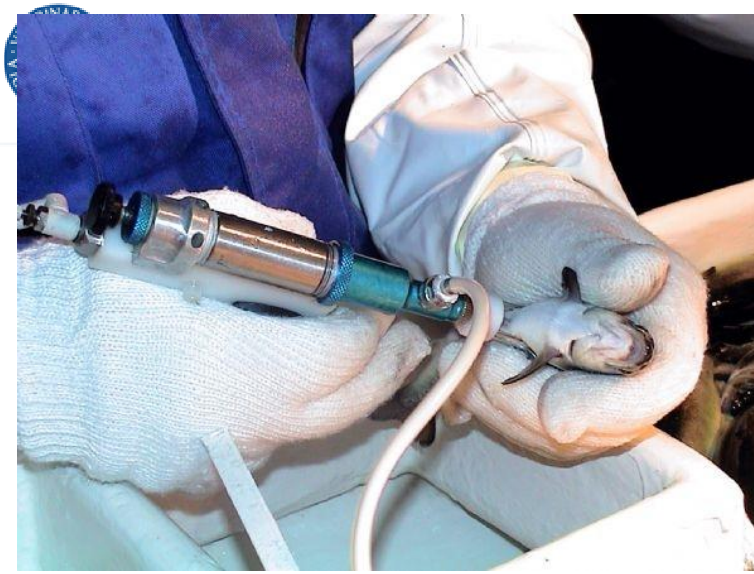
Vaksinering og bivirkninger

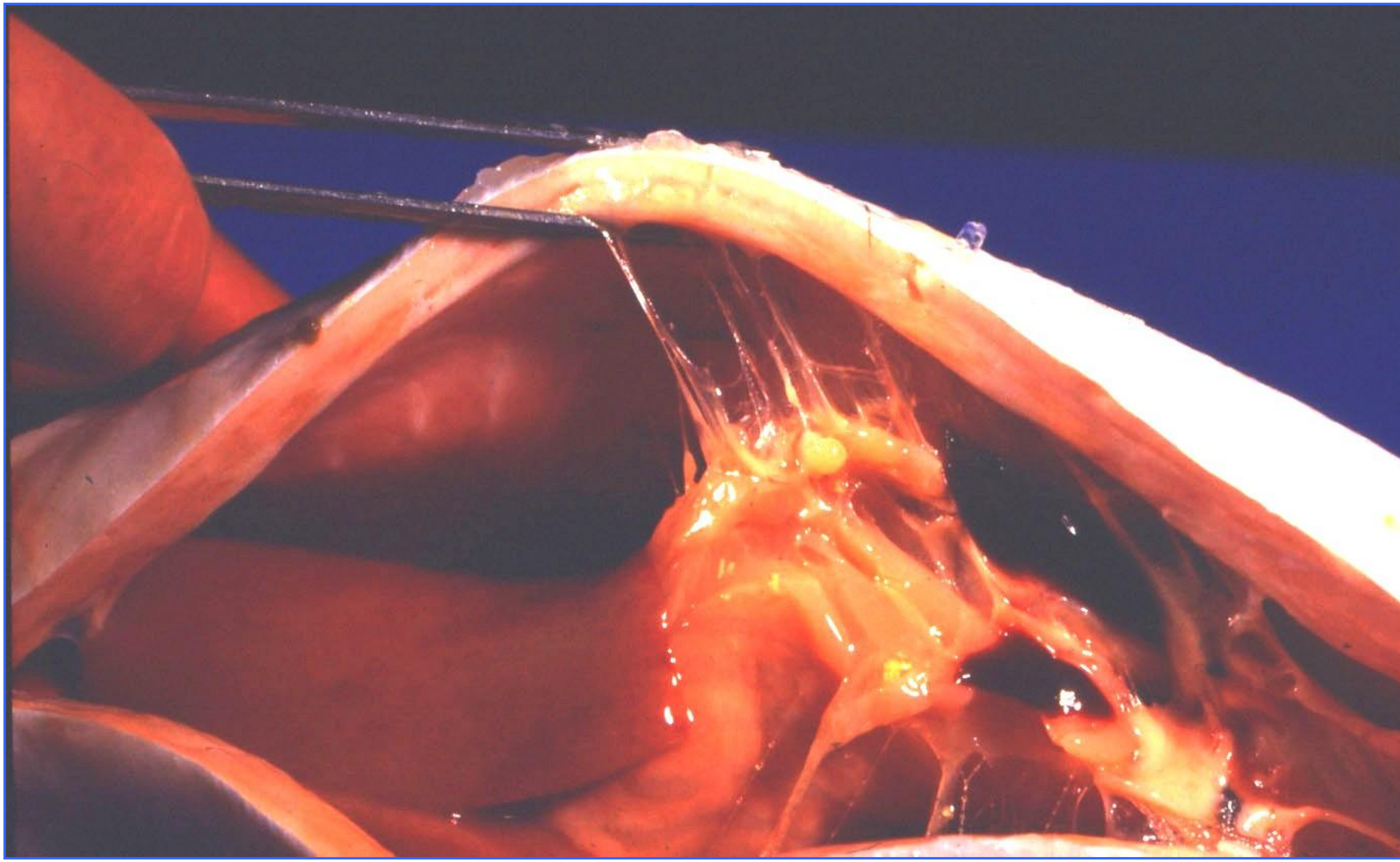
Erling Olaf Koppang

Norges veterinærhøgskole

Oslo



0,1 ml / 40 g = 200 ml / 80 kg



Journal of Fish Diseases 2005, 28, 13–22

Vaccine-associated granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white muscle

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Abstract

The purpose of this study was to investigate the nature of variably sized pigmented foci encountered in fillets of farmed Atlantic salmon, *Salmo salar* L. The material was sampled on the fillet production line and on salmon farms from fish with an average size of 3 kg from various producers. The fish had been routinely vaccinated by injection. Gross pathology, histology, immunohistochemistry using antisera against major histocompatibility complex (MHC) class II β chain and transmission electron microscopy (TEM) were used to characterize the changes. Macroscopically, melanized foci were seen penetrating from the peritoneum deep into the abdominal wall, sometimes right through to the skin, and also embedded in the caudal musculature. Histological investigation revealed muscle degeneration and necrosis, fibrosis and granulomatous inflammation containing varying numbers of melano-macrophages. Vacuoles, either empty or containing heterogeneous material, were frequently seen. The presence of abundant MHC class II^{+} cells indicated an active inflammatory condition. TEM showed large extracellular vacuoles and leucocytes containing homogeneous material of lipid-like appearance. The results showed that the melanized foci in Atlantic salmon fillet resulted from an inflammatory condition probably induced by vaccination. The described condition is not known in wild salmon and in farmed salmon where injection vaccination is not applied.

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Keywords: Atlantic salmon, inflammation, melano-macrophage, major histocompatibility complex class II, mineral oil, vaccine.

Introduction

Various pathological conditions may be associated with abnormal pigmentation in tissues and organs. Such pigments may either be of exogenous or endogenous origin. Endogenous pigments include derivates of lipids, haemoglobin, porphyrins and melanin. The term melanosis is used to describe the presence of melanin in abnormal locations (Thomson 1984). In vertebrates, melanin is synthesized by melanocytes and organized in melanosomes, which are lysosome-related intracellular organelles (Orlow 1995; Raposo, Fevrier, Stoorvogel & Marks 2002). Mammalian melanocytes originate from the embryonic neural tube (Sulaimon & Kitchell 2003) and it has been observed that such cells can migrate into inflamed tissue (Thomson 1984).

Inflammatory reactions and tissue regeneration in salmonids seem similar to those of mammals (Finn & Nielson 1971), but have in addition been associated with the involvement of so-called melano-macrophages (Roberts 1975; Agius & Roberts 2003). The origin of melanosomes in melanin-containing viscerally located cells in fish is not clear (Agius & Roberts 2003), but Sichel, Scalia, Mondio & Corsaro (1997) suggested that melanogenesis in poikilothermic vertebrates may occur in mesenchyme-derived cells of the haematopoietic lineage. Although teleost melano-macrophages have been ascribed macrophage-like properties, their functions and significance are

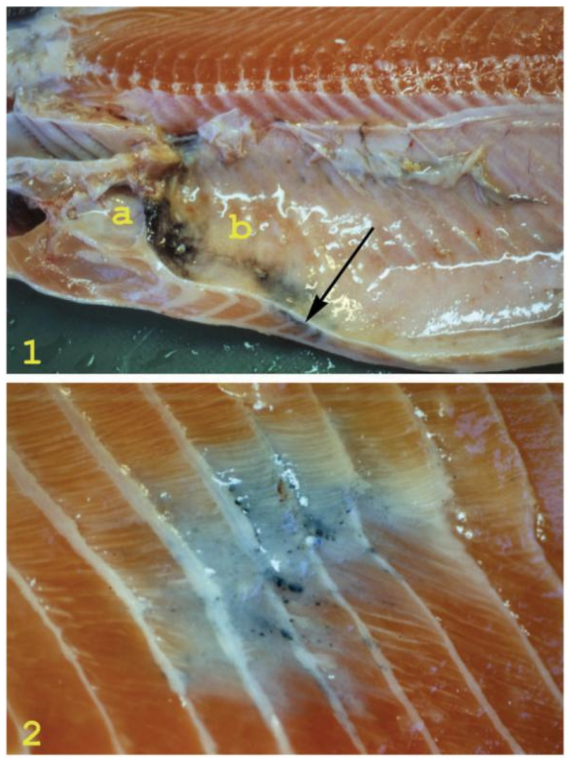


Figure 1 Gross pathological changes in the carcass of an Atlantic salmon. The pericardial cavity (a) is normal, but severe melanization is apparent in the abdominal cavity (b). Melanized musculature subjacent to the peritoneum is seen on the cut surface (arrow).
Figure 2 A melanized area in the musculature of an Atlantic salmon. The peritoneum is removed and darker foci are seen in a dark to grey area involving five myosepta. The lesion is situated laterally in the fish, covering the area of the lateral organ. Note the contraction in the musculature, disrupting the curves of the intramuscular septa.

Journal of Fish Diseases 2005, 28, 13–22 E O Koppang et al. *Vaccine-associated pathology in salmon muscle*

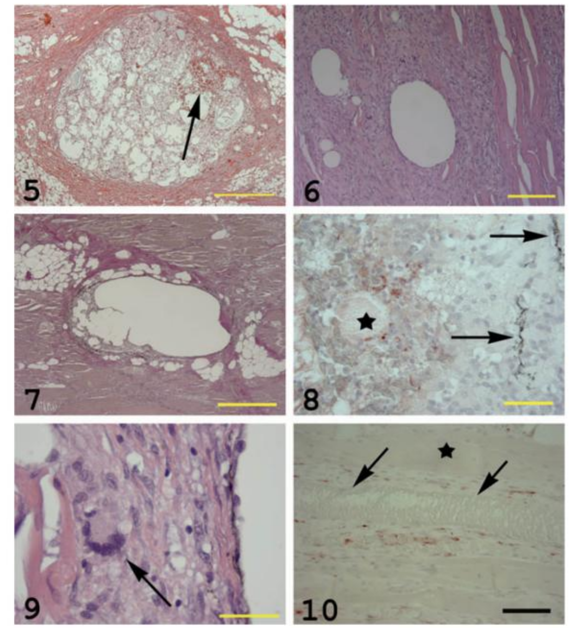
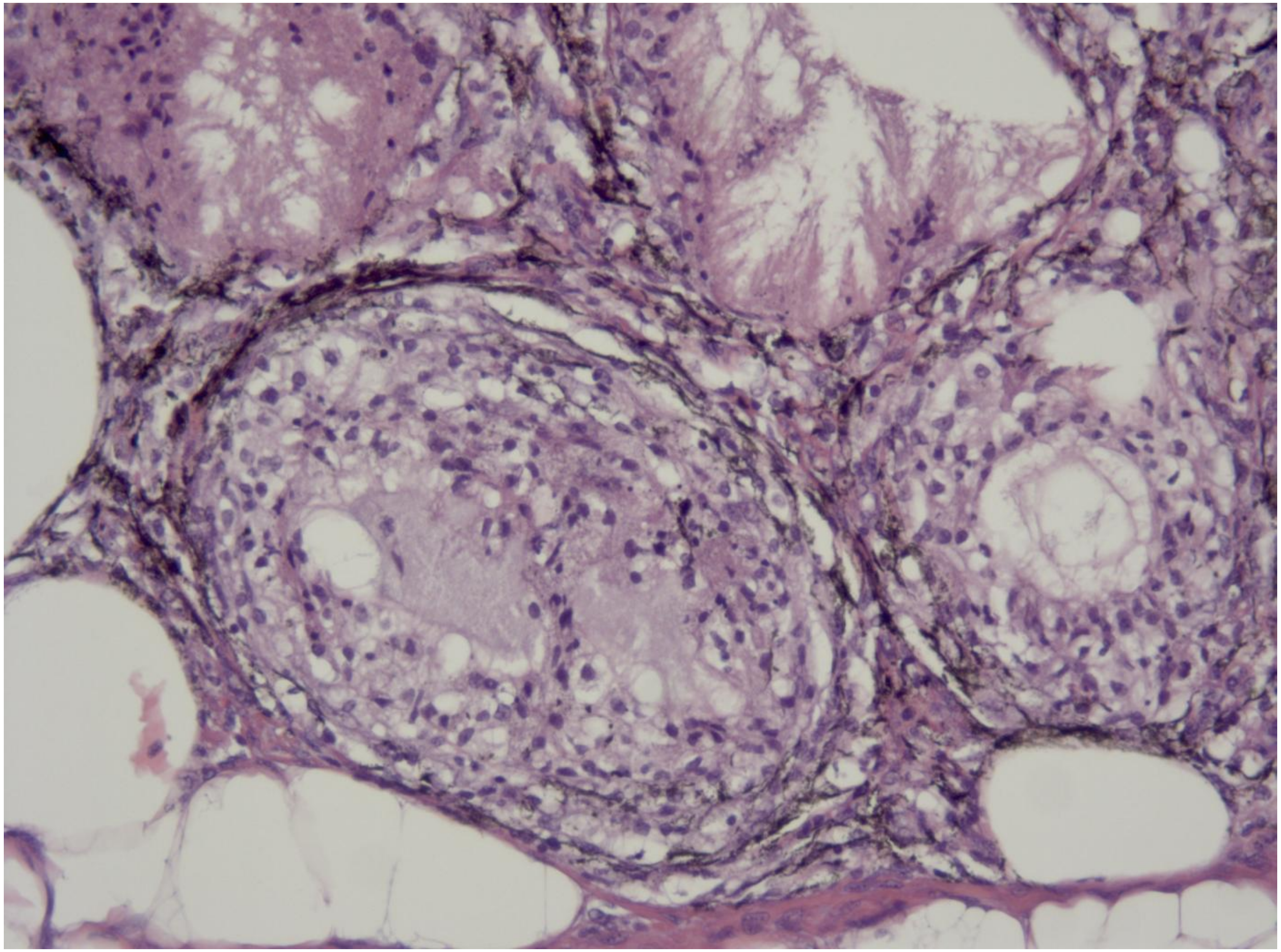
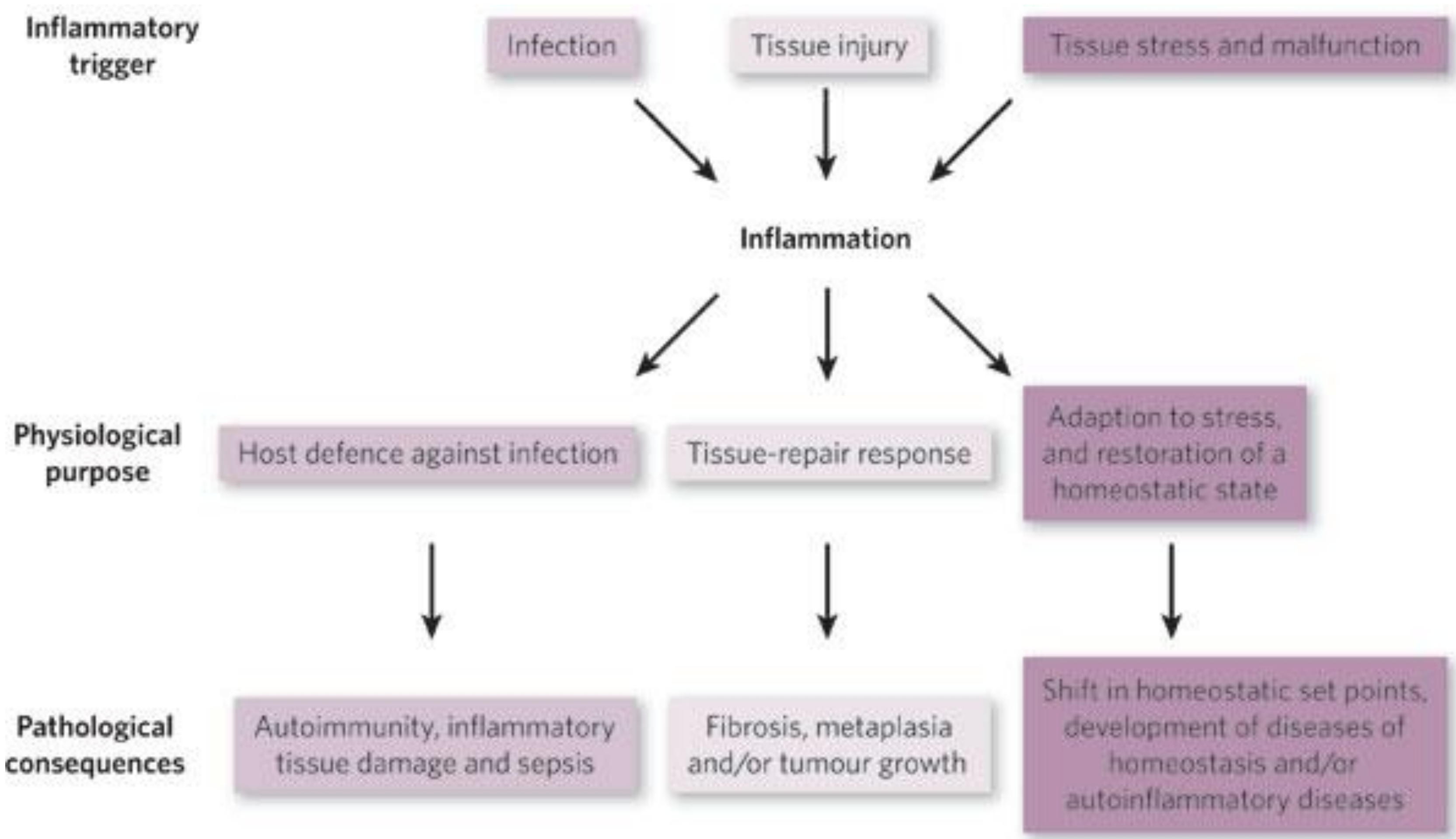


Figure 5 A large vesicle embedded in an intermyotomal septum containing macrophage-like cells, debris and a fresh haemorrhage (arrow) (H&E, bar = 500 μm).
Figure 6 Empty vesicles surrounded by granulomatous tissue embedded in the white musculature. Note adjacent, seemingly unaffected muscle cells (H&E, bar = 200 μm).
Figure 7 Vesicles embedded in the white musculature surrounded by fibrogranulomatous tissue (red staining) (EVG, bar = 500 μm).
Figure 8 Reaction against oil (red staining) in a vesicle as shown in Fig. 5. Homogeneous masses (asterisk) and macrophage-like cells show positive reactions. Note the melano-macrophages in the vesicle wall (arrows) (oil red O, bar = 50 μm).
Figure 9 High magnification of the wall of a vesicle as seen in Fig. 6. The wall contains a multinucleated giant cell (MGC)(arrow), epithelioid-like cells, small vacuoles and is lined towards the lumen of the greater vesicle with melanosome-containing cells, probably swollen melano-macrophages (H&E, bar = 40 μm).
Figure 10 Muscle cells infiltrated with MHC class II^{+} cells. One muscle cell is unaffected (asterisk). One fibre shows severe degeneration (arrowhead), whereas one is invaded by MHC class II^{+} cells (red reaction) (MHC class II immunostain, haematoxylin counterstain, bar = 100 μm).



Kronisk manifestasjon


Inflammatory trigger
Infection
Tissue injury
Tissue stress and malfunction
Inflammation
Physiological purpose
Host defence against infection
Tissue-repair response
Adaption to stress, and restoration of a homeostatic state
Pathological consequences
Autoimmunity, inflammatory tissue damage and sepsis
Fibrosis, metaplasia and/or tumour growth
Shift in homeostatic set points, development of diseases of homeostasis and/or autoinflammatory diseases

Induction of Lupus-associated Autoantibodies in BALB/c Mice by Intraperitoneal Injection of Pristane

By Minoru Satoh and Westley H. Reeves

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Summary

Intraperitoneal injection of pristane (2,6,10,14 tetramethylpentadecane) is a standard technique for obtaining monoclonal antibody-enriched ascitic fluid. However, pristane also induces plasmacytomas and an erosive arthritis resembling rheumatoid arthritis in BALB/c mice, probably as a consequence of enhanced interleukin 6 production. We report here that the production of autoantibodies characteristic of systemic lupus erythematosus (SLE) is a further consequence of injecting pristane in BALB/c mice. Anti-Su antibodies appeared as early as 1–2 mo after a single injection of 0.5 ml pristane, followed by anti-U1RNP and anti-Sm antibodies after 2–4 mo. Within 6 mo of pristane injection, 9 of 11 BALB/c mice had developed anti-Su, anti-U1RNP, anti-U2RNP, anti-Sm, and possibly anti-U5RNP antibodies. Autoantibodies were not produced by 20 BALB/c mice of the same age and sex that were not injected with pristane. Thus, autoantibodies characteristic of lupus were induced in mice that are not usually considered to be genetically susceptible to the disease. The induction of autoantibodies associated with SLE by pristane may be relevant to understanding the role of abnormal cytokine production in autoantibody production and the pathogenesis of autoimmune disease. Furthermore, the induction of high titer autoantibodies by pristane dictates caution in the use of ascitic fluid as a source of monoclonal antibodies, since the polyclonal autoantibodies induced by pristane may copurify with the monoclonal antibody secreted by an injected hybridoma.

Intraperitoneal administration of pristane (2,6,10,14 tetramethylpentadecane) before the injection of hybridoma cells is a standard technique for obtaining ascitic fluid containing a high concentration of mAbs. In addition to its effects on hybridoma cell growth, pristane-induced alterations in cytokine production have been implicated in the pathogenesis of plasmacytomas (1–3) and erosive arthritis resembling rheumatoid arthritis (4, 5). While characterizing a slowly growing murine hybridoma secreting an IgM mAb, we observed that ascitic fluid from several pristane-primed BALB/c mice injected with hybridoma cells contained polyclonal IgG autoantibodies to Su, U1RNP, U2RNP, and/or Sm. Further investigation revealed that the autoantibodies were a consequence of pristane priming itself, and were unrelated to the hybridoma cells or their secreted monoclonal IgM. Thus, intraperitoneal injection of pristane induced lupus-like autoimmunity in a strain of mouse not usually thought to be prone to autoimmune disease.

Materials and Methods

Cell Lines. The K562 (human erythroleukemia) and L929 (murine fibroblast) cell lines were obtained from the American Type Culture Collection (ATCC; Rockville, MD) and maintained in RPMI 1640 or MEM, respectively, supplemented with 9% FCS, L-glutamine, and penicillin/streptomycin.

Sera and mAbs. Prototype human autoimmune sera containing anti-Su, anti-U1RNP, anti-Sm, or other specificities, were reported previously (6–8). Additional sera with anti-U1RNP/Sm antibodies were obtained from patients with systemic lupus erythematosus (SLE) followed at the University of North Carolina Hospitals (Chapel Hill, NC) or the Keio University Hospital (Tokyo, Japan). Murine mAbs 2.73 (anti-U1-70K) (9), and 9A9 (anti-U1-A and U2-B″) (10) were provided by Dr. Yoshihiko Takeda (Medical College of Georgia, Augusta, GA) and Dr. W.J. van Venrooij (University of Nijmegen, The Netherlands), respectively. mAbs Y2 (anti-Sm B′/B and D) (11), 22G12 (anti-Sm B′/B) (12), and 2G7 (anti-Sm-D) (13) were provided by Dr. Robert A. Eisenberg (University of North Carolina).

Pristane Priming. 6–8-wk-old female BALB/c ByJ mice were obtained from The Jackson Laboratory (Bar Harbor, ME) and maintained at our animal facility. Eleven mice, ages 4–5 mo, received a single intraperitoneal injection of 0.5 ml of pristane (Sigma Chemical Co., St. Louis, MO). Sera were collected every 4 wk from the tail vein. 20 age- and sex-matched BALB/c ByJ mice that were not injected with pristane served as controls.

Immunoprecipitation. Immunoprecipitation using cell extract from K562 or L929 cells was performed as described previously (7, 8). Briefly, the cells were labeled for 14 h with [^{35}S]methionine/cysteine (25 μCi/ml), lysed in 0.5 M NaCl NET/NP-40 buffer

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Immunology

Anti-nuclear antibody production and immune-complex glomerulonephritis in BALB/c mice treated with pristane

(systemic lupus erythematosus/lupus nephritis/autoantibodies/autoimmunity/small nuclear ribonucleoproteins)

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ABSTRACT **The pathogenesis of systemic lupus erythematosus is thought to be primarily under genetic control, with environmental factors playing a secondary role. However, it has been shown recently that intraperitoneal injection of pristane (2,6,10,14-tetramethylpentadecane) induces autoantibodies typical of lupus in BALB/c mice, a strain not usually considered to be genetically susceptible to the disease. In this study, the induction of autoimmune disease by pristane was investigated. BALB/c mice receiving pristane were tested for autoantibody production and histopathological evidence of glomerulonephritis. Six of 11 mice developed IgM anti-single-stranded DNA antibodies shortly after receiving pristane and 4 developed IgM anti-histone antibodies, but anti-double-stranded DNA antibodies were absent. IgG anti-DNA and anti-histone antibodies were absent. In contrast, the lupus-associated anti-nuclear ribonucleoprotein/Sm and anti-Su autoantibodies produced by these mice were predominantly IgG. In addition to autoantibodies, most of the mice developed significant proteinuria. Light microscopy of the kidney showed segmental or diffuse proliferative glomerulonephritis. Electron microscopy showed subepithelial and mesangial immune-complex deposits and epithelial foot process effacement. Immunofluorescence revealed striking glomerular deposition of IgM, IgG, and C3 with a mesangial or mesangiocapillary distribution. Thus, pristane induces immune-complex glomerulonephritis in association with autoantibodies typical of lupus in BALB/c mice. These data support the idea that lupus is produced by an interplay of genetic and environmental factors and that unlike the MRL or (NZB × W)F$_1$ mouse models, in which genetic susceptibility factors are of primary importance, environmental factors are of considerable importance in the autoimmune disease of pristane-treated BALB/c mice.**

Systemic lupus erythematosus (SLE) is a multisystem autoimmune disease characterized by anti-nuclear antibodies, immune-complex glomerulonephritis, arthritis, and other manifestations. Anti-double-stranded (ds) DNA autoantibodies are highly specific for SLE and may play a key role in the pathogenesis of immune-complex nephritis in lupus (1, 2). However, autoantibodies to glomerular antigens (3) and/or dysregulated cytokine production (4, 5) may also be involved. Human SLE is influenced strongly by major histocompatibility complex-linked and -nonlinked genes (6–8). Multiple genetic loci that accelerate the onset of autoantibody production and/or nephritis also have been identified in murine lupus models (9, 10). The importance of environmental factors in the pathogenesis of lupus is less clear. However, the role of environmental exposures in autoantibody production is underscored by the recent demonstration that intraperitoneal (i.p.) injection of pristane (2,6,10,14-tetramethylpentadecane) induces autoantibodies characteristic of SLE, including anti-Su and anti-nuclear ribonucleoprotein (nRNP)/Sm, in BALB/c mice, a strain not usually considered to be predisposed to autoimmunity (11). Titers of these autoantibodies are comparable to those found in MRL/*lpr* mice (12). The present data show that in addition to IgG anti-Su and anti-nRNP/Sm autoantibodies, pristane induces IgM anti-single-stranded (ss) DNA, anti-histone antibodies, and immune-complex glomerulonephritis in the "nonautoimmune" BALB/c strain.

MATERIALS AND METHODS

Administration of Pristane. Eleven 4- to 5-month-old and 10 2.5-month-old female BALB/c ByJ mice (The Jackson Laboratory) received a single i.p. injection of 0.5 ml of pristane (Sigma) (11). Sera were obtained at 1, 2, and 4 weeks and monthly thereafter. Urine samples were tested monthly for protein concentration by using Albustix reagent strips (Miles).

ELISAs for Anti-nRNP/Sm, Su, ssDNA, and Histone Autoantibodies. Anti-Su and anti-nRNP/Sm antigen-capture ELISAs were performed as described (12) with 1:250 diluted murine serum and alkaline phosphatase-conjugated goat anti-mouse IgG or IgM antibodies. Antibodies to heat-denatured calf thymus DNA (ssDNA, from Sigma) and to total calf thymus histones (United States Biochemical) were detected by ELISAs as described (13, 14) with a 1:500 dilution of murine sera and alkaline phosphatase-conjugated goat anti-mouse IgG or IgM antibodies.

Light and Electron Microscopy. Six months after receiving pristane, BALB/c and control mice not receiving pristane were anesthetized and fixed by perfusion through the left ventricle (15). The inferior vena cava was nicked below the renal veins, and 20 ml of saline was perfused slowly, followed by 10 ml of 2.5% (vol/vol) glutaraldehyde in 0.1 M sodium cacodylate, pH 7.4/4 mM $CaCl_2$. For light microscopy, 3-μm sections of aldehyde-fixed renal cortex were stained with hematoxylin and eosin as described (16). For electron microscopy, aldehyde-fixed renal tissue was postfixed with osmium tetroxide, dehydrated in ethanol, and embedded in Epon 812. Thin sections (60 nm) were stained with lead citrate and uranyl acetate and examined by electron microscopy (16).

Immunofluorescence. Kidneys were excised from pristane-primed or control mice and snap-frozen in isopentane chilled in liquid N_2. Cryostat sections (4 μm) were stained with a 1:40 dilution of fluorescein isothiocyanate (FITC) or rhodamine-conjugated goat anti-mouse IgM, IgG, IgG1, IgG2a, IgG2b, or IgG3 antibodies (Southern Biotechnology Associates) or with FITC-conjugated rabbit anti-mouse C3 antiserum (Organon

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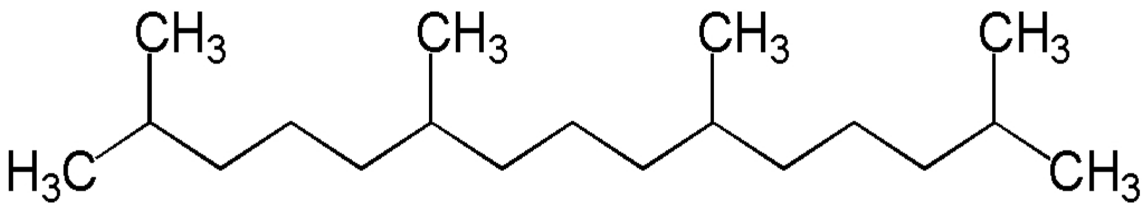
Abbreviations: nRNP, nuclear ribonucleoprotein; SLE, systemic lupus erythematosus; ds, double stranded; ss, single stranded; IL, interleukin.
‡To whom reprint requests should be addressed.

Pristane (2,6,10,14-tetramethylpentadecane, TMPD)

- C19 isoprenoid alkane ($C_{19}H_{40}$)
- A component of mineral oil



- A single intraperitoneal injection (ip) induces plasmacytoma and chronic destructive arthritis in mice
- **Induction of lupus-like autoimmune syndrome**

Antinuclear antibodies, anti-Sm/U1RNP, ribosomal P, dsDNA, immune complex glomerulonephritis

(Satoh M and Reeves WH, J Exp Med 1994, Satoh M et al., Proc Natl Acad Sci USA 1995)

Norwegian School of Veterinary Science

Aktuell publikasjon

Vaksinering av oppdrettsfisk – sjukdomsvern med attåtsmak

Vaksinering av oppdrettslaks er viktig for å unngå tapsbringande infeksjonssjukdomar. Utvikling av effektive vaksiner og vaksinestrategiar har redusert antibiotikabruken i oppdrettsnæringa i Noreg frå eit uakseptabelt høgt nivå for 15 år sidan til eit i dag som er klart lågare enn i liknande animalske produksjonar. Samstundes har produksjonen av oppdrettsfisk mangedobla seg. Dei vanlegaste nytta oljebaserte vaksinane vert formulert som vatn-i-olje, eller såkalla "incomplete Freund's adjuvans". Ulike bakterie- og virusantigen vert sette til formuleringa. Vaksinen vert injisert intraperitonealt i månadane før eller under smoltifiseringa. Her gjev han ein depoteffekt som initerar og oppretthald ein langvarig immunitet gjennom resten av produksjonssyklusen.

ORIGINALREFERANSER:

Koppang EO, Bjerkås E, Bjerkås I, Sveier H, Hordvik I. Vaccination induces major histocompatibility complex class II expression in the Atlantic salmon eye. Scand J Immunol 2003; 58 (1): 9-14.

Koppang EO, Haugarvoll E, Hordvik I, Poppe TT, Bjerkås I. Granulomatous uveitis associated with vaccination in the Atlantic salmon. Vet Pathol 2004; 41 (2): 122-30.

Koppang EO, Haugarvoll E, Hordvik I, Aune L, Poppe TT. Vaccine-associated granulomatous inflammation and melanin accumulation in Atlantic salmon, *Salmo salar* L., white muscle. J Fish Dis 2005; 28 (1): 13-22.

Tilhøve kring vaksiner i bruk

Vaksinane sine immunogene eigenskapar fylgjer av betennelsesreaksjonar framkalla av olja i vaksinen og samanblanda virus- og bakterieantigen, som skal gje eit spesifikt vern.

Det er publisert ei rekkje artiklar som tek for seg opptak og distribusjon av bakterieantigen hjå laks etter vaksinering. Det er særleg bakterielt lipopolysakkarid (LPS) og A-lag frå *Aeromonas salmonicida* subspecies *salmonicida* som har vorte undersøkt. Resultata viser at i tillegg til å persistere ved injeksjonslocus, vert antigen i hovudsak distribuert til hovudnyre (beinfisk sitt permanente pronephros) og milt. Ein reknar med at antigena vert prosessert og presentert for T-celler i desse lymfatiske organa, slik at eit spesifikk immunrespons kan initierast.

Når det gjeld opptaket og distribusjonen av oljekomponentar i vaksinen, er publiserte undersøkingar hjå fisk fråverande. Olja som vert nytta er i stor utstrekning ei blanding kvite mineraloljer, som er biprodukt frå petroleumsindustrien (1). Det er samansette av metta hydrokarbonkjeder, anten rette- og greina kjeder eller ringstrukturar, av varierande lengder som er svært resistente mot biokjemisk nedbryting. Den relative konsentrasjonen av metta ringstrukturar, rette- og greina kjeder avgjer olja sine biologiske eigenskapar. Korte hydrokarbon, med ei kjedelengdeblanding frå 15 til 25 karbonatom, har synt seg å vere meir potente enn lange hydrokarbon, med ei kjedelengdeblanding frå 25 til 50 karbonatom, til å initiere betennelsesresponsar og verke som adjuvans. For å finne informasjon om distribusjonen og metabolismen til mineraloljene må ein konsultere litteratur publisert etter undersøkingar på varmblodige dyr. I forsøk på rotter vart radiomerka hydrokarbon injisert og distribusjonen undersøkt. Sidan hydrokarbona er feittløyselege, var det ikkje uventa at dei over tid vart lokalisert og akkumulert i lever og feittvev (2). Nedbrytninga var svært langsam. Frå forsøk hjå høns er det publisert interessante studiar der hydrokarbon etter vaksinering vart analysert ved hjelp av gasskromatografi/massespektrometri. Her vart det synt at dyra kvitta seg med hydrokarbona gjennom egg. Dersom hønene ikkje verpa, var oli

Presisering om vaksinering av oppdrettsfisk

I NVT nr. 7, 2005 har professor og fagleg medarbeidar i NVT Øystein Evensen eit innlegg under emnet Debatt, med tittelen "*Vaksinering av oppdrettsfisk*", der han kommenterer artikkelen vår "*Vaksinering av oppdrettsfisk – sjukdomsvern med attåtsmak*" i NVT nr. 4, 2005. Me takkar for interessa, men ser oss nøyt til å oppklara eit par mistydingar.

Me vil for det fyrste påpeike at hovudpoenget vårt i innlegget i NVT nr. 4 er at olje-emulsjonar (1, 2) til bruk i adjuvansar bør undersøkjast for om dei kan distribuerast systemisk i atlantisk laks etter intraperitonal injisering.

Dette spørsmålet er grunna i mange studiar på pattedyr der det er funne patologiske forandringar i ulike vev etter eksponering for ulike hydrokarbon (3). Studiar frå desse eksperimenta vart referert då det er manglande kunnskap på dette området innan fisk, noko Evensen også stadfestar i innlegget sitt i NVT nr. 7, 2005. Det er også kjent hjå dyr og menneske at absorberte hydrokarbon primært vert distribuert via lymfekarsystemet til lymfeknutar og lever, sekundært til feittvev (4).

Undersøkingane våre publisert i Journal of Fish Diseases (JFD), og omtala i NVT nr. 4, viste patologiske funn som kan gje indikasjon på at den ovanfor omtala distribusjonen også kan vera tilfellet hjå fisk. Men me presiserar i JFD-publikasjonen at kjemiske studiar må utførast for å vera sikre på dette.

Sidan dei publiserte funna kan gje tilstrekkeleg mistanke om at adjuvansen kan vera distribuert i kroppen også på fisk, må det vurderast å utføre farmakokinetiske studiar med omsyn på adjuvansen (åleine eller i kombinasjon med antigenet) (5).

Poenget er såleis at me ikkje berre har basert innlegget vårt i NVT nr. 4, 2005 på funna som er publisert i JFD slik Evensen hevdar i kommentaren sin. Dette er det også gjort greie for med ei rekkje referansar til andre publikasjonar.

Erlend Haugarvoll og Erling Olaf Koppang kommer her med en presisering av innlegget de skrev i NVT nr. 4 om vaksinering av oppdrettsfisk.

Referanser

1. Hoc K. Adjuvans i vaksiner til dyr. Norsk Veterinærtidsskrift 1996; 108: 227-33.
2. Kuroda Y, Nacionales DC, Akaogi J, Reeves WH, Satoh M. Autoimmunity induced by adjuvant hydrocarbon oil components of vaccine. Biomed Pharmacother 2004; 58: 325-37.
3. Haugarvoll E, Koppang EO. Vaksinering av oppdrettsfisk – sjukdomsvern med attåtsmak. Norsk Veterinærtidsskrift 2005; 117: 286-90.
4. EMEA/CVMP/069/95-FINAL. Committee for veterinary medicinal products mineral hydrocarbons summary report. http://www.emea.eu.int/pdfs/vet/mrls/006995en.pdf. 1995.
5. EMEA/CPMP/VEG/17/03/2004v/Consultation. Guideline on adjuvants in vaccines. http://www.emea.eu.int/pdfs/human/vwp/301703en.pdf. 2004.

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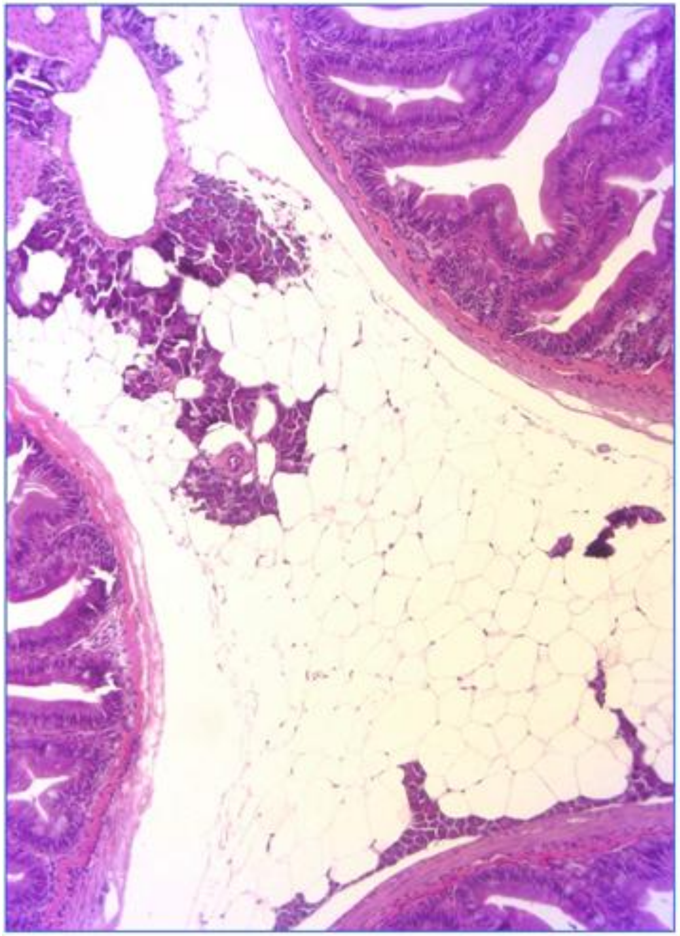
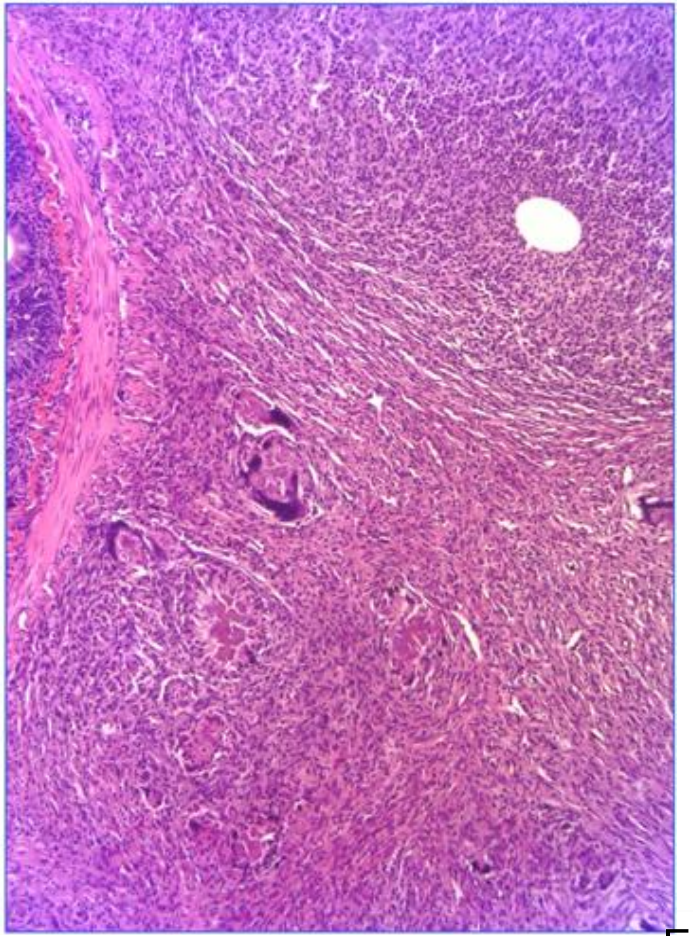
Peritoneal Tissue from Unvaccinated vs. Vaccinated Salmon

Unvaccinated



Vaccinated

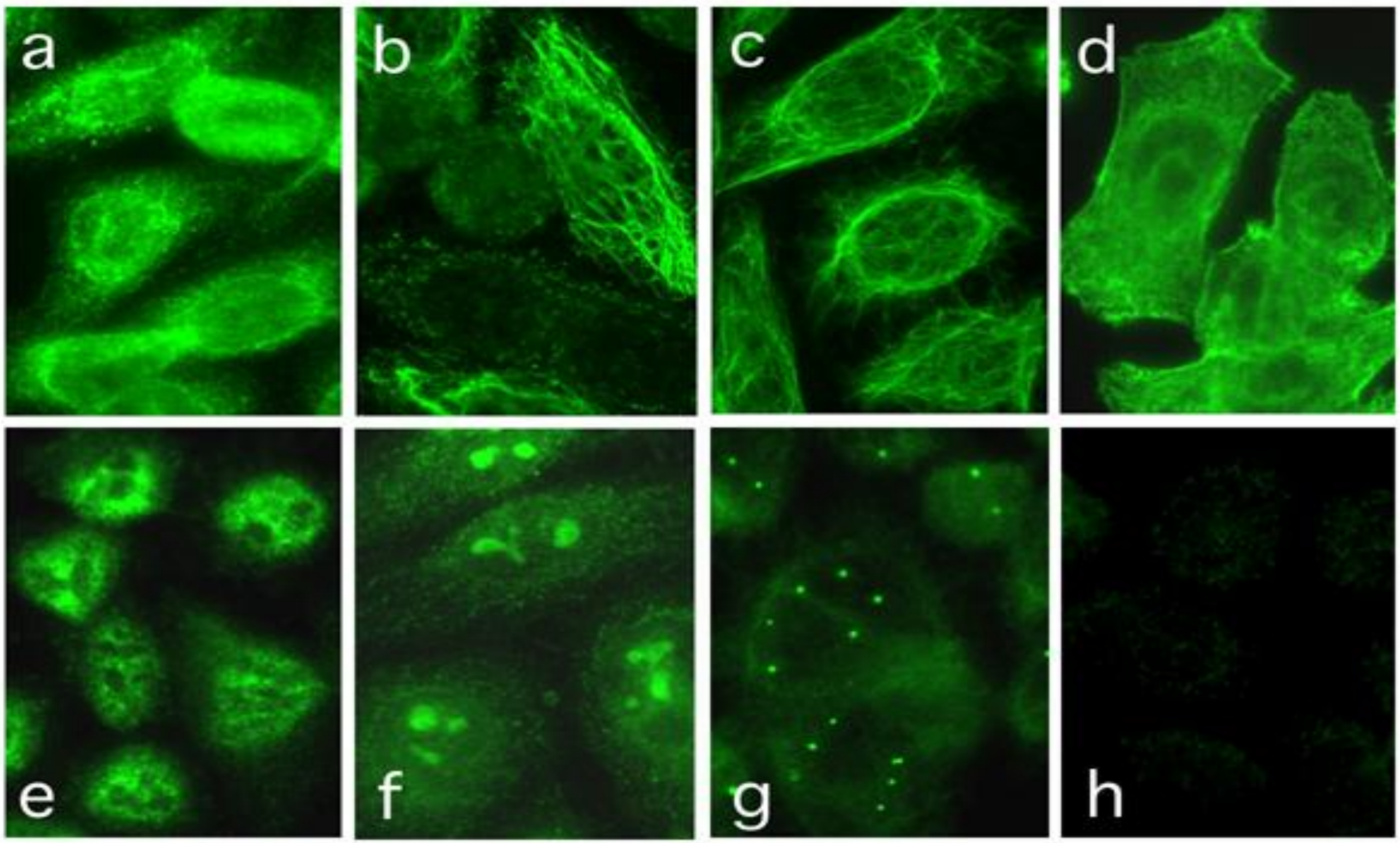


EO Koppang

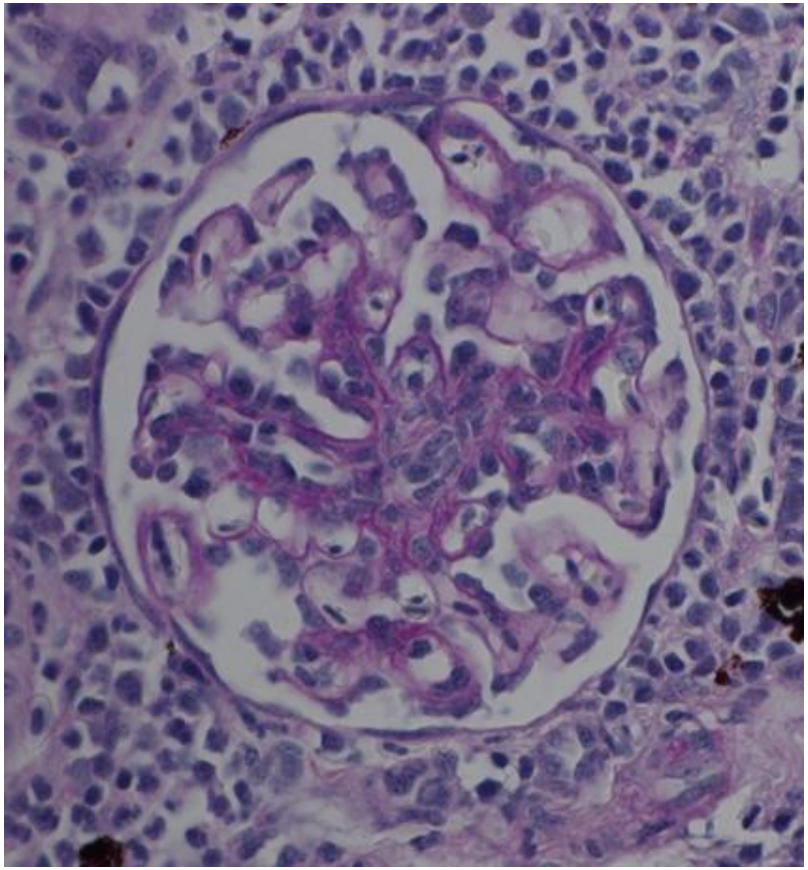
Background of Groups of Salmon

	n		Vaccine	Adjuvant	Vaccination - sample
A	10	Experimental	No	No	NA
B	10	Experimental	Yes	Mineral oil	2 y
C	20	Farmed (cave)	No	No	NA
D	55	Farmed	Yes	Mineral oil	1.5 y
E	20	Farmed	Yes	Mineral oil	2 y
F	19	Farmed	Yes	Mineral oil	2.3 y
G	20	Farmed	Yes	Mineral oil	1.3 y
H	20	Farmed	Yes	Animal/veg oil	1.3 y
I	20	Farmed	Yes	Mineral oil	1.5 y
J	11	Wild	No	No	NA

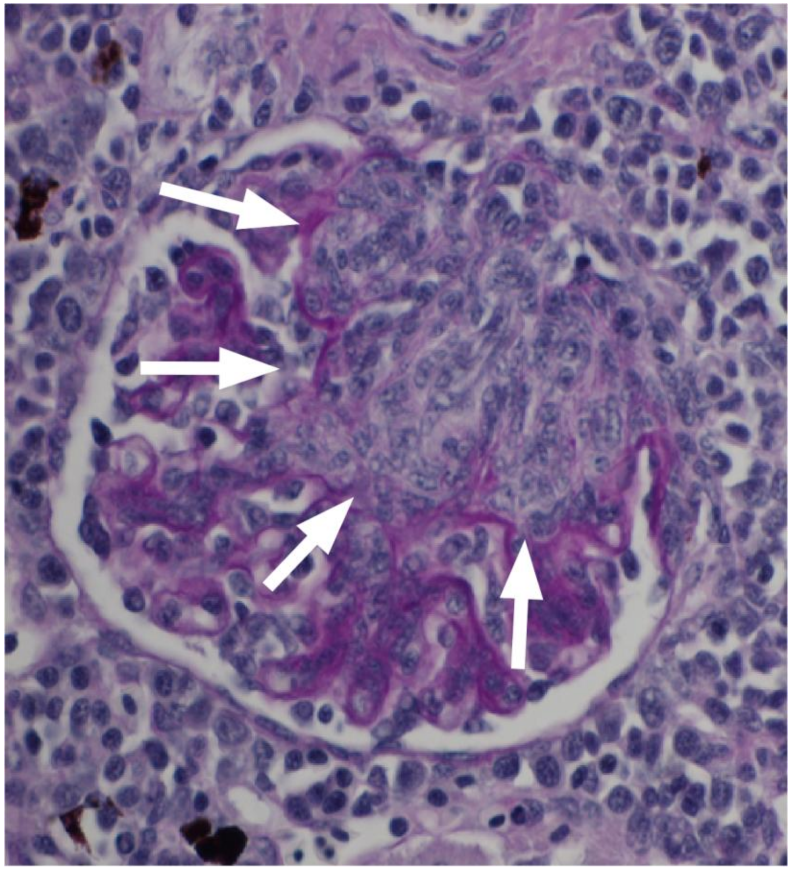
Autoantibodies in Vaccinated Salmon by Immunofluorescence



Focal Endocapillary Proliferation in Glomeruli from Vaccinated Salmon (PAS)



Unvaccinated

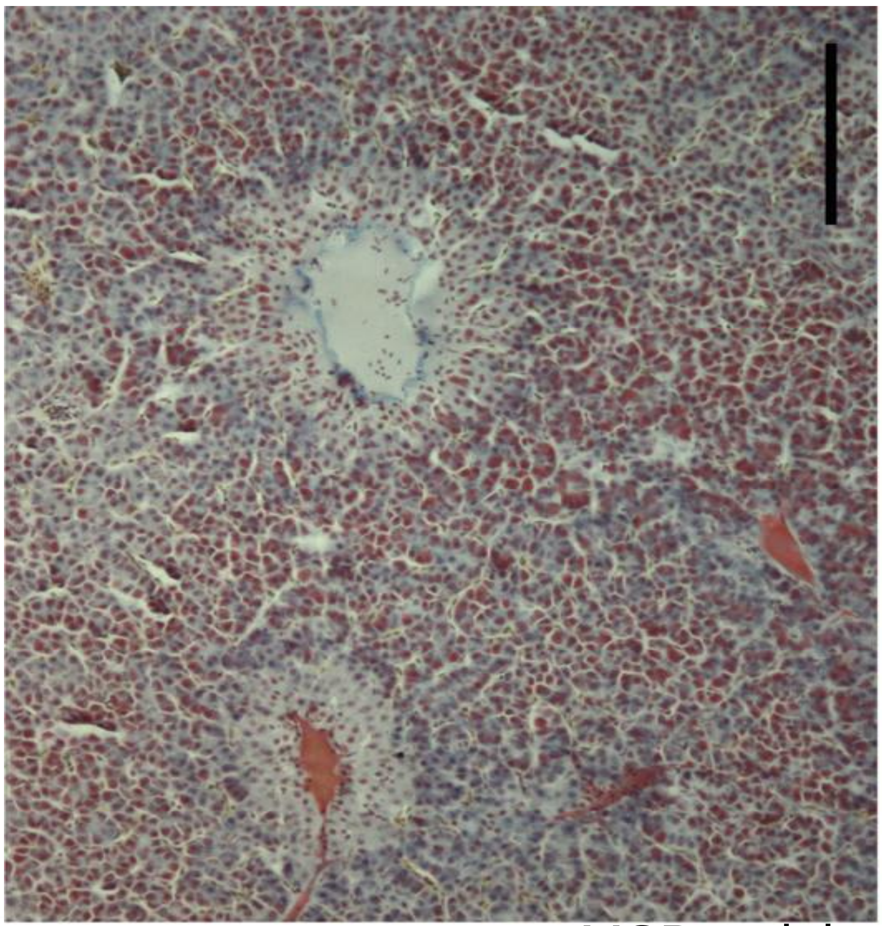


Vaccinated

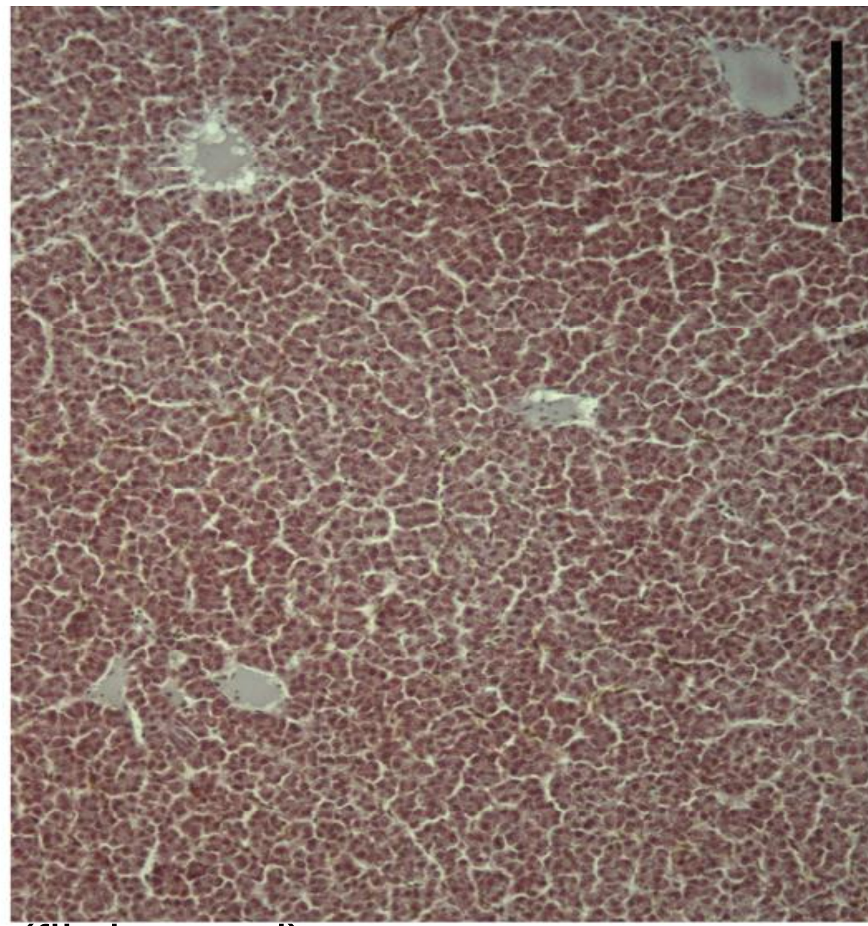
Norwegian School of Veterinary Science

Levertromber(12 måneder)

Vaksinert

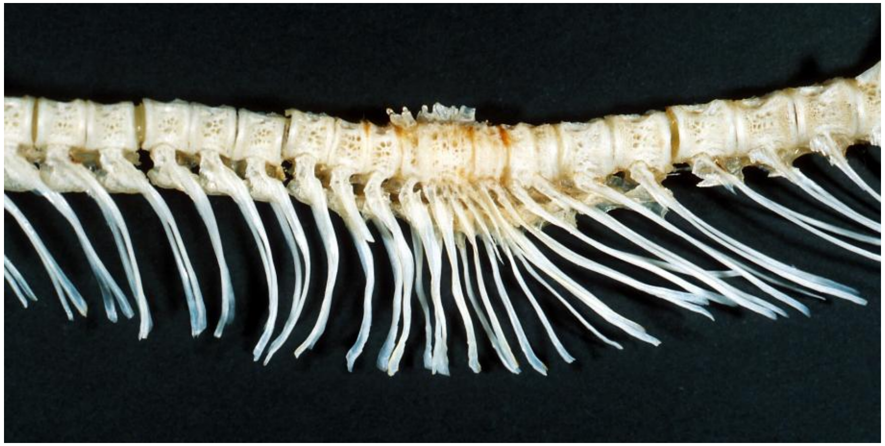


Uvaksinert



MSB staining (fibrin = red)

Mus 7 måneder etter pristan-injeksjon



Spine deformity in Atlantic salmon

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Manifestations of systemic autoimmunity in vaccinated salmon

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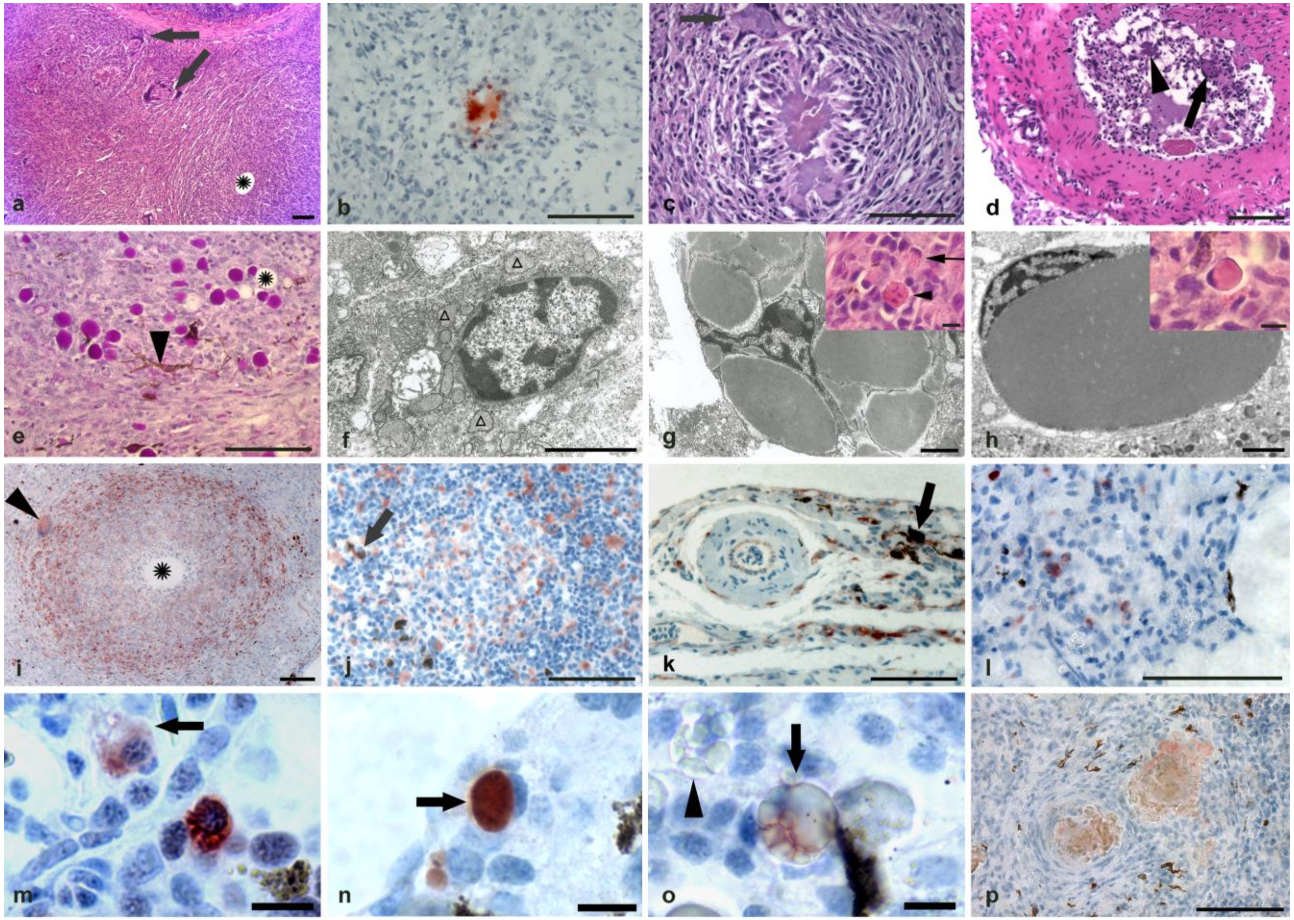
ABSTRACT

The development of systemic autoimmunity may result as an undesired side-effect following vaccination, and this condition was recently shown to occur in farmed salmon (*Salmo salar*). Several of previously reported side-effects following vaccination of fish should therefore be reviewed in the light of this condition. Here, organs and pathological changes in three separate groups of fish severely affected by vaccination were investigated by different morphological methods ($n=84$). Granulomas or microgranulomas were observed at the injection site and in several organs. Mott cells were observed in all tissues examined. Pannus-like changes with lymphocyte infiltrates were observed in spines. In conclusion, the reactions following vaccination were of a systemic nature that may be explained by a pathogenetic mechanism caused by systemic autoimmunity.

1. Introduction

The successful prevention of a series of different infectious diseases in man and animals may be obtained by vaccines, but frequently, concerns are raised regarding their safety. Undesired side-effects following vaccine injections include fever, rash, flu-like symptoms, lymphadenopathy and swelling at the site of inoculation, but long-term effects such as hypersensitivity, induction of infection and autoimmunity are considered rare [1]. In general, the lack of a standardised vaccine safety assessment system is striking [2]. According to The European Agency for the Evaluation of

may develop into autoimmune diseases [1]. In addition, the dosage:body-weight ratio in salmon vaccination is considerably higher than that of mammals [7]. All these factors argue for higher frequency and severity of vaccine-induced side-effects in salmon compared to farmed mammals and humans. IL-1, a cytokine that plays a key role in the pathogenesis of systemic autoimmunity in adjuvant-injected mice [6], is induced in vaccinated salmon [8]. Recipient fish may over time develop mild to severe pathological changes as a consequence of vaccination, and observed adverse reactions include impaired growth rate, decreased carcass quality, spinal deformities, uveitis and inflammatory reactions in the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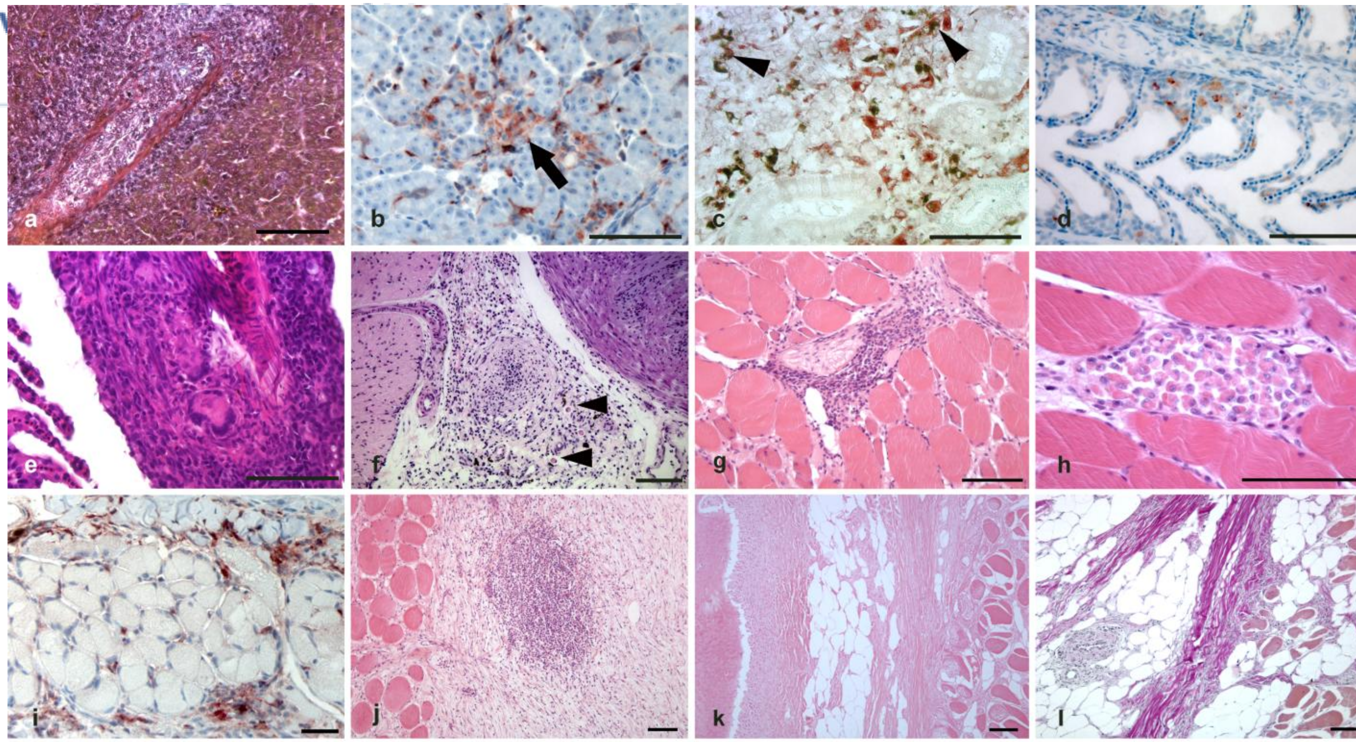
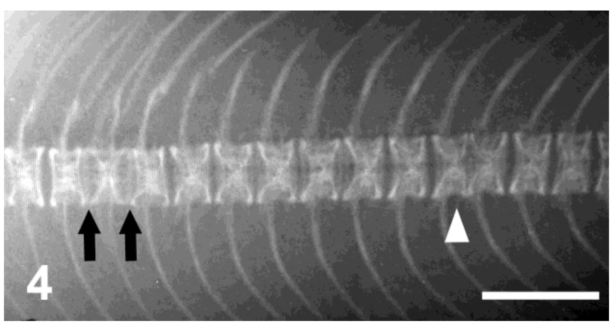
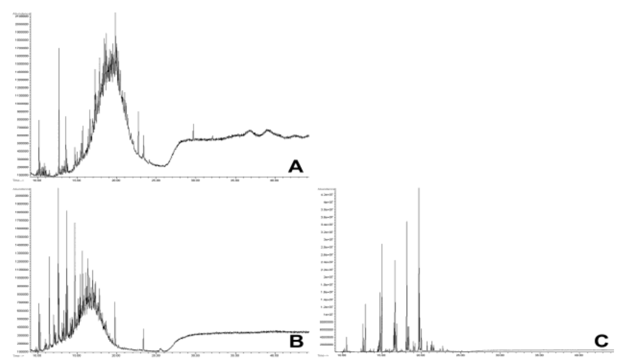


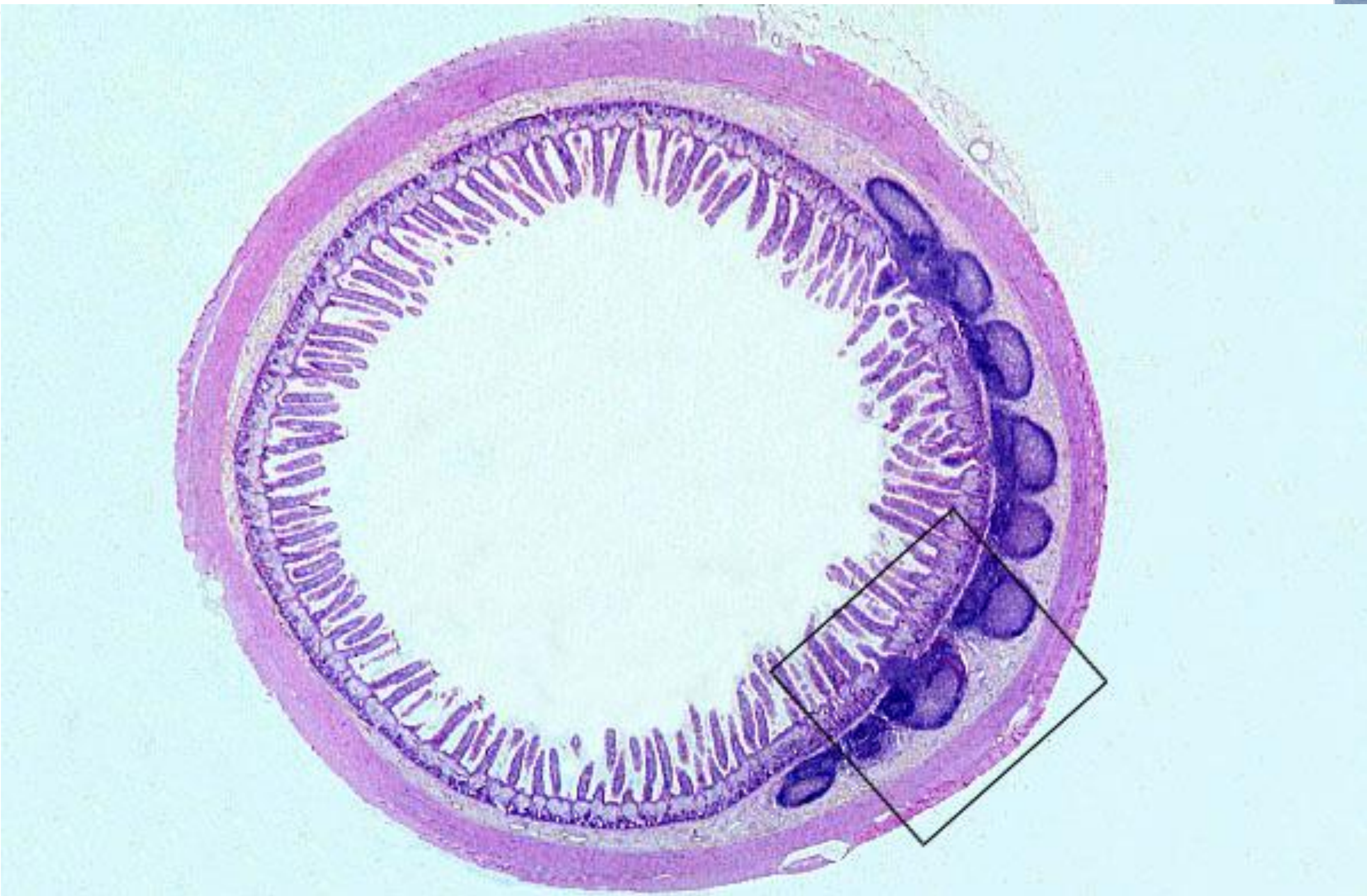


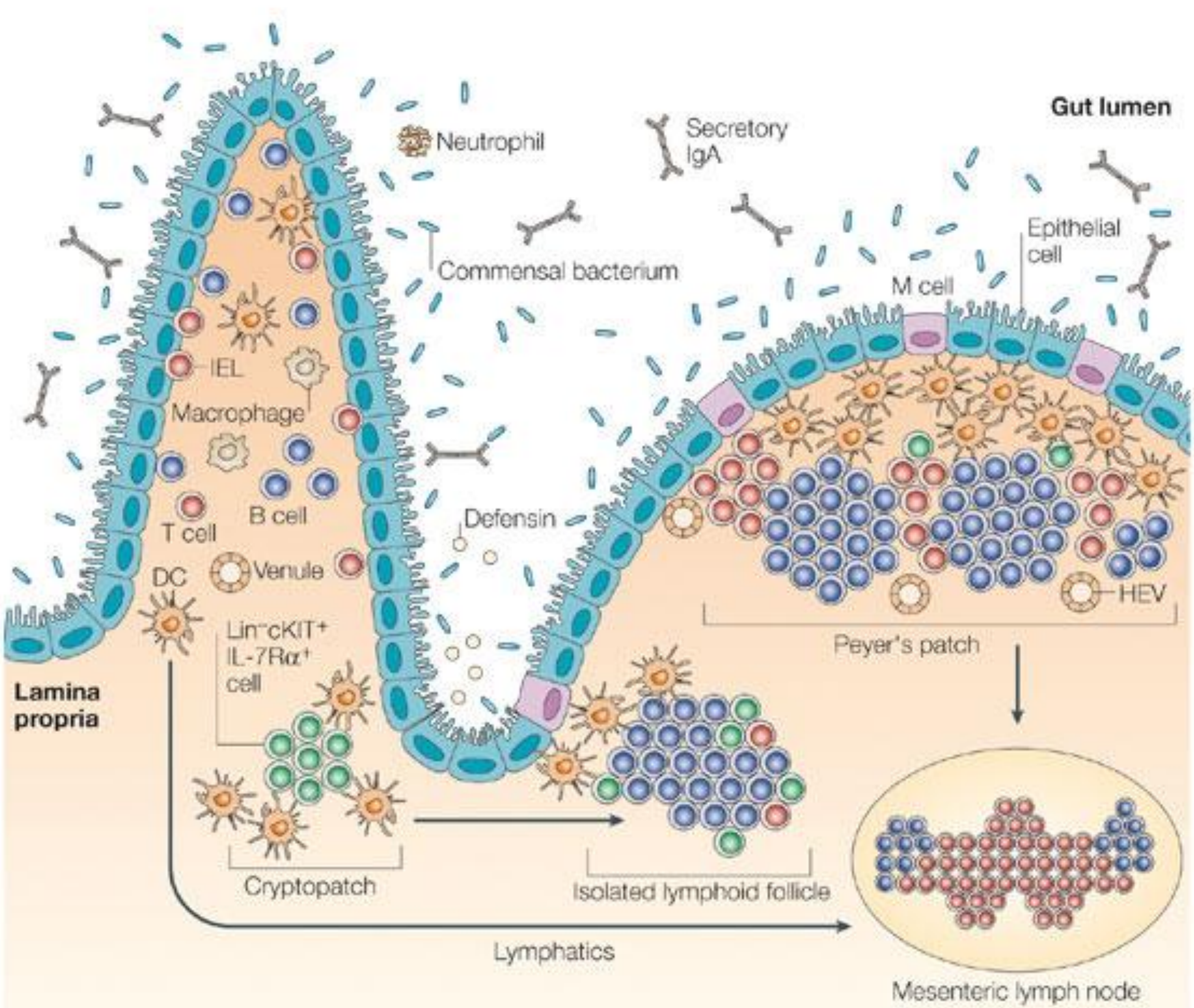


Kan dette forklare observert assosiasjon? (Berg et al. 2006 og Aunsmo et al. 2008)

Fremtidens vaksine

- Billig
- Uten bieffekter
- Lett å administrere
- Sikker – gir god beskyttelse
- Utgjør ingen risiko for konsument
- Kombinasjoner av løsninger?





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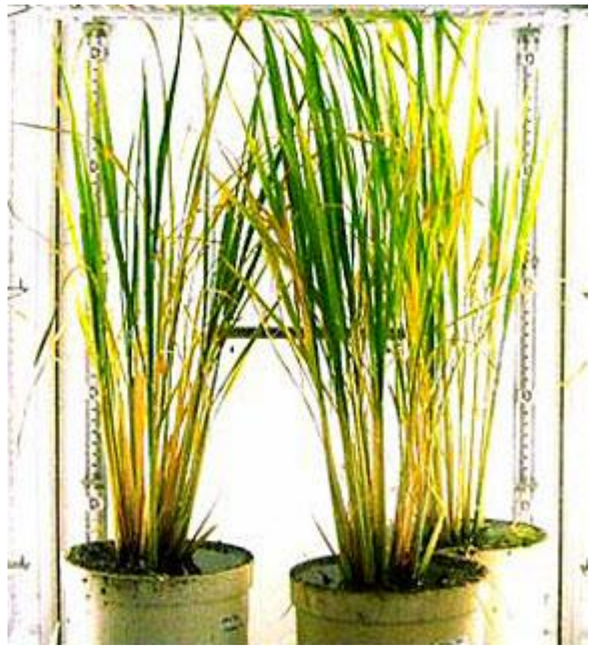
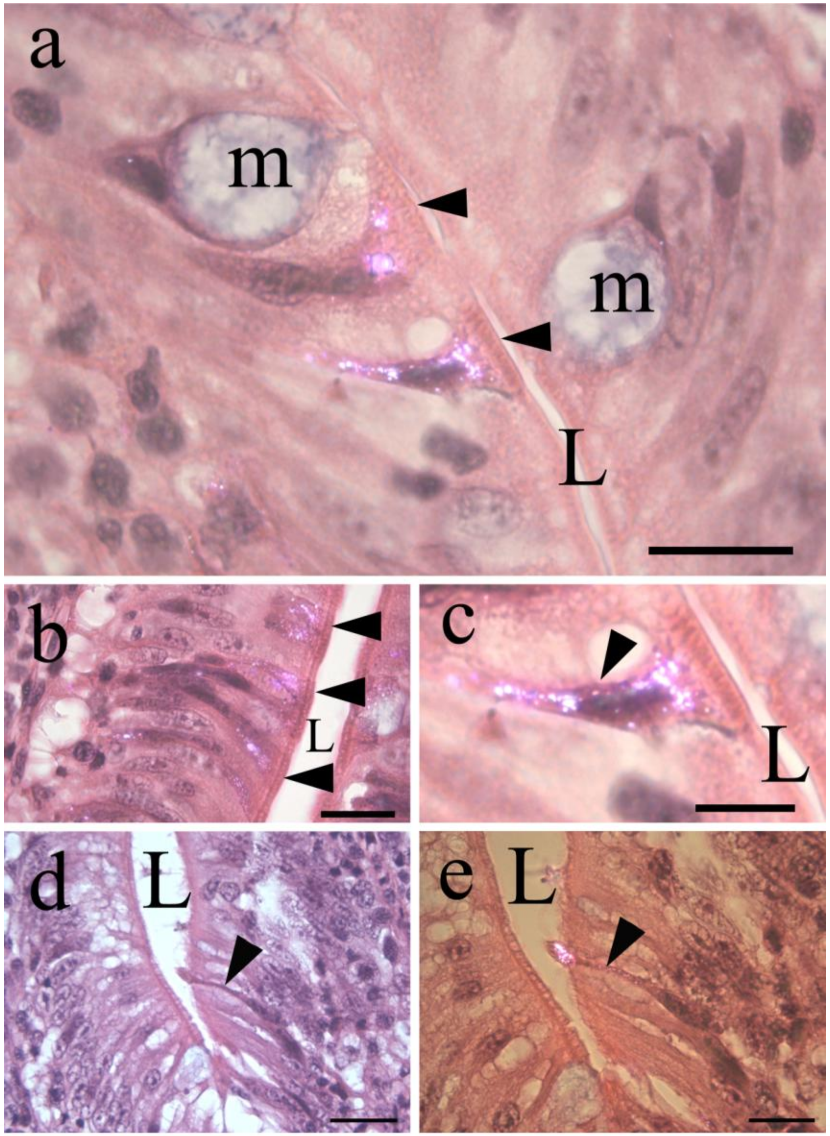
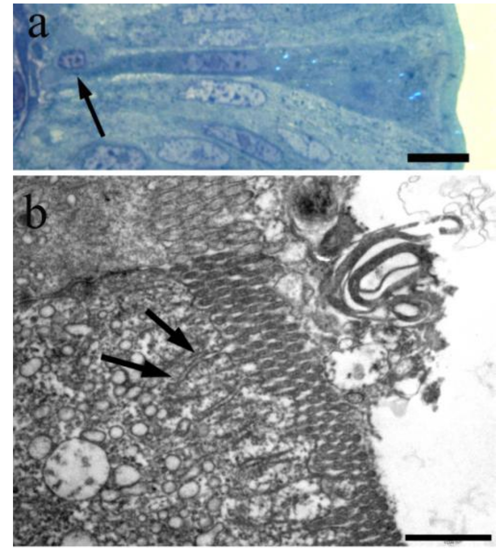
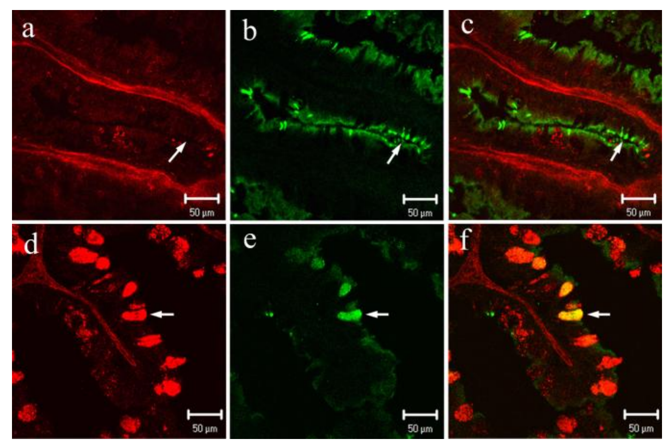


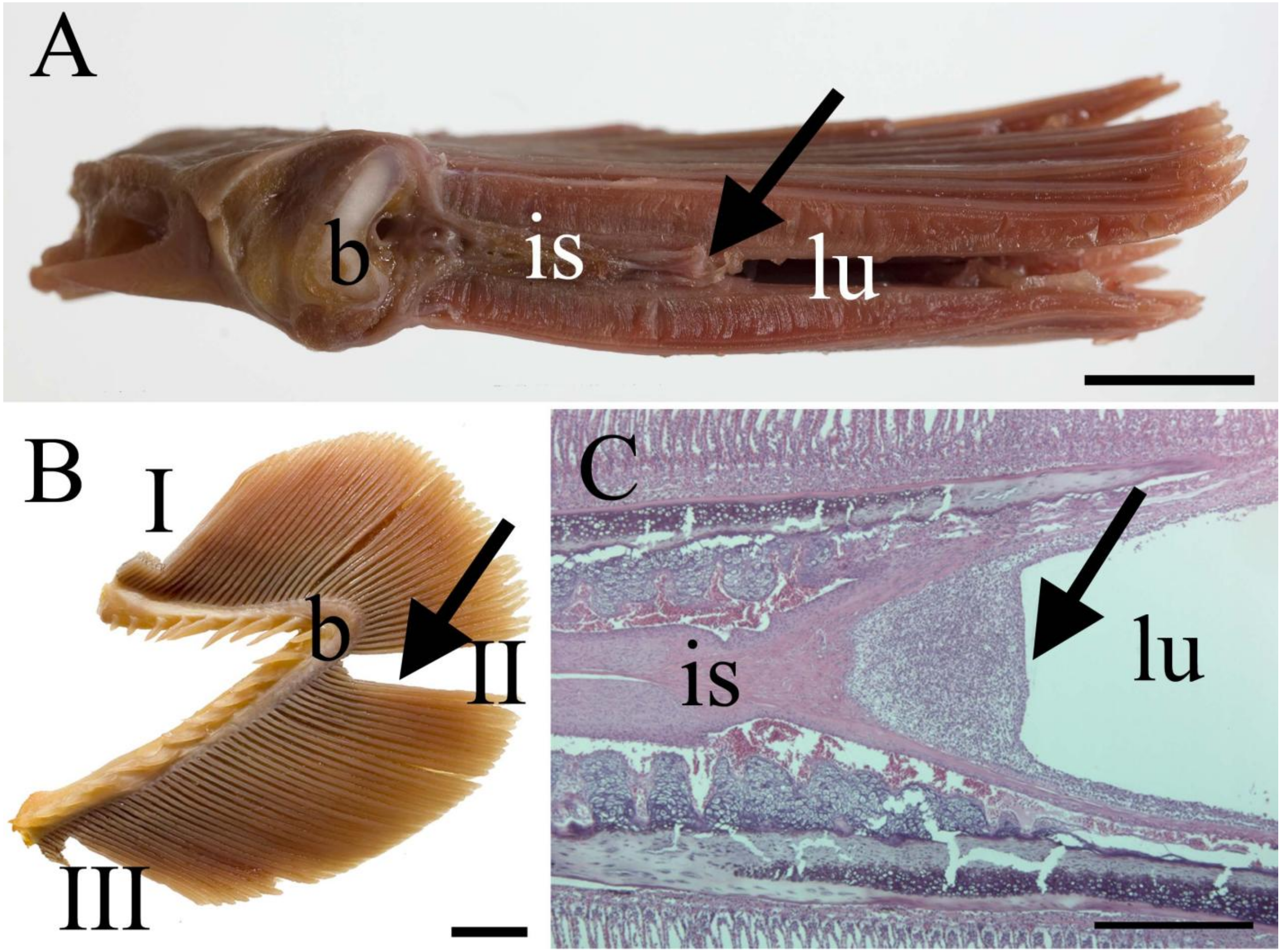
Professor Hiroshi Kiyono, Universitetet i Tokyo: Vaksiner i ris!







Fuglem et al., Dev. Comp. Immunol. 2010; 34: 768-774.



Koppang et al., Journal of Anatomy, 2010