Thibaud Roman • Jacques Charlemagne

The immunoglobulin repertoire of the rainbow trout (Oncorhynchus mykiss): definition of nine Igh-V families

Received: 21 February 1994

Abstract  An Igh-V library was constructed from the head kidney cytoplasmic RNA of an 8.5-month-old non-immunized rainbow trout, Oncorhynchus mykiss, using the 5' RACE polymerase chain reaction. Six new Igh-V segments were identified, bringing to nine the number of Igh-V families actually defined in that species. A phylogenetic analysis shows that these nine Igh-V families can be classified into three major groups. The first includes the Igh-V1, Igh-V3, Igh-V4, and Igh-V7 families, and is homologous to the human and mouse Group III Igh-V families. The second includes the Igh-V5, Igh-V8, and Igh-V9 families and is more closely related to the Group I and Group II human and mouse Igh-V families. The third group includes the Igh-V2 and Igh-V6 families, which are not closely related to any other vertebrate Igh-V gene. Six Igh-J segments were characterized. They can recombine with Igh-V segments belonging to different families and there is a high level of junctional diversity between the Igh-V and Igh-J segments.

Introduction

In mammals, antibody diversity is generated by the random assembly of multiple V, (D), and J germline-encoded segments forming the variable domain of heavy (H) and light (L) chains. This diversity is further amplified by somatic genetic events, such as the addition of N and P elements, point mutations, or gene conversions (Tonegawa 1983; Honjo et al. 1985). The mammalian Igh-V segments form a number of distinct families, according to their mutual relationships, and the Igh-V genes are believed to have evolved by the duplication of a limited number of Igh-V-like regions until a potential level of diversity was reached (Hood et al. 1985; Tutter and Riblet 1989).

The basic structure of Ig polypeptides is well conserved in all jawed vertebrates, including the most primitive sharks, which are the direct descendants of the first Palaeozoic cartilaginous gnathostomes that emerged in the Silurian period. However, the organization of the Ig loci in cartilaginous and bony animals is fundamentally different. In primitive sharks (Heterodontus francisci) and more advanced orders (Carcharhynus plumbeus, Raja erinacea), the Igh and Ig1 genes consist of multiple [V-(D)-J-C] clusters, each constituting an independent locus (Hinds and Litman 1986; Harding et al. 1990). These multiple clusters are scattered throughout the genome, and it seems unlikely that combinatorial rearrangements occur between segments belonging to independent clusters. Another interesting particularity of the shark Igh loci is that no Igh-V pseudogenes have ever been found in spite of extensive investigations (Kokubu et al. 1988). In Osteichthians (including bony fish and tetrapods), the single Igh locus is organized according to the mammalian model. This has been clearly established in several teleost fish species (Amemiya and Litman 1991), Xenopus laevis (Du Pasquier 1989) and the chicken (Reynaud et al. 1989). However, in the chicken, all Igh-V segments except the last 3' segment are pseudogenes and the Igh-V diversity depends on gene conversion events, which occur at a high rate, from the donor wtIgh-V genes to the single functional Igh-V acceptor segment (Reynaud et al. 1989). The same gene conversion system also operates at the chicken Igλ locus (Reynaud et al. 1985).

Actinopterygian fish synthesize a single IgM-like class of immunoglobulin, and very little is actually known about the molecular antibody diversity in these species. Several species are known to have multiple Igh-V families. The most extensive analysis has been done in the channel catfish Ictalurus punctatus, where six Igh-V families have been found, containing between them about 100 Igh-V
Fig. 1 Alignments of 11 nucleotide sequences defining nine rainbow trout Igh-V families. Sequences Igh-V1.1 and Igh-V1.2 correspond to the RTVH 43l and RTVH 253 germline clones described by Matsunaga and co-workers (1990) and Andersson and Matsunaga (1993). The Igh-V4-Igh-V9 sequences are from the present work. Spaces introduced to optimize homology between sequences are indicated by dots. The division into FR and CDR regions (brackets) is suggested by comparison with known Igh-V sequences (Kabat et al. 1987).

Materials and methods

Preparation of a rainbow trout Igh-V library

Total cytoplasmic RNA from the head kidney cells of an 8.5-month-old non-immunized trout was used to build an Igh-V library by anchored polymerase chain reaction (PCR; Loh et al. 1989), using a commercial kit (5' RACE System for Rapid Amplification of cDNA Ends; Life Technologies SARI, Eragny, France), essentially following the manufacturer instructions. Briefly, first-strand cDNA was synthesized by using a specific downstream primer complementary to the trout Cgl reverse transcriptase (Superscript II; Life Technologies). The RNA template was removed and the first-strand cDNA purified and derivatized, using TdT and dCTP. The second cDNA strand was then synthesized, using Taq DNA polymerase (Perkin Elmer, St. Quentin Yvelines, France) and the manufacturer's upstream 5' RACE anchor primer. The ds cDNA [about 570 base pairs (bp)] was amplified, using a nested downstream primer located 5' to the Chl1 region (CHL1: 5'-CATTGCACACCCAGGTGTCACA and M-MLV reverse transcriptase; SuperScript II, Life Technologies). The RT-PCR product was cloned into the pBluescript KS- vector (Stratagene, San Diego, CA).

Analysis of the VDJ junctions

First-strand cDNA was synthesized, using total head kidney cell cytoplasmic RNA from a 6-month-old non-immunized trout and oligo d(T)12-18 (Pharmacia LKB Biotechnology, St. Quentin Yvelines, France) and an upstream primer. This was then cloned into the Bam H1 and Sal I sites of the pBluescript KS- vector (Stratagene, San Diego, CA).