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ORIGINAL ARTICLE

Amino acid sequence variations of signaling lymphocyte activation molecule and mortality caused by morbillivirus infection in cetaceans

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ABSTRACT

Morbillivirus infection is a severe threat to marine mammals. Mass die-offs caused by this infection have repeatedly occurred in bottlenose dolphins (*Turiops truncatus*) and striped dolphins (*Stenella coeruleoalba*), both of which belong to the family Delphinidae, but not in other cetaceans. However, it is unknown whether sensitivity to the virus varies among cetacean species. The signaling lymphocyte activation molecule (SLAM) is a receptor on host cells that allows morbillivirus invasion and propagation. Its immunoguloblin variable domain-like (V) region provides an interface for the virus hemagglutinin (H) protein. In this study, variations in the amino acid residues of the V region of 26 cetacean species, covering almost all cetacean genera, were examined. Three-dimensional (3D) models of them were generated in a homology model using the crystal structure of the marmoset SLAM and measles virus H protein complex as a template. The 3D models showed 32 amino acid residues on the interface that possibly bind the morbillivirus. Among the cetacean species studied, variations were found at six of the residues. Bottlenose and striped dolphins have substitutions at five positions (E68G, I74V, R90H, V126I, and Q130H) compared with those of baleen whales. Three residues (at positions 68, 90 and 130) were found to alternate electric charges, possibly causing changes in affinity for the virus. This study shows a new approach based on receptor structure for assessing potential vulnerability to viral infection. This method may be useful for assessing the risk of morbillivirus infection in wildlife.

Key words cetacean, morbillivirus, receptor, signaling lymphocyte activation molecule.

Marine mammals face many risks from recent climate change, environmental contaminants and infectious diseases. Morbillivirus infection is one of the most severe threats to marine mammals such as cetaceans and seals. The morbillivirus is a member of the family Paramyxoviridae. Four species, namely measles virus (MV) in humans, rinderpest virus (RPV) in cows, peste des petits ruminants virus in sheep and goats, and canine distemper virus in dogs, have been identified in terrestrial mammals (1). Since the late 1980 s, morbilliviruses have repeatedly caused mass die-offs of marine mammals (2, 3). Two novel morbillivirus species, phocine distemper virus and cetacean morbillivirus (CMV), have been isolated from affected dead seals and cetaceans, respectively (4–6).

The first isolation of CMV was from several harbor porpoises (*Phocoena phocoena*) with pathological evidence

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List of Abbreviations: 3D, three-dimensional; C, constant-2 domain-like; CMV, cetacean morbillivirus; H, hemagglutinin; MV, measles virus; RPV, rinerpest virus; SINE, short interspersed element; SLAM, signaling lymphocyte activation molecule; V, variable domain-like.

of the virus stranded on the Irish coast in 1988 (5). CMV induced a severe mass die-off of the striped dolphin (*Stenella coeruleoalba*) population on the Mediterranean coast of Spain, which rapidly spread throughout the western Mediterranean sea, including the coasts of France, Italy, Greece and Turkey (6). Retrospective studies showed that CMV killed several thousand bottlenose dolphins (*Tursiops truncatus*) along the Atlantic coast of the USA in 1987–1988, and several hundred dolphins in the Gulf of Mexico during 1993–1994 (7, 8). Thus, in the past few decades, CMV has caused mass die-offs in cetaceans, particularly in dolphins belonging to the Delphinidae family. However, it is still unknown whether sensitivity to the morbillivirus differs among various cetacean species.

All morbilliviruses are lymphotropic and lymphoid tissues are the major sites of viral replication. SLAM (CD150) is the major cellular receptor for the entry and propagation of morbilliviruses in humans, cows and dogs (9, 10). It is expressed on various immune cells such as activated T cells, B cells, mature dendritic cells and thymocytes (11). The distribution of SLAM explains the viral lymphotropism and immunosuppressive nature of the morbillivirus and strongly suggests that SLAM is the universal receptor for entry of morbilliviruses into mammalian host cells. Because previous studies of CMV-infected cetaceans have shown that these viruses are also mainly distributed in lymphoid tissues, and because the pathology in these animals is reportedly very similar to that of other morbillivirus-infected animals, cetacean SLAM on immune cells are thought to function as receptors for CMV (12, 13). SLAM is the principal member of the SLAM family, which belongs to the immunoglobulin superfamily, and shares a membrane-distal immunoglobulin V region and a membrane-proximal immunoglobulin C region in its extracellular space (14). It acts as a self-ligand and forms a homophilic dimer by weak binding via the V domain (15). The V domain also has an interface for binding morbilliviruses (16). The viral H protein has a strong affinity for the V domain of SLAM, which is 400-fold greater than that for self-ligand interaction (17). Binding between SLAM and the H protein triggers subsequent cell fusion events via viral fusion protein, allowing viral invasion of host cells (18–20). Substitution experiments on the amino acid residues of the human SLAM interface have shown that some residue substitutions lead to a loss or a reduction of, or sometimes an increase in, viral infectivity, indicating that these residues are key for both binding affinity and viral infectivity (16, 18, 19). Taken together, these findings raise the possibility that, among cetaceans, there are variations in the amino acid residues on the interfaces of SLAMs and that some of them may be key residues for binding affinity and sensitivity to the virus.

We previously determined the complete nucleotide sequences of SLAMs from three different marine mammal groups, namely cetaceans, pinnipeds and sirenians, and generated 3D homology models using the human NK, T, and B cell antigen (NTB-A) molecule, a SLAM family protein, as a template (21, 22). We suggested that some amino acid residues on the interface region of these marine mammal SLAMs are key residues for both viral binding and host-virus specificity (21). The crystal structure of the complex of MV-H protein and the V region of marmoset (*Saguinus oedipus*) SLAM (marSLAM) was recently determined (18). Crystal structural analysis showed that this complex contains four important binding sites for the virus (sites 1–4), and that an electric interaction plays an important role in the binding.

In the present study, we examined variations in amino acid residues in the interface region of SLAM in various species of cetaceans, including six families of toothed and three families of baleen whales. Their 3D models were generated by homology modeling using the crystal structure of the marSLAM-MV-H complex and NTB-A as templates. In comparing the key residues contributing to viral binding, we discuss the sensitivity of cetaceans to CMV infection and a possible means of assessing the risk of morbillivirus infection in cetaceans.

MATERIALS AND MEHODS

Cetacean tissue samples

Tissue samples were collected from 19 species of toothed whales and 7 species of baleen whales (Table 1). Species identification was based on traditional morphologic classifications (23). Beluga whale (*Delphinapterus leucas*) tissue was taken from a dead captive animal (Port of Nagoya Public Aquarium). Tissues from common minke (*Balaenoptera acutorostrata*), sperm (*Physeter macrocephalus*), Bryde's (*Balaenoptera brydei*) and Sei whales (*Balaenoptera borealis*) were collected legally in 2000 or 2001 during the Western North Pacific Phase II (JARPN II) of the Japanese Whale Research Program, which was authorized by the government of Japan. JARPN II is in full compliance with the relevant international treaty, namely the International Convention for the Regulation of Whaling. Other tissues were taken from dead stranded whales along the coast of Japan. They were collected as early as possible during postmortem investigations conducted by the National Museum of Nature and Science and stored at −20 or −80°C until use.

Table 1. List of cetacean samples

Species	Scientific name	Accession no.
Odontoceti		
Delphinidae		
Bottlenose dolphin	*Tursiops truncatus*	AB612203
Indo-Pacific bottlenose dolphin	*Tursiops aduncus*	AB612204
Striped dolphin	*Stenella coeruleoalba*	AB612205
Melon-headed whale	*Peponoceplala electra*	AB612206
Pygmy killer whale	*Feresa attenuata*	AB612207
False killer whale	*Pseudorca crassidens*	AB612208
Risso's dolphin	*Grampus griesus*	AB612209
Short-finned pilot whale	*Globicephala macrorhynchus*	AB612210
Phocoenidae		
Harbor porpoise	*Phocoena phocoena*	AB612211
Finless porpoise	*Neophocaena phocaenoides*	AB612212
Dall's porpoise	*Phocoenoides dalli*	AB612213
Monodontidae		
Beluga	*Delphinapterus leucas*	AB612214
Ziphiidae		
Stejneger's beaked whale	*Mesoplodon stejnegeri*	AB612215
Blainville's beaked whale	*Mesoplodon densirostris*	AB612216
Ginkgo-toothed beaked whale	*Mesoplodon ginkgodens*	AB612217
Hubb's beaked whale	*Mesoplodon carlhubbsi*	AB612218
Kogiidae		
Pygmy sperm whale	*Kogia breviceps*	AB612219
Dwarf sperm whale	*Kogia sima*	AB612220
Physeteridae		
Sperm whale	*Physeter macrocephalus*	AB612221
Mysticeti		
Balaenopteridae		
Fin whale	*Balaenoptera physalus*	AB612222
Common minke whale	*Balaenoptera acutorostrata*	AB612223
Bryde's whale	*Balaenoptera brydei*	AB612224
Sei whale	*Balaenoptera borealis*	AB612225
Humpback whale	*Megaptera novaeangliae*	AB612226
Balaenidae		
North Pacific right whale	*Eubalaena japonica*	AB612227
Eschrichtiidae		
Gray whale	*Eschrichtius robustus*	AB612228

Determination of nucleotide sequences of the interface on cetacean signaling lymphocyte activation molecules

Deoxyribonucleic acid was extracted from whale tissues using a commercial DNA extraction kit (DNeasy Tissue Kit; Qiagen, Boston, MA, USA). For detection of the SLAM exon 2 coding the V region, the forward primer (dol-SLAM-DF3: 5′-GTGAGGGCTCAACGAATTGC-3′) was designed based on the nucleotide sequences of SLAM in killer whales (*Orcinus orca*; AB428367) and Pacific white-sided dolphins (*Lagenorhynchus obliquidens*; AB428366). The reverse primer was dol-SLAM-DR2 (5′-CATAGAGCTTCAGCTGCAGG-3′) (21). Using this primer set, PCR was performed. Amplification was carried out in a thermal cycler under the following conditions: 96°C for 1 min; 30 cycles of 96°C/20 s, 57°C/30 s, and 72°C/1 min; followed by a final 10 min 72°C extension. The nucleotide sequences of the DNA fragments produced were determined using the dye terminator method.

Homology modeling of the three-dimensional structure of signaling lymphocyte activation molecule

The Protein Data Bank entries for marSLAM in the complex with the measles virus H protein (3ALW: A–D chains, 3ALX: A–D chains, 3ALZ: B chain (18)) and for human NTB-A (21F7: A–D chains (22)) were used as the template structures. Three-dimensional models were constructed using the MODELLER 9.10 program (24) and visualized using PyMOL 1.5.0.3 (Schrödinger LLC) and PovRay (Persistence of Vision).

RESULTS

Variations in the deduced amino acid residues of cetacean signaling lymphocyte activation molecules

Polymerase chain reaction amplifications of DNA samples from all 26 cetacean species studied produced DNA fragments approximately 300 bp in length. The sequence data have been submitted to the GeneBank database; the accession numbers assigned are listed in Table 1. Among the cetaceans, variations were found at the nucleotide level at 30 nucleotide positions in the amplified and sequenced DNA fragments. Replacements of the deduced amino acid residues were detected at 13 residue positions (amino acid residue positions 32, 50, 51, 53, 66, 68, 71, 74, 82, 90, 103, 126 and 130; Fig. 1).

Three-dimensional models of cetacean signaling lymphocyte activation molecule interfaces with morbillivirus

A 3D model of the killer whale SLAM with its complete amino acid sequence was generated. This model showed that it contains a pair of globular immunoglobulin-like V and C regions, and that the V region consisted of two β-sheets, as in previous models (21) (Fig. 2a). The front sheet in the present models contains four anti-parallel β-strands and appears to provide an interface for the morbillivirus (Fig. 2b). Comparison with the interface of marmoset SLAM, shown in Figure 2c, indicates that the 3D structures of the interfaces of killer whale and marmoset are similar. The 12 residues involved in the four binding sites in the marSLAM-MV-H complex (Site 1: 77, 90, Site 2: 61, 63, and 123, Site 3: 127-131, and Site 4: 75 and 119) are depicted in different colors with black side chains in the representation of the marmoset SLAM interface (Fig. 2c). In addition to the twelve residues on the cetacean interface that correspond with those of marmoset SLAM, the residues with protruding side chains are likely components of virus binding. Thus, the 32 amino acid residues on the cetacean interface of the 3D model are thought to contribute to morbillivirus binding (Fig. 2b). When the 32 residues were compared among cetacean groups, variations were found at the six residue positions 68, 74, 82, 90, 126 and 130 (Table 2, bold and underlined). The substitutions at two positions, 74 and 126, are between similar characteristic residues, that is,

Position number of residues*	40	50	60	70	80	90	100	110	120	130
Killer whale	PVMILGRLGSSVLLPLTSDGISKSMNKSIHILVTMAGSPIDTVKKKIVSLDLRKGDSPHHLENGYEFHPENMSLRILKSRKEDEGWYFMSLEENISVQQF									
Bottlenose dolphin	**********	**********	**********	V**********	**********	**********	L**********	**********	**********	H*
Indo-Pacific bottlenose dolphin	**********	**********	**********	V**********	**********	**********	L**********	**********	**********	H*
Striped dolphin	**********	**********	**********	V**********	**********	**********	L**********	**********	**********	H*
Melon-headed whale	**********	**********	**********	**********	**********	**********	L**********	**********	**********	**********
Pygmy killer whale	**********	**********	**********	**********	**********	**********	L**********	**********	**********	**********
False killer whale	**********	**********	**********	**********	**********	**********	L**********	**********	**********	**********
Risso's dolphin	Q*********	**********	**********	**********	**********	Y*********	L**********	**********	**********	**********
Short finned pilot whale	**********	**********	**********	**********	**********	**********	L**********	**********	**********	**********
Harbor porpoise	**********	**********	**********	E**G**T**********	**********	R**********	L**********	**********	**********	H*
Finless porpoise	**********	**********	**********	E**G**T**********	**********	R**********	L**********	**********	**********	**********
Dall's porpoise	**********	**********	**********	E**G**T**********	**********	R**********	L**********	**********	**********	**********
Beluga	**********	**********	**********	E**G**I**********	**********	R**********	L**********	**********	**********	**********
Stejneger's beaked whale	**********	**********	**********	E*****I**********	**********	**********	L**********	**********	**********	V*****
Blainville's beaked whale	L*********	**********	**********	E*****I**********	**********	**********	L**********	**********	**********	V*****
Ginkgo-toothed beaked whale	**********	**********	**********	E**T**I**********	**********	**********	L**********	**********	**********	V*****
Hubb's beaked whale	**********	**********	**********	E*****I**********	**********	**********	L**********	**********	**********	V*****
Pygmy sperm whale	**********	**********	N**********	E*****I**********	**********	R**********	L**********	**********	**********	**********
Dwarf sperm whale	**********	**********	N**********	E*****T**********	**********	R**********	L**********	**********	**********	**********
Sperm whale	**********	**********	**********	E*****I**********	**********	R**********	L**********	**********	**********	V*****
Fin whale	**********	**********	E**********	E**V**I**********	**********	R**********	L**********	**********	**********	V*****
Common minke whale	**********	**********	EE*********	E**V**I**********	**********	R**********	L**********	**********	**********	V*****
Bryde's whale	**********	**********	E**********	E**V**I**********	**********	R**********	L**********	**********	**********	V*****
Sei whale	**********	**********	E**********	E**V**I**********	**********	R**********	L**********	**********	**********	V*****
Humpback whale	**********	**********	E**********	E**V**I**********	**********	R**********	**********	**********	**********	**********
North Pacific right whale	**********	**********	E**********	E*****I**********	**********	R**********	L**********	**********	**********	V*****
Gray whale	**********	**********	EE*********	E**V**I**********	**********	R**********	L**********	**********	**********	V*****

Fig. 1. Deduced amino acid sequences of the SLAM-V region in 26 cetacean species. The position number of the residues is that of the complete SLAM protein of the killer whale. The sequence data of the killer whale was obtained from Reference 21 (Accession no. AB428367) and the other sequences were determined in the present study. The residues corresponding to the PCR primers are not shown.

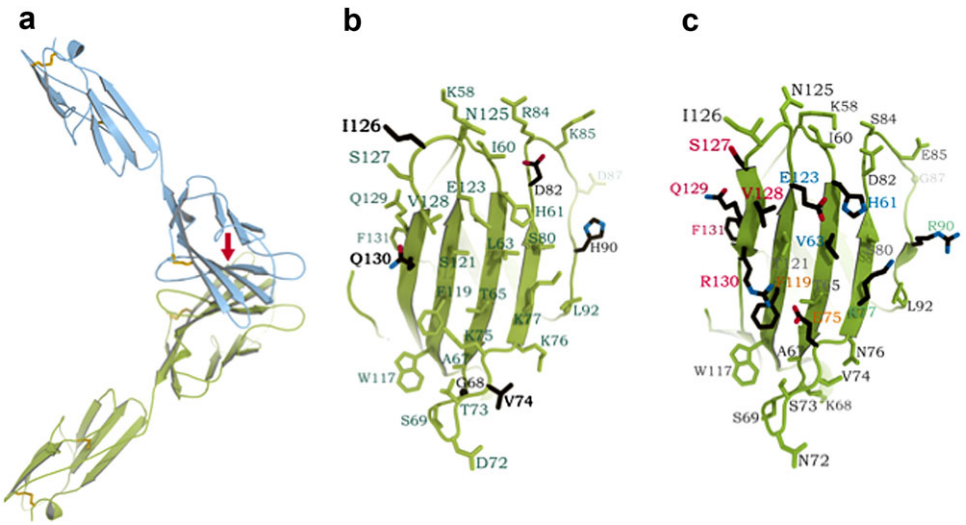

Fig. 2. Ribbon diagram of the 3D model of the killer whale SLAM extracellular region. In (a), the blue and green models show the two SLAM extracellular domains that form a homophilic dimer. The β-strands are indicated by blue and green arrows and the disulfide bonds are shown as yellow bars. The thick red arrow indicates the direction of view of the interface for morbillivirus binding as shown in (b). In (b), the position numbers of the amino acid residues that possibly interact with the viral H protein are shown. The side chains are shown with the atoms colored (black for carbons, blue for nitrogens and red for oxygens). For a reference, the marmoset SLAM interface is shown in (c). The position numbers of the residues involved in the four binding sites of marSLAM-MV-H complex are differently colored as follows: Site 1, mint green; Site 2, cyan; Site 3, red; Site 4, yellow and all with black side chains.

hydrophobic and uncharged residues, whereas the other four substitutions at of the interface were accompanied by charge alterations. The SLAM interfaces of toothed and baleen whales are depicted in Figure 3 and show that the six residue positions are located at the edge of the interfaces (Fig. 3).

Cetacean signaling lymphocyte activation molecule substitutions traced in the phylogeny

The six substitutions of the residues on the cetacean phylogenetic tree were plotted based on the SINE method, which is widely accepted in the classification of the cetaceans (Fig. 4) (25–27). The cow (*Bos taurus*, accession no. AF329970), a member of the order Cetartiodactyla in which the cetaceans are nested (25), was used as the outgroup. The remaining 26 of the 32 residues on the interface were found to be completely identical in cetaceans and cows. The 32 residues were nearly identical among baleen whale SLAMs, although substitutions were frequently found in the residues of toothed whales, particularly in Delphinidae (Fig. 4). Compared with the residues in baleen whales such as the gray whale, five substitutions (E68G, I74V, R90H, V126I and Q130H) were found in bottlenose and striped dolphins; mass die-offs have been recorded in both of these species. Three of these substitutions (E68G, R90H and Q130H) result in charge changes of the interface (Fig. 4).

DISCUSSION

In this study, we compared the SLAM-V domains of various cetacean species; species not studied included river dolphins such as the Indian river dolphin (*Platanista gangetica*) and La Plata dolphin (*Pontoporia blainvillei*) and the pygmy right whale (*Caperea marginata*; Table 1). These domains potentially function as binding interfaces for morbilliviruses. Three-dimensional homology models of cetacean SLAMs showed that 32 amino acid residues possibly bind to the virus on the interface and that, among the cetacean species studied, variations occur at six amino acid residue positions (Table 2). These six positions are located at the edge of the interface, suggesting that the residues in the central region play an essential role in a primary immunological function of SLAM and cannot change (Fig. 3).

Because the nucleotide or amino acid sequences of the SLAM-V region are too short to reconstruct a reliable phylogenetic tree, we plotted the substitutions on a phylogenetic tree constructed using the SINE method (Fig. 4), which classifies cetaceans in a manner consistent with their morphological features (25–27). Compared with baleen whales, five residue substitutions were found in bottlenose and striped dolphins, the species in which most cetacean mass die-offs have reportedly occurred (6–8). Three of the five residues (G68, H90 and H130) introduced an alteration in the charge. SLAM genes are

Table 2. Amino acid residues on cetacean SLAM interfaces that are possibly involved in regulating the binding and specificities of morbillivirus

	Toothed whales						Baleen whales†		
	Delphinidae	Phocoenidae	Monodontidae	Ziphiidae	Koggidae	Physteridae	Balaenopteridae†	Balanidae†	Eschrichtiidae†
58	K	K	K	K	K	K	K	K	K
60	I	I	I	I	I	I	I	I	I
61‡	H	H	H	H	H	H	H	H	H
63‡	L	L	L	L	L	L	L	L	L
65	T	T	T	T	T	T	T	T	T
67	A	A	A	A	A	A	A	A	A
68	**G**	**E**	**E**	**E**	**E**	**E**	**E**	**E**	**E**
69	S	S	S	S	S	S	S	S	S
72	D	D	D	D	D	D	D	D	D
73	T	T	T	T	T	T	T	T	T
74	**V**	**T**	**I**	**I**	**I or T**	**I**	**I or V**	**I**	**I**
75‡	K	K	K	K	K	K	K	K	K
76	K	K	K	K	K	K	K	K	K
77‡	K	K	K	K	K	K	K	K	K
80	S	S	S	S	S	S	S	S	S
82	**D or Y**	**D**	**D**	**D**	**D**	**D**	**D**	**D**	**D**
84	R	R	R	R	R	R	R	R	R
85	K	K	K	K	K	K	K	K	K
87	D	D	D	D	D	D	D	D	D
90‡	**H**	**R**	**R**	**H**	**R**	**R**	**R**	**R**	**R**
92	L	L	L	L	L	L	L	L	L
117	W	W	W	W	W	W	W	W	W
119‡	F	F	F	F	F	F	F	F	F
121	S	S	S	S	S	S	S	S	S
123‡	E	E	E	E	E	E	E	E	E
125	N	N	N	N	N	N	N	N	N
126	**I**	**I**	**I**	**V**	**I**	**V**	**I or V**	**V**	**V**
127‡	S	S	S	S	S	S	S	S	S
128‡	V	V	V	V	V	V	V	V	V
129‡	Q	Q	Q	Q	Q	Q	Q	Q	Q
130‡	**Q or H**	**Q or H**	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**	**Q**
131‡	F	F	F	F	F	F	F	F	F

Bold and underlined: residue positions that vary among cetacean species.
†, Baleen whales. ‡, Residues on cetacean SLAMs that correspond to those in the binding sites in the complex of marSLAM V and MV-H.

comparatively well conserved among mammals and the 32 amino acid residues on the interface have high homology between evolutionarily closely related animals. For example, the SLAMs in two pinniped species, the spotted seal (*Phoca largha*, AB428368) and walrus (*Odobenus rosmarus*, AB428369), are completely identical in those residues. The SLAMs in seals and canines (*Canis lupus familiaris*, AF325357) only differ in two residues, and those two residues are substituted with similar characteristic residues (21, 28). This similarity may explain why mass die-offs of Baikal and Caspian seals have been caused by canine distemper virus infection (29–31). Considering these findings, the substitutions in the two dolphins belonging to the Delphinidae family appear to occur frequently. This suggests that the affinity of such dolphin SLAMs for the morbillivirus is higher than that of other cetacean SLAMs (Table 2, Fig. 3). That affinity may intensify viral infectivity and tissue-to-tissue propagation of the virus in the host animal. McCarthy *et al.* reported no amino acid residue variations among regional populations of the European harbor seal (*Phoca vitulina*) in the SLAM exon 2 and exon 3 regions (32), although the mortality rates varied in mass die-offs occurring in 1988 and 2002 in these seals (4, 33). Such homology of SLAM genes within the same species is unsurprising, because the present study has shown that SLAM amino acid sequences are relatively highly conserved at genus or family levels. Other ecological or epidemiological factors may have affected the variation in mortality rate of European harbor seal populations. However, it is interesting that, among seal populations, a single-nucleotide polymorphism is present only in exon 2

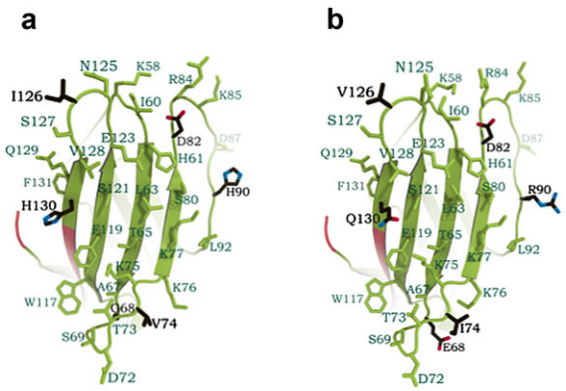

Fig. 3. Interfaces of the 3D models of bottlenose dolphin SLAM (a) and gray whale SLAM (b). The position numbers of the amino acid residues that possibly interact with the viral H protein are shown. The amino acid residues that differ between these two cetacean SLAMs are indicated in black, whereas shared residues are in blue. Their side chains are shown with the atoms colored (black for carbons, blue for nitrogens and red for oxygens). The amino acid residues corresponding to the PCR primers are indicated by red ribbons.

encoding the V region (32). Accumulation of single-nucleotide polymorphisms may promote alteration in the amino acid sequence and in features of the SLAM interface.

Hashiguchi *et al.* have recently reported that four binding sites (sites 1–4) are important in the crystal structure of the complex between the marSLAM-V domain and MV-H protein (18). The 12 residues on the marmoset SLAM interface are reportedly involved in the binding sites (18). All of the residues on the cetacean SLAM interfaces corresponding to these twelve marmoset SLAM residues were found in the possible virus-binding 32 residues (Fig. 2, Table 2). Among the three residue positions with charge alterations among cetaceans (68, 90 and 130), the residues at 90 and 130 of marSLAM are involved in the four sites. At site 1, the positively charged residue R90 of marSLAM interacts with D507, a negatively charged residue of MV-H, which is conserved in all morbilliviruses, including CMV. Residue R130 of marSLAM is involved in both sites 3 and 4. Replacement of residue R130 with S130 dramatically impairs the ability to bind to MV-H (19). At site 4, R130 binds to another SLAM residue, the negatively charged E75, via a salt bridge. On the other hand, cetacean SLAMs have residues K75, H/R90 and H/Q130 instead of the E75, R90 and R130 of marSLAM. Because these cetacean residues are differently charged from those of marSLAM, they may interact with CMV in a different manner than that between the MV-H and marSLAM complex. However, the difference among cetacean species in these residues may affect the affinity of the SLAM interface for CMV. To determine the effect of each substitution in cetacean SLAM on its binding affinity for CMV, crystal structural analyses of the complex of cetacean SLAM-V and CMV-H, or binding affinity experiments between the two proteins, are needed. Further, a CMV infection experiment using cetaceans, or alternatively using knockin mice expressing cetacean SLAMs, will give further insight into the role of cetacean SLAMs in CMV infectivity among cetaceans (34).

The similarity of the 32 residues on the interface of cetacean SLAMs to those of cow SLAMs is noteworthy. When the residues of cow SLAM were compared with those of human or dog SLAM, 11 and 8 substitutions were observed, respectively (28). However, those of baleen whales such as the gray whale have only two substitutions (V74I and H130Q) that differ from those of cows (Fig. 4). This similarity is expected because cetaceans and artiodactyla such as cows and sheep are evolutionarily related. Interestingly, the 32 residues of the bottlenose dolphin have only three substitutions (E68G, R90H and V126I) that differ from those of the cow, although they have five substitutions (E68G, I174V, R90H, V126I and Q130H) that differ from those of the gray whale (Fig. 3). This is because two substitutions (I74 and Q130) that occurred in whale SLAMs after divergence from cows reverted to the same residues as those of the cow (V74 and H130) in dolphin SLAMs (Fig. 4). The strong similarities of the residues on the interface may indicate that CMV can use cow SLAM. In addition, RPV may also be able to infect cetaceans. However, it is unlikely that cetaceans would encounter RPV in nature. This possibility should be studied in future.

In addition to other cellular factors involved in viral replication, many ecological factors, including distribution, migration, population density and reproductive behavior, are probably important in the epidemiology of mass die-offs. Environmental pollutants may have synergistic effects on cetacean mortality. However, among the numerous possible factors, we focused on the affinity between the morbillivirus and its receptor to estimate the

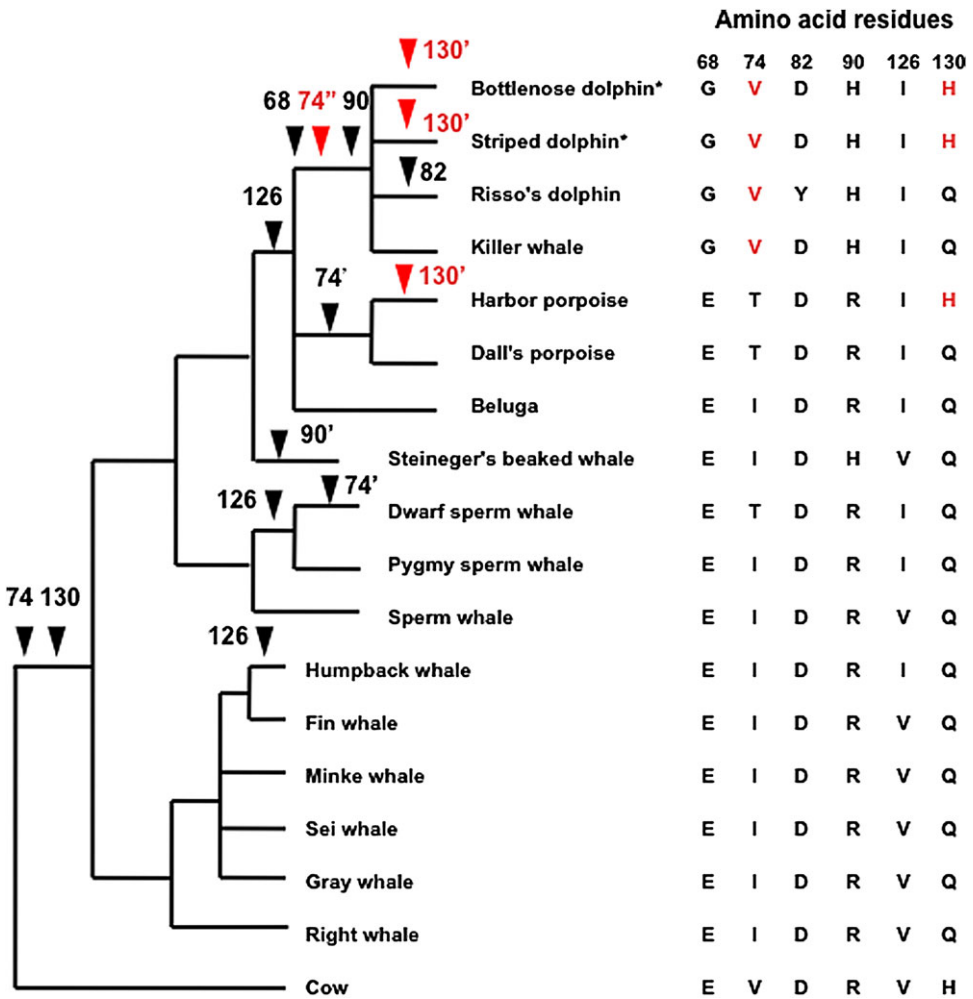

Fig. 4. Residue substitutions at six positions plotted on a phylogenetic tree based on the SINE method. The topology of the phylogenetic tree was based on previous reports using the SINE method (25–27). Information for the cow (*Bos taurus*, accession no. AF329970) was used as the outgroup. The arrowheads and numbers at the branches of the tree indicate the occurrence of substitutions and numbers of amino acid residues: 68, E68G (CGA); 74, V74I (ATC); 74', I74T (ACC); 74", I74V (GTC); 82, D82Y (TAT); 90, R90H (CAC); 90', R90H (CAT); 126, V126I (ATT); 130, H130Q (CAA); and 130', Q130H (CAC) (the changed nucleotides are underlined). The two substitutions that reverted (74" and 130') are shown in red. Dolphins in which mass die-offs have been recorded are indicated with asterisks.

potential vulnerability of cetaceans to viral infection. Further studies on SLAM structure in various forms of wildlife may be useful for assessing their risk of morbillivirus infection in nature.

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DISCLOSURE

All authors have no conflicts of interest to disclose.

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