Epidermal Growth Factor Binding Sites on Human Erythrocytes in Donors With Different ABO Blood Groups

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Analysis of epidermal growth factor (125I-EGF) binding to human red cells revealed the presence of two classes of binding sites with apparent equilibrium dissociation constants (app.Kd) in the 10^-9 - 10^-8 M and in the 10^-8 M range, respectively. The number of binding sites/cell ranged between 600 and 2,400 for the high-affinity binding site and between 7,200 and 23,000 for the low-affinity site. No differences were seen in the apparent Kd values for both types of binding sites between red cells obtained from donors with different ABO-blood groups. An increase in the number of high affinity EGF binding sites was observed in donors with blood group A-erythrocytes as compared to red cells taken from donors with blood groups O and B.

Key words: epidermal growth factor (EGF) binding, human erythrocytes, ABO blood groups

INTRODUCTION

Binding sites for the epidermal growth factor (EGF) have been described for a great variety of different cell types (for a review see [1]). Specific binding to plasma cell membranes is thought to be due to interaction of the growth factor with its receptor, a 170 kDa membrane protein whose structure has been studied in great detail especially in A 431 cells [1]. The extracellular portion of the EGF receptor contains several oligosaccharide chains which, at least in the case of A 431 cells, has been shown to contain terminal blood group A antigen-like sugar residues [2,3]. A recent study indicates that modification of these terminal sugar residues may alter both interaction of EGF with its high affinity binding site as well as the kinase activity of the receptor [4].

The detection of the presence of binding sites for EGF on human erythrocytes (see Results) prompted us to investigate the ABO-blood group dependence of EGF binding to these cells. Several human red cell membrane proteins and membrane lipids are known to bear oligosaccharide chains with ABH-antigen-like properties [5]. We hypothesized that, in analogy to the EGF receptor of A 431 cells, the putative EGF receptor of human erythrocytes might well be equipped with ABH-antigen-like sugar residues. Since erythrocytes with different ABH-antigens are believed to differ only in the composition of the protein and lipid attached sugar chains, donors with different ABO-blood groups may serve as "biological variants" for the study of the influence of ABH-antigens on binding of EGF to these cells.

MATERIALS AND METHODS

Venous blood anticoagulated by heparin was drawn from apparently healthy donors. Red cells were washed four times with 10 vol of a solution containing 140 mM NaCl, 5 mM KCl, 5 mM glycylglycine, 5 mM glucose, 0.2 mM MgCl2, 1.25 mM CaCl2 (pH 7.5), referred to as Na buffer throughout.

Cells were counted under a light microscope (Neubauer chamber); 50 µl of packed red cells were incubated (in triplicates) for 3 hr at 4°C with 12 concentrations of 125I-EGF (Amersham-Buchler, Braunschweig, Germany, specific activity 0.76 Ci/mmol) in the range between 1.0 x 10^-9 - 2 x 10^-7 M, the total reaction volume amounting to 500 µl. The reaction was started by manual addition of the red cell suspension and stopped by centrifugation for 10 min at 10,000g in an Eppendorf-Hermle

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centrifuge Model Z 380 (Eppendorf, Hamburg, Germany). Cells were quickly washed three times at 4°C with ice-cold Na buffer. Radioactivity of the pellets (total binding) was assessed by gamma-counting. Unspecific binding as determined in the presence of an excess of unlabelled EGF \(1.6 \times 10^{-8} \text{M}\) was found to be less than 30% of total binding for both types of binding sites.

Pipetting was performed by a Tecan Robotic Sample processor Model 5052 (dual arm system, Tecan AG, Frankfurt, Germany) as described in [6]. For all experiments given in Results the following binding equation for two independent classes of binding sites [7] was fitted to the data by nonlinear regression analysis yielding estimates of the binding parameters for a high- and a low-affinity binding site.

\[
B = \frac{B_{m1} \times c_f}{K_{d1} + c_f} + \frac{B_{m2} \times c_f}{K_{d2} + c_f} + C \times c_f
\]

where \(B_{m1}, K_{d1}\) are the high-affinity binding site parameters, \(B_{m2}, K_{d2}\) are the low-affinity binding site parameters, \(c_f, c_b\) are free and bound concentration of ligand, and \(C\) is the constant (correcting for unspecific binding). The above equation gives an estimate of the specific binding of a ligand to two independent binding sites (first two terms on the right side of the equation). The constant \(C\) is used to correct for any linear portion of total binding (indicative of unspecific binding) remaining after fitting the data to the parameters for two saturating binding curves.

RESULTS

Nonlinear regression analysis revealed the presence of two types of binding sites for the epidermal growth factor (EGF) on human erythrocytes (Fig. 1): a high-affinity binding site with an apparent equilibrium dissociation constant in the \(10^{-10}-10^{-8} \text{M}\) range (0.2–4.3 \(\times 10^{-9} \text{M}\) and about 1,000 sites/cell (580–2,400) and a low-affinity binding site with an apparent equilibrium dissociation constant in the \(10^{-8} \text{M}\) range (1.8–5.7 \(\times 10^{-8} \text{M}\) and around 10,000 sites/cell (7,200–23,000).

No considerable differences in app. \(K_d\) values were observed between donors with different ABO-blood groups for both types of binding sites. A\(_1\) erythrocytes exhibited a higher number of binding sites for EGF as compared to red cells taken from individuals with blood groups O and B (Tab. I), the increase being more prominent for the high affinity binding site (Tab. I).

DISCUSSION

The results of the present study indicate that human red cells bear two (hitherto unknown) classes of independent binding sites for epidermal growth factor (EGF), in accordance with results obtained on several other cell types [1]. A recent report indicates that the most abundant membrane protein present in human erythrocytes, the band 3 protein, is a fairly good substrate for the EGF receptor associated tyrosine kinase [8]. In addition, we observed in human erythrocytes a Ca inward transport susceptible towards inhibition by the phorbol ester TPA [9]. Thus, there is both direct and indirect evidence that EGF elicits intracellular responses in human erythrocytes.

The EGF receptor of A 431 cells has been shown to bear ABH-blood group antigen-like sugar structures [2,3] which may influence binding of the growth factor to its receptor [4]. In the study of Defize et al. [4], the lack of blood group antigen A on A431 cells was associated with an increased number of high affinity receptors for EGF. In contrast, in the present study, the amount of high-affinity binding sites for EGF on human erythrocytes was higher in blood group A\(_1\) as compared to blood group B and O erythrocytes (Table I). However, observations re-
Table I. EGF Binding to Erythrocytes of Healthy Donors With Different ABO Blood Groups

<table>
<thead>
<tr>
<th>Blood group</th>
<th>High-affinity site</th>
<th>Low-affinity site</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B&lt;sub&gt;max&lt;/sub&gt;</td>
<td>app. K&lt;sub&gt;d&lt;/sub&gt;</td>
</tr>
<tr>
<td>0 (n = 6)</td>
<td>960 ± 680</td>
<td>1.7 ± 1.2</td>
</tr>
<tr>
<td>A&lt;sub&gt;1&lt;/sub&gt; (n = 4)</td>
<td>1,830 ± 900</td>
<td>1.5 ± 2.0</td>
</tr>
<tr>
<td>B (n = 3)</td>
<td>950 ± 390</td>
<td>1.3 ± 0.3</td>
</tr>
</tbody>
</table>

*Red cells were treated with different concentrations of EGF for 3 h at 4°C as described in Methods. Mean values ± S.D. Data were derived from nonlinear regression analysis by using the mathematical equation given in Methods.

REFERENCES