SHORT COMMUNICATIONS

THE ENDOGENOUS HEAT-STABLE GLUCOCORTICOID RECEPTOR STABILIZING FACTOR AND THE H-2 LOCUS

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SUMMARY

It has recently been suggested that the level of the endogenous glucocorticoid receptor stabilizing factor in mouse liver is regulated by the major histocompatibility H-2 complex (Katsumata et al. [1]). We have developed an assay for the activity of the endogenous heat-stable factor in mouse liver and have assayed this factor in liver cytosols prepared from two pairs of two H-2 congenic mouse strains. Our results show that the amount of the endogenous factor is the same in all four mouse strains and that it is not regulated by the H-2 locus.

Katsumata et al. [1] recently reported differences in dexamethasone binding in liver cytosols prepared from mice of four congenic and recombinant strains. C57BL/10, B10.A, B10.A(2R) and B10.A(5R). These strains have almost identical genetic backgrounds and differ only in the H-2 complex and closely linked loci. Katsumata et al. [1] found that plots of dexamethasone binding versus concentration of liver cytosol were linear for C57BL/10 and B10.A(5R) mice whereas similar binding plots for strains B10.A and B10.A(2R) were not. The authors suggested that the differences in binding between the strains could be due to differences in the levels of an endogenous modifier that was determined by the H-2 complex. The small molecular weight, heat-stable transformation inhibitor reported by Cake et al. [2, 3] and Bailly et al. [4] and the heat-stable factor reported by Sando et al. [5, 7] were suggested as possible candidates for the endogenous modifier.

We [8] have recently examined in detail the characteristics of the endogenous heat-stable factor prepared from rat liver, which both stabilizes the binding capacity of the unbound glucocorticoid receptor and inhibits transformation of the bound receptor to the DNA-binding form. The receptor stabilizing and transformation inhibiting activities of the endogenous heat-stable factor in rat liver cytosol inhibit transformation but do not affect viability of already transformed glucocorticoid-receptor complexes to bind to nuclei or to DNA-cellulose. In our previous work [8] we expressed our results in terms of inhibitory activity calculated from similar concentration dependency curves. Table 1 presents our results in terms of inhibitory activity calculated from similar concentration dependency curves. Table 1 presents the units of inhibitory activity for the four mouse strain factors calculated from the data presented in Fig. 1. There is no significant difference in the activities of the mouse liver heat-stable, glucocorticoid receptor stabilizing factor.

Variations at the H-2 locus have been associated with susceptibility to glucocorticoid-induced cleft palate [9, 10]. Goldman et al. [11] previously suggested that the level of glucocorticoid receptor is also regulated by the H-2 complex. We subsequently showed [12], however, that specific glucocorticoid binding activity is not mediated by the H-2 locus, and Francke and Gehring [13] have recently demonstrated that the structural gene for the glucocorticoid receptor is located on chromosome 18, not with the H-2 locus on chromosome 17. Katsumata et al. [1] have further examined the relationship of glucocorticoid binding to the H-2 complex and proposed that the level of an endogenous modifier may be regulated by the H-2 complex. The results presented herein show that the difference in dexamethasone binding observed by Katsumata et al. [1] cannot be explained by different levels of the endogenous heat-stable factor and that the level of the endogenous factor is not regulated by the H-2 complex.
Fig. 1. Effect of heated mouse liver supernatants on glucocorticoid receptor inactivation and transformation. A, various amounts of heated mouse liver preparations were incubated with filtered rat liver cytosol at 20°C for 45 min and the specific binding capacity was assayed as previously described [8]. B, heated mouse liver supernatants were incubated with filtered rat liver cytosol, prebound with [3H]-triamcinolone acetonide, at 15°C and the binding to DNA-cellulose was measured after 60 min as previously described [8]. Three different preparations for each strain are presented in part A and 1 preparation from each strain is presented in part B. O, B10.A; □, A.BY; ▲, A/J; ■, C57BL/10J.
Table 1. Relationship of inhibitory units for mouse liver glucocorticoid receptor stabilising factor to H-2 and genetic background

<table>
<thead>
<tr>
<th>Background</th>
<th>H-2 haplotype</th>
<th>H-2 haplotype</th>
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<tbody>
<tr>
<td></td>
<td>H-2a</td>
<td>H-2b</td>
</tr>
<tr>
<td>A</td>
<td>A/J 5.9 ± 1.4</td>
<td>A.BY 5.5 ± 0.67</td>
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<tr>
<td></td>
<td>(5.6)</td>
<td>(4.9)</td>
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<tr>
<td>B</td>
<td>B10.A 5.8 ± 1.3</td>
<td>C57BL/10J 5.8 ± 0.45</td>
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<td></td>
<td>(6.2)</td>
<td>(7.7)</td>
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</table>

One unit of inhibitory activity is defined as the amount of heat-stable factor preparation required to inhibit receptor inactivation or transformation by 50%, in a 0.5 ml incubation containing 0.25 ml of filtered cytosol and 0.25 ml of factor preparation. The amount of factor preparation required to inhibit inactivation or transformation by 50%, was calculated from the individual experiments shown in the scatter plots of Fig. 1. The numbers in the Table represent mean factor inhibitory units for receptor inactivation ± standard error for each of the four strains. Three animals were used to determine each mean. The factor inhibitory units for receptor transformation using one animal for each of the four strains is given in parentheses.

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REFERENCES