

Inhibition of Human Polymorphonuclear Leukocyte Function by Components of Human Colostrum and Mature Milk

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To compare the effect of human colostrum (days 1 to 3 postpartum) and mature milk (days 170 ± 24 postpartum) on the function of polymorphonuclear leukocytes (PMNL), Ficoll-Hypaque-separated PMNL from the blood of 60 healthy volunteers were incubated with whole colostrum, colostrum lipid, and colostrum aqueous phase from 30 mothers, or with mature whole milk and its separated components from 30 mothers, and tested for resting and zymosan-stimulated oxidative metabolism, functional activity, and the presence of Fc receptors. Stimulated oxygen consumption, quantitative nitroblue tetrazolium dye reduction, [$1-^{14}\text{C}$]glucose utilization, and Fc receptors were significantly ($P < 0.05$ to $P < 0.001$) less in PMNL exposed to whole human colostrum or colostrum lipid than in non-lipid-exposed cells or cells exposed to the aqueous phase of colostrum. In contrast, PMNL exposed to whole mature milk or to its lipid or aqueous phase caused no significant decrease in any of these parameters when compared to nonexposed cells. In assays of phagocytosis, colostrum PMNL or blood PMNL exposed to colostrum lipid had a significant ($P < 0.001$) decrease in their ability to ingest [*methyl*- ^3H]thymidine-labeled *Staphylococcus aureus* when compared to non-lipid-exposed PMNL. Blood PMNL exposed to lipid from mature milk had no decrease in ability to ingest *S. aureus*. Analysis of total lipid and total and individual fatty acid content revealed a uniform increase in all components in mature milk when compared to colostrum. Lipid or lipid-soluble material present in human colostrum but not mature milk causes inhibition of phagocytosis and respiratory burst-related activities of PMNL.

We have previously demonstrated that human colostrum contains a large number of polymorphonuclear leukocytes (PMNL) and that these cells are defective in respiratory burst and phagocytic activities when compared to PMNL isolated from the blood of healthy donors or postpartum women (5). Morphologically, the most striking feature of PMNL from colostrum is the large amount of ingested and surface-adherent lipid as shown by oil red O staining and transmission electron microscopy (5). The purpose of this study was to determine whether defects in human PMNL from blood could be induced by acellular whole colostrum and mature milk or by their lipid and aqueous components.

MATERIALS AND METHODS

Collection. As part of a prospective study of the role of human milk in infant nutrition and health, 30 mothers were enrolled at our university hospital before delivery. Colostrum (days 2 to 4 postpartum) and mature milk (days 170 ± 24 postpartum) samples were collected with a breast pump (type SM B; Egnell,

Cary, Ill.). All specimens were obtained between 8 and 10 a.m., before nursing. None of the mothers had fever or evidence of mastitis. The study was approved by the Committee for the Protection of Human Subjects of the University of Texas Health Science Center at Houston.

Separation of PMNL from human blood. Each time colostrum was obtained, human leukocytes were isolated from 90 ml of venous blood (10 U of heparin per ml of blood) from healthy volunteers by dextran sedimentation as previously described (10). For each experiment, PMNL from the blood of healthy adults served as controls. After sedimentation, the leukocyte-rich plasma phase was centrifuged for 10 min at $400 \times g$. The supernatant was discarded, and the cell pellet was resuspended in modified Hanks balanced salt solution (HBSS) (pH 7.4) containing gelatin in a final concentration of 0.1%. This solution was layered over Ficoll-Paque (Pharmacia Fine Chemicals, Uppsala, Sweden) and centrifuged at $600 \times g$ for 30 min. The supernatant was decanted, and the remaining pellet was washed once with modified HBSS and centrifuged for 3 min at $300 \times g$. The supernatant was discarded, and the erythrocytes were removed by hypotonic lysis for 45 s in 20 ml of cold deionized water; isotonicity was restored by the addition of 20 ml of 1.7% NaCl

TABLE 1. Characterization of human colostrum and mature milk by volume and PMNL content

Days postpartum	No. of samples	Vol (ml)	Cells $\times 10^4$ /ml	PMNL $\times 10^4$ /ml
2-4	35	21 \pm 4	156 \pm 80	87.4 \pm 10
170 \pm 24	12 ^a	70 \pm 10	2.7 \pm 1.0	0.61 \pm 0.02

^a An additional 24 samples had no cells and were not averaged into these data.

solution. The cells were centrifuged and resuspended in modified HBSS to give a final concentration of 10^7 PMNL per ml. Differential counts showed $99 \pm 1\%$ (mean \pm standard deviation) PMNL. Plastic ware or siliconized glassware was used in all experiments.

Cells were counted with a ZBI Coulter Counter (Coulter Electronics, Hialeah, Fla.). Slides for differential cell counts were prepared by use of a cytocentrifuge (Shandon Southern Products, Ltd., Cheshire, England), and differential cell counts were performed by fluorescent microscopy (Zeiss; Schares Instrument Corp., Houston, Tex.) after supravital staining with acridine orange (4) and by observation under light microscopy after Wright staining. Several specimens were prepared for observation by electron microscopy. Cell viability was determined by trypan blue exclusion after cells were separated from whole blood and again after centrifugation of the PMNL after exposure to milk or milk components.

Fractionation of human milk specimens. The volume of each human milk specimen was measured after it was placed in a sterile plastic container. A crematocrit was performed to determine the percent of lipid present, and then penicillin (15 U/ml) and streptomycin (15 μ g/ml) (GIBCO Laboratories, Grand Island, N.Y.) were added. Our previous studies have demonstrated no effect on PMNL function by these antibiotics (6). The human milk specimen was then centrifuged at $600 \times g$ for 15 to 20 min. The lipid layer was removed by aspiration, and any remaining lipid was removed with cotton swabs. The aqueous portion was pipetted into a separate tube. The aqueous fraction was then centrifuged at $14,000 \times g$ for 20 min, and any remaining lipid was removed.

Effect of colostrum and mature milk on PMNL separated from blood. Purified PMNL were divided and mixed separately for 30 min at 37°C with saline, acellular colostrum, acellular mature milk, and lipid and aqueous fractions from colostrum and mature milk. The mixtures were centrifuged at $600 \times g$, and the cells were washed with HBSS and then resuspended in HBSS. The metabolic and functional activities of these cells were determined.

HMPS activity. Hexose monophosphate shunt (HMPS) activity of PMNL isolated from human blood and human milk was determined by measuring the production of $^{14}\text{CO}_2$ in the oxidation of [$1\text{-}^{14}\text{C}$]glucose (specific activity, 3.0 mCi/mmol; New England Nuclear Corp., Boston, Mass.) in resting and zymosan-stimulated (16 particles per cell) cells. Each reaction mixture contained 10^6 PMNL. The $^{14}\text{CO}_2$ liberated during the course of the incubation was collected and quantitated as previously described (5). Three controls with no cells were included in each experiment, and the background counts obtained from controls were subtracted from experimental determinations. Unstimulated and stimulated cells were assayed in triplicate.

Results are reported as counts per minute per 10^6 PMNL after incubation for 30 min.

Oxygen consumption. Oxygen consumption was determined with a YSI model no. 53 oxygen monitor (Yellow Springs Instrument Co., Yellow Springs, Ohio) attached to an Omniscrite chart recorder (Houston Instruments, Houston, Tex.). A 3-ml volume consisting of 2.15 ml of HBSS (1.9 ml in chambers containing zymosan) (pH 7.4), 0.05 ml of 20 mM KCN, 0.3 ml of serum, 0.25 ml of zymosan (30 particles per PMNL in chambers designated as stimulated), and 0.5 ml of PMNL solution (10^7 cells per ml) was placed into a water-jacketed plastic well at 37°C . The cell suspensions were stirred continuously, and the oxygen consumed in both the unstimulated and stimulated chambers was measured simultaneously. Readings were initiated after a 5-min equilibration period. Results are expressed as microliters of oxygen consumed by 5×10^6 PMNL in 30 min.

Measurement of NBT reduction. Nitroblue tetrazolium dye (NBT) reduction by PMNL was quantitated by a modification of the method of Baehner and Nathan (1). To polypropylene tubes (72 by 75 mm), 0.45 ml (0.4 ml in tubes containing zymosan) of HBSS, 0.05 ml of 20 mM KCN, 0.4 ml of 1% NBT in 0.85% NaCl, and 0.05 ml of zymosan (16 particles per PMNL in tubes designated as stimulated) were added. The tubes were preincubated in a shaking water bath at 37°C for 15 min; then a sample containing 2.5×10^6 PMNL was added to each tube. After 15 min, the reaction was stopped by the addition of 2 ml of 0.5 N HCl. The mixture was transferred to glass tubes (13 by 100 mm) and centrifuged at $2,000 \times g$ for 10 min at 4°C . The supernatant was discarded, and 1 ml of pyridine was added to the pellet. The tube was placed in a boiling water bath under an exhaust hood for 10 min and then centrifuged for 15 min at $2,000 \times g$. The optical density of the pyridine extract was determined on a Stasar III spectrophotometer (Gilford Instruments, Oberlin, Ohio) at 515 nm against a pyridine blank. The results are expressed as the change in absorbance after 15 min for 2.5×10^6 PMNL.

Assay of phagocytosis of [$\text{methyl-}^3\text{H}$]thymidine-labeled *Staphylococcus aureus*. The kinetics of attachment and internalization of radiolabeled *S. aureus* were measured by a modification of the method of Verhoef (12). *S. aureus* 502A was labeled with [$\text{methyl-}^3\text{H}$]thymidine (specific activity, 6.7 mCi/mmol; New England Nuclear) by overnight incubation in 10 ml of Mueller-Hinton broth containing 20 μCi of label. On the mornings of the assay, bacteria were centrifuged for 10 min at $1,600 \times g$ and washed twice with sterile phosphate-buffered saline (PBS). After being resuspended in PBS, the concentration of bacteria was adjusted spectrophotometrically and determined exactly by using total CFU. The bacteria were opsonized in 10% human serum for 15 min at 37°C . At the start of

TABLE 2. Metabolic activities of PMNL separated from human blood immediately after incubation with human colostrum, human milk, or their components

Stimulus	HMPS ^a	O ₂ consumption
None (resting)	585 ± 92	2.4 ± 0.8
Zymosan	5,900 ± 269	14.7 ± 1.2
Whole colostrum (days 2-4 postpartum)	1,239 ± 161 ^b	4.2 ± 1.5
Colostrum lipid	1,515 ± 275 ^c	3.4 ± 0.6
Colostrum aqueous	920 ± 120	2.9 ± 0.3
Whole mature milk (170 ± 24 days postpartum)	657.6 ± 97.4	ND ^d
Mature lipid	597.2 ± 70.3	1.5 ± 0.6
Mature aqueous	529 ± 55.0	1.0 ± 0.5

^a Data for HMPS activity are mean ± standard error of the mean (SEM) counts per minute per 10⁶ PMNL. Data for oxygen consumption are mean ± SEM microliters of O₂ consumed per 5 × 10⁶ PMNL in 30 min.

^b *P* < 0.05.

^c *P* < 0.01 compared to resting cells.

^d ND, Not done.

the phagocytic assay, 10⁷ PMNL and opsonized bacteria were mixed at approximately a 10:1 bacteria to PMNL ratio. A 0.2-ml sample of PBS-human milk lipid or aqueous phases was added to separate 1.8-ml phagocytic mixes at the start of the assay, and the mixes were incubated at 37°C on a rotary rack. Four 100-μl samples were removed at 3, 10, and 20 min and placed into duplicate 3-ml solutions containing either cold PBS for determination of total leukocyte-associated counts or into PBS containing 1 μg of lysostaphin (Schwarz/Mann, Orangeburg, N.Y.) per ml for determination of lysostaphin-resistant leukocyte-associated counts. After a 30-min incubation period at 37°C, these samples were centrifuged at 160 × *g* for 5 min to separate leukocyte-associated counts from free counts. The pellets were washed twice with cold PBS, resuspended in 1 ml of water, and added to 10 ml of Scintiverse (Fisher Scientific Co., Pittsburgh, Pa.) for counting in a liquid scintillation counter. At the end of the assay period (20 min), duplicate 100-μl samples were removed from each of the original mixes and centrifuged at 1,600 × *g* for 30 min. These pellets were resuspended in 1 ml of water and counted in 10 ml of Scintiverse to determine total counts in the mix per 100-μl sample. The total bacterial populations that were leukocyte associated (% uptake) and lysostaphin sensitive (% adherent) were calculated at each sampling time:

% uptake =

$$\frac{\text{total leukocyte-associated counts}}{\text{total counts in mix}} \times 100$$

% adherent =

$$1 - \left(\frac{\text{lysostaphin-resistant counts}}{\text{total leukocyte-associated counts}} \right) \times 100$$

Leukocyte Fc receptor analysis. High-affinity Fc receptor analysis was performed as previously described (9) by incubation of PMNL from colostrum and blood with bovine erythrocytes sensitized with immunoglobulin G (IgG)-rich rabbit antibodies to bovine erythrocytes. Pretreatment of PMNL from blood consisted of

mixing equal volumes of PMNL (10⁷/ml) in HBSS with the lipid and aqueous fractions of colostrum for 30 min at 37°C, followed by centrifugation, washing, and resuspension of the cells. Viability was determined by trypan blue exclusion. PMNL that formed rosettes with three or more antibody-sensitized erythrocytes were considered positive for Fc receptors.

Fatty acid and total lipid analysis. Total lipids and fatty acids were determined by a modification of the procedures of Southgate (11) and Read and Sarraf (8). To a 1-ml sample of milk or colostrum was added an internal standard solution containing 1 mg of pentadecanoic acid (C₁₅) and 5 ml of a chloroform-methanol (2:1) solution. After thorough mixing, the mixture was centrifuged and the organic layer was filtered through cotton. The extraction was repeated twice, and the organic layers were combined and dried in a Brinkmann SC/27R sample concentrator. The residue was redissolved in 15 ml of the chloroform-methanol solutions, and this extract was used for the total lipids and fatty acid analyses. For total lipids, a 10-ml portion of the sample extract was pipetted into a weighed sample vial and evaporated in the Brinkmann concentrator. To remove final traces of moisture, the sample was placed in a 110°C oven for 10 min. After cooling, the sample was weighed. By this specific procedure, total lipids can be calculated from the following equation:

Total lipids (g/dl) =

$$\left(\frac{X - 0.67 \text{ mg}}{0.6677 \text{ ml}} \right) \left(\frac{10 \text{ ml} - g}{\text{mg} - \text{dl}} \right)$$

where *X* = weight of lipids in milligrams.

Fatty acids were determined by drying a 3-ml portion of the sample extract in a 50-ml round bottom flask. Methanolic-HCl (25 ml) was added to the flask and brought to boiling. After refluxing the solution for 4 h, the solution was cooled, and a cold saturated solution of NaHCO₃ (10 ml) was added. The mixture was then extracted with hexanes (10 ml), and the organic layer was concentrated to about 0.1 ml. The fatty acids (as their methyl esters) were identified and quantified by gas chromatography performed on a

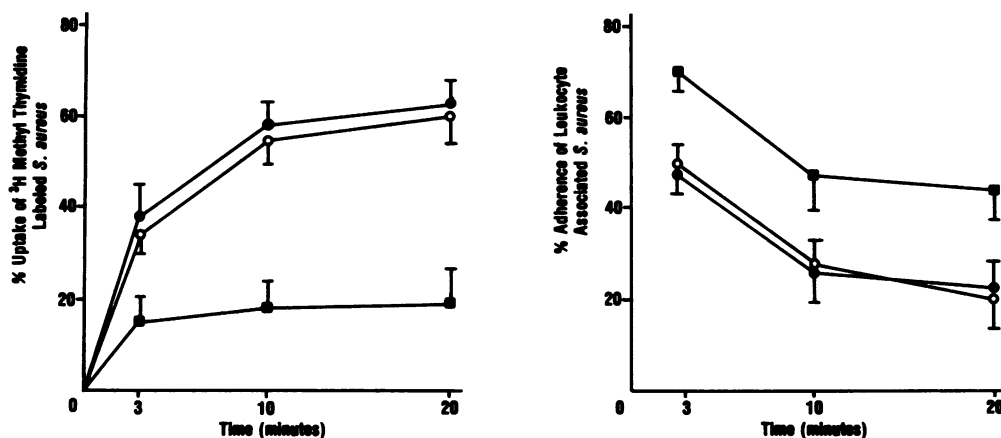


FIG. 1. Uptake and adherence of [methyl-³H]thymidine-labeled *S. aureus* by PMNL from the blood of healthy control volunteers incubated with saline (control, ●), colostrum aqueous (○), or colostrum lipid (■). The percentage of the total bacterial population that was leukocyte associated (left) or leukocyte adherent (right) was calculated after incubation for 3, 10, and 20 min. Data are means \pm standard error of the mean (SEM) of eight experiments. The total uptake of bacteria was significantly ($P < 0.05$ to $P < 0.02$) greater by control PMNL and cells exposed to colostrum aqueous than by PMNL exposed to colostrum lipid at 3, 10, and 20 min. The percentage of adherent bacteria was significantly ($P < 0.05$ to $P < 0.03$) lower at 3, 10, and 20 min by control PMNL and cells preincubated with colostrum aqueous than by PMNL preincubated with colostrum lipid.

Hewlett-Packard 5700 gas chromatograph equipped with a flame ionization detector, digital integrator, and a 6-ft (1.83-m) glass column packed with 10% Silar 5CP on 80/100 Gas Chrom Q (Applied Science Laboratories, State College, Pa.). The analysis was performed by temperature programming the column from 110 to 260°C at 8°C/min. Injector and detector temperatures were 250 and 300°C, respectively. Quantitative values were determined by comparing the ratios of the areas of the gas chromatography peaks for the endogenous fatty acids to that of the internal standard with values obtained from standard curves generated by the method of standard addition.

Statistical analysis. All results are expressed as mean \pm standard error. The statistical significance of differences between groups was determined by using paired *t*-test analysis.

RESULTS

Cells counts and morphology. Table 1 characterizes the volume collected and the number of cells and PMNL isolated at one collection time from each mother on days 2 to 4 postpartum (colostrum) and days 170 \pm 24 postpartum (mature milk). All colostrum specimens had cells for a

TABLE 3. Zymosan-stimulated metabolic activities of PMNL from blood after a 30-min preincubation with human milk or human milk components

Preincubation	HMPS (<i>n</i> = 16) ^a	O ₂ consumption (<i>n</i> = 4) ^b	NBT reduction (<i>n</i> = 12) ^c
None	585 \pm 92	2.4 \pm 0.8	0.02 \pm 0.002
Saline	5,900 \pm 269	14.7 \pm 1.2	0.227 \pm 0.018
Whole colostrum	3,875 \pm 951 ^d	12.0 \pm 1.0	0.118 \pm 0.038 ^e
Colostrum lipid	3,978 \pm 404 ^d	8.6 \pm 1.8 ^d	0.118 \pm 0.012 ^e
Colostrum aqueous	6,052 \pm 491	17.5 \pm 2.9	0.255 \pm 0.039
Saline	4,061.9 \pm 832.4	11.2	0.263 \pm 0.049
Whole mature milk	4,351.4 \pm 843.9	ND ^f	0.252 \pm 0.046
Mature lipid	4,101.6 \pm 789.6	12.6	0.250 \pm 0.040
Mature aqueous	4,454.0 \pm 890.4	11.8	0.258 \pm 0.048

^a Data are mean \pm SEM counts per minute per 10⁶ PMNL.

^b Data are mean \pm SEM microliters of O₂ consumed per 5 \times 10⁶ PMNL in 30 min.

^c Data are mean \pm SEM absorbance at 515 nm per 2.5 \times 10⁶ PMNL.

^d $P < 0.01$.

^e $P < 0.05$ compared to PMNL exposed to zymosan.

^f ND, Not done.

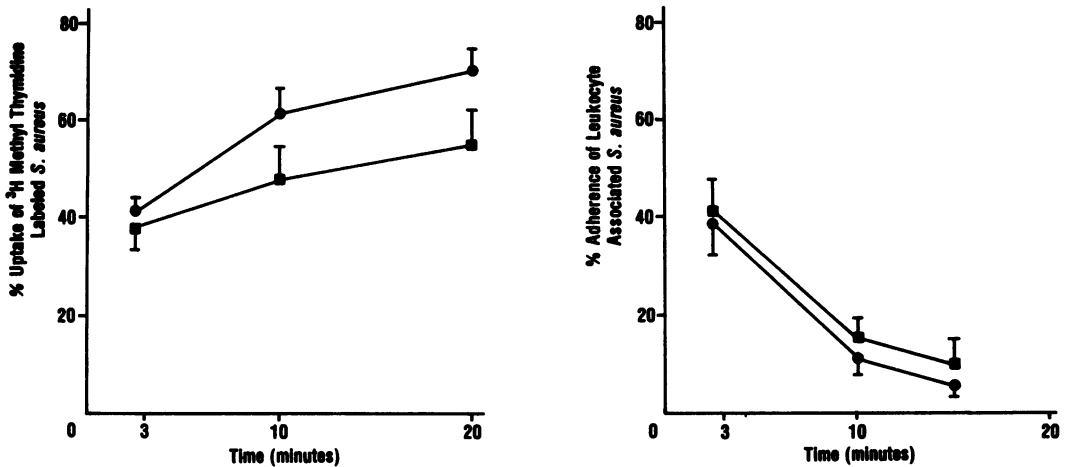


FIG. 2. Uptake and adherence of [methyl-³H]thymidine-labeled *S. aureus* by PMNL from the blood of healthy control volunteers incubated with saline (control, ●) or mature milk lipid (■). The percentage of the total bacterial population that was leukocyte associated (left) or leukocyte adherent (right) were calculated after incubation for 3, 10, and 20 min. Data are means \pm SEM of eight experiments. There was a significant ($P < 0.01$) decrease in the uptake of *S. aureus* in 10 and 20 min by PMNL exposed to mature milk lipid when compared to control cells exposed to saline. There was no significant difference in the adherence of bacteria to control PMNL or to cells exposed to mature milk lipid.

mean of $156 \pm 80 \times 10^4$ cells per ml of milk collected. A mean of 56% of these cells was PMNL, or $87.4 \pm 10 \times 10^4$ PMNL per ml. Of the 36 mature milk specimens collected, 12 had cells present. Of these 12 specimens, a mean of $2.7 \pm 1.0 \times 10^4$ cells per ml were present, with a mean PMNL concentration of 23% or $0.61 \pm 0.02 \times 10^4$ PMNL per ml. The remainder of the cells were macrophages or, to a lesser extent, lymphocytes. Four cell specimens from colostrum observed by electron microscopy showed ingested fat globules within the cytoplasm.

PMNL activity immediately after incubation with human milk or human milk components. Table 2 shows the metabolic activities of PMNL isolated from blood immediately after incubation with human colostrum and mature milk and their lipid and aqueous components. Exposure of PMNL to zymosan caused a significant ($P < 0.001$) increase in metabolic activity over resting cells as measured by HMPS activity ($5,900 \pm 269$) and oxygen consumption (14.7 ± 1.2). When human colostrum or its components were added to PMNL as possible stimulants, a significant ($P < 0.01$) increase in HMPS activity was produced by whole colostrum ($1,239 \pm 161$) and colostrum lipid ($1,515 \pm 275$) compared to resting cells (585 ± 92). There was no significant difference in PMNL exposed to colostrum aqueous, whole mature milk, mature lipid, or mature aqueous compared to resting cells.

Figure 1 shows the uptake and adherence of radiolabeled *S. aureus* by PMNL isolated from the blood of healthy volunteers and incubated

with saline (control), colostrum aqueous, and colostrum lipid. The total uptake (Fig. 1, left) of bacteria by PMNL was significantly ($P < 0.05$ to $P < 0.02$) greater by control PMNL and cells exposed to colostrum aqueous than by PMNL exposed to colostrum lipid at 3, 10, and 20 min. The percentage of adherent bacteria (Fig. 1, right) was significantly ($P < 0.05$ to $P < 0.03$) lower at 3, 10, and 20 min by control PMNL and cells preincubated with aqueous than by PMNL preincubated with colostrum lipid.

Preincubation of PMNL with human milk. Table 3 shows the zymosan-stimulated metabolic

TABLE 4. Fc receptors in human PMNL from blood, colostrum, and blood PMNL exposed to human colostrum and whole mature milk

PMNL	Antibovine erythrocytes ^a	
	Lot 1	Lot 2
Control PMNL	58.6 ± 9.0	86.5 ± 2.2
Colostrum PMNL	17.8 ± 5.9^b	
Control PMNL preincubated with		
Whole colostrum	19.6 ± 5.0^b	
Colostrum lipid	21.8 ± 8.0^b	
Colostrum aqueous	56 ± 8.3	
Whole mature milk		83.8 ± 2.1
Mature lipid		85.1 ± 2.1
Mature whole milk		84.0 ± 1.0

^a Data represent means \pm SEM.

^b $P < 0.05$ compared to control PMNL.

activities of PMNL from blood after a 30-min preincubation with human milk or human milk components. The amount of glucose consumed (counts per minute per 10⁶ PMNL) by PMNL exposed to saline (5,900 ± 269) was significantly (*P* < 0.01) greater than that consumed by PMNL exposed to whole colostrum (3,875 ± 951) or to colostrum lipid (3,978 ± 404). Oxygen consumption was significantly (*P* < 0.01) greater in cells exposed to saline than in cells exposed to colostrum lipid (14.7 ± 1.2 versus 8.6 ± 1.8 μl/5 × 10⁶ PMNL in 30 min). NBT reduction (absorbance at 515 nm by 2.5 × 10⁶ PMNL) was significantly (*P* < 0.05) greater in PMNL exposed to saline (0.227 ± 0.18) than in cells exposed to either whole colostrum (0.118 ± 0.038) or colostrum lipid (0.118 ± 0.012). There was no significant difference in HMPS activity, O₂ consumption, or NBT dye reduction in cells exposed to colostrum aqueous, whole mature milk, or mature lipid or aqueous.

Figure 2 shows uptake and adherence of bacteria by control PMNL and cells exposed to the lipid phase of mature human milk. There was a significant (*P* < 0.01) decrease in uptake of *S. aureus* at 10 and 20 min by PMNL exposed to mature milk lipid compared to control cells exposed to saline. This decrease in uptake was not as marked as that seen in PMNL exposed to colostrum lipid. There was no significant difference in adherence of bacteria to control PMNL or to cells exposed to mature milk lipid.

Trypan blue dye exclusion testing showed PMNL to be 95 to 100% viable after separation from blood and again after centrifugation, after they had been exposed to milk or its components, just before metabolic or functional testing.

Fc receptors. Table 4 shows the Fc receptor expression by PMNL from human blood, PMNL from human colostrum, and human blood PMNL preincubated with whole colostrum, whole mature milk, and their components. There was a significant (*P* < 0.05) decrease in Fc receptors on colostrum cells (17.8 ± 5.9%), blood PMNL preincubated with whole colostrum (19.6 ± 5.0%), and colostrum lipid (21.8 ± 8.0%) compared to PMNL exposed to saline (58.6 ± 9%, control). In other sets of experiments with a different lot number of antibody, Fc receptors were not significantly different in PMNL exposed to mature milk or its components compared to control PMNL (86.5 ± 2.2%).

Crematocrit and lipid and fatty acid analysis of human milk. The mean crematocrit value was 4.4 ± 0.3% for colostrum specimens (*n* = 30) compared to 5.7 ± 0.6% for mature milk samples (*n* = 29). Table 5 shows the total lipid and fatty acid content of human colostrum and mature milk specimens used in the assays of PMNL

TABLE 5. Total lipid and total fatty acid content of human colostrum and mature milk^a

Days postpartum	Total lipid (g/dl)	Total fatty acid (mg/ml)	Individual fatty acid content (mg/ml (% of total fatty acid))									
			C ₆	C ₁₀	C ₁₂	C ₁₄	C ₁₆	C _{16:1}	C ₁₈	C _{18:1}	C _{18:2}	
2 (<i>n</i> = 5)	1.04 ± 0.3	11.2 ± 5.1	0	0.009 ± 0.02 (0.04)	0.03 ± 0.01 (0.80)	0.56 ± 0.23 (4.4)	2.9 ± 1.3 (26.3)	0.44 ± 0.2 (3.9)	1.18 ± 0.47 (11.0)	4.17 ± 2.0 (36.5)	1.80 ± 0.71 (17)	
3-5 (<i>n</i> = 30)	2.04 ± 0.2	18.4 ± 2.0	0	0.02 ± 0.01 (0.08)	0.3 ± 0.1 (1.6)	0.8 ± 0.1 (4.6)	4.4 ± 0.5 (23.1)	0.8 ± 0.1 (4.1)	1.7 ± 0.2 (9.3)	7.6 ± 0.8 (41.1)	2.7 ± 0.4 (15.5)	
170 ± 24 (<i>n</i> = 29)	4.1 ± 0.3	23.6 ± 2.0	0.003 ± 0.002 (0.01)	0.2 ± 0.03 (0.9)	1.3 ± 0.1 (5.5)	1.6 ± 0.2 (7.2)	4.9 ± 0.4 (21.0)	0.8 ± 0.1 (3.5)	1.7 ± 0.2 (7.2)	9.2 ± 0.9 (38.4)	3.9 ± 0.4 (16.3)	

^a Data expressed as means ± SEM.

function grouped by days postpartum. Total lipid, total fatty acid, and individual fatty acid contents all increased with postpartum time. The total lipid content was almost twice as high (4.1 ± 0.3 g/dl) in mature milk specimens than in specimens from mothers obtained on days 3 to 5 postpartum (2.04 ± 0.2 g/dl). Likewise, total fatty acid content of mature milk (23.6 ± 2.0 mg/ml) was over twice that of colostrum obtained on day 2 (11.2 ± 5.1 mg/ml). The individual fatty acid contents of C₈, C₁₀, C₁₂, C₁₄, C₁₆, C_{16:1}, C₁₈, C_{18:1}, and C_{18:2} were uniformly higher in mature milk compared to colostrum.

DISCUSSION

Fresh human milk contains a variety of substances, including host defense factors such as immunoglobulins, mainly of the IgA class, components of complement, nonspecific immune factors, such as lactoferrin and lysozyme, and viable leukocytes and products of cell breakdown (3). These substances are present either to protect the infant against bacterial and viral infections during an immunologically deficient period of life or to function as defense mechanisms for the mammary gland. The role that each of these factors may play in protecting the suckling neonate against gastrointestinal or respiratory tract infections is not known.

It has been demonstrated that human colostrum contains a large number and variety of cells, the majority of which are PMNL (5). The total number of cells per milliliter and the percentage of PMNL dramatically decrease in mature milk compared to colostrum. In our study, 24 of the 36 (67%) mothers from whom mature milk was collected (mean postpartum day 170) had no cells detectable in their milk specimens. We have previously demonstrated that milk PMNL in human colostrum were significantly less active metabolically and functionally than PMNL isolated from blood. These defects in oxidative metabolism of human colostrum PMNL appeared to result from ingestion of colostrum lipid with altered membrane characteristics, such as Fc receptor availability, and altered metabolic reserves.

The present study demonstrates that human colostrum and the lipid phase of colostrum stimulate a respiratory burst activity of separated blood PMNL, and that preincubation of these cells with acellular colostrum or colostrum lipid results in production of defects similar to those seen in PMNL from human colostrum. In addition, these cells could not internalize radiolabeled *S. aureus* as efficiently as separated blood PMNL not exposed to colostrum. Human acellular whole mature milk did not produce these defects in separated blood PMNL. We demonstrated differences in the lipid content of human

colostrum compared to that of mature milk. Our data suggest that the lipids of lipid-soluble component of colostrum stimulate metabolic activity of PMNL, whereas the lipid component of mature milk does not. The lipid in colostrum is virtually all fatty acid, whereas fatty acid accounts for only half the total lipid in mature milk. This high fatty acid concentration as a fraction of total lipid content of colostrum may be important in this modulation of PMNL functions.

It is unclear what advantage there might be in depressing the usual functional activities of the colostrum PMNL. It seems reasonable to predict that PMNL could serve as effector cells capable of phagocytosis and killing of invading bacteria and as secretory cells capable of releasing enzymes, singlet oxygen, superoxide anions, and hydroxyl radicals which could be lethal to invading bacteria. However, given the defects of colostrum PMNL function, these cells probably do not act as effector cells for the neonate. They could benefit the neonate by transporting and releasing IgA in the gastrointestinal tract after physiological changes or neutrophil degranulation (2, 7, 13). Our data do not provide evidence for a role of colostrum PMNL in protecting the suckling neonate.

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