

The Cells of Human Colostrum

II. Synthesis of IgA and β 1c

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Extract

The production of IgG, IgA, IgM, β 1c, and the secretory piece (SP) by the cells of human colostrum was investigated by radioimmuno-electrophoresis. The synthesis of IgA and β 1c by the cells was demonstrated. No evidence, however, for the formation of IgG or IgM by colostrum cells was found. Similarly, no radiolabeling was found over the free SP immunoprecipitin lines, but labeling was detected over the bound SP immunoprecipitin lines. This may have been due to covalent binding of the SP with radiolabeled α chains of secretory IgA.

Speculation

IgA produced by the cells of human colostrum may be used in the formation of secretory IgA and may be an important source of antibodies in colostrum.

Introduction

In 1968, *in vitro* studies concerning the functions of the cells of human colostrum were reported from our laboratory [16]. Colostral cells were found to be primarily macrophages with a significant population of lymphocytes. It was demonstrated that the lymphocytes transformed in response to the presence of phytohemagglutinin or specific antigens and that the macrophages were phagocytic and possessed an abundant number of lysosomes.

To define further the immune functions of the cells of human colostrum, the ability of the cells to synthesize IgG, IgA, IgM, and β 1c was tested. The cells were cultured with ¹⁴C-labeled amino acids, and the radioactive amino acids incorporated into the newly synthesized proteins from the cultures were detected by immunoelectrophoresis and autoradiography [10].

Materials and Methods

Cell Collection

Prepartum and postpartum colostrum was collected from eight volunteer patients by means of a breast pump. Whole colostrum diluted 1:3 with Hanks' balanced salt solution (HBSS) was centrifuged at 300 \times g for 5 min. The sedimented cells were resuspended and washed three times in 5-ml volumes of HBSS. The washed cell pellet was resuspended in 1 ml of HBSS containing 1% neutral red and the viable cells were counted. A concentration of 1-5 \times 10⁶ mononuclear cells in a volume of 0.1-0.5 ml was added to 1 ml of the culture medium.

Peripheral blood leukocytes were collected and prepared for culture as follows. Leukocyte-rich plasma was removed from heparinized blood; it was diluted with 1 volume of HBSS and centrifuged at 300 \times g for

5 min. The cell sediment was washed twice and the cell concentration was adjusted to $1-5 \times 10^7$ cells in 0.1- to 0.5-ml volumes of HBSS.

Culture Medium

The culture medium [28] consisted of HBSS plus 0.5% dextrose, 0.5% ovalbumin, 1% BME-vitamins (100 \times concentrate), penicillin G (100 U/ml), and an amino acid mixture [13] from which L-lysine and L-isoleucine were omitted. The pH was adjusted to 7.4 with sodium bicarbonate. Uniformly labeled ^{14}C -L-lysine (247 mCi/mm) and ^{14}C -L-isoleucine (236 mCi/mm) [29] were added to the medium at a concentration of 1 $\mu\text{Ci/ml}$.

Cell Culture and Harvesting Procedures

Colostrum cells were cultured in Leighton tubes at 37 $^\circ$ in 5% CO_2 and 95% air without movement for 48 h. The cells were ruptured by three cycles of alternate freezing (-20°) and thawing. The resulting preparations were dialyzed against 1-liter volumes of 0.015 M NaCl for 48 h. The dialysates were concentrated by lyophilization. Immediately before use, the samples were reconstituted with distilled water to 0.1 ml.

Antisera

The specificity of each antiserum was tested by immunodiffusion and immunoelectrophoresis. Rabbit antisera specific for human serum IgG, IgA, and IgM [30] and goat antihuman $\beta 1\text{c}/\beta 1\text{a}$ [31] were obtained commercially. Rabbit antihuman secretory IgA was prepared by using secretory IgA isolated from pooled human nasal washings [34]. This antiserum was rendered specific for the antigenic determinants of secretory piece (SP) by absorption with normal adult human serum and lyophilized whole human spleen.

Immunoelectrophoresis

Immunoelectrophoresis was performed according to the micromethod of SCHEIDEGGER [15] with 0.85% agarose in pH 8.2, 0.045 M barbital buffer. Appropriate carriers (unlabeled human serum or colostrum) were added to the antigen wells to facilitate the development of reproducible immunoprecipitin lines [10, 25]. Three microliters of the carrier protein and 9 μl of the concentrated culture fluids were added to the antigen wells.

Autoradiography

Photographic sheet film [32] was placed in contact with the dried, stained slides for 2 weeks at room temperature. The film was developed with DK 60a [33] for 4 min. The autoradiographs were compared with the stained slides. An autoradiographic reaction cor-

responding to an immunoprecipitin line indicated that synthesis of the protein had occurred *in vitro* unless otherwise demonstrated by the control experiments.

Control Experiments

The following control experiments were performed: 1) human peripheral leukocytes cultured in the radioactive amino acid medium served as positive controls for synthesis of immunoglobulins [4, 26]; 2) normal human serum, cell-free human colostrum or colostrum cells previously ruptured by freezing and thawing were incubated in the radioactive medium; immunoelectrophoresis of the cultures was performed with the previously described antisera to exclude the possibility of adsorption of ^{14}C -labeled amino acids onto proteins [25]; 3) protein synthesis was inhibited by adding 300 μg of puromycin [27] or 2 μg of actinomycin D [24] to the cell cultures.

Results

Control Experiments

Evidence of the *in vitro* production of IgA, IgM, and less frequently IgG by human peripheral blood leukocytes was demonstrated as anticipated from previous studies [4, 26]. The variability of IgG synthesis in peripheral blood leukocyte cultures has been noted by CHESIN *et al.* [4].

Human serum, cell-free human colostrum, or ruptured colostrum cell preparations incubated in the radioactive medium showed no labeling of immunoglobulin or $\beta 1\text{c}$ precipitin lines. An absence of or a marked decrease in radioactive immunoprecipitin lines was detected with puromycin or actinomycin D-treated cultures.

Analysis of Colostrum Cell Cultures

No radiolabeled immunoprecipitin lines were found with antisera to IgG or IgM (table I). Radioactive immunoprecipitin lines were found in some of the anti- $\beta 1\text{c}/\beta 1\text{a}$ preparations. A distinct, evenly labeled immunoprecipitin line was demonstrated in all the anti-IgA preparations (table I).

Heavy radiolabeling of the entire precipitation line developed with antihuman secretory IgA was found in all instances (table I; fig. 1). The labeling of immunoprecipitin lines formed with antiserum IgA was more intense than labeling of lines formed with antiserum IgA.

When anti-SP was used, an immunoprecipitin line corresponding to the anodal portion of secretory IgA was demonstrated. Occasionally, an additional line of greater anodal mobility formed. Autoradiography

of the SP immunoprecipitin lines showed light but definite radioactive labeling that corresponded to the location of the fast portion of secretory IgA (table I). These reactions also appeared in certain preparations to which carrier protein (colostrum) was not added. No reactions were found over the more anodic SP immunoprecipitin lines.

Table I. Synthesis of proteins by human colostrum cells *in vitro*

Protein	Radiolabeled immunoprecipitin line
IgG	0/8 ¹
IgM	0/8
IgA	
Serum form	8/8
Secretory form	8/8
Secretory piece	8/8 ²
β 1c	2/5

¹ Number positive/total patients examined.

² Positive reactions may be due to binding of SP with radiolabeled α chains.

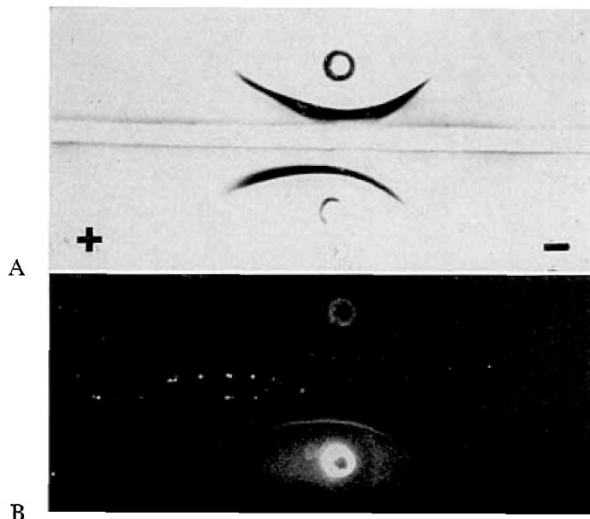


Fig. 1. A stained immunoelectrophoretic pattern (A) and a print of the corresponding autoradiograph (B) developed with antiserum to IgA in the trough and fluids from colostrum cells cultured with ¹⁴C-labeled amino acids in the antigen wells. Radiolabeling of the secretory IgA immunoprecipitin line is evident.

Discussion

The synthesis of IgA and β 1c by the cells of human colostrum was demonstrated by these experiments. No evidence, however, for the formation of IgG or IgM by colostrum cells was found. In this regard, it appears that colostrum cells differ from peripheral blood leukocytes, which produce IgG and IgM as well as IgA [4, 26], but are similar to the lymphoid cells found in the salivary glands, which primarily produce IgA [12].

It has been shown previously that β 1c is produced by macrophages but not by lymphocytes [18, 19]. Since the macrophages are the predominant cells in human colostrum, it is likely that they were responsible for the synthesis of β 1c in the cell cultures examined in this study. This possibility, however, is as yet unproven.

The immunoglobulin synthesized by colostrum cells is also the principal one found in human colostrum [1, 5, 6]. While the IgA in serum is primarily a monomer comprised of two α chains and two light chains, the IgA in colostrum and other exocrine secretions usually consists of two 7S IgA molecules bound to a 4.5S protein [3, 11, 14, 17, 21, 22]. This type of IgA is designated as secretory IgA [7, 20, 21] and the 4.5S protein is designated as the transport [17] or secretory piece (SP) [7, 21].

Evidence that cells of the human mammary gland produce IgA but no other immunoglobulins was first reported in 1964 [9]. Explants of mammary gland tissues from humans cultured *in vitro* with ¹⁴C-labeled amino acids were found to synthesize IgA in the absence of labeling of other immunoglobulins. Although no studies of the formation of the other components of secretory IgA by human mammary tissues have appeared, synthesis of these components in mice has been investigated. ASOFKY and HYLTON [2] demonstrated that lactating mammary glands of conventionally reared mice synthesized secretory IgA (11S), whereas lactating mammary glands of gnotobiotic mice lacking serum IgA produced a smaller protein (4S), but no IgA. They concluded that lactating mammary tissue from mice synthesizes a protein similar to the SP of human colostrum IgA.

The electrophoretic mobilities of the bound and free forms of the SP in colostrum have been described recently [7]. The bound form is located in the fast portion of secretory IgA, while the free form migrates more anodically. Bound SP was found consistently in our cultures of colostrum cells and the free form was demonstrated occasionally. We hypothesized that SP may be synthesized by colostrum cells, since SP immunoprecipitin lines developed from colostrum cells cultured with radioactive amino acids were radioactive. Since the position of the radiolabeled SP lines corresponded

to the bound rather than the free form of that protein we could not prove that SP was produced. An alternate explanation of these observations is that the labeling of the SP immunoprecipitin line was due to the existence of radioactive α chains bound covalently to SP. Using this interpretation, one can infer that α chains produced by colostrum cells were used to form secretory IgA and that the SP was not produced by the colostrum cells but was absorbed onto the surface of the cells or was contained by them. This interpretation agrees with the findings of most other investigators [8, 23] which indicate that SP is produced by the epithelial cells and not by the lymphoid cells of exocrine glands. Further experiments will be required to ascertain if either the secretory type of IgA or the SP is synthesized by the cells of human colostrum.

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