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The phagocytic activity of leucocyte in Japanese flounder, *Paralichthys olivaceus*, by chemokine gene injection using expression vector

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INTRODUCTION

Chemokines are a superfamily of small related protein molecules, these are secreted by a variety of cells and that have, among their diverse biological properties, the ability to recruit a wide range of immune cells to the sites of infection and disease. Chemokine functions such as integrin activation, chemotaxis, lipid mediator biosynthesis, superoxide radical production, and granule enzyme release have been reported.[1,2] There are two groups, one is the CXC-chemokine and the other is the CC-chemokine family. Previously, we isolated a putatively identified chemokine gene by EST analysis from Japanese flounder, *Paralichthys olivaceus*, belonging to the CC-chemokine family.[3] Although, a number of chemokine genes were identified in earlier studies,[4-6] functional characterization has not been conducted. In this study, we studied the function of a chemokine gene on phagocytic activity, following plasmid DNA injection method.

MATERIALS AND METHODS

Isolation of chemokine gene by EST analysis has been reported previously. Japanese flounder CC-chemokine cDNA was ligated with an expression vector that contained human cytomegalovirus (CMV)-promoter (pTARGET Mammalian Expression Vector (Promega), shown in Fig. 1 at 4℃ overnight. The ligation product was used to transform JM109, a *dam*-host strain of *Escherichia coli* (Nippon gene). Plasmid DNA (pCMV-CCC) for injection was prepared by the alkaline lysis method described by Sambrook *et al.*[7] A total of 45 Japanese flounder (body weight = 150 g) were anesthetized with MS-222 (3-aminobenzoic acid ethyl ester, Sigma) and injected using a tuberculin syringe and a 26 1/2G needle with 25 μ g of pCMV-CCC dissolved in 100 μ l of phosphate-buffered saline (PBS). A control group of fish was injected with 100 μ l of PBS and 25 μ g of the pTARGET vector dissolved in 100 μ l of PBS. The depth of penetration of the needle into the target tissue was 2 to 5mm. The epaxial muscle was the injection site for the CC-chemokine gene with expression vector. Phagocytosis was examined as described previously by Yoshida *et al.*[8] Phagocytic cells were isolated by Percoll (Amersham) gradient's adjusted to 10^7 cells/ml in a RPMI 1640 medium (Nissui) containing 10 % FBS. Cells were allowed to adhere to a glass coverslip (22 mm X 22 mm) for 1 h, non-adherent cells were removed by washing with HBSS. Latex particles (Difco; 0.85 μ m; 10^9 particles/ml) were suspended in RPMI 1640 medium (10% Japanese flounder serum) and were applied to the phagocytic cell cover slip. This was incubated for 2 h at 20℃. The number of adhered cells was about 5 X 10^5 cells per coverslip, and the number of phagocytic cells per 300 adhered cells was counted microscopically. The phagocytic activity was determined by the ratio of number of ingesting cells to the total cells, this was expressed as phagocytic index.

RESULTS AND DISCUSSION

Phagocytic activity, a non-specific immune-response, induced by pCMV-CCC was highest on the 3rd day (41 %), this was 37.8 % and 35 % during 1st and 5th day, respectively. Control experiments with PBS and pTARGET induced activity was significantly lower than that of pCMV-CCC ($P < 0.005$) (Fig. 2). Therefore, phagocytic activity, of the group injected with pCMV-CCC was higher found to be than the groups injected with either PBS or pTARGET vector.

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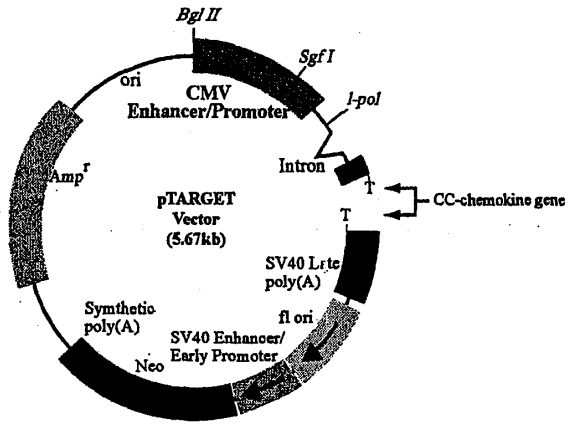

Fig.1 The circle map of pTARGET Mammalian Expression vector used in this study.

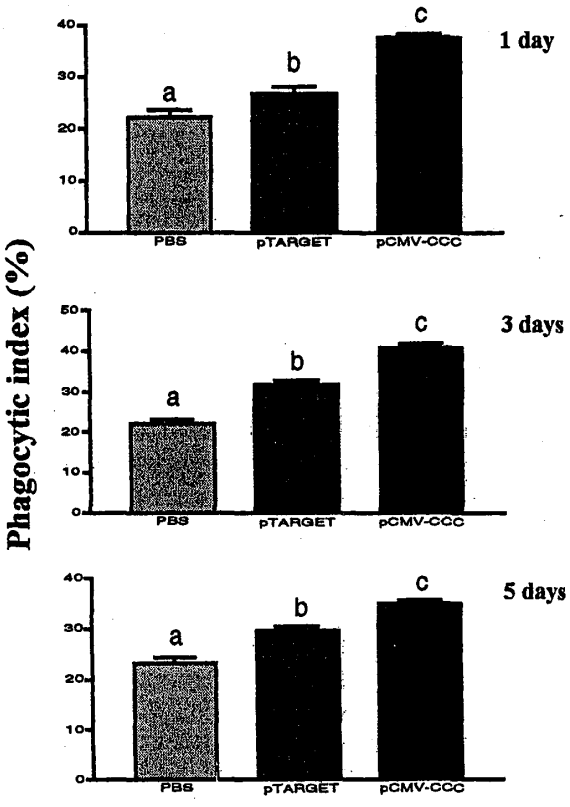

Fig.2 The phagocytic activities in kidney macrophages of carp injected with PBS, pTARGET and pCMV-CCC. Data are measurements in triplicate from five fish of different groups, 1, 3 and 5 days post injection. "a", "b and "c" indicates $P < 0.05$.

It is reported that antigen-specific antibody production and protective immunity can be induced expression of proteins following deliverly plasmid DNA containing genes encoding antigenic proteins into live animals.[9,10]

Therefore, we investigated one of the non-specific immune-response by measuring the phagocytic activity at 1, 3 and 5 days post DNA injection. Phagocytic activity at 1, 3 and 5 days post -injection increased significantly following the injection of pCMV-CCC, rather than that of PBS or pTARGET injection. This suggests that the expressed chemokine increased the activity of phagocytic cells. These functional aspects of chemokine in teleost's have not yet been reported. From the above observations, it is clear that chemokine has an important role in non-specific immune system. The CC-chemokine of Japanese flounder reported in this study has a potentially similar function to that of its mammalian counterpart.

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