Effect of Shock on the Morphofunctional State of Alveolar Macrophages

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The number of macrophages, their functional activity, and ultrastructure are studied in bronchoalveolar lavage of rats exposed to traumatic or burn shock. It is found that the intensity of nonspecific, stereotypic, and phase macrophagal reactions correlates with the severity of shock. Responses to damaging factors and adaptive alterations varying in the intensity and occurrence at different observation periods are revealed.

Key Words: *shock; alveolar macrophages; structure and function*

Structural and metabolic rearrangements occurring in healthy and diseased lungs are linked with the functional state of alveolar macrophages (AM) [3,4, 7,14]. There is considerable evidence confirming the key role of posttraumatic respiratory insufficiency in the pathogenesis and thanatogenesis of posttraumatic syndrome; however, the information on variations of the number and morphofunctional state of AM is scarce and contradictory. It was found that the number and structure of AM in bronchoalveolar lavage change at the peak of shock reaction [1,8, 11,12]. Meanwhile, the role of AM in the genesis of the shock process remains unclear. This study is an attempt to analyze functional and morphological changes in AM induced by etiologically different variants of shock in relation to the severity of posttraumatic syndrome.

MATERIALS AND METHODS

Experiments were performed on outbred male rats. Traumatic shock was produced by the method of Cannon and burn shock by the method of Kochetygov under light hexenal anesthesia. The animals were sacrificed at various periods (1 h—14 days) after shock. Bronchoalveolar lavage (BAL) was prepared as described elsewhere [13]; cells were counted in Goryaev's chamber and analyzed on smears stained by the method of Romanowsky—Giemsa. Alveolar macrophages were isolated by centrifugation on a Ficoll-Verograffin gradient. Cells were counted, and the percent of viable cells was calculated. The functional state of AM was assessed in spontaneous and induced tests with nitro blue tetrazolium (sNBT and iNBT, respectively) [2]. The phagocytizing activity of AM was determined using a 24-h culture of *E. coli* [9]. For electron microscopy BAL was mixed with an equal volume of 2.5% glutaraldehyde, postfixed with 1% osmium tetroxide, dehydrated in raising concentrations of ethanols, and embedded in Epon 812. Sections were contrasted with uranyl acetate and studied in a PMU electron microscope. The result were analyzed by parametric statistical methods.

RESULTS

Analysis of the results obtained showed that mechanical or thermal trauma induces phase and stereotypic reactions of AM correlating with the severity of posttraumatic syndrome. Three periods can be distinguished in these changes: torpid phase, natural termination of the process, and development of posttraumatic inflammatory complications in the lungs.

At the peak of torpid phase of traumatic and burn shock the number of AM in BAL increased

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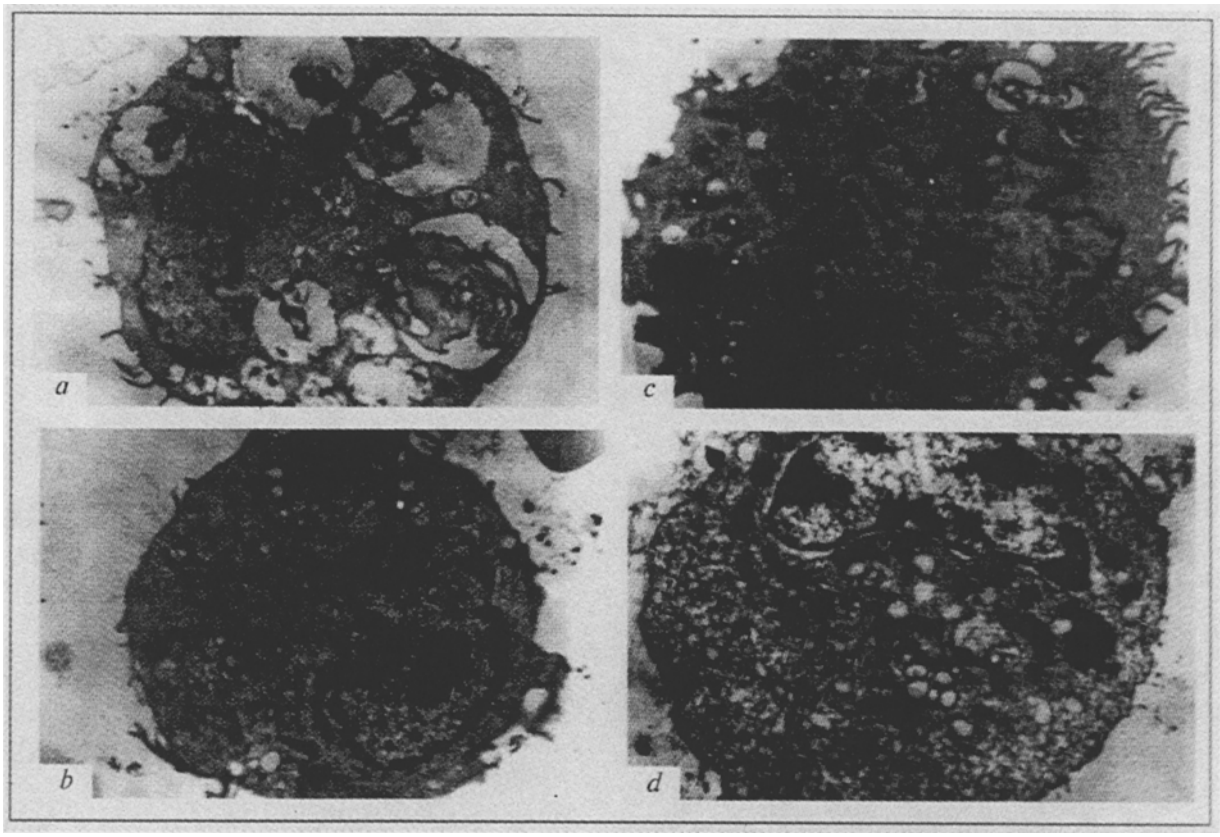

Fig. 1. Variants of alveolar macrophages after shock. *a*) degenerating macrophages, ×5400; *b*) transitory form, ×6750; *c*) actively phagocytizing macrophage, ×5400; *d*) macrophage with the signs of enhanced biosynthesis, ×8100.

considerably, the increase in the number of dead cells being more pronounced. The relative parameters of sNBT also increased, changes in the iNBT parameters being less pronounced, while the stimulation coefficient and phagocytizing activity of AM decreased (Table 1). Examination of BAL smears showed

TABLE 1. Changes in Number and Functional Activity of Alveolar Macrophages After Traumatic and Burn Shock (Deviation from the Control Value, %)

Parameter	Observation period		
	torpid phase	termination of shock	posttraumatic complications
Total count	437.8±73.5	219.4±14.9	238.9±25.2
	182.8±36.9	22.6±2.7	206.5±18.0
Number of nonviable cells	619.0±86.4	371.4±22.9	236.0±39.0
	547.1±20.3	14.3±3.3	156.6±28.3
sNBT	193.6±20.7	63.1±14.1	-69.3±13.7
	201.4±17.9	25.3±1.5	-39.7±2.6
iNBT	10.1±2.8	84.4±13.7	-36.1±4.4
	23.2±3.7	12.4±0.9	-32.4±1.1
Coefficient of stimulation	-52.5±4.5	4.6±3.1	129.1±23.5
	-56.8±4.1	9.4±3.6	44.3±11.0
Phagocytizing activity	-43.9±13.1	-25.6±5.7	-29.3±8.5
	-35.1±9.2	-35.6±14.0	-34.0±9.8
Phagocytic index	-34.9±10.1	-10.6±2.4	-0.4±1.2
	-24.2±8.3	-12.3±3.1	-6.9±0.6

Note. Numerator is traumatic shock; denominator is burn shock.

TABLE 2. Cell Content of Bronchoalveolar Lavage at Early Periods of Posttraumatic and Burn Disease

Observation period	Total cell count, $\times 10^6$/g	Alveolar macrophages								Neutrophils		Lymphocytes		Others	
		total count		small		large		degrading							
		abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.	abs.	rel.
Control	5.3±0.4	4.9±0.4	92.7±1.0	1.5±0.2	29.0±0.4	2.6±0.3	50.7±3.8	0.7±0.1	13.0±3.0	0.2±0.03	4.5±0.6	0.1±0.01	2.0±0.5	0.04±0.01	0.8±0.1
Posttraumatic disease															
Torpid phase	12.0±2.0*	9.8±1.3*	83.3±3.0*	3.3±0.1	28.0±4.0	4.8±0.9*	39.5±0.5*	1.9±0.3*	15.5±0.5	0.4±0.1	3.5±0.5	0.5±0.1*	4.5±0.5*	1.1±0.4*	9.0±2.0*
Termination phase	5.9±1.6	4.9±1.4	81.3±1.2*	1.6±0.2	28.0±2.2	2.2±0.5	40.0±2.3*	0.8±0.3	12.6±2.3	0.4±0.03*	7.3±1.5*	0.3±0.1	5.6±0.1*	0.3±0.05*	5.6±1.4*
Aftershock period	8.8±0.3*	7.1±0.5*	80.6±1.7*	2.9±0.3*	33.3±2.9	3.6±0.3	40.6±1.9*	0.6±0.2	6.6±0.7*	0.9±0.3*	11.0±1.3*	0.5±0.1*	5.3±1.5*	0.3±0.01*	2.6±0.1*
Burn disease															
Torpid phase	13.3±2.4*	10.6±1.8*	80.0±1.0*	3.2±1.1	34.3±4.5	4.3±0.7*	33.6±3.1*	1.7±0.5*	12.0±1.3	0.5±0.1	5.6±1.4	0.6±0.3	4.3±1.2	1.3±0.2*	10.0±0.1*
Termination phase	6.4±1.4	4.9±0.6	78.5±6.5	1.9±0.4	31.5±1.5	2.2±0.3	35.5±3.5*	0.7±0.1	11.5±1.5	0.9±0.5	15.0±5.0*	0.2±0.1	4.0±2.0	0.1±0.01*	2.5±0.5*
Aftershock period	7.9±2.4	6.3±0.6	79.0±2.0*	2.4±0.2*	30.6±1.2	3.1±0.5	38.3±1.6	0.8±0.1	10.0±0.3*	1.0±0.3*	13.6±1.1*	0.4±0.1*	5.0±1.0*	0.1±0.03*	2.3±0.5*

Note. absolute values, $\times 10^6$/g; relative values, %. *$p<0.05$ compared with the control.

that an increase in the total number of cells is paralleled by that in all subpopulations of AM, while their relative contents varied in physiological range or decreased (Table 2). It is noteworthy that the number of small (young) and degrading (vacuolized) AM increased.

Electron microscopy studies showed that the population of AM is heterogeneous, being represented by various structural and metabolic variants of macrophages described for other pulmonary conditions [5,6]. In the torpid phase, the number of AM with ultrastructural manifestations of degeneration (large vacuoles with flocculent content and autophagosomes) increased (Fig. 1, *a*). Small AM with a kidney-shaped nucleus, moderately developed cytoplasmic reticulum, few surface protrusions, and small round lysosomes were seen (Fig. 1, *b*). These cells can be regarded as transitory forms between the precursor cells and mature macrophages. The number of typical actively phagocytizing AM with pseudopodia, invaginations of the nuclear membrane, pinocytotic vesicles, and lysosomes varying in size and electron density markedly decreased as compared with the control (Fig. 1, *c*).

Natural termination of shock in surviving animals coincided with less pronounced changes in the number of AM and an increase in the intensity of iNBT and stimulation coefficient. The total AM count and that of nonviable AM in BAM were higher, while the phagocytizing activity and phagocytizing index were lower than in the control. A tendency toward normalization of the total count and the subpopulation ratios was observed in parallel with the rise of absolute and relative neutrophil and lymphocyte counts. Electron microscopy revealed an increase in the number of young and active AM with high phagocytizing and digesting activities as well as macrophages with well-developed rough cytoplasmic reticulum, numerous mitochondria, free ribosomes, and polysomes (Fig. 1, *d*), which may be indicative of enhanced synthetic processes in the cell.

The specific feature of the aftershock period was the development of inflammatory processes in the lungs manifesting themselves as small foci of interstitial infiltration, acinous foci of serous-desquamative pneumonia, and suppurative inflammation. The total count of AM and that of nonviable AM increased again, although to a lesser extent than in the torpid phase, and the parameters of iNBT and sNBT decreased with a simultaneous rise of potential reserve, while the phagocytizing index varied in the normal range. The total cell and AM counts showed a tendency toward normalization, which was accompanied by a significant decrease in the per-

centage of AM. An increase in the neutrophil and lymphocyte populations was paralleled by growing counts of young, typical and actively phagocytizing AM, while the count of degrading macrophages dropped.

Shock of various etiologies induced changes in the total count of alveolar macrophages, the number of their subpopulations, and proportions of their structural and metabolic forms. At certain periods after shock, these changes are adaptive and are aimed at restoring the number and functional potential of AM, as evidenced by the increase in the number of young AM in the lungs. However, an increase in the amount of degenerating cells and activated macrophages can be regarded as an alterative factor, since AM may be involved in tissue destruction due to accumulation of degradation products, enhanced synthesis of biologically active substances, catabolic enzymes, exocytosis of lysosomal hydrolases, etc. [4,10,15]. Various manifestations of posttraumatic respiratory insufficiency and the course of posttraumatic process may depend on the balance between damaging and protective reactions which occur in the lungs and are associated with the morphofunctional state of AM.

REFERENCES

1. I. R. Vazina, S. I. Pylaeva, and O. A. Vasil'chuk, *Byull. Eksp. Biol. Med.*, **97**, No. 5, 542-544 (1984).
2. M. P. Gracheva, *Zh. Mikrobiol.*, No. 2, 87-88 (1984).
3. T. N. Kop'eva, G. V. Barmina, O. M. Grobova, and L. M. Voronina, *Arkh. Pat.*, No. 9, 5-12 (1992).
4. D. N. Mayanskii, *Chronic Inflammation* [in Russian], Moscow (1991).
5. G. I. Nepomnyashchikh, V. N. Efremov, V. P. Tumanov, and L. M. Nepomnyashchikh, *Byull. Eksp. Biol. Med.*, **101**, No. 1, 105-109 (1986).
6. V. V. Polosukhin, S. M. Egunova, S. G. Chuvakin, and A. P. Bessonov, *Immunologiya*, No. 4, 51-56 (1994).
7. L. K. Romanova, *Arkh. Pat.*, No. 11, 22-27 (1991).
8. N. G. Kharlanova, Yu. M. Lomov, and E. A. Bardakhch'yan, *Byull. Eksp. Biol. Med.*, **115**, No. 1, 82-86 (1993).
9. E. N. Shlyakhov and L. P. Andriesh, *Immunology: A Reference Book* [in Russian], Kishinev (1985).
10. J. D. Brain, *Reticuloendothelial System: A Comprehensive Treatise*, Vol. 7b, New York - London (1985), pp. 315-337.
11. R. F. Jacobs, D. R. Dorsey, A. F. Tryka, and D. R. Tabor, *Exp. Lung Res.*, **14**, No. 3, 359-374 (1988).
12. L. D. Loose, R. Megirian, and J. Turinsky, *Infect. Immun.*, **44**, No. 3, 554-558 (1984).
13. Q. N. Myrvik, E. S. Leake, and B. Farris, *J. Immunol.*, **86**, No. 2, 128-132 (1961).
14. M.-C. Snella, T. Venaille, P. Holt, and R. Rylander, *Agents Actions*, **22**, No. 3-4, 261-269 (1987).
15. D. E. Tracey, *Reticuloendothelial System: a Comprehensive Treatise*, Vol. 4, New York - London (1983), pp. 77-101.