Correlation between Glucocorticoid Receptor and Cytolytic Response of Murine Lymphoid Cell Lines

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ABSTRACT

By fusion between murine thymoma lines which are either homozygous (r⁺/r⁺ or r⁻/r⁻) or hemizygous (r⁺/r⁻) for the glucocorticoid receptor structural gene r, hybrid clones have been obtained which carry one, two, three, or four copies of the r⁺ allele. These hybrids contain different amounts of normal glucocorticoid receptor as the result of a r⁺ gene dosage effect. Measurements of the cytolytic response of these hybrids to dexamethasone indicate a tight correlation between receptor content and sensitivity.

INTRODUCTION

The usefulness of glucocorticoid receptor measurements in human leukemia cells in predicting the response to glucocorticoid therapy is a controversial issue (3, 11). Early studies (6, 7) indicated a good correlation between the presence of glucocorticoid receptors in leukemic cells and the therapeutic as well as the in vitro inhibitory effects of glucocorticoids. However, more recent studies find little correlation between receptor levels and glucocorticoid sensitivity of cells in vitro or known clinical sensitivity of the various leukemias and lymphomas (2, 5).

The quantitation of glucocorticoid receptors in leukemic lymphocytes presents several pitfalls. First, the cytosol technique used in early studies appears unreliable because of poor recovery and inactivation of the receptors in the extract. The interpretation of the results may be further complicated by the fact that one is dealing with a functionally heterogeneous population of lymphocytes. Moreover, the presence of the glucocorticoid administered as part of the therapy is a potential difficulty since it may obscure the results in 2 respects: the drug may occupy a fraction of the receptors, thereby reducing the number of available binding sites; and prolonged glucocorticoid treatment could select for resistant leukemic cells either containing a nonfunctional receptor which has retained affinity for glucocorticoid or containing a normal receptor but defective in another function necessary for the response.

The system we are investigating does not present any of these complications. We have used a whole-cell assay to determine glucocorticoid receptors in cloned murine lymphoid hybrid cell lines which have not been exposed to glucocorticoids. In that model system, we find a tight relationship between glucocorticoid receptor content and the cytolytic response to the steroid.

MATERIALS AND METHODS

The parental cell lines and the procedures for growth, dexamethasone sensitivity tests, and karyotyping have been described elsewhere (1). Cell fusions were induced by polyethylene glycol and carried out essentially as described by Davidson and Gerald (4). All parental lines carry either a TG⁺ or a BrdUrd resistance marker to allow the selection of hybrids in hypoxanthine-aminopterine-thymidine medium (8).

The [1,2-³H]dexamethasone-binding assays were performed on whole cells as described by Pfahl et al. (10).

RESULTS AND DISCUSSION

The mouse thymoma line W7 contains approximately 30,000 dexamethasone-binding sites per cell and is highly sensitive to the cytolytic effect of glucocorticoids (1). Derivatives of the W7 line have been selected as being partially resistant to low concentrations of the steroid and were found to contain one-half the parental amount of receptors. Evidence has been presented elsewhere (1) that, while the parental W7 line is normally diploid for the receptor structural gene (r⁺/r⁺), its partially resistant derivatives appear functionally hemizygous at that locus (r⁺/r⁻). By selection for full resistance to high concentrations of dexamethasone, derivatives of the W7 line can also be isolated which do not contain any detectable glucocorticoid receptor (r⁻/r⁻). Properties of W7 lines carrying the TG or BrdUrd markers and some of their variants are shown in Table 1. The receptors of the hemizygous lines, MS1 and EO24, appear normal; they have the same affinity for dexamethasone as that of the parental W7 lines (see Table 1) and, as described elsewhere (9), the receptor-steroid complexes are transferred into the nucleus to the same extent as in the case of the homozygous (r⁺/r⁺) W7TB and W7TG lines.

Chart 1A illustrates the sensitivity of these lines to varying low concentrations of dexamethasone; the 2 cell lines containing a reduced amount of receptor, MS1 and EO24, have an intermediate sensitivity to the steroid, not unexpected since they were selected for partial resistance in the range of 5 to 8 x 10⁻⁹ M dexamethasone. The fact that variants containing a reduced quantity of normal receptor were selected in those
Table 1

<table>
<thead>
<tr>
<th>Cell line</th>
<th>Selective marker</th>
<th>Receptor alleles</th>
<th>No. of r* alleles/cell</th>
<th>Receptor sites/cell</th>
<th>Kx × 10^8</th>
</tr>
</thead>
<tbody>
<tr>
<td>W7TB</td>
<td>BrdUrd</td>
<td>r*/<em>r</em></td>
<td>2</td>
<td>26,000 ± 300^a</td>
<td>1.4 ± 0.2^b</td>
</tr>
<tr>
<td>W7TG</td>
<td>TG</td>
<td>r*/<em>r</em></td>
<td>2</td>
<td>30,100 ± 1,100</td>
<td>1.3 ± 0.1</td>
</tr>
<tr>
<td>MS1</td>
<td>BrdUrd</td>
<td>r*/<em>r</em></td>
<td>1</td>
<td>14,700 ± 1,100</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>EO24</td>
<td>TG</td>
<td>r*/<em>r</em></td>
<td>1</td>
<td>13,200 ± 200</td>
<td>1.2 ± 0.2</td>
</tr>
<tr>
<td>AN6</td>
<td>BrdUrd</td>
<td>r*/<em>r</em></td>
<td>0</td>
<td>&lt;100</td>
<td></td>
</tr>
<tr>
<td>SL3</td>
<td>TG</td>
<td>r*/<em>r</em></td>
<td>0</td>
<td>&lt;100</td>
<td></td>
</tr>
<tr>
<td>W7TB × W7TG</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>4</td>
<td>57,500 ± 3,900^a</td>
<td>1.4 ± 0.2^b</td>
</tr>
<tr>
<td>W7TB × EO24</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>3</td>
<td>44,600^c</td>
<td>1.7^c</td>
</tr>
<tr>
<td>W7TG × MS1</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>3</td>
<td>41,000^c</td>
<td>1.8^c</td>
</tr>
<tr>
<td>W7TB × SL3</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>2</td>
<td>33,700 ± 1,700</td>
<td>1.5 ± 0.4</td>
</tr>
<tr>
<td>MS1 × EO24</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>2</td>
<td>33,200 ± 1,500</td>
<td>1.7 ± 0.2</td>
</tr>
<tr>
<td>W7TG × AN6</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>2</td>
<td>30,700^f</td>
<td>1.2^c</td>
</tr>
<tr>
<td>MS1 × SL3</td>
<td></td>
<td>r*/<em>r</em> × r*/<em>r</em></td>
<td>1</td>
<td>12,700 ± 1,500^d</td>
<td>1.5 ± 0.3^d</td>
</tr>
</tbody>
</table>

^a Mean ± S.E.
^b Mean ± S.E. for 2 independent hybrid clones.
^c Single determinations.
^d Mean ± S.E. for 4 independent hybrid clones.

Conditions is suggestive of a relationship between receptor content and sensitivity.

To further investigate this correlation, we have constructed a series of hybrids containing different amounts of normal receptor. In this case, no selective pressure was imposed for any level of resistance to dexamethasone; the selection of these hybrids was based on the presence of the TG and BrdUrd resistance markers in the parental lines. These hybrid clones had never been exposed to glucocorticoids before being assayed for receptor or being tested for their level of sensitivity to dexamethasone.

By fusion between W7 lines and their variants, we have obtained hybrid clones containing 1 (r*/*r* × r*/*r*), 2 (r*/r* × r*/r* or r*/r* × r*/r*), 3 (r*/r* × r*/r*), or 4 (r*/r* × r*/r*) copies of the r* allele. All hybrids were karyotyped and found to contain a typically tetraploid number of 80 ± 1 (S.E.) chromosomes, with less than 10% of the cells having fewer chromosomes. As shown in Table 1, the receptor content of these hybrids reflects the r* gene dosage effect.

Chart 1B shows the cytolysis response of these hybrid cell lines and illustrates that their level of sensitivity is determined both by the dexamethasone concentration and by the intracellular receptor content. The tetraploid line containing a single dose of receptor (1r*/4n) is the most resistant, and sensitivity increases with the number of receptors per cell. Even at concentrations of dexamethasone of 5 × 10^-8 M or above (data not shown), which saturate all receptor sites, the hybrids with fewer receptor sites fail to show complete sensitivity. These results indicate that in these cells the number of receptor-steroid complexes limits the cytolytic response. The reason why the intracellular concentration of receptor-steroid complexes should determine the level of the response cannot be ascertained at this time because the molecular mechanism of action of steroid receptors is unknown. However, the widely

Chart 1. Sensitivity to dexamethasone of parental and hybrid cell lines. The cellular material present in cultures after 8 doublings was monitored by turbidity conditions is suggestive of a relationship between receptor content and sensitivity.

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at 660 nm (1). The results obtained for the cultures with dexamethasone are expressed as percentages of the A660 reached in the control without steroid. A, parental lines: O, W7TB; •, W7TG; - , EO24; , MS1; A, AN6; Δ, SL3. B, hybrid lines: X, W7TB × W7TG; □, W7TB × EO24; ■, W7TG × MS1; ○, W7TG × AN6; ●, MS1 × EO24; 9, W7TB × SL3; Δ, MS1 × SL3.
accepted working hypothesis in this field assumes that the response is triggered by the interaction of steroid-receptor complexes with nuclear acceptor sites. In terms of that model, one explanation of our data would be that in cells containing a reduced amount of receptors the number of steroid-receptor complexes is insufficient to saturate all the nuclear acceptor sites.

The comparison of Chart 1, A and B, reveals that the diploid parental lines containing 2 \( r^+ \) alleles (2\( r^+ \)/2\( n \)) are more sensitive to dexamethasone than their tetraploid hybrid containing 4 \( r^+ \) alleles (4\( r^+ \)/4\( n \)). This effect might be due to the fact that the tetraploid cell does not have exactly twice the volume of its diploid parents and, therefore, that the intracellular receptor concentrations may not be identical. This point remains to be clarified.

Our observations indicate a tight correlation between the glucocorticoid receptor content of these mouse thymoma cells and their sensitivity to the steroid. It is conceivable that the reason why such a tight correlation is evident in this model system is that it avoids the pitfalls and complications which may have obscured such a correlation in leukemic cells of patients. However the cells used in this study are of murine origin. The lymphocytolytic response in mice, a glucocorticoid-sensitive species, may not be directly comparable to the inhibitory effects of glucocorticoids in humans, and the therapeutic benefits of glucocorticoids could involve other effects as well.

REFERENCES

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