Advances in Brief

N-Methyl-N-nitrosourea Alters Thymocyte Subset Distribution and Targets Immature CD4^-8^+ Cells for Lymphoma Development

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Abstract

The majority of N-methyl-N-nitrosourea (MNU)-induced lymphomas in AKR/J mice express a CD4^-8^ phenotype. The CD4^-8^ subset in normal thymus contains functionally mature medullary cells and immature cycling cells. This study demonstrates that MNU-induced lymphomas correspond to the immature CD4^-8^ subset. In addition, specific changes in the distribution of thymocyte subsets defined by CD4 and CD8 expression were observed after MNU treatment. Cortical thinning and selective depletion of immature CD4^-8^ and CD4^-8^ subsets occur immediately after treatment. In contrast, immature CD4^-8^ progenitors and mature medullary CD4^-8^ and CD4^-8^ subsets are relatively resistant to cytotoxicity. Normal thymic architecture and subset distribution are restored within 2 weeks after which selective expansion of the immature CD4^-8^ subset occurs. The data suggest that MNU induces neoplastic conversion in progenitor cells corresponding to the CD4^-8^ or immature CD4^-8^ stages of thymocyte maturation.

Introduction

MNU^1^ is a potent carcinogen that induces various types of neoplasms depending on the dosage administered and the experimental animal species tested. In mice, injection of MNU results in a high incidence of thymic lymphomas (1). The AKR strain is particularly susceptible to MNU-induced lymphogenesis. When 6-week-old AKR mice are given a single injection of MNU (75 mg/kg), approximately 80% develop thymic lymphoma prior to 180 days of age (1-3). Untreated AKR mice have a high incidence of spontaneous thymic lymphoma at 8-12 months of age resulting from somatic integration of endogenous recombinant murine leukemia viruses referred to as mink cell focus-forming virus due to their expanded host range (4,5). In contrast to spontaneous lymphomas, MNU-induced lymphomas of AKR mice appear prior to 6 months of age and do not contain mink cell focus-forming proviral integrations (6,7) suggesting that distinct mechanisms underlie the development of spontaneous versus MNU-induced lymphoma. This notion is consistent with the finding that in contrast to spontaneous lymphomas which are phenotypically heterogeneous, the majority of MNU-induced lymphomas express a CD4^-8^ phenotype (8). The present study was undertaken, in part, to determine if MNU-induced lymphomas represent neoplastic counterparts of mature CD4^-8^ cells or a recently described compartment of immature cycling CD4^-8^ cells that express high levels of heat-stable antigen and contain progenitors of the CD4^-8^ subset (9-11). Immunofluorescence studies were performed to determine the fate of thymocyte subsets after MNU-induced depletion, during regeneration, and throughout the latent period prior to development of lymphoblastic foci. The data provide new insight regarding changes in thymocyte subset distribution and the specific population targeted for neoplastic transformation after MNU treatment.

Materials and Methods

Mice and MNU Treatment. AKR/J mice were purchased from The Jackson Laboratory (Bar Harbor, ME). Lymphomas were induced by a single i.p. injection of MNU. This dose has been shown to induce thymic lymphomas in >80% of AKR/J mice by 6 months of age (2, 6-8).

Cell Suspensions, Immunofluorescence Staining, and Flow Cytometry. Single cell thymocyte suspensions were prepared and stained for direct and indirect immunofluorescence assays by standard techniques (6, 8). To deplete CD4-bearing cells, thymocytes were incubated with an optimal dilution of anti-CD4 mAb in the presence of rabbit complement (Low-Tox M; Cedarlane, Hornby, Ontario, Canada) as described (12). Fluorescin isothiocyanate-conjugated anti-CD8 (clone 53-6.7), phycoerythrin-conjugated anti-CD4 (clone GK 1.5) and phycoerythrin-conjugated streptavidin were purchased from Becton Dickinson (Mountain View, CA). The J11d hybridoma, producing mAb that recognizes an epitope of HSA, was obtained from Dr. M. Bevan (11). Immunofluorescence was analyzed on a fluorescence-activated cell sorter (FACS IV; Becton-Dickinson) using a 488-nm argon ion laser. Dead cells were excluded from analysis by forward light scatter. Data were collected using a three decade log amplifier, stored in the list mode of a Consort 40 PDP/11-based computer system (Becton-Dickinson FACS) and, for single color analysis, displayed as histograms with the log fluorescence intensity on the x-axis and relative cell number of the y-axis. For two color analysis, contour plots were generated with quadrants based on the profile of unstained cells and cells stained with a single mAb.

Results and Discussion

MNU-induced Alterations in Thymic Cellularity and Histology. Profound changes in thymic architecture and cellularity were observed shortly after MNU treatment. The number of viable cells recovered from MNU-treated mice was reduced by approximately 90% throughout the first week. Thereafter, thymic regeneration took place, resulting in a normal thymocyte yield by 14 days. In agreement with a previous report (13), histological analysis revealed substantial cortical thinning and hypocellularity with sparing of the medulla at 2 days (Fig. 1B). By 7 days, restoration of a discrete cortical region was evident (Fig. 1C) and at 2 weeks, regeneration of normal thymic architecture was complete (Fig. 1D). Thereafter, the regenerated thymus maintained a normal histological appearance until an increase in the ratio of blasts to small lymphocytes became apparent in the thymic cortex of some mice by 6 to 8 weeks after MNU injection. By 10 weeks, large areas of homogeneous lymphoblastic cells characterized by a high mitotic index were routinely observed (data not shown).

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2 To whom requests for reprints should be addressed.

3 The abbreviations used are: MNU, N-methyl-N-nitrosourea; HSA, heat-stable antigen; mAb, monoclonal antibody.
Alterations in Thymocyte Subset Distribution within 1 Week after MNU Treatment. MNU induced dramatic shifts in the relative proportions of thymocyte subsets defined by CD4 and CD8 expression. Table 1 summarizes the distribution of thymocyte subsets in normal and MNU-treated mice at various intervals. Most striking is the marked depletion of the immature CD4⁺CD8⁺ population which, at 3 days, represented only 20% of total thymocytes. This finding is consistent with the cortical thinning observed on histological survey. However, all immature subsets are not equally sensitive to the cytotoxic effects of MNU since a slight increase was observed in the proportion of cells in the CD4⁺CD8⁻ precursor compartment at 2 and 3 days after treatment.

Sparing of the medullary compartment after MNU treatment was reflected by the relative increase in CD4⁺CD8⁻ and CD4⁻CD8⁺ subsets, although an unexpected shift in their ratio (from 1:1...
to 3:1) was observed on day 3. These data suggest that the CD4⁻8⁻ subset is more sensitive to the cytotoxic effects of MNU than is the CD4⁻8⁻ subset. Recent studies have shown that the CD4⁻8⁻ population contains a component of immature cells that express high levels of cell surface HSA (15, 16). To determine if immature and mature CD4⁻8⁻ cells differ in sensitivity to MNU-mediated cytotoxicity, CD4⁻bearing cells were eliminated from thymocyte suspensions by incubation with anti-CD4 biotinylated anti-J1 Id plus phycoerythrin-conjugated avidin (ordinate). Contour plots were generated from two color immunofluorescence analysis simultaneous expression HSA (detected by J11d mAb) and CDS molecules.

Table 1  Thymocyte subset distribution at intervals after MNU treatment

<table>
<thead>
<tr>
<th>Interval post-MNU</th>
<th>No. of mice</th>
<th>CD4⁻8⁻</th>
<th>CD4⁺8⁻</th>
<th>CD4⁺8⁺</th>
</tr>
</thead>
<tbody>
<tr>
<td>2 days</td>
<td>4</td>
<td>9.5 ± 0.9</td>
<td>52.7 ± 6.12</td>
<td>24.2 ± 1.8</td>
</tr>
<tr>
<td>3 days</td>
<td>5</td>
<td>7.5 ± 0.9</td>
<td>22.9 ± 2.2</td>
<td>52.8 ± 3.5</td>
</tr>
<tr>
<td>5 days</td>
<td>4</td>
<td>5.7 ± 0.8</td>
<td>72.8 ± 4.6</td>
<td>13.4 ± 2.5</td>
</tr>
<tr>
<td>7 days</td>
<td>3</td>
<td>4.0 ± 0.6</td>
<td>75.5 ± 2.2</td>
<td>9.3 ± 1.4</td>
</tr>
<tr>
<td>2 wk</td>
<td>4</td>
<td>1.4 ± 0.1</td>
<td>85.8 ± 0.3</td>
<td>5.8 ± 0.5</td>
</tr>
<tr>
<td>6 wk</td>
<td>3</td>
<td>2.3 ± 0.4</td>
<td>80.3 ± 2.3</td>
<td>10.6 ± 2.5</td>
</tr>
<tr>
<td>10 wk</td>
<td>3</td>
<td>2.1 ± 0.2</td>
<td>31.3 ± 12.9</td>
<td>1.5 ± 0.7</td>
</tr>
<tr>
<td>Normal thymus</td>
<td>9</td>
<td>3.2 ± 0.8</td>
<td>79.9 ± 3.3</td>
<td>8.8 ± 2.5</td>
</tr>
</tbody>
</table>

Fig. 2. HSA expression on CD4⁻8⁻ and CD4⁻8⁺ thymocytes from untreated (A) and MNU-treated (B) mice. After eliminating CD4⁻bearing thymocytes by anti-CD4 plus complement treatment, the remaining cells were assessed for simultaneous expression HSA (detected by J11d mAb) and CDS molecules. Contour plots were generated from two color immunofluorescence analysis of reactivity with fluorescein isothiocyanate-conjugated anti-CD8 (absissa) and biotinylated anti-J1 Id plus phycoerythrin-conjugated avidin (ordinate).
yond the CD4+8− differentiation stage has not been observed.

We cannot rule out the possibility that the target population for MNU-induced lymphomagenesis resides in a bone marrow pre-T-cell compartment. However, this seems unlikely in view of a report showing that thymectomy prevented the appearance of MNU-induced T-cell neoplasms (20). Implanted neonatal thymic grafts into thymectomized recipients failed to restore thymic lymphoma development unless the grafted thymus was exposed to MNU. Furthermore, after exposure of the thymic implants to MNU, the resulting lymphomas in several animals were derived from the donor thymus (20). These findings implicate intrathymic cells as the target population in MNU-induced lymphoma. Taken together with the results in the present report, the data indicate that conversion to a malignant phenotype takes place in thymocytes at the CD4+8− or immature CD4+8−HSA

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### References

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