

Complement expression in common carp (*Cyprinus carpio* L.) during infection with *Ichthyophthirius multifiliis*

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Abstract

A real-time PCR assay for determination of the complement response to infection with the ectoparasite *Ichthyophthirius multifiliis* in carp is presented. Specific primers were designed for selected genes representing the three pathways of the carp complement system. The investigated complement molecules were C1r/s, C3, C4, C5, factor I, factor B/C2-A (Bf/C2-A), mannose-binding lectin (MBL) and MBL-associated serine protease (MASP). The expression of the selected genes was analyzed on RNA extracts from skin, liver, and whole blood from carp at 3, 12, 24, 36, and 48 h post-infection (pi) with *I. multifiliis*. A pronounced up-regulation of Bf/C2-A, in skin, blood, and liver (250-, 60-, and 4-fold respectively), was observed at later sampling points pi (24–48 h). In addition, an intermediate (from 5 to 13-fold) down-regulation of MASP was observed in skin and liver samples at 36 and 48 h pi with respect to control fish. MBL was expressed only in liver and no variation in the transcription level of this lectin was observed. Complement factor C3 was significantly up-regulated in liver (4-fold up-regulation, 24 h pi). The presented results indicate that infection with the parasite *I. multifiliis* in carp to a large extent stimulates the expression of complement molecules. Moreover, the dramatic and early up-regulation of Bf/C2-A in skin indicates a role of this molecule as an acute-phase reactant. Furthermore, our study confirms the role of fish skin as an important extra-hepatic site of expression of complement molecules as well as an active regulator of complement expression. Expression of some of the components of the complement system in blood suggests that leukocytes in carp act as an important extra-hepatic source of complement molecules.

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1. Introduction

The complement system is a major effector system of innate immunity. It is composed of a series of soluble plasma proteins and receptors, which play an essential role in alerting the host immune system of the presence of potential pathogens, as well as in their clearance [1]. The majority of the complement proteins are synthesized as

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inactive precursors in the liver, although extra-hepatic synthesis of complement proteins is also well documented and different cell types have been associated to complement production [2]. Complement activation in fish occurs, as in mammals, via three overlapping pathways (Fig. 1): the classical complement pathway (CCP), the alternative com-

plement pathway (ACP), and the lectin complement pathway (LCP) [1,3]. Activation of the CCP is triggered by binding of antibody to the C1 complex, formed by C1q and two serine proteases (C1r and C1s), or by direct binding of the C1q component to the pathogen surface [4–6]. The ACP does not contain any specific recognition molecules for

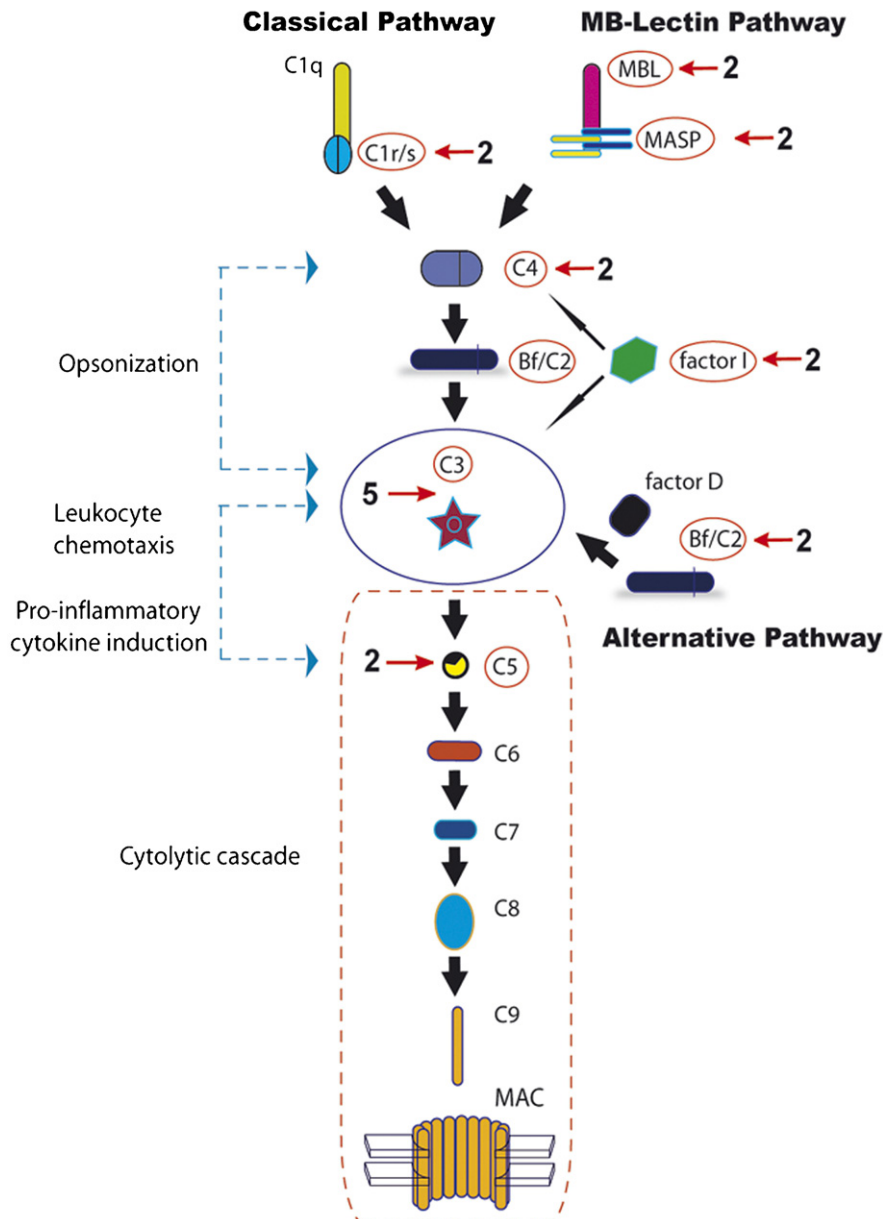


Fig. 1. Schematic representation of the three complement pathways in *C. carpio*. The small red arrows indicate the number of isoforms described for the signaled molecule in carp. The blue arrows on the left indicate the main functions of the central molecules of the complement system. The red circles indicate the complement molecules targeted in this study. A red dashed line groups the molecules involved in the cytolitic cascade.

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