Changes in Normal and Abnormal Colony Formation of Thyroid Cells in vivo, When Transplanted at 1 Day or 6 Weeks after X-Irradiation in situ

H. WATANABE and J. H. HENDRY

Department of Cancer Research
Research Institute for Nuclear Medicine and Biology
Hiroshima University
Kasumi 1-2-3 Minami-ku
Hiroshima 734, Japan

Cancer Research Campaign Department of Experimental Radiation Oncology, Paterson Institute for Cancer Research
Christie Hospital (NHS) Trust
Manchester M20 9BX, U.K.
(Received, February 7, 1994)
(Revised Received: May 30, 1994)
(Accepted, June 3, 1994)

Thyroid cells were given 5.5 Gy X-rays in situ and transplanted after 1 day or after 6 weeks to form colonies in fat pads. The colony-forming efficiency unexpectedly decreased by a factor of about 2 with this increase in delay time before transplantation. In addition, there was a concomitant marked increase above the control levels in the proportion of new structures of thyroid origin containing abnormal follicles. These quantitative and qualitative increases in injury may be related to the gradual expression of thyroid abnormalities following irradiation in situ, and they may have implications for the recovery of functional tissue subunits in other organs.

INTRODUCTION

Cellular recovery in slowly-renewing tissues after irradiation has been studied variously using chromosomal (1–5), colony (4, 6) and cell growth (7) endpoints. In only one tissue, the liver, have experiments been performed which showed that the gradual loss of chromosomal lesions expressed as micronuclei occurred concomitantly with an increase in the colony-forming efficiency of cells (4). Also, the relative lack of recovery in several systems measured after neutron irradiation (1–3, 5, 7) indicates the importance of repair as opposed to repopulation in
the recovery phenomena. In the thyroid, the frequency of induced chromosomal lesions was shown to decline by 6 weeks after X-irradiation (3). Studies for comparison are now reported of the colony-forming efficiency of cells transplanted from the thyroid to fat-pad sites for growth at the beginning and end of a 6 week period after irradiation in situ. Such studies provide further evidence for the cellular basis of tissue responses (e.g. 8, 9, 10).

MATERIALS AND METHODS

Experiments were performed in Hiroshima, Japan, and in Manchester, U.K. Male F344 (CDF F344/Cr 1BR) rats were obtained from the Charles River Company (Atsugi, Japan; Margate, U.K.). The donors were 10 weeks old, and the recipients were either 4 weeks old (Hiroshima) or 6 weeks old (Hiroshima and Manchester).

The thyroids of donor rats anaesthetised using sodium pentobarbitol were given 5.5 Gy X-rays at 1.2 Gy/min (Shimazu SHT-250M-3 200 kVp, 25 mA, hvl = 1.2 mm Cu, Hiroshima; Pantak 300 kVp, 10 mA, hvl = 2.3 mm Cu, Manchester) through a 1 cm circle in a 3 mm thick lead sheet. The dose of 5.5 Gy was chosen because this is the dose used previously in Manchester to study loss of chromosome aberration (3), and also it is similar to the dose of 5 Gy used in carcinogenesis studies (11).

The transplantation assay is described in detail elsewhere (11, 12). Briefly, the donor thyroids from 30 10-week old rats were minced and incubated with collagenase, pronase, and DNase in M199 medium. Nine serial dilutions of the resultant viable single-cell suspensions in brain homogenate were inoculated into the fat pads of a total of 25 recipient animals. The recipients were thyroidectomised 1 day before inoculation, and put on a low iodine diet, to produce maximal growth of the transplanted cells in each of usually 5 inoculated fat-pad sites (one medioventral and 4 laterodorsal). After 4 weeks the fat pads were stained with haematoxylin and examined for positive growth. The frequencies of positive growth among the inoculated sites for the various cell inocula were analysed using a double-logarithmic transformation in the DRFIT computer program (13) to calculate the number of cells containing one clonogen i.e. the reciprocal of the colony-forming efficiency, and the slope of the dilution curve which is a measure of whether the data are compatible with single-cell transplantation kinetics (e.g. 14).

It was noticed that some of the structures were abnormal. This was characterised by lack of colloid, and the presence of multilayers of cells forming structures of varying sizes (e.g. Figure 1a, right structure). This was in contrast to normal-appearing follicles which were lined with a single layer of cuboidal cells resting on a basement membrane (Figure 1a, left structure).

The normal and abnormal structures were examined histologically. Some of the histological sections were restained using the periodic-acid-Schiff reaction (15), which is known to stain the colloid in normal thyroid follicles (16). The epithelial nature of the structures was assessed using an anti-rat-EGF monoclonal antibody (Otsuka Pharmaceutical Co). AutoProbe-LM and Inten SE-11 (Janssen Life Science Products, Belgium) were used for immunogold-silver staining. A thyroglobulin antibody was used to identify the thyroid origin of the structures. The sections were incubated for 30 minutes at room temperature with a rabbit anti-human thyroglobulin
antibody (A251 DAKO A/S, Denmark) diluted 1:500. Then the sections were sequentially incubated with a biotin-labelled secondary antibody and an alkaline phosphatase conjugated streptavidin complex using new fuchsin as the chromogen substrate (DAKO LSAB kit alkaline phosphatase system 40, K0628, DAKO Co. Carpintia).

Table 1. Number of cells containing 1 clonogen, and the type of follicular structures produced, using different delay times before assay.

<table>
<thead>
<tr>
<th>Recipients</th>
<th>Cells/clone (x10^6)</th>
<th>Structure: All normal follicles</th>
<th>Cell surviving fraction: All structures</th>
<th>Normal structures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>+</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(a) 4 weeks old,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiroshima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day: control</td>
<td>380 ± 50</td>
<td>36/36+</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5.5 Gy</td>
<td>720 ± 160</td>
<td>23/56</td>
<td>0.53 ± 0.12</td>
<td>0.22 ± 0.05</td>
</tr>
<tr>
<td>6 weeks: control</td>
<td>410 ± 60</td>
<td>34/34</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5.5 Gy</td>
<td>1560 ± 430</td>
<td>8/41</td>
<td>0.26 ± 0.07</td>
<td>0.052 ± 0.014</td>
</tr>
<tr>
<td>(b) 6 weeks old,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hiroshima</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 day: control</td>
<td>1120 ± 410</td>
<td>29/30</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5.5 Gy</td>
<td>1470 ± 160</td>
<td>9/33</td>
<td>0.76 ± 0.24</td>
<td>0.21 ± 0.07</td>
</tr>
<tr>
<td>6 weeks: control</td>
<td>930 ± 180</td>
<td>37/38</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>5.5 Gy</td>
<td>1980 ± 530</td>
<td>8/22</td>
<td>0.47 ± 0.14</td>
<td>0.17 ± 0.05</td>
</tr>
<tr>
<td>(c) 6 weeks old,</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Manchester</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>control</td>
<td>270 ± 90</td>
<td>-</td>
<td>1.0</td>
<td></td>
</tr>
<tr>
<td>5.5 Gy, 1 day</td>
<td>610 ± 160</td>
<td>-</td>
<td>0.44 ± 0.10</td>
<td></td>
</tr>
<tr>
<td>5.5 Gy, 6 weeks</td>
<td>1080 ± 220</td>
<td>-</td>
<td>0.25 ± 0.09</td>
<td></td>
</tr>
</tbody>
</table>

(a) and (b), 1 experiment for each age of recipients. Slope of the dilution curve not significantly different from 1.0 in 3 out of the 4 groups in each experiment.
(c) 3 experiment pooled. Slope of the dilution curve significantly less than 1.0 in all cases.
+ frequency of positive growth in the inoculated sites. ++ structures containing at least 1 normal follicle.
Standard error quoted.
RESULTS

Using 4 week-old recipient rats in Hiroshima, it was calculated that around 400 cells contained one clonogen (Table 1). The surviving fraction was around 0.5 when the assay was performed at 1 day after 5.5 Gy, and surprisingly was about half this value when an interval of 6 weeks was left before assay. A feature also noticed was the appearance of abnormal follicles in some of the structures produced after irradiation (Figure 1a). The follicular structures in the Hiroshima studies were examined further. The colloid was PAS positive (Fig. 1b), and the cell cytosol was EGF positive (Fig. 1c). These are characteristics of normal thyroid follicles developing even in animals fed an iodine-reduced diet. The abnormal follicles lacked colloid, and were PAS negative as expected. Both normal and abnormal structures were of thyroid origin, as detected by the staining procedure for thyroglobulin (Fig. 1d). More abnormal follicles were observed after irradiation with the longer delay before assay, so that the proportion of structures...
containing all-normal follicles was reduced (see Section (a), Table 1). This had the effect of producing a greater decline in survival with increasing delay time, with respect to clonogens producing all-normal structures.

The studies in Hiroshima were repeated using 6 week-old recipients (Table 1), because of the known lower colony-forming efficiency of cells in older rats (15), and the use of 6 week-old recipients in Manchester (see below). With the older recipients, a higher number of cells was required to form a follicular structure, and there was a tendency towards higher values of surviving fraction. However, the features of a reduction in surviving fraction with an increase in delay time before assay, the presence of abnormal structures after irradiation, and the increase in their frequency with an increase in delay time before assay, were again noted but were not as marked as when the younger recipients were used.

The studies in Manchester using 6 week-old rats (Table 1) confirmed the effect in another Institute, but the results were more consistent with the Hiroshima data using 4 week-old rats, regarding colony-forming efficiency and its decline with increasing delay time. However, the slope of the dilution curve was consistently less than 1.0, which might be related to another feature noted in these studies, namely the presence of lymphocytic infiltration in the structures in some cases. Also, in view of this a detailed study of the structure morphology was not attempted.

DISCUSSION

The colony-forming efficiencies in these studies of at least one clonogen in 300 cells were lower than found in previous studies, where there was 1 clonogen in about 80 cells (11, 12). This may be due to factors such as the different substrains of rats or the different low levels of iodine in the various diets. The lymphocytic infiltration noted in some of the studies has been recorded previously in dysplastic mammary outgrowths produced in a similar assay (18). However, the surviving fractions assessed at 1 day after 5.5 Gy (0.44–0.76) are similar to the value of about 0.5 which can be read from a graph of similar data for a range of doses (19).

The decrease in surviving fraction by up to a factor of 2 when the delay before assay was increased from 1 day to 6 weeks, was unexpected because of the reported increase between time zero and 1 day (19) and the data for chromosome aberrations (3). Data for Wistar rats in Manchester showed that after 5.5 Gy the frequency of cells with bridges and fragments was 34±4% when stimulation of the cells to divide and express the lesions was made immediately after irradiation, 25±4% at 1 day, and 15±3% at 6 weeks, compared to a control level of 10±2% (3). If these aberrations are considered to be cell lethal, the aberration yields above the control can be translated into surviving fractions of 73±5% (time zero, ie \((90-24)/90\)%), 83±5% (1 day), and 94±4% (6 weeks). This expected increase in surviving fraction is in contrast to the measured decrease for the colony-forming cells. One possible explanation for the discrepancy may relate to the fact that the colony data pertain to less than 1% of the cells, whereas the chromosomal data reflect the response of the majority of the cells which are capable of dividing at least once. If the colony-forming cells are a subpopulation more sensitive to
radiation-induced differentiation under conditions in situ, their number may show such a decline. This idea regarding subpopulations of progenitor cells in the thyroid relates to the detection of cells in the follicular epithelium with varying proliferative potential (20). A radiation-induced decrease in the number of progenitor cells has been detected in haemopoietic tissue (21), but not in other organ systems such as kidney (22) or liver (23).

Abnormal thyroid structures with reversed polarity have been observed previously in suspension cultures eg (24). However, the first observations of abnormal thyroid follicular structures in vivo induced by irradiation are presented here with also the presence of mixed structures containing both and abnormal structures. In the combined controls in the Japanese studies the incidence of these was very low (2 out of 138 structures) but it rose to values of 64% (57/89) at 1 day after 5.5 Gy and 75% (47/63) at 6 weeks. This implies the induction of stable mutants. A similar effect regarding an increased incidence of abnormalities has been noted regarding ductal dysplasias in mammary glands using a transplantation technique (10).

The quantitative and qualitative increase in follicle injury described here may be related to the gradual expression of gross thyroid abnormalities after irradiation in situ. Also, the findings reported here may have parallels in other organ systems. There is evidence for example that cellular recovery in the kidney is not reflected in terms of organ function (22). It has already been suggested that such a difference may reflect the inability of surviving irradiated cells to produce functionally-normal replacement structures during tissue recovery (25).

ACKNOWLEDGEMENTS

These studies were supported in part by the Cancer Research Campaign, UK. Thanks to Ann Kaye for her help in the preparation of this manuscript.

REFERENCES