

## Fish Cytokines with

**Chris Secombes**, Professor, FIBiol, Head, Scottish Fish Immunology Research Centre and School of Biological Sciences, University of Aberdeen, Zoology Building, Tillydrone Avenue, Aberdeen, AB24 2TZ, Scotland, UK.  
c.secombes@abdn.ac.uk



### What is known about cytokines in lower vertebrates?

**Chris Secombes:** Whilst the bioactivity of a variety of fish and amphibian cytokines had been known for several decades, attempts to purify and sequence these peptides and proteins met with little success (1). Approaches that relied on cross-hybridisation using RNA/DNA probes and cross-reactivity of antisera were also attempted, and often showed reactivity but the problem was knowing whether this was due to detection of the homologous molecules or spurious reactions with unrelated molecules. Logic dictated that if cross-reactivity was detectable, then the homology should be sufficient to allow homology cloning with primers designed to conserved regions of the molecule of interest. However, again only little success was achieved using this approach. The main exceptions were those cytokines that were particularly well conserved, and typically involved in development of the organism, as with the fibroblast growth factors (FGF) and transforming growth factor beta's (TGF- $\beta$ ), or had high transcript level, such as interleukin (IL)-1 $\beta$ . Within the last decade increasing numbers

of expressed sequence tag (EST) studies have been performed with fish tissues and cells, and interrogation of the produced databases has been successful in discovering relatively large numbers of fish cytokine genes. By using particular signature motifs it has even been possible to find fish cytokines that are candidate members of particular cytokine families but that have no clear homology to any known mammalian cytokine. Lastly, with the sequencing of several fish genomes (Fugu, Tetraodon, zebrafish and more recently medaka)(2) and an amphibian genome (Xenopus), it has been possible to undertake extensive searches using known cytokine gene sequences. Also, gene synteny approaches have been used to find genes in particular locations within genomes, where their homology was too low to allow discovery by other means. Both approaches have been particularly successful. Indeed, analysis of particular loci within fish genomes is still yielding new cytokine genes to date, some of which are again without clear homologues in other vertebrate groups.

### How many cytokines and cytokine-related molecules have been identified in fish until today?

**Chris Secombes:** Fish are the dominant vertebrates, in terms of biomass and numbers of species (some 24,000 species of teleost fish alone). Due to their diversity it is difficult to be sure that discovery of a cytokine gene in one species will necessarily mean that it is present in all fish species. Nevertheless,

it will be assumed that where clear homology exists between fish and mammalian cytokine genes, that these are molecules likely to be present in all fish species. It is much more difficult to make such conclusions about genes discovered in a particular species that have little homology to known genes, where

gene duplication with a species, genera or all fish species could equally well explain their occurrence. A further complicating factor in fish is that in some groups, as with the salmonids and cyprinids, whole or partial genome duplication events are known to have occurred in their past giving rise to two loci for many genes, including cytokine genes.

Currently a large number of interleukins are known within teleost fish species, including IL-1 $\beta$ , IL-2, IL-6, IL-8, IL-10, IL-11, IL-12 ( $\alpha$  and  $\beta$ ), IL-15, IL-16, IL-17, IL-18, IL-20, IL-21, and genes with homology to IL-22/26. With some genes, as with IL-1 $\beta$  in rainbow trout, multiple loci and alleles are present (3). Many of these genes have been located within the Fugu/ tetraodon or zebrafish genomes, and often show good conservation of gene synteny with neighbouring genes from fish to mammals. Of the known mammalian interleukins currently undiscovered in fish, much effort has been spent searching for the Th2 cytokines (eg IL-4, IL-5, IL-9, IL-13) but to no avail (4). Similarly, genes such as IL-1 $\alpha$  and IL-1ra have not been discovered to date,

and perhaps represent genes that duplicated after the fish-tetrapod divergence.

Both type I and type II interferon (IFN) genes have been found in fish. The exact number of the former is still being confirmed, and as with birds does not appear to be as extensive as in mammals. Curiously, all the fish type I IFN genes have four introns (5), potentially representing the ancestral state. A single type II IFN (IFN- $\gamma$ ) gene is present and an adjacent related gene (6). The function of the latter is not yet known but it is present in several different fish groups, and potentially all teleost fish. The presence of IFN- $\gamma$  together with IL-2, is good evidence that Th1 cells exist in fish.

Of the cytokines involved in development as well as immune responses, such as the TGF- $\beta$ 's and FGFs, these are fairly well documented in fish with three known TGF- $\beta$  isoforms (7) as in higher vertebrates and multiple FGF isoforms, including a novel member FGF24 (8) unknown in tetrapods.

Other cytokines involved in inflammation will be discussed below.

### Are inflammatory processes in fish different from humans?

**Chris Secombes:** In terms of cytokines present that may constitute a cytokine cascade in response to bacterial infection, the processes appear to have many similarities. TNF- $\alpha$  has been well described, and the function of the recombinant protein(s) studied (9). It can induce the expression of other proinflammatory cytokines such as IL-1 $\beta$ , IL-8, certain CC chemokines and immune relevant genes such as COX2. It can also induce leucocyte migration and increase phagocyte activity. Whilst no evidence of TNF- $\beta$  has been found in fish, recently a molecule with similarities to LT- $\beta$  has been described (10), that is in tandem to TNF- $\alpha$  in the genome (11).

Similarly the function of IL-1 $\beta$  is

relatively well studied in teleost fish. As in mammals, the protein appears to be produced as a precursor that is cleaved to give rise to a mature peptide (12), however an ICE cut site is not obvious in fish IL-1 $\beta$ s despite being well conserved in fish IL-18. Based on modelling of the protein (13), a predicted mature peptide has been produced as a recombinant molecule and shown to induce COX2 and MHC class II expression, as well as having a positive effect on its own expression in cultured cells (14). Similarly, parental administration increases COX2, lysozyme and IL-1 $\beta$  expression in the gill and head kidney, as well as increasing phagocyte activity (15,16). Peptides derived from the mature protein have also

been found to have biological activity in some species (13,17). Lastly, a large number of chemokine genes have been discovered recently in fish, of the CC and CXC types (18,19). Both inducible and constitutive CC chemokines exist, with some 18 different sequences found in rainbow trout, clustered into 12 clear groups. Interestingly some

of these groups have no mammalian counterparts, as seen with the trout CK2-4 and CK6 molecules. Both "ELR+" and "ELR-" CXC chemokines have been discovered in fish (although the ELR motif is not absolutely conserved), with homologies to CXCL2, CXCL8, CXCL9/10/11, CXCL12 and CXCL14.

### Is it possible to use your findings for therapeutic intervention within the fish industry?

Since most of the fish cytokine genes have only been discovered recently, few studies have yet addressed their potency in terms of use as vaccine adjuvants or immunostimulants, although clearly this application potentially exists. Two such studies have been carried out with IL-1 $\beta$  where inclusion in bacterins has increased the antibody response seen post-vaccination, in carp and sea-bass (20,21). Administered by itself, IL-1 $\beta$  or derived peptides has also

been shown to have short-term effects on disease resistance against viral and bacterial pathogens (22,23). One alternative application of the use of cytokine molecules, is as a marker of immunostimulant effectiveness. Again such studies are only just beginning, but already there are several reports that such treatments (eg with CpGs or ergosan) clearly up-regulate expression of many key cytokines (IL-1 $\beta$ , IL-8, TNF- $\alpha$ )(24,25).

### REFERENCES

1. Secombes CJ et al. In Aquatic Genomics 277, 2003
2. Clark MS. In Aquatic Genomics 1, 19, 2003
3. Wang T et al. Fish & Shellfish Immunol 16, 335, 2004
4. Bird S et al. Current Pharmaceutical Design in press
5. Robertsen B. Fish & Shellfish Immunol 20, 172-191, 2006
6. Igawa D et al. Molec Immunol 43, 999, 2006
7. Laing KJ et al. Fish & Shellfish Immunol 10, 261, 2000
8. Draper BW et al. Development 130, 4639, 2003
9. Zou J et al. Dev Comp Immunol 27, 813, 2003
10. Kono T et al. Molecular Immunology in press
11. Savan R et al. Immunogenetics 57, 140, 2005
12. Hong S et al. Fish & Shellfish Immunol 16, 453, 2004
13. Koussounadis AI et al. Current Pharmaceutical Design 10, 3857, 2004
14. Hong S et al. Vet Immunol Immunopathol 81, 1, 2001
15. Hong S et al. Dev Comp Immunol 27, 801, 2003
16. Buonocore F et al. Marine Biotechnol 7, 609, 2005
17. Peddie S et al. J Fish Diseases 25, 351, 2002
18. Laing KJ et al. Immunol 41, 793, 2004
19. Baoprasertkul P et al. Molec Immunol 42, 1355, 2005
20. Yin Z et al. Fish & Shellfish Immunol 10, 375, 2000
21. Buonocore F et al. Marine Biotechnol 6, 53, 2003
22. Peddie S et al. Diseases of Aquatic Organisms 56, 195, 2003
23. Hong S et al. Dev Comp Immunol 27, 801, 2003
24. Jørgensen JB et al. Fish & Shellfish Immunol 11, 673, 2001
25. Peddie S et al. Vet Immunol Immunopathol 86, 101, 2002