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Localization of IgA and IgM in Human Colostral Elements Using Immunoelectron Microscopy

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In addition to the free form, IgA is associated with cellular and noncellular elements present in human colostrum. To resolve the existing controversy as to the cell type(s) containing IgA, we used immunoelectron microscopy with horseradish peroxidase-labeled $F(ab')_2$ or Fab' fragments of anti-IgA or anti-IgM to determine the distribution of these immunoglobulins in colostral elements. IgA and IgM were localized in phagocytic vacuoles of polymorphonuclear leukocytes and macrophages in the vicinity of the cell membrane. In neutrophilic leukocytes, both immunoglobulins were occasionally found in phagocytic vacuoles distributed throughout the cytoplasm. Although the *in vitro* phagocytic activity of colostral cells was low, they retained the ability to ingest colloidal gold particles which were subsequently localized in phagocytic vacuoles that also contained IgA or IgM. IgA and IgM were not detected in lymphocytes, and plasma cells were not found in human colostrum. Numerous noncellular colostral globules of various shapes and sizes also contained IgA and IgM. These observations indicate that IgA and IgM were acquired by phagocytic cells and noncellular globules and were not actively synthesized by lymphoid cells present in human colostrum.

KEY WORDS: Colostral cells; IgA; phagocytes; milk.

INTRODUCTION

Various biological functions of colostral cells have been reported, including phagocytosis and killing of microorganisms, synthesis, ingestion and release of immunoglobulins, and production of interferon (for review, see Refs. 1–4). The cells in human colostrum are represented by large numbers of polymorphonuclear cells (PMN) and macrophages and small numbers of lymphocytes, epithelial cells, and epithelial cell fragments (5–9). In addition to cells, noncellular globules have been observed (7, 10).

Various combinations of immunoglobulins and other milk proteins have been detected within human colostral cells (7) but there is a controversy concerning the origin of these immunoglobulins. While active synthesis of IgA (11) and the production of IgA specific for enteric bacteria have been reported (12, 13), other findings suggest that colostral PMN and macrophages acquire colostral proteins, including immunoglobulins, by ingestion (7, 10, 14–16). Properties of colostral cells have been previously studied by transmission electron microscopy (6, 8, 17). In this study we employed immunoelectron microscopy to investigate the distribution of IgA and IgM in organelles of human colostral cells.

MATERIALS AND METHODS

Preparation of Antibodies and Their Conjugation with Horseradish Peroxidase (HRP). Secretory IgA (S-IgA) was purified from human colostrum and IgM was purified from the sera of patients with Waldenström's macroglobulinemia as previously described (18, 19). Antisera specific for the heavy chains of IgA and IgM were obtained by absorption on immunosorbent columns of cyanogen bromide-activated Sepharose 4B coupled with relevant proteins. Specificity was evaluated by immunodiffusion and immunoelectrophoresis against normal human serum, colostrum, and purified IgA, IgM, and IgG (20) and by radioimmunoassay (21).

The γ-globulin fraction of anti-α chain was digested with pepsin and the $F(ab')_2$ fragments, isolated by Sephadex G-200 chromatography, were labeled

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with HRP, Type VI (Sigma Chemical Corp., St. Louis, MO) (22). Fab′ fragments specific for μ chain were prepared by digestion with pepsin, reduction with 2-mercaptoethanol, and alkylation with iodoacetamide. Fab′ fragments, isolated by Sephadex G-200 chromatography, were then labeled with HRP and used for immunocytochemical staining (22). Fab′ fragments obtained from nonimmunized rabbit IgG were labeled with HRP and used as a control. Activated HRP was prepared by oxidizing HRP in 0.3 *M* sodium bicarbonate buffer (pH 8.1) with 0.04 *M* sodium meta-periodate in distilled water, according to Nakane *et al.* (23).

Immunocytochemical Procedures. Human colostrum from healthy donors was collected into sterile, conical tubes within 3 days after delivery. Immediately after collection, colostral samples were diluted 1:2 with phosphate-buffered saline (PBS) and centrifuged at 400*g* for 10 min. Pellets of colostral cells were fixed with 1% glutaraldehyde in 0.1 *M* phosphate buffer for 15 min at room temperature, then washed overnight at 4°C with several changes of PBS. To inhibit endogenous peroxidase activity in colostral cells, suspensions were incubated with a 0.1% phenylhydrazine · HCl solution in 0.06 *M* phosphate buffer and 0.36% NaCl for 2 hr at 37°C (24). After seven washings, colostral cells were incubated with 0.1 *M* lysine buffer to block free aldehyde groups, then washed three times with PBS. The colostral cells and globules were incubated overnight (15–24 hr) at 4°C with HRP-labeled $F(ab')_2$ fragments of anti-α chain or Fab′ fragments of anti-μ chain in the presence of 0.025% Triton X-100 (Eastman Kodak Co., Rochester, NY) as described (25, 26). After brief washing, these cells were immersed for 20 min at room temperature in 3,3′-diaminobenzidine tetra · HCl (DAB) (Sigma) (0.3 mg/ml) in 0.05 *M* Tris–HCl buffer (pH 7.6) containing 0.01 *M* sodium azide free of H_2O_2 and then in 0.005% H_2O_2 for 5–10 min (27). After washing, the cells were fixed with 2% osmium tetroxide in distilled water for 60 min at room temperature, rinsed, dehydrated in ethanol, and embedded in Spurr medium (Ladd Research Industries, Burlington, VT). Thin sections, either unstained or stained with uranyl acetate and lead citrate, were examined with an electron microscope (TEM 300, Philips North America, Atlanta, GA).

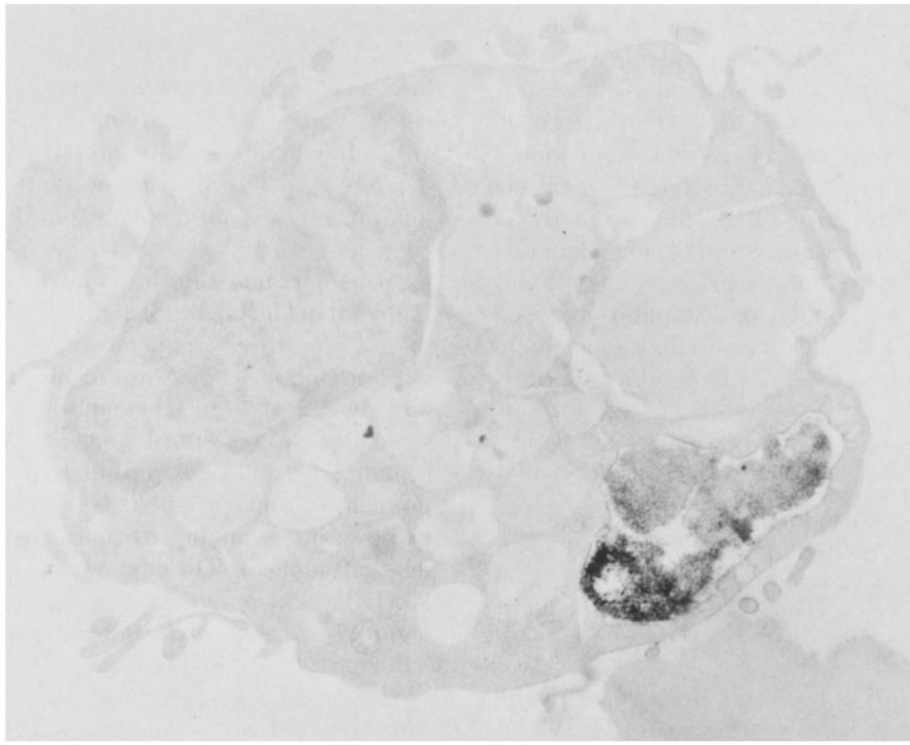

Fig. 1. Colostral macrophage incubated with HRP-labeled $F(ab')_2$ fragments of anti-IgA. IgA is located in a large phagocytic vacuole near the cell membrane. × 15,000, without counterstaining.

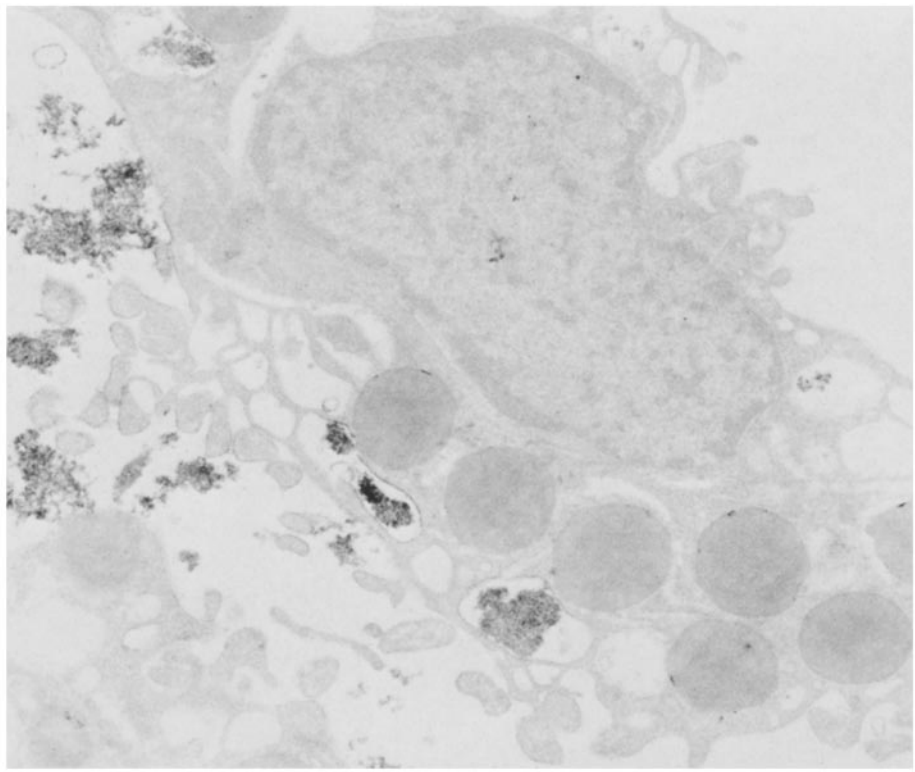

Fig. 2. Colostral macrophage incubated with HRP-labeled Fab′ fragments of anti-IgM. IgM is located within phagocytic vacuoles that do not fuse with dense bodies and also extracellularly. × 15,000, without counterstaining.

Blood from healthy donors was collected into sterile, heparinized syringes and mononuclear cells were isolated as previously described (20). Lymphocytes were cultured at 37°C in humidified air with 5% CO_2 at a concentration of 1×10^6 cells/ml in RPMI 1640 medium (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (Reheis Corp., Kankakee, IL), 2 m*M* L-glutamine, 100 μg/ml penicillin, and 100 units/ml streptomycin (GIBCO) in the presence of 10 μl/ml pokeweed mitogen (PWM) (GIBCO). Unstimulated or PWM-transformed lymphocytes (harvested after 7 days in culture) were processed as described for colostral cells.

Each experiment included the following controls: (a) determination of endogenous peroxidase activity in colostral cells and globules by the DAB reaction; (b) inhibition of endogenous peroxidase activity followed by the DAB reaction; (c) inhibition of peroxidase activity followed by incubation with HRP-labeled Fab′ fragments of normal rabbit IgG; and (d) inhibition of peroxidase activity followed by incubation with activated HRP. Transformed or unstimulated human peripheral blood lymphocytes were used as positive or negative controls, respectively.

Examination of Phagocytic Activity. Washed colostral cells were suspended in RPMI-1640 with 10% FBS. Colloidal gold solution (Range, Anderson Laboratory, Inc., Fort Worth, TX) was concentrated by boiling to one-ninth its volume. Two parts of RPMI 1640 with 10% FBS and one part of colloidal gold solution were added to pelleted cells as described (28) and incubated overnight at 37°C in 5% CO_2 in humidified air. To remove partially adherent cells, the mixture was vigorously pipetted, then washed, and the cells were pelleted and fixed as described above. Cells were subsequently incubated with HRP-labeled $F(ab')_2$ or Fab′ fragments of anti-α- or anti-μ-chain antibodies and processed in the same manner as described above.

RESULTS

Ultrastructural Characteristics of Colostral Cells. PMN in colostrum were round cells with

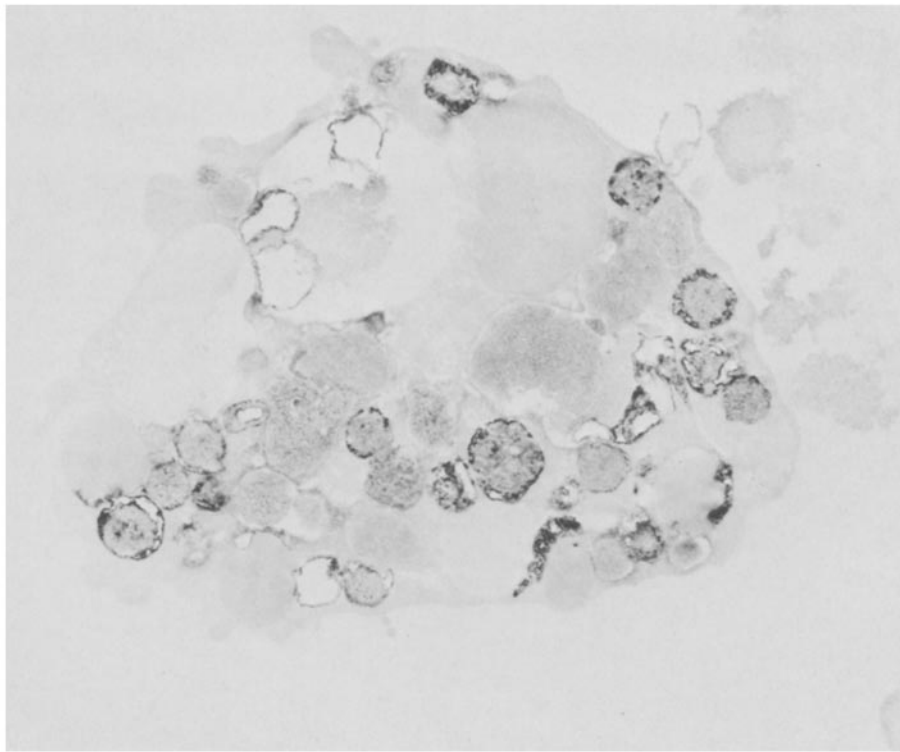

Fig. 3. Colostral PMN incubated with HRP-labeled $F(ab')_2$ fragments of anti-IgA. IgA is found in large numbers of phagocytic vacuoles. × 12,300, without counterstaining.

multilobulated nuclei and peripherally condensed chromatin. Previous studies in this laboratory have shown these cells to be neutrophilic; no eosinophilic cells were detected (7). The cytoplasm, with a few cytoplasmic processes, contained two or three large, homogeneous lipid droplets, small numbers of spherical or rod-shaped dense bodies, and large numbers of phagocytic vacuoles of various sizes, shapes, and densities. The number of dense bodies varied in each cell and tended to decrease as the number of phagocytic vacuoles increased.

The colostral macrophages had indented or irregularly shaped nuclei and many cytoplasmic processes. The cytoplasms contained relatively well-developed Golgi apparatuses, strands of endoplasmic reticulum, small scattered mitochondria, homogeneous lipid droplets, spherical dense bodies, and phagocytic vacuoles of various sizes. Some phagocytic vacuoles appeared lucent and others contained granular or amorphous substances with various densities. Vacuoles were often present near complicated cytoplasmic processes at the outer border of these cells.

Donne's corpuscles (foamy macrophages) had a characteristically large size and contained numerous lipid droplets and phagocytic vacuoles with amorphous substances giving the cytoplasm its foamy appearance.

The small numbers of lymphocytes observed in colostrum displayed structural properties similar to those in peripheral blood. No plasma cells were found in colostrum.

Round, ring-like, or peculiarly shaped noncellular globules were also present in colostrum. These elements were found to have medium densities and were composed of finely granular or homogeneous substances. The sizes of the globules ranged from less than 10 to larger than 80 μm in diameter. Spherical dense bodies and vacuoles of various sizes and contents were also present in some globules. Endogenous peroxidase activity was present in spherical or rod-shaped dense bodies and in phagocytic vacuoles of colostral PMN but not in lymphocytes, macrophages, or noncellular globules.

The inhibition of endogenous peroxidase fol-

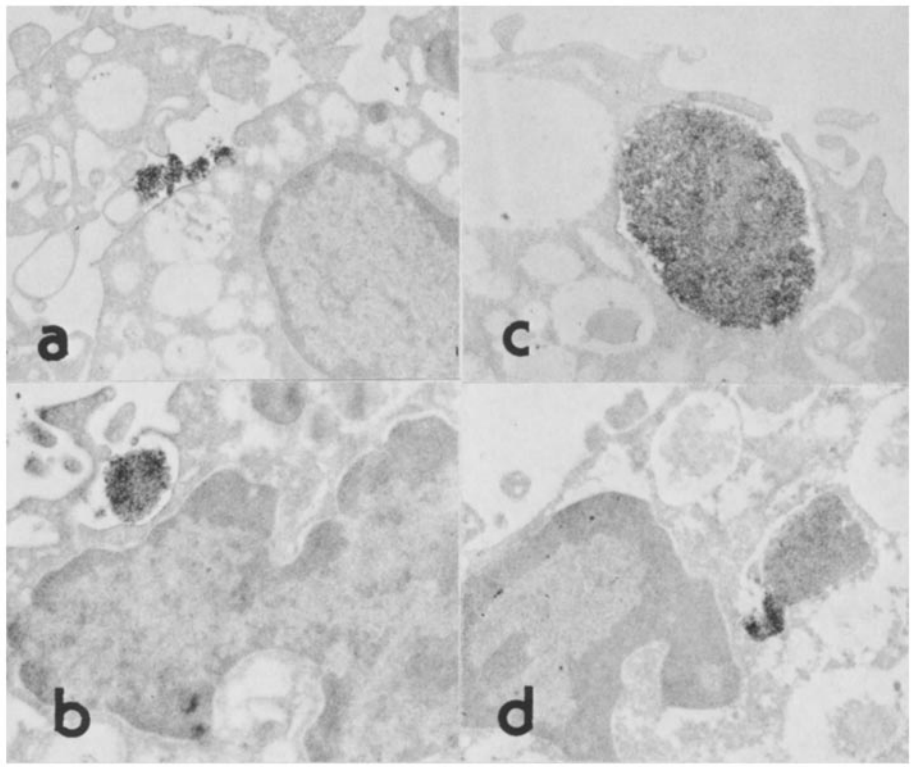

Fig. 4. Ingestion of IgA or IgM by colostral macrophage and PMN. (a) Macrophages incubated with HRP-labeled Fab′ fragments of anti-IgM. IgM is found in the vicinity of, and attached to, the cell membrane. × 11,200, without counterstaining; (b) Macrophages incubated with HRP-labeled Fab′ fragments of anti-IgM. IgM is detected in the extracellular space surrounded by cytoplasmic processes. × 12,300, without counterstaining; (c) Macrophages incubated with HRP-labeled $F(ab')_2$ fragments of anti-IgA. IgA is surrounded by two cytoplasmic processes. × 15,000, without counterstaining; (d) PMN incubated with HRP-labeled $F(ab')_2$ fragments of anti-IgA. IgA is located in a phagocytic vacuole in the cytoplasm. × 12,300, without counterstaining.

lowed by the DAB reaction showed that this activity was no longer present in PMN. Incubation with HRP-labeled Fab′ fragments prepared from normal rabbit IgG, or with activated HRP after inhibition of endogenous peroxidase activity, did not result in positive reaction products in colostral cells or in noncellular globules.

Distribution of IgA and IgM in Colostral Elements. After incubation with HRP-labeled anti-α- or anti-μ-chain reagents, IgA and IgM were localized in phagocytic vacuoles near the cell membrane of macrophages (Figs. 1 and 2). No staining was observed in the nuclear envelope, Golgi apparatus, or endoplasmic reticulum.

A small number of neutrophils contained numerous phagocytic vacuoles which stained for IgA (Fig. 3) and a few which stained for IgM. There was no difference in the localization patterns of IgA and IgM, but these immunoglobulins were found more frequently in macrophages than in neutrophils. In certain areas representing sections through complicated invaginations of the macrophage or PMN cell surface, IgA and IgM were present in extracellular spaces near the cell membranes, in concavities incompletely surrounded by cytoplasmic processes, and inside the cytoplasm (Figs. 4 a–d).

Both IgA and IgM were localized throughout noncellular globules, but in some cases, they were observed only in the periphery (Fig. 5).

To determine the ability of colostral cells to phagocytize, they were incubated with a solution of colloidal gold. Electron-dense particles were present within phagocytic vacuoles and between extended cytoplasmic processes of both PMN and macrophages (Figs. 6 and 7). The coincidental appearance within phagocytic vacuoles of colloidal

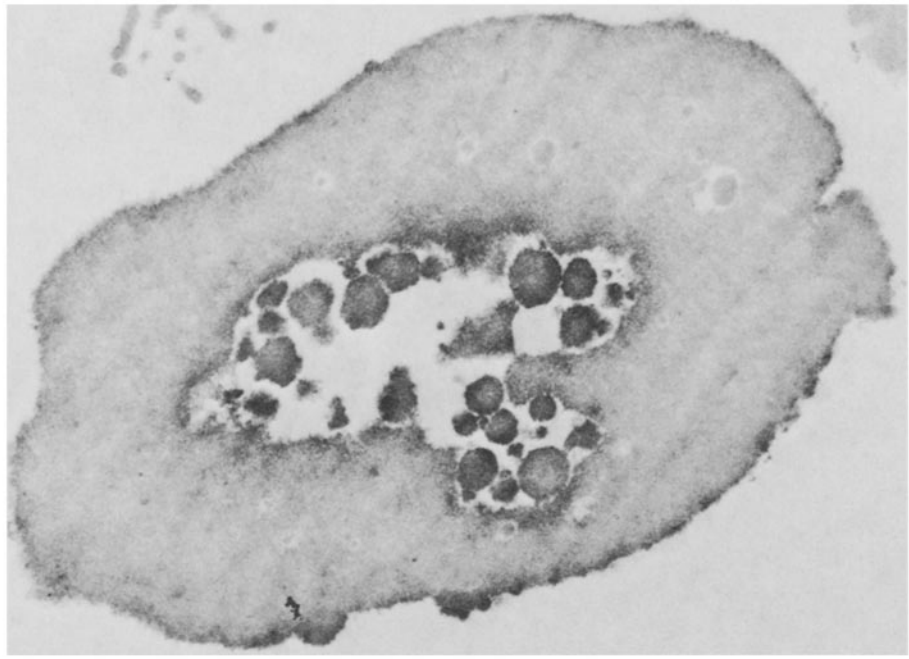

Fig. 5. Noncellular globule incubated with HRP-labeled $F(ab')_2$ fragments of anti-IgA. IgA is found within the globule. × 15,000, without counterstaining.

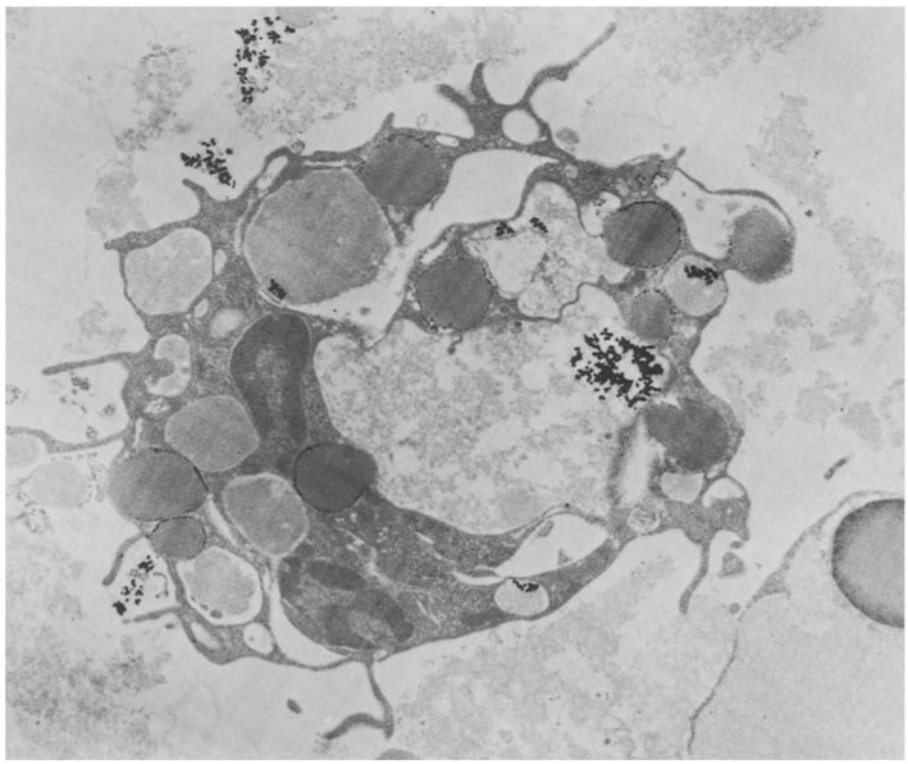

Fig. 6. Colostral PMN incubated with colloidal gold. Electron-dense colloidal gold particles are seen in several phagocytic vacuoles and between two cytoplasmic processes. × 10,200, without counterstaining.

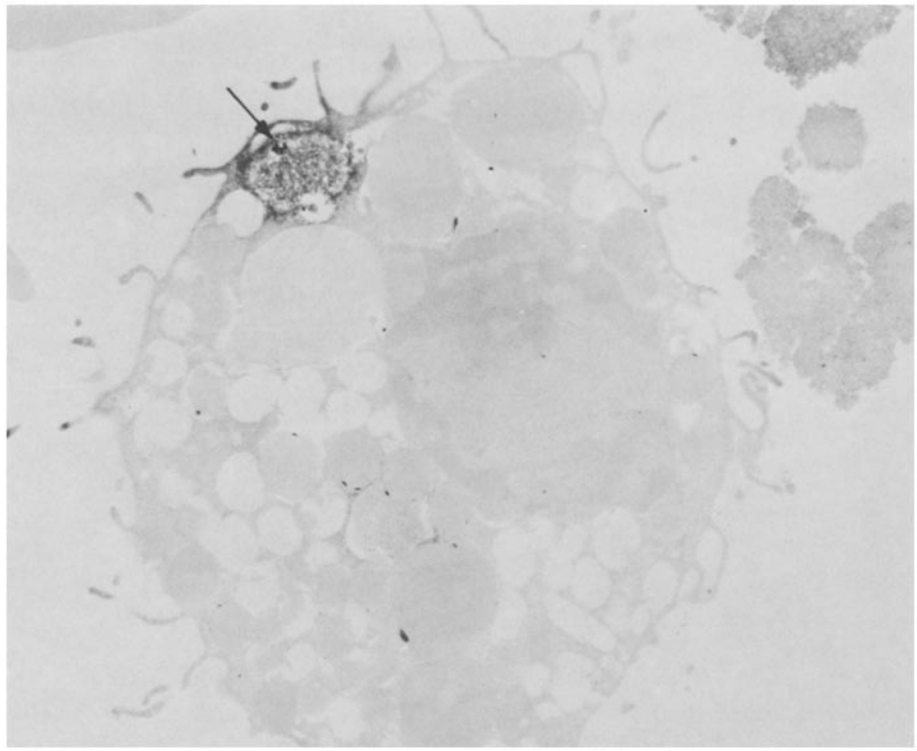

Fig. 7. Colostral macrophage incubated with colloidal gold and then reacted with HRP-labeled anti-μ-chain reagent. Coincidental localization of IgM and colloidal gold particles (arrow) are seen in a phagocytic vacuole. × 10,200, without counterstaining.

gold and IgM or IgA was disclosed by subsequent incubation of such cells with HRP-labeled anti-μ- or anti-α-chain reagents.

No IgA or IgM was detected in colostral lymphocytes. However, incubation with HRP-labeled anti-α-chain or anti-μ-chain reagents revealed the presence of IgA and IgM in the endoplasmic reticulum of PWM-transformed peripheral blood lymphocytes (Fig. 8) used as positive controls.

DISCUSSION

The results presented here indicate that IgA and IgM are found in colostral PMN, macrophages, and noncellular globules but not in lymphoid cells. Furthermore, they provide ultrastructural evidence for the distribution of these immunoglobulins within phagocytic vacuoles. Immunoglobulins and other milk proteins were previously detected in colostral phagocytes by immunofluorescence (7) plaque assay (10, 16) and by measurement of IgA released from cultured cells (14, 15). The mode of acquisition of intracellular IgA and IgM by phagocytic cells remains speculative. It has been demonstrated that a subpopulation of human PMN and macrophages displays surface receptors for polymeric IgA (29, 30) and intracytoplasmic IgA has been detected in a small number of PMN from healthy individuals and in a higher percentage of PMN from peripheral blood of patients with IgA nephropathy (Berger's disease) (31). In such cells, IgA apparently occurred in the form of immune complexes because of the coincidental presence of C3 (31). It is possible that colostrum contains increased numbers of cells with receptors for IgA, which is subsequently internalized and results in an intense intracellular staining patterns in some samples. Our previous analyses of the molecular form of IgA released from lysed colostral elements indicate that 93% of IgA is associated with secretory component (SC) and occurs almost exclusively in a polymeric form (10). Furthermore, the coincidental presence of IgM, IgA, SC, lactoferrin, α-lactalbumin, and both κ and λ chains within single colostral elements, as demonstrated by paired immunofluorescence (7), indicates that these proteins were acquired from colostrum

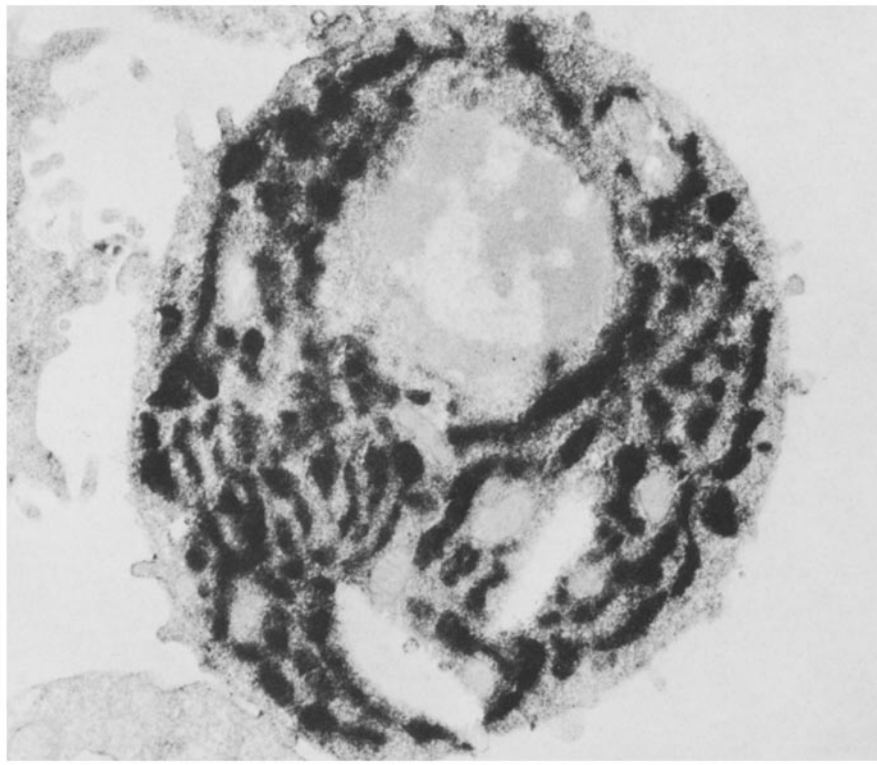

Fig. 8. Transformed lymphocytes incubated with HRP-labeled Fab' fragments of anti-IgM. IgM is detectable in the endoplasmic reticulum. × 18,750, without counterstaining.

rather than plasma. The endocytosis of colloidal gold demonstrates that colostral PMN and macrophages retain their phagocytic activity.

During pregnancy, large numbers of macrophages, neutrophils, lymphocytes, and plasma cells are observed in the mammary gland interstitium. After delivery, these cells, except for plasma cells, pass through spaces between myoepithelial and epithelial cells and appear in the lumen of acini and ducts of the mammary gland and free in colostrum (32–34). Plasma cells in the mammary gland interstitium are rich in immunoglobulins, particularly IgA (35), but other cell types seem to be devoid of it (36, 37).

The fate of IgA and IgM ingested by colostral macrophages and PMN is unknown. It is well documented that peripheral blood PMN contain lysosomal proteases that are capable of cleaving IgM, IgA, and IgG (38–40). However, our previous studies of the properties of IgA released from colostral cells by lysis indicate that this immunoglobulin occurs primarily in a polymeric, SC-associated form with only a small proportion of monomers; low molecular weight fragments were not detected (10). It is unclear whether the failure to detect IgA cleavage products is due to the intrinsic resistance of IgA, further increased by its association with SC (41, 42), to the lysosomal proteolytic enzymes present in colostral phagocytic cells, or due to the failure of vacuoles containing ingested IgA to fuse with lysosomes. The appearance of undigested IgA but not IgG in bile may also be explained by different intracellular processing by hepatocytes of vesicles that contain IgA as compared to vesicles which contain other glycoproteins and fuse with lysosomes (43). Apparently the different membrane receptors for IgA and other glycoproteins play decisive roles in the intracellular processing of the ingested material. Depletion of lysosomes, which are not regenerated in PMN, may also result in their inability to digest ingested IgA.

IgA-containing noncellular globules, which are numerous in colostrum, have not been extensively examined in other studies. Globules contaminate all cell populations and have been observed to form direct (IgM) and indirect (IgA) hemolytic plaques against erythrocytes coated with antigens of common enteric bacteria (10, 16). Although globules

have been considered to be aggregates of secreted protein (44), their origin still remains obscure. Because of the presence of IgA and SC, a glycoprotein synthesized by epithelial cells, it seems likely that they are of epithelial origin. Furthermore, DR antigens have been detected in association with milk globules, indicating that they may be derived from epithelial cells (45).

Although the protective role of free colostral IgA has been documented (for review see Ref. 46, 47), the function of IgA found within phagocytic cells remains speculative. It has been suggested that colostral macrophages serve as vehicles for transport of ingested IgA which is released later in the digestive tract of the neonate (14, 46). During the first 2 days of lactation, colostrum contains approximately 10^6 cells/ml (4, 5, 9); 500 ng of IgA have been measured per 10^6 colostral elements (10). Colostrum collected 1–2 days postpartum contains on the average 12 mg of IgA/ml (46). Therefore, when the levels of free as opposed to cell-associated IgA are compared, it is clear that the amount of IgA released from colostral elements constitutes only a trivial amount (approximately 0.004%) of the total IgA in colostrum. However, it is possible that the cell-associated IgA may have certain functional advantages (48).

ACKNOWLEDGMENTS

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REFERENCES

1. Hanson LÅ, Winberg J: Breast milk and defense against infection in the newborn. Arch Dis Child 47:845–848, 1972
2. Goldman AS, Smith CW: Host resistance factors in human milk. J Pediat 82:1082–1090, 1973
3. Welsh JK, May JT: Anti-infective properties of breast milk. J Pediat 94:1–9, 1979
4. Pittard WB III: Breast milk immunology. Am J Dis Child 133:83–87, 1979
5. Smith CW, Goldman AS: The cells of human colostrum. I. In vitro studies of morphology and function. Pediat Res 2:103–109, 1968
6. Smith CW, Goldman AS, Yates RD: Interaction of lymphocytes and macrophages from human colostrum. Exp Cell Res 69:409–415, 1971
7. Crago SS, Prince SJ, Pretlow TG, McGhee JR, Mestecky J: Human colostral cells. I. Separation and characterization. Clin Exp Immunol 38:585–597, 1979
8. Ho FCS, Wong RLC, Lawton JWM: Human colostral and breast milk cells. A light and electron microscopic study. Acta Paediat Scand 68:389–396, 1979
9. Ogra SS, Weintraub DL, Ogra PL: Immunological aspects of human colostrum and milk: Interaction with the intestinal immunity of the neonate. Adv Exp Med Biol 107:95–107, 1978
10. Laven GT, Crago SS, Kutteh WH, Mestecky J: Hemolytic plaque formation by cellular and noncellular elements of human colostrum. J Immunol 127:1967–1972, 1981
11. Murillo GJ, Goldman AS: The cells of colostrum. II. Synthesis of IgA and β1C. Pediat Res 4:71–75, 1970
12. Goldblum RM, Ahlstedt S, Carlsson B, Hanson LÅ, Jodal U, Lindin-Janson G, Sohl-Åkerlund A: Antibody-forming cells in human colostrum after oral immunisation. Nature 257:797–798, 1975
13. Ahlstedt S, Carlsson B, Hanson LÅ, Goldblum RM: Antibody production by human colostral cells. I. Immunoglobulin class, specificity and quantity. Scand J Immunol 4:535–539, 1975
14. Pittard WB, Polmar SH III, Fanaroff AA: The breast milk macrophage: A potential vehicle for immunoglobulin transport. J Reticuloendothel Soc 22:597–603, 1977
15. Weaver EA, Goldblum RM, Davis CP, Goldman AS: Enhanced immunoglobulin A release from human colostral cells during phagocytosis. Infect Immun 34:498–502, 1981
16. Lodinova R, Tlaskalova-Hogenova H: Role of breast feeding for the nutrition and immunologic development of the infant. *In* Infectious Diarrhea in Infancy and Its Nutritional Consequences, JA Bellanti (ed). International Center for Interdisciplinary Studies of Immunology (in press)
17. Wooding FBP, Peaker M, Linzell JL: Theories of milk secretion: Evidence from the electron microscopic examination of milk. Nature 226:762–764, 1970
18. Mestecky J, Kulhavy R, Kraus FW: Studies on human secretory immunoglobulin A. II. Subunit structure. J Immunol 108:738–747, 1972
19. Schrohenloher RE, Mestecky J: Recombination of the polypeptide chains of a Waldenström's IgM binds IgG. J Immunol 111:1699–1711, 1973
20. Crago SS, Mestecky J: Secretory component: Interaction with intracellular and surface immunoglobulins of human lymphoid cells. J Immunol 122:906–911, 1979
21. Kutteh WH, Prince SJ, Phillips JO, Spenney JG, Mestecky J: Properties of immunoglobulin A in serum of individuals with liver diseases and in hepatic bile. Gastroenterology 82:184–193, 1982
22. Wilson MB, Nakane PK: Recent developments in the periodate method of conjugating horseradish peroxidase (HRPO) to antibodies. *In* Immunofluorescence and Related Staining Techniques, W Knapp, K Holubar, G Wick (eds). Amsterdam, Elsevier North-Holland Biomedical Press, 1978, pp 215–224
23. Nakane PK, Kawaoi A: Peroxidase-labeled antibody. A new method of conjugation. J Histochem Cytochem 22:1084–1091, 1974
24. Straus W: Factors affecting the sensitivity and specificity of the cytochemical reaction for the anti-horseradish peroxidase antibody in lymph tissue sections. J Histochem Cytochem 28:645–652, 1980
25. Yokota S, Fahimi HD: The peroxisome (microbody) membrane: Effects of detergents and lipid solvents on its ultrastructure and permeability to catalase. Histochem J 10:469–487, 1978

26. Yokota S, Fahimi HD: Immunocytochemical localization of catalase in rat liver. J Histochem Cytochem 29:805–812, 1981
27. Graham RC, Karnovsky MJ: The early stages of absorption of injected horseradish peroxidase in the proximal tubules of mouse kidney: Ultrastructural cytochemistry by a new technique. J Histochem Cytochem 14:291–302, 1966
27. Vincent RA, Spicer SS: Giant dense bodies in fibroblasts cultured from beige mice with the Chediak-Higashi Syndrome. AM J Pathol 105:270–278, 1966
29. Spiegelberg HL, Lawrence DA, Henson P: Cytophilic properties of IgA to human neutrophils. Adv Exp Med Biol 45:67–74, 1974
30. Fanger MW, Shen L, Pugh J, Bernier GM: Subpopulations of human peripheral granulocytes and monocytes express receptors for IgA. Proc Natl Acad Sci USA 77:3640–3644, 1980
31. Tomino Y, Sakai H, Endoh M, Kaneshige H, Nomoto Y: Detection of immune complexes in polymorphonuclear leukocytes by double immunofluorescence in patients with IgA nephropathy. Clin Immunol Immunopathol 24:63–71, 1982
32. Helminen HJ, Ericsson JLE: Studies on mammary gland involution. I. On the ultrastructure of the lactating mammary gland. J Ultrastruct Res 25:193–213, 1968
33. Seeling LL Jr. Beer AE: Transepithelial migration of leukocytes in the mammary gland of lactating rats. Biol Reprod 17:736–744, 1978
34. Salazar H, Tobon H: Morphologic changes of the mammary gland during development, pregnancy and lactation. *In* Lactogenic Hormones, Fetal Nutrition and Lactation, JB Josimovich (ed). New York, John Wiley & Sons, 1974, pp 221–277
35. Brandtzaeg P: The secretory immune system of lactating human mammary glands compared with other exocrine organs. Ann NY Acad Sci 409:353–382, 1983
36. Kraehenbühl JP, Racine L, Galardy RE: Localization of secretory IgA, secretory component, and α chain in the mammary gland of lactating rabbit by immunoelectron microscopy. Ann NY Acad Sci 254:190–202, 1975
37. Fujimura M, Chen ST, Sudo T, Kumaya T, Saito T: Cellular sites of immunoglobulin. VII. Localization of immunoglobulins in female mammary gland. Acta Histochem Cytochem 14:163–167, 1981
38. Folds JD, Prince HE, Spitznagel JK: Limited cleavage of human immunoglobulins by elastase of human neutrophil polymorphonuclear granulocytes. Possible modulator of immune complex disease. Lab Invest 39:313–321, 1978
39. Tax A, Korngold L: Comparison of the effect of elastase on human secretory IgA and serum IgA. J Immunol 107:1189–1191, 1971
40. Prince HE, Folds JD, Spitznagel JK: Interaction of human polymorphonuclear leukocyte elastase with human IgM. *In vitro* production of an Fabμ-like fragment. Mol Immunol 16:301–306, 1979
41. Underdown BJ, Dorrington KJ: Studies on the structural and conformational basis for the relative resistance of serum and secretory immunoglobulin A to proteolysis. J Immunol 112:949–959, 1974
42. Lindh E: Increased resistance of immunoglobulin dimers to proteolytic degradation after binding of secretory component. J Immunol 114:284–286, 1975
43. Underdown BJ, Schiff JM, Nagy B, Fisher MM: Differences in processing of polymeric IgA and asialoglycoproteins by the rat liver. Ann NY Acad Sci 409:402–410, 1983
44. Feldman DG: Origin and distribution of virus-like particles associated with mammary tumors in DBA strain mice. I. Virus-like particles in mammary gland tissue. J Natl Cancer Inst 30:477–501, 1962
45. Ceriani RL, Thompson K, Peterson JA, Abraham S: Surface differentiation antigens of human mammary epithelial cells carried on the milk fat globule: Proc Natl Acad Sci 74:582–586, 1977
46. Hanson LÅ, Brandtzaeg P: The mucosal defense system. *In* Immunologic Disorders in Infants and Children, ER Stiehm, VA Fulginiti (eds). Philadelphia, WB Saunders, 1980. pp 137–164
47. Glass RI, Svennerholm A-M, Stoll BJ, Khan MR, Hossain KMB, Huq MI, Holmgren J: Protection against cholera in breast-fed children by antibodies in breast milk: N Engl J Med 308:1389–1392, 1983
48. Weaver EA, Tsuda H, Goldblum RM, Goldman AS, Davis CP: Relationship between phagocytosis and immunoglobulin A release from human colostral macrophages. Infect Immun 38–1073–1077, 1982