saline, LIP-PBS or LIP-CLOD and bled at the indicated time points. TPO plasma levels was evaluated as described in Materials and Methods. *p<0.001 significantly different from saline-treated mice. Bonferroni test (two-tailed), n= 7 mice/ group.

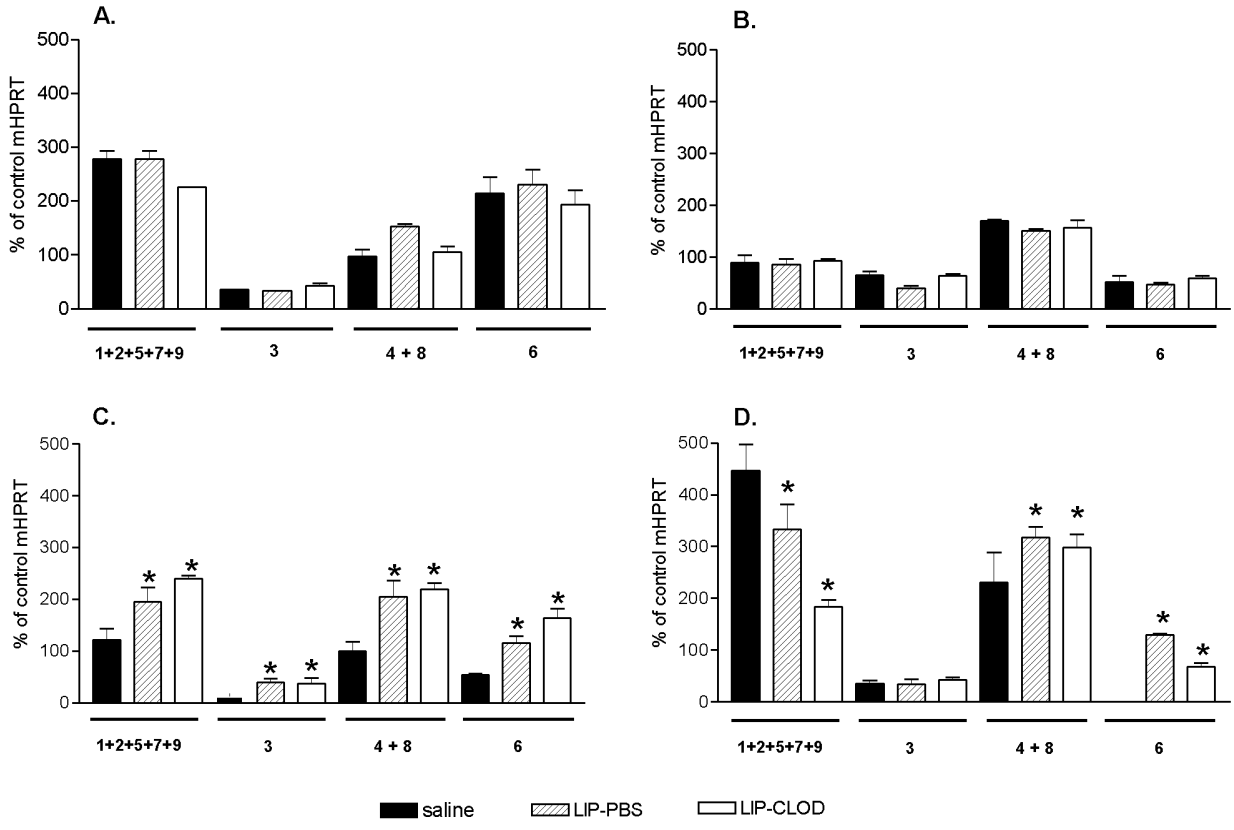

Fig. 5 TPO gene transcription following macrophage depletion. Three groups of mice were injected with saline, LIP-PBS or LIP-CLOD. After 24 hr of treatment the animals were sacrificed and a reverse transcriptase-PCR procedure was performed to determine relative quantities of mRNA TPO isoforms (Iso) in kidney (**A**), bone marrow (**B**), liver (**C**) and spleen (D) of the treated mice as described in Materials and Methods. *p<0.001 significantly different from saline-treated mice. Bonferroni test (two-tailed), n= 3 mice/ group.

provokes an enhancement of both megakaryocytopoiesis and thrombocytopoiesis (3). In fact, we showed that the depletion of splenic and hepatic macrophages in mice exhibited a consistent increase of BM and splenic megakaryocyte mass and a subsequent elevation of platelet count (3), suggesting a role for the macrophages in the regulation of megakaryocyte and platelet production. In view of this, we considered worthwhile to investigate the potential changes in the compartment of megakaryocyte progenitor cells (CFU-MK) and their correlation with plasma TPO and TPO transcription after macrophage depletion.

The assessment of CFU-MK performed in this study suggests a regulatory role of macrophages on the MK compartment. In fact, the number of MK colonies upon CSF-GM and IL-3/IL-11/TPO stimulus was significantly higher in the hematopoietic organs of LIP-CLOD-treated mice, and correlated inversely with the number of macrophage in each organ. These results indicate that the increase in the amount of MK induced by macrophage depletion (3) is not only due to the growth and development of pre-existing MK progenitor cells but also to an increase in the pool of megakaryocyte committed progenitors.

The spleen seemed to be more reactive to the effects of liposomes since MK colony expansion was also detected, although in a lesser extent, in the LIP-PBS group. The lack of impermeable vascular barriers between macrophages and the liposomes entering this organ (55) could make the spleen more sensitive to their action (9,56). Moreover, it has been reported that empty liposomes may block the macrophage action for certain periods of time (14) influencing macrophage function to some extent. On the contrary, the bone marrow structural characteristics seem to hamper the liposome effects on resident phagocytes. This point is consistent with the immunohistochemical and flow cytometry findings previously reported (3,20,23).

Alterations in the frequency and lineage distribution of progenitors responsive to CSF-GM stimulus were evident in both the bone marrow and spleen of macrophage-depleted mice. The analysis of these colonies revealed that there was a bias in the formation of a specific hematopoietic lineage. Indeed, an increase in the frequency of MK colonies and a reduction in the number of CFU-GM was observed in the hematopoietic organs of LIP-CLOD-treated mice compared to their control counterparts.

Whether macrophages or macrophage-derived products, regulate directly the commitment of the common myeloid progenitor (CMP) to generate the megakaryocyte/erythrocyte-restricted progenitors (MEPs), granulocyte/monocyte-restricted progenitors (GMPs) (1,33) or the proliferative capacity of the MEPs or GMPs populations, remains obscure and cannot be elucidated from our present results. Nevertheless, all these observations highlight the importance of macrophage-derived signalling in the negative regulation of the MK compartment and, in consequence, the formation of mature blood thrombocytes.

Since the depletion of splenic and hepatic macrophages also affect the megakaryopoyesis in bone marrow cells, we can speculate that this effect could be due to a soluble factor(s) and not to a cell to cell contact. In addition, if this is the case, it does not necessarily imply to be a direct effect.

Although it is well known that macrophages can produce a variety of important soluble factors for the hematopoyesis, we decided to evaluate changes in plasma levels of TPO and the gene expression in organs. TPO is one of the main growth factor that promotes the proliferation of committed MK progenitors, the maturation of MK and the blood platelet levels by the interaction with the c-mpl receptor (4). The TPO levels are elevated in thrombocytopenic animals and inversely related to the platelet number (34) and its concentration is regulated by the platelet and megakaryocyte mass. A similar relationship exists between erythropoietin and red blood cells during anemia (5). However, in humans, high levels of circulating TPO have been also reported in patients with reactive thrombocytosis (28), or with aplastic anemia where thrombocytopenia is associated with MK hypoplasia (19).

Our *in vivo* kinetic studies revealed a 1.5- and 1.3-fold increase in the TPO plasma concentrations at 12 and 24 hr after macrophage depletion, returning to control values at 48 hr. Given that similar platelet counts were detected at all time points in all experimental groups, we reasoned that TPO augmentation could be responsible for the stimulation of MK precursors in the bone marrow and spleen, while the fall of the plasma TPO to control levels might be ascribed to the increased MK mass that scavenge the circulating protein through the c-mpl receptor. This is in close relation with our previously reported data (3). In fact, we showed a significant increase in the reticulated platelet (immature platelets) count at 48 hr of LIP-CLOD injection which was followed by a raise in the platelet count at 96 hr of macrophage depletion.

TPO synthesis is detected in several organs throughout the body, but the main sources are liver and kidney (15,37,38). Nowadays, controversial data regarding mRNA TPO regulation exist. In fact, it has been demonstrated that this gene is constitutively expressed in the liver and kidney, and that TPO circulating levels are regulated by the combined platelet and megakaryocyte mass (42). In addition, TPO mRNA content on the liver was unaffected by platelet counts (42,53). Several studies support this issue, and revealed no signs of regulation of TPO transcription in liver or kidney of severe thrombocytopenic mice induced by radiation,

chemotherapy or anti-platelet antibodies treatment, or markedly thrombocythemic by platelet transfusions (13,52). Conversely, other evidences support TPO gene regulation. In fact, it has been shown that bone marrow stromal cells produce TPO and that transcriptional regulation occurs in this organ in response to platelet demand (25,40). In a recent report it has been shown that the level of circulating TPO can be altered under pathophysiologic conditions (10). Moreover, Wolber *et al*. reported that transcription of hepatic TPO increase during LPS-induced reactive thrombocytosis in mice (60), however, thrombocytopenic patients with liver cirrhosis show decreased hepatic TPO mRNA expression (61). In addition, *in vitro* studies with the human hepatic cell line HepG2 demonstrated that interleukin-6 (IL-6) was able to induce TPO production (59).

In this paper we have also shown an increase of all isoforms of mRNA TPO in the liver in both liposome-treated groups, suggesting a regulation of the genetic expression. However, in spleen our results suggest that liposomal treatment may also modulate TPO transcription through a mechanism of alternative splicing, since a reduction of the isoforms 1+2+5+7+9 appeared to be counteracted by an augmentation of the 4+8 and 6 transcripts. This finding is not in agreement with previously reported data (12,21) where no signs of variations in TPO isoforms encoding non secreted proteins were evident. Stoffel *et al.* (52) also reported that the expression profile of Iso 1, Iso 2, Iso 3 and Iso 4 remained unchanged in the liver and kidney of the thrombocytopenic mice. However, these authors used primers that could not make possible the detection of the five new types of isoforms (Iso 5 to 9) described by Wu (63), all of which were identified in this work.

The physiological role (s) of spliced forms is unclear. Isoform 1 is the most abundant and biologically active species and Iso 2, Iso 3 and Iso 4 have been reported to be efficiently expressed intracellularly but poorly secreted *in vitro*. Hoshi *et al* demonstrated that TPO-4 fusion protein has bioactivity comparable to TPO-1 (27). In addition, Wu *et al* showed that TPO-4 remains in the cytoplasm while TPO-1 is secreted (63). This raises the possibility that truncated TPO variants are stored in the cytoplasm after their synthesis, and released upon cellular stimulation. Since the liposomal effect is exerted mainly in liver and spleen, organs with high reticuloendothelial system (RES) activity, we propose that a product(s) derived from liposome-engulfing macrophages might induce TPO gene upregulation in these organs. However, transcriptional TPO upregulation correlates with an increase of plasma levels of TPO only in those animals where macrophages had been removed effectively. The underlying mechanism of this effect remains elusive and more precise studies are needed to clarify this point.

Given these results, we have, at least, two options. One is based on the possibility that LIP-CLOD treatment could enhance TPO mRNA translation or, on the other hand, that it could promote the secretion of the intracellular pool of TPO.

Collectively, the observations made on the hematopoietically active organs clearly indicate a regulatory role of the macrophage populations on the megakaryocytopoiesis. At present, we can speculate that this effect could be due to several and concurrent factors. Quantitative differences in the BM and spleen macrophage population might lead to imbalanced cell-cell interactions and/or differences in local cytokine concentrations, including stimulatory molecules like TPO (15,36,58), Oncostatin M (57) or stem- cell derived factor (54), or their counterparts inhibitory ones: tumor necrosis factor-α (30), macrophage inflammatory proteins-1α (24), transforming growth factor-ß1 (30,47). In such a scenario, concomitantly with an increase in the hepatic TPO synthesis, the splenic and bone marrow committed MK progenitors may be hypersensitive to the TPO proliferative signal increasing the MK mass in both organs.

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