Human Colostrum Has Anti-inflammatory Activity in a Rat Subcutaneous Air Pouch Model of Inflammation

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ABSTRACT. An animal model was used to examine the effect of human colostrum on an acute inflammatory process *in vivo*. Subcutaneous air pouches on the backs of outbred rats were injected with carrageenan as an inflammatory challenge, normal saline, pooled aqueous human colostrum, or carrageenan plus colostrum concurrently. Oral dexamethasone or indomethacin was administered to some animals before and during challenge as anti-inflammatory agents. Polymorphonuclear leukocyte (PMN) counts in pouch fluid were determined 6 h postchallenge. Carrageenan challenge resulted in a significant acute inflammatory response [48.8 ± 4.9 × 10^6 PMN/pouch (mean ± SEM, n = 46)] compared with normal saline controls [0.9 ± 0.2 × 10^6 (n = 31, p < 0.001 versus carrageenan)] or with colostrum [4.3 ± 0.8 × 10^6 PMN/pouch (n = 11, p < 0.001 versus carrageenan)]. The concurrent injection of colostrum plus carrageenan challenge significantly reduced the PMN response compared with carrageenan alone [18.8 ± 2.9 versus 48.8 ± 4.9 × 10^6 PMN/pouch (carrageenan plus colostrum versus carrageenan, n = 41 versus 46, p < 0.001)]. This degree of suppression of PMN influx was not significantly different from that seen with indomethacin treatment but was significantly more than that seen with dexamethasone treatment. The decreases in PMN counts observed most likely reflect suppression of the acute inflammatory response because a significant amount of PMN lysis in colostrum was not observed *in vitro* and the accumulation of PMN granule contents was not seen in pouch fluid from colostrum-treated animals *in vivo*. These data directly demonstrate for the first time that human colostrum has a biologically significant effect on the inflammatory process *in vivo*. (*Pediatr Res* 34: 208–212, 1993)

Abbreviations

Carr, 0.2% solution of carrageenan in PBS
Col, pooled aqueous human colostrum
PMN, polymorphonuclear leukocyte
Carr + Col, concurrent challenge with Carr and Col
HBSS, Hanks' balanced salt solution without calcium or magnesium

Human colostrum contains an average of 1.5 × 10^9 PMN/L over the first 4 d of lactation and nearly twice this number of

total leukocytes (1, 2). Human newborns, assuming an average oral intake of about 0.1 L/kg/d over this period, will consume more than 2 billion PMN and as many mononuclear cells. These cells may offer the newborn some benefit by augmenting its immature host defenses. However, leukocytes in colostrum and breast milk function poorly compared with circulating white blood cells (3–9), making it difficult to understand how they could augment host defenses. However, if the primary role of these maternal cells were to protect a mother's breasts as this new secretory pathway was established, then these cells may offer little immunologic benefit to the infant directly. If the PMN in colostrum continued to function normally, they might pose a threat to the infant's gastrointestinal tract mucosa because they could mediate inflammation and local tissue injury (10–13).

We hypothesize that antileukocyte activity in colostrum is needed to protect the newborn in this unique physiologic setting of mucosal exposure to exogenous PMN and that this antileukocyte activity results in a biologically significant anti-inflammatory effect. *In vitro* data directly demonstrate that human colostrum and milk have antileukocyte activity. Human milk and colostrum inhibit many peripheral blood (3–5, 14–16) and milk-derived leukocyte (3–9) functions *in vitro*. Human colostrum can protect cell monolayers from injury by activated PMN, and this protection is probably mediated by several antioxidant components (17, 18).

Inhibition of PMN activity by colostrum might be expected to result in suppression of acute inflammatory responses. Acute inflammation is a dynamic process involving tissue and circulating leukocytes, and the best evidence of a biologically significant anti-inflammatory effect is based on data from epidemiologic or *in vivo* experimental data. An anti-inflammatory effect of human colostrum or breast milk *in vivo* has not been described; however, in humans, several epidemiologic phenomena suggest such an effect. Common respiratory and gastrointestinal infections are less frequent and severe in infants who are breast-fed (19, 20). This protection may be mediated by specific anti-infective factors in milk, such as secretory immunoglobulin, lysozyme, lactoferrin, and oligosaccharides (21, 22). However, these infections represent the most common stimulus for inflammation in this population, and an anti-inflammatory effect of breast-feeding might be indistinguishable from an anti-infective effect. Enteric infection with pathogens that typically produce intestinal inflammation, such as *Shigella*, are commonly noninflammatory in breast-fed infants (23, 24). Necrotizing enterocolitis also has been shown to be significantly less likely to occur in breast-fed infants (25). Although indirect, these associations suggest the possibility of anti-inflammatory activity in breast milk. Reviewing the available data, other investigators have found support for the hypothesis that breast milk is anti-inflammatory (19, 20).

A well-described animal model of acute inflammation is used to examine whether human colostrum can alter the cascade of

events in acute inflammation leading to PMN accumulation locally and to compare the magnitude of this effect with those of known potent anti-inflammatory agents.

MATERIALS AND METHODS

Collection of colostrum. Colostrum used for these studies was the lactational product obtained from inpatient mothers from 1 to 4 d postpartum after informed consent was obtained. No mothers had symptoms of mastitis. Mothers were not excluded because of premature delivery. The entire contents of one breast were obtained aseptically using an electric breast pump (Egnell, Cary, IL). Colostrum was immediately chilled and transported to the laboratory on ice. Specimens were then promptly separated by ultracentrifugation (LS-70, Beckman Instruments, Inc., Palo Alto, CA) at 81 000 × g for 30 min at 4°C. The aqueous fraction was collected by puncturing the centrifuge tube with an 18-gauge needle and draining this fraction dropwise into a sterile plastic container. The cream layer and cell pellet were discarded, and the samples were frozen at −70°C until used. Samples of aqueous colostrum from at least six donors were thawed and pooled. Each pool of colostrum was filter-sterilized (0.45 µm) and stored at −70°C until used. Seven pools were used for these experiments. Individual donor colostrum was prepared similarly but not pooled.

Challenge materials. All challenge materials were prepared aseptically. PBS was prepared with 10 × PBS (Whittaker, M. A. Bioproducts, Walkersville, MD) and charcoal-filtered, nonsterile water, then autoclaved. Normal saline for injection (Baxter Healthcare Corp., Deerfield, IL) was aspirated from a sealed bag immediately before use. Carrageenan (type IV, Sigma Chemical Co., St. Louis, MO) was dissolved in PBS at 2 g/L using vigorous agitation and autoclaving and was stored at 4°C. Carrageenan solutions (Carr) were agitated and warmed to 20°C before use. Carrageenan solutions with visible precipitate were reautoclaved and cooled before use.

Rat model of acute inflammation. The protocol described was approved by the Animal Welfare Committee of the University of Texas Health Science Center Houston. Female outbred Sprague-Dawley rats (Sasco, Omaha, NE), weighing 125-150 g, were housed six to a cage in a temperature- and ventilation-controlled environment and given standard rat food and water *ad libitum.* On d 0 after inducing CO_2 necrosis, a 20-mL subcutaneous air pouch was formed in the midline of the back of each rat by injection of air, and pouches were refilled with 10 mL of air in a similar manner. Tails were marked for identification on d 3. On d 6, 5 mL of a challenge material, carrageenan, normal saline, or colostrum, were injected into the pouch. The effect of colostrum on inflammation was tested by injecting 5 mL of colostrum into the pouch immediately after carrageenan injection (Carr + Col). Animals were observed periodically for signs of local or systemic distress. Six h later, rats were killed using CO_2 overdose and pouch fluid was promptly collected. Pouches were injected with 3 mL of heparinized PBS (10^6 U/L) and vigorously kneaded for 15 s. Skin overlying the pouch was shaved with electric clippers (Oster, no. 40 blade, Milwaukee, WI), and the pouch was opened with sharp dissection. Pouch fluid was drained and expressed into a plastic cup. The volume of fluid obtained was measured, and samples of pouch fluid were aliquoted. One aliquot of pouch fluid was diluted 1:10 with 10% formalin in PBS, and cell number determined using a hemocytometer. Differential cell counts were performed using cytocentrifuge-prepared slides (Shandon, Cheshire, England) of a fresh aliquot of pouch fluid, stained with a modified Wright's stain (Diff-Quik, Baxter, Miami, FL). In some cases, an aliquot of fluid was frozen at −70°C for assay of PMN granule contents at a later time. Scrapings and histologic examination of the pouch wall after pouch fluid collection showed no adherent white blood cells (wbc) on the pouch wall surface. The number of PMN per pouch was calculated as follows:

$$\text{PMN/pouch} (\times 10^6) = \text{Volume (mL)}$$
$$\times \text{cell number} (\times 10^6/\text{mL}) \times \text{wbc/cells} \times \text{PMN/wbc}$$

Anti-inflammatory agents. Dexamethasone (Decadron elixir) and indomethacin (Indocin oral suspension) were gifts of Merck Sharp & Dohme (West Point, PA). Either agent, dexamethasone (10 mg/L) or indomethacin (100 mg/L), was added to the animals' water supply for 3 d before and during challenge with carrageenan.

Cell stability in colostrum. Human PMN were purified from heparinized whole blood of healthy adult volunteers by centrifugation over a Ficoll-Hypaque gradient, dextran sedimentation, and hypotonic lysis (26) and resuspended at 10^{11} cells/L in HBSS. Autologous plasma diluted with one volume of HBSS was collected from the tops of the gradients after centrifugation and was filter-sterilized (0.45 µm). Thawed pooled aqueous colostrum was similarly diluted and filtered. Triplicate samples prepared with 0.2 mL of PMN suspension and 3 mL of colostrum or autologous plasma were incubated in 5% moist CO_2 at 37°C. Aliquots were taken at various time points, and absolute and differential cell counts were performed as for pouch fluid.

Leukocyte granule marker assays. Aliquots of frozen pouch fluid were thawed and sonicated (W185, Heat Systems-Ultrasonic, Plainview, NY) at 40 W for 30 s on ice after the addition of equal volumes HBSS-0.1% Triton X-100. Lysate supernatants were collected after centrifugation at 15 000 × g for 20 min at 4°C and stored at −70°C until used. β-Glucuronidase activity was determined as previously described (27) by incubating 0.05 mL of lysate supernatant with 0.05 mL 2 mM 4-methylumbelliferyl-β-D-glucuronide (Sigma) in 100 mM acetate buffer (pH 3.5)-0.1% Triton X-100 at 37°C for 60 min, and then stopping the reaction with 2.4 mL of 50 mM glycine buffer (pH 10.4)-5 mM EDTA. Fluorescence of the samples was measured in a fluorescence spectrophotometer (650-10S, Perkin-Elmer, Oak Brook, IL), using 365-nm excitation and 450-nm emission wavelengths. The correlation between fluorescence and PMN number was excellent ($r = 0.997$) over a range of 0.1 to 35 × 10^9 purified human PMN/L in HBSS.

Fractionation of colostrum. A pool of aqueous colostrum was thawed and diluted into two portions; one portion was fractionated whereas the other was not. The fractionated portion was separated by molecular size filtration into three fractions containing components with >100 000-D, 100 000- to 10 000-D, and <10 000-D molecular mass ranges. This portion was partially passed through a filter retaining molecules > 100 000 D (YM100, Diaflow ultrafilters, Amicon, Beverly, MA) under nitrogen at 4°C with constant stirring, and the filtrate was collected. The retentate was washed once with one volume of normal saline when its volume reached 10% of original, and then this retentate was rediluted to the original starting volume with normal saline to produce the >100 000-D fraction. The YM100 filtrate was refiltered and washed using a 10 000-D cutoff filter (YM10), and the filtrate collected. The 100 000- to 10 000-D fraction was prepared from the washed YM10 retentate in the manner described previously. The YM10 filtrate was lyophilized (Cryolyzer, New Brunswick Scientific Co., Inc., New Brunswick, NJ) and reconstituted with normal saline to the original sample volume to produce the <10 000-D fraction. The unfractionated portion of colostrum and the prepared fractions were stored together during this procedure and were frozen together at −70°C on completion.

Data analysis. Unless otherwise noted, data are expressed as means ± SEM, and differences among groups were compared using t test, with $p < 0.05$ taken as a significant difference. Values of n represent the numbers of animals used to examine each experimental condition. Linear regression analysis was used to compare PMN granule marker activity and PMN counts.

RESULTS

Response to challenge materials in pouch fluid. Combined data from all experiments showing pouch fluid characteristics for animals challenged with normal saline, colostrum, carrageenan, and carrageenan plus colostrum are shown in Table 1. Animals with pouches injected with normal saline had no visible change in behavior, no signs of local inflammation in the skin overlying the pouch, and little PMN influx into pouch fluid. Pouch fluid cell counts were $0.9 \pm 0.2 \times 10^6$ PMN/pouch for normal saline ($n = 31$) over many experiments. Injection of colostrum resulted in no changes in rat behavior, no signs of local inflammation, and a modest PMN influx. Pouch fluid PMN counts were $4.3 \pm 0.8 \times 10^6$ PMN/pouch for colostrum ($n = 11$) ($p < 0.001$ for Col *versus* Carr or normal saline). In a single pilot trial, the use of whole or acellular colostrum resulted in more PMN influx (10.1 or 9.8×10^6 PMN/pouch, respectively) than aqueous colostrum (4.5×10^6 PMN/pouch). Injection of either of these cream-containing materials into the pouch resulted in the accumulation of many lipid-laden white blood cells, which made accurate cell counting difficult. Additional experiments were performed only with Col.

Approximately half the animals challenged with carrageenan showed decreased activity or ruffled fur but very few showed warmth or tenderness over the pouch. These animals consistently demonstrated the accumulation of large numbers of PMN in pouch fluid. Pouch fluid PMN counts 6 h after carrageenan challenge were $48.4 \pm 4.9 \times 10^6$ PMN/pouch ($n = 46, p < 0.001$ *versus* normal saline or Col). This significant cellular influx consisted almost exclusively of PMN ($81 \pm 2\%$). No significant differences in PMN responses were seen when comparing carrageenan prepared in PBS or normal saline (data not shown).

Effects of aqueous colostrum on acute inflammation. Injection of colostrum immediately after carrageenan did not perceptibly alter the occurrence of behavioral changes compared with carrageenan-challenged animals. The addition of colostrum to the pouch consistently and significantly suppressed the influx of PMN into pouch fluid in response to carrageenan. Pouch fluid PMN counts were reduced by an average of 61% over the course of many experiments [18.8 ± 2.9 *versus* $48.8 \pm 4.9 \times 10^6$ PMN/pouch (Carr + Col, $n = 41$, *versus* Carr, $n = 46$; $p < 0.001$)].

Relative activities of colostrum and anti-inflammatory agents. Treatment with an anti-inflammatory agent before and during challenge with carrageenan significantly suppressed PMN influx into the pouch. Pouch fluid characteristics for animals concurrently challenged with carrageenan with or without anti-inflammatory agents or colostrum are shown in Table 2. Pouch fluid PMN counts for dexamethasone-treated animals were reduced by an average of 64% [18.9 ± 3.3 *versus* $53.2 \pm 6.6 \times 10^6$ PMN/pouch (Carr + dexamethasone *versus* Carr, $n = 16$ each, $p < 0.001$ *versus* Carr)]. Pouch fluid PMN counts for indomethacin-treated animals were reduced by an average of 79%, $8.7 \pm 3.0 \times 10^6$ PMN/pouch (Carr + indomethacin, $n = 13, p < 0.001$ *versus* Carr). Pouch fluid PMN counts for colostrum-treated animals were reduced by an average of 85%, $8.0 \pm 2.9 \times 10^6$ PMN/

pouch (Carr + Col, $n = 13$, $p < 0.001$ *versus* Carr). Treatment with colostrum or indomethacin was significantly more effective in reducing PMN influx into pouch fluid after carrageenan challenge than dexamethasone ($p < 0.05$).

Variability in carrageenan activity. Six carrageenan preparations were used during the course of these experiments, and variability in the inflammatory activity of these preparations was seen. Pouch fluid PMN counts ranged from $82.7 \pm 9.7 \times 10^6$ PMN/pouch ($n = 9$) for the most active preparation to $13.2 \pm 8.2 \times 10^6$ PMN/pouch ($n = 4$) for the least active preparation. This variation may be related to the poor-solubility of carrageenan in water. Precipitation was seen in the preparation with the least activity, and this precipitate cleared and activity improved after reautoclaving the preparation.

Variability of colostral anti-inflammatory activity. A comparison of PMN responses in paired carrageenan and carrageenan plus colostrum-treated animals was made for each carrageenan preparation used. Colostrum treatment resulted in mean decreases in pouch fluid PMN counts of 22 to 85% in each carrageenan preparation examined, and these differences were significant for three of the six preparations. Three of four preparations with seven or more animals in each carrageenan and colostrum plus carrageenan group produced significant differences, demonstrating that larger groups tended to show significant differences, as expected. Three of four carrageenan preparations with PMN responses of less than 50×10^6 PMN/pouch failed to show significant differences between carrageenan and carrageenan plus colostrum-treated animals, demonstrating that a less inflammatory carrageenan preparation more often failed to demonstrate significant suppression.

Seven pools of aqueous colostrum were used during the course of these experiments. PMN responses among paired carrageenan and carrageenan plus colostrum-treated animals were compared for each colostrum pool used. Six of seven colostrum pools produced mean decreases in pouch fluid PMN counts of 50 to 84%, whereas one pool showed a 10% decrease. Significant suppression of PMN influx was present in four of seven pools.

In a single experiment, aqueous colostrum samples from four individual donors were compared with a colostrum pool prepared from other donors. The pooled colostrum reduced the PMN influx in response to carrageenan by 52%, whereas the individual donor samples reduced the influx by 26, 27, 33, and 74%. These data indicate that anti-inflammatory activity in colostrum may vary considerably among single samples from single donors. The consistent anti-inflammatory activity in several pools of colostrum suggests that pooling six or more individual donor's colostrum samples minimizes the variability among single donor samples.

PMN stability in colostrum. The possibility that lysis of PMN in the presence of colostrum was responsible for the decreased PMN counts observed was evaluated. When human PMN were incubated in either pooled aqueous colostrum or autologous plasma, cell counts decreased from 6.45 to 6.35×10^9 PMN/L in plasma (average of triplicate determinations) and from 7.83 to 6.90×10^9 PMN/L in colostrum over 6 h of incubation. Cell counts at 24 h of incubation were 3.18×10^9 PMN/L in plasma

Table 1. *Acute inflammatory responses to challenge materials and anti-inflammatory effect of Col in rat air pouch model of inflammation*

Pouch fluid	Normal saline ($n = 31$)	Col ($n = 11$)	Carr ($n = 46$)	Carr + Col ($n = 41$)
Volume (mL)	2.6 ± 0.2†	5.7 ± 0.3†	3.5 ± 0.1	7.5 ± 0.3†
Cell count (10^6/mL)	3.0 ± 0.8†	1.6 ± 0.2†	20.0 ± 1.9	5.7 ± 0.7†
WBC/WBC + RBC	0.83 ± 0.05	0.93 ± 0.04	0.91 ± 0.02	0.87 ± 0.03
PMN/WBC	0.23 ± 0.05†	0.50 ± 0.06†	0.81 ± 0.02	0.51 ± 0.04†
PMN/pouch (10^6)	0.9 ± 0.2†	4.3 ± 0.8†	48.8 ± 4.9	18.8 ± 2.9†

* Values are mean ± SEM of data from all experiments on pouch fluid at 6 h postchallenge with normal saline for injection, Col, Carr, or Carr + Col. WBC, white blood cells; RBC, red blood cells.
† $p < 0.001$ *vs* Carr.

Table 2. *Anti-inflammatory effects of Col compared with systemic dexamethasone or indomethacin**

Pouch fluid	Carr ($n = 16$)	Carr + Col ($n = 13$)	Carr + Dex ($n = 16$)	Carr + Ind ($n = 13$)
Volume (mL)	3.4 ± 0.2	7.8 ± 0.7†	1.9 ± 0.2†‡	3.4 ± 0.2‡
Cell count (10^6/mL)	19.5 ± 2.8	2.4 ± 0.5†	13.3 ± 2.4‡	3.7 ± 1.0†
WBC/WBC + RBC	0.98 ± 0.01	0.97 ± 0.02	0.93 ± 0.05	0.97 ± 0.01
PMN/WBC	0.86 ± 0.02	0.42 ± 0.05†	0.90 ± 0.05‡	0.58 ± 0.11§
PMN/pouch (10^6)	53.2 ± 6.6	8.0 ± 2.9†	18.9 ± 3.3†‡	8.7 ± 3.0†

* Values are mean ± SEM of rat air pouch fluid parameters at 6 h postchallenge with Carr alone, or with Carr plus Col, Carr plus systemic dexamethasone (Dex), or Carr plus indomethacin (Ind). WBC, white blood cells; RBC, red blood cells.
† $p < 0.001$ vs Carr.
‡ $p < 0.05$ vs Carr + Col.
§ $p < 0.05$ vs. Carr.

and 6.70×10^9 PMN/L in colostrum. The stability of human PMN in colostrum or serum *in vitro* appears somewhat different, but this difference would not account for the large decreases in PMN counts seen in animals receiving carrageenan plus colostrum.

PMN granule marker assay on pouch fluid. An initial experiment using lysozyme as a PMN granule content marker revealed high levels of lysozyme activity in aqueous colostrum and in colostrum-treated pouch fluid and poor correlation with PMN counts in pouch fluid. Aqueous colostrum contained modest β-glucuronidase activity, and this PMN granule marker correlated well with PMN count in carrageenan and carrageenan plus colostrum-treated pouch fluid. In a single experiment, mean β-glucuronidase activity was 7.6 ± 2.8 fluorescence units per 25 μL (units) ($n = 2$) in normal saline-treated pouch fluid samples, 34.2 ± 1.7 units ($n = 2$, $p = 0.02$ versus normal saline) in colostrum-treated pouch fluid samples, 95.1 ± 14.1 units ($n = 7$) in carrageenan-treated pouch fluid samples, and 54.9 ± 5.4 units ($n = 5$, $p = 0.04$ versus Carr) in carrageenan plus colostrum-treated pouch fluid samples. If lysis of PMN was occurring in colostrum-treated pouches, increasing amounts of PMN granule contents would be seen as cells arrived and were lysed. This action would result in increased β-glucuronidase activity per observed leukocyte in colostrum-treated pouch fluids, independent of any added β-glucuronidase activity due to the colostrum itself. In pouch fluid from carrageenan-challenged animals, the relationship between β-glucuronidase activity and the observed PMN count was 2.28 ± 0.56 units per 10^6 PMN/mL ($r = 0.875$, $n = 7$, $p = 0.01$) using linear regression analysis. In samples from animals challenged with carrageenan plus colostrum, the same relationship was 2.84 ± 0.50 units per 10^9 PMN/L ($r = 0.956$, $n = 5$, $p = 0.01$). The difference between these two slopes was not significant ($p = 0.49$), suggesting that PMN granule contents were not accumulating in carrageenan plus colostrum-treated pouches as more PMN arrived.

Anti-inflammatory activity of molecular sizing fractions of aqueous colostrum. Separation of a pool of aqueous colostrum by molecular sizing filtration yielded three fractions. Treatment with unfractionated colostrum from the same pool decreased PMN influx after carrageenan challenge from 25.7 ± 6.6 × 10^6 PMN/pouch to 2.4 ± 0.3 × 10^6 (Carr + Col, $n = 3$, versus Carr, $n = 4$, $p = 0.03$). Treatment with the colostrum fraction with components > 100 000 D produced a PMN influx of 30.6 ± 3.8 × 10^6 PMN/pouch ($n = 3$, $p = 0.6$ versus Carr). Treatment with the fraction with components from 100 000 to 10 000 D produced a PMN influx of 20.2 ± 0.8 × 10^6 PMN/pouch ($n = 3$, $p = 0.5$ versus Carr). Treatment with the fraction with components < 10 000 D produced a PMN influx of 6.9 ± 4.0 × 10^6 PMN/pouch ($n = 3$, $p = 0.08$ versus Carr). Although the number of animals is small and significant suppressive activity of any fraction cannot be shown, these data suggest that anti-inflammatory activity in colostrum may be segregated to the low molecular weight fraction and that proinflammatory activity may be segregated to the high molecular weight fraction.

DISCUSSION

The rat air pouch model used in these studies is able to contain colostrum, challenge materials, and products of the inflammatory process for prolonged periods in contact with an organized tissue surface *in vivo*. Compared with other rodent models, this model can demonstrate inflammatory responses with good sensitivity (28-30). The model has been used previously to demonstrate the effects of known anti-inflammatory agents (31). Our experiments used observations of PMN numbers in pouch fluid as an end point because this parameter is closely associated with acute inflammation *in vivo* and can be estimated accurately and reproducibly in the laboratory. Our conclusions would not be altered if leukocyte influx instead of PMN influx into pouch fluid was used as the primary end point.

Carrageenan was used as the inflammatory challenge in these experiments because it is well studied and predictable in animal models of inflammation, and in preliminary trials it produced an impressive PMN response at an early point in acute inflammation. The diverse properties of carrageenan have been previously reviewed (32). Although gross signs of inflammation were infrequent, carrageenan challenge produced a significant cellular influx at 6 h postchallenge that was consistent with an acute inflammatory response. We conclude that carrageenan injection induces a prompt local acute inflammatory response in the rat air pouch.

For colostrum to protect newborns from inflammation, others have suggested that it first must be a poor inducer of inflammation (25). In these experiments, the inflammatory activity of colostrum was intermediate compared with the activity of normal saline or of carrageenan. Colostrum was less inflammatory than laboratory-prepared PBS that was used as a negative control in a single preliminary trial (data not shown) and that probably contained small amounts of endotoxin. We conclude that the aqueous phase of human colostrum is a poor inducer of acute inflammation in this model. The modest cellular response to aqueous colostrum injection might be related to the known chemoattractant activity of the major milk protein casein. The persistence of fluid in colostrum-injected pouches may reflect the higher osmotic activity of colostrum compared with the other challenge materials.

Pooled aqueous human colostrum significantly suppressed PMN influx after carrageenan challenge in these experiments. The degree of suppression was equivalent to those of potent anti-inflammatory agents given systemically. The decreases seen in the presence of colostrum are most likely due to suppression of a component of the inflammatory cascade leading to PMN accumulation, because incubation of PMN in colostrum *in vitro* and correlation of leukocyte granule contents and cell counts in pouch fluid samples revealed no signs of significant PMN lysis. We conclude that colostrum can significantly alter acute inflammation in response to carrageenan challenge in the rat air pouch.

Whether decreases in the inflammatory response seen with colostrum treatment are the result of a specific anti-inflammatory activity was not examined. If colostrum possessed a specific

anticarrageenan effect, then decreased PMN influx would be expected with colostrum treatment. However, in a small number of animals, suppression of PMN influx in response to heat-killed *Staphylococcus aureus* in tryptic soy broth also was observed when colostrum was injected with the staphylococcal preparation, as well as when an anti-inflammatory agent was given (data not shown). Although colostrum possesses secretory IgA and lysozyme, which might decrease the inflammatory response to staphylococci, it is unlikely that anti-inflammatory effects after carrageenan and staphylococcal challenge are the result of separate specific interactions with these stimuli. Therefore, we believe the aqueous phase of colostrum is a potent suppressor of acute inflammation in this *in vivo* model. These data directly support the hypothesis that human colostrum is anti-inflammatory *in vivo* and indirectly supports the hypothesis that colostrum may protect newborns from exposure to PMN.

Variation in the anti-inflammatory activity among individual donor's colostrum is evident and suggests that this anti-inflammatory activity is related to specific components in colostrum that vary among mothers. The identity of these anti-inflammatory components in colostrum is unclear. Fractionation of colostrum by molecular size filtration suggested that colostral anti-inflammatory factors may segregate with low molecular mass components (less than 10 000 D). These data also could be interpreted as reflecting partition of proinflammatory colostral components, such as casein, to the high molecular mass fraction. Digestion of colostrum with proteinase K did not eliminate the suppression of PMN influx in one experiment, and PAGE of the digested colostrum showed disappearance of proteins with molecular masses greater than 50 000 D (data not shown). The suppressive activity of this digested colostrum was greater than undigested colostrum, suggesting a loss of proinflammatory activity with protease treatment. Proinflammatory or chemotactic activity in colostrum may obscure some anti-inflammatory activity seen in this model, and this chemotactic activity may be due to casein. Lactoferrin is a milk component that can decrease the production of proinflammatory cytokines such as IL-1 and tumor necrosis factor by mononuclear cells (33), as well as the production of complement component C3 convertase (34), and so could suppress inflammation. However, lactoferrin should partition to the 10 000- to 100 000-D colostrum fraction and should be proteinase K–susceptible and thus is unlikely to be the active component in this model. Additional studies to delineate and characterize the mediators of this anti-inflammatory effect are needed.

These data show that human colostrum has an anti-inflammatory effect at the surface of a subcutaneous pouch in rats. If human milk is important in prevention of diarrheal disease and damage from PMN in infants, we would hypothesize that it also has an anti-inflammatory effect at the mucosal surface of the gastrointestinal tract in newborns. Because this is not a model of the mucosal inflammation, the biologic significance of this anti-inflammatory effect is unclear. We believe these results suggest the need for examination of the effects of human milk colostrum on inflammation in more biologically relevant settings.

In summary, a rat subcutaneous air pouch model of inflammation using carrageenan as an inflammatory stimulus was used to test the effects of the aqueous phase of colostrum on inflammation. Pooled aqueous colostrum had little acute inflammatory activity, and when present in the pouch with carrageenan, it suppressed acute inflammation as effectively as systemically administered dexamethasone or indomethacin. These data are the first experimental evidence of an anti-inflammatory effect of colostrum *in vivo*. They are consistent with *in vitro* data suggesting that human milk or colostrum has antileukocyte activity and epidemiologic data suggesting anti-inflammatory effects.

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