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Antibodies
β1c
colostral cells
human colostrum
IgA
lymphocytes
secretory piece
macrophages

The Cells of Human Colostrum
II. Synthesis of IgA and β1c

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Extract

The production of IgG, IgA, IgM, β1c, and the secretory piece (SP) by the cells of human colostrum was investigated by radioimmunoelectrophoresis. The synthesis of IgA and β1c by the cells was demonstrated. No evidence, however, for the formation of IgG or IgM by colostral 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 β1c was tested. The cells were cultured with ^{14}C-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 $1–5 \times 10^6$ 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× 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 μCi/ml.

Cell Culture and Harvesting Procedures

Colostral cells were cultured in Leighton tubes at 37° 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 β1c/β1a [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 corresponding 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 colostral 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 CHESSIN *et al.* [4].

Human serum, cell-free human colostrum, or ruptured colostral cell preparations incubated in the radioactive medium showed no labeling of immunoglobulin or β1c precipitin lines. An absence of or a marked decrease in radioactive immunoprecipitin lines was detected with puromycin or actinomycin D-treated cultures.

Analysis of Colostral 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-β1c/β1a 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 antisecretory 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 colostral 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]
β1c	2/5

[1] Number positive/total patients examined.
[2] Positive reactions may be due to binding of SP with radiolabeled α chains.

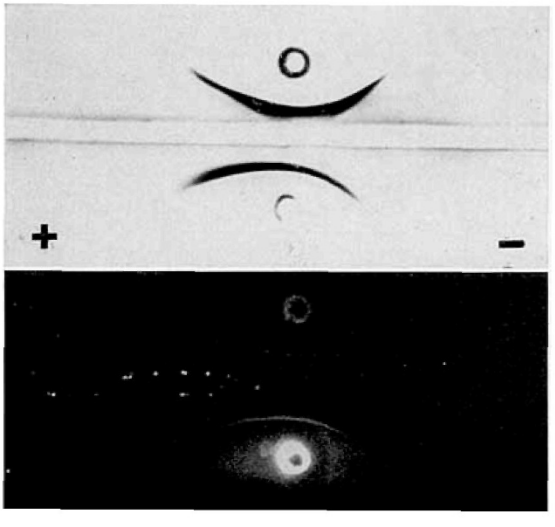

Fig. 1. A stained immunoelectrophoretic pattern (A) and a print of the corresponding autoradiograph (B) developed with antisecretory IgA in the trough and fluids from colostral cells cultured with ^{14}C-labeled amino acids in the antigen wells. Radiolabeling of the secretory IgA immunoprecipitin line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

Discussion

The synthesis of IgA and β1c by the cells of human colostrum was demonstrated by these experiments. No evidence, however, for the formation of IgG or IgM by colostral cells was found. In this regard, it appears that colostral 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 β1c 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 β1c in the cell cultures examined in this study. This possibility, however, is as yet unproven.

The immunoglobulin synthesized by colostral 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 ^{14}C-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. Asofsky 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 synthesize a protein similar to the SP of human colostral 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 colostral cells and the free form was demonstrated occasionally. We hypothesized that SP may be synthesized by colostral cells, since SP immunoprecipitin lines developed from colostral 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 colostral cells were used to form secretory IgA and that the SP was not produced by the colostral 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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