LACTOFERRIN-INDUCED STIMULATION OF Fcμ and Fcγ RECEPTOR EXPRESSION ON THE SURFACE OF HUMAN THYMUS LYMPHOCYTES IN VITRO

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Antigens common to a number of highly specialized tissues (hetero-organic antigens) are known to be present in the epithelial tissue of the thymus. For instance, the myoid cells of the thymus contain antigens common with antigens of muscle tissue [12], and antigens common with antigens of several types of surface epithelium have been found in the cytoplasm of the cells of Hassall's corpuscles and cells of the epithelial reticulum [1]. The thymus has been shown to contain lactoferrin and a secretory component [3, 4], which are present in the secretions produced by epithelium of the mammary, salivary, and lacrimal glands and of the alimentary and respiratory tracts, the endometrium of the uterus, and certain other organs [13, 14]. Neutrophils also are important producers of lactoferrin [14]. The presence of receptors for lactoferrin has been demonstrated on macrophages and lymphocytes of the peripheral lymphoid organs [7, 10] and thymus [5]. The ability of theophylline, adenosine, and the supernatant after decantation of thymocytes to stimulate expression of lactoferrin receptors on thymocytes [5] provides the basis for a method of isolating this lymphocyte subpopulation of the thymus and studying the concrete mechanisms of action of lactoferrin on it. Another possible way of shedding light on this problem is by studying the action of lactoferrin on thymus lymphocyte subpopulations which can be identified through the presence of certain markers on their surface, such as, for example, those for IgM and IgG receptors (T_μ and T_γ cells of the thymus).

The aim of the present investigation was accordingly to study the effect of lactoferrin in vitro on expression of receptors for IgM and IgG on thymus lymphocytes.

EXPERIMENTAL METHOD

Thymus lymphocytes from children undergoing surgery at the age of 13 years for a congenital heart defect (11 cases) were investigated by an immunofluorescence method.

Lymphocytes freed from stroma of the thymus were washed twice in Eagle's medium with the addition of 10% bovine serum, a suspension containing $2 \cdot 10^7$ cells in 1 ml was prepared, and it was allowed to stand overnight at 4°C in an excess of medium. Next day the thymocytes were washed, incubated for 1 h at 37°C in 0.1 ml of a solution of human IgM or IgG (concentration 400 μg/ml), washed again, and then treated for 45 min at 37°C with FITC-labeled globulin fractions against the corresponding immunoglobulin class. The number of thymocytes with receptors for the Fc-fragment of IgM and IgG (T_μ and T_γ cells) was counted per 1000 lympyocytes, during simultaneous observations in blue-violet light and with a phase-contrast system. Cells in the control were treated with labeled globulin fractions against human IgM or IgG only. In cases when cells binding the anti-IgM- or anti-IgG-preparation were found in the suspension, their number (usually not more than 0.2%) was subtracted from the number of T_μ or T_γ cells discovered in that particular thymus.

When the effect of lactoferrin on the ability of thymocytes to express receptors for the Fc fragment of IgM and IgG was studied the cells were incubated for 1 h at 37°C in 0.1 ml of lactoferrin solution (concentration 400 μg/ml), and the number of T_μ and T_γ cells was counted after washing.

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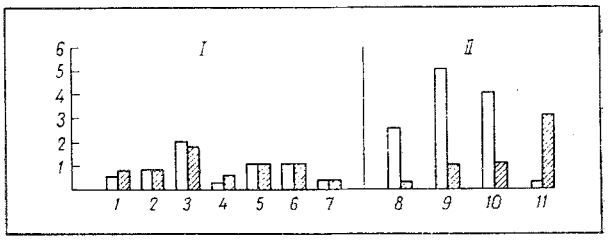

Fig. 1. Relative numbers of T_μ and T_γ cells in human thymus. Here and in Figs. 2 and 3: I) group of donors (1-7) with equal numbers of T_μ and T_γ cells in the thymus; II) group of donors (8-11) with different initial numbers of T_μ and T_γ cells in the thymus. Unshaded columns — number of T_μ cells in thymus: shaded columns — number of T_γ cells in thymus.

IgM and IgG were isolated from the serum of myeloma patients by the usual method on DEAE-cellulose.

Lactoferrin was isolated from human colostrum by gel-filtration on Sephadex G-200 [6].

EXPERIMENTAL RESULTS

The results showed that the number of T_μ cells in the human thymus can vary considerably, between 0 and 6%. Individual differences also were found in the number of T_γ cells, which varied in the cases studied from 0 to 3%. Analysis of the data showed that, depending on the ratio between the numbers of T_μ and T_γ cells in the thymus, all the cases investigated could be divided into two groups. In most cases a low level of T_μ and T_γ cells was observed in the human thymus, with about equal numbers of each (Fig. 1). In four cases considerable differences were found in the number of T_μ and T_γ cells. In three cases there were more T_μ than T_γ cells, and in one case the opposite situation was found: a low level of T_μ cells in the thymus accompanied by a raised level of T_γ cells (Fig. 1).

The results of a study of the effect of lactoferrin on T_μ cells of the human thymus are given in Fig. 2. They show that under the influence of lactoferrin on the thymocytes of the group I donors an increase in the number of T_μ cells was observed in all seven cases. In the control, when the thymocytes were incubated without the addition of lactoferrin for 1 h at 37°C there was no change in the number of T_μ cells. By contrast, when the initial level of T_μ cells was higher (group II), the number of T_μ cells in the control experiments was lower than initially in all three cases (Fig. 2). Treatment of the thymocytes with lactoferrin in two cases led to a decrease in the number of T_μ cells (Fig. 2, Nos. 8 and 9), and in one case (donor No. 10) it was unchanged compared with the initial number of T_μ cells in the thymus of this donor. It must also be pointed out that in group II, in one case in which the initial number of T_μ cells was low, after treatment of the thymocytes with lactoferrin the number of these cells increased (Fig. 2, Nos. 11).

The results of the study of the effect of lactoferrin on T_γ cells of the human thymus are given in Fig. 3. According to these results, as a result of the action of lactoferrin on thymocytes of the group I donors an increase in the number of T_γ cells was observed in four cases, in two other cases the number of T_γ cells was reduced, and in one case their number was unchanged.

Under the influence of lactoferrin on thymocytes of the group II donors an increase in the number of T_γ cells was observed in all four cases (Fig. 3). In the control, i.e., when thymocytes were incubated without the addition of lactoferrin, the number of T_γ cells was unchanged in all cases, and for that reason the results of these experiments are not shown in Fig. 3.

The results of this investigation thus show that lactoferrin, a hetero-organic antigen of the thymus, possesses immunomodulating activity, which is manifested as stimulation of expression of receptors for the Fc fragment of IgM and IgG on the surface of its lymphocytes. According to these results, if the initial number of T_μ cells in the thymus is low lactoferrin stimulates expression of receptors for the Fc fragment of IgM on the thymocytes, and thus promotes differentiation of T_μ cell precursors in the thymus. By contrast, if the initial number of T_μ cells is high, lactoferrin either inhibits expression of Fc_μ receptors on T_μ cells, for their number decreased in response to its action by an even greater degree than in

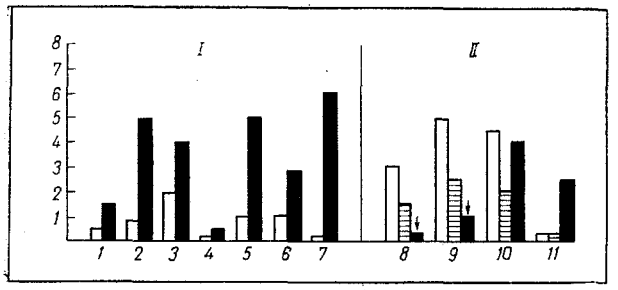

Fig. 2. Effect of lactoferrin on T_μ cells of the human thymus. Unshaded columns — initial number of T_μ cells in thymus; horizontally shaded columns — number of T_μ cells in control (after incubation for 1 h in medium at 37°C), black columns — number of T_μ cells after incubation with lactoferrin.

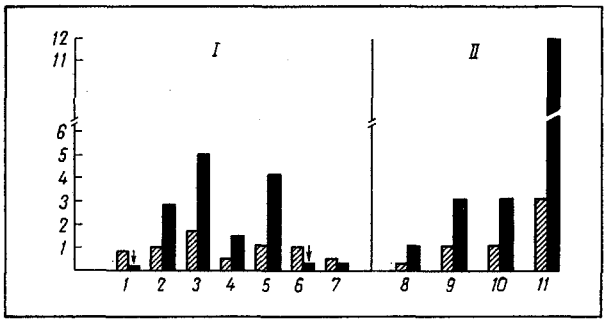

Fig. 3. Effect of lactoferrin on T_γ cells in the human thymus. Obliquely shaded columns — initial number of T_γ cells in thymus, black columns — number of T_γ cells after incubation with lactoferrin.

the control (incubation of cells in medium without the addition of lactoferrin), or it has no effect on the ability of the T_μ cells and their precursors to express Fc receptors for IgM. This dependence of the effect of lactoferrin on the initial number of T_μ cells in the thymus is evidence that its influence on this thymocyte subpopulation is regulatory in character and is aimed at maintaining the number of T_μ cells in the thymus at a certain level.

By contrast the character of the effect of lactoferrin on T_γ cells is independent of their initial number in the thymus, and it is evidently determined by certain features of their state (for example, their stage of differentiation). As a rule, under these circumstances, lactoferrin increased the number of T_γ cells, and only in a few rarer cases was its action aimed at lowering the level of these cells in the thymus.

The results are thus evidence that lactoferrin is a factor in the internal medium of the thymus which, together with its other factors, participates in differentiation of the T_μ and T_γ cells of the organ and regulates their relative numbers in the thymus.

We know that lactoferrin not only has a bacteriostatic action and participates in Fe^{++} ion transport, but it also influences the functional activity of macrophages. It has been shown that it inhibits the ability of macrophages to produce a factor stimulating colony formation between these cells and neutrophils [8, 9]. Meanwhile lactoferrin stimulates the suppressor activity of macrophages which, in turn, inhibits antibody synthesis by B lymphocytes [11]. Since receptors for lactoferrin are found not only on macrophages [10], but also on B cells [7], it can be postulated that lactoferrin can modulate the functional activity of these cells directly. Our observations showing the presence of receptors for lactoferrin on thymocytes suggest that these receptors also are present on T cells of the peripheral lymphoid organs and that lactoferrin influences the function of these cells. Thus it follows from all the available data and the results of the present investigation that lactoferrin takes part in the regulation of different components of the immune system, at the level both of its central organ, the thymus, and of the peripheral lymphoid organs.

It was noted above that the thymus contains antigens characteristic of muscle tissue and of the surface and secretory epithelium. The results of the present investigation, indicating that lactoferrin influences maturation of the T_μ and T_γ cells of the thumus, suggests that the

function of hetero-organic antigens is as follows: like other factors of the thymus they have an influence on differentiation of the various subpopulations of its lymphocytes, including the T_{μ} and T_{γ} cells of the organ.

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EFFECT OF POLYPEPTIDES FROM THE THYMUS, BONE MARROW, AND BURSA OF FABRICIUS ON IMMUNOGENESIS AND HEMOSTASIS IN NEONATALLY THYMECOTIMIZED AND ANTENATALLY BURSECTOMIZED CHICKENS

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Polypeptide factors from the thymus (thymosin, thymalin, etc.), bone marrow (antibody producer stimulator, hemalin, etc.), and bursa of Fabricus (bursilin) are known to act on different stages of lymphocyte differentiation [4, 6, 8, 9]. Thymalin has been shown to activate differentiation of T lymphocyte precursors selectively into mature T cells and also to correct disturbances of immunity and hemostasis in thymectomized rats [6, 10]. Injection of polypeptide factors from the bursa of Fabricius into neonatally bursectomized chickens normalizes the immune response to T-dependent and T-independent antigens and abolishes disturbances in the regulation of hemostasis [4]. Experiments on mice, rats, and guinea pigs have shown that hemalin stimulates the formation of B lymphocytes and increases their number in the circulation, in the thymus, and in the spleen of thymectomized animals, and also stimulates proliferative processes in the bone marrow [7].

Neonatal thymectomy in birds leads to selective blocking of differentiation of T lymphocytes and to disturbances of cell-mediated immunity [13], whereas bursectomy in ovo is accompanied by profound changes in humoral immunity, which are much more marked than those after neonatal bursectomy [12].

Considering that the state of immunity of an organism is reflected in the course of its hemostatic responses [3] it was decided to assess the level of immunity, hemostasis, and fi-

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