Coenzymes Q Levels in Liver, Spleen, and Blood of Mice with Friend Leukemia Virus Infection

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SUMMARY

The coenzymes Q levels of blood, spleen, and liver of mice infected with Friend leukemia virus were compared with those of control groups at 10, 20, 30, and 40 days after infection. A significant increase in the total coenzymes Q level in the blood was observed. This increase reaches about fivefold the normal level, 30 days after the infection. The total coenzymes Q concentration in the liver does not change appreciably, but the relative concentration of coenzyme Q₁₀ begins to increase 10 days after infection. No change in coenzymes Q level or in the homolog present was observed in the spleen of the infected mice.

INTRODUCTION

The coenzymes Q are essential components of the electron transport system in mitochondria and are therefore of extreme importance to the energy-producing systems of the cell. The concentration of these coenzymes has been studied in the blood and tissues of normal humans and experimental animals, as well as in those with various pathological processes, in order to elucidate whether these quantities are related to the progression of the diseases. In the case of neoplasia, the level of coenzymes Q in the liver of a few cancer patients has been studied (5) and was found to be no different from that of normal individuals. Similar results were obtained when the livers of tumor-bearing rats and the blood of cancer patients were studied (10). A significant decrease in the total concentration of coenzymes Q was found in the livers of rats with Walker carcinoma (13) and in mitochondria of ascites hepatoma in rats (12). Recently, it was reported that the heart muscle of 8 cancer patients showed a highly significant increase of coenzymes Q concentration, compared with that of normal controls (2).

We report here a comparative study between the levels of coenzymes Q in the liver, spleen, and blood of mice with FLV² and those of normal controls, during the progress of the infection. FLV induces splenomegaly and hepatomegaly in susceptible mice associated with the appearance of blastic (mainly erythroblastic) cells in the peripheral blood and diffuse cellular infiltration into the spleen and liver causing fatal hemorrhage (4).

We attempted to determine the level of each coenzyme Q homolog present in the coenzyme Q fraction to evaluate whether the biosynthetic pattern of the coenzymes Q had been modified during the progress of the disease.

MATERIALS AND METHODS

 Animals. Male Swiss-Webster mice weighing 20 g were obtained from 1 commercial breeder. The mice were kept in a room artificially illuminated during daylight hours and at uniform humidity, with a temperature of 72 ± 2°F. Food and water were available ad libitum throughout the experiments. Fifty animals were used in each experimental and each control group.

 FLV. A large batch of FLV was prepared as recommended by Rowe et al. (9) and was stored at −60°. Preliminary testing established that 0.2 ml of this preparation, diluted and injected i.v. into Swiss-Webster mice, produced splenomegaly (mean spleen weight, more than 1000 mg at 20 days after challenge) and a mortality rate higher than 90%.

 Extraction of the Coenzymes Q Fraction from Tissue and Blood. The tissues (liver or spleen) of all animals in a group were removed and homogenized at various time intervals after infection with FLV. Duplicate determinations on 10 g of wet tissue were carried out. The coenzymes Q fraction was extracted from these tissues and purified by a previously described method (1).

 The coenzymes Q were extracted from blood by the method of Redalieu et al. (8), and the extracts were purified as described earlier (1). Twenty ml of pooled blood were collected from all animals in a group at various time intervals after infection with FLV. Determinations were carried out in duplicate.

 Determination of the Total Coenzymes Q Content in Purified Extracts. The modified Craven test described by Redalieu et al. (8) was used. The purified extracts contain the mixture of all the coenzyme Q homologs present, and the Craven test quantitatively determines (by spectrophotometric means) the total amount of these materials in the sample. The

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2The abbreviation used is: FLV, Friend leukemia virus.
results are expressed as the concentration of the predominant coenzyme Q (coenzyme Q₉ in mice) per g, wet tissue.

Estimation of Coenzyme Q Individual Homolog in the Purified Extracts. The individual homologs were separated by reversed-phase paper chromatography followed by densitometric quantitation according to previously described procedure (1). This method allows the direct estimation of individual coenzyme Q homolog present in the extract. We calculated the total coenzymes Q concentration by adding the results for the individual homologs, and the results are expressed as µg/g, wet tissue.

Standards. Coenzyme Q₁₀ was obtained from Cudahy Laboratories, Omaha, Neb.; coenzyme Q₉ was isolated from rat livers and purified as reported (1); coenzymes Q₈ and Q₇ were kindly supplied by Dr. Karl Folkers, Institute for Biomedical Research, Austin, Texas; and coenzyme Q₆ was obtained from Sigma Chemical Company, St. Louis, Mo. All solvents were purified prior to use.

General. Coenzymes Q estimations were carried out by the modified Craven test and by the densitometric technique, 10, 20, 30, and 40 days after infection with FLV. The experiment was repeated 5 times, and the results were statistically analyzed.

In this work, special care was taken to minimize the individual variations so often found during investigation of coenzymes Q levels (3, 5, 7). For this purpose, the determinations were carried out on pooled tissues (livers, spleens, or blood) from 50 animals in a group.

RESULTS AND DISCUSSION

The results of this study indicate that in the blood of mice infected with FLV there is a statistically valid, notable increase in coenzymes Q concentration as the disease progresses. As Table 1 shows, this increase eventually reaches about 5-fold the normal level of these coenzymes. Moreover, while the blood of normal mice contains only coenzyme Q₉, the blood of mice infected with FLV also contains coenzyme Q₁₀. The low absolute concentration (in µg/ml) of coenzymes Q in the blood precluded the quantitative estimation of the individual homologs.

As shown in Table 2, the level of total coenzymes Q in the liver of mice with FLV does not differ appreciably from the liver levels in normal mice. However, in the liver of leukemic mice, the relative concentration of coenzyme Q₁₀ begins to increase 10 days after infection with FLV to levels detectable by the methods used. Since the liver is known to biosynthesize its own coenzymes Q, and since coenzymes Q₉ and Q₈ are the predominant homologs in mice liver, this observation may point to a shift in the biosynthetic pattern of these coenzymes.

The total concentration of coenzymes Q in the spleen of mice with FLV is appreciably the same as the spleen levels of normal mice (Table 3). Furthermore, the same homologs (coenzymes Q₉ and Q₁₀) seem to be biosynthesized during the progress of the disease.

Shichiri et al. (10) reported no difference in the coenzymes Q level in the liver of tumor-bearing rats (transplantable ascites hepatoma) and that of normal controls. Thus the results of our investigation of total coenzymes Q levels in the liver of mice infected with FLV are in complete agreement with theirs. This is of interest, since the 2 studies involved different tumor systems in different animal species.

A wide variation in the total coenzymes Q concentration in the blood of 30 "normal" individuals has been reported (8). Since a similar wide variation was found in the blood of 23 cancer patients with a variety of neoplastic conditions (10), it was concluded that on the average there was no significant

<table>
<thead>
<tr>
<th>Day after FLV infection</th>
<th>Group</th>
<th>Total coenzymes Q (µg/ml)</th>
<th>Significance level of difference between FLV and control groups</th>
<th>Individual homologs present</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Q₉</td>
<td>Q₁₀</td>
</tr>
<tr>
<td>10</td>
<td>FLV</td>
<td>0.5 ± 0.2</td>
<td>Not significant</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.6 ± 0.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>20</td>
<td>FLV</td>
<td>1.8 ± 0.3</td>
<td>0.005</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.5 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>30</td>
<td>FLV</td>
<td>2.5 ± 1.6</td>
<td>0.01</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.5 ± 0.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>FLV</td>
<td>3.3 ± 1.6</td>
<td>0.005</td>
<td>X</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>0.7 ± 0.1</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a Control group, mice given i.v. injections of 0.2 ml of 0.9% NaCl solution (50 mice in a group); FLV group, mice given injections of FLV in 0.2 ml of 0.9% NaCl solution (a minimum of 50 mice in each group).

b Total coenzymes Q concentration was estimated spectrophotometrically by Craven's method.

c Mean ± S.D. of 6 experiments.
Table 2

*Estimation of coenzymes Q concentration in liver of mice infected with FLV*

The livers of 50 animals in a group were homogenized, and the coenzymes Q content was determined as described in "Materials and Methods." The Craven method estimates the total coenzymes Q concentration in the sample, irrespective of the homologs present. The densitometer method determines the concentration of the individual homologs present.

<table>
<thead>
<tr>
<th>Day after FLV infection</th>
<th>Group</th>
<th>Total coenzymes Q (µg/g, wet wt)</th>
<th>Individual homologs&lt;sup&gt;b&lt;/sup&gt; (µg/g, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Craven method</td>
<td>Densitometric method</td>
</tr>
<tr>
<td>10</td>
<td>FLV</td>
<td>50.0 ± 7.5&lt;sup&gt;e&lt;/sup&gt;</td>
<td>46.1 ± 9.4</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>52.0 ± 8.4</td>
<td>53.9 ± 9.4</td>
</tr>
<tr>
<td>20</td>
<td>FLV</td>
<td>51.3 ± 9.9</td>
<td>49.6 ± 6.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>53.3 ± 9.7</td>
<td>54.7 ± 15.0</td>
</tr>
<tr>
<td>30</td>
<td>FLV</td>
<td>54.3 ± 8.6</td>
<td>53.2 ± 6.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>40.3 ± 9.1</td>
<td>40.6 ± 11.6</td>
</tr>
<tr>
<td>40</td>
<td>FLV</td>
<td>40.3 ± 7.0</td>
<td>41.9 ± 4.9</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>38.4 ± 7.2</td>
<td>36.7 ± 21.6</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control group, mice given i.v. injections of 0.2 ml of 0.9% NaCl solution (50 mice in a group); FLV group, mice given injections of 0.2 ml of FLV in 0.9% NaCl solution (a minimum of 50 mice in each group).

<sup>b</sup> Estimated by densitometry on paper chromatogram.

<sup>c</sup> Mean ± S.D. of 6 experiments.

Table 3

*Estimation of coenzymes Q concentration in spleen of mice infected with FLV*

The spleens of 50 animals in a group were homogenized, and the coenzymes Q content was determined as described in "Materials and Methods." The Craven method estimates the total coenzymes Q concentration in the sample, irrespective of the homologs present. The densitometer method determines the concentration of the individual homologs present.

<table>
<thead>
<tr>
<th>Day after FLV infection</th>
<th>Group</th>
<th>Total coenzymes Q (µg/g, wet wt)</th>
<th>Individual homologs&lt;sup&gt;b&lt;/sup&gt; (µg/g, wet wt)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Craven method</td>
<td>Densitometric method</td>
</tr>
<tr>
<td>10</td>
<td>FLV</td>
<td>8.2 ± 1.8&lt;sup&gt;e&lt;/sup&gt;</td>
<td>12.1 ± 5.1</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>12.5 ± 8.5</td>
<td>13.6 ± 8.3</td>
</tr>
<tr>
<td>20</td>
<td>FLV</td>
<td>11.7 ± 3.2</td>
<td>14.9 ± 5.5</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>13.9 ± 3.9</td>
<td>17.8 ± 3.2</td>
</tr>
<tr>
<td>30</td>
<td>FLV</td>
<td>8.5 ± 3.4</td>
<td>14.2 ± 7.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6.5 ± 1.4</td>
<td>9.3 ± 1.9</td>
</tr>
<tr>
<td>50</td>
<td>FLV</td>
<td>14.4 ± 5.3</td>
<td>17.5 ± 9.2</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>6.3 ± 6.8</td>
<td>12.1 ± 4.2</td>
</tr>
</tbody>
</table>

<sup>a</sup> Control group, mice given i.v. injections of 0.2 ml of 0.9% NaCl solution (50 mice in a group); FLV group, mice given i.v. injections of 0.2 ml of FLV in 0.9% NaCl solution (a minimum of 50 mice in each group).

<sup>b</sup> Estimated by densitometry on paper chromatogram.

<sup>c</sup> Mean ± S.D. of 6 experiments.

difference between the blood level of total coenzymes Q in cancer patients and that in normal individuals. The presence of coenzymes Q in blood is mainly associated with the cell fraction, and it has been reported (11) that the erythrocytes do not contain appreciable amounts of coenzymes Q. Puera et al. (6) demonstrated cytochemically that the coenzymes Q concentration decreases in the cells of the erythropoietic system with the maturity of the cell. Since FLV infection involves the erythroblastic cells and since the coenzymes Q concentration in the leukemic blastic blood cells has not been studied, it could then be possible that the high level of coenzymes Q observed by us during FLV infection may be due
to the increased number of these cells as the disease progresses. The need for an extension of coenzymes Q blood-level studies to other neoplastic systems is clearly indicated since these results may be of potential clinical importance. These studies are the subject of our continuing investigations.

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REFERENCES

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