Qualitative and quantitative cytometric analysis of peripheral blood leukocytes in carps (*Cyprinus carpio*)

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ABSTRACT: The studies were performed involving qualitative and quantitative cytometric analysis of peripheral blood leukocytes in healthy carps at various stages of their ontogeny, i.e., in 3- to 29-month old carps. Three populations of leukocyte line cells were distinguished, which differed in forward scatter (FSC) and side scatter (90º, SSC) of laser light. The most abundant leukocyte pool was present in the eldest (23- to 29-month old) fish. Lower numbers of the cells were observed in the youngest (3- to 9-month old) carps while the lowest levels of the cells were detected in carps of the moderate age (11- to 21-month old). The leukocyte populations, distinguished on grounds of FSC/SSC characteristics, were suggested to correspond to lymphocytes/thrombocytes (low FSC, low SSC), granulocytes (high FSC, high SSC) and monocytes (high FSC, low SSC). ***For free full paper in pdf format see http://www.vri.cz/vetmed.asp***

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INTRODUCTION

Flow cytometry represents one of the most modern diagnostic techniques. It permits rapid and multiparameter appraisal of physical and metabolic properties of cells and their components. With increasing frequency, the technique is applied also in morphological and functional studies on fish cells even if specific surface markers of cells in the animals only begin to be recognised. In the fish, the technique of flow cytometry has been employed, i.a., in genetic, pathogenetic studies, in studies on immunopathological processes (Chilmończyk and Monge, 1999, Chilmończyk *et al*., 1995; Perez *et al*., 1994; Rodriguez *et al*., 1991, 1993, 1995), in quantitative analysis of immune system cells and qualitative analysis of some aspects of cell-mediated immunity (Bly *et al*., 1990; Chilmończyk and Monge, 1999; Chilmończyk *et al*., 1995, 1997; DeLuca *et al*., 1983; Ellsaesser *et al*., 1985; Evans *et al*., 1987; Hamdani *et al.*, 1998; Lamas and Ellis, 1994; Morgan *et al*., 1993; Pettersen *et al*., 1995; Secombes and Fletcher, 1992; Siegl *et al*., 1998; Slierendrecht *et al*., 1995; Voccia *et al*., 1994).

Present study aimed at qualitative and quantitative cytometric analysis of peripheral blood leukocytes in carps at various stages of ontogeny.

MATERIAL AND METHODS

The studies were performed on 33 healthy carps, 3 to 29 months of age, 30 g to 1 000 g in body weight, in the following experimental groups: Group I of 13 carps, 3 to 9 months of age, Group II of 10 carps, 11 to 21 months of age, Group III of 10 carps of 23 to 29 months of age. Blood of carps was studied. Blood samples were taken from tail vein to tubes containing heparin (50 IU per ml blood), in field conditions, immediately after taking the fish out of the pond. Laboratory tests were performed 60 to 80 min after blood sampling, i.e. immediately after delivery of the blood samples to the laboratory. General condition and health status of the fish were examined using routine methods of medical-veterinary diagnosis.

Qualitative and quantitative cytometric analysis of peripheral blood leukocyte populations was performed according to Vowells *et al*. (1995), as adapted to fish. Samples of a full, heparinised blood (50 IU/ml blood) was suspended in 29 volumes of 0.3% NaCl an the suspension was left for 30 min. Samples of the suspension of 100 µl volume were mixed each with 300 µl of Ortho-mune Lysing Reagent (Ortho Diagnostic System, Germany) and, then, after 20 min absolute numbers of leukocytes, lymphocytes, monocytes and neutrophilic granulocytes

were scored using Cytoron Absolute flow cytometer (Ortho Diagnostic System, Germany). Results of the tests, presented in Table 1 in absolute numbers, were subjected to statistical analysis using Student's *t*-test, at $p = 0.05$.

RESULTS AND DISCUSSION

The cytometric separation of peripheral blood leukocytes in carps using no monoclonal antibodies represents a technique which allows for an objective quantitative appraisal of leukocytes and their populations. The studies, performed with and without the oxygen metabolism stimulator, PMA, permitted to distinguish three populations of leukocyte line cells, which differed in a forward light scatter (FSC) and a side scatter (SSC), at the angle of 90°. The bi-dimensional analysis of cells using FSC/SSC system opened potential for differentiation of cells in respect to their size (FSC) and in respect to their shape and inner granules (SSC). The studies showed (Table 1) that the pool of leukocyte was most abundant in the eldest fish, i.e., in carps aging 23 to 29 months. The youngest carps, aging 3 to 9 months, demonstrated a significantly lower number of the cells and their lowest levels were seen in the carps aging 11 to 21 months. Among leukocytes, the cells characterised by low FSC/low SSC (81.0–82.2/ 22.3–23.6, respectively) formed the highest proportion in the eldest (23- to 29-month old) carps and comprised approximately 50% cells in the remaining experimental groups (i.e., in carps aging 3 to 9 months and those aging 11 to 21 months). The next in sequence of numerical force were cells which exhibited high FSC and high SSC (161.8–171.4 and 128.7–139.4, respectively), which were more numerous in the youngest (3- to 9-month old) fish and in 11- to 21-month old fish but were less frequent in the eldest carps (aging 23 to 29 months). The least abundant fraction was formed by cells of high FSC and low SSC (150.9–152.8 and 44.8 to 61.3, respectively). The highest numbers of the cells were detected in carps 11 to 21 months of age. The cells were less frequent in 3- to 9-month old carps and least frequent in the eldest, 23- to 29-month old carps. Except of the cells of low FSC and low SSC or high FSC and high SSC in carps of Groups I and II, the inter-group differences in content of individual types of cells were significant (Table 1).

Considering the FSC/SSC characteristics, the three distinct populations of leukocytes could be suggested to correspond to lymphocytes/thrombocytes (low FSC/low SSC), granulocytes (high FSC/high SSC) and monocytes (high FSC/low SSC), respectively. It should, however, be stressed that the cytometric profile of leukocyte populations defined in our studies differed from the data presented by other authors (Chilmończyk and Monge, 1999; Chilmończyk *et al*., 1995; Evans *et al*., 1987; Hamdani *et al*., 1998; Morgan *et al*., 1993; Siegl *et al*., 1998). Cytometric analysis of frontal kidney and of peripheral blood in rainbow trout (*Oncorhynchus mykiss*) demonstrated presence of two populations of cells, i.e., of lymphocytes and of granulocytes (Chil-mończyk and Monge, 1999; Chilmończyk *et al*., 1995; Morgan *et al*., 1993; Siegl *et al*., 1998) while in the spleen apart from erythrocytes only lymphocytes were detected (Siegl *et al*., 1998). In turn, Evans *et al*. (1987) in frontal kidney of a catfish (*Ictalurus punctatus*) demonstrated two populations of cells, including erythrocytes. The divergent data most probably should be linked to different species of the studied fish and to various applied procedures of testing.

Taking into account the ontogenetic aspect, the obtained results of quantitative studies demonstrated that the numerical values supplied by flow cytometry differ basically from those determined in our own studies using traditional techniques (Stosik, 1995). It should be noted that differences observed in the values have been significant and that they could not be qualified as unequivocally correlated with ontogenetic development of studied carps. In the case of leukocyte number determined by flow cytometry, absence of quantitative differ-

Table 1. Number of leukocytes as well as number and percentage of cell subpopulations detected by flow cytometry in healthy carps

Group of carps	Number of leukocytes	Number (%) of		
		lymphocytes	granulocytes	monocytes
I	41 279.2 ± 4 637.9*	22 197.9 ± 2 196.7* (53.8 ± 5.3)*	18 297.4 ± 1 368.4** (44.3 ± 3.9)**	783.9 ± 53.2** (1.9 ± 0.1)**
II	37 663.6 ± 2 963.5*	21 889.3 ± 2 358.2* (58.1 ± 6.2)*	14 846.5 ± 1 532.7** (39.4 ± 3.6)**	927.8 ± 67.1** (2.5 ± 0.3)**
III	55 701.4 ± 6 119.6	46 107.7 ± 4 253.4 (82.8 ± 7.4)	8 935.4 ± 716.3 16.5 ± 1.3)	658.3 ± 48.5 (1.2 ± 0.1)

* significant decrease as compared to value for Group III
** significant increase as compared to value for Group III

ences has been disclosed in the youngest carps, aging 3 to 9 months. Number of the cells in older carps has been significantly lower (around 30% in carps aged 11 to 21 months, around 15% in carps aged 23 to 29 months). The carps aged 3 to 9 months and 11 to 21 months have demonstrated significantly lower numbers of lymphocytes (around 45%). Within monocytes, around fivefold elevated numbers of the cells were demonstrated in carps aged 3 to 9 months, an around fourfold decrease in in their number in carps aged 23 to 29 months and absence of changes in their number in the fish aged 11 to 21 months. On the other hand, in the fish of all experimental groups, i.e., aging 3 to 29 months, a markedly higher level of neutrophilic granulocytes has been disclosed (eightfold, fivefold and twofold, respectively, for carps aged 3–9 months, 11–21 months and 23–29 months).

CONCLUSIONS

The studies have proved that bi-dimensional cytometric analysis of cells in FSC/SSC system allows to obtain objective quantitative data on numerical force of leukocytes and their subpopulations (lymphocytes, granulocytes, monocytes), using no monoclonal antibodies. Using the cytometric technique, carps of various age demonstrated 37 663.6 to 55 701.4 leukocytes, including 21 889.3 to 46 107.7 lymphocytes (58.1 to 82.8%), 8 935.4 to 18 297.4 granulocytes (16.0 to 44.3%), 658.3 to 927.8 monocytes (1.2 to 2.5%). The cytometric data on leukocyte number or content of their subpopulations did not correlate with stages of ontogeny of the studied fish.

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