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Homologs of CD83 from Elasmobranch and Teleost Fish1,2

Yuko Ohta,* Eric Landis,† Thomas Boulay,‡ Ruth B. Phillips,§ Bertrand Collet,¶ Chris J. Secombes,¶ Martin F. Flajnik,*, and John D. Hansen3¶

Dendritic cells are one of the most important cell types connecting innate and adaptive immunity, but very little is known about their evolution. To begin to study dendritic cells from lower vertebrates, we isolated and characterized CD83 from the nurse shark (Ginglymostoma cirratum (Gici)) and rainbow trout (Oncorhynchus mykiss (Onmy)). The open reading frames for Gici-CD83 (194 aa) and Onmy-CD83 (218 aa) display ~28–32% identity to mammalian CD83 with the presence of two conserved N-linked glycosylation sites. Identical with mammalian CD83 genes, Gici-CD83 is composed of five exons including conservation of phase for the splice sites. Mammalian CD83 genes contain a split Ig superfamily V domain that represents a unique sequence feature for CD83 genes, a feature conserved in both Gici- and Onmy-CD83. Gici-CD83 and Onmy-CD83 are not linked to the MHC, an attribute shared with mouse but not human CD83. Gici-CD83 is expressed rather ubiquitously with highest levels in the epigonal tissue, a primary site for lymphopoiesis in the nurse shark, whereas Onmy-CD83 mRNA expression largely paralleled that of MHC class II but at lower levels. Finally, Onmy-CD83 gene expression is up-regulated in virus-infected trout, and the promoter is responsive to trout IFN regulatory factor-1. These results suggest that the role of CD83, an adhesion molecule for cell-mediated immunity, has been conserved over 450 million years of vertebrate evolution. The Journal of Immunology, 2004, 173: 4553–4560.

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2 The sequences presented in this article has been submitted to GenBank under accession numbers Y1833667, AY263793-797, AY437982-983, and AY650049-050.
3 Abbreviations used in this paper: DC, dendritic cell; IgSf, Ig superfamily; UTR, untranslated region; EST, expressed sequence tag; ORF, open reading frame; Gici, Ginglymostoma cirratum; Onmy, Oncorhynchus mykiss; gDNA, genomic DNA; BAC, bacterial artificial chromosome; IHNV, infectious hemopoietic necrosis virus; IRF, IFN regulatory factor; CHO, Chinese hamster ovary; ISRE, IFN-stimulated regulatory element.

Materials and Methods
cDNA cloning of fish CD83

A nurse shark PBL ZAPI cDNA library (Stratagene, La Jolla, CA) was used as the template for a degenerate PCR approach for amplifying unique V-set genes. Three different degenerate primers (reverse/complementary for D/S/N-X-G-Y-V-X-C) the successful primer corresponded to (A/G)CA-II-RTA-III-NCC-III-RTC corresponding to the F strand of IgSf V-set domains were used in conjunction with an anchored T3 primer
(within the ZAP vector) for the amplification of potential V domains. Primers were used in the following PCR profile for amplification: 94°C for 1 min followed by 35 cycles of 94°C for 20 s, 43°C for 25 s (0.1°C increase per cycle), and 72°C for 30 s. The PCR samples were then extended for an additional 10 min at 72°C to facilitate TA-cloning (pTOPO; BD Clontech, Palo Alto, CA). Aliquots of the amplified cDNA library were diluted in 5 mM Tris (pH 8.0) and denatured at 98°C for 10 min before usage as a template to release the cDNA templates from the phagemids. Restriction digests were performed, and 60 random inserts in the proper size range (250–400 bp) were sequenced via automated sequencing (Applied Biosystems 310; Applied Biosystems, Foster City, CA). One particular clone showed strong similarity to V-set domains and was used as the basis for nested 3′ anchored PCR to amplify the full-length gene from the library. Nested PCR was performed using two gene-specific primers corresponding to the putative 5′-untranslated region (UTR) (forward, 5′-CAT GATCTTCTAAAACGTTG-3′) and ATG codon (forward 2, 5′-AACAGTGT TGGAAGAGGATGATG-3′) with the anchored 17 primer corresponding to the 3′ region of all inserts within the library. Full-length cDNAs were amplified and cloned into pTOPO and sequenced in both directions (Applied Biosystems 377).

Rainbow trout CD83 was obtained via a BLASTn search of the trout expressed sequence tags (ESTs) within GenBank using sequence information from the Ginglymostoma cirratum (Gicy)-CD83 open reading frame (ORF). The location of the rainbow trout CD83 ORF was then used to amplify cDNA encoded as previously described (15). The 3′ end of Oncorhynchus mykiss (Onmy)-CD83 was amplified from a directional PBL cDNA library (UniZap; Stratagene) using a vector (CT3) and an Onmy-CD83 gene-specific primer (CD83-ER1; 5′-CCAGGGACAGCTTGACTT-3′) in anchored PCR. Products were cloned and sequenced to derive a consensus 5′ ATG primer, Primers (Onmy-CD83-5′-UTR-R.21801, 5′-TTCCTCCTTATGTTCAAGTATAC-3′) were used to amplify the full-length clones of CD83 cDNA from splicen cDNA derived from the homoyzous strains of trout, OSU-142, HotCreek, and Arlee (22). Naive spleens from the OSU-142, HotCreek, and Arlee clonal lines were generously provided by Dr. G. Thorgaard (Washington State University). Products were cloned (pTOPO) and sequenced.

The following accession numbers were used for assembling the flounder, zebrafish, chicken, rat, and bovine CD83-like sequences: flounder, AU091120; zebrafish, BI671547/BI517226; chicken, B1384417/BU457418/BU452135/BM440129/A9818465; rat, XM_225224; and bovine, BE755455/ B51096.21. Both PCR and direct sequencing were used to confirm the alternate splice forms for trout. DNA was isolated from positive BAC clones using the Qiagen (Valencia, CA) columns. Both PCR and direct sequencing were used to confirm the positions of the rainbow trout CD83 cDNA clones as previously described (25). This strain has the same chromosome number as the OSU clonal line (n = 30). Onmy-CD83 BAC DNA was labeled with Spectrum Orange (Vysis, Downers Grove, IL) using nick translation kit (Vysis). Human placental DNA (0.2 μg) and Cot-1 DNA (1 μg; prepared from rainbow trout gDNA) were added to the probe mixture for blocking. Hybridizations were conducted at high stringency and exposed for 6 days whereupon the hybridization pattern for the nurse shark MHC (23). It should be mentioned that the Onmy-CD83 gene is amplified from the pTOPO vector (http://genome.ucsc.edu/cgi-bin/hgBlat).

**Genomic structure**

Long-range PCR (Elongase; Invitrogen Life Technologies, Carlsbad, CA) was used to deduce the exon/intron structure for Gicy-CD83. PCR conditions consisted of the following profile: 2 min at 95°C followed by 35 cycles of 94°C for 30 s, 57°C for 30 s, and 68°C for either 3, 6, or 9 min depending on the primer pairs. Products were then cloned into pBLUNT (Invitrogen Life Technologies) for verifying the sequence and to determine the exon/intron splice site and phase. Intronic size estimates were based upon standard 1% agarose gel electrophoresis of PCR products minus exon contributions. The following primers were used: exon 1 to exon 2: exon 1F.21789, 5′-ATGGTTTACCTTAAAGAAGT-3′, and exon 2R.21790, 5′-TTCCTCACATCTTACTGTAAC-3′; exon 2 to exon 3: exon 2F.21455, 5′-TGCGAAGTTCACTGAAATGTTG-3′, and exon 3R.21700, 5′-TTGACACTGATCCCTCAAC-3′; plus nested exon 3R.21699; 5′-ACATGAGACACTGGATAGAAGT-3′; exon 3 to exon 4: exon 3F.21452_5′-GACTTGTTGAAAGATCACTGTG-3′, and Onmy-CD83-STOP.R.5′-TTCCTCCTTATGTTCAAGTATAC-3′) were then used to amplify full-length clones of CD83 cDNA from splicen cDNA derived from the homoyzous strains of trout, OSU-142, HotCreek, and Arlee (22).

**Southern blotting for Onmy-CD83**

For Southern blotting, 15 μg of rainbow trout gDNA was digested, electrophoresed, and transferred to nylon as previously described (15). The blot was hybridized with a radiolabeled cDNA probe corresponding to the Onmy-CD83 V region. Blots were washed at a final stringency of 0.4× SSC/0.4% SDS at 68°C and then exposed to film for 2 days at −80°C.

**Linkage analysis for Gicy-CD83**

The isolation of gDNA from 39 nurse shark pups (multiple paternity) has been previously described (24). Five micrograms of gDNA was digested to completion with EcoRI, which produced a useful restriction fragment poly-morphism using the Gicy-CD83 probe. The PCR-generated probe corresponded to the entire ORF for Gicy-CD83 (primers, exon 2F.21455 and exon 3R.21701; see above for details) and procedures for Southern blotting under high-stringency conditions with randomly primed probes have been described previously (24). Segregation patterns for Gicy-CD83 were then compared with previously identified patterns for the nurse shark MHC (23).

**Onmy-CD83 in situ hybridization and karyotyping**

The V region of trout CD83 was used as a probe to screen high-density filters corresponding to 4.5 times coverage of a bacterial artificial chromosome (BAC) genomic library from the OSU-142 all-female clonal line of trout. DNA was isolated from positive BAC clones using the Qiagen (Valencia, CA) columns. Both PCR and direct sequencing were used to confirm positive BAC clones harboring Onmy-CD83. Peripheral blood was cultured in the presence of rainbow trout lymphoplasma and murine IL-2 (25). This strain has the same chromosome number as the OSU clonal line (n = 30). Onmy-CD83 BAC DNA was labeled with Spectrum Orange (Vysis, Downers Grove, IL) using nick translation kit (Vysis). Human placental DNA (0.2 μg) and Cot-1 DNA (1 μg; prepared from rainbow trout gDNA) were added to the probe mixture for blocking. Hybridizations were conducted at high stringency and exposed for 6 days whereupon the hybridization pattern for the nurse shark MHC (23). It should be mentioned that the Onmy-CD83 gene is amplified from the pTOPO vector (http://genome.ucsc.edu/cgi-bin/hgBlat).

**Northern blot hybridization and RT-PCR**

RNA isolation and Northern blotting protocols have been previously described (24, 28). Briefly, 15 μg of total RNA was electrophoresed under alkaline conditions and transferred to Nytran+ (Fisher Scientific, Atlanta, GA) using 20× standard saline citrate phosphate/EDTA. Two different probes were amplified from the pGicy-CD83 plasmid to confirm the expression pattern for Gicy-CD83, corresponding to the V domain exon 2F.21455, and exon 3R.21699 (see genomic structure for details). The other to the cytoplasmic domain plus the 3′-UTR (exon 5F.21800, 5′-GTCAACAAGCAAACCCATGTTCC-3′ and 3′-UTR-R.21801, 5′-GTTTATGTTGAAATCCAAGCTT-3′). Amplified fragments were purified and then randomly primed (Invitrogen Life Technologies) with [32P]dCTP (Amer sham Biosciences, Piscataway, NJ). Nonincorporated nucleotides were removed using G-50 spin columns (Invitrogen Life Technologies) before hybridization. The Gicy-MHC class IIA and NDPK probes have been previously described. For Onmy-CD83 Northern blotting, 12 μg of total DNA was electrophoresed, transferred to nylon (Nyttran), and hybridized overnight using an Onmy-CD83 V domain probe (VaVb). The blot was washed at high stringency and exposed for 6 days whereupon the filter was stripped and probed with a probe specific to a MHC class IIA (exon 2, 28× exposure) and then with EtTu-1 (12× exposure). The protocols for acute infectious hemopoietic necrosis virus (IHN) infection have been described previously (29). Briefly, trout weighing 200 g were infected by injection (i.p.) with 1×106 PFU of IHNV, and tissues were harvested on specified days. Controls consisted of saline-injected fish.

**Promoter and reporter assays**

The promoter region for Onmy-CD83 blotting was obtained using the promoter trapping kit (Genome Walking; Bio S&T, Montreal, Quebec, Canada) in conjunction with specific reverse primers found within exon 1 (exon 1R, 5′-CTTGAGCGGGGAGGCAGCT-3′) of Onmy-CD83. Two different promoters were amplified from the OSU-142 Onmy-CD83 BAC clone that was used for physical mapping. Products were cloned into pBlunt (Invitrogen Life Technologies), sequenced, and assessed for potential transcription factor binding sites using the MatInspector Professional software suite.
An amino acid alignment (Fig. 1A) was generated to display features that have been conserved during vertebrate evolution for CD83. The leaders for all sequences are predicted to be ~19–20 aa via pSort (http://psort.nibb.ac.jp/). The N-terminal cleavage site (T.LK) for the Gici-CD83 leader was confirmed using N-terminal amino sequencing of Gici-CD83-Ig fusion proteins (J. D. Hansen, unpublished data). The Gici-CD83 mature ORF displays conservation of critical residues found within CD83, and overall, the sequences display ~20%. Both trout and flounder share 37% similarity with Gici-CD83 and 63% similarity (45% identity) between themselves for the entire ORF. The cysteine residues in strands B and F that forms a disulfide bond within most Ig domains, as well as the invariant tryptophan in the C strand, are present in CD83. One unique feature shared by all CD83 sequences is the presence of a cysteine residue (also found in CD90) within the A strand of the IgSF V domain. However, Gici-CD83 does not possess the conserved cysteine residue between the G strand and the transmembrane region. The relevance of this additional cysteine residue is not known, but mammalian CD83 is monomeric, and thus, the additional cysteine residue does not play a role in dimerization.

Immunoprecipitation of human and murine CD83 under both reducing and nonreducing conditions demonstrated that CD83 proteins are heavily glycosylated (broad 45-kDa band), because the predicted molecular masses for the mature proteins are roughly only 20 kDa (3). The relative locations of two of the three N-linked glycosylation sites (N-X-S/T) are conserved in evolution, suggesting that fish CD83 proteins are also heavily glycosylated. The third mammalian N-glycosylation site in the G strand is absent in fish. Conserved O-linked glycosylation sites (XPXX, glycosylated if X = S or T) were not observed within extracellular domain for any of the CD83 sequences. The transmembrane and cytoplasmic domains display ~17–41% amino acid identity among the various CD83-like sequences and teleost, rat, and avian cytoplasmic domains are much longer than those of the other vertebrates. The trout, flounder, and chicken cytoplasmic domains had to be aligned separately from the other sequences due to their low sequence identity. A role for the cytoplasmic domain has yet to be reported for CD83, but it has been shown that the conserved serine and threonine residues in mice and humans are not phosphorylated (3). Neither ITIM nor ITAM motifs are found in any of the sequences, although several tyrosine residues are found especially within the chicken cytoplasmic domain. Finally, cysteine residues present in the fish CD83 (also in rat and chicken) cytoplasmic domains warrant further investigation. Cysteine residues were also found in the human CD83 pseudogene (6p22:27636584–27637508) upon BLAT (http://genome.ucsc.edu/cgi-bin/hgBlat) inspection.

Phylogenetic analysis of the CD83 V domains

To compare the fish CD83 V domains with other members of this family present as cell surface proteins on blood cells, we conducted a phylogenetic analysis of CD83, CD7, CD8, CD28, CD83, and CD152. As shown in the V region neighbor-joining tree (Fig. 2), all of the CD83 sequences cluster as one group as supported by bootstrap analysis. In addition, the fish and higher vertebrates form their own separate clusters within the CD83 branch, with the chicken sequence found between the two groups, proving that these sequences represent homologs.

Genomic structure

We used long-range PCR to determine the exon/intron organization for Gici-CD83 and found that Gici-CD83 possesses the same exon/intron pattern as mammalian CD83. The Gici-CD83 gene is composed of five exons and spans roughly 18 kbp from the ATG initiation codon through the polyadenylation site (Fig. 3). Exon 1...
encodes the 5′-UTR and the first 13 aa of the leader; exons 2 and 3 encode the IgSf V domain; exon 4 encodes the remainder of the V domain (last 10 residues) and the transmembrane domain; and exon 5 encodes the cytoplasmic domain and 3′-UTR. In all cases, splice junctions correlated with consensus 5′ and 3′ donor and acceptor splice sites. Overall, the Gici-CD83 genomic structure is nearly identical with that of mammals including conservation of phase for the four intron splice sites. Finally, we also determined that the Onmy-CD83 V region is composed of two exons, but the intron between the Onmy-CD83 Va and Vb exons is much smaller (150 or 763 bp, phase 0) than that for Gici or murine CD83.

FIGURE 1. CD83 protein alignment.
Amino acid sequences were aligned using ClustalX and by manual adjustment. A, Alignment of CD83 genes from various vertebrate species. Solid vertical lines represent the borders between the leader, extracellular domain, transmembrane region, and the cytoplasmic domain. Presumed β strands (A–G) are indicated with horizontal lines above the amino acid translation. The locations of exon/intron boundaries are denoted by underlining adjacent amino acids (i.e., GL in the nurse shark). Conserved cysteine residues involved in the V domain structure are shaded, and the location of conserved N-linked glycosylation sites are boxed. Identical (•) and similar (◦) amino acids are also shown within the alignment. B, Amino acid alignment of Onmy-CD83 from three different homozygous trout. Two different Onmy-CD83 genes were found in the Arlee and Hotcreek clonal lines. A second partial (2P) OSU-142 CD83 gene was obtained from the OSU-142 CD83 BAC clone (AY437982).

MHC linkage analysis
In mammals, amphibians, and birds, MHC class I, II, and III regions are closely linked. However, in all teleost fish, the MHC class I and II regions are not linked and reside on different chromosomes (31). Recently, the genomic architecture for the elasmobranch MHC was examined. In two different shark species, class I, II, and III genes were shown to be linked, strongly suggesting that the primordial organization of the MHC was similar to that found for all other vertebrates, and the lack of linkage for the bony fish MHC is a derived characteristic (23, 32, 33).

The human CD83 gene maps near the human MHC (6p23, telomeric of myelin oligodendrocyte glycoprotein) (6, 13). In mice, this association is not found, because CD83 maps outside of the...

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MHC on chromosome 13 (6, 13). To determine whether the Gici-CD83 gene is linked to the shark MHC, we studied segregation of Gici-CD83 in a nurse shark family of 39 pups. According to RFLP analysis with a classical class IA probe, pups were sorted into at least 13 groups (A–M). These groups represent the combination of maternal and paternal alleles; therefore, a maximum of four groups can be obtained from a single-copy gene. The increased number of groups is due to multiple paternities (at least five fathers in this family) (23). Because most of the groups contain only a few pups, we used groups of more than three pups (A, D, and H) for the linkage analysis. Furthermore, groups A and D appear to share the same paternal allele p2. In Fig. 5, a 6.6-kb band is absent in the maternal lane; therefore, the 6.6-kb band represents a paternal band. In all cases, only 50% of the pups carry the 6.6-kb band (i.e., 1/2H1101150% recombinant between two paternal chromosomes), strongly suggesting that Gici-CD83 is not linked to the shark MHC.

Recently, the rainbow trout chromosomes and linkage groups harboring the trout MHC have been determined (34). Interestingly, a duplication event has led to the development of two class I regions on chromosomes 14 and 18, whereas a compact class II region containing both DAA and DAB was found on chromosome 17. In addition, ABCB2 (TAP1), a transporter gene located in mammalian class II region, was found on chromosome 3. In this study, we determined that Onmy-CD83 is found near the centromere on the short arm of chromosome 9 (9p) using in situ hybridization (Fig. 6). Therefore, as in sharks and mice, Onmy-CD83 is not linked to the trout MHC, and thus the linkage of the two human CD83 genes (CD83 and the CD83 pseudogene) to the MHC is most likely a derived feature.

Tissue-specific expression of Gici- and Onmy-CD83

We then examined the tissue-specific expression of Gici-CD83 by Northern blotting using two probes, one designed for the V domain and the other encompassing the cytoplasmic tail and 3′-UTR. Both probes showed identical results with ubiquitous expression and highest levels within the brain, epigonal tissue, gills, intestine, PBLs, spleen, and testis (Fig. 7A). A nurse shark MHC class IIA probe was then used as a comparative marker for the expression analysis, because some DC lineages express high levels of MHC class II, consistent with their role in Ag presentation. As expected, nurse shark MHC class IIA also displayed strongest expression within the gills, intestine, PBLs, and spleen. Transient transfection of N terminus Flag-tagged Gici-CD83 and subsequent FACS analysis, demonstrated surface expression on CHO cells (data not shown), thus supporting that Gici-CD83 is a type-I transmembrane protein. We then examined the expression of Onmy-CD83 in selected trout tissues. Onmy-CD83 (Fig. 7B) was primarily expressed within the spleen and testis by Northern blotting (V domain probe) at much lower levels in comparison to MHC class IIA (DAA).
Expression of *Onmy*-CD83 was also found in the thymus, pronephros (bone marrow equivalent), and mesonephros. RT-PCR demonstrated that all tissues examined were positive for *Onmy*-CD83, including skin (data not shown). Finally, we determined that two *Onmy*-CD83 genes are up-regulated during acute IHNV (a fish rhabdovirus) infection in the spleen, pronephros, and intestine at day 6 postinfection (Fig. 7C). Mammalian CD83 gene expression is largely controlled by SP1 and NF-κB (7–9), with no apparent involvement of IRFs. *Onmy*-CD83 up-regulation occurred as early as 24 h postinfection and remained induced up to 192 h postinfection (data not shown). Recently, we showed that STAT-1 and members of the class I pathway are also up-regulated during acute IHNV infection (29). In that study, STAT-1, ABCB2 (TAP1), and PSMB9A (LMP2) were clearly induced by 24 h postinfection, implicating the involvement of type-I IFNs.

In mice, CD83 tissue mRNA expression was found in the heart, brain (high), spleen (high), lung, and muscle, and weakly within the kidney, but similar to Gici-CD83 and *Onmy*-CD83, prolonged exposure or RT-PCR demonstrated expression within all tissues examined (6). In humans, CD83 mRNA expression was also detected in the brain, lungs, and mitogen-activated B lymphocytes, and within some T cell populations. Flow cytometric analysis has shown that CD83 expression is limited to cells of hemopoietic lineages, especially to cells of DC lineages (3, 35). Interestingly, CD83 expression has also been associated with a rather unique murine cell type, one that possesses both B cell CD markers and DC morphology and function, because they were found to be potent allo-APCs in MLRs (36). Thus, it will be interesting to see whether a similar cell type can be found in *fish*. Because DCs have a diverse tissue distribution, the above results (Fig. 6) are consistent with CD83 expression by DC lineages in the nurse shark. Recently, the primary and secondary lymphoid tissues of the nurse shark have been examined (37). Based upon expression of key markers including RAG1 and TdT, the epigonal tissue and thymus were found to be the primary lymphoid tissues throughout the life of the nurse shark. Interestingly, the nurse shark spleen strongly

**FIGURE 5.** The nurse shark CD83 gene is not linked to the MHC. M, Maternal RFLP pattern. Individual pups (numbered) corresponding to MHC segregating groups H, D, and A are shown. Recombinants are noted by the presence or absence of the 6.6-kbp band.

**FIGURE 6.** Fish CD83 is not linked to the MHC. *Onmy*-CD83 resides on the short arm of rainbow trout chromosome 9 as visualized by in situ hybridization using CD83*^+^* BAC clones as probes. The trout MHC class I and II regions (boxed) are found on chromosomes 3, 14, 17, and 18 (34).
comprises the mammalian splenic architecture in possessing distinct/organized T and B cell zones and the presence of MHC class II+ DC networks. In salmonids, MHC class IIA and -B mRNA and protein expression are mainly limited to lymphoid tissues including the thymus, kidney, spleen, and intestine (28, 38). Additionally, Atlantic salmon class IIB protein expression was primarily localized to cells of the white pulp with a few positive cells (likely melanomacrophages) in the red pulp. This suggests that salmonid splenic APCs are found in the same location as that for sharks and mammals.

Figures

**Figure 8.** Deletion analysis of the Onmy-CD83 promoter. **A**, 5′-Flanking region for Onmy-CD83. Numbers on the left and right borders are relative to the inferred ATG for Onmy-CD83. Putative transcription factor binding sites are in bold with the name of site above. Numbers in parentheses indicate the distance from the putative ATG. The TATA-box is underlined. Peak promoter activity for the human CD83 promoter (7) was compared with the putative trout IRFs and NF-κB binding motifs known to be implicated in the regulation of immune relevant genes were found within the putative -CD83 promoter region (103 to 100, and the interaction of trout IRFs with the TATA-box (Fig. 8A). The location of the TATA-box is −261 bp upstream of the putative ATG. Cotransfection of trout IRF-1 with the −CD83 promoter (Fig. 8B) of the Onmy-CD83 promoter were cloned into the pGL3-Basic luciferase reporter vector to assess their activity in CHO cells upon cotransfection with trout IRF-1 and/or IRF-2 (39) as determined by 5′-RACE analysis. Poly(I:C) treatment of CHO cells cotransfected with the IRF-1 expression constructs suggests that this region (−103 to −3) may be involved in basal transcription. Poly(I:C) treatment of CHO cells transfected with the various pGL3-OmCD83 constructs did not result in enhanced luciferase activity (data not shown), suggesting that the Onmy-CD83 promoter promoters do not physically interact with sites found within the Onmy-CD83 promoter. In direct contrast, enhanced luciferase activity (6- to 7-fold induction) was found for pGL3-OmCD83-1-3 constructs cotransfected with the IRF-1 expression construct but not IRF-2 (Fig. 8C). The enhanced luciferase activity was not found upon cotransfection of pGL3-OmCD83-3 with IRF-1, indicating that trout IRF-1 interacts with a site located between −103 to −3 relative to the Onmy-CD83 ATG. A true consensus IRF-1 site (SAAAGYGAAAC; −85) is identical with the IRF-1 and -2 physical binding site as is found in higher vertebrates (41, 42).
promoter, our results imply that \textit{Onmy}-CD83 mRNA expression is likely controlled by innate mechanisms including NF-kB and type-I IFNs. Taken together, the expression patterns for \textit{Gici}-C D83 and \textit{Onmy}-83 are consistent with the expression of CD83 in mammals.

\textbf{Concluding remarks}

We have used a degenerate primer strategy for the isolation of a primordial gene that, based upon our analysis, corresponds to a CD83 homolog of the nurse shark. We then performed a broad comparison of CD83 from a variety of vertebrates that suggest that all CD83 proteins are heavily glycosylated, consistent with the identification of CD83 as a sialic acid-binding Ig-like lectin protein. Finally, expression analysis indicates that CD83 is largely expressed within immunologically important tissues in fish, and that expression is up-regulated during acute viral infection in trout. This represents the first isolation of a putative fish marker for DC lineages, thus opening the door for studies examining DC biology in elasmobranchs and teleosts, as well as the evolution of adaptive immunity and Ag presentation.

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\textbf{References}