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Relationship Between Phagocytosis and Immunoglobulin A Release from Human Colostral Macrophages

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Macrophages and neutrophils that contain mainly secretory immunoglobulin A (IgA) comprise the majority of cells in human colostrum. These cell populations were separated and analyzed for their ability to release total IgA and secretory IgA when stimulated to phagocytose. Colostral macrophages phagocytosed opsonized bacteria and nonopsonized latex particles; at the same time, IgA was released. Neutrophils poorly phagocytosed opsonized bacteria but actively phagocytosed latex particles. In contrast to the macrophages, the neutrophils did not release IgA, even after active phagocytosis of latex. Consequently, colostral macrophages are the main source of IgA released from colostral leukocytes when these cells are exposed to organisms or particles that are phagocytosed. A function for colostral neutrophils which sequester IgA is proposed.

Several investigators have presented evidence that human colostral cells contain immunoglobulin A (IgA) (1, 3, 5), particularly secretory IgA (SIgA) (3). It has been demonstrated in vitro that IgA is released from these cells over time (3), which has led to speculation that colostral cells may function in immunoglobulin transport from mothers to their newborns. The exact role of IgA in these modified colostral cells in the protection of the recipient neonate is not known, however. Because of the phagocytic nature of these leukocytes, we hypothesized that these IgA-laden cells would adhere to the mucosa of the upper alimentary tract of the infant and would release a large portion of their IgA during phagocytosis of colonizing microorganisms. Those pathogens would then be unable to adhere to the epithelium, and colonization would be prevented. Such a mechanism would be particularly important in protecting the mucosa between feedings, when little of the fluid-phase IgA would remain.

Recently, we reported evidence for the phagocytic induction of IgA release by human colostral leukocytes (7). When unfractionated colostral cells phagocytized inert latex particles, serum-opsonized *Candida albicans*, or serum-opsonized, live *Escherichia coli* O7KL, about 40% of the IgA was released within 30 to 60 min. The requirement for phagocytosis was confirmed by the lack of IgA release when nonopsonized microorganisms were used.

In those initial experiments, the identity of the phagocytic cells that released SIgA was not determined. In this report, we present evidence that human colostral macrophages release IgA during phagocytosis.

MATERIALS AND METHODS

Subject selection and cell preparations. The research was approved by the Institutional Review Board of the University of Texas Medical Branch, Galveston, Tex. Nine donors ranging in age from 17 to 31 years were recruited. Colostrum was collected on day 2 or 3 by means of an electric pump (Egnell, Inc., Cary, Ill.). Colostrum, diluted 1:2 with Hanks balanced salt solution, was centrifuged at 300 × *g* for 15 min. The cell pellet was resuspended and washed three times in 5-ml volumes of cold Dulbecco modified Eagle medium–10% fetal calf serum. Only samples which contained >95% viable cells as shown by trypan blue exclusion were used (eight of nine samples collected). The washed cells were resuspended in Dulbecco culture medium (final concentration, 10^6 cells per ml) and held at 4°C. Differential counts were made on small portions of the cell preparations by use of a nonspecific esterase stain (8) that distinguishes macrophages from other leukocytes.

Adherence-detachment from gelatin-serum substrate. Donors were selected for high counts of neutrophils (60 to 80%) or macrophages (45 to 55%). All experiments employed 60-mm-diameter polystyrene dishes (Falcon no. 3002). Gelatin–serum-coated dishes were prepared as previously described (4). Briefly, 1.0 ml of a 0.25% swine skin gelatin (Sigma Chemical Co., St. Louis, Mo.) solution was added to the dishes. This was allowed to dry, and the dishes were stored at 4°C. Before use, 40% fetal calf serum in Dulbecco modified Eagle medium was placed on the dishes, which were then incubated for 30 min at 37°C. The dishes were washed once in Hanks balanced salt solution. Cells (4 ml of 10^6 cells per ml) in Dulbecco modified Eagle medium–10% fetal calf serum were added to the dishes

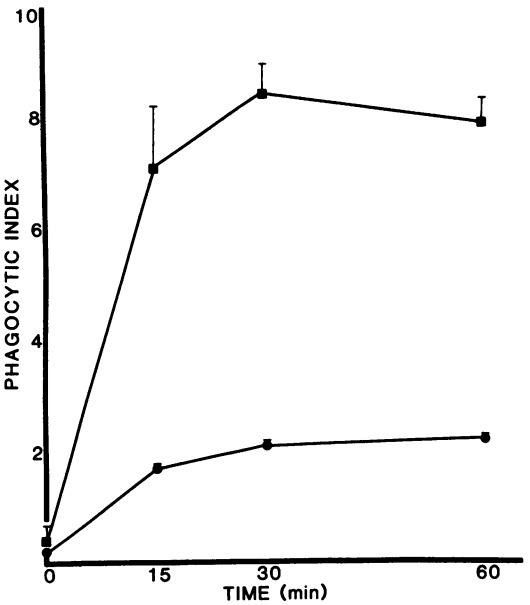

FIG. 1. The phagocytic index (number of bacteria per 100 leukocytes divided by 100) for macrophages plus *E. coli* O7KL (■) and neutrophils plus *E. coli* O7KL (●). Data are presented as means ± standard error of the mean.

and incubated at 37°C in 5% CO_2 for 1 h. The plates were washed twice; nonadherent cells were collected by centrifugation. Adherent cells were detached by the addition of 3 mM EDTA for 30 min. About 95% of the nonadherent cells were nonspecific esterase negative (neutrophils); 87 to 95% of the cells that detached from the gelatin-serum substrate were nonspecific esterase positive (macrophages). Both separated cell populations were used in subsequent experiments.

Phagocytosis. *E. coli* O7KL obtained from 24-h cultures was suspended in sterile phosphate-buffered saline. The organisms were preopsonized by incubating them at 37°C for 2 h with IgA-deficient human cord blood serum. The bacteria were then washed twice in phosphate-buffered saline and resuspended to a final concentration of 5×10^8 bacteria per ml. Latex particles (1 to 2 μm in diameter; Difco Laboratories, Detroit, Mich.) were washed twice in cold sterile phosphate-buffered saline and resuspended to 5×10^8 particles per ml.

A 0.5-ml volume of the cell preparation was placed in each of eight sterile plastic tubes with 0.3 ml of RPMI 1640 with HEPES (*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid) buffer culture medium. Four of the tubes were incubated with latex particles or opsonized *E. coli*; phosphate-buffered saline (0.2 ml) was added to the remaining four tubes. The preparations were incubated at 37°C for 0, 15, 30, or 60 min in a shaking water bath. Colostral cell preparations incubated with latex beads were stained for nonspecific esterase (5); leukocyte-associated *E. coli* were detected by staining with acridine orange (2). The phagocytic index was calculated by dividing the number of bacteria per 100 leukocytes by 100.

Assays for IgA. At the end of each incubation period, the tubes were centrifuged at 300 × *g* for 5 min. The supernatant fluids and cells were separated. The cells were lysed in 1 ml of Tween 20 (0.5% solution). The lysates and supernatants were assayed for total IgA and SIgA by an immunofluorescent method as previously described (3, 7).

RESULTS

Phagocytic activity. In these experiments, human colostral macrophages ingested *E. coli* O7KL to a significantly greater degree ($P < 0.001$) than colostral neutrophils (Fig. 1). However, nonopsonized latex particles were readily phagocytized by enriched neutrophil preparations. Phagocytic index values for colostral neutrophils were comparable to those obtained with mixed colostral leukocytes and enriched colostral macrophages (Table 1).

IgA release during phagocytosis. The principal type of IgA in both the colostral macrophages and neutrophils was SIgA, as noted in our previous study (7). Neutrophils contained more IgA than macrophages; however, this appeared to be due to a release of some IgA by macrophages when they attached to gelatin-serum substrates (data not shown).

In the presence of serum-opsonized *E. coli* O7KL, about 50% of the remaining intracellular IgA was released from macrophages within 30 to 60 min (0 versus 60 min; $P < 0.002$) (Fig. 2). The decrease in intracellular IgA correlated with a simultaneous increase in extracellular IgA ($r = -0.95$). In contrast, there was no significant release of IgA observed in neutrophil preparations (Fig. 2). Previous experiments (Fig. 1) showed that isolated neutrophils did not phagocytose opsonized *E. coli* as well as macrophages. Consequently, to determine whether the lack of IgA release was correlated with reduced phagocytosis in neutrophils, these purified cells and purified macrophages were incubated with latex beads. As shown in Table 1, both neutrophils and macrophages readily phagocytosed latex. However, approximately 40% of the intracellular IgA was released in macrophages (0 versus 60 min; $P < 0.05$) (Fig. 3), but colostral neutrophils released only 3% of their intracellular IgA (Fig. 3). Neither macrophages nor neutrophils released IgA in the absence of opsonized *E. coli* or latex.

DISCUSSION

These experiments suggest that certain populations of human colostral macrophages obtained by detachment from gelatin-serum substrates release about 50% of their intracellular IgA during phagocytosis of human serum-opsonized *E. coli* O7KL or nonopsonized latex parti-

TABLE 1. Phagocytosis of latex beads by unfractionated colostral leukocytes, purified colostral macrophages, and purified colostral neutrophils

Cell population	Phagocytic index[a] at the following incubation times (min):			
	0	15	30	60
Unfractionated leukocytes	0.3 ± 0.1	3.2 ± 0.3	3.4 ± 0.3	3.4 ± 0.3
Purified macrophages	0.2	2.8	3.3	3.4
Purified neutrophils	0.2 ± 0.0	2.2 ± 0.1	3.9 ± 0.1	3.6 ± 0.1

[a] Phagocytic index = number of intracellular particles per 100 leukocytes/100. Data are expressed as means ± standard error the mean.

cles. The kinetics of this release were similar to those observed in unfractionated colostral leukocytes (7). Release was apparent by 15 min and reached a maximum by 30 to 60 min of incubation. The release of IgA by these cells during phagocytosis is of particular interest, since macrophages are known for their long life span and adaptability to many organ sites. It would appear that these maternal cells could reside on the mucosa of the alimentary tracts of infants for substantial periods, and, as we previously hypothesized (7), they would release much of their IgA during phagocytosis.

In contrast, colostral neutrophils did not release IgA upon exposure to opsonized *E. coli*. The degree of phagocytosis of opsonized *E. coli* by these neutrophils was, however, quite limited as compared to the macrophages. It is unclear from these experiments whether the decrease in phagocytosis of *E. coli* was due to an artifact in the separation technique, the source of opsonins, or an inherent feature of the cells. To ascertain whether failure to release IgA was due to lack of phagocytosis, enriched neutrophil or macrophage preparations were incubated with latex particles, which were readily ingested by

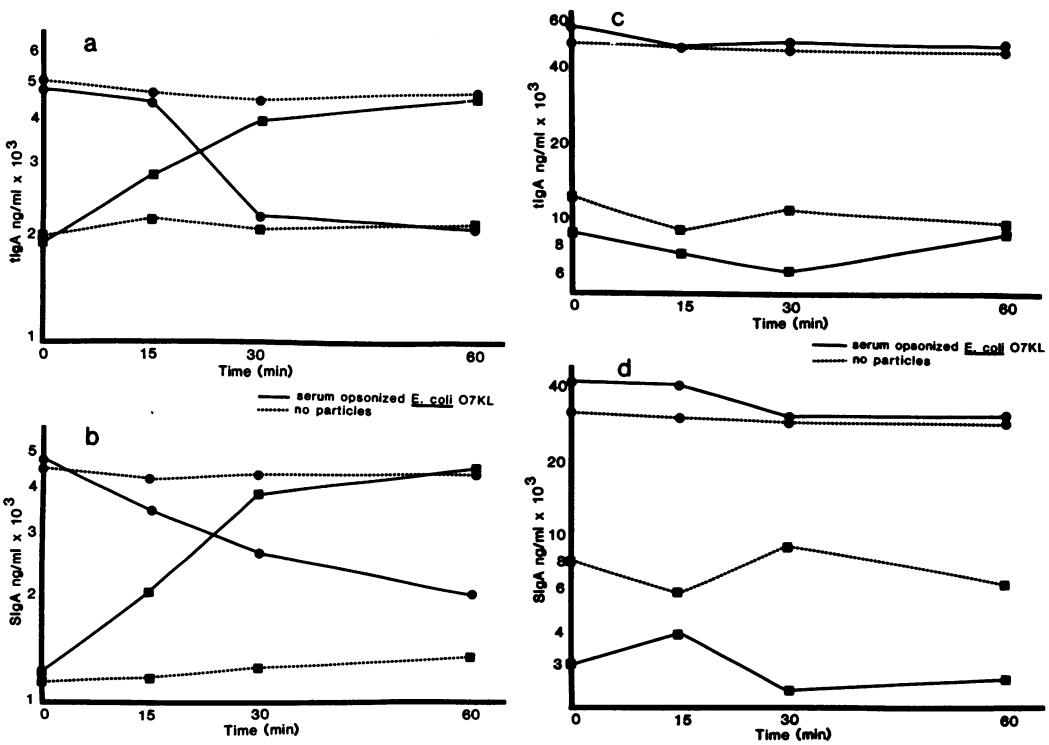

FIG. 2. Release of total IgA (a and c) and SIgA (b and d) from colostral macrophages (a and b) or colostral neutrophils (c and d) incubated with serum-opsonized *E. coli* O7KL. IgA content was determined for experimental supernatants (■——■), experimental lysates (●——●), control supernatants (■-----■), and control lysates (●-----●).

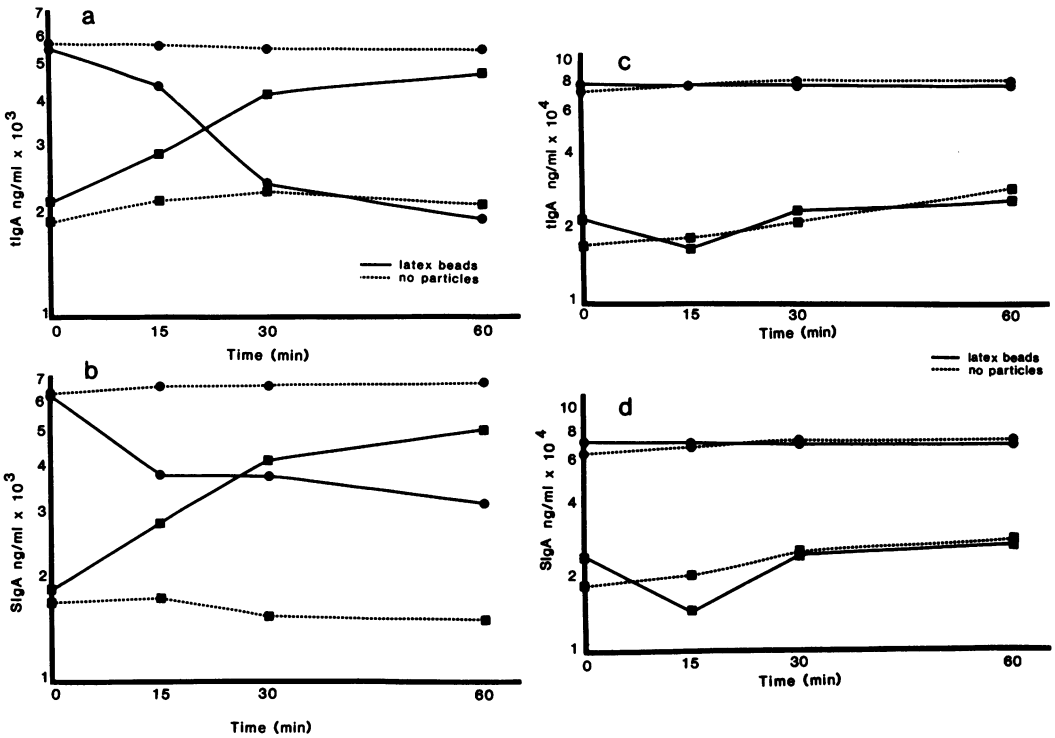

FIG. 3. Release of total IgA (a and c) and SIgA (b and d) from colostral macrophages (a and b) or colostral neutrophils (c and d) incubated with nonopsonized latex beads. See legend to Fig. 2 for explanation of symbols.

both types of cells. The results of these experiments indicate no release of IgA by the neutrophils. Thus, whereas IgA was promptly released from the macrophages, our observation suggests that colostral neutrophils do not release IgA during phagocytosis.

The data presented in this paper are the first to suggest that colostral leukocytes are composed of at least two populations of cells with differing responses to phagocytosed particles. Colostral macrophages obtained by detachment from gelatin-serum substrates contain IgA and release this IgA during phagocytosis. As originally hypothesized (7), these cells may adhere to the mucosa of the upper alimentary tract of the neonate and may release a large portion of their IgA during phagocytosis of colonizing microorganisms. If this released IgA combined with the microorganisms, they would be unable to adhere and colonization would be prevented (6).

In contrast, although esterase-negative nonadherent cells (colostral neutrophils) contain IgA, they do not release this IgA during phagocytosis in vitro. This surprising finding has led us to modify our original hypothesis (7), which suggested that the majority of colostral leukocytes would release IgA. Consequently, we suggest that colostral neutrophils may travel farther down the alimentary tracts of infants and release their IgA when they encounter appropriate stimuli. Although the principal type of IgA in colostral leukocytes is SIgA, which has been shown to be resistant to proteolysis (6), immunoglobulin sequestered in neutrophils would be further protected from proteolysis until their membranes were lysed. Again, once the SIgA is released, it may combine with microorganisms, thereby preventing their adherence and colonization. Further experimentation will be necessary to determine what, if any, stimuli will lead to release of IgA from enriched populations of colostral neutrophils.

We have advanced evidence that colostrum contains leukocyte populations with distinct adherence and phagocytic properties. Experiments are in progress in our laboratory to further define the functions of these populations of human colostral leukocytes in in vitro systems. These experiments will bear on the possibility that these maternal leukocytes provide a unique cell-associated SIgA that may serve to protect the recipient infant.

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