CHANGES OF FATTY ACID COMPOSITION AND OXIDATIVE METABOLISM OF HUMAN NEUTROPHILS MIGRATING INTO AN INFLAMMATORY EXUDATE

A. CARLETTO,[1] P. BELLAVITE,[2] P. GUARINI, D. BIASI,[1] S. CHIRUMBOLO,[2] P. CARAMASCHI,[1] L. M. BAMBARA,[1] and R. CORROCHER[1]

[1]*Institute of Medical Pathology and*
[2]*Institute of Clinical Chemistry and Microscopy*
University of Verona, Italy

Abstract—The peripheral blood neutrophils and the neutrophils accumulated into a skin-window experimental exudate were compared for their ability to release superoxide anion (O_2^-) and for their fatty acid composition, determined by capillary gas-chromatography. The basal O_2^- release and the phorbol myristate acetate (PMA)-induced O_2^- release were not significantly different in the two neutrophil populations, while in response to formyl-methionyl-leucyl-phenylalanine (fMLP) the exudate cells showed an activity that was two fold higher than that of blood cells. The most significant changes of fatty acid composition of exudate versus blood cells were the following: i) increase of C16:0 (palmitic acid) from 21.3 ± 1.2% to 23.5 ± 1.3% (+ 10.2%) of total fatty acids ($p < 0.001$), ii) increase of C18:2 (linoleic acid) from 9.3 ±1.7% to 11.0 ± 2.1% (+ 18.3%) ($p = 0.005$), iii) decrease of C20:4 (arachidonic acid) from 12.8 ± 1.6% to 10.6 ± 1.2% (−17.3%) ($p < 0.001$), whereas C18:1 (oleic acid) did not change. The total content of saturated or unsaturated fatty acids did not change. In exudate cells, a strong negative correlation was found between palmitic acid content and O_2^- release in response to both fMLP and PMA ($r = -0.52$, $p < 0.02$ and $r = -0.49$, $p < 0.05$, respectively) whereas arachidonic acid correlated positively, but weakly, with O_2^- ($r = 0.40$, $p = 0.07$ and $r = 0.47$, $p = 0.05$, with fMLP and PMA as stimulants respectively). A positive correlation was also found between the arachidonic acid content of blood cells and the number of cells that migrated into the inflammatory exudate. These results indicate that the process of extravasation from blood into the exudate causes specific and consistent modifications of the fatty acid composition of neutrophils and suggest that these modifications have a role in the activation and the regulation of the O_2^- forming system.

INTRODUCTION

Under normal conditions, neutrophil oxidative metabolism is very low, but once they are activated by soluble or particulate agents, these cells may increase their

0360-3997/96/0400-0123$09.50/0

metabolic activity in order to produce and release a battery of reactive oxygen metabolites, a phenomenon called respiratory burst. The duration and the extent of the respiratory burst may vary according to the state of sensitivity and of responsiveness of receptor, transduction and effector mechanisms of the cells. These regulations are operating in a number of pathophysiological circumstances. For example, when the neutrophils are recruited to sites of inflammation, they come in contact with chemotactic substances originating from bacteria (e.g. formylpeptides, endotoxins), from serum (C5a), or from tissue cells (NAP-1/IL-8, PAF, LTB4, substance P), and these substances induce a state of preactivation, so-called primed state. Similar changes may be obtained also in vitro by exposing the cells to substimulatory doses of chemotactic peptides or to cytokines such as interferon-g and tumor necrosis factor (1–7).

The molecular mechanisms of priming have not been fully clarified yet, and the current knowledge indicates that several biochemical phenomena are involved, such as increase of membrane receptors (8–10), changes in the levels of intracellular calcium (11–12), modifications of the terminal NADPH oxidase (13–15). In addition, a role of cellular lipids in the regulation of activation or activity of the oxidase has been suggested by the stimulating effect on the solubilized and isolated oxidase exhibited by phosphatidylethanolamine (16), by phosphatidylserine (17) and by phosphatidic acid (18–20). More important, the NADPH oxidase in cell-free systems is activated by arachidonic acid and other unsaturated fatty acids (21–23).

A way to get new insights into the priming phenomenon is to compare the functional and molecular characteristics of leukocytes circulating in blood in a resting state with those of leukocytes obtained from a site of physiological or pathological inflammation. In a series of previous studies we used the Senn's skin-window model of inflammation to this purpose, and we were able to show that the activation of exudate neutrophils with fMLP and substance P results in enhanced production of oxygen metabolites as compared to the production by peripheral blood neutrophils and that these differences are associated with modifications of cell adhesiveness and of receptors number, but not with changes of Ca++ and cAMP intracellular responses (10, 24, 25).

The object of the present work was to characterize the in vivo primed neutrophils as regards their fatty acid content, compared with the composition of neutrophils harvested from peripheral blood, in order to investigate whether a correlation exists between the lipid composition of neutrophils and the changes of oxidative metabolism occurring in association with the extravasation, chemotactic movement, and sojourn into the exudate. Migrated neutrophils were then assayed for their ability to respond to suitable stimulants with an enhanced production of superoxide anion and this variable was correlated with fatty acid composition. The results indicate that significant changes in the content of spe-

cific fatty acid moieties occur in exudate cells and that some of these changes may have a role in relation with the considered cell functions.

MATERIALS AND METHODS

Reagents. FMLP, PMA and reference fatty acids were purchased from Sigma Chemical Company, St. Louis, Missouri; cytochrome c from Boehringer, Mannheim, Germany; purified serum bovine albumin and human albumin from Behring Institut, Marburg, Germany; Percoll was from Pharmacia, Uppsala. Sterile 96-well microtiter plates with flat-bottomed wells (Linbro type) were from Flow Laboratories. The microplates were pre-coated with fetal bovine serum (Flow Laboratories) in order to abolish nonspecific cell activation as described (26). Hank's balanced salt solution (without calcium and magnesium) (HBSS) and Dulbecco's phosphate buffered saline (PBS) were from Gibco Ltd, Paisley, Scotland; the composition of HBSS was 0.4 g/l KCl, 0.06 g/l KH_2PO_4, 8 g/l NaCl, 0.09 g/l Na_2HPO_4, 1 g/l D-glucose (pH 7.4); the composition of PBS was 0.2 g/l KCl, 0.2 g/l KH_2PO_4, 0.047 g/l $MgCl_2$, 8 g/l NaCl, 1.15 g/l Na_2HPO_4 (pH 7.4). Other materials and reagents were of the highest purity available.

Cell Preparation. Neutrophils were purified from blood and skin window exudates from healthy subjects, after obtaining their informed consent. Blood neutrophils were prepared from Na_2ethylene diamine tetraacetate-anticoagulated blood by centrifugation over Percoll gradients (Pharmacia, Uppsala, Sweden) (27). Inflammatory exudates were obtained from a 1 cm^2 skin abrasion made on the forearm with a rotating sterile abrasive cylinder operated by a milling cutter. The abrasion did not cause bleeding as only the epidermis was removed and the wet, transuding surface of derma was exposed. Exudate neutrophils were isolated using disposable chambers purchased from Far-Italia (Verona, Italy) added with 1 ml of autologous serum as previously described (24).

Superoxide Measurement. A microplate assay of O_2^- production was performed according to previously reported procedures (26, 28), with the following modifications. The assay medium was H-A supplemented with 0.5 mM $CaCl_2$ and 1 mM $MgSO_4$ (H-ACM). The microplate wells were supplemented with 25 μl of 0.6 mM cytochrome c and with either 25 μl of the stimulant dissolved in H-ACM (fMLP or PMA at the final concentration of 5×10^{-7} M and 10 ng/ml respectively) or with 25 μl of H-ACM (resting assays). Assays were currently done in triplicate for each experimental condition. The plate was brought to 37°C, and 50 μl of the neutrophil suspension (2×10^5 cells), pre-warmed at 37°C, were added to each well. The plate was then incubated for 10 minutes in a humidified thermostat at 37°C. When required, the plate was rapidly transferred into a microplate reader (Reader 400, SLT Labs Instruments) and the reduction of cytochrome c was measured at 550 nm using 540 nm as reference wavelength, and using 0.037 optical density units as standard for 1 nmol of reduced cytochrome c (26).

Fatty Acid Determination. Analysis of fatty acids was performed on aliquots of neutrophil preparations containing 10^7 cells, by extraction of total lipids with 4.5 ml of isopropanol/chloroform (11/7, v/v) added with 0.45 mM 2,6-di-ter-p-cresol (BHT) as antioxidant. A gas chromatographic (Hewlett Packard 5890 chromatograph, Hewlett Packard, Palo Alto California, USA) method was employed as previously described (29), based on fatty acid direct transesterification technique (30). Analyses were performed in duplicate on each sample. Peak identification and quantification were made with reference fatty acids commercially available. As internal standard heptadecanoic acid (C17:0) was used. The heights of the peaks were measured and the quantification was performed with the aid of a PC Vectra QS/16S equipped with the software HP-3365 ChemStation (Hewlett

Packard) working in Microsoft Windows 3.0. Fatty acid composition data were expressed as mg/100 mg fatty acid methyl esters. The mean % recovery was 90.6 ± 2.7 (range 85%-94%).

Data Analysis. Statistical analysis was performed on a PC Apple Macintosh SE/30 equipped with the software Systat 5.0, working in Windows 3.0 (Microsoft). Values are expressed as mean ± standard deviation and the correlation between different parameters was studied using the Pearson correlation coefficient.

RESULTS

Cell preparations obtained from 24-h skin window exudates in a group of 22 normal subjects were composed almost exclusively by neutrophils (>98%, the remaining being monocytes and some rare eosinophils), with a mean yield of 67.2 ± 23.9 × 10^6 per subject. Moreover, over 95% of cells were trypan blue negative. The number of neutrophils migrated to the skin-window was positively correlated with the content of arachidonic acid of the circulating leukocytes (Fig. 1). No correlations between cell migration and other fatty acids were found.

The oxidative metabolic responses of neutrophils isolated from the exudate were compared, in a simultaneous assay, with those of the peripheral blood

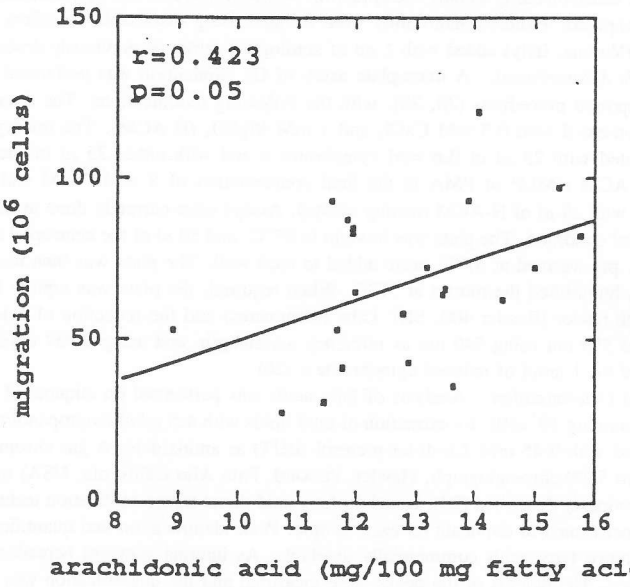

Fig. 1. Correlation between arachidonic acid content of blood neutrophils and the number of cells which migrated into the skin window exudate.

neutrophils obtained from the same subject. In order to ascertain the stimulus specificity of activated responses and to investigate the mechanism involved in priming, we challenged the cells with two neutrophil agonists that are known to interact at different levels with the stimulus-response coupling machinery. As reported in table I, exudated cells showed a markedly increased superoxide release in response to fMLP, while both the resting (basal) activity and the PMA-induced response were not significantly different in the two cell populations. No differences of the response to PMA were found using a range of PMA concentrations from 1 ng/ml up to 40 ng/ml (data not shown). These results demonstrate that the skin window cells used in these studies are actually primed, and that this priming is factor-specific.

The fatty acid content of the exudate and primed cells is shown in table II, where the composition of blood cells is also reported as a reference. No changes have been observed in the total content of saturated or unsaturated fatty acids, neither oleic acid, the most abundant monounsaturated fatty acid, changed after cell exudation. However, some important and significant changes of the most representative fatty acids were noted: i) an increase of palmitic acid (+ 10.2%) and of linoleic acid (+ 18.3%), ii) a small decrease of stearic acid (− 7.8%) and a marked decrease of arachidonic acid (− 17.1%), iii) a decrease of long-chain saturated fatty acids, but this change is relevant only in relative terms, being quantitatively small because these species represent only about 1 percent of total fatty acids.

The changes of fatty acid composition of the cells obtained from two different sites could be due, in principle, either to phenomena strictly associated to the functional activity or regulation of the cells or, alternatively, to a remodelling of membrane composition due to the stay of exudate cells for up to 24 h in a medium with a possible different composition in fatty acids with respect

Table I. Superoxide Release by Neutrophils of Blood and of Skin Window Inflammatory Exudate

Stimulant	Superoxide release nmoles/10 min/10^6 cells ± S.D. (n = 22)		
	Blood cells	Exudate cells	p[a]
none	0.92 ± 0.82 (0.0–3.0)	1.17 ± 0.99 (0.0–3.0)	n.s.
3×10^{-7} M fMLP	11.04 ± 3.55 (6.0–17.0)	25.05 ± 7.92 (13.3–42.4)	<0.001
10 ng/ml PMA	28.33 ± 9.4 (19.5–49.6)	28.80 ± 9.91 (16.0–54.7)	n.s.

[a] The paired Student t test comparing values of blood neutrophils with those of exudate neutrophils from each subject are reported.

Table II. Fatty Acids Content of Neutrophils from Blood and from Skin Window Inflammatory Exudates

	Fatty acids (% of total, w/w) ± S.D. (n = 20)		
	Blood cells	Exudate cells	p[a]
SATURATED			
TOTAL	44.40 ± 2.35	44.78 ± 1.56	n.s.
C12:0	0.27 ± 0.15	0.26 ± 0.06	n.s.
C14:0	0.77 ± 0.23	1.16 ± 0.26	<0.001
C16:0 (palmitic)	21.35 ± 1.19	23.54 ± 1.26	<0.001
C18:0 (stearic)	18.84 ± 2.19	17.36 ± 1.62	<0.015
C20:0	0.38 ± 0.02	0.43 ± 0.06	n.s.
C22:0	1.28 ± 0.19	0.97 ± 0.15	<0.001
C24:0	1.92 ± 0.26	1.38 ± 0.20	<0.001
C26:0	0.37 ± 0.14	n.t.	
MONOUNSATURATED			
TOTAL	29.91 ± 2.74	30.04 ± 1.67	n.s.
C16:1w9	0.52 ± 0.24	0.68 ± 0.55	n.s.
C18:1w9 (oleic)	27.94 ± 2.19	28.56 ± 1.50	n.s.
C20:1w9	1.01 ± 0.23	0.91 ± 0.14	n.s.
C22.1w9	1.34 ± 0.51	0.79 ± 0.26	0.03
POLYUNSATURATED			
TOTAL	24.93 ± 1.93	24.13 ± 2.38	n.s.
C18:2w6 (linoleic)	9.32 ± 1.71	11.03 ± 2.09	<0.005
C18:4w3	1.22 ± 0.24	0.88 ± 0.13	<0.001
C20:2w6	0.98 ± 0.29	0.87 ± 0.26	n.s.
C20:4w6 (arachidonic)	12.81 ± 1.63	10.62 ± 1.24	<0.001
C20:5w3	0.60 ± 0.03	0.53 ± 0.19	n.s.
C22:6w6	1.26 ± 0.32	1.09 ± 0.40	n.s.

[a] The paired Student t test comparing values of blood neutrophils with those of exudate neutrophils from each subject are reported.

to plasma. The latter possibility seems not to be the case, because we found an almost identical fatty acid content in plasma and in the supernatant of the exudate after cell centrifugation (table III). The only difference was in the content of C:12, but this fatty acid species is quantitatively irrelevant and does not change in exudate versus blood cells (table II).

We then calculated the correlations between fatty acid composition and superoxide production in exudate neutrophils, taking into consideration these variables in the same cells from each subject. The superoxide release of resting

Table III. Fatty Acids Content of Serum and of Supernatant of Skin Window Inflammatory Exudates

	Fatty acids (% of total, w/w) ± S.D. (n = 20)		
	Plasma	Exudate	p[a]
SATURATED			
TOTAL	33.89 ± 1.88	34.71 ± 3.43	n.s.
C12:0	0.11 ± 0.04	0.23 ± 0.19	<0.01
C14:0	1.01 ± 0.32	1.26 ± 0.73	n.s.
C16:0 (palmitic)	22.93 ± 1.74	22.98 ± 2.68	n.s.
C18:0 (stearic)	7.97 ± 0.77	8.39 ± 0.78	n.s.
C20:0	0.40 ± 0.11	0.41 ± 0.08	n.s.
C22:0	0.82 ± 0.18	0.80 ± 0.20	n.s.
C24:0	0.71 ± 0.16	0.71 ± 0.20	n.s.
MONOUNSATURATED			
TOTAL	24.71 ± 4.2	24.52 ± 4.26	n.s.
C16:1w9	1.65 ± 1.12	1.54 ± 1.04	n.s.
C18:1w9 (oleic)	23.62 ± 3.53	23.49 ± 3.75	n.s.
C20:1w9	0.26 ± 0.13	0.23 ± 0.04	n.s.
C22.1w9	0.22 ± 0.15	0.23 ± 0.12	n.s.
POLYUNSATURATED			
TOTAL	40.75 ± 4.55	40.20 ± 5.53	n.s.
C18:2w6 (linoleic)	27.33 ± 4.59	27.18 ± 4.81	n.s.
C18:4w3	0.47 ± 0.23	0.45 ± 0.18	n.s.
C20:2w6	0.32 ± 0.18	0.25 ± 0.07	n.s.
C20:4w6 (arachidonic)	9.82 ± 2.83	9.53 ± 2.08	n.s.
C20:5w3	0.67 ± 0.21	0.69 ± 0.20	n.s.
C22:6w6	2.17 ± 0.57	2.13 ± 0.57	n.s.

[a]The paired Student t test comparing values of serum with those of cell-free exudate from each subject are reported.

cells was so low that no possible correlations could be calculated with respect to the fatty acids content. Therefore, the only consistent data were obtained on PMA- and fMLP-activated cells and the drawn correlation was between fatty acid content and the activation of the metabolism (Fig. 2). Among the fatty acids species reported in Table II, the majority were not correlated with oxidative metabolism and neither correlation with the total content of unsaturated and saturated fatty acids was found. However, palmitic acid showed a significant negative correlation with PMA- and fMLP-activated respiratory burst (Fig. 2A and 2B): the superoxide production was higher in cells having a low content of

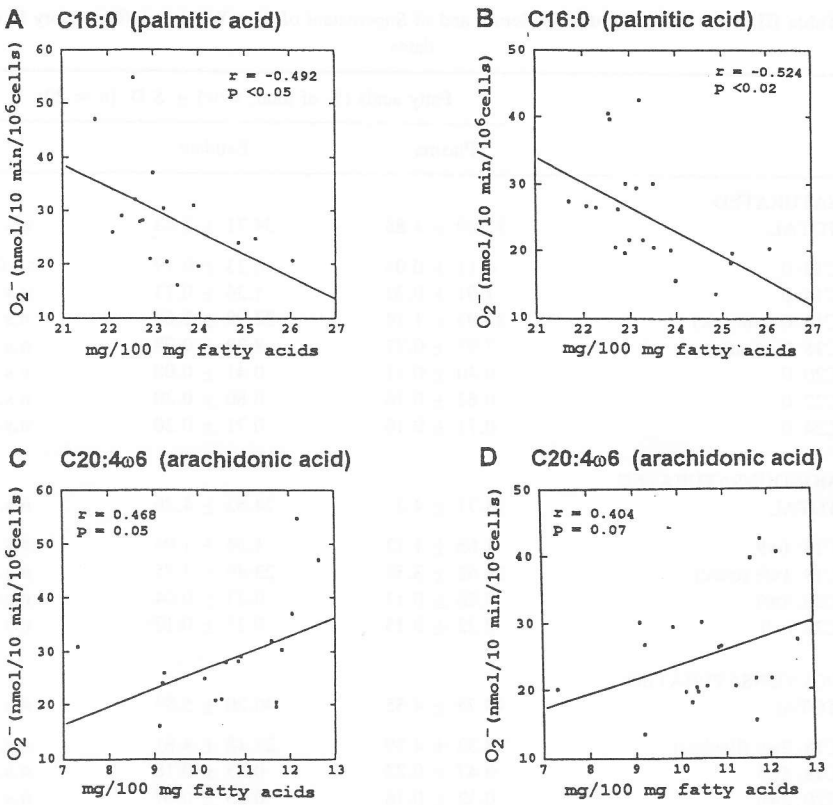

Fig. 2. Linear regression plot of fatty acid content and PMA-stimulated (A and C) and fMLP-stimulated (B and D) O_2^- release in human neutrophils.

palmitic acid. On the other hand, arachidonic acid showed a correlation having a positive trend with both PMA and fMLP as stimulants (Fig. 2C and 2D), although the latter did not reach statistical significance.

DISCUSSION

Once recruited into inflammatory focus, neutrophil granulocytes undergo to profound biochemical and functional modifications, including the priming of free radical-generating oxidative metabolism. A series of experimental evidence indicates that membrane fatty acid composition and metabolism have essential importance in the activation and regulation of neutrophil superoxide production:

i) various cytokines affect arachidonic acid metabolism and it has been postulated that this may be the biochemical basis for priming (31–34); ii) arachidonic acid may have a second messenger role in the stimulus-response coupling of receptor-activated neutrophils (35–37); iii) arachidonic acid acts directly on the assembled NADPH oxidase in the membranes and in the endosomes and increases its catalytic activity by increasing the affinity for NADPH (38, 39); iv) phospholipase A2 inhibition suppresses the release of arachidonic acid as well as the O_2^- production and decrease the priming effect (40, 41).

In a recent work (42), we have shown that the production of superoxide anion by peripheral blood neutrophils in response to fMLP is in direct correlation with the arachidonic acid content of the neutrophils of the same donor and in inverse relationship with linoleic acid and palmitic acid content, suggesting that arachidonic acid and other specific cellular fatty acids should be regarded as potential regulatory factors involved in the determination of cell activability.

In the present work, we have taken advantage of a well-established model of experimental inflammation in order to characterize the major changes of fatty acid composition which are associated with one of the most important functions of the neutrophil leukocytes, i.e. the process of extravasation and accumulation into the exudate. As shown by Fig. 1, the content of arachidonic acid of resting (blood) cells seems to be important for the migration process. This might be explained either by an higher flexibility of the cell membrane and/or by an increased availability of this fatty acid, which is utilized in the metabolic processes activated during migration. The latter hypothesis is supported by the marked decrease of arachidonic acid following exudation (table I).

The remodelling of fatty acid composition of the cells involves specific moieties and in two directions: palmitic and linoleic acid increase, while arachidonic acid markedly decreases in exudate cells. The decrease of arachidonic acid content in skin window cells is in keeping with the proposed role of its metabolism in leukocytes. Human neutrophils contain a cytosolic phospholipase A2 that is activated in agonist-stimulated neutrophils, yelding arachidonic acid as one of the major products (43, 44). Free arachidonic acid is either released from the cells or associated to the membranes, or metabolized through either the cyclooxygenase or lipoxygenase pathways, yelding various metabolites as thromboxane B2, hydroxyeicosatetraenoic acids and leukotrienes. These metabolites are, in turn, potent stimuli for chemotaxis and promote priming of NADPH oxidase (45).

The bulk of arachidonic acid in mammalian cells is esterified in the fatty acyl chains of glycerophospholipids, almost exclusively in the 2-acyl position (46). Phospholipase A2 activity is therefore responsible for controlling free arachidonic acid levels. However, the actual free fatty acid level in a tissue actually represents a balance between the liberation of the fatty acid by hydrol-

ysis and its re-esterification into complex lipids by acyltransferases. Therefore, our data demonstrate that during the process of chemotaxis and in the time employed by extravasated neutrophils into the exudate, the rate of arachidonic acid hydrolysis and utilization is greater than the rate of its synthesis and esterification.

We have looked for a possible correlation between the arachidonic acid content and superoxide formation by exudate cells, a finding that was expected on the basis of previous studies carried out on blood cells (42). The data showed a positive trend of this correlation, using PMA as stimulant, and a weak positive correlation using fMLP. A possible interpretation of this weak correlation might be the fact that the pool of arachidonic acid of exudate cells is reduced and functionally unstable due to the chemotactic activation of the cells, and therefore the resulting data are quite dispersed between different samples. In any case, the positive slope of the correlation suggests that the arachidonic acid availability may be a positive factor in the regulation of the respiratory burst also in inflammatory cells. In fact, the phospholipase A2 enzymes are well known to be highly sensitive probes of the physical state of the substrate and the presence of arachidonoyl-rich domains favor the phospholipase attack to the membranes (47).

Linoleic acid is an essential fatty acid which is metabolized by a series of desaturase and elongase enzymes to form arachidonic acid. The increase of this fatty acid moiety in the exudate cells is undoubtedly a sign of increased incorporation into cell membranes, which, in turn, may be a first step of the biochemical pathway leading to arachidonic acid synthesis. This increase of linoleic acid does not seem to affect the oxidative metabolism, because there is no correlation between the relative amount of this fatty acid and the rate of superoxide generation.

Finding an increase in palmitic acid content of inflammatory- and primed-neutrophils was unexpected and is paradoxical, because this fatty acid correlates negatively with the respiratory burst, as shown both in this study and in a previous report on blood cells (42). The present data show that during the exudation process in vivo, which causes cell priming, the palmitic acid consistently increases, but when the cells are stimulated in vitro, those having a higher content of this saturated fatty acid show a lower respiratory burst. Therefore, it is conceivable that palmitic acid cellular content exerts a regulatory effect on the final steps of the NADPH oxidase activation, but not on the priming process. This hypothesis is also in agreement with the finding that the negative correlation between palmitic acid and superoxide production was found both with fMLP and PMA, two agents which trigger the oxidase through different transduction pathways, while priming did not involve the response to PMA. Putative levels of these regulations are palmitoylation processes or protein kinase C activity. The latter enzyme is sensitive to the specificity of the fatty acyl

moieties of diacylglycerol: dicylglycerols containing at least one unsaturated fatty acid are fully active on this enzyme, while diacylglycerols containing two saturated fatty acids such as dipalmitin and distearin are far less effective (48).

In conclusion, the changes of the fatty acid composition occurring in neutrophils as a consequence of the accumulation in an inflammatory focus appear to be quite specific, regard both saturated and unsaturated species, and some of these changes appear to have functional regulatory significance. The view that changes in the phospholipid and fatty acid composition of plasma membranes under some conditions could modulate the function of the activated oxidase, thereby exerting regulatory effects on superoxide generation, is confirmed and strengthened by these results.

The study of lipid composition and its relation with the behavior of inflammatory cells may have practical applications in medicine, because several lines of evidence show that the fatty acid composition of cell membranes and even the arachidonic acid metabolism may be modified by dietary supplementation (49–52), that alterations of arachidonic acid metabolism and increase of free radicals generation occur in patients with rheumatoid arthritis and systemic lupus erythemathosus (53, 54), and that altering dietary essential fatty acids is of benefit in various inflammatory diseases such as rheumatoid arthritis, psoriasis, and atopic eczema (55–60).

Acknowledgments—This work was supported by a grant from Ministero Università Ricerca Scientifica e Tecnologica and from Public Health Department, Regione Veneto. The skillful technical assistance of Fabio Poli is also acknowledged.

REFERENCES

1. McCall, C. E., D. A. Bass, L. R. Dechatelet, A. S. Link, Jr., and M. Mann. 1979. In vitro responses of human neutrophils to N-formyl-methionyl- leucyl- phenylalanine, correlation with effects of acute bacterial infection. *J. Infect. Dis.* **140:**277–286.
2. Van Epps, D.E. and M. L. Garcia. 1980. Enhancement of neutrophil function as a result of prior exposure to chemotactic factor. *J. Clin. Invest.* **66:**167–175.
3. Zimmerli, W., P. D. Lew, H. J. Cohen, and F. A. Waldvogel. 1984. Comparative superoxide-generating system of granulocytes from blood and peritoneal exudates. *Infect. Immun.* **46:**625–630.
4. Berton, G., L. Zeni, M. A. Cassatella, and F. Rossi. 1986. Gamma interferon is able to enhance the oxidative metabolism of human neutrophils. *Biochem. and Biophys. Res. Com.* **138:**1276–1282.
5. Briheim, G., O. Stendahl, B. I. Coble, and C. Dahlgren. 1988. Exudate polymorphonuclear leukocytes isolated from skin chambers are primed for enhanced response to subsequent stimulation with the chemoattractant fMet-Leu-Phe and C3 opsonized yeast particles. *Inflammation* **12:**141–152.

6. Follin, P., M. P. Wymann, B. Dewald, M. Ceska, and C. Dahlgren. 1991. Human neutrophil migration into skin chambers is associated with production of NAP-1/IL8 and C5a. *Eur. J. Haematol.* **47:**71–76.
7. H. L. Nurcombe, R. C. Bucknall, and S. W. Edwards. 1991. Neutrophils isolated from the synovial fluid of patients with rheumatoid arthritis, priming and activation in vivo. *Ann. Rheum. Dis.* **50:**147–153.
8. Zimmerli, W., B. Seligmann, and J. I. Gallin. 1986. Exudation primes human and guinea pig neutrophils for subsequent responsiveness to the chemotactic peptide N-formylmethionyl-leucylphenylalanine and increases complement component C3bi receptor expression. *J. Clin. Invest.* **77:**925–933.
9. Tennenberg, S. D. and J. S. Solomkin. 1990. Activation of neutrophils by cachectin/tumor necrosis factor, priming of n-formyl-methionyl-leucyl-phenylalanine-induced oxidative responsiveness via receptor mobilization without degranulation. *J. Leukocyte Biol.* **47:**217–223.
10. Biasi, D., M. L. Bambara, A. Carletto, P. Caramaschi, M. C. Serra, P. Guzzo, C. Santonastaso, and P. Bellavite. 1993a. Molecular mechanisms of priming. Exudate human neutrophils are primed to fMet-Leu-Phe but have normal intracellular calcium and cAMP responses. *Bull. Mol. Biol. Med.* **18:**225–235.
11. Axtell, R. A., R. R. Sandborg, J. E. Smolen, P. A. Ward, and L. A. Boxer. 1990. Exposure of human neutrophils to exogenous nucleotides causes elevation in intracellular calcium, transmembrane calcium fluxes, and an alteration of a cytosolic factor resulting in enhanced superoxide production in response to FMLP and arachidonic acid. *Blood* **75:**1324–1332.
12. Zimmerli, W., A. M. Reber, and C. A. Dahinden. 1990. The role of formylpeptide receptors, C5a receptors, and cytosolic-free calcium in neutrophil priming. *J. Infect. Dis.* **161:**242–249.
13. Guthrie, L. A., L. C. McPhail, P. M. Henson, and R. B. Johnston. 1984. Priming of neuthrophils for enhanced release of oxygen metabolites by bacterial lipopolysaccharide. Evidence for increased activity of the superoxide-producing enzyme. *J. Exp. Med.* **160:**1657–1671.
14. Paty, P. B., R. W. Graeff, S. J. Mates, and T. K. Hunt. 1990. Superoxide production by wound neutrophils. Evidence for increased activity of the NADPH oxidase. *Arch. Surg.* **125:**65–69.
15. Cassatella, M. A., F. Bazzoni, R. M. Flynn, S. Dusi, G. Trinchieri, and F. Rossi. 1990. Molecular basis of interferon-g and lipopolysaccharide enhancement of phagocyte respiratory burst capability. Studies on the gene expression of several NADPH oxidase components. *J. Biol. Chem.* **265:**20241–20246.
16. Gabig, T. G. and B. M. Babior. 1979. The O_2^- forming oxidase responsible for the respiratory burst in human neutrophils. Properties of the solubilized enzyme. *J. Biol. Chem.* **254:**9070–9074.
17. Tamura, M., T. Tamura, S. R. Tyagi, and J. D. Lambeth. 1988. The superoxide-generating respiratory burst oxidase of human neutrophil plasma membrane. Phosphatidylserine as an effector of the activated enzyme. *J. Biol. Chem.* **263:**17621–17626.
18. Bellavite, P., F. Corso, S. Dusi, M. Grzeskowiak, V. Della Bianca, and F. Rossi. 1988. Activation of a NADPH-dependent superoxide production in plasma-membrane extracts of pig neutrophils by phosphatidic acid. *J. Biol. Chem.* **263:**8210–8214.
19. Agwu, D. E., L. McPhail, S. Sozzani, D. A. Bass, and C. E. McCall. 1991. Phosphatidic acid as a second messenger in human polymorphonuclear leukocytes. Effects on activation of NADPH oxidase. *J. Biol. Chem.* **88:**531–539.
20. McPhail, L. C., D. Qualliotine-Mann, D. E. Agwu, and C. E. McCall. 1993. Phospholipases and the activation of the NADPH oxidase. *Eur. J. Haematol.* **51:**294–300.
21. Bromberg, Y. and E. Pick. 1984. Unsaturated fatty acids stimulate NADPH-dependent superoxide production by cell-free system derived from macrophages. *Cell. Immunol.* **88:**213–221.

22. McPhail, L. C., P. S. Shirley, C. C. Clayton, and R. Snyderman. 1985. Activation of the respiratory burst enzyme from human neutrophils in a cell-free system. Evidence for a soluble cofactor. *J. Clin. Invest.* **75:**1735–1739.
23. Clark, R. A., K. G. Leidal, D. W. Pearson, and W. M. Nauseef. 1987. NADPH oxidase of human neutrophils. Subcellular localization and characterization of an arachidonate-activatable superoxide-generating system. *J. Biol. Chem.* **262:**4065–4074.
24. Biasi, D., L. M. Bambara, A. Carletto, M. Caraffi, M. C. Serra, S. Chirumbolo, and P. Bellavite. 1993b. Factor-specific changes of the oxidative metabolism of exudate human neutrophils. *Inflammation* **17:**13–23.
25. Bellavite, P., A. Carletto, D. Biasi, P. Caramaschi, F. Poli, F. Suttora, and L. M. Bambara. 1994. Studies of skin-window exudate human neutrophils. Complex patterns of adherence to serum-coated surfaces in dependence on FMLP doses. *Inflammation* **18:**575–587.
26. Bellavite, P., S. Chirumbolo, C. Mansoldo, G. Gandini, and P. Dri. 1992. A simultaneous assay for oxidative metabolism and adhesion of human neutrophils. Evidence for correlations and dissociations of the two responses. *J. Leukocyte Biol.* **51:**329–335.
27. Metcalf, J. A., J. I. Gallin, W. M. Nauseef, and R. K. Root. 1986. *In* Laboratory Manual of Neutrophil Function. Raven Press, New York.
28. Bellavite, P., S. Chirumbolo, G. Lippi, P. Guzzo, and C. Santonastaso. 1993. Homologous priming in chemotactic peptide stimulated neutrophils. *Cell Biochem. Function* **11:**93–100.
29. Girelli, D., M. Azzini, O. Olivieri, P. Guarini, M. T. Trevisan, A. Lupo, P. Bernich, G. Panzetta, and R. Corrocher. 1992. Red blood cells and platelet membrane fatty acids in non-dialyzed and dialyzed uremics. *Clin. Chim. Acta* **211:**155–166.
30. Lepage, G. and C. C. Roy. 1986. Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid Res.* **27:**114–120.
31. Dipersio, J. F., P. Billing, R. Williams, and J. C. Gasson. 1988. Human granulocyte-macrophage colony-stimulating factor and other cytokines prime human neutrophils for enhanced arachidonic acid release and leukotriene B4 synthesis. *J. Immunol.* **140:**4315–4322.
32. Corey, S. J. and P. M. Rosoff. 1991. Unsaturated fatty acids and lipoxygenase products regulate phagocytic NADPH oxidase activity by a nondetergent mechanism. *Journal of Laboratory and Clinical Medicine,* **118:**343–351.
33. Bauldry, S. A., C. E. McCall, S. L. Cousart, and D. A. Bass. 1991. Tumor necrosis factor-a priming of phospholipase A2 activation in human neutrophils. An alternative mechanism of priming. *J. Immunol.* **146:**1277–1285.
34. Darmani, H., J. Harwood, and S. K. Jackson. 1993. Interferon-g-stimulated uptake and turnover of linoleate and arachidonate in macrophages: A possible pathway for hypersensitivity to endotoxin. *Cell. Immunol.* **152:**59–71.
35. McPhail, L. C., C. C. Clayton, and R. Snyderman. 1984. A potential second messenger role for unsaturated fatty acids, Activation of Ca_2^+-dependent protein kinase C. *Science* **224:**622–624.
36. Beaumier, L., N. Faucher, and P. H. Nacchache. 1987. Arachidonic acid-induced release of calcium in permeabilized human neutrophils. *FEBS Lett.* **221:**289–292.
37. Abramson, S. B., J. Leszczynska-Piziak, and G. Weissmann. 1991. Arachidonic acid as a second messenger. Interactions with a GTP-binding protein of human neutrophils. *J. Immunol.* **147:**231–236.
38. Rubinek, T. and R. Levy. 1993. Arachidonic acid increases the activity of the assembled NADPH oxidase in cytoplasmic membranes and endosomes. *Biochim. Biophys. Acta* **1176:**51–58.
39. Henderson, L. M., S. K. Moule, and J. B. Chappel. 1993. The immediate activator of the NADPH oxidase is arachidonate, not phosphorylation. *Eur. J. Biochem.* **211:**157–162.

40. Maridonneau-Parini, I., S. M. Tringale, and A. I. Tauber. 1986. Identification of distinct activation pathways of the human neutrophil NADPH-oxidase. *J. Immunol.* **137:**2925–2929.
41. Forehand, J. R., R. B. Johnston, Jr., and J. S. Bomalaski. 1993. Phospholipase A2 activity in human neutrophils. Stimulation by lipopolysaccharide and possible involvement in priming for an enhanced respiratory burst. *J. Immunol.* **151:**4918–4925.
42. Bellavite, P., P. Guarini, D. Biasi, A. Carletto, M. T. Trevisan, P. Caramaschi, L. M. Bambara, and R. Corrocher. 1995. Correlations between the intensity of fMLP-dependent respiratory burst and cellular fatty acid composition in human neutrophils. *Br. J. Haematol.* **89:**271–276.
43. Alonso, F., P. M. Henson, and C. C. Leslie. 1986. A cytosolic phospholipase in human neutrophils that hydrolyzes arachidonoyl-containing phosphatidylcholine. *Biochem. Biophys. Acta* **878:**273–280.
44. Ramesha, C. S. and D. L. Ives. 1993. Detection of arachidonoyl-selective phospholipase A2 in human neutrophil cytosol. *Biochim. Biophys. Acta* **1168:**37–44.
45. Sandborg, R. R. and J. E. Smolen. 1988. Biology of disease. Early biochemical events in leukocyte activation. *Lab. Invest.* **59:**300–320.
46. Irvine, R. F. 1982. How is the level of free arachidonic acid controlled in mammalian cells? *Biochem. J.* **204:**3–16.
47. Stubb, C. D. and A. D. Smith. 1984. The modification of mammalian membrane polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim. Biophys. Acta* **779:**89–137.
48. Mori, T., Y. Taki, R. Minakuchi, B. Yu, and Y. Nishizuka. 1980. Inhibitory action of chlorpromazine, dibucaine, and other phospholipid-interacting drugs on calcium-activated phospholipid-dependent protein kinase. *J. Biol. Chem.* **255:**8378–8380.
49. Cathcart, E. S., C. A. Leslie, S. N. Meydani, and K. C. Hayes. 1987. A fish oil diet retards experimental amyloidosis, modulates lymphocyte function, and decreases macrophage arachidonate metabolism in mice. *J. Immunol.* **139:**1850–1854.
50. Olivieri, O., M. Negri, M. De Gironcoli, A. Bassi, P. Guarini, A. M. Stanzial, M. L. Guadagnin, S. Ferrari, and R. Corrocher. 1988. Effects of dietary fish-oil on malondialdehide production and glutathion-peroxidase activity in hyperlipidemic patients. *Scand. J. Clin. Lab. Invest.* **48:**659–665.
51. Fletcher, M. P., and V. A. Ziboh. 1990. Effects of dietary supplementation with eicosapentaenoic acid or gamma-linolenic acid on neutrophil phospholipid fatty acid composition and activation responses. *Inflammation* **14:**585–697.
52. Jannace, P. W., R. H. Lerman, J. I. Santos, and J. J. Vitale. 1992. Effects of oral soy phosphatidylcholine on phagocytosis, arachidonate concentrations, and killing by human polymorphonuclear leukocytes. *Am. J. Clin. Nutr.* **56:**599–603.
53. Smith, D. M., J. A. Johnson, and R. A. Turner. 1989. Alterations in arachidonic acid metabolism and chemotactic response in polymorphonuclear leukocytes from patients with rheumatoid arthritis. *Clin. Exp. Rheumatol.* **7:**471–477.
54. Suryaprabha, P., U. N. Das, G. Ramesh, K. Vijay Kumar, and G. Sravan Kumar. 1991. Reactive oxygen species, lipid peroxides and essential fatty acids in patients with rheumatoid arthritis and systemic lupus erythematosus. *Prost. Leuk. Ess. Fatty Acids* **43:**251–255.
55. Kremer, J. M., J. Bigaunoette, A. V. Michalek, M. A. Timchalk, L. Leninger, R. I. Rynes, C. Huyck, J. Zieminski, and L. E. Bartholomew. 1985. Effects of manipulation of dietary fatty acids on clinical manifestations of rheumatoid arthritis. *Lancet* **i:**184–187.
56. Tate, G. A., B. F. Mandell, R. A. Karmali, M. Laposata, D. G. Baker, H. R. Shumacher, and R. B. Zurier. 1988. Suppression of monosodium urate crystal-induced acute inflammation by diets enriched with gamma-linolenic acid and eicosapentaenoic acid. *Arthritis Rheum.* **31:**1543–1551.

57. Belch, J. J. F., D. Ansell, R. Madhok, A. O'Dowd, and R. D. Sturrock. 1988. Effects of altering dietary essential fatty acids on requirements for non-steroidal anti-inflammatory drugs in patients with rheumatoid arthritis, a double blind placebo controlled study. *Ann. Rheum. Dis.* **47:**96–104.
58. Corrocher, R., S. Ferrari, M. De Gironcoli, A. Bassi, O. Olivieri, P. Guarini, A. M. Stanzial, A. L. Barba, and L. Grigolini. 1989. Effects of fish-oil supplementation on erythrocyte lipid pattern, malondialdehyde production and glutathion-peroxidase activity in psoriasis. *Clin. Chim. Acta* **179:**121–132.
59. Schena, D., G. C. Chieregato, M. De Gironcoli, D. Girelli, O. Olivieri, A. M. Stanzial, R. Corrocher, A. Bassi, S. Ferrari, P. Perazzoli, P. Guarini, and A. Barba. 1989. Increased erythrocyte membrane arachidonate and platelet malondialdehyde (MDA) production in psoriasis: normalization after fish-oil. Acta Dermatologica Venereologica (Stockholm) 146 (suppl.), 42–44.
60. Burton, J. L. 1989. Dietary fatty acids and inflammatory skin disease. *Lancet* **i:**27–31.