LACTOFERRIN RECEPTORS ON HUMAN THYMIC LYMPHOCYTES. STIMULATION OF EXPRESSION BY ADENOSINE, THEOPHYLLINE, AND SUPERNATANT OF THYMIC LYMPHOCYTES

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Lactoferrin, a glycoprotein with molecular weight of 75,000 daltons, was first discovered in colostrum, and later in other excretory fluids such as saliva, tears, and mucus covering the epithelium of the respiratory and alimentary tracts, the endometrium of the uterus, and certain other organs. Lactoferrin has been shown to be synthesized by the epithelial cells of these organs [13], and also by blood neutrophils [10, 12]. The study of the function of lactoferrin has shown that it has a bacteriostatic action and also participates in Fe^{++} transport [12, 13]. Immunomodulating activity has recently been stated to be present in lactoferrin: It stimulates the suppressor function of macrophages, leading to inhibition of antibody synthesis [6, 9].

The cellular and structural elements of the thymus are known to contain heteroorganic antigens common with some highly specialized tissues [1]. The discovery of large numbers of cells synthesizing lactoferrin in the thymus suggested that it also belongs to the system of heteroorganic antigens [2]. Accordingly, and also in connection with discovery of lactoferrin receptors on macrophages and lymphocytes of pheripheral lymphoid organs [8], it is interesting to examine whether thymus lymphocytes also possess receptors for lactoferrin.

The aim of this investigation was to study interaction between lactoferrin and thymus lymphocytes and the effect of certain preparations modulating the intracellular cAMP concentration (theophylline, adenosine, levamisole), on this process, by an immunofluorescence method. To determine the ability of thymus lymphocytes to affect suppression of lactoferrin receptors the action of a supernatant obtained after incubation of thymocytes in medium for 3 h at 37°C on this process was studied.

EXPERIMENTAL METHOD

An immunofluorescence study was undertaken of thymus lymphocytes from children undergoing operations for the correction of congenital heart defects at the age of 13-14 years (13 cases). The lymphocytes were washed twice in Eagle's medium containing 10% inactivated bovine serum, a suspension containing $20 \cdot 10^6$ cells/ml was prepared, and this was allowed to stand overnight at 4°C in an excess of medium. After washing, the cells were incubated for 1 h at 37°C in 0.1 ml of lactoferrin solution (400 μg/ml), washed twice, and treated for 1 h at 37°C with antibodies against lactoferrin (0.1 ml, concentration 400 μg/ml). The cells washed off with medium were incubated for 45 min at 37°C in 0.1 ml of antibodies against rabbit IgG, labeled with fluorescein isothiocyanate (FITC). The number of labeled cells was counted in 500 small lymphocytes revealed during simultaneous observation in blue-violet light and with a phase-contrast system. To prove the specificity of interaction of the thymus lymphocytes with lactoferrin, the cells were incubated for 1 h at 37°C with lactoferrin, ovalbumin, or *Salmonella typhi* Vi-antigen (this last preparation was generously supplied by N. S. Sergeeva, Laboratory of Immunology of Enteric Infections, N. F. Gamaleya Research Institute of Epidemiology and Microbiology), washed, treated again with lactoferrin, after which the number of lymphocytes

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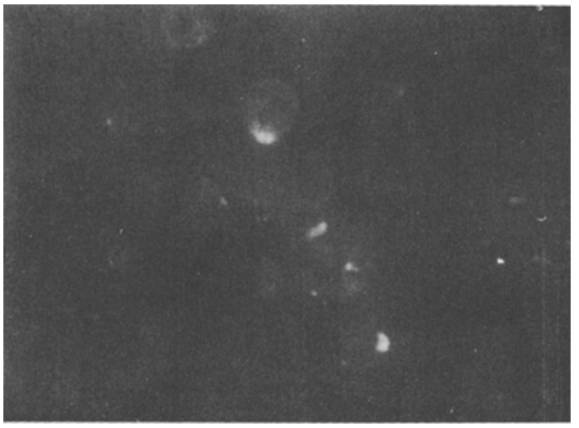

Fig. 1. Detection of lactoferrin receptors by immunofluorescence method on human thymic lymphocytes. Lymphocytes incubated consecutively with human lactoferrin, rabbit antibodies against lactoferrin, and FITC-labeled antibodies against rabbit IgG.

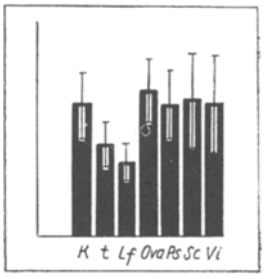

Fig. 2. Effect of antigens on ability of thymic lymphocytes to bind lactoferrin. K) Original number of lymphocytes binding lactoferrin. t) After incubation in medium for 1 h at 37°C, Lf) after retreatment with lactoferrin, Ova) after incubation of lymphocytes with ovalbumin, Ps) with group A streptococcal polysaccharide, Sc) with secretory components, Vi) with *S. typhi* Vi-antigen. Ordinate, number of thymic lymphocytes binding lactoferrin (in %).

with lactoferrin receptors was determined. Because the thymus contains a secretory component [3] and an antigen common with cells of the cambial layer of certain epithelia, cross-reacting with streptococcal polysaccharide [11], the effect of these heteroorganic antigens on the ability of thymus lymphocytes to bind lactoferrin also was studied. In the control thymus lymphocytes were treated with rabbit antibodies against lactoferrin and with labeled antibodies against rabbit IgG only or with antibodies against rabbit IgG only. To study the effect of theophylline on expression of lactoferrin receptors, $2 \cdot 10^7$ lymphocytes were incubated for 1 h at 37°C in 0.1 ml of a 3 mM solution of theophylline, washed twice in excess of medium, after which the number of lymphocytes binding lactoferrin was determined. In the same way thymus lymphocytes were incubated in 0.1 ml of a 0.5 mM solution of adenosine, 0.1 ml of levamisole solution (24.5 g/ml), or 0.2 ml of supernatant of thymus lymphocytes. To obtain the supernatant the lymphocytes were incubated for 3 h at 37°C at the rate of $2 \cdot 10^6$ cells to 0.2 ml of medium containing 10% inactivated bovine serum. The cells were then removed by centrifugation and the supernatant was filtered and kept at −20°C.

Lactoferrin and secretory components were isolated from human colostrum by the method described previously [4]. To remove any secretory IgA present as an impurity, the preparation of secretory component was absorbed with antiserum against serum IgA, treated with glutaraldehyde. Antibodies against lactoferrin were isolated from rabbit antiserum with the aid of an immunosorbent, namely pure lactoferrin treated with glutaraldehyde. In the same way, antibodies against this immunoglobin were isolated from the serum of a donkey immunized with rabbit IgG and conjugated with FITC. Group A streptococcal polysaccharide was isolated by the formamide method [7].

TABLE 1. Stimulation of Expression of Lactoferrin Receptors on Thymic Lymphocytes by the Action of Adenosine, Theophylline, and Supernatant of Thymic Lymphocytes (in %)

No. of investigation	Control	Treatment with				
		t	Te	A	D	Le
1	0	0,7	4	5	2	3
2	0,3	2	4	4	15	1
3	3	3	6	11	7	2
4	1,2	0	2,5	5	17	0,5
5	2	0	12,6	—	12	1,6
6	1,2	0,5	5	7	5	1
7	0,5	1,4	1,2	7	2,5	0
8	10	9	16	5	—	18
9	12	5	23	10	—	0
10	6	2	5	1	5	5
11	6	6	6	5	7	2
12	5	5	5	5	20	3
13	2	—	2	2	2	1
Mean	3,7±0,9	2,6±0,8	7,1±0,8	5,6±2	8,5±2	2,9±1,3

Legend. Control) Original number of cells binding lactoferrin, t) on incubation of lymphocytes in medium for 1 h at 37°C without addition of preparations; Te) after treatment of lymphocytes with theophylline, A) with adenosine, D) with thymocyte supernatant, Le) with levamisole.

EXPERIMENTAL RESULTS

On consecutive treatment of human thymus lymphocytes with lactoferrin, rabbit antibodies against lactoferrin, and FITC-labeled antibodies against rabbit IgG fluorescence of single punctate structures, sometimes in the shape of a cap (Fig, 1), was observed on the surface of individual cells (by their morphology, small lymphocytes). The number of lymphocytes binding lactoferrin averaged 3.7 ± 0.9%. In control experiments, in which lymphocytes were treated with antibodies against lactoferrin and antibodies against rabbit IgG only, labeled cells were absent or did not exceed 0.3% in number. Similar results were obtained on treatment of the lymphocytes with FITC-labeled antibodies against rabbit IgG only. On consecutive double treatment of the cells with lactoferrin (incubation for 1 h, washing off, and reincubation with lactoferrin) a decrease was observed in the number of lymphocytes binding lactoferrin compared with the control, in which the cells were incubated only once with lactoferrin (Fig. 2). Preincubation of thymic lymphocytes with other heteroorganic antigens (secretory components, group A streptococcal polysaccharide), and with foreign antigens (ovalbumin, *S. typhi* Vi-antigen) did not affect the ability of the lymphocytes to bind lactoferrin (Fig. 2).

The results of the study of the effect of preparations modulating the intracellular cAMP concentration and of the supernatant of thymic lymphocytes are given in Table 1. They show that incubation of lymphocytes in medium for 1 h without the addition of the preparations led to a small (not statistically significant) reduction in the number of lymphocytes binding lactoferrin. Under the influence of theophylline, expression of lactoferrin receptors was found to be stimulated, with an increase in the number of cells binding lactoferrin to 7.1 ± 1.97% on average. In four cases the number of lymphocytes binding lactoferrin was unchanged by theophylline. Under the influence of adenosine, the number of cells binding lactoferrin was increased in six of the 12 cases studied to 5.6 ± 2% on average. In two cases the number of lymphocytes with receptors for lactoferrin was reduced, and in four cases adenosine had no effect on the ability of the lymphocytes to bind lactoferrin. Absence of a stimulating effect of theophylline and adenosine, incidentally, was observed as a rule in the same cases, i.e., during their action on lymphocytes from the same thymus. As Table 1 shows, supernatant of thymus lymphocytes had a marked stimulating effect on expression of lactoferrin receptors: The number of cells with lactoferrin receptors was increased by the action of the supernatant on average to 8.5 ± 2% and the increase was observed in eight of the 11 cases studied: In three cases the number of lymphocytes binding lactoferrin was unchanged by the action of the supernatant. The results showed that levamisole does not affect the ability of thymus lymphocytes to bind lactoferrin: An increase in the number of cells binding lactoferrin during the action of levamisole was observed in three cases, a decrease in four cases, and no effect in five cases.

The investigation thus showed that the human thymus contains a subpopulation of lymphocytes capable of binding lactoferrin, evidently because of the presence of special receptors on their surface. Evidence in support of the specific (i.e., receptor) character of lactoferrin binding by thymic lymphocytes is given by the absence of an additive effect on retreatment of the cells with lactoferrin, and also by the fact that preincubation of the cells with other heteroorganic and foreign antigens does not affect the number of lymphocytes binding lactoferrin. Expression of lactoferrin receptors depends on the intracellular cAMP concentration, for preincubation of thymic lymphocytes with preparations raising the intracellular cAMP concentration (adenosine, theophylline) leads to an increase in the number of cells binding lactoferrin. Meanwhile levamisole, which lowers the intracellular cAMP concentration [5], does not affect expression of lactoferrin receptors on thymic lymphocytes. According to the results, supernatant of thymic lymphocytes had the strongest stimulating effect. The fact that the effect of adenosine, theophylline, and supernatants of thymic lymphocytes is manifested in the course of 1 h indicates that what happens in this case is unmasking of pre-existing receptors and not their synthesis *de novo*. Potential ability to bind lactoferrin is evidently a property of many cells, but the corresponding receptor on undifferentiated lymphocytes is masked and is expressed only in the course of their functional maturation. Ability of lactoferrin receptors to undergo expression under these circumstances is determined by certain differentiating factors of the thymus, including those secreted by lymphocyte subpopulations in the organ itself. The possibility of increasing the number of cells with lactoferrin receptors with the aid of substances facilitating intracellular accumulation of cAMP, and also of thymocyte supernatant, can be used for the enrichment of this subpopulation during its isolation for the purpose of studying its function and determining the effect of this heteroorganic antigen on thymic lymphocytes. It must also be noted that the presence of lactoferrin receptors may be a marker for the detection and study of the corresponding lymphocyte subpopulation in the thymus and peripheral lymphoid organs under normal and various pathological conditions.

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