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Macrophage activity stimulated by chitosan in vitro

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Chitosan is well known as a wound healing promotor when applied topically on the wound and it could increase immune response and resistance to infection when given systemically. Macrophages play a major role in increasing non-specific immune response and resistance to infection, and also supply various mediators for succeeding systemic immunoresponse and wound healing. There are however very few reports describing the direct effects of chitosan on macrophages. The purpose of this study was to investigate the changes on phagocytic activity and cytokine production of macrophages after direct stimulation by chitosan.

Macrophages were stimulated by chitosan particles with mean diameters of 3.2 and 5.0 μm. Phagocytic activities were evaluated by flowcytometric analysis and microscopic observation. Macrophage phagocytic activities were increased significantly by chitosan particles with 5.0 μm. Mannan, a mannose receptor agonist, evidently showed inhibitory effects on macrophage phagocytic activity when supplemented at concentrations of 2.5mg/ml. This result indicates that chitosan directly accelerates the phagocytic activity of macrophages, and that this activation is mediated by mannose receptors on macrophages. Production of IL-1β, IL-6, GM-CSF and IFN-γ in the culture media of macrophages stimulated by chitosan were assayed. Significant increase of IL-1β, IL-6 and GM-CSF activities were found in the culture media. These results suggest that the cytokines produced by macrophages act as an enhancer of immunity through activated T- and B- cells when chitosan is applied systemically.

In conclusion, this study showed that chitosan may directly increase macrophage functions, such as phagocytic activity mediated by mannose receptor and the production of cytokines IL-1β, IL-6 and GM-CSF.

Pathological Studies of Captive Dolphins in an aquarium

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Lesions and causes of death in captive dolphins were examined for the purpose of prevention of diseases and care of captive dolphins. Twenty-five dolphins, including 20 bottlenose dolphin2 (*Tursiops tryncatus*), 3 harbor porpoises (*Phocoena phocoena*) and 2 finless porpoises (*Neophocaena phocaenoides*), (13 males, 12 female ; approximately 6 months to 23 years old) that had been kept in 3 aquariums in Hokkaido and one aquarium in the Chubu district

for more than 4 months and died in the last 9 years were used for the pathological study. The main lesions were as follows: skin lesions in 18 dolphins, pneumonia in 15 dolphins, ruminal ulcers in 9 dolphins, and enteritis in 7 dolphins. Other lesions were proliferative cholangiohepatitis in 4 animals, and encephalitis and meningoencephalitis in 2 dolphins. Nine dolphins (36%) died of septicemia. The results proved that septicemia, skin lesions and pneumonia often occur in captive dolphins.

Aspiration pneumonia was found in 8 of 15 dolphins with pneumonia. Bacterial and fungal encephalitis was observed in 2 dolphins.

Aspiration pneumonia was composed of mild focal fibrinous bronchopneumonia to severe diffuse necrotizing pneumonia, and the severity of the lesion varied depending on the case. Three dolphins died of severe aspiration.

Foreign body accumulation in the rumen was observed in 6 of 8 dolphins kept in an open pen. Parakeratosis and ulcers were found in their rumina. The mucosal lesions were severe in the rumen, containing sharp or firm foreign bodies. As severe foreign body accumulation in the upper alimentary tract caused death by starvation in one dolphin, swallowing of foreign bodies seems to be life-threatening for some dolphins.

Calcium deposition in the bronchiolar mucosa was observed in 24 dolphins. The lesion was observed only in the bronchiolar mucosa, and no significant elevation of the serum calcium concentration was seen in those dolphins. Cellular debris occasionally accumulated on the bronchiolar mucosa and was incorporated in the mucosa. From these findings, it was assumed that the calcium deposition resulted from distrophic calcification on the exudate, cellular debris and foreign bodies in the bronchiolar mucosa. However, the lesion was considered to be incidental and pathologically insignificant.

Fungal infection were often seen, particularly in mucosal lesions (rumen 7, esophagus 3, laryngopharynx 3, and nasal sinus 2). The fungal infection caused severe, systematic infection such as pneumonia, nephritis and encephalitis in 2 dolphins treated with steroids for a long time. Therefore, the findings suggested that long-term steroid therapy in dolphins can induce severe fungal infection.

Pathological Studies of GM_1 Gangliosidosis in a Shiba Dog

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GM_1 gangliosidosis is a lysosomal storage disease caused by deficient activity of lysosomal acid beta-galactosidase. This genetic disorder is inherited as an autosomal recessive trait.

It has been identified and studied in humans, cats, cattle, dogs, and sheep. In this study, we the described morphological manifestations of GM_1 gangliosidosis in a Shiba dog and compared this case and other cases with GM_1 gangliosidosis.

A 6-month-old Shiba dog showed progressive clinical signs of a predominantly cerebellar ataxia and resting and intentional head tremors. Clinical signs, including ataxic abasia, hypermetria, tetraplegia, corneal clouding and visual deficits were also prominent at 10 months of age. Vacuolated lymphocytes suggesting a storage disorder were seen in the peripheral blood. At