ROLE OF MACROPHAGE BREAKDOWN PRODUCTS IN THE ALVEOLAR PHAGOCYTOSIS RESPONSE

L. I. Privalova, B. A. Katsnel'son, and A. V. Osipenko

UDC 612.112.3:612.215.3

Peritoneal macrophages of Wistar rats obtained after intraperitoneal injection of mineral oil were disintegrated by repeated freezing and thawing. In response to intratracheal injection of intact rats and rats receiving four daily inhalations of titanium dioxide dust, these macrophage breakdown products (MBP) caused a substantial increase in the number of alveolar macrophages and neutrophilic leukocytes in washings from the lungs; the mean value of the neutrophil/alveolar macrophage ratio, moreover, was several times greater than in rats receiving intratracheal injections of physiological saline. The response to dust particles of low cytotoxicity plus exogenous MBP thus very closely resembled that usually observed after inhalation of a cytotoxic (quartz, for example) dust. By increasing the contribution of neutrophils to phagocytosis of inhaled particles, MBP lead to a substantial decrease in the mean dust load on the single alveolar macrophage, although the total number of phagocytosed particles is increased. Preponderant involvement of granulocytes, especially neutrophils, compared with peritoneal macrophages also was found in the peritoneal exudate of rats receiving MBP or quartz intraperitoneally, whereas injection in this way did not affect alveolar phagocytosis. MBP stimulated migration of the blood leukocytes and promoted the more efficient utilization of oxygen by the macrophages. The possible role of MBP as a factor regulating the phagocytic response of the body at various stages is discussed.

KEY WORDS: *macrophages; neutrophils; alveolar phagocytosis.*

Consequent upon the connection between the character of the phagocytic response of the lungs to dust and the degree of damage to the free alveolar macrophages it has been suggested that macrophage breakdown products (MBP) play the leading role in the autoregulation of this response. It has been suggested that MBP cause the liberation of an increased number of alveolar macrophages and, in particular, of neutrophils into the lumen of the deep respiratory passages [5]. Other workers also have put forward hypotheses to explain the role of macrophagal products in the regulation of alveolar phagocytosis [8, 9].

In this investigation an attempt was made to obtain direct confirmation of the effect of MBP on this response.

EXPERIMENTAL METHOD

To ensure that aseptic MBP were obtained, peritoneal macrophages were used; these are cells of the same type as alveolar macrophages, i.e., they are derivatives of the blood monocytes [11]. Peritoneal macrophages constituted 82-86% of all the cells in the exudate obtained 45 h after intraperitoneal injection of sterile mineral oil into male Wistar rats. After repeated washing they were broken up by freezing and thawing in physiological saline three times. The effect of MBP on the rate of O_2 consumption by peritoneal macrophages *in vitro* was studied in a cuvette with a covered platinum electrode on the Lp-60 polarograph

Department of Work Hygiene, Sverdlovsk Institute of Work Hygiene and Occupational Diseases. Department of Pathological Physiology, Sverdlovsk Medical Institute. (Presented by Academician of the Academy of Medical Sciences of the USSR L. M. Shabad.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 342-345, March, 1977. Original article submitted June 16, 1976.

TABLE 1. Effect of Intratracheal Injection of MBP on Alveolar Phagocytosis (M ± m)

Experimental conditions	Agent injected	Number of cells in washings ($\times 10^6$)			Ratio of neutrophils/alveolar macrophages
		total	alveolar macrophages	neutrophils	
Without inhalation of dust	Physiological saline	4,47±0,51	3,02±0,53	0,39±0,14	0,13±0,03
	MBP	12,89±1,42	5,31±0,51	5,09±0,66	0,96±0,01
	P	<0,01	<0,01	<0,001	<0,001
After four inhalations of TiO_2 dust	Physiological saline	8,66±1,20	6,46±0,71	1,56±0,77	0,21±0,10
	MBP	16,47±1,08	9,24±0,59	6,04±1,09	0,75±0,20
	P	<0,01	<0,01	<0,01	<0,001

[6] (incubation in Hanks's medium) during the action of MBP in a dose equivalent to $2 \cdot 10^6$ disintegrated cells per 10^6 peritoneal macrophages of syngeneic rats. The effect of MBP on migration of the blood neutrophils of rats was studied by the leukocyte film culture method [4]; the dose of MBP under these circumstances was equivalent to $300 \cdot 10^6$ disintegrated cells to 1 ml of medium. In the experiments *in vivo* Wistar rats received three intraperitoneal injections of MBP in doses equivalent to $150 \cdot 10^6$ cells/100 g body weight, at intervals of 24 h, or a single intratracheal injection of the same dose in a volume of 1 ml; control rats received injections of physiological saline. Under hexobarbital anesthesia the lungs were removed 24 h later so that the respiratory passages could be flushed out in order to count the total number of "free" cells; the washings were then centrifuged and the cell composition of the residue studied in a film [1, 7]. Some rats of all groups were exposed daily to the action of finely dispersed TiO_2 dust for 5 h daily with a mean dust concentration in the chamber of 50 mg/m^3. Intraperitoneal injections of MBP or physiological saline were given on the 4th, 5th, and 6th days of inhalation of dust, and intratracheal injections immediately after the fourth inhalation. The cell composition of the peritoneal exudate also was investigated after the third intraperitoneal injection, at 24-h intervals, of MBP, 1 ml of physiological saline, and a suspension of 30 mg TiO_2 or SiO_2 in 1 ml physiological saline.

EXPERIMENTAL RESULTS

As Table 1 shows, the usual sharp predominance of free alveolar macrophages over neutrophils was observed in the lungs of the control rats 24 h after intratracheal injection of physiological saline. After intratracheal injection of MBP the number of all the cells was significantly increased, but the greatest increase was in the number of neutrophils, so that the neutrophil/alveolar macrophage ratio was significantly increased. A similar shift took place among the increased number of cells in the respiratory passages of rats receiving inhalations of TiO_2. By itself, TiO_2 caused only a small increase, which was not significant, in the neutrophil/alveolar macrophage ratio, as is characteristic of the action of dusts of low cytotoxicity.

As confirmation of the lower cytotoxicity of TiO_2 than of SiO_2 (the most characteristic feature of the alveolar phagocytosis of which is an increase in this ratio [5]), besides the results obtained *in vitro* [10], the difference discovered in these experiments between the percentages of clearly degenerated peritoneal macrophages in the exudate after injection of these dusts can be cited (6.0 ± 1.7 and 50.0 ± 2.4%, respectively; $P < 0.001$). As will be clear from Table 1, under the influence of exogenous MBP the quantitative and qualitative features distinguishing the response of alveolar phagocytosis of this relatively unaggressive dust acquired the typical features of the response to inhalation of SiO_2: The greatest mobilization of all the cells was observed and there was a significant increase in the ratio of neutrophils to alveolar macrophages.

However, any dust can have a harmful action on the macrophage: In rats receiving inhalations of TiO_2 and intratracheal injections of physiological saline, for instance, among the alveolar macrophages there were 16.0 ± 1.2% which were clearly degenerated, whereas in rats receiving physiological saline only there were only 9.6 ± 0.7% ($P < 0.001$). Presumably the response of an increase in the number of cells in the respiratory passages with a minimal shift toward neutrophils, evoked by inhalations of dust without the additional injection of MBP, is the response to some increase in the endogenous production of MBP as a result of contact of the free alveolar macrophages, always present in the respiratory passages, with TiO_2.

The total number of dust particles inside the free neutrophils in a rat inhaling TiO_2 calculated relative to the whole volume of washings was $(1.40 \pm 0.85) \cdot 10^6$. Under the influence of MBP it rose to $(6.34 \pm 1.83) \cdot 10^6$ ($P < 0.05$), although the mean number of particles per neutrophil did not change significantly. Besides the probable role of the total contribution of the neutrophils to dust phagocytosis as the reserve of the cell clearance of particles, it can be shown that it is also responsible for the decrease in the mean "dust load" of each alveolar macrophage. For instance, in rats not exposed in the dust chamber, the mean number of mineral particles inside an alveolar macrophage was reduced almost by half by MBP (from 2.7 ± 0.7 to 1.4 ± 0.2; $P < 0.05$), whereas the total number of particles phagocytosed by both types of cells was increased by 1.23 times. In rats inhaling TiO_2, the dust particles in $30.2 \pm 5.7\%$ of active alveolar macrophages were too numerous to be counted: Under the influence of MBP the proportion of these macrophages fell to $14.9 \pm 4.2\%$ ($P < 0.05$). Under natural conditions increased formation of MBP is the result of phagocytosis of living or nonliving particles with a well marked noxious action on alveolar macrophages. For this reason, the redistribution of phagocytic activity observed in these experiments, leading to a total increase in the number of phagocytosed particles despite a simultaneous decrease in the dose of the harmful factor per alveolar macrophage (without any increase in the dose per neutrophil), must prevent death of the alveolar macrophages, i.e., must increase the efficiency of phagocytosis. At the same time, it limits the further formation of MBP and prevents a chain increase in the response beyond the required intensity.

After intraperitoneal injection of MBP no significant changes in alveolar phagocytosis were observed, but the cell composition of the peritoneal exudate changed appreciably. The combined total of eosinophils and neutrophils after injection of physiological saline amounted to $15.5 \pm 1.9\%$ of all cells, after injection of TiO_2 it fell to $9.7 \pm 2.8\%$ (the difference is not significant), it increased after injection of MBP to $52.3 \pm 3.3\%$ ($P < 0.001$ compared with the value after injection of physiological saline), and after injection of SiO_2 to $32.0 \pm 2.8\%$ ($P < 0.001$ compared with the value after injection of TiO_2). After injection of SiO_2 the preferential attraction of the granulocytes was presumably connected with the effect of MBP formed under the influence of cytotoxic particles, although under these circumstances the percentage of both neutrophils and eosinophils was increased, whereas after injection of exogenous MBP only the percentage of neutrophils was increased. However, considering that eosinophils were characteristic of the peritoneal exudate (even that obtained in response to injection of physiological saline) but were virtually absent in washings from the lungs, the common feature for the lungs and peritoneal cavity is that under the influence both of exogenous MBP and of cytotoxic dust the percentage of neutrophils was significantly increased, i.e., they were attracted to the site of administration (and, correspondingly, of formation) of MBP in even greater numbers than peritoneal or alveolar macrophages. This attraction probably takes place against the concentration gradient of a certain soluble factor which stimulates migration of the phagocytes. It was in fact found that when the supernatant obtained by centrifugation of the same MBP (10 min, 15,000 rpm) was added to a leukocyte film culture, the migration index was increased on the average by 7.2 ± 1.28 times compared with the control ($P < 0.05$); the effect of whole MBP was stronger still.

Further evidence of the positive effect of MBP on the functional state of the phagocyte is given by the polarographic data. Although the rate of the endogenous effect of the peritoneal macrophages (2.47 ± 0.02 µatoms O_2/min in the control) showed only a tendency to increase in the presence of MBP (2.62 ± 0.25 µatoms O_2/min), at the same time there was an appreciable fall in the critical pO_2 level, restricting respiration: 22.5 ± 2.4 and 14.5 ± 2.1 mm Hg, respectively ($P < 0.02$). This shift, which gives a better idea of the oxygen utilization of the cell, can be interpreted as adaptive [2]. The writers previously obtained the analogous polarographic characteristics of the effect of MBP *in vivo* on the O_2 consumption of rat bone marrow tissue [3].

It has also been shown that MBP increase the number of splenic colony-forming units (CFU) in the hematopoietic organs of mice, directing differentiation of the CFU in the spleen mainly toward granulocytopoiesis. The more rapid maturation of monocytes and granulocytes in the bond marrow and the development of a true increase in the number of monocytes and neutrophils in the blood, replacing the redistributive neutrophilic leukocytosis, have also been shown in rats under the influence of MBP. In conjunction with these findings, the results of the experiments described in this paper suggest that on destruction of the macrophage a factor (or factors) is liberated which controls the phagocytic response of the body and, in particular, the response of alveolar phagocytosis at its various stages: activating the phago-

cytic cell and attracting it (to begin with, neutrophils) to the site of formation of MBP; hence determining the more or less substantial contribution of neutrophils to phagocytosis as an additional mechanism of protection associated with the favorable redistribution of the functional load among the cells, providing a warning of the need to mobilize extra numbers of neutrophils and also, possibly, of monocytes from the depots through the circulating blood; finally, increasing and maintaining the reserves of the pool of phagocytic cells through the appropriate influence on hematopoiesis.

LITERATURE CITED

1. A. V. Bykhovskii and G. S. Komovnikova, in: The Pathogenesis of the Pneumoconioses. Proceedings of an All-Union Symposium [in Russian], Sverdlovsk (1970), pp. 341-361.
2. E. A. Kovalenko and L. N. Grinberg, Patol. Fiziol., No. 5, 12 (1972).
3. L. I. Privalova, A. V. Osipenko, and V. N. Frash, Byull. Éksp. Biol. Med., No. 12, 1480 (1976).
4. V. L. Skuratov, A. V. Osipenko, and V. N. Frash, Fiziol. Zh. (Ukr.), No. 1, 95 (1974).
5. S. I. Starikova, B. A. Katsnel'son, G. V. Aronova, et al., Byull. Éksp. Biol. Med., No. 9, 113 (1970).
6. I. M. Épshtein, Med. Tekhnika, No. 5, 56 (1967).
7. C. W. LaBelle and H. Brieger, Arch. Environ. Health, 1, 423 (1960).
8. A. G. Heppleston, in: "Inhaled particles, III," Proceedings of an International Symposium Organized by the British Occupational Hygiene Society, London, 1970, edited by W. H. Walton, Gresham Press, Old Woking (1971), pp. 357-369.
9. K. H. Kilburn, Ann. N. Y. Acad. Sci., 221, 276 (1974).
10. W. Klosterkotter, Beitr. Silikose-Forsch., 6, 125 (1965).
11. L. D. Leder, Blut, 16, 86 (1967).

INVESTIGATION OF THE EFFECT OF CERTAIN CHLORINATED HYDROCARBONS ON THE COMPOSITION OF THE HEPATOCYTE POPULATION OF THE RAT LIVER

N. N. Belyaeva, T. I. Bonashevskaya, T. L. Marshak, and V. Ya. Brodskii

UDC 612.35.014.46:547.222

Hepatocytes in the liver of albino rats poisoned by inhalation of dichloropropane and trichloropropane were investigated cytophotometrically and karyometrically. With respect to the effect on polyploidization of the hepatocyte nuclei trichloropropane was found to be more toxic than dichloropropane. The development of polyploidization is determined by the dose of the toxic agent and the exposure to it: The smaller the dose the shorter the time required for the effect to take place.

KEY WORDS: *ploidy of hepatocytes; chlorinated hydrocarbons; binuclear cells; dose; exposure.*

The action of the chlorinated hydrocarbon 1,2,3-trichloropropane (TCP) on the ploidy of rat liver hepatocytes was studied previously [2] in subacute experiments involving inhalation of TCP for 7 days. In the present investigation, besides TCP another member of this group of compounds was used, namely 1,2-dichloropropane (DCP), a compound widely used in industry and agriculture. The morphological changes arising after exposure to DCP consisted essen-

Laboratory of Morphology, A. N. Sysin Institute of General and Communal Hygiene, Academy of Medical Sciences of the USSR. Laboratory of Cytology, N. K. Kol'tsov Institute of Developmental Biology, Academy of Sciences of the USSR, Moscow.

(Presented by Academician of the Academy of Medical Sciences of the USSR A. M. Chernukh.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 3, pp. 345-348, March, 1977. Original article submitted July 7, 1976.