COMPARATIVE ANALYSIS OF IMMUNOCOMPETENT CELL SUBSETS AND SECRETORY IgA SYSTEM OF NEONATAL AND MATERNAL MILK

V. P. Chernyshov, I. I. Slukvin, and I. K. Galanina

UDC 612.664.017.1

KEY WORDS: neonatal milk; maternal milk; immunocompetent cell subsets; local immunity; ontogeny.

The leading role in defense against the majority of infectious agents and the formation of physiological interaction between the body and micro-organisms colonizing it in the neonatal period is played by protective mechanisms of the mucous membranes, one of the most important of which is what are known as immune factors. Attempts which have so far been undertaken to analyze local immunity in the newborn have not given a complete picture of the characteristics of cellular factors of immunity of the mucous membranes in the neonatal period, mainly due to the difficulties involved in obtaining material for investigation. Considering that the immune system of the mucous membranes includes lymphoid tissue associated with the intestine, lymphoid tissue associated with the bronchi, immunocompetent cells, and organized lymphoid follicles in the conjunctiva, salivary glands, upper respiratory passages, genitourinary tract, and mammary glands [7], we chose the mammary gland as a model with which to analyze the development of local immunity in the neonatal period.

The aim of this investigation was to study the character of development of local immunity in the neonatal period by studying the distribution of regulatory lymphocyte subsets, Ia-positive cells, free secretory component (Sc), and secretory IgA (SIgA) in maternal milk and in the secretion of the neonatal mammary gland (neonatal milk), formed during a sex crisis in an infant, assuming that milk formation in the neonatal mammary gland is induced by the same hormonal factors as in the maternal mammary gland [1].

EXPERIMENTAL METHOD

Observations were made on seven healthy full-term neonates aged 5-12 days with distinct manifestations of a sex crisis and on their seven mothers. The surface markers of immunocompetent cells in the blood and milk were analyzed by means of monoclonal antibiotic (from NEN, West Germany) against human Ia-antigen, monocytes, and cytotoxic (suppressors) and helper (inducers) T lymphocytes. The milk samples were diluted twice with medium 199 and centrifuged at 400 g for 15 min to remove fat; they were then washed twice with culture medium at 200 g

TABLE 1. Subpopulations of Immunocompetent Cells (in %) in Maternal and Neonatal Blood and Milk (M ± m)

Subpopulations of immunocompetent cells	Maternal		Neonatal		
	blood (1)	milk (2)	blood (3)	milk (4)	p
T lymphocytes					
Helpers (inducers)	33,8±4,1	30,8±2,9	31,8±2,5	30,3±4,7	
Cytotoxic (suppressors)	20,6±2,8	23,5±2,7	19,8±2,5	23,2±2,0	
Ia-positive cells	17,0±1,5	30,6±2,5	11,6±0,8	39,0±2,6	$p_{1-2}<0,001$ $p_{1-3}<0,01$ $p_{2-4}<0,05$ $p_{3-4}<0,0001$
Monocytes	6,1±0,8	33,8±1,6	8,5±1,3	35,2±5,8	$p_{1-2}<0,0001$ $p_{3-4}<0,001$

Laboratory of Immunology, Kiev Research Institute of Pediatrics, Obstetrics, and Gynecology, Ministry of Health of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR E. M. Luk'yanova.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 340-342, September, 1987. Original article submitted February 16, 1987.

0007-4888/87/0009-1277$12.50

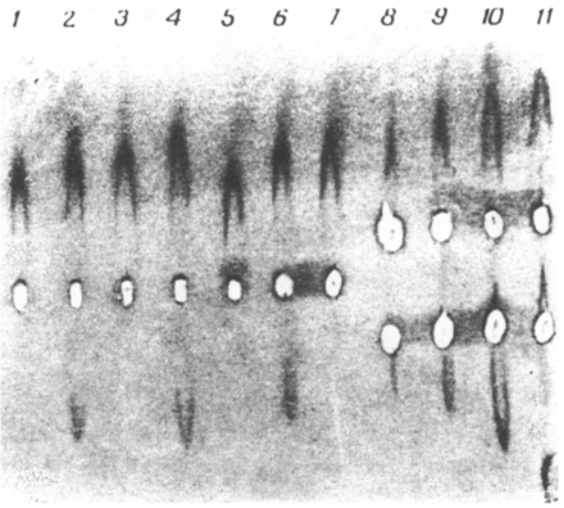

Fig. 1. Determination of SC and SIgA separately in neonatal and maternal milk by two-directional rocket immunoelectrophoresis. Conditions of electrophoresis: gel contains 1% agar (Difco, USA) and 0.6% agarose (chemapol, Czechoslovakia) in the ratio of 1:1, 1.5% antiserum to SIgA, Veronal–Medinal buffer, pH 8.6, ionic strength 0.025, with 0.001M EGTA. Wells contained 4 μl of standard preparation for the test sample. Electrophoresis for 15 h with gradient of 6 V/cm. 1, 3, 5, 7) Neonatal milk (dilution 1:8); 2, 4, 6) maternal milk (dilution 1:8); 8, 9, 10, 11) four twofold dilutions of standard preparations of Sc (above) and SIgA (below).

for 10 min, and mononuclear cells were isolated from maternal milk and maternal and fetal blood is a Ficoll–Verografin density gradient (d = 1.077). The isolated cells, numbering 4×10^5–5×10^5 in 0.2 ml of medium 199 with 5% fetal calf serum (IEM, Minsk) were incubated for 30 min with monoclonal antibodies, taken in optimal doses. Considering the small volume of the sample of neonatal milk and the relatively higher level of lymphocytes in it, the mononuclear cells of neonatal milk were labeled without preliminary isolation. After being washed twice, the cells were resuspended in 0.1 ml medium and incubated with 0.1 ml of diluted (1:50) fluorescein-conjugated $F(ab')_2$ immunoglobulin fragment of sheep serum against mouse immunoglobulins (from NEN). The cells were washed twice and resuspended in medium 199 with 30% viscerol. All operations were carried out at 4°C. The cells were spread out on a slide and studied under the LYUMAM I-1 microscope. Altogether 100-200 cells were counted. Only mononuclears were counted in neonatal milk. Luminescence in the control did not exceed 3-5%.

Sc and SIgA in milk were determined separately by the two-dimensional rocket immunoelectrophoresis method [5]. A Soviet-produced monospecific serum against human SIgA (I. I. Mechnikov Central Research Institute of Vaccines and Sera), the globulin fraction of which was obtained by salting out twice with ammonium sulfate, was used as the antiserum. The antiserum was carbamylated with NaCNO [3]. The samples were diluted eightfold before being introduced into the wells. Because of the marked excess of Sc over SIgA in neonatal milk and the impossibility of choosing optimal concentrations of antiserum to determine them simultaneously, the SIgA level in neonatal milk was estimated by radial immunodiffusion, using an anti-α-serum (IEM, Gorkii), and a commercial standard SIgA. Saliva from a 10-year-old child with selective IgA deficiency was used as the Sc standard. Samples of milk taken for analysis of Sc and SIgA were defatted by centrifugation for 30 min at 15,000g and 4°C, quickly frozen, and kept at −20°C. The results were subjected to statistical analysis by Student's t test and determination of the coefficient of correlation.

EXPERIMENTAL RESULTS

The total number of cells in the maternal and neonatal milk was $(1.2 \pm 0.2) \times 10^6$ and $(0.99 \pm 0.3) \times 10^6$/ml respectively. Comparison of the distribution of regulatory lymphocyte subsets in the mammary gland secretions and blood revealed a tendency for the number of cytotoxic (suppressor) lymphocytes to rise and the number of helper (inducer) lymphocytes to fall

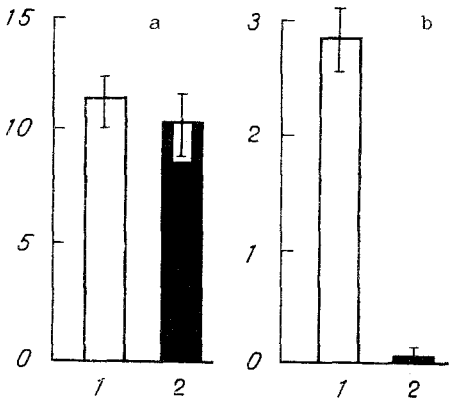

Fig. 2. Content of free Sc (in conventional units/liter) and SIgA (in g/liter) in maternal (1) and neonatal (2) milk. a) Sc, b) SIgA.

in both maternal and neonatal milk, but these differences were not statistically significant (Table 1). In maternal and neonatal milk the number of cells carrying monocytic antigen was considerably greater than in blood. Mathematical analysis showed no correlation between the percentage distribution of cell subsets in the blood and mammary gland secretions of neonates and mothers, confirming the view that local immunity is to some degree autonomous in the neonatal period already. The principles of distribution of the Ia-positive cells that were found are particularly interesting. Whereas in neonatal blood there were significantly fewer of these cells than in maternal blood, the opposite tendency was observed with respect to secretions (Table 1). The level of Ia-positive cells, as we know, is an indicator of functional activity of the immune system [2]. The neonatal period is evidently characterized by activation of local immunity when functional activity of systemic immunity is depressed. A close correlation was found between the SIgA level and the relative percentage of Ia-positive cells in maternal milk ($r = 0.915$, $p < 0.01$). This correlation can be explained by the participation of Ia-antigens in the activation of helper T cells, regulating antibody formation. A significant negative correlation was found between the free Sc level and the percentage of cytotoxic (suppressor) lymphocytes in maternal milk ($r = -0.908$, $p < 0.01$). The presence of this correlation was probably due to the need to create optimal conditions for SIgA synthesis. For instance, SIgA production is promoted by a high Sc level, for the latter plays the most important role in IgA transport [14] and is evidently a factor in the selective localization of precursors of IgA-synthesizing cells [6]. Meanwhile it is logical to suggest that a low suppressor T cell level would predispose to SIgA synthesis. Any direct effect of Sc on suppressor cells, or vice versa, is unlikely. It is more probable that the same factors, most probably hormonal, cause an increase in Sc production and a fall in the suppressor level, as a result of which favorable conditions are created for SIgA synthesis. No correlation could be found between cellular and secretory factors in neonatal milk, such as are characteristic of maternal milk. This fact is evidently explained by the relative immaturity of local immunity in the neonatal period, which is also reflected in a low SIgA level, accompanied by a high Sc level in neonatal milk (Figs. 1 and 2).

Activation primarily of the immune system of the mucous membranes thus takes place in the neonatal period, whereas functional activity of systemic immunity is depressed compared with that in adults. Meanwhile, in the newborn local immunity is relatively immature, as shown by the low SIgA level and absence of the correlations between Sc and cytotoxic (suppressor) lymphocytes, SIgA, and Ia-positive cells, characteristic of maternal milk. The distinctive features of the immune system of the mucous membrane in the neonatal period and its definite autonomy make it essential to use parameters characterizing local immunity when the immune status of neonates is assessed.

LITERATURE CITED

1. V. K. Zubovich, The Hormonal Crisis in the Newborn [in Russian], Minsk (1978).
2. N. V. Medunitsin, Usp. Sovrem. Biol., 100, No. 3, 410 (1985).
3. N. Axelsen and B. Weeke, A Manual of Quantitative Immunoelectrophoresis, Oslo (1973).
4. P. Brandtzaeg, Recent Advances in Mucosal Immunity, ed. by W. Strober et al., New York (1982), p. 267.

5. T. Kosaka, T. Asahina, and Kobayashi, J. Immunol., 40, 597 (1980).
6. W. Strober, R. Krakauer, and H. L. Klaeveman, N. Engl. J. Med., 294, 351 (1976).
7. T. B. Tomasi, The Immune System of Secretions, Englewood Cliffs, New Jersey (1976).

PRODUCTION OF MONOSPECIFIC ANTISERA TO IgA OF LABORATORY ANIMALS BY CASCADE IMMUNIZATION WITHOUT PRELIMINARY ANTIGEN ISOLATION

M. M. Lyubinskaya and E. V. Chernokhvostova

UDC 615.373.03:[57.083.33:57.082

KEY WORDS: mouse, rat, and guinea pig IgA; antiserum; precipitation lines.

Mice, rats, and guinea pigs are widely used in laboratory practice to study the principles governing the formation of the immune response and, in particular, to evaluate vaccine preparations. In infections of the mucous membranes a definite role for local immunity has been demonstrated, and its study requires determination of serum and secretory IgA and of antibodies bound with them. This raises the problem of obtaining monospecific antisera to the IgA of these laboratory animals.

The traditional method of obtaining antisera, based on isolation and purification of a protein antigen, is unsuitable for the present task because of the small volume of material, especially when the serum IgA level is low (guinea pigs), and the absence of an affinity adsorbent which is sufficiently specific for IgA. Isolation of IgA from secretions in which it is the dominant component is difficult because of the difficulty of obtaining secretions in sufficient amounts for fractionation. Various methods not requiring purification of the antigen, and based on the use of immune precipitates [1, 4, 6, 7, 11] or of other insoluble immune complexes [3, 9, 10], which include the required antigen, have been suggested. These methods, however, can yield monospecific antisera only in the early stages of immunization [4], for during long-term immunization with the same precipitate, obtained with a polyspecific antiserum, antibodies to impurities appear.

The method of cascade immunization suggested in this paper does not require isolation and purification of the antigen, but envisages the use of immune preparation including IgA. The specificity of the antisera is achieved by successive 2-3-stage (cascade) immunization with exchange of the antiserum-producing animals and source of IgA by means of which the precipitate for immunization is obtained.

To obtain the first-order antiserum to mouse IgA, a serum fraction obtained on DEAE-cellulose by elution with 0.01 M phosphate buffer, pH 8.0, was used. This fraction, according to the results of immunochemical testing, contained only IgG, but the serum obtained by immunization with this fraction gave a line not only of IgG, but also of IgA (Fig. 1a). To prepare immune precipitates, a mouse coprofiltrate was used: this was obtained by washing segments of the small intestine with a solution containing protease inhibitors [5], followed by tenfold concentration by precipitation with ammonium sulfate at 50% saturation.

To obtain antiserum to rat IgA two secretions were used: bile, obtained through a cannula inserted into the bile duct of the anesthetized animal, and saliva, stimulated by subcutaneous injection of 1 mg pilocarpine.

To obtain antiserum to guinea pig IgA we used tear fluid, secretion of which was stimulated by application of a piece of red pepper to the conjunctiva of the eye, and colostrum, clarified by centrifugation at 10,000 rpm.

To remove antibodies to IgG present as impurities in antisera to IgA, adsorbents prepared from the IgG fractions of mouse, rat, and guinea pig sera were used. The fractions were obtained on DEAE-cellulose, as described above for mouse serum.

Laboratory for the Study of Antibody Structure and Function. G. N. Gabrichevskii Moscow Research Institute of Epidemiology and Microbiology, Ministry of Health of the RSFSR. (Presented by Academician of the Academy of Medical Sciences of the USSR B. A. Lapin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 9, pp. 343-344, September, 1987. Original article submitted July 10, 1986.

 0007-4888/87/0009-1280$12.50