# Gas-bubble lesions in stranded cetaceans

## Was sonar responsible for a spate of whale deaths after an Atlantic military exercise?

There are spatial and temporal links between some mass strandings of cetaceans — predominantly beaked whales — and the deployment of military sonar[1–3]. Here we present evidence of acute and chronic tissue damage in stranded cetaceans that results from the formation *in vivo* of gas bubbles, challenging the view that these mammals do not suffer decompression sickness. The incidence of such cases during a naval sonar exercise indicates that acoustic factors could be important in the aetiology of bubble-related disease and may call for further environmental regulation of such activity.

Fourteen beaked whales were stranded in the Canary Islands close to the site of an international naval exercise (Neo Tapon, 2002) held on 24 September 2002. Strandings began about 4 hours after the onset of mid-frequency sonar activity. We necropsied eight Cuvier's beaked whales (*Ziphius cavirostris*), a Blainville's beaked whale (*Mesoplodon densirostris*) and a Gervais' beaked whale (*Mesoplodon europaeus*), six of which were very fresh. These animals showed severe, diffuse vascular congestion and marked, disseminated microvascular haemorrhages associated with widespread fat emboli within vital organs. Intravascular bubbles were present in several organs, although definitive evidence of gas embolism *in vivo* is difficult to determine after death[4]. No pathogenic bacteria were isolated from the carcasses.

These lesions are consistent with acute trauma due to *in vivo* bubble formation resulting from rapid decompression (as occurs in decompression sickness)[4,5]. Bubble formation in response to sonar exposure might result from behavioural changes to normal dive profiles (such as accelerated ascent rate), causing excessive nitrogen supersaturation in the tissues (as occurs in decompression sickness); alternatively, bubble formation might result from a physical effect of sonar on *in vivo* bubble precursors (gas nuclei) in nitrogen-supersaturated tissues[6,7].

The beaked whales found in the Canary Islands are not the only stranded cetaceans to provide evidence of bubble-associated tissue injury. In strandings that occurred in Britain between October 1992 and January 2003, three out of 24 Risso's dolphins (*Grampus griseus*), three out of 342 common dolphins (*Delphinus delphis*) and one out of 1,035 harbour porpoises (*Phocoena phocoena*) necropsied, as well as the only Blainville's beaked whale studied, contained gas bubbles in their blood vessels and gas-filled cavities in their parenchymous organs.

The livers of these animals were the most consistently affected organ, with macroscopic gas-filled cavities (diameter, 0.2–6.0 cm) occupying 5–90% of the volume (Fig. 1) and having variable degrees of fibrotic encapsulation. Intrahepatic spherical non-staining cavities (gas bubbles) of diameter 50–750 μm were associated with compression of hepatic tissue, distension of portal blood vessels, and sometimes with haemorrhage, acute hepatocellular necrosis or fibrosis, indicating that this damage was inflicted before death. One of the *D. delphis* specimens also had bilateral acute renal infarcts associated with gas bubbles.

The cavitary lesions described here are new to marine-mammal pathology. Their presence in fresh carcasses in the absence of bacterial isolates, and the apparent progression through the stages of pericavitary fibrosis, are inconsistent with decompositional bubbles from bacterial activity. The coexistence of ante mortem gas bubbles and gas-filled fibrosed cavities suggests that *in vivo* bubble formation and expansion is the proximate aetiology of this disease process. Bubble formation may either have initiated in the liver and kidneys ('autochthonous' bubble formation) or in other tissues (fatty tissue, for example) before haematogenous transfer to the liver and kidneys as gas emboli.

Nitrogen bubbles and emboli can develop in decompression sickness in humans and experimental animals as a result of expansion of pre-existing gas nuclei within nitrogen-supersaturated tissues[5]. Anatomical, physiological and behavioural adaptations may mitigate against *in vivo* formation of nitrogen bubbles in marine mammals[8–12], although there is empirical evidence of nitrogen supersaturation in cetaceans[8]. Some deep-diving species are predicted to undergo up to 300% nitrogen tissue supersaturation[7]. Static diffusion in nitrogen-supersaturated tissues is therefore a plausible mechanism for bubble development and is consistent with a greater prevalence of cases in deep-diving species such as Risso's dolphins and beaked whales.

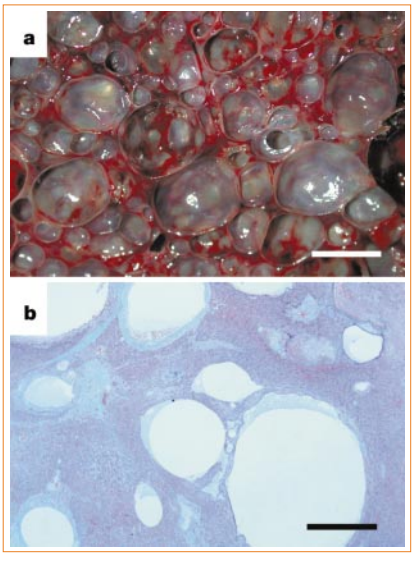


**Figure 1** Gas-filled cavities in the liver of a stranded common dolphin (*Delphinus delphis*). **a,** Cut surface of the liver, showing that cavitary lesions have extensively replaced the normal tissue. Scale bar, 10 mm. **b,** Photomicrograph of liver section, showing multiple cavities (gas bubbles) within the portal tracts and hepatic parenchyma. Scale bar, 750 μm.

Further investigation is needed into the physical and behavioural effects on cetaceans exposed to sonar, and the relation of these effects to bubble growth *in vivo* and to strandings. Necropsies should aim to compare fat and gas emboli in stranded cetaceans suspected of having been exposed to sonar with results from unexposed stranded controls. In a wider conservation sense, our findings need to be taken into account in considering the regulation and limitation of the adverse impact of anthropogenic sonar on cetaceans.

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Immunology

# Hepatitis A virus link to atopic disease

Atopic diseases, including asthma, allergic rhinitis and atopic dermatitis, are caused by both environmental and genetic factors. Here we show that infection by hepatitis A virus (HAV) may protect individuals from atopy if they carry a particular variant of the gene that encodes TIM-1 (also known as HAVcr-1) — the cell-surface receptor used by HAV to infect human cells[1]. Exposure to HAV is associated with poor hygiene, large family size and attendance at day-care centres, all factors that are also inversely associated with atopy[2–6]. Our discovery indicates that interaction between HAV and TIM-1 genotype may contribute to the aetiology of atopic diseases, and provides a mechanism to account for the hygiene hypothesis.

Using a congenic positional cloning strategy, we identified *TIM-1* as a candidate gene for atopy and asthma in a region of mouse chromosome 11, which is homologous to a segment of human chromosome 5q31–33 that has been linked to atopy[7,8]. TIM-1 is expressed by activated $CD4^+$ T cells during the development of helper-T-cell (Th2) responses and regulates cytokine production[7]. We therefore investigated whether the interaction between HAV and TIM-1 on lymphocytes can modify T cells in a way that protects against atopy, and whether polymorphisms in TIM-1 can alter susceptibility to atopy[7].

By sequencing complementary DNA from human lymphocytes, we identified a six-amino-acid insertion (ins) at residue 157, termed 157insMTTTVP (one-letter amino-acid notation), as well as two single-amino-acid changes, 195delT (where 'del' signifies a deletion) and A206T. The insertion 157insMTTTVP is located at the centre of an extracellular mucin-like region that is required for efficient HAV uncoating[9] and, because 157insMTTTVP lengthens this critical region by 12–14%, this variation may affect the efficiency of viral entry (Fig. 1).

To determine the effect of the insertion 157insMTTTVP on the occurrence of atopy, we carried out a cross-sectional study of 375 individuals who were evaluated by history and tested serologically for atopy and prior HAV infection. To correct for potentially confounding effects of population admixture, we used stratified Mantel–Haenszel $\chi^2$ tests to quantify the association between atopy and 157insMTTTVP in the total sample. We found that HAV seropositivity protects against atopy, but only in individuals with the 157insMTTTVP variant of TIM-1 ($P = 0.0005$; Table 1).

The protective effects of HAV therefore depend upon a common *TIM-1* allele that is carried by 63% of Caucasians, 46% of Asians and 64% of African Americans in this population (see supplementary information). As allelic variation in TIM-1 does not affect HAV-infection rates in our population ($\chi^2 = 1.567$, $P = 0.211$), we conclude that the interaction of HAV with TIM-1 genotype seen here is not due to variation in the rate of seroconversion following HAV exposure.

Before 1970, the seroprevalence of antibodies against HAV approached 100% in Western countries[4], and infection with HAV may have protected many individuals against atopy[3]. However, modernization has led to a reduction in average family size and significant improvements in public health, causing anti-HAV seroprevalence to fall to 25–30%, while the prevalence of atopic disease has doubled[4]. Our finding that TIM-1 is associated with atopy in HAV-seropositive individuals indicates that exposure to a specific pathogen may influence the expression of atopy — so a declining prevalence of HAV infection could contribute to an increase in atopy by association with *TIM-1*. It will be necessary to determine whether HAV exposure must occur during childhood to have a protective effect, whether HAV can mitigate the severity of existing atopic disease, and whether HAV vaccination can reproduce the effects of natural HAV infection.

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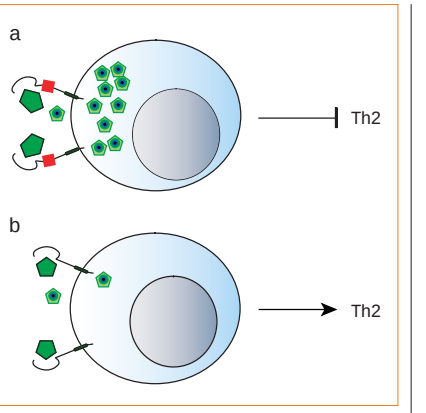


**Figure 1** Possible mechanisms of interaction between the cell-surface TIM-1 receptor and hepatitis A virus (HAV), and the effect of HAV on cytokine production. **a,** A variant of TIM-1 ('hook') that carries a 6-amino-acid insert (red), 157insMTTTVP, may increase binding of HAV (large pentagons) to the receptor, thereby enhancing HAV viral uncoating (small pentagons) and infection of TIM-1-expressing T cells. This could lead to deletion of certain lymphocyte subsets, such as Th2 cells, or reduce Th2-cell differentiation, causing a reduction in atopy and asthma. **b,** Alternatively, HAV may bind less efficiently to the form of TIM-1 without the insertion, resulting in less HAV infection and hence more Th2-cell development and more atopy. The mechanism that underpins this interaction between TIM-1, its 157insMTTTVP region and HAV could relate to viral uncoating[9], the extent and duration of HAV viraemia[10], or to a direct effect on Th1/Th2-subset differentiation.

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**Table 1 TIM-1 insert and protection against atopy**

| *HAV status* | *157insMTTTVP genotype* | *Total* | *Atopic* | *Non-atopic* | *$\chi^2$ (P)* | *Odds ratio (95% CI)* |
|---|---|---|---|---|---|---|
| Seronegative ($n = 198$) | Insertion | 120 | 83 (69%) | 37 (31%) | 0.463 (0.496) | 1.285 (0.708–2.439) |
| | No insertion | 78 | 50 (64%) | 28 (36%) | | |
| Seropositive ($n = 123$) | Insertion | 65 | 31 (48%) | 34 (52%) | 11.978 (0.0005) | 0.257 (0.116–0.570) |
| | No insertion | 58 | 46 (79%) | 12 (21%) | | |

Comparison of allele distributions across subjects using the Cochran–Mantel–Haenszel $\chi^2$ test with racial stratification, two-sided tests of significance ($P$), and number of subjects within each genotype (see supplementary information). The six-amino-acid insertion 157insMTTTVP is associated with atopy in individuals seronegative for hepatitis A virus but not in seropositive individuals. HAV does not independently affect atopy ($\chi^2 = 0.513$, $P = 0.474$). Subgroup analysis of Caucasians and Asians confirms this association in both groups ($P = 0.024$ and $P = 0.036$, respectively), and Breslow–Day tests of the homogeneity of the odds ratios demonstrate no significant difference between racial strata (see supplementary information). This table excludes data from 54 individuals with an intermediate atopic phenotype (see supplementary information).