GENERAL PATHOLOGY AND PATHOLOGICAL PHYSIOLOGY

Granulomatous Inflammation of the Liver in Mice with Gadolinium Chloride-Blocked Kupffer Cells

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(CBA×C57Bl/6) F_1 mice injected with zymosan intravenously developed granulomas in the liver; the number of granulomas in mice pretreated with gadolinium chloride, a selective blocker of Kupffer cells, was half that in the untreated animals. Kupffer cells isolated from the liver 5 days after zymosan injection, i.e., during the period when granuloma generation was at its height, displayed a high capacity for stimulating both the luminol-dependent chemiluminescence of blood leukocytes (which is associated with the generation of reactive oxygen species) and the colony-forming activity of bone marrow cells; this capacity was much lower in mice pretreated with gadolinium chloride. It is shown that granulomatous inflammation of the liver is directly dependent on the activity of Kupffer cells.

Key Words: *granulomatous inflammation; Kupffer cell; macrophage; gadolinium chloride; zymosan*

We showed previously that characteristic granulomas consisting predominantly of mononuclear phagocytes are formed in the liver and lungs of mice and rats injected intravenously with zymosan granules that persistently irritate the macrophages residing in these organs [2,3]. However, the precise mechanisms of formation of these granulomas are unknown.

It was found recently that gadolinium chloride ($GdCl_3$) selectively depresses Kupffer cells (KC) but has little or no effect on macrophages of the other compartments [7]. $GdCl_3$ not only blocks the phagocytic activity of hepatic macrophages, but also causes a selective elimination of large macrophages located in the periportal zone of the hepatic lobule. The liver begins to be repopulated by fresh macrophages not earlier than 4 days after an intravenous injection of $GdCl_3$. After the repopulation is completed, the new macrophages show the same sensitivity toward $GdCl_3$ as the first KC pool [9]. These observations led us to undertake a study of granuloma generation in $GdCl_3$-induced KC depression in order to define the role of KC in this process.

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MATERIALS AND METHODS

Male (CBA×C57Bl/6) F_1 mice weighing 18-22 g were used. They were divided into four groups. The first three groups consisted of normal mice and mice treated with $GdCl_3$ (Gd-mice) or zymosan (Z-mice), while group 4 comprised mice treated with zymosan 2 days after $GdCl_3$.

$GdCl_3$ (Aldrich) was injected via the tail vein in a dose of 10 mg/kg in 0.2 ml of 0.85% NaCl. Normal (control) mice received 0.85% NaCl in the same volume. A suspension of zymosan granules

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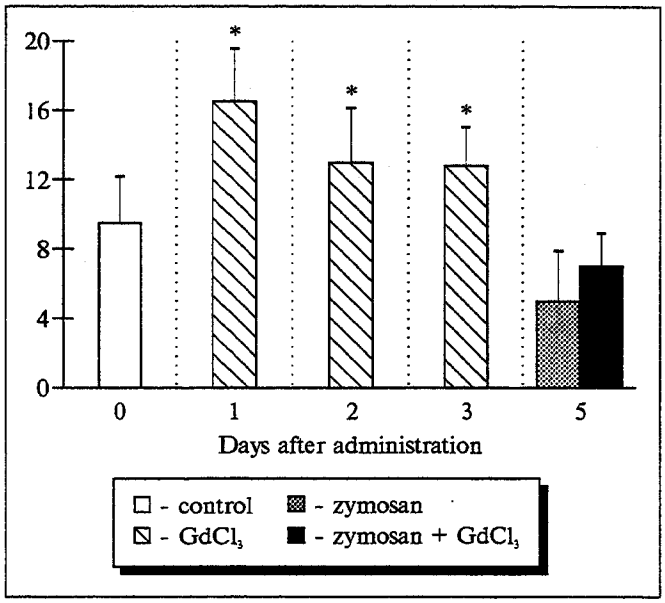

Fig. 1. Elimination half-time of colloidal carbon from the blood of mice. Ordinate: elimination half-time, min. *$p<0.05$ in comparison with control mice.

was injected intravenously in a dose of 10 mg/100 g of body weight, the mice were killed 2 or 5 days later [4], and the total number of granulomas was counted per mm^2 of hematoxylin-eosin-stained liver sections. In addition, the average granuloma size was measured in μ^2, as was, in percent, the relative area of the section involving the granulomas. The engulfing function of KC was assessed by the speed at which colloidal carbon (Gunter-Wagner) particles were eliminated and by

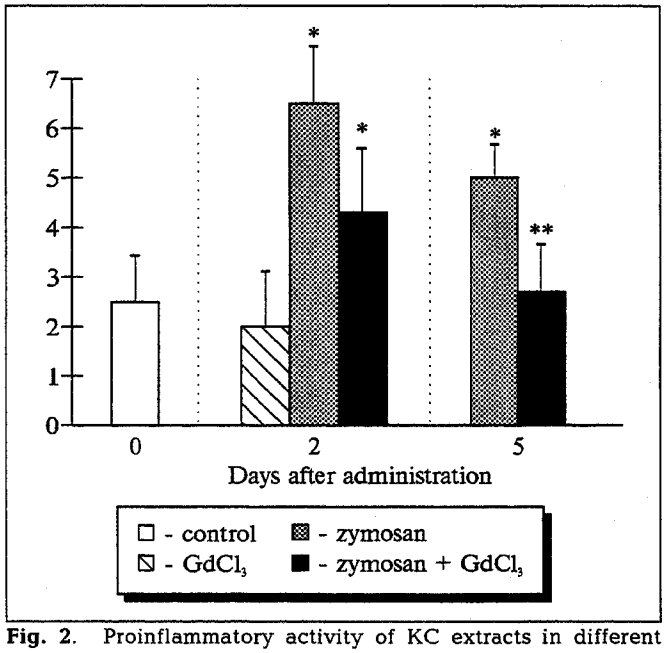

Fig. 2. Proinflammatory activity of KC extracts in different groups of mice. Ordinate: chemiluminescence intensity ($\times10^{-3}$ cpm/granulocyte). *$p<0.01$ in comparison with the control group; **$p<0.05$ in comparison with zymosan-treated mice.

the number of carbon-laden KC per field of vision at a ×400 magnification.

Kupffer cells were isolated from the liver by the method of Nabors *et al.* in our modification [1]. P-100F colloidal iron (particle size 0.8-1 μ) was used to load the cells. After the cells had been disintegrated in hypotonic medium, the material was centrifuged at 10,000 g at 4°C for 20 min, and protein was determined in the supernatant by Lowry's method. The proinflammatory activity of KC was evaluated by the ability of their extracts to elicit a chemiluminescent response from syngeneic leukocytes. For this, 0.7 ml of Hanks' medium and 0.1 ml of a solution containing 10^{-4} M luminol (Sigma) were added to 0.1 ml of whole murine blood. Background chemiluminescence was recorded, after which 0.1 ml of the supernatant was added to the same cell of the chemiluminometer (SKIF-0301). After the chemiluminescence intensity had been recorded every 2 minutes for 1 h, the peak amplitude of the chemiluminescent response was determined and expressed in cpm/granulocyte.

The colony-stimulating activity of KC was evaluated by the ability of their extracts (100 μg protein/ml) to induce growth of granulocyte-macrophage colonies. For this, the extracts were added to a culture containing 10^5 cells of normal syngeneic bone marrow in RPMI-1640 medium supplemented with 20% fetal calf serum, 2×10^{-2} M 2-mercaptoethanol, L-glutamine (200 mg/liter), gentamicin (80 mg/liter), and 0.8% methylcellulose [8]. Culturing was continued for 7 days at 37°C in a humid atmosphere with 5% CO_2 in a gas-flow incubator. Cell conglomerates containing at least 50 cells were counted as colonies. The colony-stimulating activity of the extracts was expressed as the number of granulocyte-macrophage colonies per 10^5 nucleated bone marrow cells.

The numerical data were statistically analyzed by Student's *t* test.

RESULTS

The elimination of colloidal carbon from the blood of Gd-mice was greatly delayed. The depression was most marked after 24 h and was still recorded on days 2 and 3 postadministration. Zymosan speeded up blood clearance, which reached its maximum on day 5 and was 2.5 times higher than in normal mice. After zymosan injection into Gd-mice, their clearing ability became almost normal, but did not reach the level recorded for Z-mice (Fig. 1). After 2 days, the livers of Gd-mice contained only one-third as many carbon-laden KC as did those of control animals (11.3±3.14 *vs.* 31.5±2.56 per visual field).

Granulomas were already present in the livers of normal mice on day 2 after zymosan injection. Thereafter, the area they occupied rapidly increased to reach 29.7±1.59% of the entire area of the liver section. This resulted from increases in both the number and size of the granulomas. The number of granulomas in zymosan-treated Gd-mice was only half that in Z-mice, although the granulomas were virtually of the same mean size in both groups (Table 1).

The chemiluminescent response of syngeneic neutrophils to KC extracts from normal mice ranged from 2.10×10^{-3} to 3.13×10^{-3} cmp/granulocyte (on average, $2.49\pm0.53\times10^{-3}$). In the Gd-mice, these figures were practically the same as in the normal mice. When KC were taken from Z-mice, the response ranged from 4.71×10^{-3} to 5.37×10^{-3} cmp/granulocyte - values that are twice as high as in the normal and Gd-mice. In contrast, the chemiluminescent response in Gd+Z-mice was significantly lower than in Z-mice, ranging from 2.8×10^{-3} to 3.41×10^{-3} cpm/granulocyte, reaching, on average, only 62.1% of that in Z-mice ($p<0.05$) (Fig. 2).

After the addition of KC extracts from normal mice to the bone-marrow cell culture, 32.2± ±2.53 granulocyte-macrophage colonies formed. Similar values of colony-stimulating activity were obtained following the addition of KC extracts from Gd-mice (Fig. 3). On the other hand, extracts from Z-mice caused a marked increase in colony formation. Thus, extracts prepared 2 days after zymosan injection led to a 3.9-fold rise in the number of colonies and those prepared after 5 days, to a 2.5-fold rise. The colony-stimulating activity of KC extracts from Gd+Z-mice differed little from that of extracts from Z-mice if the extracts were prepared 2 days after zymosan injection, but was 4.2 times lower if they were prepared after 5 days.

Thus, characteristic granulomas developed in the liver of mice after zymosan administration. We showed previously [4] that granuloma formation peaks on days 5 and 6 postinduction. $GdCl_3$-pretreated mice developed a KC depression accompanied by inhibition of granuloma generation. The development of granulomas went along with a rise in the phlogogenic activity of KC, whose extracts

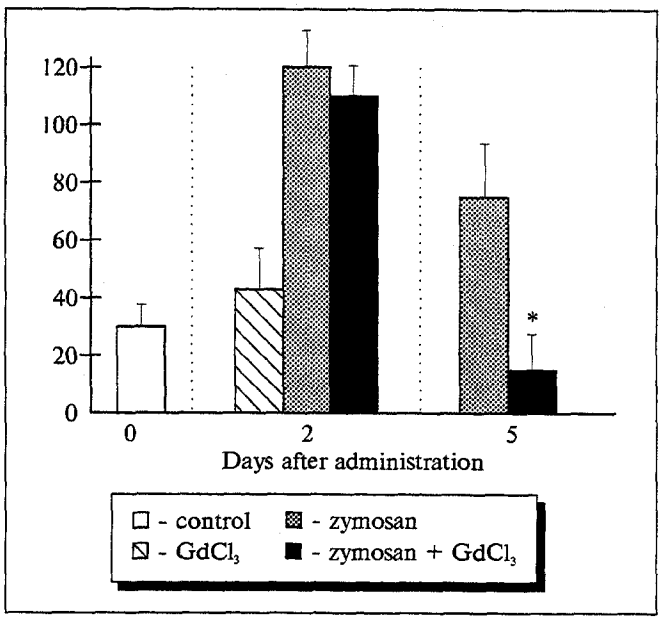

Fig. 3. Colony-stimulating activity of KC extracts in different groups of mice. Ordinate: number of granulocyte-macrophage colonies/10^5 bone marrow cells. *$p<0.01$ in comparison with zymosan-treated mice.

caused blood leukocytes to exhibit a markedly increased luminol-dependent chemiluminescence, which is associated with the generation of reactive oxygen metabolites [5,6]. This activity was appreciably lower when granulomas were induced in animals with $GdCl_3$-induced KC depression.

The involvement of KC in inflammation depends not only on the total phlogogenic activity of these cells *in situ* but also on their hematopoiesis-stimulating function. It is noteworthy in this context that when granuloma formation was at its peak, KC extracts showed a much greater ability to stimulate the growth of granulocyte-macrophage colonies from bone-marrow precursors, this ensuring a sufficient inflow of newly formed inflammatory cells to the granuloma with the blood. On the other hand, granuloma generation in $GdCl_3$-treated animals was accompanied by a much smaller increase in the colony-stimulating activity of KC extracts.

In general, pretreatment with $GdCl_3$ led to a selective elimination of KC and to a shrinking of their phagocytic pool. Moreover, the KC lost their sensitivity to zymosan so that the number of granulomas in the liver decreased by half. The reduction

TABLE 1. Intensity of Granulomatous Inflammation in Intact and $GdCl_3$-Treated Mice after Zymosan Injection

Mice	No. of granulomas per mm²	Mean granuloma size, mm²	Relative area of granulomas, %
Z-mice ($n=6$)	16.1±2.17	0.089±0.01	29.7±1.59
Gd+Z-mice ($n=5$)	7.35±0.78*	0.085±0.007	13.9±0.79*

Note. *$p<0.05$ in comparison with Z-mice.

in granuloma numbers was accompanied by a weakening of the proinflammatory and colony-stimulating activities of KC.

The results of this study confirm that KC or, more precisely, their responsiveness to the phlogogenic stimulus determine the development of hepatic granulomas.

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