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Tissue chambers — a useful model for in vivo studies of cytokine production in the pig

Eva Wattrang^{a,*}, Per Wallgren^b, Lisbeth Fuxler^a,
Marie Lindersson^a, Caroline Fossum^a

^a Department of Veterinary Microbiology, Division of Immunology, Swedish University of Agricultural Sciences, BMC, Box 588, S-751 23 Uppsala, Sweden

^b National Veterinary Institute, Box 7073, S-750 07 Uppsala, Sweden

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Abstract

An in vivo tissue chamber model was developed to enable studies of local cytokine production and cellular events during inflammatory and immune reactions in the pig. Tissue chambers made of sialistic rubber tubing were surgically implanted in the subcutaneous tissue and samples of tissue chamber fluid (TCF) and inflammatory cells were collected by aspiration with a syringe. To evaluate the model for local cytokine production, two cytokine inducers, polyribinosinic-polyribocytidylic acid (poly I:C) and fixed Aujeszky's disease virus infected PK15 cells (ADV-PK15), were injected into the tissue chambers and samples of TCF were collected 0, 4, 8, 12, 24 and 48 h post injection.

Poly I:C injections induced local production of interferon- α (IFN- α) as well as tumor necrosis factor (TNF) in the TCF but kinetic differences in the production of the cytokines were noted. Poly I:C also induced an increase in cell numbers in the TCF, mainly due to increased neutrophil numbers. Injections of ADV-PK15 induced local IFN- α production in the TCF as long as the pigs were serologically negative to ADV.

Immunofluorescence and in situ hybridization techniques could be applied for characterization of TCF cells. Moreover, cells recovered from the tissue chambers were viable and could be used in functional in vitro tests. Taken together, this tissue chamber model could prove very useful in in

* Corresponding author at: Immunology (V), BMC, Box 588, S-751 23 Uppsala, Sweden. Tel: +46-18174534; fax: +46-18174382.

vivo studies of inflammatory/immune responses and cytokine production in the pig. © 1997 Elsevier Science B.V.

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1. Introduction

Cytokines are crucial for initiation of immune reactions as well as for modulation of the ensuing immune response. The availability of reagents for detection of cytokines has increased considerably during recent years, also for domestic species such as the pig (Murtaugh, 1994, Myers and Murtaugh, 1995). Porcine cytokines and their regulation are however not solely of interest in the field of veterinary medicine, since swine are increasingly used in models of human disease (Misfeldt and Grimm, 1994) and are also potential donors for xenotransplantation (Sachs, 1994). The production of several porcine cytokines can be induced and studied *in vitro* (La Bonnardière et al., 1994; Murtaugh, 1994) but results obtained under such conditions are not necessarily reflecting the *in vivo* situation. Thus, reliable and practical models for *in vivo* studies of porcine cytokines would be beneficial for a better understanding of the initiation and regulation of immune responses.

Several approaches to study local immune responses *in vivo* such as cannulation of lymphatic vessels (Miyasaka and Trnka, 1986); vacuum blisters (Wisselink et al., 1993) and various types of tissue chambers (Higgins et al., 1987; Vaudaux et al., 1992; Dawson et al., 1993), have been made. In the tissue chamber models, perforated chambers create artificial, fluid filled, compartments in e.g. the subcutaneous tissue (Clarke, 1989). A variety of such chambers have been used in several species, most of the models being developed for pharmacological studies, but some types have also been used for other purposes. For instance, tissue chambers have been used to monitor cellular and cytokine responses to infections and other inflammatory stimuli in mice (Dawson et al., 1993); guinea-pigs (Vaudaux et al., 1992); and horses (Higgins et al., 1987).

In the present study, partially perforated chambers made of sialistic rubber tubing were implanted subcutaneously in pigs. This particular design of tissue chambers was adopted from a bovine model used for pharmacokinetic studies of antibacterials (Bengtsson et al., 1984; Bengtsson, 1990). Similar chambers have also been used in rabbits, dogs, rats and sheep (Calnan et al., 1972; Chisholm et al., 1973; Piercy, 1978). The fluid in such chambers is considered a product of the young capillary network of the granulation tissue invading the chamber and communicates with the general interstitial fluid compartment. The diffusion rate out of and into the chamber fluid is rapid for small molecules, such as sodium, while it is slower for larger molecules, such as albumin (Bergan, 1981).

To evaluate the present porcine model two types of cytokine inducers were used, a soluble and a particulate. Both inducers, i.e. polyribinosinic-polyribocytidylic acid (poly I:C) and fixed Aujeszky's disease virus (ADV) infected cells, have earlier been used for *in vivo* interferon (IFN) induction in pigs (Vengris and Maré, 1972; Loewen and

Derbyshire, 1986; Artursson et al., 1995). In addition to monitoring the induced, short-term cytokine and cellular responses in the tissue chamber fluid, the model was also evaluated for functionality and endurance.

2. Materials and methods

2.1. *Experimental animals*

Nine SPF-barrows were included in the study which was approved by the Ethical Committee for Animal Experiments, Uppsala, Sweden. The animals were housed under SPF-conditions with two pigs per pen at the National Veterinary Institute, Uppsala, Sweden. The experiment was divided in two sets with four pigs in the first and five pigs in the second set. The animals were 10 weeks of age when they were operated on, and the study was ended when they were 8 to 9 months of age.

2.2. *Tissue chambers*

The tissue chambers used were made out of sialistic rubber tubing (AB Labassco, Partille, Sweden), sealed in both ends with sialistic rubber plugs (AB Labassco). Two sizes of chambers were used, the smaller was 60 mm in length with an inner diameter of 10 mm, while the larger was 70 mm long with an inner diameter of 12 mm. About 35 to 45 holes, 2 mm in diameter, were punched in the tubing at the ends of each chamber, leaving the midsections (35 mm in length for the smaller size and 40 mm for the larger size) of the tissue chambers unperforated.

2.3. *Surgical technique*

The pigs were anaesthetized with azaperone (2 mg kg⁻¹ body weight i.m.; Stresnil*, Jensen Pharmaceutica, Beerse, Belgium) in combination with methomidate (10 mg kg⁻¹ body weight i.p.; Hypnodil*, Jensen Pharmaceutica) and the operation sites were shaved and washed according to routine surgical procedures. The tissue chambers were inserted through a skin incision, where a subcutaneous tunnel was created by blunt dissection using a pair of Pean's forceps. The skin wound was stitched with interrupted sutures, using polyamide C (1 USP; Suturamid*, Ethicon, Nordsterstedt, Germany) and the sutures were removed 10 days post surgery.

In the first set of pigs two chambers were implanted per pig, each of two pigs had one chamber on the lateral neck and one in the flank region and two pigs had one in each flank. In the second set of pigs four chambers were implanted in each pig, one on each side of the neck and in each flank. The tissue chambers used in the neck region were of the smaller size in the first set of pigs, while in the second set all tissue chambers used were of the larger size.

2.4. *Bacteriological and histological examination*

Samples for bacteriology were enriched and cultivated under aerobic as well as anaerobic conditions. Isolated bacteria were identified according to routine procedures at the division of Bacteriology, National Veterinary Institute (Uppsala, Sweden).

At autopsy, 5 or 7 months post surgery, samples from tissue chambers and tissue surrounding these as well as lymph nodes were collected. The samples were fixed with formalin, sectioned and hematoxylin-cosin stained.

2.5. Collection of tissue chamber fluid (TCF)

The pigs were immobilized by snout restraining and samples of TCF were collected by puncturing the skin and the midsection of the chamber wall with a 0.6 mm diameter needle and aspirating with a sterile syringe. The TCF was subsequently transferred to sterile blood collecting tubes with 15 mg EDTA K₃ as additive (B-D Vacutainer, Meylan Cedex, France). The total number of nucleated cells per ml TCF was determined in samples stained with Türk's solution using a Fuchs-Rosenthal hemacytometer (KEBO AB, Stockholm, Sweden) before the samples were centrifuged and the cell free supernatant collected and stored at -20°C until further analysis. The identity and functional capacity of cells recovered from TCF was examined as described below.

2.6. Experimental design

If not otherwise indicated, one tissue chamber was injected with a cytokine inducer and another chamber was injected with an equal amount of sterile, pyrogen free physiological saline solution (NaCl; Baxter Medical AB, Kista, Sweden) for each treated animal. Injections were made after collection of the first TCF sample (0), whereafter TCF samples were collected at 4, 8, 12, 24 and 48 h post-injection, if not otherwise indicated in the text. In most experiments an untreated animal was included, where samples of TCF were collected from one chamber at the same time points as for the treated animals.

2.7. Preparation of cytokine inducers

Poly I:C (double strand, sterile; Pharmacia, Uppsala, Sweden) was dissolved in NaCl according to the manufacturer's instructions and 2.4 mg of poly I:C in a volume of 1.2 ml was injected per chamber.

ADV infected porcine kidney (PK-15; Flow Laboratories, Irvine, Scotland, UK) cells (ADV-PK15) were prepared according to Artursson et al. (1995), with slight modifications. In brief, confluent monolayers of PK15 cells were trypsinized and transferred to siliconized tubes ($1 \times 10^6 \text{ ml}^{-1}$ of complete Dulbecco's modification of Eagle's medium; DMEM with 10% FCS) and thereafter infected with ADV ($10 \text{ ID}_{50} \text{ ml}^{-1}$; strain Bartha), for 12 h. The ADV-PK15 cells were subsequently washed in PBS and fixed in 1% glutaraldehyde. The ADV-infected cells were then further inactivated by treatment with 0.5 J UV-irradiation per cm^2 . Non infected PK15 cells were prepared in parallel. All cell preparations were verified to be free of infectious ADV particles. According to an enzyme linked limulus amoebocyte lysate test (COATEST® Endotoxin, Chromogenix AB, Mölndal, Sweden) the cell preparations were also free from endotoxin. The cells were suspended in approximately 1 ml of NaCl and 30×10^6 ADV-PK15 cells or uninfected PK15 cells were injected in each tissue chamber.

2.8. Cytokine analyses

TCF as well as serum samples were analyzed for their content of IFN- α , IFN- γ and TNF, respectively. The levels of IFN- α were determined by a dissociation-enhanced lanthanide fluoroimmunoassay (DELFLIA) for porcine IFN- α described by Artursson et al. (1995). IFN- γ was determined with a similar DELFLIA based on two monoclonal antibodies (mAbs) B37 and G47 (Vandenbroeck et al., 1993), kindly provided by B. Charley and C. La Bonnadière (Laboratoire de Virologie et Immunologie Moléculaires, INRA, Jouy en Josas, France). The tracer antibody G47 was labeled with Eu lanthanide chelate (9 Eu Ig⁻¹). The sensitivity of this immunoassay was 30 pg IFN- γ and the linear range of detection continued to 30000 pg IFN- γ according to a standard of recombinant porcine IFN- γ (Ciba-Geigy Ltd., Basel, Switzerland). TNF activity was measured with a bioassay on PK15 cells as described by Bertoni et al. (1993) with slight modifications. A standard of human recombinant TNF- α (Genzyme Diagnostics, Cambridge, MA, USA) was included on every test plate and 1 U TNF was defined as the dilution which destroyed 50% of the PK15 cells, as determined after staining with crystal violet.

2.9. Identification of cells in the TCF

Differential cell counts were carried out on hematoxylin-eosin stained cytospin (Shandon Southern Products Ltd., Astmoor, Runcorn, UK) preparations of TCF. In some experiments the identity of the TCF cells was further examined by labeling with mAbs and flow cytometric analysis (FACScan; Becton Dickinson). Before labeling, the red blood cells in the TCF were lysed with 0.17 mM Tris-0.14 M ammonium chloride buffer (pH 7.2) and the remaining cells were incubated in PBS with 0.02% NaN₃ (PBS-NaN₃) for 30 min at 37°C to remove cytophilic antibodies (Watson, 1976). The indirect immunofluorescence was carried out by incubation with either of the following primary murine mAbs: PT90A, PT36B, H42A (Veterinary Medical Research and Development Inc., Pullman, WA, USA) directed to porcine CD4, CD8 and MHC Class II molecules, respectively, as well as with MIL2, MIL3, MIL4 and 74-22-15 (kindly provided by M. Bailey and K. Haverson, Division of Molecular and Cellular Immunology, Department of Veterinary Clinical Sciences, University of Bristol at Langford, UK). According to Haverson et al. (1994) MIL2 is directed to an antigen present on neutrophils and monocytes/macrophages, MIL3 is directed to an antigen present on the majority of neutrophils and eosinophils while MIL4 recognises most eosinophils and less than 50% of blood neutrophils. Both MIL3 and 4 antigens are also found on some small lymphocytes. The mAb 74-22-15 is a type-specific monocyte/granulocyte marker (Pescovitz et al., 1984). As secondary antibody, fluorescein-conjugated goat anti-mouse Ig antibodies (Becton Dickinson, San Jose, CA, USA) was used. All reagents were diluted in PBS-NaN₃ and all incubations were carried out for 30 min on ice.

2.10. *In situ* hybridization of TCF cells

In situ hybridization using ³⁵S-labeled cRNA probes for porcine IFN- α (Lefèvre and La Bonnadière, 1986) and - β mRNA (Artursson et al., 1992) was carried out as earlier

described by Artursson et al. (1995) with slight modifications according to Sandberg et al. (1994). Erythrocytes among the TCF cells were lysed as described above whereafter the TCF cells were fixed in 2% paraformaldehyde (Merck, Darmstadt, Germany) in PBS and transferred to slides which were air dried and stored at -80°C until use. Hybridization was performed at high stringency for 18 h at 50°C with probes in 50% formamide and $2 \times \text{SSC}$, followed by RNase treatment. The slides were covered with NTB-2 nuclear track emulsion (Eastman-Kodak, Rochester, USA) and developed after 7 days, for IFN- α probes, or 10 days, for IFN- β probes.

2.11. Functional tests for cells in the TCF

The ability of TCF cells to respond with proliferation and IL-2 production after stimulation with the mitogen concanavalin A (Con A, $5 \mu\text{g ml}^{-1}$; Pharmacia) was determined for cells cultured in microtiter plates (Nunc, Roskilde, Denmark) at a concentration of 2 to 4×10^6 cells per ml growth medium, i.e. RPMI 1640 (Flow Laboratories) with 20 mM HEPES buffer, supplemented with L-glutamine (2 mM), penicillin (200 IU ml^{-1}), streptomycin ($100 \mu\text{g ml}^{-1}$), 2-mercaptoethanol (5×10^{-5} M) and 5% FCS (Myoclone[®]; Gibco, Scotland, UK), as earlier described for porcine blood mononuclear cells (Edfors-Lilja et al., 1991). The proliferation was determined as the average incorporation of radioactive thymidine (^3H -thymidine; $18.5 \text{ kBq per well}$, specific activity $185 \text{ GBq mmol}^{-1}$; Amersham International, Amersham, UK) and is expressed as stimulation index (SI) values, i.e. cpm values in Con A stimulated cultures divided by cpm values in control cultures. The IL-2 activity is expressed as percentage of a laboratory standard of porcine IL-2 included in the assay as described in detail by Edfors-Lilja et al. (1991).

The IFN- α producing capacity of TCF cells was determined in cultures stimulated with fixed ADV-PK15 cells prepared as described above (0.5×10^5 ADV-PK15 cells per well). After 18 h at 37°C , the culture supernatants were collected and the IFN- α content determined in the earlier mentioned DELFIA (Artursson et al., 1995). The IFN- α producing capacity was also determined by this method in whole blood cultures (Wallgren et al., 1994) (heparinized blood diluted 1:5 in growth medium) established from some of the experimental animals.

The capacity of TCF cells (1×10^6 cells ml^{-1} Hank's balanced salt solution) to phagocytose zymosan particles (Sigma, St. Louis, MO, USA), opsonized with normal SPF pig serum, was assessed in a luminol (2×10^{-4} M 3-aminophthal-hydrazide; Aldrich, Steinheim, Germany) enhanced chemiluminescence assay. The light emission of the samples was measured every minute over 30 min in a microplate luminometer (MicroLumat LB 96 P; EG and G Berthold, Bad Wildbad, Germany).

2.12. Detection of antibodies to ADV

Antibodies to the glycoprotein gII (gB) of ADV in serum or TCF were detected by a competitive ELISA (SVANOVIR[®] EIA-kit; SVANOVA BIOTECH, Uppsala, Sweden). Samples were titrated in two-fold dilutions until considered negative according to the manufacturer's instructions.

3. Results

3.1. *Patho-anatomical and histological evaluation of the tissue chamber model*

After an initial post-operative swelling of the surrounding tissues, the implanted chambers seemed to be well tolerated by the animals and the operation wounds healed rapidly. To allow the initial inflammatory reactions to decline the tissue chambers were left in position for 3–4 weeks before the first samples were collected.

In the first set of pigs two out of eight, and in the second set eight out of 20, implanted tissue chambers became infected. The infections were in eight of these chambers detected close to surgery, before or at the first occasion of sampling. The other two chambers became infected during the course of the experiments. From all but one of the infected chambers *Staphylococcus hyicus* was isolated, from the other chamber *Streptococcus equisimilis* was isolated. These local infections developed into abscesses that healed without residues after removal of the chamber. Infected tissue chambers were excluded from the experiments and removed from the pig. The anatomical location of the tissue chambers, i.e. flank or lateral neck, did not influence the incidence of post operative infections, sample collection procedure or the cellular and cytokine responses in the TCF.

When examined grossly at section, 5 or 7 months post surgery, the chambers were surrounded by subcutaneous connective tissue and enclosed in a tight capsule of connective tissue. Strands of connective tissue grew from the capsule through the holes in the chamber wall to a mass of granulation tissue inside the ends of the chamber. A strand or clot of fibrin filled the unperforated parts of the tissue chamber. Histologically, the tissue inside the chamber consisted of partially organized hematomas with connective tissue and blood vessels. A moderate infiltration of inflammatory cells was seen in the connective tissue with mostly neutrophils and macrophages and occasionally also poly-nucleated giant cells. The capsule surrounding the tissue chamber consisted of a broad inner layer of collagen rich connective tissue and a thin peripheral layer of connective tissue with numerous blood vessels and a moderate infiltration of mononuclear cells. The regional lymph nodes were generally not reactive but in some cases a slight hypertrophy with occasional active lymphoid follicles were noted.

3.2. *Total numbers and characterization of cells in the tissue chamber fluid*

The volume of TCF obtained at each sampling varied between 1 ml and 2 ml, regardless of size of the chamber and the same sample volume of TCF could still be recovered seven months after implantation. Repeated sample collection, as often as four times in 12 h, did not decrease the acquired volume of TCF but the amount of red blood cells in the fluid increased during this procedure. The number of nucleated cells in TCF obtained from untreated chambers was in mean $2.0 \pm 1.2 \times 10^6 \text{ ml}^{-1}$ (\pm SD, $n = 16$). Histologically, these cells were determined to be mostly polymorphonuclear leukocytes ($45 \pm 10\%$ neutrophilic granulocytes, $11 \pm 10\%$ eosinophilic granulocytes) while $24 \pm 8\%$ were identified as monocytes and $19 \pm 9\%$ as lymphocytes.

Further characterization of the cells in the TCF was carried out by means of immunofluorescence labeling and flow cytometric analysis. The cells were gated to

exclude contaminating erythrocytes and cell debris, and subsequently divided into mononuclear and polymorphonuclear cell populations on the basis of their forward and right angle light scatter. Among the mononuclear cells in the TCF from untreated chambers, $22 \pm 5\%$ were $CD4^+$ ($n = 5$), $25 \pm 11\%$ were $CD8^+$ ($n = 5$) and $33 \pm 15\%$ were MHC Class II⁺ ($n = 7$). When the monocyte/granulocyte marker, mAb 74-22-15 was used, $34 \pm 10\%$ of the gated mononuclear cells and $94 \pm 1\%$ of the gated granulocytes in the TCF of untreated chambers were positively labeled ($n = 5$). The corresponding percentage of monocytes identified by their histology among mononuclear cells in these tissue chamber samples was $26 \pm 13\%$.

3.3. Detection of cytokines in the TCF after injection with poly I:C

Fluid collected from all five chambers injected with poly I:C contained low levels of IFN- α after 4 h (Fig. 1). The levels of IFN- α peaked at 12 h and were still elevated at 48 h post injection. The TNF response was more prompt than that observed for IFN- α , reaching the peak value at 4 h post injection. The TNF levels remained elevated at 8 and 12 h after poly I:C injection but had returned to basal levels by 24 h. No IFN- γ was detected in TCF or serum after injection of poly I:C.

The cytokine responses remained local, i.e. IFN- α and TNF were not detected in control chambers of poly I:C treated pigs. Only trace amounts of IFN- α (1.3 units at 4 h) were detected in serum from one of the poly I:C injected pigs and no TNF activity could be detected in serum from any of these animals.

3.4. Influence of repeated sample collection on the cytokine levels in TCF after injection with poly I:C

To study the influence of frequent sample collection on the pattern of the cytokine responses the above results were compared with those obtained in experiments with fewer sample collections, i.e. at 0, 8 and 24 h. The levels of IFN- α were, at 8 h (Fig. 2a), of similar magnitude in both types of experiments in three out of four chambers,

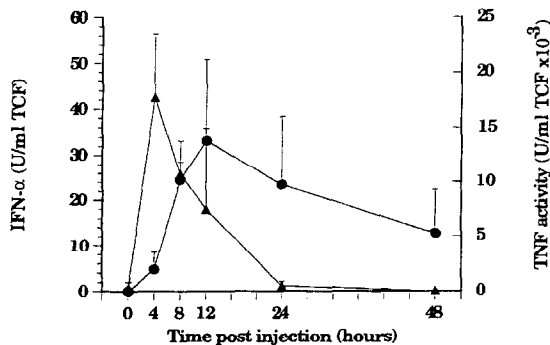


Fig. 1. Levels of IFN- α (filled circles) and TNF (filled triangles) in TCF after injection of poly I:C. TCF samples were collected at the indicated time points and the values are expressed as mean values \pm SD, $n = 5$.

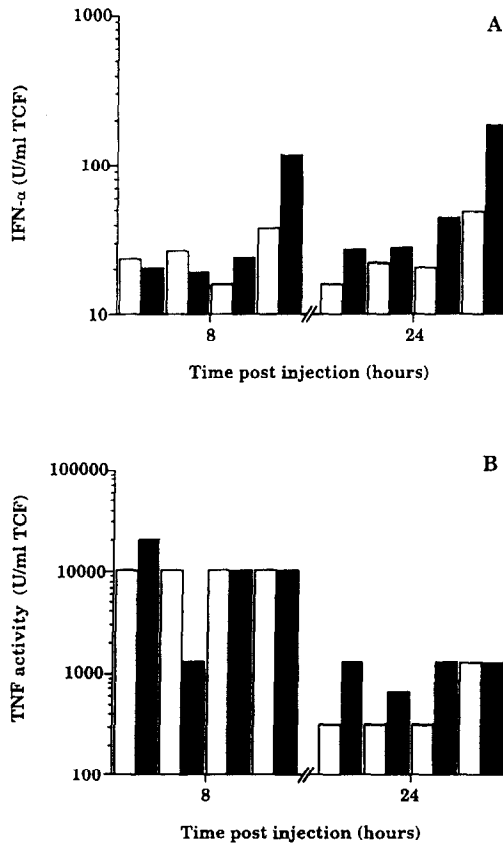


Fig. 2. Levels of IFN- α (a) and TNF (b) detected in TCF obtained at 8 and 24 h after poly I:C injection in chambers from which samples were collected at 0, 4, 8, 12 and 24 h post injection (open bars) or at 0, 8 and 24 h post injection (filled bars). The values are expressed as individual values for each tissue chamber.

while the concentrations of IFN- α at 24 h in general were highest in chambers with less frequent sample collection. One pig displayed higher levels of IFN- α , both at 8 and 24 h, during the experiment with few sample collections. Similarly, at 24 h the TNF activity (Fig. 2b) was higher in fluid from three of four chambers when the sample collection was less frequent.

3.5. Detection of cytokines in the TCF of chambers injected with virus infected cells

Fixed, ADV infected cells of the porcine kidney cell line PK15 were used as a non-infective, non-soluble IFN- α inducer. IFN- α was detected in the TCF of four out of five chambers after a primary injection of ADV-PK15 (Table 1). Both the kinetics and levels of IFN- α produced varied between pigs. It should however be noted that the right flank chamber of Pig No. 261, in which very high IFN- α levels were detected, was found to be infected with *Staph. hyicus* during this experiment. This is the only case

Table 1
 IFN- α levels (U ml^{-1}) and antibody titers[†] to ADV in TCF and serum recorded at indicated time points after injection of ADV-PK15 in the tissue chambers

Fig No.	Chamber	1st injection, Day 0										2nd injection, Day 7													
		ADV-PK15					IFN- α in TCF					ADV-PK15					IFN- α in TCF								
			4 h	8 h	12 h	24 h	48 h	TCF	Serum		4 h	8 h	12 h	24 h	48 h	TCF	Serum		4 h	8 h	12 h	24 h	48 h	TCF	Serum
261	Right flank	+	12	353	313	76	10	neg.	neg.	-						neg.	neg.	-	0	0	0	0	0	0	neg.
	Left flank	-	0	0	0	0	0	neg.	neg.	+	0.6	0	15	8	8	neg.	neg.	+	0.6	0	15	8	8	8	neg.
	Left neck	+	0	0	0	0	0	neg.	neg.	-	0	0	0	0	0	neg.	neg.	-	0	0	0	0	0	0	neg.
	Left flank	-	0	0	0	0	0	neg.	neg.	+	0	0	0.7	3	5	neg.	neg.	+	0	0.7	3	5	0	0	neg.
287	Right flank	+	6	20	57	75	77	neg.	neg.	+	0	0	0	0	0	neg.	neg.	+	0	0	0	0	0	0	1:256
	Left flank	-	0	0	0	0	0	neg.	neg.	-	0	0	0	0	0	neg.	neg.	-	0	0	0	0	0	0	1:32
	Right neck	-	0	0	0	0	0	neg.	neg.	-	0	0	0	0	0	neg.	neg.	-	0	0	0	0	0	0	1:4
289	Right flank	+	8	10	12	10	4	neg.	neg.	+	0	0	0	0	0	neg.	neg.	+	0	0	0	0	0	0	1:16
	Left flank	+	n.t.	n.t.	33	45	25	neg.	neg.	+	0	0	0	0	0	neg.	neg.	+	0	0	0	0	0	0	1:16

[†] The IFN- α content in TCF was measured with the DELFIA described in Materials and methods. [†] Antibodies to ADV were detected with the ELISA-kit described in Materials and methods. + / - The symbols mark if the indicated tissue chamber was injected with ADV-PK15 (+) or not (-). n.t. not tested. neg. sample considered negative for the presence of antibodies to ADV.

were an infected chamber was used in an experiment and all cellular data from this chamber have been omitted. When a second injection of ADV-PK15 was administered one week after the first, IFN- α was detected in the TCF of both injected chambers. In contrast, IFN- α was not detected in any of three chambers injected with ADV-PK15 2 weeks after the first injection.

Blood mononuclear cells from the experimental pigs were tested for *in vitro* IFN- α production in response to ADV-PK15 on two occasions before the animals were exposed to ADV-PK15 *in vivo*. Pig Nos. 287 and 289 were found to produce higher levels (17; 195 and 137; 40 U ml⁻¹, respectively) of IFN- α *in vitro* than Pig Nos. 261 and 358 (7; 0 and 1; 0 U IFN- α , respectively). Thus, the pigs which produced the lowest levels of IFN- α *in vivo* also produced the lowest levels of IFN- α *in vitro* (cf. Table 1).

No IFN- γ was detected in serum or TCF collected during the first two weeks after injection of ADV-PK15. Three weeks after the primary injection of virus infected cells, IFN- γ was detected in the ADV-PK15 injected chamber of one of the pigs (No. 287 right flank, 2 ng IFN- γ ml⁻¹). At slaughter, i.e. approximately 2 months after the primary injection of ADV-PK15, both experimental pigs displayed IFN- γ in serum as well as in all tissue chambers (No. 287 right flank, 10 ng; left flank, 8 ng; serum, 7 ng IFN- γ ml⁻¹; No. 289 right neck, 1 ng; right flank, 0.8 ng; left flank, 0.8 ng; serum, 2 ng IFN- γ ml⁻¹). TNF activity was low or undetectable in the TCF of ADV-PK15 injected chambers. Injection of PK15 alone did not induce production of IFN- α , IFN- γ or TNF.

3.6. Development of antibodies to ADV after local administration of ADV-PK15

All pigs were seronegative to ADV at the first injection of virus infected cells and none of the pigs had gained detectable levels of antibodies to ADV in serum or in TCF one week after the first injection of ADV-PK15 (Table 1). However, when the second injection was administered 2 weeks after the first injection of ADV-PK15, the pigs had antibodies to ADV both in serum (titer \geq 1:32) and in the TCF (titer \geq 1:4). All of the pigs injected with ADV-PK15 had seroconverted at least 3 weeks after the primary injection. At slaughter of Pigs Nos. 287 and 289, approximately 2 months after the primary injection of ADV-PK15, higher antibody titers to ADV were detected in serum and TCF compared with those recorded at the time of the second injection of ADV-PK15 (No. 287 right flank, 1:512; left flank, 1:32; serum, 1:64; No. 289 right neck, 1:1024; right flank, 1:2048; left flank, 1:1024; serum, 1:128).

3.7. Alterations in cell numbers in the TCF

Alterations in TCF cell numbers were studied after injections of poly I:C, ADV-PK15 or PK15 cells and compared to those in chambers injected with NaCl or only subjected to sample collections (Fig. 3). No significant ($n = 5$) alterations in total TCF cell number were found during repeated sample collection. In chambers injected with NaCl however, the total number of nucleated cells tended to increase during sample collection ($P < 0.05$ for 4, 8 and 12 h vs. 0 h, $n = 11$) but was not significantly different from the corresponding values for non-treated chambers. After poly I:C injection a pronounced

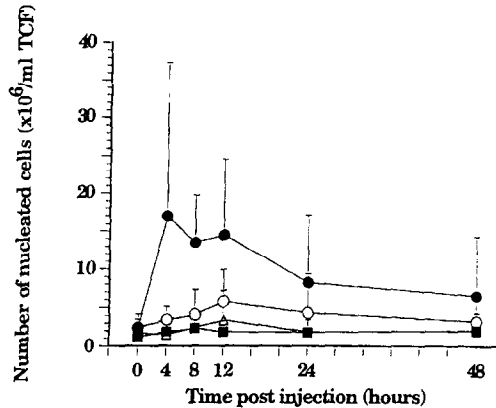


Fig. 3. The total number of nucleated cells in the TCF obtained from chambers injected with NaCl (open circles, $n = 11$); poly I:C (filled circles, $n = 5$); ADV-PK15 (filled squares, $n = 6$); or from untreated chambers (open triangles, $n = 5$). TCF samples were collected at the indicated time points and the values are expressed as mean values \pm SD.

increase in the total number of nucleated cells in TCF obtained at 4 to 12 h ($P < 0.05$ for 4, 8 and 12 h vs. 0, 24 and 48 h) was noted in most treated chambers. These cell numbers were also significantly higher than in untreated chambers ($P < 0.01$ at 4 h and $P < 0.05$ at 8, 12 and 24 h) as well as in chambers injected with NaCl ($P < 0.01$ at 4 and 8 h, $P < 0.05$ at 12 h) at these time points.

The proportion of neutrophilic granulocytes increased during sample collection both in untreated tissue chambers (50 ± 11 , 56 ± 13 , 61 ± 16 , $63 \pm 15\%$ neutrophils at 0, 4, 8 and 12 h, respectively, mean values \pm SD, $n = 5$), in chambers injected with NaCl (51 ± 18 , 59 ± 19 , 68 ± 13 , $76 \pm 13\%$ neutrophils at 0, 4, 8 and 12 h, respectively, mean values \pm SD, $P < 0.05$ for 0 h vs. 4, 8 and 12 h, $n = 13$) and in chambers injected with poly I:C (43 ± 9 , 78 ± 17 , 78 ± 14 , $78 \pm 9\%$ neutrophils at 0, 4, 8 and 12 h, respectively, mean values \pm SD, $P < 0.05$ for 0 h vs. 4, 8 and 12 h, $n = 5$). Injections of ADV-PK15 or PK15 (not shown in the figure) did not induce any other changes in the total numbers or composition of cells in the TCF than those observed in untreated or NaCl injected tissue chambers.

When tested one week after the chambers had been injected with poly I:C, ADV-PK15 or PK15 cells, the total number of cells and proportions of different cell populations ($2.0 \pm 1.8 \times 10^6$ nucleated cells ml^{-1} TCF, $48 \pm 13\%$ neutrophilic granulocytes, $13 \pm 11\%$ eosinophilic granulocytes, $21 \pm 8\%$ monocytes, $18 \pm 8\%$ lymphocytes, mean value \pm SD, $n = 27$) were not significantly different from those recorded before the treatments.

3.8. Further identification of cells in the TCF during experiments

Administration of soluble (poly I:C) and non-soluble (ADV-PK15) IFN- α inducers into the chambers resulted in a local cytokine production which in the case of poly I:C was accompanied by an influx of nucleated cells. In an attempt to further identify these

cells indirect IF labeling with the mAbs MIL2, MIL3 and MIL4, directed to different surface antigens on porcine myeloid cells, was carried out on TCF cells in some of the experiments (Fig. 4). Although only a limited number of observations were made, the results indicate that the proportion of MIL4⁺ granulocytes decreased ($P < 0.05$ for 0 h vs. 8 and 24 h, $n = 6$) and the proportion of MIL2⁺ granulocytes tended (n.s.) to increase in TCF after sample collection, regardless of treatment. Further, the proportions of granulocytes expressing MIL2, 3 and 4, respectively, showed a small variation between chambers at all time points as reflected by the low SD values. Among mononuclear TCF cells, the proportions of MIL3 and 4 positive cells remained constant during the experiments (data not shown). The proportion of MIL2⁺ mononuclear cells were however approximately doubled after poly I:C injection ($32 \pm 9\%$, $63 \pm 3\%$ and $62 \pm 18\%$ at 0, 8 and 24 h, respectively, mean values \pm SD, $n = 3$) compared with the other treatments ($29 \pm 4\%$, $29 \pm 10\%$ and $29 \pm 11\%$ at 0, 8 and 24 h, respectively, mean values \pm SD, $n = 6$).

In addition, RNA-RNA in situ hybridization with probes specific for porcine IFN- α and IFN- β was applied on samples of cells recovered from the TCF collected from Pig Nos. 261 and 358 after injection of ADV-PK15 cells. Although the majority of samples were negative, occasional cells (approximately 1 per 0.5×10^6), heavily labeled either for IFN- α or IFN- β mRNA were detected in samples collected 4 to 24 h after injection.

3.9. Functional *in vitro* tests of cells in the TCF

The viability and functional capacity of cells recovered from the TCF was analyzed by some *in vitro* functional tests, i.e. Con A induced proliferation and IL-2 production, *in vitro* IFN- α induction with ADV-PK15 and a luminol-enhanced chemiluminescence assay. The mitogen induced proliferation of TCF cells, expressed as SI values ranged

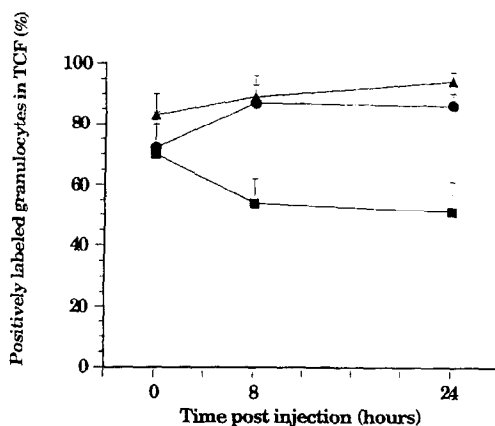


Fig. 4. The percentage of MIL2⁺ (filled circles); MIL3⁺ (filled triangles); and MIL4⁺ (filled squares) granulocytes recovered from TCF at the indicated time points. The proportions of cells positive for the markers were determined by means of immunofluorescence labeling and flow cytometric analyses. The values are expressed as mean values \pm SD, $n = 9$.

from 1 to 56 (SI 8 ± 14 , mean value \pm SD, $n = 14$). The IL-2 activity (%) in supernatants from these cultures ranged from 1 to 175% ($81 \pm 67\%$, mean value \pm SD, $n = 14$). The IFN- α production of TCF cells *in vitro* showed a large variation, ranging from 0 to 199 units IFN- α (51 ± 76 U ml⁻¹, mean value \pm SD, $n = 7$). Further, granulocytes recovered from TCF exhibited phagocytic activity as determined by light emission in the chemiluminescence assay, (1130 ± 556 cps as mean peak value \pm SD, $n = 5$). Thus, viable cells could be recovered from TCF and used in *in vitro* experiments.

4. Discussion

A tissue chamber model for *in vivo* studies of local inflammatory responses, such as cytokine production and cell recruitment was established in the pig. The tissue chambers were well tolerated by the animals and the pigs could be housed under normal conditions. Further, sample collection was easily performed and was not affected by the growing of the pigs (from 30 to 200 kg) which enabled the use of the experimental animals over long periods of time. Moreover, the content of cytokines in the TCF could be monitored at short intervals since the amount of fluid recovered from each chamber remained constant even when collected frequently.

The main problem observed during the study was the occurrence of post-operative infections in the chambers. In total, 36% of the implanted chambers became infected with bacteria commonly present on the skin of pigs. However, only two chambers became infected during the experiments, which indicates that infections seldom were introduced by the sample collection. Instead, it is more likely that the close proximity between chamber and the skin wound augmented the risk for infections, especially in the second set when the pigs had four chambers implanted and thus rested on the wounds before they had healed. By only implanting tissue chambers on one side of the pigs, post surgical infections of the wound appeared to be less frequent according to the first and second set of the present study, possibly because the animals could rest on the non operated side. When a similar model was used on larger animals, *i.e.* cattle (Bengtsson *et al.*, 1984), the frequency of infected tissue chambers was initially about 10%. This figure was further reduced to almost nil, by extending the distance between the tissue chamber and the skin wound (Bengtsson, personal communication, 1996) and by introducing antibiotic treatment of the animals during the pre- and post-operative period (Bengtsson, 1990). Consequently, precautions could be taken to reduce the risk of post-operative infections also in the porcine model. For instance, it might be beneficial to use larger/older pigs to operate on.

The cytokine responses were studied in TCF after administration of fixed ADV-infected PK15 cells or poly I:C which both are known to induce the production of Type I IFNs. In addition, poly I:C can induce the production of several other cytokines such as TNF and IL-6 (Bunning *et al.*, 1990; North *et al.*, 1991; Pyo *et al.*, 1993). In the present experiments, the kinetics of IFN- α and TNF could be monitored simultaneously in tissue chambers injected with poly I:C. The highest levels of TNF were achieved in the first sample and then gradually decreased while the IFN- α levels were increasing during the first three occasion of sampling. Thus, the sample collection procedure marginally

affected the kinetic and magnitude of the cytokine responses. The poly I:C induced IFN- α production in TCF was however delayed compared with that earlier recorded after systemic administration of this inducer. The highest IFN levels were then recorded already 2 to 4 h after intravenous injection of poly I:C in pigs (Vengris and Maré, 1972; Loewen and Derbyshire, 1986). This discrepancy is probably due to a more rapid degradation of the inducer when administered systemically. In fact, rapid hydrolysis of poly I:C by serum ribonucleases has been observed in humans (Nordlund et al., 1970). A further advantage of the tissue chamber model could therefore be that metabolization of substances, in for instance the liver, could be avoided or delayed.

The kinetics of the IFN- α responses induced by injection of ADV-PK15 in the tissue chambers resembled that induced by poly I:C while the magnitude of the response varied more between individuals. An individual variation in the IFN- α production was similarly observed after intra-dermal administration of ADV-PK15 in pigs (Artursson et al., 1995) and is well documented for IFN- α production of porcine leukocytes when induced in vitro (Edfors-Lilja et al., 1994; Watrang et al., 1994). Indeed, leukocytes obtained from the pig which did not produce any detectable amounts of IFN- α in TCF after the primary injection of ADV-PK15, responded poorly to IFN- α induction in vitro. When a second injection of ADV-PK15 was administered in the tissue chambers 2 weeks after the primary injection, the IFN- α response was abolished. This inhibition of the IFN- α responses was most likely due to the presence of antibodies to ADV in the TCF at this time, since antibodies to certain viral glycoproteins can inhibit IFN- α production in vitro (Lebon, 1985; Charley and Laude, 1988; Ankel et al., 1994). In contrast, markedly higher IFN- α levels were noticed after ADV-PK15 induction in the bacterially infected tissue chamber. The magnitude of the IFN- α response can be increased in vitro by addition of other cytokines to the cell cultures (Cederblad and Alm, 1991; Charley et al., 1994; Watrang et al., 1995) and enhanced IFN production has been observed during bacterial infections in man (Ikossi-O'Connor and Chadha, 1984). Thus, 'priming' effects on the IFN- α producing cells by inflammatory cytokines could explain the high IFN- α levels in the infected tissue chamber.

No IFN- γ was detected in samples collected during the first 48 h after injection of the inducers. However, IFN- γ was recorded in the TCF 2 weeks after injection of ADV-PK15 in one of the pigs and in serum and TCF of both experimental pigs 2 months after the injections. These systemic and long-lasting IFN- γ responses suggest an on-going immune response, possibly triggered by locally preserved antigens. In contrast, the IFN- α and TNF responses induced in the experiments stayed localized to the tissue chamber in question, allowing the animal to be its own control during short term experiments.

Besides the cytokine responses, the number and composition of nucleated cells in the TCF were studied. During frequent sample collection the total number of TCF cells remained rather constant although the proportion of neutrophilic granulocytes increased. Immunofluorescence labeling confirmed the shift in TCF cell composition, determined by histologic characterization of the TCF cells. For instance, the proportion of granulocytes expressing the MIL2 antigen, which among granulocytes only is present on neutrophils (Haverson et al., 1994), increased. Moreover, the proportion of granulocytes expressing the MIL4 antigen, which is present on the majority of eosinophils but only on

50% of the neutrophils (Haverson et al., 1994), decreased after sample collection. The most prominent changes in TCF cell numbers were however the rapid increases in neutrophil number and proportional increase of MIL2⁺ mononuclear cells observed after injection of poly I:C. These alterations were probably mediated by locally produced cytokines. For instance, TNF increases the expression of adhesion molecules on endothelial cells as well as on leukocytes and thus contributes to the accumulation of neutrophilic granulocytes (Vassalli, 1992).

Although only a limited number of observations on cell identification were undertaken, the results clearly indicate that the present tissue chamber model could be used for studies of cell recruitment. In addition, cells recovered from the TCF were viable and could be used in several functional assays. Taken together, the tissue chamber model offers a novel opportunity to study for example immune and inflammatory responses in their natural context i.e. in the presence of cytokines and accessory cell populations. Further, *in vitro* findings can be verified during experimentally modulated *in vivo* conditions by local or systemic treatments such as depletion of cell populations or administration of antibodies or pharmaceuticals.

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References

- Ankel, H., Capobianchi, M.R., Castilletti, C. and Dianzani, F., 1994. Interferon induction by HIV glycoprotein 120: role of the V3 loop. *Virology*, 205: 34–43.
- Artursson, K., Gobl, A., Lindersson, M., Johansson, M. and Alm, G., 1992. Molecular cloning of a gene encoding porcine IFN- β . *J. Interferon Res.*, 12: 153–160.
- Artursson, K., Lindersson, M., Varela, N., Scheynius, A. and Alm, G.V., 1995. Interferon- α production and tissue localization of interferon- α/β producing cells after intradermal administration of Aujeszky's disease virus-infected cells in pigs. *Scand. J. Immunol.*, 41: 121–129.
- Bengtsson, B., 1990. The tissue cage as a model for studies on extravascular distribution of antibacterials. Dissertation, Swedish University of Agricultural Sciences.
- Bengtsson, B., Luthman, J. and Jacobsson, S.-O., 1984. Evaluation of a tissue cage model for use in cattle. *Acta Vet. Scand.*, 25: 480–494.
- Bergan, T., 1981. Pharmacokinetics of tissue penetration of antibiotics. *Rev. Inf. Dis.*, 3: 45–66.
- Bertoni, G., Kuhnert, P., Peterhans, E. and Pauli, U., 1993. Improved bioassay for the detection of porcine tumor necrosis factor using a homologous cell line: PK(15). *J. Immunol. Methods*, 160: 267–271.
- Bunning, R.A., Russell, R.G. and Van Damme, J., 1990. Independent induction of interleukin 6 and prostaglandin E2 by interleukin 1 in human articular chondrocytes. *Biochem. Biophys. Res. Commun.*, 166: 1163–1170.
- Calnan, J.S., Ford, P.M., Holt, P.J.L. and Pflug, J.J., 1972. Implanted tissue cages — a study in rabbits. *Br. J. Plastic Surg.*, 25: 164–174.

- Cederblad, B. and Alm, G.V., 1991. Interferons and the colony stimulating factors IL-3 and GM-CSF enhance the interferon- α response in human blood leukocytes induced by herpes simplex virus. *Scand. J. Immunol.*, 34: 549–555.
- Charley, B. and Laude, H., 1988. Induction of alpha interferon by transmissible gastroenteritis corona virus: Role of transmembrane glycoprotein E1. *J. Virol.*, 62: 8–11.
- Charley, B., Lavenant, L. and Lefèvre, F., 1994. Coronavirus transmissible gastroenteritis virus-mediated induction of IFN-mRNA in porcine leukocytes requires prior synthesis of soluble proteins. *Vet. Res.*, 25: 29–36.
- Chisholm, G.D., Waterworth, P.M., Calnan, J.S. and Garrod, L.P., 1973. Concentration of antibacterial agents in interstitial tissue fluid. *Br. Med. J.*, 1: 569–573.
- Clarke, C.R., 1989. Tissue-chamber modeling systems-applications in veterinary medicine. *J. Vet. Pharmacol. Therap.*, 12: 349–268.
- Dawson, J., Rordorf-Adam, C., Geiger, T., Towbin, H., Kunz, S., Nguyen, H., Zingel, O., Chaplin, D. and Vosbeck, K., 1993. Interleukin-1 (IL-1) production in a mouse tissue chamber model of inflammation. I. Development and initial characterisation of the model. *Agents Actions*, 38: 247–254.
- Edfors-Lilja, I., Bergström, M., Gustavsson, U., Magnusson, U. and Fossum, C., 1991. Genetic variation in Con A induced production of interleukin 2 by porcine peripheral blood mononuclear cells. *Vet. Immunol. Immunopathol.*, 27: 351–363.
- Edfors-Lilja, I., Watrang, E., Magnusson, U. and Fossum, C., 1994. Genetic variation in parameters reflecting immune competence of swine. *Vet. Immunol. Immunopathol.*, 40: 1–16.
- Haverson, K., Bailey, M., Higgins, V.R., Bland, P.W. and Stokes, C.R., 1994. Characterization of monoclonal antibodies specific for monocytes, macrophages and granulocytes from porcine peripheral blood and mucosal tissues. *J. Immunol. Meth.*, 170: 233–245.
- Higgins, A.J., Lees, P. and Sedgwick, A.D., 1987. Development of equine models of inflammation. *Vet. Rec.*, 120: 517–522.
- Ikossi-O'Connor, M.G. and Chadha, K.C., 1984. The effect of malignant epithelial tumors, surgical therapy, and bacterial sepsis upon various parameters of inteferon system. *J. Surg. Oncol.*, 26: 27–34.
- La Bonnardière, C., Lefèvre, F. and Charley, B., 1994. Interferon response in pigs: molecular and biological aspects. *Vet. Immunol. Immunopathol.*, 43: 29–36.
- Lebon, P., 1985. Inhibition of Herpes simplex virus type 1-induced interferon synthesis by monoclonal antibodies against viral glycoprotein D and by lysosomotropic drugs. *J. Gen. Virol.*, 6: 2781–2785.
- Lefèvre, F. and La Bonnardière, C., 1986. Molecular cloning and sequencing of a gene encoding biologically active porcine α -interferon. *J. Interferon Res.*, 6: 349.
- Loewen, K.G. and Derbyshire, J.B., 1986. Interferon induction with polyinosinic:polycytidylic acid in the newborn piglet. *Can. J. Vet. Res.*, 50: 232–237.
- Misfeldt, M.L. and Grimm, D.R., 1994. Sinclair miniature swine: an animal model of human melanoma. *Vet. Immunol. Immunopathol.*, 43: 167–175.
- Miyasaka, M. and Trnka, Z., 1986. Lymphocyte migration and differentiation in a large animal model: the sheep. *Immunol. Rev.*, 91: 87–114.
- Murtaugh, M.P., 1994. Porcine cytokines. *Vet. Immunol. Immunopathol.*, 43: 37–44.
- Myers, M.J. and Murthaugh, M.P., 1995. Cytokines in animal health and disease. In: M.J. Myers, and M.P. Murthaugh (Editors). *Book Cytokines in Animal Health and Disease*. Marcel Dekker Inc., NY.
- Nordlund, J.J., Wolff, S.M. and Levy, H.B., 1970. Inhibition of biologic activity of poly I:poly C by human plasma. *Proc. Soc. Exp. Biol. Med.*, 133: 439–444.
- North, R.J., Dunn, P.L. and Havcill, E.A., 1991. A role for tumor necrosis factor in poly (I:C)-induced hemorrhagic necrosis and T-cell-dependent regression of a murine sarcoma. *J. Interferon Res.*, 11: 333–340.
- Pescovitz, M.D., Lunney, J.K. and Sachs, D.H., 1984. Preparation and characterization of monoclonal antibodies reactive with porcine PBL. *J. Immunol.*, 133: 368–375.
- Piercy, D.W.T., 1978. Distribution of trimethoprim/sulphadiazine in plasma, tissue and synovial fluids. *Vet. Rec.*, 102: 523–524.
- Pyo, S., Gangemi, J.D., Ghaffar, A. and Mayer, E.P., 1993. Poly I:C-induced antiviral and cytotoxic activities are mediated by different mechanisms. *Int. J. Immunopharmacol.*, 15: 477–486.
- Sachs, D.H., 1994. The pig as a potential xenograft donor. *Vet. Immunol. Immunopathol.*, 43: 185–191.

- Sandberg, K., Eloranta, M.-L. and Campbell, I.L., 1994. Expression of alfa/beta interferons (IFN- α/β) and their relationship to IFN- α/β -induced genes in lymphocytic choriomeningitis. *J. Virol.*, 68: 7358–7366.
- Vandenbroeck, K., Martens, E., D'Andrea, S. and Billiau, A., 1993. Refolding and single-step purification of porcine interferon-gamma from *Escherichia coli* inclusion bodies. Conditions for reconstitution of dimeric IFN-gamma. *Eur. J. Biochem.*, 215: 481–486.
- Vassalli, P., 1992. The pathophysiology of tumor necrosis factors. In: W.E. Paul, C.G. Fathman and H. Metzger (Editors). *Annual Review of Immunology*, Annual Reviews Inc., Palo Alto, CA, 411–452.
- Vaudaux, P., Grau, G.E., Huggler, E., Schumacher-Perdreau, F., Fiedler, F., Waldvogel, F.A. and Lew, D.P., 1992. Contribution of tumor necrosis factor to host defense against staphylococci in a guinea pig model of foreign body infections. *J. Infect. Dis.*, 166: 58–64.
- Vengris, V.E. and Maré, C.J., 1972. Swine interferon II. Induction in pigs with viral and synthetic inducers. *Can. J. Comp. Med.*, 36: 288–293.
- Wallgren, P., Wilén, I.-L. and Fossum, C., 1994. Influence of experimentally induced endogenous production of cortisol on the immune capacity in swine. *Vet. Immunol. Immunopathol.*, 42: 301–316.
- Watson, D.L., 1976. The effect of cytophilic IgG₂ on phagocytosis by ovine polymorphonuclear leukocytes. *Immunology*, 31: 159–165.
- Watrang, E., Cederblad, B. and Fossum, C., 1995. Lectins inhibit the Aujeszky's disease virus induced interferon- α production of porcine peripheral blood mononuclear cells. *J. Interferon Cytokine Res.*, 15: 301–308.
- Watrang, E., Edfors-Lilja, I., Andersson, L. and Fossum, C., 1994. Genetic and stress-mediated influence on Aujeszky's disease virus induced interferon- α production in porcine leukocytes. *Acta Vet. Hun.*, 42: 331–336.
- Wisselink, M.A., Koeman, J.P. and Willemsse, T., 1993. Leukocyte mobilization to skin lesions in dogs. *Am. J. Vet. Res.*, 54: 1598–1601.