A-T cells are also known to be hypersensitive to ionizing radiation as a convenient aid for the laboratory diagnosis of A-T. Since the action of the antitumor antibiotic neocarzinostatin was tested, using colony-forming ability as the criterion for survival. All the A-T strains were significantly more sensitive to killing by neocarzinostatin than were the control strains. The average D₀ for the A-T strains following neocarzinostatin treatment was 14.6 ng/ml, as compared to 37.9 ng/ml for the normal strains. The two A-T heterozygous strains showed intermediate sensitivity with an average D₀ of 26.9 ng/ml.

Neocarzinostatin sensitivity of A-T cells could therefore serve as a convenient aid for the laboratory diagnosis of A-T. Since A-T cells are also known to be hypersensitive to ionizing radiation and bleomycin, it would appear that they are primarily hypersensitive to DNA-breaking agents.

INTRODUCTION

A-T² is a human autosomal recessive disease featuring cerebellar degeneration, oculocutaneous telangiectases, immunodeficiency, cancer proneness, and radiation sensitivity (see Ref. 27 for review). Cultured cells from A-T patients show increased spontaneous chromosomal breakage and are hypersensitive to the clastogenic and cytotoxic effects of ionizing radiation and bleomycin (19, 27, 35) that produce mainly single-strand breaks and alkali-labile sites in the DNA (37, 39). The A-T fibroblast strains tested in our laboratory were found to be hypersensitive to the cytotoxic action of another DNA-breaking agent, the antitumor antibiotic NCS. This drug has been shown previously to inhibit DNA synthesis and to induce single- and double-stranded breaks in the DNA in vivo and in vitro (2, 3, 11, 16, 23, 28, 36).

RESULTS

The D₀ values obtained for each cell strain following treatment with NCS (Table 1) were consistent over repeated experiments. Eight A-T strains derived from donors belonging to 6 sibships showed a significantly higher sensitivity to cytotoxicity of NCS, their average D₀ being 14.6 ng/ml, as compared to an average D₀ of 37.9 ng/ml for 3 normal strains. The D₀ values of all the Jerusalem A-T strains were very close [15.0 ± 0.3 ng/ml (S.D.)]; however, strain AT3BI showed a somewhat higher sensitivity (D₀, 11.5 ± 0.3 ng/ml). The D₀ for the 2 heterozygous strains (26.4 ± 0.4 ng/ml) was close to the midpoint between normals and A-T homozygotes. An evident difference between the normal and the A-T homozygous strains was observed with regard to the slope and shoulder size of the survival curves (Chart 1), while the 2 heterozygous strains formed a third distinct curve.

DISCUSSION

The difference in sensitivity to NCS between normal and A-T fibroblasts resembles their different sensitivities to X-rays (1, 34, 40). In both cases, the D₀ values obtained with A-T cells were roughly 2.5-fold smaller than those obtained with normal cells. Both the control and the A-T strains established in our institution constitute homogeneous groups with regard to NCS sensitivity, and the variation between strains in each group is relatively small (Table 1; Chart 1). It is of interest that strain AT3BI, which showed a somewhat higher sensitivity to NCS than did the Jerusalem A-T strains, was also found to be more sensitive to bleomycin than was another British A-T strain, AT5BI (19). The Jerusalem A-T strains are derived from patients of North African and Middle Eastern origin, and their ethnic extraction is probably entirely different from that of previously described A-T strains from Europe and North Amer-
Table 1

<table>
<thead>
<tr>
<th>Local designation</th>
<th>International designation for A-T cell lines</th>
<th>Age of donor (yr)</th>
<th>Sex</th>
<th>Country of origin</th>
<th>Colony-forming efficiency (%) (^{\circ})</th>
<th>(D_0) (ng/ml) of NCS</th>
<th>No. of experiments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>F-107</td>
<td></td>
<td>28</td>
<td>M</td>
<td>Israel</td>
<td>31.9 ± 2.1 (^{\circ})</td>
<td>38.6 ± 1.2</td>
<td>5</td>
</tr>
<tr>
<td>F-187</td>
<td></td>
<td>29</td>
<td>M</td>
<td>Israel</td>
<td>30.8 ± 2.3</td>
<td>37.1 ± 0.8</td>
<td>4</td>
</tr>
<tr>
<td>F-196</td>
<td></td>
<td>11</td>
<td>M</td>
<td>Israel</td>
<td>33.2 ± 1.5</td>
<td>30.0 ± 0.9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>A-T homozygotes</td>
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<td></td>
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<tr>
<td>F-112</td>
<td>AT17UE-F</td>
<td>21</td>
<td>F</td>
<td>Morocco</td>
<td>29.8 ± 2.4</td>
<td>15.1 ± 0.1</td>
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</tr>
<tr>
<td>F-113</td>
<td>AT18UE-F</td>
<td>18</td>
<td>F</td>
<td>Morocco</td>
<td>25.5 ± 1.2</td>
<td>14.7 ± 0.3</td>
<td>3</td>
</tr>
<tr>
<td>F-131</td>
<td>AT19UE-F</td>
<td>9</td>
<td>F</td>
<td>Morocco</td>
<td>18.1 ± 1.7</td>
<td>15.5 ± 0.2</td>
<td>4</td>
</tr>
<tr>
<td>F-143</td>
<td>AT20UE-F</td>
<td>5</td>
<td>M</td>
<td>Iran</td>
<td>22.3 ± 2.0</td>
<td>15.0 ± 0.3</td>
<td>2</td>
</tr>
<tr>
<td>F-144</td>
<td>AT21UE-F</td>
<td>9</td>
<td>M</td>
<td>Iran</td>
<td>24.3 ± 1.8</td>
<td>15.3 ± 0.5</td>
<td>4</td>
</tr>
<tr>
<td>F-169</td>
<td>AT22UE-F</td>
<td>3</td>
<td>F</td>
<td>Israel (Arab)</td>
<td>22.8 ± 1.7</td>
<td>14.5 ± 0.4</td>
<td>2</td>
</tr>
<tr>
<td>F-182</td>
<td>AT23UE-F</td>
<td>14</td>
<td>M</td>
<td>Morocco</td>
<td>30.7 ± 2.0</td>
<td>15.2 ± 0.2</td>
<td>3</td>
</tr>
<tr>
<td></td>
<td>A-T heterozygotes</td>
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<tr>
<td>F-198</td>
<td>AT60UE-F</td>
<td>39</td>
<td>M</td>
<td>Morocco</td>
<td>29.0 ± 1.3</td>
<td>26.7 ± 0.4</td>
<td>3</td>
</tr>
<tr>
<td>F-218</td>
<td>AT61UE-F</td>
<td>48</td>
<td>M</td>
<td>Morocco</td>
<td>28.3 ± 1.8</td>
<td>27.2 ± 0.6</td>
<td>3</td>
</tr>
</tbody>
</table>

\(^{\circ}\) Mean ± S.D.

\(^{\circ}\) Mean ± S.D. not including AT3BI: 15.0 ± 0.3 ng/ml.

The uniform system for designation of A-T cell strains was agreed upon at the International Workshop on A-T held at MRC Cell Mutation Unit, The University of Sussex, Brighton, England, November 1980 (B. A. Bridges and D. G. Harnden, eds. Ataxia-Telangiectasia: A Cellular and Molecular Link Between Cancer, Neuropathology and Immune Deficiency. London: John Wiley and Sons, 1982).

A-T homozygotes constitute a cancer-prone population (31) but are otherwise healthy, and identification of these individuals may contribute to the understanding of the epidemiology of cancer. To date, there is no satisfactory laboratory method for A-T heterozygote detection. Paterson et al. (26) reported on intermediate hypersensitivity of several A-T heterozygous strains to hypoxic-γ-irradiation; however, other heterozygous strains tested showed normal sensitivity. We are currently testing additional series of heterozygous strains, in order to see whether similar variability also applies to sensitivity to NCS.

Chart 1. Survival of human skin fibroblasts following treatment with NCS for 1 hr at 37°. For reasons of clarity, only 3 A-T strains are shown. For technical details, see "Materials and Methods." Normal strains: F-107 (O); F-187 (D); F-196 (A). A-T strains: F-131 (•); F-144 (○); F-182 (A). A-T heterozygous strains: F-198 (□); F-218 (□).

NCS, an antitumor antibiotic (14) composed of an acidic peptide moiety (20) and a nonprotein chromophore (22), acts via the release of the chromophore, which in turn interacts with the DNA (17). As a result of NCS action, the DNA is degraded both in vitro (2, 36) and in vivo (2, 3, 23, 28). This degradation is coupled to the release of DNA bases, particularly thymine (11, 15), and to the production of apurinic-apyrimidinic sites as intermediates (4). It has recently been shown that direct deoxyribose damage is involved in DNA strand scission by NCS (10). The hypersensitivity of A-T cells to NCS is additional evidence of the unusual response of A-T cells to DNA-breaking agents. However, cells from A-T patients have been shown to rejoin DNA breaks induced by ionizing radiation and bleomycin.


Cellular Hypersensitivity to Neocarzinostatin in Ataxia-Telangiectasia Skin Fibroblasts

Yosef Shiloh, Einat Tabor and Yechiel Becker


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