A Case Report of Spontaneous Polyarthritis in *Cynomolgus* Monkeys

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ABSTRACT. Veterinary x-ray photography and examinations of synovial fluid, blood and urine were conducted on a *Cynomolgus Monkey* from China (5 years old) which exhibited macroscopically visible systemic joint swelling after the quarantine period. The presence of inflammatory cells in the synovial fluid obtained by arthrocentesis, high counts of neutrophils, monocytes and large unstained cells and the elevated serum CRP level suggested that the lesions in this animal were due to polyarthritis.

KEY WORDS: *Cynomolgus Monkey*, polyarthritis, spontaneous.

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On October 24, 2011, a *Cynomolgus Monkey* kept as a stock animal at Ina Research Inc. was found in a feverish condition. This animal was imported from China on January 13, 2011 and underwent legally required quarantine at Ina Research Inc. No appreciable clinical signs were observed in this animal during the quarantine period. Thereafter, this animal exhibited swelling of the fingers, knees and tail and a feverish condition, but did not receive any particular therapeutic treatment. Since deformity of the pelvis (Fig. 1) and loss of mobility were observed on February 16, 2012, veterinary clinical examinations were conducted to determine the cause of the swelling of the joints and to verify if the swelling was due to polyarthritis or not [1–5]. Two additional animals with no visible systemic joint swelling were also examined for comparison to clearly identify the observed lesions.

A total of 3 *Cynomolgus Monkeys* of the same batch from China (Breeder: Hainan Jingang Biotech Co., Ltd.) were used in this study, i.e., 1 healthy male (Animal ID No. 1), 1 healthy female (Animal ID No. 2) and 1 female with joint swelling (Animal ID No. 3).

The animals were fasted from the afternoon 1 day prior to each examination. Blood samples for analysis of arthritis-related serum biomarkers, hematology and clinical chemistry were collected from the femoral vein of unanesthetized animals using sterile, disposable polypropylene syringes fitted with sterile, disposable needles. These samples were divided into 4 aliquots as follows: approximately 3 ml into polyester resin tubes with procoagulant film (Venoject® II Vacuum Blood Collecting Tube, Terumo Corporation, Tokyo, Japan) to obtain serum for analysis of arthritis-related biomarkers, 0.9 ml into tubes containing 0.1 ml of 3.2 w/v% sodium citrate to obtain plasma for the coagulation tests, approximately 1 ml into tubes containing EDTA-2K to obtain

whole blood for the other hematological parameters and approximately 3 ml into tubes containing heparin sodium to obtain plasma for clinical chemistry. Blood samples for collection of serum and plasma were centrifuged (approx. $1,600 \times g$, 10 min., 4°C). Blood samples for collection of serum were allowed to stand at room temperature for 30 to 60 min prior to centrifugation.

Serum samples obtained were sent refrigerated to The Corporation for Production and Research of Laboratory Primates where C-reactive protein (CRP) levels, rheumatoid factor (RF) and antinuclear antibody (ANA) were analyzed.

The prothrombin time and activated partial thromboplastin time were determined using an Automated Blood Coagulation Analyzer CA-510 (Sysmex Corporation, Hyogo, Japan). ADVIA 120 Hematology System (Siemens Healthcare Diagnostics K.K., Tokyo, Japan) was used to analyze the following hematological parameters: the red blood cell count, hemoglobin concentration, hematocrit value, mean corpuscular volume, mean corpuscular hemoglobin, mean corpuscular hemoglobin concentration, reticulocyte count, platelet count, white blood cell count, and neutrophil, lymphocyte, monocyte, eosinophil, basophil and large unstained cell counts. In addition, blood smear slides were prepared from all animals and stained with May-Grunwald-Giemsa. Clinical Analyzer Model 7180 (Hitachi High-Technologies Corporation, Tokyo, Japan) was used for the following blood chemistry parameters: aspartate aminotransferase, alanine aminotransferase, alkaline phosphatase, lactate dehydrogenase, creatinine kinase, glucose, total bilirubin, urea nitrogen, creatinine, total cholesterol, triglycerides, phospholipids, inorganic phosphorus, calcium, uric acid, sodium, potassium, chloride, total protein, albumin and albumin-globulin ratio.

After blood sampling, animals were anesthetized with a combination of ketamine (Veterinary Ketamine®, Daiichi Sankyo Pharma Co., Ltd., Tokyo, Japan) at 5 mg/kg and xylazine (Celactal®, Bayer Yakuhin, Ltd., Osaka, Japan) at 1 mg/kg by intramuscular injection. Radiographs of the lumbar area and extremities (including the tail) where joint swelling was observed in the model animal were taken from all animals (so that the same areas as in the model animal were radiographed in the animals with no visible joint swell-

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Fig. 1. Swelling at the joints (A: finger, B: knees, C: tail) and pelvic deformity (D).

Fig. 2. Changes in the articular surfaces (A: knee joints, B: tail joints).

ing) at the following settings: voltage 55 kV, current 5.0 mA for the lumbar area and voltage 55 kV, current 2.0 mA for the extremities; intensifying screen, present; grid, none; and film, HR-S (Fuji Film Co., Ltd., Tokyo, Japan).

After radiography, 3 ml of sterile physiological saline

Fig. 3. Synovial fluid from a normal animal (A) and an animal with swelling joint (B).

(Otsuka Normal Saline: Otsuka Pharmaceutical Factory, Inc., Tokushima, Japan) were injected into the joint cavity by arthrocentesis from the lateral surface of the knee joint using sterile, disposable polypropylene syringes fitted with sterile, disposable 23 G needles. Then, different sets of sterile, disposable polypropylene syringes containing heparin sodium (Novo-Heparin 10,000 units/10 ml for Injection:

Mochida Pharmaceutical Co., Ltd., Tokyo, Japan) fitted with sterile, disposable 23 G needles were inserted into the joint cavity from the lateral surface of the knee joint to collect the synovial fluid. The collected synovial fluid was stained with new methylene blue and examined under a microscope. An analgesic, Meloxicam (Metacam®: Boehringer Ingelheim Japan, Tokyo, Japan), was administered intramuscularly at 1 mg/kg prior to anesthesia and after the experiment for pain control.

Fresh urine (urine samples accumulated for 4 hr after the start of urine collection) and cumulative urine (the entire amount of urine accumulated over 24 hr after the start of urine collection) were used for urinalysis. At least 1 ml of fresh urine and at least 3 ml of cumulative urine were taken using urine pipettes and analyzed. After sampling, the remaining urine was measured to determine the total urine volume using graduated cylinders. Fresh urine samples were analyzed to determine the pH, protein, glucose, ketone bodies, bilirubin, occult blood and urobilinogen using urine test strips (MULTISTIX®SG-L: Siemens Healthcare Diagnostics K.K.) and an Automatic Urine Analyzer Clinitek 500 (Siemens Healthcare Diagnostics K.K.), while the urine specific gravity was measured using a Urine S.G. Refractometer URC-JE (Atago Co., Ltd., Tokyo, Japan). The fresh urine samples were then centrifuged (approx. $1,600 \times g$, 10 min., 4°C) to obtain sediments which were examined microscopically after Sternheimer staining. After visually examining the color, the cumulative urine samples were centrifuged (approx. $1,600 \times g$, 10 min., 4°C), and the supernatant was examined using the Clinical Analyzer Model 7180 (Hitachi High-Technologies Corporation) to determine urinary NA, K and CL.

Animal ID No. 3 that exhibited joint swelling showed roughening of the articular surfaces of the knees and tail on

Table 1. Percentage of inflammatory cells count

ID No.	Total cell count (No. of cells)	Inflammatory cell count (No. of cells)	Incidence of inflammatory cells (%)
1	117	2	1.7%
2	76	0	0.0%
3	184	40	21.7%

Table 2. Analysis of serum biomarkers

ID No.	CRP (mg/dl)	Rheumatoid factor (U/ml)	Antinuclear antibody (Titer)
	Acceptable range: ≤ 0.30	Acceptable range: ≤ 20	Acceptable range: < 20
1	0.13	0	< 20
2	0.04	0	< 20
3	2.96	0	< 20

radiograph (Fig. 2), high inflammatory cell percentages and an elevated white blood cell count and numerous nucleated cells in the synovial fluid (Fig. 3 and Table 1), elevated CRP level (Table 2) and white blood cell count as well as high neutrophil, monocyte and large unstained cell counts in hematology (Table 3). There were no statistically significant differences in the RF or ANA level, clinical chemistry values or urinalysis values.

No abnormal findings were noted in either the other animals.

The findings in Animal ID No. 3 suggested that there was no multiple organ inflammation, but only localized inflammation in the joints. Although the radiographs showed the presence of deformities in the articular surfaces, no disease-

Table 3. Hematology

ID No.	WBC ($10^3/\mu\text{l}$)	RBC ($10^6/\mu\text{l}$)	HGB (g/dl)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dl)	PLT ($10^3/\mu\text{l}$)
1	8.380	6.180	14.60	49.50	80.00	23.60	29.50	298.0
2	10.990	4.610	11.60	38.60	83.80	25.20	30.10	519.0
3	29.130	5.730	12.50	43.90	76.60	21.70	28.40	644.0

ID No.	%Neut (%)	%Lymph (%)	%Mono (%)	%Eos (%)	%Baso (%)	%LUC (%)	%Retic (%)	PT (sec)
1	34.20	58.40	5.30	1.00	0.50	0.60	1.50	7.90
2	63.70	31.10	3.00	1.50	0.50	0.30	1.60	7.80
3	77.20	16.50	5.20	0.20	0.50	0.50	1.60	9.80

ID No.	#Neut ($10^3/\mu\text{l}$)	#Lymph ($10^3/\mu\text{l}$)	#Mono ($10^3/\mu\text{l}$)	#Eos ($10^3/\mu\text{l}$)	#Baso ($10^3/\mu\text{l}$)	#LUC ($10^3/\mu\text{l}$)	#Retic ($10^9/\mu\text{l}$)	APTT (sec)
1	2.870	4.900	0.440	0.080	0.040	0.050	91.90	21.70
2	13.220	6.450	0.630	0.310	0.100	0.060	74.50	23.10
3	22.490	4.800	1.500	0.050	0.140	0.150	91.30	26.80

RBC: red blood cell count. WBC: white blood cell count. HGB: hemoglobin concentration. HCT: hematocrit value. MCV: mean corpuscular volume. MCH: mean corpuscular hemoglobin. MCHC: mean corpuscular hemoglobin concentration. PLT: platelet count. Neut: neutrophil. Lymph: lymphocyte. Mono: monocyte. Eos: eosinophil. Baso: basophil. LUC: large unstained cell counts. Retic: reticulocyte count. PT: Prothrombin time. APTT: activated partial thromboplastin time.

specific biomarkers were detected since the uric acid, RF and ANA values were not elevated. Therefore, it can be surmised that the joint swelling observed in the model animal was due to persistent, localized inflammation. However, since the method used to analyze the RF and the ANA is for human subjects, the possibility of autoimmune origin cannot be ruled out.

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