

SHORT COMMUNICATION

OPPOSITE EFFECTS OF MAST CELL DEGRANULATION BY COMPOUND 48/80 ON PERITONEAL INFLAMMATION IN SWISS AND CBA MICE

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The murine strains differ in the number of peritoneal mast cells. Degranulation of peritoneal mast cells by single injection of compound 48/80 (1.2 mg/kg) followed by zymosan-induced (2 mg/ml, 0.5 ml/mouse) peritoneal inflammation caused either inhibition or enhancement of an early influx (at 4 h of peritonitis) of exudatory leukocytes in Swiss and CBA mice, respectively. These opposite effects correspond with statistically significant differences in the number of peritoneal mast cells in the intact Swiss (11×10^3) and CBA (39×10^3) mice.

Key words: *peritoneal mast cells, compound 48/80, inflammation, histamine, degranulation, peritoneal leukocytes*

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Abbreviations: C48/80 – compound 48/80, MC – mast cell, PMN – polymorphonuclear leukocytes, PTL – peritoneal leukocytes

INTRODUCTION

Zymosan-induced peritoneal inflammation is commonly used for investigations of cellular and molecular mechanisms of peritonitis [2]. Mast cells (MCs) are the principal initial effector cells in inflammation and allergic reactions. This effects may be attributed to the wide range of cytokines, vasoactive amines, and other mediators released after activation [10, 13]. The role of MCs can be established by experiments on genetically MC deficient W/W^v mice and their control counterparts [5, 7] and on the pharmacologically treated naive mice. The treatment with cromolyn stabilizes MC granules [17] while compound 48/80 (C48/80) induces their degranulation [1, 12]. The results of thorough experiments of Ajueborg et al. [1] showed that Swiss mice *ip* injected with a single dose of C48/80 are almost completely depleted of peritoneal MCs 72 h later. Peritonitis induced at this time by *ip* zymosan injection is significantly different from that in the control animals with intact MCs. In particular, the number of exudatory polymorphonuclear leukocytes (PMNs) at 4 h of peritonitis in mast cell-depleted (MCx) mice is significantly lower than that in their control counterparts (MC) [1]. We recorded the same phenomenon in the Swiss mice from another supplier while quite opposite results were obtained during experiments on CBA mice subjected to the same procedure. The aim of the present investigations was to find reasons for this discrepancy.

MATERIALS and METHODS

Animals

Swiss male mice (4–6 weeks old, 26–30 g) were purchased from the Animal Laboratory (Warszawa) while CBA, C57BL, Outbred (Out), and Balb/c male mice (4–6 weeks old, 25–28 g) were purchased from the Animal Department of Collegium Medicum (Kraków), and from Animal Department of Genetics IZ (Kraków). All mice were housed 4–5 per cage in the laboratory room with a fixed light-darkness conditions (12:12) with water and standard diet *ad libitum*.

Treatment

Some mice received a single dose of C48/80 (1.2 mg/kg *ip*, Sigma, St. Louis, MO, USA) dissolved in phosphate-buffered saline (PBS) (C groups) or with PBS (PBS groups). Groups of 3–5 animals were killed by cervical dislocation at time 0 (intact) or 30 min, 8, 24, 48, and 72 h after C48/80 administration while PBS-treated controls were killed at 72 h only. At 72 h, remaining C48/80-pretreated mice were injected *ip* with zymosan A (2 mg/ml, 0.5 ml/mice, Sigma, St. Louis, MO, USA) in order to induce peritoneal inflammation [2], and were designated as CZ groups. As a control, intact animals were injected with zymosan only (Z group). Mice from CZ and Z groups were killed at 0, or 30 min, 4 and 8 h after zymosan-induced peritonitis.

Sampling

The peritoneal cavity of each animal from the C, PBS, CZ, and Z groups was lavaged with 1 ml of saline and the lavage/exudate fluid was used for cell counts and cytospin preparations or centrifuged at 400 g for 15 min and frozen at –20°C for histamine and chemotactic factor assays. Total and differential cell counts were done with a hemocytometer and a light microscope following staining with Turk's solution (0.01% crystal violet in 3% acetic acid) and on May-Grunwald Giemsa-stained cytospin preparations. The MCs were counted and regarded as “full” (F-MC) or partly degranulated (D-MC). The former were round shaped filled completely or almost completely with dark violet granules while the latter were at least partly disintegrated with some granules extruded. The MCs completely devoid of stainable granules were uncountable by this method. For this reason the method applied here underestimates the total MCs number. Similar subjective methods of estimation of the degree of MC degranulation were also used by other investigators [3, 14, 16].

Histamine content was measured by Histamine ELISA kit (ICM Pharmaceuticals, Inc., Costa Mesa, CA) according to the procedure recommended by the manufacturer.

Statistics

Experiments on kinetics of zymosan-induced inflammation with or without previous 72 h treatment of C48/80 were repeated three times and the results were pooled. One-way analysis of variance

(ANOVA), multifactor analysis of variance, and Student's *t*-test were used for statistical evaluation of the data. Differences were considered statistically significant at $p < 0.05$.

RESULTS and DISCUSSION

Figure 1 shows that murine strains differ significantly in the percentage of MCs among the peritoneal leukocytes (PTLs) (Fig. 1a) and in the total number of MCs (Fig. 1b). The lowest numbers were recorded in Swiss mice while the highest in CBA mice (0.2% ; $11 \pm 2.7 \times 10^3$ and 1.6% ; $39 \pm 4 \times 10^3$ MCs/mice, respectively). These two contrasting strains were used for further experiments.

The strain differences of peritoneal MCs numbers were recorded in rats with smallest values in BN rats than in Wistar and SD rats [15]. Murine strains differ also in the number of conjunctival MCs which were much more abundant on histological sections from C3H/HeN than from C57BL/6

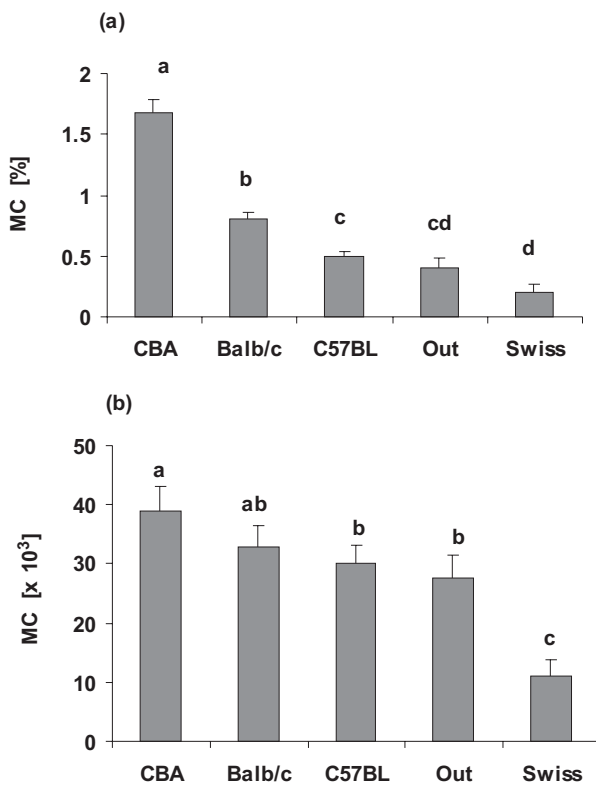


Fig. 1. Percentages of mast cells (MCs) among peritoneal leukocytes (a) and total number (b) of MC in the peritoneal cavities of several strains of mice. The mean values sharing the same letters do not differ significantly while those with various letters are statistically significantly different according to ANOVA

and ASW/J and practically absent in genetically MC deficient W/W^v mice [8].

Figure 2 shows comparisons of the kinetics of appearance of some inflammation-related factors in the peritoneal cavities of Swiss and CBA mice *ip* injected with C48/80. It became apparent that in both strains this compound induced peritoneal inflammation, as evidenced by an influx of PMNs with a peak at 24 h (Fig. 2a). The multifactor analysis of variance showed a similarity of kinetics of PMN influx in Swiss and CBA mice (Fig. 2a). Similar influx of PMNs was described by Li et al. [8] who reported that inflammatory reaction peaked 6–24 h after C48/80 administration.

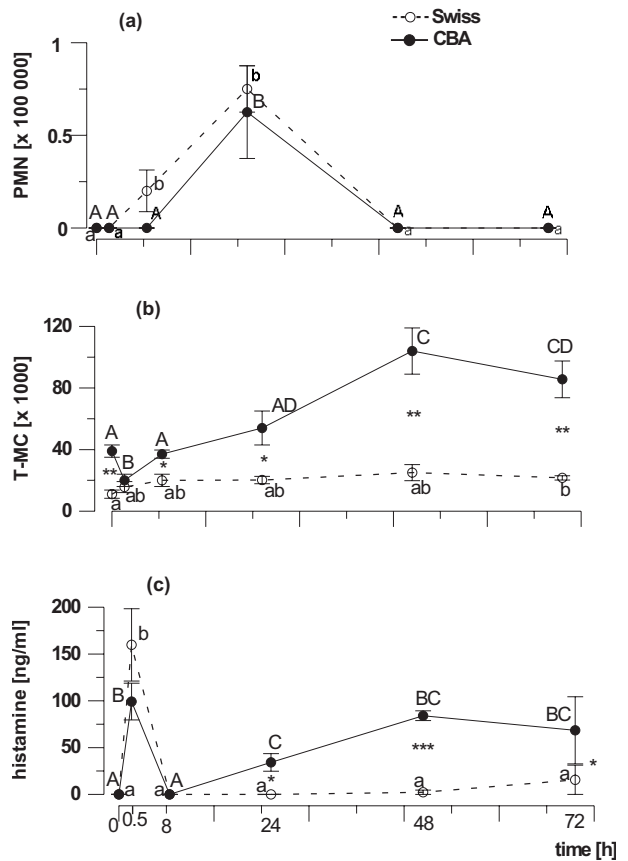


Fig. 2. Inflammation-related factors in Swiss (open symbols and broken line) and CBA (solid symbols and lines) mice injected *ip* with C48/80. (a) numbers of exudatory polymorphonuclear leukocytes (PMNs); (b) total numbers of peritoneal MCs (T-MC); (c) levels of histamine in peritoneal fluid. Means \pm SE from 3–5 animals. The mean values sharing the same letters do not differ significantly while those with various letters are statistically significantly different according to ANOVA. Asterisks between values significantly different according to Student's *t*-test: * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$

The multifactor analysis of variance showed statistically significant interstrain differences in MC numbers (Fig. 2b) and histamine levels (Fig. 2c). The number of countable MCs increased only slightly in Swiss mice, while it changed considerably in CBA animals, with a maximum at 48 h after injection (Fig. 2b). The level of histamine was undetectable in intact animals, in both strains it sharply increased immediately after C48/80 injection and come back 8 h later. Then it stayed close to zero in Swiss mice while increased again in CBA animals reaching a second peak at 48 h and remaining at this level at 72 h (Fig. 2c). This second wide peak of histamine paralleled with the pronounced influx/proliferation of MCs observed in CBA strain.

It should be noted that 72 h after PBS injection into the control animals the MC numbers and histamine levels were the same as those in the intact animals of both strains. This indicates that the effects described above resulted from C48/80 itself but not from the stressing puncture and/or PBS injection.

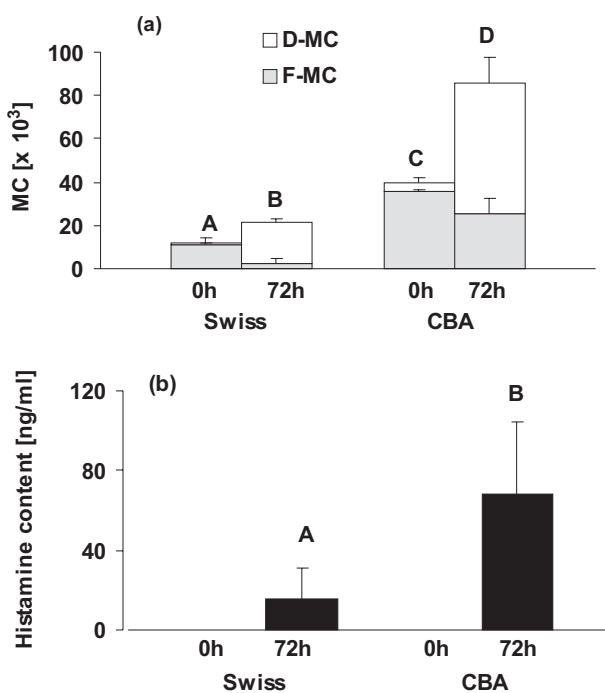


Fig. 3. Strain-specific effects of C48/80 on (a) numbers of mast cells (MCs), full (F-MCs, dotted parts of bars) or partly degranulated (D-MCs, empty parts of bars) and (b) levels of histamine (solid bars) in Swiss and CBA mice at 0 and 72 h after *ip* injection with C48/80. Means \pm SE ($n = 3-5$ mice). The mean values sharing the same letters do not differ significantly while those with various letters are statistically significantly different according to ANOVA

In conclusion, the first part of the latest experiments shows that the local injection of the well-known MC degranulator, C48/80, induces peritoneal inflammation in both Swiss and CBA mice, MC degranulation and influx and/or local proliferation of these cells which is only slight in Swiss mice but very pronounced in CBA animals. The mechanism of MC accumulation in the site of inflammation is poorly understood. Local proliferation of MCs was described previously [6]. Influx of MCs may be caused by MC chemoattractants including stem cell factor, transforming growth factor γ_1 , complement components C1q, C3a, C5a, platelet-activating factor (PAF) and adenine nucleotides [9, 11].

As summarized in Figure 3, in both strains investigated here intraperitoneal injection of C48/80 induced MC degranulation, an increase in their numbers, and an increased histamine level. At 72 h a majority of MCs were more or less degranulated in both strains while only 12% and 30% of them (in Swiss and CBA mice, respectively) were almost completely filled with strongly stained granules (Fig. 3a). At this time the level of histamine (being close to zero in intact animals) was low in Swiss mice (15.6 ng/ml) while it was quite pronounced (68.5 ng/ml) in CBA animals (Fig. 3b).

In other words, at 72 h after injection of C48/80, the local environment of peritoneal cavity is considerably different in Swiss and CBA mice. MCs are almost completely degranulated and histamine level is relatively low in Swiss animals (character-

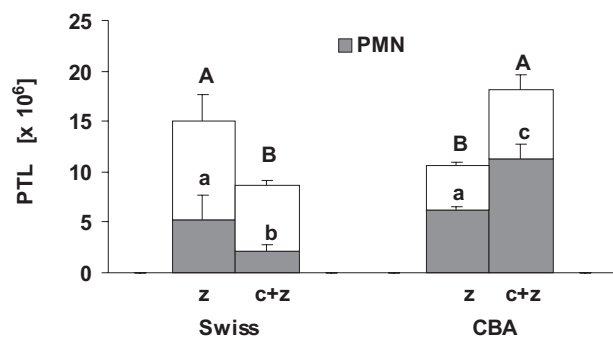


Fig. 4. Effects of mast cell degranulation on total numbers of PTLs and among them PMNs (dotted parts of bars) at 4 h after *ip* zymosan injection in Swiss and CBA mice. Z – mice injected with zymosan only; CZ – mice injected with zymosan 72 h after C48/80 pretreatment. Means \pm SE ($n = 3-5$ mice). The mean values sharing the same letters do not differ significantly while those with various letters are statistically significantly different according to ANOVA

zed by low MCs number in intact). In contrast, at 72 h after C48/80 injection in CBA mice (which possess a high number of MCs as intact), there is an abundant population of MCs, some of them being filled with intensively stained granules. Consequently, at this time a histamine level is significantly higher in CBA than in Swiss mice. Therefore, it is not surprising that the C48/80 injection affects the subsequent zymosan-induced peritonitis in a different way in Swiss mice than in CBA animals.

Figure 4 compares the numbers of peritoneal leukocytes (PTLs) and, among them, PMNs, at 4 h of zymosan-induced peritonitis in Swiss and CBA mice pretreated with C48/80 with those in the animals injected with zymosan only. It turned out that following C48/80 pretreatment, the numbers of PTLs and PMNs were statistically significantly decreased in Swiss mice but increased in CBA mice.

The decreased number of PMNs in C48/80-treated Swiss mice was recorded previously by Ajueborg et al. [1], who focused only on this time point of zymosan-induced inflammation. As shown in Figure 5, we recorded some inflammation-related factors after 30 min, 4 h and 8 h of zymosan-induced peritonitis in Swiss and CBA mice with and without C48/80 pretreatment. It turned out that in both strains the differences in the PTL and PMN numbers between C48/80-treated and untreated mice were statistically significant only at 4 h after zymosan injection while they were insignificant at shorter (30 min) and at longer (8 h) time intervals. In other words, the effects of C48/80-induced MC degranulation are transient and restricted to the specific stages of zymosan-induced inflammation (Fig. 5a, b).

Zymosan itself influences more CBA than Swiss mice peritoneal MCs (Fig. 5c, c') and in both strains some of them degranulate rapidly as evidenced by the decreased number of "full" MCs at 30 min after the treatment with zymosan only (Fig. 5d, d'). A quite different picture is observed in the animals treated with zymosan after C48/80 pretreatment (Figs 5c, c', d, d'). In Swiss mice the numbers of countable cells decrease till 4 h after zymosan injection (perhaps due to their complete degranulation) and then they return to the initial level (Fig. 5c) in parallel with the increased number of the "full" MCs (Fig. 5d). In CBA mice the initial total MC number is very high, it decreases rapidly till 4 h and then recovers (Fig. 5c'). The number of

the "full" MC is low till 4 h and then it significantly increases (Fig. 5d'). It seems that in C48/80-pretreated Swiss and CBA mice the second proinflammatory stimulus, zymosan, induces complete degranulation of peritoneal MCs followed by their slow recovery by reconstitution of the granular content [6] or by influx of new MCs from other sites [4, 12].

The rapid release of histamine is evident soon after zymosan treatment of both Swiss and CBA mice (Fig. 5e, e'). In Swiss mice pretreated with C48/80 the level of histamine increases much later, at 8 h after zymosan injection, in parallel with the recovery of MCs (Fig. 5e). In contrast, in the same group of CBA animals, the initial histamine level is rather high and it decreases completely by 4 h after zymosan treatment (Fig. 5e').

Effects of C48/80-pretreatment on MCs/histamine during the subsequent zymosan-induced peritonitis are very complex. The numbers of zymosan-induced exudatory PTLs and PMNs are different at 4 h but similar at 8 h in animals with and without C48/80 pretreatment. It suggests the existence of some regulatory mechanisms leading to the recovery of the process disregulated by C48/80 treatment. Such a phenomenon of full recovery of inflammatory process following its temporary impairment was recorded in mice treated with cromolyn (a well-known stabilizer of MC granules) or in those with pharmacological blockade of histamine receptors [7].

The results obtained here indicate that the procedure of MC depletion by C48/80-treatment should be optimized for each particular murine strain. In some strains (e.g. Swiss) the MCs are almost completely degranulated at 72 h after treatment. The same procedure induces different effects in other murine strains (e.g. CBA) inducing not only their degranulation but also the massive influx and/or local proliferation. The effects of C48/80 on the local microenvironment of peritoneal cavity are strain-dependent and perhaps related to the initial number of the local MCs. The difference in numbers and/or state of cellular cycle/activation of MCs may induce various effects on some stages of the subsequent inflammatory reactions.

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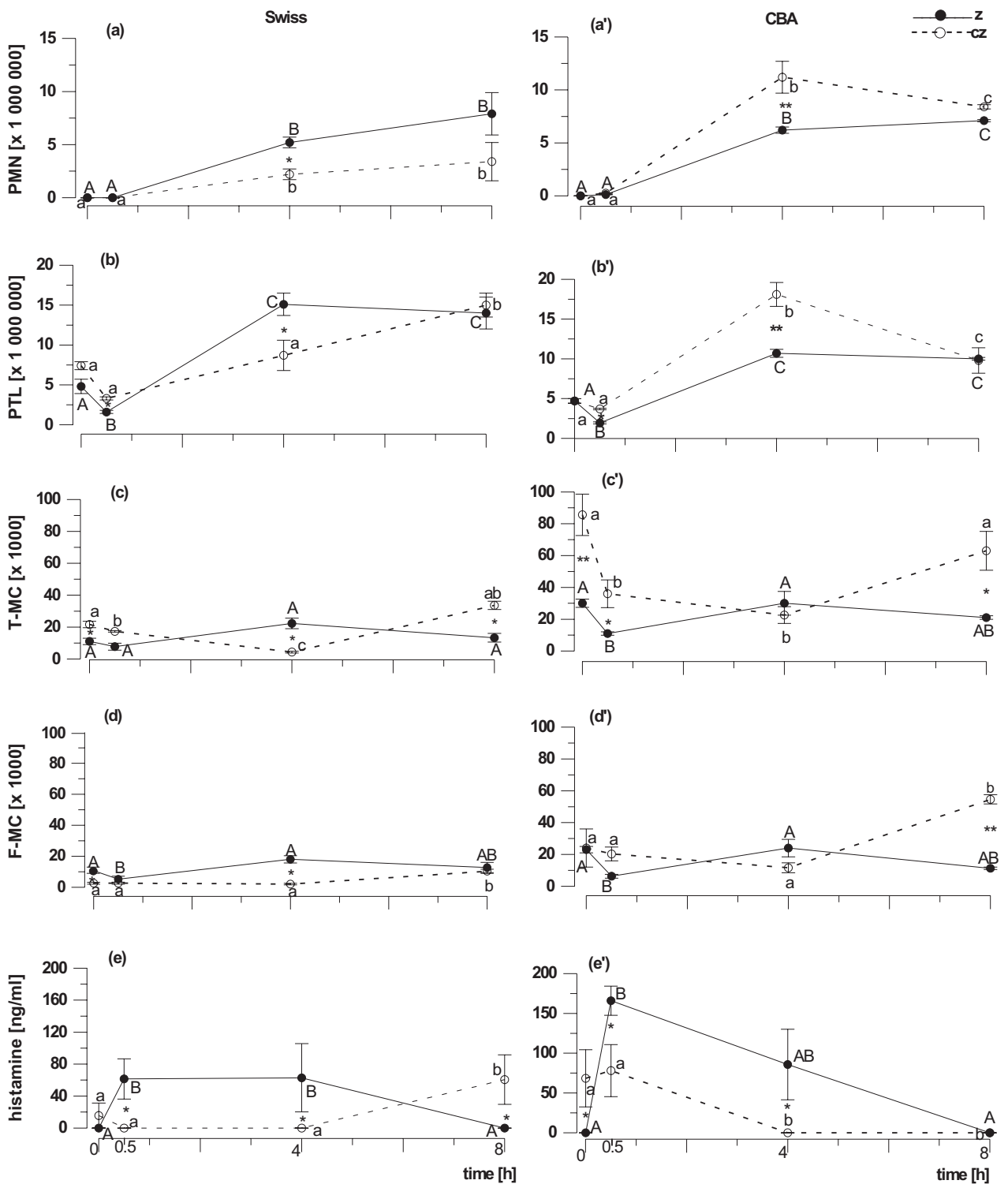


Fig. 5. Inflammation-related factors in Swiss (left panel, (a)–(e)) and CBA (right panel, (a')–(e')) mice injected *ip* with zymosan (z) without (solid symbols and lines) or with (empty symbols and dotted lines) pretreatment with C48/80 (cz). (a), (a') numbers of exudatory PMNs; (b), (b') total numbers of exudatory leukocytes PTLs; (c), (c') total numbers of peritoneal MCs (T-MC); (d), (d') numbers of full peritoneal MCs (F-MC); (e), (e') levels of histamine in peritoneal fluid. Means \pm SE from 3–5 animals. The mean values sharing the same letters do not differ significantly while those with various letters are statistically significantly different according to ANOVA. Asterisks between values significantly different according to Student's *t*-test: * $p < 0.05$, ** $p < 0.01$

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