Local Pathological Reactions Induced in Pigs and Cats by Adjuvant ISA-70 Containing Inactivated Newcastle Disease Virus Antigen

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ABSTRACT. Gross and microscopic changes at the injection site in pigs and cats were investigated for 8 weeks after an intramuscular injection of oil adjuvant ISA-70 emulsion, containing inactivated Newcastle disease virus antigen. In pigs, an egg-sized and discolored lesion with pinpoint to miliary-sized nodules was observed at 2 to 8 weeks post injection (PI). In cats, there was partial thickening of the subcutaneous tissue at 2 to 8 weeks PI. Histopathologic changes in pigs were fundamentally similar to those in cats, however, the local tissue reactions in pigs were severer and more protracted than those in cats. The lesions in pigs were characterized by formation of large-sized cysts and well-developed encapsulation of the cysts with epithelioid cells.—**KEY WORDS:** adjuvant, local tissue reaction, oil granuloma.

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Several oil-adjuvanted inactivated vaccines are currently marketed for use in chickens, pigs and cats. It has been demonstrated that these oil-adjuvanted vaccines induce high levels of circulating antibodies and provide protection against challenge with virulent strains [2–4, 6–10, 12, 13]. The local tissue reactions induced by these vaccines, however, have not been described in detail. In previous papers [14, 15], we described pathological changes at the intramuscular injection site in guinea pigs, rats and chickens induced by a new oil adjuvant emulsion, called oil adjuvant ISA-70 (SEPPIC, Cosmetics / Pharmacy Division, Paris), containing inactivated Newcastle disease virus (NDV) antigen. In the chickens injected with oil emulsion (OE) prepared with ISA-70 and NDV antigen, the acute inflammatory reaction was mild and the granulomatous lesion was not so extensive as those in guinea pigs and rats. These findings indicate a variation in the response to OE among animal species.

The present report describes the pathological changes in pigs and cats at the intramuscular injection site due to OE prepared with ISA-70 containing inactivated NDV antigen. The purpose of the investigation is to compare the local tissue reactions in different animal species to OE using same antigen.

One-month-old conventional pigs and 3- to 5-month-old Japanese domestic cats, that had been clinically shown as normal, were injected into the femoral muscle with 0.5 ml of ISA-70 OE containing inactivated NDV antigen. ISA-70, consisting of nine parts of mineral oil and one part of an emulsifier (AMOE: anhydro mannitol octadecenoate ether), was emulsified with the NDV antigen at a 7:3 volume ratio by manual shaking for a brief period just before injection as described [14, 15]. Two pigs and two cats were electrocuted or euthanatized with ketamine hydrochloride at the following times post injection (PI); 72 hrs and 2, 4 and 8 weeks. They were necropsied to examine the gross and microscopic changes. Samples taken from the injection site (muscle) were fixed in 10% neutral buffered formalin and embedded in paraffin by the standard method. The sections were stained with hematoxylin and eosin (HE), and with Masson trichrome. The

frozen sections were stained for fat with Sudan III.

Grossly, in pigs, the inocula were localized in both the muscle and subcutaneous tissue with edema at 72 hrs PI. At 2 to 8 weeks PI, an egg-sized and yellowish-white discoloration lesion was observed in the muscle of the injection site. A large number of pinpoint to miliary sized whitish nodules were scattered in the lesion and whitish material exuded from the cut section. In cats, there were partial thickening of the subcutaneous tissue and discoloration of the muscle of the injection site at 2 weeks PI and later. These gross lesions reached its peaks at 4 weeks PI. The lesions at 8 weeks PI showed a decrease in severity compared with that seen at 4 weeks PI. No whitish

Table 1. Comparison of main histopathological changes at the injection site in pigs and cats intramuscularly injected with ISA-70 emulsion containing inactivated Newcastle disease virus antigen

Time	Histopathological changes	Severity of changes	
		Pigs	Cats
PI			
72 hrs	Size of cysts ^{a)}	220, 120	60, 10
	Acute inflammation	++ ^{b)} , +	+, +
2 weeks	Size of cysts	220, 350	150, 150
	Acute inflammation	–, +	+, –
	Epithelioid cells	+++ ^{b)} , +++	+, +
	Lymphocytes	+, ++	+, –
	Plasma cells	–, +	–, –
4 weeks	Size of cysts	625, 610	215, 160
	Neutrophils	+, +	+, –
	Epithelioid cells	+++ ^{b)} , +++	++, ++
	Lymphocytes	++, ++	++, +
	Plasma cells	+, +	+, +
	Fibrosis	++, ++	+, ++
8 weeks	Size of cysts	590, 850	120, 170
	Neutrophils	+, +	–, –
	Epithelioid cells	+++ ^{b)} , ++	+, +
	Lymphocytes	++, ++	+, +
	Plasma cells	++, ++	+, +
	Fibrosis	++, ++	+, ++

a) The maximum diameter of cysts (μm).

b) –; No change, +; Slight, ++; Moderate, +++; Severe.

nodules were present throughout the experimental period.

The histopathologic changes to ISA-70 emulsion containing inactivated NDV antigen at the injection site in pigs and cats are compared in Table 1. In both animals, acute exudative inflammatory changes with infiltration of macrophages and neutrophils were noted in the subcutaneous and interstitial connective tissue in the muscle at 72 hrs PI. Various sized cystic spaces, due to the oil, were also observed and were surrounded by macrophages or outlined by a thin layer of connective tissue. Although some muscle fibers were separated by cystic spaces and acute inflammatory exudates, muscle fiber degeneration was not recognized in any of the animals. At two weeks PI and later, granulomatous lesions containing numerous cysts developed in the subcutaneous and interstitial connective tissue in the muscle. In pigs, marked proliferation and aggregation of epithelioid cells were observed around the various sized cysts. The cysts in pigs were comparatively large in size, measured up to 625–850 μm in diameter, at 4 to 8 weeks PI. Around the cysts, infiltration of neutrophils and macrophages, and proliferation of fibroblastic cells were also noted (Fig. 1). Infiltration of plasma cells and aggregation of lymphocytes were observed in the lesion at 2 to 8 weeks PI. In pigs, encapsulation of the cysts progressed until the 8th week PI and was more evident than that in cats.

The cysts in cats were smaller in size than those in pigs and measured up to 215 μm in diameter at 4 weeks PI. The peaks of granulomatous lesions in cats reached at 4

weeks PI (Fig. 2). There was no evidence of abscess formation at the injection site in any of the animals.

Straw *et al.* [11] compared the tissue reactions in pigs caused by six different adjuvants incorporated in a *Haemophilus pleuropneumoniae* vaccine. They showed that the mineral oil adjuvants were highly irritant and caused extensive granulomatous lesion and it persisted at eight weeks after injection. It has been demonstrated that bacterial mineral oil-adjuvanted vaccines induce severer inflammatory reactions at injection site than do viral ones [1, 5]. In the present study, inactivated NDV antigen was used in order to differentiate the local tissue reactions to OE among animal species. The oil adjuvant ISA-70 emulsion used in this experiment induced less severe local tissue reactions than did such other adjuvants as Freund's adjuvants and aluminum phosphate gel in guinea pigs, rats [14] and chickens [15]. The basic pathologic changes in the local tissue in pigs and cats were the same as those in guinea pigs and rats. The present study revealed, however, that the reaction in pigs was severer and more protracted than that in cats. The granulomatous reactions containing oil cysts at the injection site reached its peak at 4 weeks PI in cats and at 8 weeks PI in pigs. The characteristic histological findings in pigs were large-sized cyst formation and well-developed encapsulation by epithelioid cells of the cysts formed at the injection site. In pigs, the oily adjuvant material in the cysts may be fused mutually with time in the lesion. The oily adjuvant material in the large cysts may be retained for a long time

Fig. 1. The ISA-70 containing NDV antigen injected site of a pig 4 weeks PI. A granulomatous lesion consisting of cysts, epithelioid cell aggregation, fibroblastic proliferation and small round cell infiltration. The cyst is large in size. $\times 130$, HE.

Fig. 2. The ISA-70 containing NDV antigen injected site of a cat 4 weeks PI. A granulomatous lesion with small-sized cyst and small round cell infiltration. $\times 130$, HE.

at the injection site and caused continued irritancy. Although the reason for large cyst formation in pigs is unclear, it may be related with the prolonged local tissue reactions.

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