Leukemia in a Rhesus Monkey (Macaca Mulatta) Following Exposure to Whole-Body Proton Irradiation

By ALAN M. SIEGAL, HAROLD W. CASEY, ROBERT W. BOWMAN, AND JOSEPH E. TRAYNOR

THE SPONTANEOUS DEVELOPMENT of leukemia in a subhuman primate is an uncommon occurrence. Whereas ionizing radiation will predictably induce leukemia in certain strains of mice and rats and is associated with human leukemia, as far as we could ascertain, there are only two previously reported cases of leukemia associated with ionizing radiation in primates.1 2 The purpose of this report is to document a case of acute granulocytic leukemia in a rhesus monkey following whole-body proton irradiation.

CASE REPORT

This animal was part of a group of 123 primates (Macaca mulatta) which was exposed to 400 Mev proton irradiation at the University of Chicago cyclotron in March 1965. The acute effects of this irradiation have been reported elsewhere. 3 The animals which survived the acute phase of the irradiation have been maintained in a chronic animal facility at Brooks Air Force Base for the observation of late irradiation effects. The animal reported herein received 100 rads of 400 Mev proton irradiation delivered at a dose rate of 16 rads/min.

Thirty-two (32) months postirradiation the animal developed anorexia and lethargy, and was noted to have slight dependent edema and weight loss. Physical examination revealed generalized lymphadenopathy, splenomegaly, and cutaneous petechiae. The CBC done at this time revealed a total white cell count of 204,500 per mm.3 The general morphologic appearance of the leukemic cells in the peripheral blood smear is illustrated in Figure 1. Essentially all leukemic cells were primitive blast types. Some cells resembled promyelocytes. The cells were large and varied from 11 to 21 μ in diameter. Demarcation between the nucleus and cytoplasm was frequently poorly defined. Nuclei were generally

From the Radiobiology and Biosciences Divisions of the USAF School of Aerospace Medicine, Aerospace Medical Division, AFSC, United States Air Force, Brooks Air Force Base, Texas.

This work was funded in part by the National Aeronautics and Space Administration, Task No. 7757-04.

Further reproduction is authorized to satisfy the needs of the U.S. Government.

The animals involved in this study were maintained in accordance with the “Guide for Laboratory Animal Facilities and Care” as published by the National Academy of Sciences—National Research Council.

First submitted May 15, 1968; accepted for publication June 30, 1968.

ALAN M. SIEGAL, Capt., USAF, MC.: Space Radiation Branch, Radiobiology Division, USAF School of Aerospace Medicine, Brooks AFB, Texas. HAROLD W. CASEY, Maj., USAF, VC: Chief, Cytology-Pathology Branch, Biosciences Division, USAF School of Aerospace Medicine, Brooks AFB, Texas. ROBERT W. BOWMAN, 1st Lt., USAF, BSC: Cytology-Pathology Branch, Biosciences Division, USAF School of Aerospace Medicine, Brooks AFB, Texas. JOSEPH E. TRAYNOR, Maj., USAF, MC.: Chief, Space Radiation Branch, Radiobiology Division, USAF School of Aerospace Medicine, Brooks AFB, Texas.
Fig. 1.—Peripheral blood smear illustrating large pleomorphic leukemic cells. A normal segmented neutrophil is visible on the left. Wright's stain × 1000.

Fig. 2.—Blast type cell from peripheral blood. This type of cell with the rounded nuclear profile contains few mitochondria (M) when compared to Figure 3. N = nucleus; G = Golgi zone × 10,800.
round, but occasional nuclei had indentions that gave a bean-shaped appearance. One to four nucleoli were distinguishable and the nuclear chromatin had a fine delicate appearance. Nuclei were surrounded by a thin rim of cytoplasm that exhibited variation in staining characteristics from deep to pale basophilia. A large number of cells had multiple cytoplasmic vacuoles. Azurophilic granules were seen in some cells. Occasional Auer rods were seen. Phagocytized erythrocytes were frequently seen in leukemic cells. Cells in mitosis were also present in peripheral smears. Myelocytes and metamyelocytes were present together with normal band and segmented granulocytes.

Electron microscopic examination of the osmium tetroxide fixed buffy coat revealed that the majority of the leukocytes could be divided into two morphologically distinct groups. These two groups of blast type cells differed primarily in nuclear shape and the number and location of mitochondria. One cell type (Fig. 2) exhibited a slightly irregular round nuclear profile with a thin rim of cytoplasm that contained round or oval mitochondria, an occasional segment of rough endoplasmic reticulum, many vesicles, ribosomes, an occasional bundle of fine filaments, and a Golgi zone. In contrast, the other cell type (Fig. 3) exhibited an indented bean-shaped nucleus in a round cellular profile. The large cytoplasmic area formed by the nuclear indentation contained many mitochondria and a Golgi zone. Distributed randomly throughout the cytoplasm were many vesicles, ribosomes, an occasional bundle of fine filaments, and an occasional segment of rough endoplasmic reticulum. Approximately 30–50 per cent of all leukocytes contained crystallloid inclusions (Fig. 4). However, non-neoplastic cells also contained crystalloids as they were observed in endothelial cells of lymph nodes and Kupffer cells of the liver. A similar crystallloid was also seen in a leukocyte of one other irradiated monkey that was caged adjacent to the leukemic monkey.

Table 1 shows the hematology data over the postirradiation period. In retrospect,
Fig. 4.—Cytoplasmic crystalloid inclusion. Similar crystalloids were seen in 30–50 per cent of all leukocytes as well as in non-neoplastic cells. In most areas these crystalline arrays were bounded by a membranous coat (arrow). N = nucleus; M = mitochondrion. × 41,300.

on reexamining the smear from 25 August 1966, abnormal cells were found with an occasional blast cell and myelocyte, nucleated red cells, and rare hypersegmented (6-lobed) polymorphonuclear leukocytes.

After discovery of the leukemia, the monkey was taken to another laboratory* and sacrificed in order to obtain material for viral and transmission studies.

Pathologic Findings

The Sedanian-anesthetized monkey was euthanized by exsanguination via the saphenous vein. Gross autopsy findings were recorded and representative specimens of all lesions, organs and tissues were placed in buffered formalin and/or Zenker’s solution. Histologic sections were prepared from paraffin embedded blocks, then stained with hematoxylin-eosin. Selected sections were also stained with Giemsa stain.

Necropsy revealed the body to be in good nutritional condition. Petechiae were diffuse in the skin and subcutaneous tissues of the entire body. All lymph nodes were enlarged. Several nodes were up to 10 times their normal size. On cut surface, enlarged nodes were greyish-white and demarcation between the cortical and medullary areas frequently was not visible. Tissues from the thymic area were edematous and contained small (less than 1 cm.) white nodules. The medullary cavity of long bones was filled with reddish-grey marrow. The spleen was uniformly enlarged to a size approximately ten times normal. The splenic capsule was taut and the surface was milky grey. On cut surface, the parenchyma was greyish-pink and had white irregular shaped nodular areas that were up to 6 mm.

*National Cancer Institute (Dr. Frank Rauscher) and the Bionetics Research Laboratories (Dr. John Landon).
Table 1.

<table>
<thead>
<tr>
<th>Date</th>
<th>Time Postradiation</th>
<th>WBC</th>
<th>Neuts</th>
<th>Lymphs</th>
<th>Monos</th>
<th>Eos</th>
<th>Hgb</th>
<th>Hct</th>
<th>Platelets (x 10⁶)</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 Mar 65</td>
<td>R + 24 hrs</td>
<td>4,400</td>
<td>56</td>
<td>43</td>
<td>1</td>
<td>1</td>
<td>13.2</td>
<td>42</td>
<td>327</td>
</tr>
<tr>
<td>19 Mar 65</td>
<td>R + 48 hrs</td>
<td>2,200</td>
<td>56</td>
<td>42</td>
<td>1</td>
<td>1</td>
<td>11.5</td>
<td>37</td>
<td>270</td>
</tr>
<tr>
<td>21 Mar 65</td>
<td>R + 4 days</td>
<td>3,250</td>
<td>47</td>
<td>48</td>
<td>3</td>
<td>2</td>
<td>12.8</td>
<td>40</td>
<td>307</td>
</tr>
<tr>
<td>24 Mar 65</td>
<td>R + 7 days</td>
<td>5,000</td>
<td>53</td>
<td>46</td>
<td>1</td>
<td>1</td>
<td>11.9</td>
<td>35</td>
<td>342</td>
</tr>
<tr>
<td>1 Apr 65</td>
<td>R + 15 days</td>
<td>4,550</td>
<td>51</td>
<td>45</td>
<td>4</td>
<td>2</td>
<td>10.5</td>
<td>34</td>
<td>283</td>
</tr>
<tr>
<td>16 Apr 65</td>
<td>R + 30 days</td>
<td>5,350</td>
<td>65</td>
<td>26</td>
<td>9</td>
<td>1</td>
<td>12.1</td>
<td>38</td>
<td>170</td>
</tr>
<tr>
<td>17 May 65</td>
<td>R + 60 days</td>
<td>7,450</td>
<td>62</td>
<td>36</td>
<td>0</td>
<td>1</td>
<td>13.6</td>
<td>42</td>
<td>232</td>
</tr>
<tr>
<td>15 Jun 65</td>
<td>R + 90 days</td>
<td>6,350</td>
<td>25</td>
<td>74</td>
<td>0</td>
<td>1</td>
<td>14.0</td>
<td>45</td>
<td>309</td>
</tr>
<tr>
<td>13 Sep 65</td>
<td>R + 180 days</td>
<td>7,850</td>
<td>17</td>
<td>80</td>
<td>0</td>
<td>3</td>
<td>13.9</td>
<td>42</td>
<td>338</td>
</tr>
<tr>
<td>25 Aug 66</td>
<td>R + 17 months</td>
<td>24,100</td>
<td>36</td>
<td>58</td>
<td>2</td>
<td>4</td>
<td>13.4</td>
<td>44</td>
<td>408</td>
</tr>
<tr>
<td>6 Dec 67</td>
<td>R + 32 months</td>
<td>204,500*</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>10.0</td>
<td>33</td>
<td>73</td>
<td></td>
</tr>
</tbody>
</table>

*92 percent blast.

in diameter. The liver was moderately enlarged. Its external surface had a greyish cast and the lobular pattern was accentuated. Subjacent and medial to the gall bladder, a 2.5 × 3.0 cm. white nodule protruded from the hepatic parenchyma.

Histologically, leukemic cells had invaded and multiplied extensively throughout the body as they were found in the lymph nodes, spleen, bone marrow, liver, kidney, urinary bladder, intestinal tract, kidneys, heart, lungs, and brain. The leukemic cells were morphologically similar in all organs and tissues (Figs. 5 and 7). These cells had discrete borders even though they were closely packed. Nuclei were generally round to oval. One to two nucleoli were present in most cells. Numerous small clumps of nuclear chromatin located

Fig. 5.—Leukemic cells in an axillary lymph node illustrating the typical appearance of leukemic cells in all tissue sections. These cells have moderate amounts of cytoplasm. Numerous mitotic figures and nucleated erythrocytes are visible. H& E × 400.
Fig. 6.—Leukemic node showing diffuse neoplastic involvement of the cortex with capsular invasion which extends into the surrounding loose areolar tissue. A portion of the capsular sinus is visible on the right. H&E × 53.

near the nuclear membrane, were present in all cells. Most cells had a moderate amount of cytoplasm. Generally, the cytoplasm had a fine granulated appearance. Occasionally, eosinophilic myelocytes were visible in the neoplastic cellular accumulation (Fig. 7-inset). Erythrophagocytosis was also occasionally seen. Mitotic figures were present in high numbers as most neoplastic areas contained 2 to 4 mitotic figures per high power field.

All lymph nodes were infiltrated with neoplastic cells which in many areas had completely replaced the node's normal components in both the cortex and medullae. In numerous nodes, leukemic cells had invaded the capsule and infiltrated the surrounding loose fatty tissues (Fig. 6). In some nodes, remnants of germinal centers were still distinguishable.

Definite thymic tissue was not identified at the histologic level; however, lymph nodes in the thymic area showed diffuse involvement with the neoplastic process.

The splenic capsule was intact; however, the internal splenic structure was markedly distorted by leukemic cells. The trabeculae were widely separated and frequently invaded by neoplastic cells. Sheets of neoplastic cells corresponded to the grey nodules seen on cut surface at autopsy. Normal splenic follicles were not present; however, remnants of these structures could be distinguished.

Bone marrow spaces in sections from both long and flat bones were essentially 100 per cent cellular. A high percentage of the marrow cells were neoplastic. In numerous areas these cells extended into perivascular spaces of the haversian system. In occasional areas the periosteum was also invaded and cellular infiltration of the surrounding muscular tissue had occurred.

The liver exhibited diffuse infiltration with heavy collars of neoplastic cells that surround both the hepatic and portal vessels (Fig. 7). The one elevated nodule seen at autopsy was composed almost entirely of neoplastic tissue, and only at the parenchymal edge were clusters of hepatic cells distinguishable.

Moderate to light infiltration of the leptomeninx of the cerebrum was present. In occasional areas leukemic cells were also present in perivascular areas of the cerebral
LEUKEMIA IN A RHESUS MONKEY

Fig. 7.—Low power view of liver illustrating leukemic cell infiltration. Dense cellular area in upper left corner comprises a portion of a solid neoplastic nodule. Smaller areas of infiltration are visible around the portal and central vein areas. H&E x 45. Inset: Eosinophilic myelocyte present in the neoplastic nodule, upper left corner. H&E x 1380.

vessels located in the grey lamina. No involvement of other parts of the brain and meninges was seen.

COMMENTS

The first reported case of primate leukemia was in 1923. Since that time, only a small number of cases of spontaneous leukemia have been reported. Only a few of these have been described in detail. However, all of these spontaneous cases were chronic lymphatic leukemia.

The role of the ionizing radiation in the etiology of this animal’s blood dyscrasia cannot be stated unequivocally. It is beyond the scope of this report to review the evidence for the leukemogenic action of ionizing radiation. However, some interesting points should be mentioned. All the cases of spontaneous leukemia in monkeys have been chronic lymphatic, whereas the three cases of radiation-related leukemia in primates have been acute granulocytic. It is also interesting to note that the peak incidence of leukemia in Hiroshima and Nagasaki occurred about 5–7 years after the explosion. The present case developed 2 1/2 years after irradiation which would correlate with the above peak incidence if one assumes the life span of a rhesus monkey to be one-third to one-half that of a human. The dose of radiation (100 rads) received by this animal was relatively low. The other experimental animals in this group received doses of 25–1200 rads under the same conditions. No abnormalities of the hematopoietic system have developed as yet in these survivors. The presence of cytoplasmic crystalloids in leukemic and normal
cells of this animal is indicative of a viral infection; however, if they are of viral origin, their relationship to the concurrent leukemia is unknown.

**SUMMARY**

A rhesus monkey (Macaca mulatta) developed acute granulocytic leukemia 2½ years after whole-body irradiation with protons. This is the third reported case of primate leukemia following irradiation.

**SUMMARIO IN INTERLINGUA**

Un simia rheso (Macaca mulatta) disveloppava acute leucernia granulocytic 2½ annos post irradiation protonic del corpore total. Isto es le tertie reportate caso de leucernia occurrente post irradiation in un primato.

**ACKNOWLEDGMENTS**

The authors wish to express their appreciation to the Veterinary Sciences Division of the School of Aerospace Medicine, especially Capt. James F. Harwell, for the detection of this primate's clinical condition. We also thank Mrs. Yvonne Balthazor, SSgt. Ralph E. Wright, and SSgt. Fred Morris for their technical assistance.

**REFERENCES**

Leukemia in a Rhesus Monkey (Macaca Mulatta) Following Exposure to Whole-Body Proton Irradiation

ALAN M. SIEGAL, CAPT., HAROLD W. CASEY, MAJ., ROBERT W. BOWMAN, 1ST LT. and JOSEPH E. TRAYNOR, MAJ.