Binding of Fc Receptors on Blood T and B Cells with IgG from Healthy Cows and Cows with Chronic Lympholeukemia

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Pure population of T and B cells was obtained by a combined method consisting in lymphocyte fractionation through Nylon wool and precipitation of E-rosettes in 17% Verograffin density gradient. Scatchard analysis showed that T and B cells isolated from the blood of healthy cattle bind similar quantities of IgG molecules. Binding of IgG to Fc receptors of T and B cell isolated from animals with chronic lympholeukemia increases. The expression of Fc receptors for IgG is changed in cattle with chronic lympholeukemia.

Key Words: *T and B cells; Fc receptors; kinetic parameters of binding; chronic lympholeukemia*

The main function of Fc receptors (FcR) expressed on blood lymphocytes is presumably their participation in the regulation of antibody production [12], but their role in the development of leukemia is not quite clear.

Quantitative studies of binding capacity of FcR expressed on cattle lymphocyte showed that these receptors were characterized by different capacity to bind IgG molecules in health and chronic lympholeukemia (CLL) [3]. It was therefore interesting to clear out, which population of functionally different T and B cells is more expended during the interaction of FcR with IgG molecules.

Our aim was to characterize surface receptors for IgG FcR fragment on cattle T and B cells by quantitative parameters of binding.

MATERIALS AND METHODS

Cattle blood lymphocytes were isolated from healthy cows and cows with CLL [2]. Lymphocytes were passed through Nylon wool (Fenwal Laboratories L. T. 242 typ. 200) as described previously [4] and T-cell- and B-cell-enriched fractions were thus obtained. B-cells in fraction 2 were isolated by E-rosette precipitation. Sheep erythrocytes were 3 times washed in normal saline and treated with fresh 2-S-aminoethyl-isothiouronium bromide (AET; Serva) solution for stabilization of E-rosettes. E-rosettes formed by T-cells were determined as described previously [2]. B-cell-rich fraction (1×10^6 cells/ml) was mixed 4:1 with AET-treated 2.5% sheep erythrocytes, incubated for 1 h at 37°C with mixing every 5 min, centrifuged at 1000 rpm for 5 min, carefully resuspended, and layered onto 17% Verograffin density gradient. After 20-min centrifugation at 2500 rpm E-rosette-forming T cells were precipitated on the bottom, while the interphase contained B-cell-rich suspension. B cells were washed 2-3 times in medium 199. E-rosettes were isolated in T-cell-rich fraction by the method of E-rosette formation, and lymphocytes containing surface immunoglobulins were isolated by the immunofluorescent method. FITC-labeled rabbit antiserum to bovine globulins (Sigma) was used in the reaction. IgG isolated from the blood of healthy cows [11] were labeled with FITC [1].

Binding of FITC-labeled IgG with Fc receptors on T and B cells were carried out as described previously

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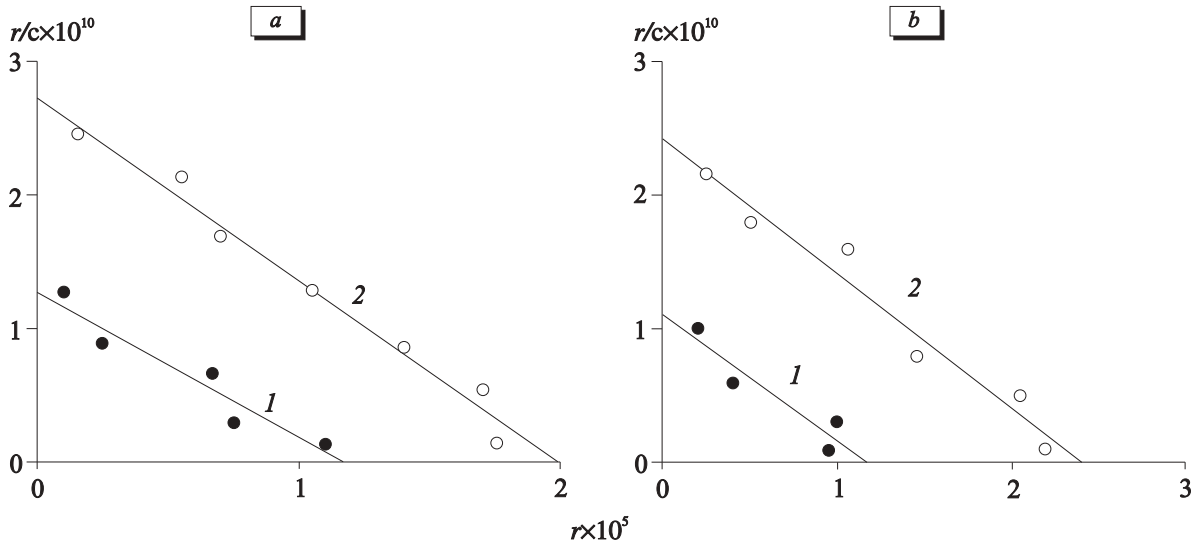

Fig. 1. Scatchard plot for specific binding of FITC-IgG with T (*a*) and B cells (*b*) from normal cows (*1*) and cows with chronic lympholeukemia (*2*). Abscissa: bound IgG (mol); ordinate: the ratio of bound moles IgG (r) to free moles IgG (c) r.

[9]. For evaluation of quantitative parameters of binding (association constant K_a and maximum number of IgG molecules bound to one cell (*n*) by lymphocyte FcR of healthy cows and cows with CLL), total binding of FITC-labeled IgG (FITC-IgG) to blood lymphocytes without nonlabeled IgG was determined. Nonspecific binding was evaluated under the same conditions in the presence of 20-fold excess of non-labeled IgG. Specific binding was determined as the difference between total and nonspecific binding. For evaluation of the relationship between fluorescence intensity and concentration of labeled IgG in cell lysate calibration curves were constructed in each experiment (IgG concentration range 0.8-28.0 nM). The results of FcR binding to FITC-IgG were estimated [9]. Fluorescence intensity in cell lysates was measured on an MPF-4 spectrophotometer (Hitachi) at λ=493 nm and fluorescence peak 520 nm.

The results were processed using Student's *t* test.

RESULTS

Homogenous populations of T and B cells were isolated for evaluation of quantitative parameters FITC-IgG binding to FcR on T- and B-cells. A simple and reproducible method of fractionation on a column with Nylon wool is most appropriate for obtaining T- and B-cell-rich suspensions. Therefore, T and B cells were separated by a combined method (fractionation on a column with Nylon wool and precipitation of E-rosettes in 17% Verografffin density gradient). Due to combined fractionation method, 89.1±1.2% T cells formed E-rosettes and 97.3±1.2% B cells contained surface immunoglobulins, *vs.* 85.0±14.1 and 95.0±2.5%, respectively, in other studies [6]. In order to evaluate quantitative parameters of FITC-IgG binding to T- and B-cell FcR, cell binding with FITC-IgG was studied at concentrations of 4 to 15 μM. Scatchard analysis showed that both T and B cells from the blood of healthy animals bound similar numbers of FITC-IgG molecules with similar affinity (Fig. 1). It was shown that both T and B cells from cows with CLL also bound similar numbers of FITC-IgG with similar affinity.

The binding capacity of T- and B-cell FcR from cows with CLL was almost 2-fold higher than that of cells from healthy animals. B cells exhibited more pronounced capacity to bind FITC-IgG (Table 1).

TABLE 1. Parameters of Serum Homologous IgG Binding to FcR of T and B Cells from Normal Cows and Cows with CLL (*M*±*m*)

Group	T cells		B cells	
	K_a, 10^5 M^{-1}	N, 10^5 mol/cell	K_a, 10^5 M^{-1}	N, 10^5 mol/cell
Normal	1.02±0.23	1.22±0.20	1.04±0.21	1.17±0.25
CLL	1.04±0.26	2.00±0.42*	0.93±0.18	2.23±0.31**

Note. *$p<0.001$, **$p<0.005$ compared to healthy cows. *n*: maximum number of IgG bound to one cell.

Published data on the presence of FcR on T and B cells are scanty and hardly comparable. This can be explained by the use of different methods for FcR detection. We used direct binding of FITC-IgG to FcR on T and B cell. We found no published data about FcR expression on T and B cell, and therefore we compared our results with the results obtained by other methods for detection of FcR on T and B cell.

Expression of FcR on the surface of T and B cells probably depends on other surface structures on lymphocyte plasma membrane [10]. Mouse splenic B cells with surface IgG, IgD, IgM, and Ia antigens express 2×10^4 FcR for IgG per cell, while B cells having only IgG express 8×10^4 FcR for IgG per cell. It was also found that about 20% T cells with surface Thy-1, Lyt-1, and Lyt-2 antigens express FcR for IgG [10]. T cells containing Lyt-2 antigen express 8×10^4 FcR for IgG, those having no Lyt-2 express 2×10^4 FcR for IgG per cell. No significant direct correlation between IgG FcR expression and surface markers of T and B cells was detected [10].

The number of FcR on T and B cells isolated from the blood of cows with CLL was almost 2-fold higher than in healthy cows. T-cell population of healthy cows and animals with CLL possesses FcR to Fc fragments of IgM and IgG (Tμ and Tγ, respectively). Tμ and Tγ cell subpopulations with these receptors formed stable E-rosettes. The biological role of Tμ and Tγ cells in cows is not yet clear. The results indicated predominance of Tγ cell subpopulation in the suspension of T cells isolated from the blood of cows with CLL. It is noteworthy that the leukemic process involves primarily Tγ cell subpopulation, which acquires more pronounced expression of FcR. It was shown previously that 22.3% blood cells of normal cows and 70.4% lymphocytes of cows with CLL carry FcR to IgG Fc fragment.

Our findings are in line with previous data [7] that the content of T cells carrying FcR for IgG was 2-4-fold higher in patients with CLL than in healthy subjects. Studies of B cells in human and animal CLL occupy a special place. Many authors showed that the number of B cells increased and their antigen repertoire changed in CLL, which determines changes in their functional capacities [5].

Hence, studies of FcR on T and B cell from cattle with CLL showed that the capacity to bind IgG markedly increased in both T and B cells in this disease, but B cells possess higher bind capacity in respect to IgG.

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