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Comparative Metabolism of Guinea Pig Peritoneal Exudate Neutrophils and Eosinophils (40801)

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Despite considerable speculation the biologic role of the eosinophil remains uncertain. In the related leukocyte the neutrophil, much biologically relevant information has been obtained by study of phagocytic and metabolic function. Leukocytes respond to phagocytosis (1) with a marked increase in oxygen consumption, activation of the hexose monophosphate (HMP) shunt, and production of active oxygen products including superoxide anion, hydroxyl radical, and hydrogen peroxide. The hydrogen peroxide is believed to react with myeloperoxidase and a halide (or similar agent) to form a potent antimicrobial system (2). Much of this information was derived from study of peritoneal exudate neutrophils elicited from a variety of animal species.

Attempts to elucidate eosinophil function by study of their phagocytic and metabolic properties have been hampered by a lack of cells, and thus have been confined to selected studies of eosinophils from patients with eosinophilia (2–9). The majority of studies have employed microscopic techniques to demonstrate diminished phagocytosis by eosinophils with respect to both the number of particles per cell and the percentage of cells ingesting particles (3–7). Eosinophils do respond to phagocytosis with significant metabolic changes; however, reports of the extent and character of these changes have been contradictory. Investigators have reported a markedly increased (9), slightly increased (3, 7), or equivalent (5) activation of the HMP shunt by eosinophils as compared to neutrophils. Hydrogen peroxide production has been reported to be equivalent in resting eosinophils and increased in phagocytizing eosinophils (3), elevated in resting eosinophils and equivalent in phagocytizing eosinophils (6), and increased in both resting and phagocytizing eosinophils (9).

I have employed peritoneal exudate eosinophils obtained from Polymyxin B-treated guinea pigs to characterize the metabolic response of eosinophils to phagocytosis. These studies were undertaken to explore possible unique features of eosinophil metabolism and to validate the use of these cells as an experimental model.

Materials and methods. Leukocyte preparation. Eosinophils were obtained by peritoneal lavage of Polymyxin B-treated animals as previously reported (10). Animals were lavaged with normal saline on a weekly or biweekly basis, and the eosinophils were purified according to the method described by Gleich and Loegering (11). Peritoneal exudate leukocytes were suspended in normal saline at a concentration of 1×10^7 leukocytes/ml, layered over Hypaque-M (Winthrop Laboratories, New York) diluted to a density of 1.142, and centrifuged for 40 min at 400*g*. The final preparations contained 85 to 95% eosinophils, with the contaminating cells being exclusively mononuclear. Guinea pig peritoneal exudate neutrophils were induced by caseinate (Nutritional Biochemicals, Cleveland, Ohio) injection according to standard methods (12). The cells were collected after 15 hr, washed twice with sterile normal saline, and any erythrocytes removed by hypotonic lysis prior to use. Trypan blue dye exclusion studies showed

[1] Dr. Pincus was a research associate of the Veterans Administration.

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greater than 90% viability of both cell populations.

Measurement of phagocytosis. Phagocytosis was studied by measuring the uptake of Oil Red O–paraffin oil particles coated with *Escherichia coli* lipopolysaccharide 026:B6 (Difco Laboratories, Detroit, Mich.) (13). All experiments were carried out in duplicate employing 1.4 × 10^7 phagocytic cells per assay.

Metabolic studies. Postphagocytic HMP activation was measured by [1-^{14}C]glucose conversion to $^{14}CO_2$ and hydrogen peroxide production was evaluated by conversion of [^{14}C]formate, as previously described (14). Briefly, the reaction mixtures were incubated at 37°C in a shaking water bath and contained 1 μmole of glucose, (0.05 μCi [^{14}C]glucose, New England Nuclear) and the indicated number of leukocytes suspended in calcium-free Krebs–Ringer phosphate buffer, pH 7.4, final volume 0.5 ml. For formate oxidation the reaction mixture additionally contained 1 μmole sodium [^{14}C]formate (0.1 μCi pretreated with 0.1 *N* HCl, New England Nuclear) in place of the radiolabeled glucose and 2500 units of dialyzed beef liver catalase (Worthington Biochemical Corp., Freehold, N.J.). Reactions were initiated by the addition of leukocytes and terminated by the addition of acid. [^{14}C]O_2 was collected in alkali and measured by liquid scintillation counting. Results are expressed as nanomoles of glucose oxidized, or nanomoles of formate converted to [^{14}C]O_2. Unless otherwise specified, all results are expressed per 10^7 neutrophils or eosinophils. For the standard assay 0.03 ml of latex beads (2 × 10^9 particles, 1.1 μdiam., Dow Chemical Co., Indianapolis, Ind.) was employed. The standard incubation time was 20 min for C-1 glucose oxidation and 60 min for formate oxidation. All experiments were performed in duplicate. Variations from the standard procedure are indicated in the table and figure legends. Preopsonized zymosan was employed as follows: Zymosan (Sigma Chemical Corp., St. Louis, Mo.) was prepared as a 10-mg/ml suspension, centrifuged, resuspended to the original volume with pooled guinea pig serum, incubated for 20 min at 37°C, washed four times with distilled water, and resuspended to the original volume before use.

Results.phagocytosis. Both eosinophils and neutrophils ingested paraffin oil particles coated with *E. coli* lipopolysaccharides. Surprisingly, peritoneal exudate eosinophils ingested particles more rapidly and reached a plateau earlier than did peritoneal exudate neutrophils (Fig. 1). When the rate was determined at 5 min, eosinophils ingested a mean of 0.92 ± 0.10 mg paraffin oil/10^7 leukocytes contrasted with 0.62 ± 0.05 mg paraffin oil/10^7 leukocytes ingested by neutrophils ($P < 0.025$, two-tailed Student's *t* test). However, the total phagocytic capacity measured by uptake at 30 min was equivalent.

Hexose monophosphate shunt activation. Both neutrophils and eosinophils responded to the presence of phagocytizable particles by marked increases in C-1 glucose oxidation, reflecting activation of the HMP shunt. The responses were highly dependent upon experimental conditions. An important variable appeared to be the number of cells present. As demonstrated in Table I, the amount of C-1 glucose oxidation per 10^7 cells increased markedly as the number of cells present in the reaction mixture decreased. The stimulation of glucose oxidation by reduction of the number of cells in the assay was more pronounced with neutrophils, as compared to eosinophils. When 1 × 10^7 cells were

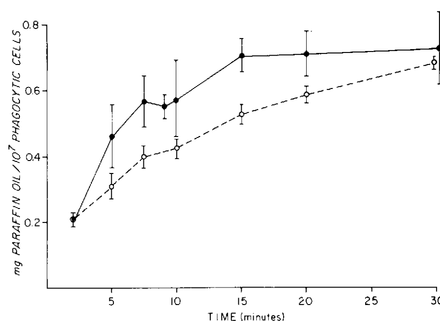

FIG. 1. Ingestion of paraffin oil particles by guinea pig eosinophils and neutrophils. Peritoneal exudate eosinophils (●) or neutrophils (○) were incubated for the indicated time as described under Materials and methods. Each point represents the mean ± standard deviation of four to nine experimental values.

TABLE 1. GLUCOSE OXIDATION BY EOSINOPHILS AND NEUTROPHILS[1]

	Leukocytes (1×10^7)		Leukocytes (3×10^6)		Leukocytes (1×10^6)	
	Resting	Phagocytizing	Resting	Phagocytizing	Resting	Phagocytizing
Eos	6.0 ± 1.2	103.9 ± 15.3	13.5 ± 3.1	142.0 ± 24.6	12.0 ± 5.0	148.0 ± 25.3
Neu	4.0 ± 1.8	74.2 ± 21.1	11.7 ± 3.2	175.0 ± 22.0	18.8 ± 6.7	209.9 ± 35.5
P	<0.025	NS	NS	<0.01	NS	<0.001

[1] Results are the mean ± standard deviation of 4 to 11 experimental values of the nanomoles of glucose oxidized per 10^7 neu or eos. Experiments were performed simultaneously according to the standard technique described under Materials and methods employing 2×10^9 latex beads and 20-min incubation periods. *P* values were determined by the Student's *t* test. NS denotes not significant, $P \geq 0.05$.

employed, eosinophils had a slightly higher resting consumption as compared to neutrophils ($P < 0.025$), but no significant differences were noted between phagocytizing neutrophils and eosinophils. Employing 3×10^6 cells resting values of eosinophils and neutrophils were not significantly different, but consumption by phagocytizing neutrophils was greater. As the cell concentration was reduced by decreasing the number of cells to 1×10^6 consumption was further increased by neutrophils, but did not increase for phagocytizing eosinophils.

The time course of HMP shunt activation was studied with both eosinophils and neutrophils at high (1×10^7) and moderate (3×10^6) cell numbers per assay. Maximal rates of glucose oxidation were noted during the first 20 min. When 1×10^6 leukocytes per assay were employed resting eosinophils oxidized lesser amounts of glucose. Phagocytizing eosinophils similarly oxidized less C-1 glucose and reached a plateau earlier (Fig. 2). Thus, when large numbers of leukocytes were employed, eosinophils and neutrophils appeared to be similar. However, when the number of cells was reduced, neutrophils oxidized glucose at a faster rate.

Two other factors were observed to significantly affect the metabolic response. If the peritoneal exudate neutrophils were collected after more than 18 hr, the cells responded less well to phagocytic stimuli and had a larger number of contaminating mononuclear cells. For this reason, all neutrophil exudates were collected at 15 hr. If the cell suspensions were held at 4°C for prolonged periods of time, the C-1 glucose oxidation response to phagocytosis was reduced. Cells chilled for 3 hr had less than half the response of cells directly processed in the routine fashion (data not shown). Polymyxin B, the eliciting agent, had no effect upon C-1 oxidation by phagocytizing eosinophils or neutrophils.

Since eosinophils were harvested both on weekly and bimonthly basis, an experiment was performed to see if prolonged incubation within the peritoneal cavity effected C-1 glucose oxidation and could thus contribute to experimental variability. Previously published information has documented that eosinophils accumulate within the peritoneal cavity (10). Cells that were harvested from animals that had been allowed to rest (i.e., received injections without lavage) for 6 weeks were compared with cells obtained after a 2-week rest period (Table II). No differences were apparent between phagocytizing eosinophils. Resting values were decreased with older leukocytes. Thus, for eosinophils the residence time within the peritoneal cavity did not appear to influence the response to phagocytosis.

The marked variation in response employing varying numbers of leukocytes suggested that the postphagocytic C-1 glucose oxidation was highly dependent upon the particle load. In order to further assess whether these changes were due to variations in cell–cell interaction and/or cell particle interaction, a series of experiments was carried out employing varying numbers of cells and varying particle to cell ratios (Fig. 3). Employing three different concentrations of cells and four different latex bead to cell ratios it can be seen that the most important determinant appeared to be

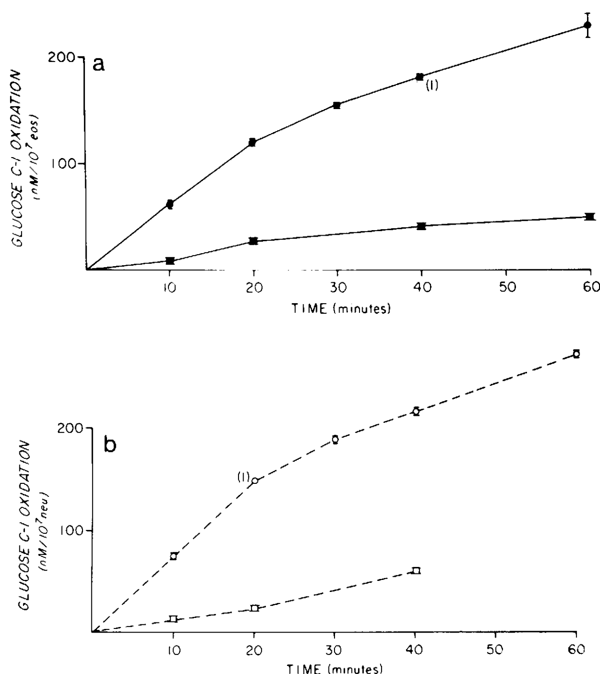

FIG. 2. Time course of C-1 glucose oxidation by eosinophils and neutrophils. (a) C-1 oxidation by phagocytizing eosinophils. Eosinophils, either 3×10^6 (●) or 1×10^7 (■), were incubated with latex particles for the indicated time according to the standard procedure as described under Materials and methods. Values represent the mean ± standard deviation of duplicate experiments of the nanomoles C-1 glucose oxidized per 10^7 eosinophils, with exceptions as noted. (b) C-1 oxidation by phagocytizing neutrophils. Neutrophils, either 3×10^6 (○) or 1×10^7 (□) were incubated with latex particles for the indicated time according to the standard procedure as described under Materials and methods. Values represent the mean ± standard deviation of duplicate experiments of the nanomoles C-1 glucose oxidized per 10^7 neutrophils, with exceptions as noted.

the number of particles per cell. For eosinophils the amount of C-1 glucose oxidized per cell remained equivalent at all particle to cell ratios tested regardless of the number of leukocytes employed in the assay. For neutrophils, the amount of C-1 glucose oxidized per cell increased when the particle to cell ratio was increased by reducing the number of cells employed or by increasing the number of latex beads. The increase at high particle to cell ratios demonstrated here was less than that seen in some other experiments and may reflect variations in the physiologic state of the neutrophils. When neutrophils were harvested after 18 hr or allowed to remain on ice for 3 hr, the effect of increases in particles per cell was accentuated.

Similar studies with preopsonized zymosan as the phagocytizable particle were also performed. At a low particle to cell ratio, the difference was not statistically significant (Fig. 4). As the number of zymosan particles increased, more C-1 glucose oxidation was observed. However, neutrophils were able to respond to a much greater degree, and differences in response were much more apparent at higher particle to cell ratios (6:1 or 10:1).

Because eosinophils and neutrophils appeared to ingest lipopolysaccharide-coated paraffin oil particles, the availability of similar particles to activate the hexose monophosphate shunt was investigated. Diisodecylphthalate was substituted for paraffin oil, and experiments employing eosinophils and neutrophils were performed simultaneously. No differences were found between neutrophils and eosinophils employing a 3×10^6 leukocytes

TABLE II. EFFECT OF INCUBATION TIME IN PERITONEAL CAVITY ON C-1 GLUCOSE OXIDATION BY GUINEA PIG EOSINOPHILS[1]

Lavage internal	Resting	Phagocytizing
2 weeks	20.9 ± 4.7	56.4 ± 0.4
6 weeks	6.1 ± 1.4	64.2 ± 12.6
P	≤0.025	NS

[1] Incubations employing 3×10^6 eosinophils were performed according to the standard method. Resting values were determined after 60 min and phagocytizing values after 20 min. Results are the mean ± standard deviation of duplicate experiments of the nanomoles glucose oxidized per 10^7 eosinophils. Eosinophils were prepared from animals that had been allowed either a 2- or 6-week rest after the previous lavage. P values for the difference between 2- and 6-week eosinophils were calculated by the paired t test.

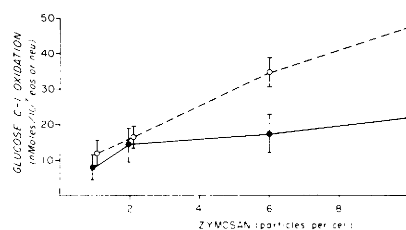

FIG. 4. The response of 3×10^6 eosinophils (●) and neutrophils (○) to increasing numbers of zymosan particles. Incubations were performed 20 min according to the standard procedure as described under Materials and methods, with the number of zymosan particles varied as indicated. Each point represents the mean ± standard deviation of the nanomoles of C-1 glucose oxidized per 10^7 eosinophils or neutrophils.

and three different amounts of phthalate particles.

Formate oxidation. Both eosinophils and neutrophils responded with marked increases in formate oxidation during phagocytosis. Eosinophils were significantly more active as compared to neutrophils, when studied under most resting and phagocytizing conditions (Table III). The results were significantly different when low (1×10^6), intermediate (3×10^6), or high (1×10^7) numbers of leukocytes were employed. However, when the number of leukocytes was further increased to 2×10^7 (employed in previous studies of human neutrophils) no differences were noted. Under these conditions (2×10^7 per assay) formate oxidation was less effectively stimulated and the n-fold increase with phagocytosis was low for both eosinophils and neutrophils. The n-fold increase (percentage stimulation) with phagocytosis was greater at low cell numbers (i.e., high particle to cell ratios) and was less pronounced in eosinophils. Formate oxidation by resting neutrophils was increased when greater numbers of cells were employed, but oxidation by resting eosinophils was similar under all conditions tested.

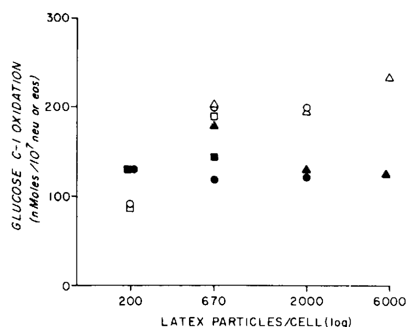

FIG. 3. The effect of changes in cell particle ratio employing varying numbers of eosinophils and neutrophils. Incubations were performed for 20 min employing the indicated number of latex beads per cell. The number of eosinophils (closed symbols) and neutrophils (open symbols) per reaction mixture was either 1×10^7 (□), 3×10^6 (○), or 1×10^6 (△). The results represent the mean of duplicate values from a representative experiment of the nanomoles of C-1 glucose oxidized per 10^7 eosinophils or neutrophils.

The time course of the formate oxidation following phagocytosis was investigated in both eosinophils and neutrophils (Fig. 4). At 10 min the formate oxidation was equivalent, but by 20 min eosinophils demonstrated significantly greater formate oxidation. The rate of formate oxidation was greater for eosinophils, and the high rate was maintained for a longer period of time as compared to neutrophils.

Discussion. Studies of the metabolic and phagocytic properties of guinea pig peritoneal exudate eosinophils were undertaken in order to gain further insight into the biologic role of the eosinophil. These cells were compared to guinea pig peritoneal exudate neutrophils in order to eliminate species differences and provide comparable cell populations. The

TABLE III. FORMATE OXIDATION BY GUINEA PIG EOSINOPHILS AND NEUTROPHILS[1]

	Leukocytes (2×10^7)		Leukocytes (1×10^7)		Leukocytes (3×10^6)		Leukocytes (1×10^6)	
	Resting	Phagocytizing	Resting	Phagocytizing	Resting	Phagocytizing	Resting	Phagocytizing
Eos	9.3 ± 0.3	22.2 ± 1.1	11.6 ± 2.7	44.5 ± 19.4	12.5 ± 4.2	139.1 ± 8.0	11.0(1)	245.5 ± 24.5
Neu	16.2 ± 7.2	26.8 ± 8.8	1.6 ± 0.6	18.5 ± 7.8	0.9 ± 1.2	49.3 ± 6.5	ND	111.3 ± 18.5
P	NS	NS	<0.005	<0.025	<0.025	<0.001	—	<0.001

[1] Experiments were performed under standard conditions as described under Materials and methods, and all incubations were for 60 min. The data represent the mean ± standard deviation of four to seven experimental values (with exceptions as noted) of the nanomoles formate oxidized per 10^7 neutrophils or eosinophils. Experiments were performed simultaneously except for 2×10^7 leukocytes. *P* denotes the *P* value determined by the two-tailed Student's *t* test and NS denotes >0.05 level of significance. ND denotes experiment not performed.

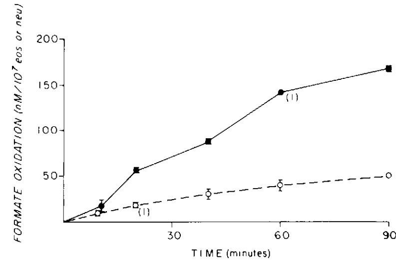

FIG. 5. Time course of formate oxidation by guinea pig eosinophils and neutrophils. 3×10^6 eosinophils (●) or neutrophils (○) were incubated under standard conditions for the time indicated. Results represent the mean ± standard deviation of duplicate experiments of the nanomoles formate oxidized per 10^7 eosinophils or neutrophils, with exceptions as noted.

peritoneal exudate cells appeared healthy without vacuolization or degranulation.

Despite previous reports of decreased phagocytosis by eosinophils, these studies did not reveal any major differences (3–6) (Fig. 1). A quantitative measure of the rate of phagocytosis is the measurement of uptake of dye-laden oil particles coated with bacterial lipopolysaccharide (13). When such particles were employed eosinophils appeared to have a slightly more rapid initial rate of phagocytosis, though at later time periods phagocytosis was equivalent. Recent studies (15) of human eosinophils and neutrophils have confirmed equivalent phagocytosis of *E. coli* and *Staphylococcus aureus*. Though particle ingestion has been documented in neutrophils and macrophages employing electron microscopy, I do not have morphologic confirmation that ingestion actually occurred when peritoneal eosinophils were employed, and it is possible that the measurements reflect adherent particles resistant to the established washing procedure.

Both eosinophils and neutrophils respond to phagocytosis with marked activation of the HMP shunt as has been previously reported (3–9). The critical nature of the cell:particle ratio was emphasized in experiments employing different cell numbers (1×10^6, 3×10^6, 1×10^7) and latex beads. When the number of leukocytes per assay was reduced, there was a marked increase in HMP activation per cell; neutrophils had an increased capacity to respond as com-

pared to eosinophils. Similarly, when the cell to particle ratio was manipulated by increasing the number of latex beads, eosinophils demonstrated little capacity to increase their response. This suggests, that under these standard conditions eosinophils were maximally stimulated whereas neutrophils had considerable reserve capacity. Since the rate of C-1 glucose oxidation by eosinophils and neutrophils is linear for the first 20 min comparisons should be made within this time frame. When preopsonized zymosan was employed, neutrophils also demonstrated a greater capacity for increased response with greater particle loads.

The information along with other data is helpful in evaluating apparently contradictory previous observations about the extent and character of the metabolic response of eosinophils to phagocytosis. Neutrophils from patients with severe infection containing toxic granulation had an elevated C-1 glucose oxidation response to phagocytosis (16). Eosinophils from patients with eosinophilia may be similarly activated (3–9). In addition, the assay conditions, especially the cell to particle ratio, are critical. When mixed populations of eosinophils and neutrophils (5–38%) are employed at low cell to particle ratios, more effective stimulation of neutrophils may be at least partially responsible for apparent elevations in C-1 glucose oxidation (3, 6). Careful attention must also be paid to the type of particle employed and the time selected for assay, since the reaction is not linear with time. Finally, there is a discrepancy between these findings and studies of human blood eosinophils, where eosinophils uniformly oxidize equivalent or greater amounts of C-1 glucose. This suggests that there may be considerable species variation in the metabolic response of eosinophils, as previously noted in studies of neutrophils (12).

The experiments reported here help resolve questions related to further characterization of hydrogen peroxide production as measured by formate oxidation. Eosinophils demonstrate elevated resting production of hydrogen peroxide only if measured at low cell concentrations, similar to previous observations (3, 6). Both eosinophils and neutrophils respond to phagocytosis with sharp increases in hydrogen peroxide production (Table III). At low cell concentrations, eosinophils are much more effective in generating hydrogen peroxide, as compared to neutrophils. This confirms the findings of some investigators (2, 9), but contradicts the findings of others (6). The explanation may be at least in part in the conditions employed. If lower numbers of cells or greater numbers of particles were employed, differences would be more apparent. Further differences between the peritoneal exudate cells employed here and the previously employed peripheral blood eosinophils may represent species variation or variation in the metabolic state of the cells.

How these findings relate to eosinophil function is uncertain. These findings demonstrate that under some conditions eosinophils may appear to phagocytize particles as well as neutrophils. This does not necessarily mean that the biologic role of the eosinophil relates to the ability to phagocytize, and several studies have shown that bacterial killing, which does require phagocytosis, is less well performed by eosinophils (2, 4, 11). Additional caution is required because some investigators have reported that latex particles have caused marked HMP activation in eosinophils without evidence of internalization of particles (12). The response of C-1 glucose oxidation to phagocytosis suggests that similar membrane-directed activation of oxidative metabolism occurs in neutrophils and eosinophils. Perhaps a more important clue is provided by the observations of elevated hydrogen peroxide generation, and the increased response to phagocytosis, most prominent at low cell concentrations. This suggests that the eosinophil is adapted for both the continuous and activated production of hydrogen peroxide. Whether this combines with the large amounts of eosinophil peroxidase to perform some necessary function remains to be determined. Further investigation should be addressed to the consequence of the clearly active oxidative metabolism of the eosinophil and the interaction of cellular

hydrogen peroxide with eosinophil peroxidase.

Summary. In order to gain further insight into eosinophil metabolism, phagocytosis and accompanying metabolic changes were studied in guinea pig peritoneal exudate eosinophils and compared to guinea pig peritoneal exudate neutrophils. Eosinophils and neutrophils ingested lipopolysaccharide-coated paraffin oil particles in similar amounts, though eosinophils had a more rapid initial ingestion rate. Postphagocytic hexose monophosphate (HMP) shunt activation was equivalent in eosinophils and neutrophils when 200 latex beads per cell were employed. However, when the particle to cell ratio was increased by reducing the number of cells or increasing the number of latex beads, neutrophils demonstrated a greater ability to respond. When the generation of hydrogen peroxide was studied employing formate oxidation, eosinophils had elevated resting production of hydrogen peroxide. Elevated phagocytosis-induced hydrogen peroxide production by eosinophils was much more evident when the particle to cell ratio was increased by decreasing the number of leukocytes. Thus, the character and extent of the postphagocytic metabolic burst is highly dependent upon experimental conditions, and comparisons between neutrophils and eosinophils are dependent upon the particular conditions selected. Nonetheless, eosinophils appear to be adapted for elevated production of hydrogen peroxide under both resting and phagocytizing conditions.

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