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TECHNICAL MANUSCRIPT 230

NATURAL RESISTANCE OF HAMSTER CELLS TO 8-AZAGUANINE

Alan Richter

JULY 1963

UNITED STATES ARMY
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NATURAL RESISTANCE OF HAMSTER CELLS TO 8-AZAGUANINE

Alan Richter

Virus and Rickettsia Division
DIRECTORATE OF BIOLOGICAL RESEARCH

Project 1L013001A91A

July 1965
ABSTRACT

In studies designed to introduce mutations into selected animal cell lines in culture, cell lines derived from the Syrian hamster were found to be naturally resistant to 8-azaguanine. Resistance to the drug was measured by plating colony-forming units at different drug concentrations and determining the concentration at which 50% of the colony-forming units formed colonies. By this technique it was shown that KB cells (human) and L cells (mouse) have approximately the same susceptibility to 8-azaguanine as reported by others for L, D98 (human), and P388 (mouse). On the other hand, both P113, a polyoma-transformed derivative of embryonic hamster kidney, and BHK21C13, an untransformed hamster cell displaying contact inhibition, proved 50 times more resistant than the cell lines of human and murine origin. The higher resistance of the hamster cells to 8-azaguanine is specific, since these cells are still susceptible to the unrelated drug 5-bromodeoxyuridine. Preliminary results suggest that the basis for natural resistance is not loss of inosinic-guanic acid pyrophosphorylase activity, because the cells form colonies under conditions in which growth depends on an exogenous supply of hypoxanthine (HAT medium). The naturally resistant hamster cells therefore resemble the mutant D98/AG described by Szybalski.
NATURAL RESISTANCE OF HAMSTER CELLS TO 8-AZAGUANINE

In our laboratory we have been characterizing several animal cell lines for drug susceptibility preparatory to the selection of specific enzyme-deficient mutants. We have been primarily interested in mutants deficient in inosinic-guanylic acid pyrophosphorylase activity that display resistance to 8-azaguanine as a consequence of enzyme loss, and in mutants deficient in thymidine kinase activity that display resistance to 5-bromodeoxyuridine.

Susceptibility was determined by plating cells in Eagle's medium containing the drug and counting the number of colonies that formed in the presence and absence of the drug. The number of cells forming colonies in the presence of the drug divided by the number of cells forming colonies in the absence of the drug is expressed in per cent as the relative plating efficiency. By plotting graphically the relative plating efficiency at different concentrations of the drug, it is possible to determine the concentration of the drug that gives a relative plating efficiency of 50% or the LD50 concentration of the drug. Data for the susceptibility of cell lines to 8-azaguanine were available in the literature for mouse strain L cells, mouse strain P388,1 and human strain D50. All of these cells were inhibited 50% by drug concentrations between 2 x 10⁻⁷ and 4 x 10⁻⁷ M 8-azaguanine.

We first tested L cells and W5 (human) cells. The data are shown in Tables 1 and 2. Each value represents the average of five dishes. By plotting the data of Tables 1 and 2 graphically, it can be shown that the LD50 concentration of 8-azaguanine for L cells is about 2 x 10⁻⁷ M and that the LD50 concentration for KB cells is about 4 x 10⁻⁷ M. Table 3 compares the data for L cells with those published in the literature for P388 and D98. The LD50 concentrations are similar.

We next examined a hamster cell called P113, which was isolated by Dr. Defendi after polyoma infection of embryonic hamster kidney. This cell line was unaffected by concentrations of 8-azaguanine that completely eliminated populations of L or KB cells. By employing increasing concentrations of the drug we found that 2 x 10⁻⁷ M 8-azaguanine was necessary to halve the plating efficiency of this cell. To test a possible association between 8-azaguanine resistance and polyoma transformation, we examined a so-called normal hamster cell, BHK21C13. However, the latter proved to be resistant at the same level of drug as the transformed line. This is shown in the fourth table. The increased resistance of the hamster cells to 8-azaguanine is specific, since these cells are still highly susceptible to the unrelated drug 5-bromodeoxyuridine. Preliminary

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* Dr. Vittorio Defendi, Wistar Institute, Philadelphia, Pa.
results suggest that the basis for natural resistance is not the loss of inosinic-guanylic acid pyrophosphorylase activity because the cells form colonies under conditions in which growth depends upon an exogenous supply of hypoxanthine in the medium (hypoxanthine, aminopterin, thymidine). Growth in HAT medium is considered to be an indirect test for the enzyme function. It thus seems that the naturally resistant hamster cells resemble the mutant described by Szybalski as D98/AG, which is resistant to 8-azaguanine but possesses the pyrophosphorylase function. Attempts to select enzyme-deficient mutants from the hamster cells are in progress.
### TABLE 1. EFFECT OF 8-AZAGUANINE ON THE RELATIVE PLATING EFFICIENCY OF L CELLS

<table>
<thead>
<tr>
<th>M Concentration of Drug</th>
<th>Cell Input, 90 per dish</th>
<th>Cell Input, 180 per dish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average No. Colonies Found Per Dish</td>
<td>Plating Efficiency</td>
</tr>
<tr>
<td>0</td>
<td>51.8</td>
<td>57.5</td>
</tr>
<tr>
<td>$1 \times 10^{-7}$</td>
<td>48.6</td>
<td>94</td>
</tr>
<tr>
<td>$3 \times 10^{-7}$</td>
<td>17.4</td>
<td>33.6</td>
</tr>
<tr>
<td>$5 \times 10^{-7}$</td>
<td>0.2</td>
<td>0.39</td>
</tr>
<tr>
<td>$8 \times 10^{-7}$</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>$1 \times 10^{-8}$</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
### TABLE 2. EFFECT OF 8-AZAGUANINE ON THE RELATIVE PLATING EFFICIENCY OF KB12 CELLS

<table>
<thead>
<tr>
<th>M Concentration of Drug</th>
<th>Cell Input, 90 per dish</th>
<th>Cell Input, 180 per dish</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average No. Colonies Found Per Dish</td>
<td>Plating Efficiency</td>
</tr>
<tr>
<td>0</td>
<td>50.7</td>
<td>61.1</td>
</tr>
<tr>
<td>1 x 10^{-7}</td>
<td>61.4</td>
<td>104</td>
</tr>
<tr>
<td>3 x 10^{-7}</td>
<td>42.0</td>
<td>71.6</td>
</tr>
<tr>
<td>5 x 10^{-7}</td>
<td>30.4</td>
<td>51.9</td>
</tr>
<tr>
<td>8 x 10^{-7}</td>
<td>9.4</td>
<td>16.0</td>
</tr>
<tr>
<td>1 x 10^{-8}</td>
<td>4.2</td>
<td>7.2</td>
</tr>
</tbody>
</table>
TABLE 3. SUSCEPTIBILITY OF DIFFERENT CELL LINES TO 8-AZAGUANINE

<table>
<thead>
<tr>
<th>Drug Concentration</th>
<th>Relative Plating Efficiency, % survival</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>L</td>
</tr>
<tr>
<td>1 x 10(^{-7}) M</td>
<td>88.4</td>
</tr>
<tr>
<td>3 x 10(^{-7})</td>
<td>30.7</td>
</tr>
<tr>
<td>5 x 10(^{-7})</td>
<td>0.31</td>
</tr>
<tr>
<td>8 x 10(^{-7})</td>
<td>0</td>
</tr>
<tr>
<td>1 x 10(^{-6})</td>
<td>0</td>
</tr>
</tbody>
</table>

\(^a\) Information from Bradley et al.\(^1\)
\(^b\) Information from Szybalska and Szybski.\(^2\)

TABLE 4. DRUG SUSCEPTIBILITY OF CELL STRAINS

<table>
<thead>
<tr>
<th>Cell</th>
<th>LD(_{50}) Concentration of Drug(^b)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>8-azaguanine, 10(^{-7}) molar</td>
</tr>
<tr>
<td>Mouse strain L</td>
<td>4.5</td>
</tr>
<tr>
<td>Human strain KB</td>
<td>4</td>
</tr>
<tr>
<td>Syrian hamster strain 113</td>
<td>200</td>
</tr>
<tr>
<td>BHK21C13</td>
<td>200</td>
</tr>
</tbody>
</table>

\(^a\) Concentration of drug permitting 50% relative plating efficiency compared with plating in absence of drug. All determinations in Eagle's medium containing 10% calf serum.
\(^b\) ND - Not determined.
LITERATURE CITED


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