Hematological Characterization of Anemia Recently Prevailing in Wild Japanese Flounder in Japan

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ABSTRACT—Hematological characteristics of the anemia prevailing in wild Japanese flounder Paralichthys olivaceus were examined in detail by cell counting and microscopy. The erythrocyte counts, MCH, and MCHC decreased correlative with the decrease in the hemoglobin concentration. Moreover, the reduction of erythrocyte volume was also observed in many anemic fish. Light microscopy showed erythrocytes with structural abnormalities, such as vacuolated, weakly stained cytoplasm and deformed outlines. In the TEM observations of erythrocytes, an insufficient accumulation of hemoglobin was observed as well. These results indicate that the anemia can be identified as a hypochromic microcytic anemia accompanied with hypoglobulia and a structural abnormality of the erythrocytes.

Key words: anemia, hematology, Paralichthys olivaceus, erythrocyte, Japanese flounder

Recently, severe anemia has been observed frequently in both wild and cultured Japanese flounder, Paralichthys olivaceus, in Japan1-3. The anemia has been characterized by the decrease in hemoglobin concentration and hematocrit value, and the frequent occurrence of abnormal immature and mature erythrocytes having vacuolated or weakly stained cytoplasm and deformation2,3. However, these characterizations have been made mainly based on the observation of blood smears, and more detailed characterizations are needed to understand the anemia. The present study has been conducted to clarify the hematological characterization of the anemia in detail.

Fig. 1. Relationships between hemoglobin concentration (Hb) and the erythrocyte count a), MCV b), MCh c) and MCHC d) in the peripheral blood of flounder (●: anemic or non anemic wild fish, △: laboratory-reared healthy fish). Regression formulae and correlation coefficients for wild fish are given; data taken from laboratory reared fish were not included in the calculation.
Hematology of wild flounder showing anemia

The peripheral blood of each fish was taken from the dorsal artery with a heparinized syringe and needle. Hemoglobin concentration (Hb) was determined by the cyanmethemoglobin method. The blood was analyzed using an electronic cell counter (Coulter Counter Z2, Coulter, FL). As erythrocytes had almost the same cell volume as leucocytes, the cell counter analysis was unable to distinguish these cells. Therefore, the volume of all cells was used as the volume of erythrocyte. This substitution was found to have minimal effect on the values in our preliminary examination, because leucocytes account for <2–3% of the total blood cells. For erythrocyte count (EC), leucocyte counts measured using a hematocytometer were then subtracted from the total cell counts measured by the cell counter. Based on the measurements, erythrocytic indices, namely the mean corpuscular cell volume (MCV), mean corpuscular hemoglobin (MCH) and mean corpuscular hemoglobin concentration (MCHC), were calculated. For light microscopic observations, blood smears were stained with May-Grunwald and Giemsa stain. For electron microscopic observations, packed blood cells were embedded in Epon 812 (Taab Laboratories, UK), ultra-thin sectioned, and stained with uranyl acetate and lead acetate.

**Results and Discussion**

The anemic fish with low Hb showed lower values of EC, MCH and MCHC than the wild and reared healthy fish (Fig. 1-a, c and d). Clear correlations were detected between Hb and these values, indicating that the anemia was hypochromic. Many of anemic fish showed smaller MCV than healthy fish, although some of the anemic fish with low Hb had similar values of MCV to the healthy, laboratory-reared fish (Fig. 1-b). In the light microscopic examination, the blood cells derived from non-anemic wild fish and laboratory-reared fish (Hb>4 g/dL) predominantly consisted of normal mature erythrocytes (Fig. 2-a). While, abnormal immature and mature erythrocytes having vacuolated and/or weakly stained cytoplasm were frequently found in the wild anemic fish (Hb<4 g/dL). There was a tendency that the ratio of the abnormal cells increased with decreasing Hb. Furthermore, the mature erythrocytes often showed an unusual spindle-like shape in the anemic fish (Fig. 2-b). These deformed erythrocytes were apparently smaller than normal erythrocytes and found mostly in fish with low MCV. Although low MCV was found in many anemic fish, some of anemic fish had values of MCV similar to the healthy fish (Fig.1-b). In the anemic fish with normal MCV, a small number of deformed or small erythrocyte and a large of immature were found together (Fig. 2-c). These observations indicate that the anemia was microcytic. In the TEM observations, the abnormal erythrocytes were characterized with deformed outlines or an insufficient accumulation of hemoglobin (Fig. 2-d, e). However, cytoplasmic vacuolation, which was often found in smear samples, was not observed in TEM. The cytoplasmic vacuolation in the blood smear seems to have resulted from the reduction of hemoglobin and cytoplasmic volume.

The present results revealed by using cell counter that the anemia was hypochromic microcytic anemia. Moreover, the TEM observations demonstrated that the abnormal erythrocytes had an insufficient accumulation of hemoglobin. From the present results together with data from previous reports, the anemia can be characterized as a hypochromic microcytic anemia accommodated by using cell counter.
nied with hypogloblobia, the occurrence of immature erythrocytes and the structural abnormality of erythrocytes.

Although the causative agent of the anemia has not been fully clarified yet, the blood-feeding monogenean parasite *Neoheterobothrium hirame* has been suggested as the most probable agent3-6). In general, anemia is one of the most common symptoms in blood-feeding monogenean infections. Moreover, hypochromic microcytic anemia has been reported from crucian carp heavily infected with the monogenean *Diplozoon nipponicum7)*. The present results support, from the point of view of hematology, the previous suggestion that *N. hirame* is the cause of the anemia. However, there have been no reports showing the induction of abnormal erythrocytes caused by infections of blood feeding monogenean. It is necessary to know how blood loss by the parasite infection leads to the structural abnormalities in erythrocytes in Japanese flounder to conclude the cause of the anemia.

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**References**