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Cell surface sialic acid affects immunoglobulin binding to macrophages

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We demonstrate that guinea pig peritoneal macrophages pretreated with neuraminidase from *Vibrio cholerae* bind more ^{125}I-IgG than non-treated cells. Estimation of binding constants (K_a and B_{max}) shows that the elevation of binding is the result of an increase in affinity and not in the number of receptors for IgG. The change of affinity is proportional to amounts of sialic acid liberated from the cells by increasing doses of neuraminidase. It is also shown that affinity of interactions of IgG with the macrophage receptor is pH dependent. These results indicate that electrostatic forces are important for IgG binding to the macrophage FcγR. The IgG-FcγR interaction can be modulated by changing the degree of sialylation of the macrophage surface glycoconjugates.

Sialic acid; Fcγ receptor; Neuraminidase; Macrophage; IgG binding

1. INTRODUCTION

Sialic acids are constituents of glycoconjugates distributed on surfaces of animal cells [1]. It is documented that alterations in the content of cell surface sialic acids have an influence on biological properties of these cells [1–5]. Macrophages are cells with very expanded cell surfaces and it is known that their surface receptors play an important role in many immunological processes [6,7]. During studies on properties of FcγR present on GPPM, we observed that pretreatment of these cells with neuraminidase from *Vibrio cholerae* potentiates their ability for IgG binding [8]. The purpose of this report is to elucidate the mechanism of the increased IgG-binding ability and to find out how the release of sialic acid affects IgG-binding parameters (K_a, B_{max}).

2. MATERIALS AND METHODS

2.1. *Macrophages*

Randomly bred guinea pigs (from Animal Farm of the Institute of Immunology and Experimental Therapy, Wrocław, Poland) weighing 300–400 g were injected intraperitoneally with 30 ml of sterilized liquid paraffin. Four days after the injection, macrophages were harvested from the peritoneal cavities using HBSS containing heparin. Erythrocytes present in the cell suspension were lysed and macrophages were washed with BSS and PBS. Macrophages were centrifuged and washed 3 times in large glass centrifuge tubes with PBS, shaking for 30 min before centrifugation. Non-adherent cells were decanted. The percentage of macrophages was determined according to the method of Askenase and Heyden [9] and was found to be 95%.

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Abbreviations: FcγR, receptor for the Fc region of IgG; NANA, *N*-acetylneuraminic acid; GPPM, guinea pig peritoneal macrophages; PBS, phosphate-buffered saline, pH 7.2; K_a, apparent association constant; B_{max}, binding capacity; HBBS, Hank's balanced salt solution

2.2. *Immunoglobulins*

Rabbit IgG used in these studies was prepared by chromatography of gamma globulins on DE-32 cellulose as described in [10]. It was previously demonstrated that rabbit IgG binds to the same receptor sites on GPPM as guinea pig IgG [8,10]. Immunoglobulins were iodinated with a carrier-free Na^{125}I (Institute of Nuclear Research, Warsaw, Poland) and Iodogen (Pierce, USA) according to [11]. Iodinated protein was separated from the unbound ^{125}I on columns loaded with 10 ml of Sephadex G-25. The iodine non-covalently bound to IgG in final preparations was determined with the uranyl acetate method [12] and did not exceed 6%.

2.3. *Treatment of GPPM with neuraminidase*

Macrophages (1×10^8 cells) were incubated in PBS for 1 h at 37°C with various doses (0.01–100 mU) of neuraminidase from *V. cholerae* (Serva, FRG). The total reaction volume was 2 ml. After incubation, cells were centrifuged, the supernatant was collected for determination of liberated sialic acid and then the cells were washed twice with cold PBS. Finally, the macrophages were suspended in the test buffer (5×10^7 cells/ml of PBS containing 0.5% BSA and 0.2% NaN_3) and then used in the binding assay.

2.4. *Determination of sialic acid*

Sialic acid was determined with periodic acid-thiobarbituric acid assay [13] in 200 μl samples taken from supernatants of macrophages treated and not treated with neuraminidase. The control cells were incubated under the same conditions as the neuraminidase-treated cells.

For determination of total cellular sialic acid, the cells were treated with 0.05 M H_2SO_4 at 80°C for 1 h [14] and then slightly alcalized at room temperature with 0.1 M NaOH to remove the O-acetyl groups [15].

2.5. *Binding assay and determination of binding parameters*

The assay is a modification of the method previously described [16]. Briefly, assays were performed at 4°C in the test buffer in 96-well flat bottom plastic plates (Costar, USA). Each well contained a constant amount of ^{125}I-IgG (0.04 μg), increasing amounts of un-

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labelled IgG (0–150 μg) and a constant number of cells (2×10^6). Non-displaced radioactivity (at the highest concentration of unlabelled IgG) of ^{125}I-IgG bound represents the non-specific binding. The final reaction volume was 200 μl. The plates were incubated for 3 h with gentle agitation. At the end of incubation, 150-μl aliquots from each well were centrifuged in a microfuge through a mixture of dibuthylphtalate and *bis*-ethylhexylphtalate (1.1 : 1, v/v) to separate the cells from the medium. Tips of the centrifuge tubes containing cell pellets were cut off and radioactivity bound to the cells was measured. All determinations were done in duplicates.

In experiments on the effect of pH on IgG-binding ability of macrophages, the final pH of the test buffer was adjusted to values between 6.0 and 8.0.

Experiments on the effect of removal of sialic acid from the cells on binding of IgG and on the effect of pH on interaction of IgG with the receptor were repeated at least 3 times.

Binding data were analyzed by weighted non-linear least squares curve-fitting procedure using computer program LIGRE (elaborated by W. Gorczyca and E. Hałas) based on assumptions of the LIGAND program [17]. One-class or two-class binding site models were used to obtain the best curve fit. In both models a non-specific binding component was also considered in analysis. Binding parameters (K_a and B_{max}), estimated for specific binding of IgG to the macrophage Fcγ receptor, are displayed in Scatchard coordinates [18].

3. RESULTS AND DISCUSSION

Pretreatment of GPPM with various amounts of neuraminidase caused an increase in binding of rabbit ^{125}I-IgG to these cells (table 1). This effect could be due to an elevation of the number of binding sites for IgG on the cells and/or to a change in the affinity of interactions after enzymatic digestion. To answer these questions, we carried out equilibrium-binding experiments and determined the values of binding constants. In all analyzed cases, the best fit of experimental data points suggested a one-class binding site model which corresponded to the linear Scatchard plots. Data from representative experiments and results of analysis are shown in fig.1 and respective values of binding constants are presented in table 2. The numbers of binding sites determined in all experiments were practically the same and were comparable with control values. This fact argues against the possibility that enzyme treatment uncovers new binding sites [1,19]. On the other hand, macrophages pretreated with neuraminidase showed higher affinity to IgG. Values of K_a were increasing with increasing amounts of sialic acid released by the enzyme from the surface of macrophages (table 2).

Table 1

^{125}I-IgG binding to GPPM not treated or pre-treated with neuraminidase

Cells	^{125}I-IgG bound (cpm)	$\frac{\text{cpm bound}}{\text{cpm added}} \times 100$
Not treated	3946	8.77
Pretreated with 0.05 mU of neuraminidase	5160	11.47
Pretreated with 2.0 mU of neuraminidase	7730	17.88

Macrophages were pretreated with enzyme as described in section 2 and then 2×10^6 cells were incubated with a constant amount of ^{125}I-IgG (0.04 μg) for 3 h at 4°C. After this time, radioactivity bound to the cells was measured. In the table, results from one of the experiments are presented

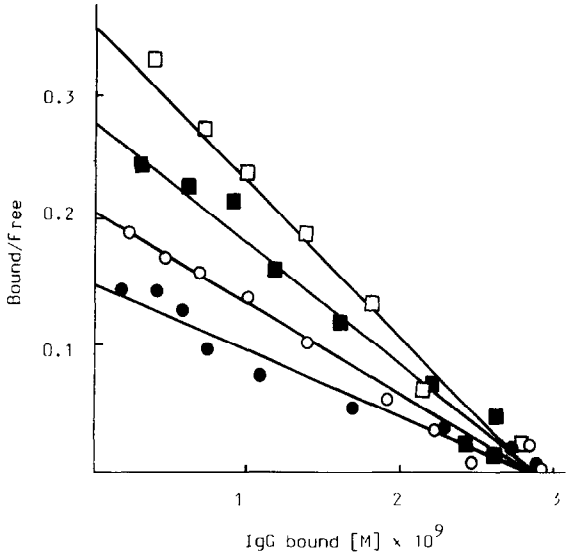

Fig.1. Scatchard plots of binding of rabbit IgG to GPPM. Cells were pretreated with 0.05 mU (—○—), 1.0 mU (—■—) or 5 mU (—□—) of neuraminidase from *V. cholerae* and then used in binding assays. In the control experiment (—●—), cells were preincubated under the same conditions as cells treated with the enzyme.

The release of the cell surface sialic acid by neuraminidase from *V. cholerae* was saturable (fig.2). The enzyme could liberate from 1×10^8 GPPM about 20 μg of sialic acid which corresponded to 3.9×10^8 molecules of NANA per cell and constituted 22% of the total cellular sialic acid content. The values of K_a and B_{max} were determined for interaction of IgG with macrophages pretreated with all concentrations of neuraminidase used.

When values of K_a were plotted against the amounts of sialic acid released by neuraminidase and analyzed using linear regression analysis, a straight line with correlation coefficient $r = 0.937$ was obtained (fig.3). This confirms our conclusion that there is a direct correlation between an increase in values of K_a and the amount of sialic acid released from the surface of GPPM.

We have previously shown that the IgG-binding ability of macrophages is pH-dependent [8]. Here we present evidence that this dependence is due to the dependence of values of K_a on pH (fig.4).

The results obtained showed that the macrophage surface sialic acid has an influence on the IgG-binding properties of the cells. Removal of electronegatively charged sialic acid molecules from the cell surface glycoconjugates changes the cell surface charge and in

Table 2

The effect of neuraminidase concentration on values of binding constants and on sialic acid liberation from GPPM

Neuraminidase concentration (mU/10^8 cells)	Apparent association constant ($\times 10^7$ M^{-1})	No. of binding sites/cell ($\times 10^5$)	Amount of sialic acid liberated ($\mu g/10^8$ cells)
0[a] (6)[c]	4.45 ± 0.38	1.75 ± 0.14	ND
0[b] (6)	5.27 ± 0.17	1.68 ± 0.14	2.26 ± 0.36
0.05 (3)	7.44 ± 0.04	1.70 ± 0.09	5.26 ± 0.35
1.00 (3)	10.08 ± 0.37	1.71 ± 0.11	13.69 ± 1.02
5.00 (3)	11.84 ± 0.30	1.71 ± 0.09	18.37 ± 1.34

[a] Control 1, cells were stored at 4°C; b Control 2, cells were preincubated under the same conditions as cells pretreated with enzyme; [c] Numbers in parentheses are the numbers of the experiments; ND, non-detectable. In the table, mean values ± SD are presented. The number of binding sites per cell were calculated from the respective values of B_{max}

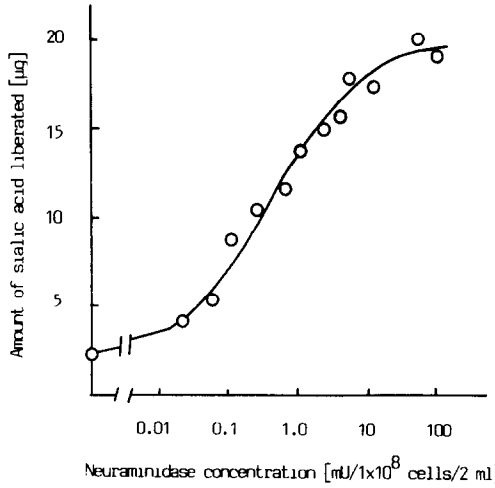

Fig.2. The release of sialic acid from GPPM by various concentrations of neuraminidase from *V. cholerae* is shown. The experimental points are averages from three experiments and the values of SD did not exceed 12%.

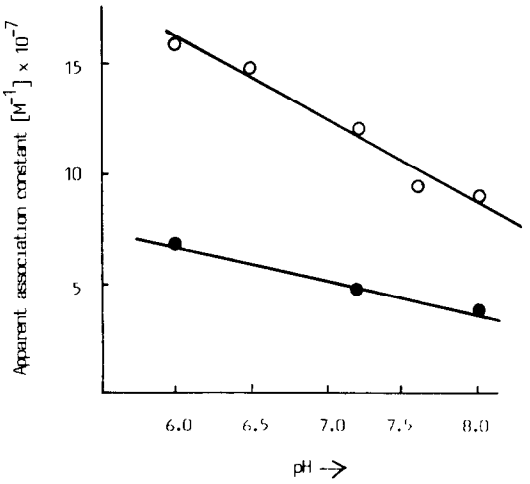

Fig.4. The effect of pH on the affinity of IgG binding to GPPM. Cells not treated (—●—) or pretreated with 5 mU of neuraminidase (—○—) were used in binding experiments at various pHs and then K_a values were estimated.

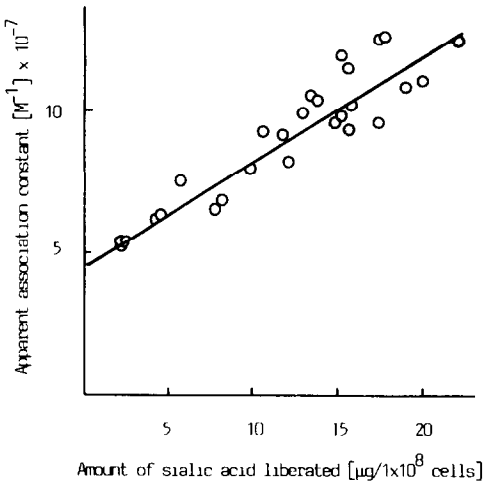

Fig.3. The effect of the amount of released sialic acid from GPPM on the affinity of interaction between GPPM and IgG. Cells were pretreated with various doses of neuraminidase, liberated sialic acid was determined and the cells were used in binding assays. Values of K_a were estimated as described in section 2.

such a way can affect immunoglobulin binding to the receptor. A possibility that the removal of sialic acid also induces some conformational changes in FcγR molecules affecting their binding abilities cannot be excluded. A linear relationship between amounts of sialic acid released and affinity increase (fig.3) rather argues against a possibility that liberation of particular sialic acid molecules (e.g. from FcγR or from glycoconjugates in the vicinity of the receptor) causes an increase in affinity of binding of IgG to the macrophage FcγR.

Heino et al. [20] have observed that maturation of monocytes to macrophages is concomitant with an increase in the number of sialoglycoproteins on the surface of cells. At the same time, the association constant of binding of IgG to the Fcγ receptor decreased [21]. It is tempting to suggest that cells, by changing the degree of sialylation of their surfaces, might be able to regulate affinity of interactions of ligands with receptors on the surfaces of these cells.

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