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Low blood cell counts in wild Japanese monkeys after the Fukushima Daiichi nuclear disaster

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In April 2012 we carried out a 1-year hematological study on a population of wild Japanese monkeys inhabiting the forest area of Fukushima City. This area is located 70 km from the Fukushima Daiichi Nuclear Power Plant (NPP), which released a large amount of radioactive material into the environment following the Great East Japan Earthquake of 2011. For comparison, we examined monkeys inhabiting the Shimokita Peninsula in Aomori Prefecture, located approximately 400 km from the NPP. Total muscle cesium concentration in Fukushima monkeys was in the range of 78–1778 Bq/kg, whereas the level of cesium was below the detection limit in all Shimokita monkeys. Compared with Shimokita monkeys, Fukushima monkeys had significantly low white and red blood cell counts, hemoglobin, and hematocrit, and the white blood cell count in immature monkeys showed a significant negative correlation with muscle cesium concentration. These results suggest that the exposure to some form of radioactive material contributed to hematological changes in Fukushima monkeys.

Following the massive earthquake that struck eastern Japan on March 11, 2011, a nuclear reactor core meltdown occurred at the Fukushima Daiichi Nuclear Power Plant (NPP). After the NPP disaster, the range of radiocesium soil concentrations in Fukushima City was 10,000–300,000 Bq/m²¹, and the cumulative radiation dose in the air measured using an integrated dosimeter for the 2-year period after April 2011 was 7.5 mSv².

Despite the occurrence of several NPP disasters, including the Chernobyl accident in 1986, no research on the health effects of radioactive material has been done on wild primates. We therefore examined the relationship between long-term exposure to radioactive material and the health effect on wild Japanese monkey (*Macaca fuscata*) populations inhabiting Fukushima City, the eastern part of Fukushima Prefecture, located 70 km from the NPP (Fig. 1). The Japanese monkey, which is endemic to Japan, has a life span of more than 20 years³. In Japanese monkey populations in Fukushima City, adult females aged 5 years or higher are pregnant in the late fall and give birth in the spring⁴.

Between April 2011 and June 2012, Hayama *et al.*⁵ investigated chronological changes in muscle radiocesium concentrations in monkeys inhabiting Fukushima City. The Japanese monkey usually forms a troop of 50–100 individuals of maternal lineage, and each troop has a home range of about 8–31 km² in snowy areas⁶. The mean muscle radiocesium concentration in monkeys captured in areas with a soil contamination level of 100,000–300,000 Bq/m² was significantly higher than that in monkeys captured in areas of 10,000–100,000 Bq/m² ($P < 0.001$)⁵.

A large number of studies have investigated the health effects of the Chernobyl disaster^{7,8}. In particular, hematological abnormalities such as a decreased blood cell count in people living in contaminated areas have been reported as a long-term effect of low-dose radiation exposure⁸. In Fukushima, the radiation dose has been reported to be correlated with the occurrence of morphological abnormalities in lycaenid butterflies⁹ and with decreased abundance of birds, butterflies, and cicadas^{10,11}.

In this study, we therefore performed a hematological study of Japanese monkeys inhabiting Fukushima City (hereafter, Fukushima) (Fig. 1), using known chronological records of radiation exposure as described above, to reveal the health effects of radiation exposure. For comparison, we examined Japanese monkeys inhabiting the Shimokita Peninsula in Aomori Prefecture, located approximately 400 km from the NPP (hereafter, Shimokita) (Fig. 1). Data from non-human primates—the closest taxonomic relatives of humans—should make a notable contribution to future research on the health effects of radiation exposure in humans.

Figure 1 | Map showing the location of Fukushima and Shimokita, where the present investigation was conducted. This map was modified from blank open license maps provided by the Geospatial Information Authority of Japan.

Results

Hematological values, muscle radiocesium concentrations, and fat indices are shown in Table 1. Total muscle cesium concentration in Fukushima monkeys was in the range of 78–1778 Bq/kg, whereas the level of cesium was below the detection limit in all Shimokita monkeys. Comparisons of hematological values between areas showed that white blood cell count (WBC), red blood cell count (RBC), hemoglobin (Hb), and hematocrit (Ht) were significantly different (two-way ANOVA with effect of areas, $p < 0.01$, Table 2). However, no significant effects of age or age by areas interaction were observed in those values (Table 2). To further analyze the 4 hematological values that showed a significant difference between Fukushima and Shimokita populations (WBC, RBC, Hb, and Ht), we performed multiple comparisons using the Tukey–Kramer method to compare Shimokita monkeys and the 2 groups of Fukushima monkeys. The results showed that the hematological values were significantly lower in all Fukushima monkeys than in Shimokita monkeys ($p < 0.001$), with no significant difference between the 2 groups of Fukushima monkeys. Platelet counts, WBC differential, and fat index were not observed to be significantly different.

When the relationships between 4 hematological values (WBC, RBC, Hb, and Ht) and muscle radiocesium concentration were assessed in individual Fukushima monkeys, WBC was observed to have a significant negative correlation in the immature group (Pearson's correlation coefficient, $r = -0.52$, $p = 0.011$, Table 3, Fig. 2), but no correlation was observed in the mature group ($r = 0.029$, $p = 0.887$). No other hematological values showed significant correlations with muscle radiocesium concentration. The 4 hematological items were correlated with each other as well.

Discussion

In the present study, WBC, RBC, Hb, and Ht were significantly lower in Fukushima monkeys than in Shimokita monkeys. Although direct comparison of this study with previous studies may be problematic, since methods and sites for blood sampling reportedly affect blood properties¹², the blood cell counts of the present Shimokita monkeys

are similar to those reported previously^{13,14}. Nigi *et al.*¹⁴ reported that Japanese monkey populations, except for the Yakushima macaque (*Macaca fuscata yakui*) subspecies, show large individual variability in blood cell counts, with no difference between the local populations. Although the number is small, previous studies that investigated blood cell counts in wild Japanese monkey populations showed no evidence for different hematological values among the populations. It is possible that the low blood cell counts in Fukushima monkeys were caused by infectious diseases or malnutrition. However, our group has been investigating and has autopsied more than 1000 monkeys captured in Fukushima City since 2008, with no findings of infectious disease specific to the area that may have reduced blood cell counts. Moreover, fat indices did not vary significantly between Fukushima and Shimokita monkeys (Tables 1, 2), indicating that the low blood cell counts was not caused by malnutrition.

Because no cesium was detected in the muscle of Shimokita monkeys, the low hematological values in Fukushima monkeys could have therefore been due to the effect of other radioactive materials. Stepanova *et al.* conducted hematological studies of Ukrainian children between 1993 and 1998 after the Chernobyl disaster of 1986¹⁵. They observed reduced blood cell counts, Hb, and platelet counts in these children, and found that the extent of the reduction in each child correlated with the level of radiocesium in the soil of the area of residence. This is similar to what we observed in the present study. Although blood cell counts varied significantly between Fukushima and Shimokita populations, no significant difference was observed between the 2 groups of Fukushima monkeys captured in areas with different levels of soil contamination. The study conducted in Ukraine that is described above also showed that WBC did not differ significantly near the border of 2 areas with different levels of soil contamination¹⁵. Further studies are needed to investigate monkeys inhabiting an area with a high soil contamination level. In addition, the muscle radiocesium concentrations in Fukushima monkeys are known to show seasonal variation, increasing 2–3-fold in winter⁵. This suggests that muscle cesium concentrations would vary greatly

Table 1 | Hematological values of Japanese monkeys captured in Fukushima and Shimokita

Hematological values	Fukushima												Shimokita				West Japan* (reference data)			
	10,000–100,000 Bq/m ² in soil						100,000–300,000 Bq/m ² in soil													
	Immature			Mature			Immature			Mature										
	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	Mean	SD	n	SD
WBC ($\times 10^2/\mu\text{l}$)	13	65.3	45.4	11	87.6	42.6	11	71.7	34.0	16	84.3	51.1	17	148.6	33.6	8	137.1	76.7	200	144
RBC ($\times 10^2/\mu\text{l}$)	14	432.6	90.4	10	484.6	72.4	10	476.7	88.8	14	443.8	99.1	17	549.2	78.2	11	518.9	53.2	198	472
Hb (g/dl)	13	10.6	1.9	10	12.0	2.0	10	11.7	2.1	14	11.2	2.5	17	14.6	2.1	11	14.2	1.5	200	14.1
Ht (%)	14	33.4	6.8	10	38.7	6.2	10	36.3	6.4	14	35.5	8.3	17	45.9	6.2	11	45.5	5.1	204	42
Pl ($\times 10^4/\mu\text{l}$)	12	40.6	33.8	7	23.3	13.5	6	16.3	9.5	13	29.9	12.9	14	39.8	10.0	8	33.8	11.6	-	-
Lymphocytes (%)	15	62.1	3.7	15	61.6	2.9	14	62.1	3	17	59.2	15.4	18	61.8	2.7	13	60.4	2.9	-	-
Granulocytes (%)	15	36.2	3.8	15	36.7	3.1	14	36.7	3.2	17	33.8	9.1	18	36.4	2.6	13	37.8	2.9	-	-
Monocytes (%)	15	1.7	1.1	15	1.1	0.8	14	1.2	0.9	17	1.2	1.1	18	1.8	1.0	13	1.8	1.1	-	-
Fat index	15	9.0	5.9	15	13.2	7.8	14	12.7	5.4	18	10.2	8.6	18	12.6	6.7	13	16.3	8.5	-	-
Cs (Bq/kg)	14	430.3	341.6	15	291.2	257.1	14	560.4	242.6	18	908.3	501.4	18	ND	-	13	ND	-	-	-

WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; Pl, platelets; Cs, muscle radiocesium concentration; ND, not detected; n, number of individuals.

*Nigri et al [Ref. 14].

Table 2 | Variation in hematological values and fat index in relation to area and age in two-way ANOVA with area and age as factors

	Mean square	F	d.f.	p
WBC				
Area	32624.89	14.94	2, 70	<0.01
Age	1083.38	0.50	1, 70	0.48
Age $\times$ Area	1714.51	0.79	2, 70	0.46
RBC				
Area	47339.27	6.93	2, 70	<0.01
Age	258.46	0.04	1, 70	0.85
Age $\times$ Area	13876.56	2.03	2, 70	0.14
Hb				
Area	76.98	18.13	2, 69	<0.01
Age	0.78	0.18	1, 69	0.67
Age $\times$ Area	6.37	1.50	2, 69	0.23
Ht				
Area	0.17	6.72	2, 86	<0.01
Age	0.01	0.47	1, 86	0.50
Age $\times$ Area	0.02	0.72	2, 86	0.49
Pl				
Area	860.60	2.65	2, 53	0.08
Age	138.10	0.43	1, 53	0.52
Age $\times$ Area	1020.18	3.14	2, 53	0.05
Lymphocytes				
Area	0.00	0.23	2, 86	0.80
Age	0.01	1.13	1, 86	0.29
Age $\times$ Area	0.00	0.21	2, 86	0.81
Granulocytes				
Area	0.00	1.20	2, 86	0.31
Age	0.00	0.11	1, 86	0.74
Age $\times$ Area	0.00	1.70	2, 86	0.19
Monocytes				
Area	0.00	2.51	2, 86	0.09
Age	0.00	0.00	1, 86	0.95
Age $\times$ Area	0.00	0.00	2, 86	1.00
Fat index				
Area	101.68	1.94	2, 86	0.15
Age	77.52	1.48	1, 86	0.23
Age $\times$ Area	104.28	1.99	2, 86	0.14

among monkeys captured in the same area, as in this study (Table 1). The biological half-life of cesium in monkeys is approximately 21 days⁵. Even if radiation damage is the cause of the low blood cell counts seen here, it is difficult to prove a causal relationship because of the time lag between uptake of the radioactive material and the appearance of radiation damage. The difficulty multiplies when comparing areas with relatively similar radiation exposure.

Despite these complex factors, a significant negative correlation was observed between WBC and muscle radiocesium concentrations in immature Fukushima monkeys (Table 3). In addition, WBC, RBC, Hb, and Ht values—which were lower Fukushima monkeys compared with Shimokita monkeys—were significantly correlated with each other, suggesting that with more samples it will be possible to verify the correlation between the 4 hematological values and the muscle radiocesium concentrations. In immature Fukushima monkeys, WBC was significantly negatively correlated with cesium concentration in the muscle, but in mature Fukushima monkeys, no correlation between hematological values and muscle cesium concentration was observed. It is possible that WBC declined because immature monkeys were more vulnerable to radioactive materials. Moysich *et al.*¹⁶ conducted an epidemiological study to investigate the risk of leukemia among Europeans affected by the Chernobyl disaster, and found that the risk was clearly higher among small children than among adults, suggesting that the hematological consequences of radiation exposure vary by age.

Table 3 | Correlation between muscle radiocesium concentration and hematological values in Fukushima monkeys

	RBC			Hb			Ht			Cs		
	n	r	p	n	r	p	n	r	p	n	r	p
Immature												
WBC	22	0.430	0.045	21	0.501	0.021	22	0.526	0.012	23	−0.520	0.011
RBC				22	0.967	<0.001	23	0.964	<0.001	23	0.011	0.959
Hb							23	0.982	<0.001	22	0.037	0.870
Ht										23	0.015	0.947
Mature												
WBC	23	0.430	0.041	23	0.415	0.049	23	0.430	0.041	26	0.029	0.887
RBC				23	0.980	<0.001	23	0.971	<0.001	23	−0.342	0.110
Hb							23	0.985	<0.001	23	−0.363	0.089
Ht										23	−0.400	0.058

Pearson's correlation coefficient (r) was calculated to show correlation between muscle radiocesium concentrations and hematological values for each age group. WBC, white blood cell count; RBC, red blood cell count; Hb, hemoglobin; Ht, hematocrit; Cs, muscle radiocesium concentration; n, number of individuals; p, p values.

The hematological changes in the Fukushima monkeys might likely be the result of exposure to some form of radioactive material, but only radiocesium concentration was measured in this study. These hematological changes might have been caused by a decline in hematopoietic function in the bone marrow because the WBC differential did not differ between the Fukushima and Shimokita monkeys. We therefore plan to investigate in a future study the underlying mechanism in detail with the aim of detecting other radioactive materials, such as ^{90}Sr . Presently, it is difficult to investigate Japanese monkeys inhabiting highly contaminated areas where entry is restricted. However, we intend to perform hematological tests and other measurements as soon as the relevant permissions can be obtained.

Low blood cell count does not necessarily mean that the health of individual monkeys is at risk. However, it may suggest that the immune system has been compromised to some extent, potentially making individual animals and the entire troop susceptible to, for example, epidemic infectious disease. It is therefore necessary to perform long-term immunological and other health-related studies of Japanese monkey populations in Fukushima.

Methods

Animals and ethics. Blood and muscle samples were collected from 92 Japanese monkeys (61 from Fukushima and 31 from Shimokita) between April 2012 and March 2013. Individuals were captured using box traps in forested areas close to farmland. Collection of the monkeys used for experiments in this study was performed after obtaining permission from the governors of Fukushima and Aomori Prefectures in accordance with the Japanese Monkey Management Plan, which was established based on the Wildlife Protection and Hunting Management Law. The methods used to capture and kill monkeys conformed to the guidelines published by the Primate Research Institute of Kyoto University¹⁷. Moreover, the Japanese monkeys inhabiting this area are not listed as endangered species on the Japanese Red List, as revised by the Ministry of the Environment in 2012¹⁸.

The mean muscle radiocesium concentration in monkeys captured in areas with a soil contamination level of 100,000–300,000 Bq/m² was significantly higher than that in monkeys captured in areas with 10,000–100,000 Bq/m² ($p < 0.001$)⁵. We therefore divided Fukushima monkeys into 2 groups, based on whether the areas of sample collection had a soil contamination level of 10,000–100,000 Bq/m² or 100,000–300,000 Bq/m². We then compared hematological values between the 2 groups of Fukushima monkeys and the Shimokita monkeys.

Among the monkeys used in this study, those individuals captured between April and June 2014 were the same individuals as those used in a previously published study⁵. This previous study aimed to establish a correlation between the changes in cumulative cesium concentration over time in the monkeys' muscles and the levels of soil contamination. However, the aim of the present study was to determine whether

Figure 2 | White blood cell counts and muscle radiocesium concentrations in immature Japanese monkeys captured in Fukushima.

there is a correlation between changes in hematological values and exposure to radiation, using muscle cesium concentration in the monkeys as an index of radiation exposure, which is clearly different from the previous study's aim. Additionally, in the case of wild animals, it is difficult to determine the long-term amount of radiation exposure. In the previously published study on cumulative radiation exposure and its effects on health, the levels of soil contamination at the capture site and the amounts of accumulated radioactivity in the body at the time of capture were used as indices of radiation exposure. Conversely, in the present study, the amount of accumulated cesium in the muscles was used as the index.

Blood and muscle samples. Blood samples were collected by 10 ml syringe and 18 gauge needle from the heart immediately after killing. Blood was mixed in a vial containing ethylenediaminetetraacetic acid (EDTA) dipotassium salt dihydrate. The methods used for blood sampling and sample transportation were consistent among monkeys and between different areas. Carcasses and blood samples were transported under refrigerated conditions to our laboratory and subjected to autopsy and hematological analysis within 2 days after blood was collected. The following measures were included in the hematology profile: WBC, RBC, Hb, Ht, platelet count, and WBC differential (lymphocytes, monocytes, and granulocytes). Hematology results were obtained using a Sysmex Hemacytometer XT-2000 (Kobe, Japan). EDTA blood smears were prepared with Giemsa staining and reviewed manually under a microscope. The body weight of each monkey was measured in grams. During necropsy, 500–1,000 g of muscle tissue were collected from the hind limb for measurement of radiocesium content. Skeletal muscle was chosen as samples because that organs of 500 g or more were required to measure with radiocesium. Collected muscle tissues were stored frozen at -30°C until used for radioactivity measurement.

Age determination and fat index. During autopsy, the status of tooth eruptions was checked and used to estimate the age of each animal, as described by Iwamoto *et al.*¹⁹. The estimated age was used to divide the animals into 2 age groups: immature (0–4 years) and mature (>5 years). Furthermore, we calculated fat indices to evaluate monkeys' nutritional status. In the previous study²⁰, the ratio of mesenteric fat weight to body weight is proportional to the percentage of body fat in Japanese monkeys. The fat index was defined as mesenteric fat weight (g) divided by body weight (g), multiplied by 1000.

Measurements of radioactivity. The radioactivity of radiocesium of muscle samples was analyzed using a germanium semiconductor spectrometer (Canberra, GC2020-7500SL-2002CSL, Meriden, CT) and a NaI (Tl) scintillation detector (Atomtex, AT1320A, Minsk, Belarus). Data were corrected to background radiation dose in the measurement environment on an as-needed basis. ^{134}Cs was detected using 604.70- and 795.85-keV gamma-ray energies, while ^{137}Cs was detected using 661.6-keV gamma-ray energies. The radioactivity of radiocesium was adjusted to the value on the day of capture based on its physical half-life. The limit of detection was 10 Bq/kg. Muscle cesium concentration was calculated as the combined concentration of ^{134}Cs and ^{137}Cs per kg fresh weight muscle.

Statistics. Data were analyzed using SPSS 10.0 for Windows (SPSS Inc., Chicago, Illinois, USA). Two-way ANOVA was used to compare hematological values and fat index with areas and age as factors. The Tukey–Kramer method was used to perform multiple comparisons of hematological values between groups. Pearson's correlation coefficient was used to examine correlations between muscle cesium concentration and hematological values.

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Author contributions

Sa.N., Se.N., N.I., T.U. and F.K. collected specimens; Y.K., S.T. and T.O. designed the study; K.O. and T.K. analyzed the data; and S.H. wrote the paper.

Additional information

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