COMPARISON OF OXYGEN UPTAKE BY POLYMORPHONUCLEAR LEUKOCYTES OF CONVENTIONAL AND PATHOGEN-FREE C57BL/6 MICE DURING PHAGOCYTOSIS

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Investigation of natural immunity on conventional, pathogen-free (PF), and germfree animals has considerably widened the scope for differential study of the effect of various factors on constitutional and adaptive protective mechanisms and, in particular, in granulocyte formation and on phagocytosis of bacteria [5, 11]. It is generally accepted that phagocytosis of inert particles takes place identically in ordinary and germfree animals [5, 11], but it has been shown that many pathogenic agents, and even viruses, can cause a marked decrease in phagocytic activity of polymorphonuclear leukocytes (PNL) and of the intensity of the oxygen uptake [6, 14]. There is no doubt that representatives of the normal microflora, and conventional pathogenic and persistent microorganisms can have a considerable effect, both positive and negative, on the formation and course of processes connected with phagocytosis [5, 12]. We know that during phagocytosis by PNL activation of oxygen uptake insensitive to cyanide takes place [13]. One result of this process is the formation of products of reduction and excitation of oxygen, such as the superoxide anion, hydrogen peroxide, the hydroxyl radical, and singlet oxygen, which are essential for bactericidal activity of PNL to take place in mammals and man [9].

In the investigation described below, oxygen uptake processes were investigated during phagocytosis of *Staphylococcus aureus* by PNL of conventional and PF mice of the C57BL/6 strain.

EXPERIMENTAL METHOD

Two groups of mice weighing 18-22 g were used. The animals of one group were kept under ordinary conditions and fed on PK 120-3 brand concentrates (Group A). Animals of the other group were the third generation of PF mice and were also free from helminths and itch-mites (group B). These mice were obtained in the Laboratory of Experimental Biological Models by decontamination with antibiotics and other antimicrobial and antiparasitic preparations. As food they received Mark PK 120-3 concentrates, sterilized by γ-ray irradiation.

PNL were isolated from mouse peritoneal exudate [8, 10, 13]. The final leukocyte concentration was $2 \cdot 10^8$ cells/ml and the cell composition was: 72-77% of PNL, 10-13% of macrophages, and 13-15% of lymphocytes. The viability of the cells by the time of the last test of each experiment was 98%. As the object for phagocytosis, a culture of *S. aureus*, killed by boiling, was used in a concentration of $5 \cdot 10^{10}$ microorganisms/ml. The bacteria were opsonized with fresh autologous mouse serum of the corresponding experimental groups for 30 min at 37°C [8]. Phagocytosis was induced by addition of a suspension of *S. aureus*, with the ratio of bacteria to PNL of 700 to 1000.

The rate of oxygen uptake by PNL was recorded polarographically by measuring the oxygen concentration with a Clark's electrode [3].

Cell respiration was measured under constant temperature conditions at 37°C.

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TABLE 1. Respiratory Activity of Intact (V_0) and Phagocytic PNL (V_{ph}) and Ratios between Them (K)

Animals	Expt. No.	No. of animals	$V_0 \cdot 10^{-5}$	$V_{ph} 10^{-5}$	$(V_{ph} - V_0) \cdot 10^{-5}$	K
			micromoles per cell per hour			
PF micr (group B)	1	8	2,68±0,44	4,95±0,52	2,26±0,27	1,9±0,40
	2	8	2,09±0,61	3,24±0,54	1,15±0,10	1,6±0,50
	3	8	2,60±0,36	4,92±0,49	2,31±0,84	1,95±0,30
Mean value for three experiments		24	2,49±0,23	4,45±0,41	1,96±0,30	1,79±0,18
Conventional mice (group A)	1	8	2,45±0,22	12,40±0,44	10,13±0,83	5,06±0,55
	2	7	2,93±0,40	13,78±0,52	10,85±0,53	4,71±0,68
	3	8	2,66±0,31	15,30±0,38	12,54±0,49	5,75±0,67
	4	7	2,51±0,11	13,15±0,25	10,64±0,30	5,24±0,26
Mean value for four experiments		30	2,63±0,11	13,74±0,36	11,11±0,35	5,22±0,27

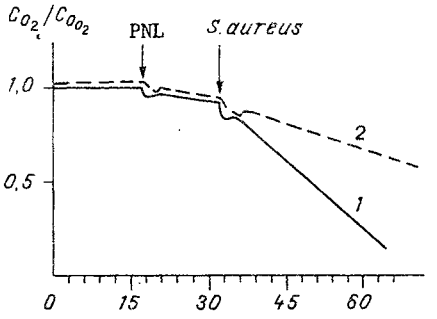

Fig. 1. Dependence of oxygen uptake (in relative units) by intact and phagocytic PNL on time. Abscissa, time (in min); ordinate: 1) PNL of conventional mice, 2) PNL of PF mice.

We know that during phagocytosis respiration of lymphocytes is not activated, and that activation of respiration of cells of the macrophage-monocyte series is considerably lower than that of PNL [9, 10, 13]. Calculations showed that if 13% of macrophages was present in the exudate, their contribution to the total oxygen activity of the exudate did not exceed 4%.

EXPERIMENTAL RESULTS

The number of leukocytes in 1 mm^3 of blood of the conventional mice was 2.4 times greater than in the blood of PF animals (13,700 ± 389 and 5630 ± 629 respectively), in agreement with previous data [4]. Differences in the blood formulas of the animals consisted mainly of an increase in the number of stab cells and juvenile PNL in the conventional mice, respectively (5 and 1%) relative to PF animals, which contained only 1% of stab cells. These figures are evidence of weakening of the hematopoietic functions of the bone marrow as a result of isolation of PF mice and of delaying of granulocytopoiesis in these animals [4].

The cell composition of the peritoneal exudates did not differ significantly, although the yield of PNL pwer animal was $(37 \pm 1) \cdot 10^5$ cells in PF mice and $(56 \pm 3) \cdot 10^5$ in conventional mice.

Data on the rate of respiration of intact PNL of mice of groups A and B are given in Fig. 1 and Table 1. These rates were virtually identical. Lengthening the incubation time to 20 and 30 min did not lead to an increase in the rate of cell respiration. Addition of bacteria to PNL from group B mice caused a very small increase in oxygen utilization. The ratio of the rate of respiration of phagocytic and intact PNL under these circumstances was 1.79 ± 0.18. A completely different picture was observed in experiments with cells from group A animals, in which stimulation of respiration during phagocytosis under these same conditions exceeded the background values by more than 5 times (Fig. 1, Table 1). The absolute rate of oxygen consumption by phagocytic cells was $(13.74 \pm 0.36) \cdot 10^{-5}$ micromoles per

TABLE 2. Effect of Opsonizing Activity of Serum of PF Animals on Phagocytosis of Bacteria by PNL of Conventional Mice

Opsonizing serum	$V_0 \cdot 10^{-5}$	$V_{ph} \cdot 10^{-5}$	$(V_{ph}-V_0) \cdot 10^{-5}$	K	Phagocytic PNL, %
	micromoles per cell per hour				
Serum of group A mice	1,53±0,24	8,41±0,33	6,90±0,48	5,50±0,94	91,1±1,7
Serum of group B mice	1,44±0,16	8,31±0,31	6,86±0,16	5,80±0,70	90,6±1,3

cell per hour, and by intact cells $(2.63 \pm 0.11) \cdot 10^{-5}$ micromoles per cell per hour. The good reproducibility of the results of the different experiments made it possible to estimate not only the ratio, but also the difference between the background and experimental values of oxygen uptake (Table 1).

The considerable differences in oxygen uptake during phagocytosis in the animals of the two groups could be connected with differences in the opsonizing activity of the autologous sera [14]. Keeping animals on a sterile diet and under conditions of germfree isolation is known to lead to a considerable decrease in the number of species represented in their microflora and to lowering of certain immunologic parameters [4]. Sera of germ-free animals differ in several parameters from sera of ordinary animals [1, 2, 5], and this could be reflected in their opsonizing activity. To test this hypothesis, bacteria, opsonized by serum of group B mice, were added to peritoneal exudate cells of group A mice.

As Table 2 shows, serum from PF animals did not differ in its opsonizing activity from serum of ordinary mice in the activation of respiration during phagocytosis test or as regards the percentage of phagocytic PNL. The level of phagocytic activity, assessed from the change in oxygen uptake, evidently is mainly determined by the level of preparation of the oxygen-absorbing systems of the cells themselves. The present experiments showed that in PF animals there is a marked decrease in activity of PNL with respect to oxygen uptake during phagocytosis. Incidentally, this parameter is closely linked with the bacterial functions of PNL. Since stem cells of germ-free mice are in an inactive state, and since PF mice have much in common with them as regards the hematopoietic parameters of cells of the myeloid series [7], it must be expected that PF mice may be used as an independent and sensitive model for estimating the effects of various factors on maturing precursors of PNL against a background of a very weak influence of antigenic stimuli.

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