NR 2002:3

Organic dust from pig environment induces activation of human T cells

Charlotte Müller-Suur

Department of Medicine,
Division of Respiratory Medicine,
Karolinska Institutet, Stockholm, Sweden

Program for Respiratory Health and Climate,
National Institute for Working Life, Stockholm, Sweden

ARBETE OCH HÄLSA | VETENSKAPLIG SKRIFTSERIE

ISBN 91-7045-634-8 ISSN 0346-7821 http://www.niwl.se/

Arbetslivsinstitutet
National Institute for Working Life

Arbete och Hälsa

Arbete och Hälsa (Work and Health) is a scientific report series published by the National Institute for Working Life. The series presents research by the Institute's own researchers as well as by others, both within and outside of Sweden. The series publishes scientific original works, dissertations, criteria documents and literature surveys.

Arbete och Hälsa has a broad target-group and welcomes articles in different areas. The language is most often English, but also Swedish manuscripts are welcome.

Summaries in Swedish and English as well as the complete original text are available at www.niwl.se/ as from 1997.

National Institute for Working Life
S-112 79 Stockholm
Sweden

ISBN 91–7045–634–8
ISSN 0346–7821
http://www.niwl.se
Printed at Elanders Gotab, Stockholm

To my family

Original articles

This thesis is based on four publications referred to by their Roman numerals:

I. Increased number of activated lymphocytes in human lung following swine dust inhalation. C Müller-Suur, K Larsson, P Malmberg, PH Larsson. *Eur Respir J* 1997; 10:376-380

II. T cell activation by organic dust in vitro. C Müller-Suur, PH Larsson, K Larsson. *Respiratory Medicine* 2000; 94:821-827

III. Lymphocyte activation after exposure to swine dust. The role of humoral mediators and phagocytic cells. C Müller-Suur, PH Larsson, K Larsson, J Grunewald. *Eur Respir J* 2002; 19:104-107.

IV. Organic dust induced IL-12 production activates T-and NK cells. C Müller-Suur, K Larsson, J Grunewald. (Submitted for publication.)

Abbreviations

ADCC	Antibody-dependent cell mediated cytotoxity
AM	Alveolar macrophage
APC	Antigen presenting cell
BAL	Bronchoalveolar lavage
BHR	Bronchial hyperresponsiveness
CD	Cluster of differentiation
EDTA	Ethylene diamine tetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FEV_1	Forced expiratory volume in one second
FCS	Fetal calf serum
G+	Gram-positive strain of bacteria
G-	Gram-negative strain of bacteria
HSC	Hematopoetic stem cell
ICAM-1	Intercellular adhesion molecule
IL	Interleukine
IFN-γ	Interferon-gamma
kD	Kilodalton
LPS	Lipopolysaccharide
MHC	Major Histocompatibility Complex
NAL	Nasal lavage
NK cells	Natural Killer cells
ODTS	Organic dust toxic syndrome
PBMC	Pheripheral blood mononuclear cells
RPMI	Roswell Park Memorial Institute 1640 medium
VC	Vital capacity
TCR	T cell receptor
TNF-α	Tumour necrosis factor alpha

Table of contents

Introduction

Background

In a number of reports from Sweden and other countries it has been shown that individuals working in an agriculture environment (farming) more frequently have respiratory symptoms and lung injuries than the population in general (Brouwer et al.,1986; Iversen et al.,1988; Donham et al.,1986; Donham et al.,1989). Fever and other systemic reactions due to acute exposure of dust are common and an increased prevalence of chronic bronchitis and other airway related disorders such as asthma have been reported (Heederik et al., 1991; Randon et al., 2001; Rylander et al.,1989; Malmberg et al.,1991).

The intensive swine housing began in Europe and Sweden in the early 1960s and it is not known how many Swedish farmers who are presently working in environments, in which they are exposed to swine dust. However, it can be estimated that thousands of farmers in Sweden are exposed to the swine farming environment in animal confinement buildings, animal breeding/livestock, and at transportation and storage of agricultural products. Confinement systems imply a large number of animals within a relatively small space. When weighing animals prior to slaughter the farmers are exposed to high levels (> 20 mg/m^3) of airborne inhalable dust (Wang et al., 1996; Wang et al., 1997; Larsson et al., 1994) during long periods of time (hours). Of the inhalable dust, particles >10 μm are trapped in the upper respiratory tract (nose, throat) whereas the respirable dust (particles < 5 μm) reaches the small airways and the alveolar region.

Organic dust

Organic dust from confinement buildings constitute a very complex mixture containing components of animal and plant origin, as well as microbial constituents and metabolites. Table 1 lists the major constituents in dust from swine confinement buildings according to Donham et al. (Donham et al.,1986; Donham et al., 1985)

Table 1. Swine dust components

Swine dander
Animal hair
Urine
Mite, or their parts
Bacteria
Bacterial endotoxin
(1-3) ß -D-glucan
Microbial protease
Pollen grains
Particles of plants
Feed grains
Hay
Silage
Fungal spores
Hyphae or sporangia from any decomposing organic material
Mycotoxins

In swine confinement buildings, the swine themselves contribute to the composition of the dust primarily by skin shedding and by release of gut epithelium and micro-organisms by excretion of faecal material (Donham et al., 1986; Attwood et al., 1987). Plant origin in dust compartments comes from feed particles (crushed feed), hay, grasses, pollen grains and other plant materials carried by the animals (Donham et al., 1986).

The air in these buildings also contains irritating gases, such as ammonia, carbon dioxide, hydrogen sulphide and methane (Malmberg et al., 1991; Crock et al., 1991) although most often in concentrations below hygienic threshold levels (von Essen et al., 1999). In addition to direct toxic or allergenic properties, these products also serve as substrates for the growth of micro-organisms. Urine and faecal material may form aerosols carrying Gram-negative bacteria (Travers et al., 1988; Clark et al., 1983; Crock et al., 1991). Micro-organisms found in swine confinement buildings are listed in Table 2 (Donham et al., 1986).

Table 2. Micro-organisms in swine confinement buildings:

Bacteria	Moulds	Yeast
Aerococcus viridans	Acremonium	Candida
Bacillus spp	Aspergillus	Cryptococcus
Escherichia coli	Alternaria	Hansenula
Klebsiella	Circinella	Rhodotorula
Micrococcus lylae	Cladosporium	Trichosporon
Pseudomonas	Fusarium	Torulopis
Rhodococcus	Geotrichum	Prototheca
Staphylococcus lentus	Mucor	
S.homini	Paecilomyces	
Xanthomonas	Penicillinum	
Yersinia	Rhizopus	
	Scopulariopsis brevicaulis	
	Stemphylium	

Dairy farmer

Dairy farmers, who have no respiratory symptoms, frequently have signs of an ongoing inflammatory airway reaction reflected by an increased number of activated lymphocytes and antibodies against mould in bronchoalveolar lavage (BAL) fluid (Cormier et al., 1984; Larsson et al., 1988). These results indicate that dairy farmers express an immune response to mould which, in the long-term, may result in allergic alveolitis (Parker et al., 1992; Von Essen et al., 1990).

Swine farmers

In symptom-free swine farmers a subclinical airway inflammatory reaction dominated by neutrophilic granulocytes has been demonstrated by analysis of BAL fluid (Larsson et al., 1992; Pedersen et al., 1996; Malmberg et al., 1991). After a few hours of work in the pig-farming environment the farmer may develop fever and cough, which disappears within 24 hours after exposure. This condition is often referred to as organic dust toxic syndrome (ODTS) (Anonymous, 1994) (May et al., 1990; Malmberg et al., 1993). The prevalence of ODTS in farmers is 6-8% (Zejda et al., 1993) and among swine farmers up to 30% are affected (Donham et al., 1990; doPico et al., 1984). The prevalence of ODTS is 30-50 times higher than that for allergic alveolitis (Malmberg et al., 1988).

Cells participating in airway inflammation caused by inhaled organic dust

BAL fluid from healthy subjects mainly consists of alveolar macrophages (90%), lymphocytes (5-10%) and only few neutrophils and eosinophils (1-2%). The mononuclear phagocytic system consists of monocytes that appear in the blood stream, and macrophages residing in the tissue. Circulating monocytes continuously differentiate into macrophages and migrate into the tissues and macrophages entering the lungs differentiate into alveolar macrophages. During this differentiation the cell enlarge and its intracellular organelles increase in number and complexity. The cells acquire increased phagocytic ability, produce

higher amounts of enzymes and start to secrete a number of soluble mediators such as TNF-α and IL-6 (Schollmeyer et al., 1990; Gordon et al., 1995). Macrophages are capable of ingesting and digesting exogenous antigens such as whole micro-organisms and insoluble particles. Lysosomes contribute with lysozymes and other hydrolytic enzymes for digestion of the internalised content (Russell et al., 1995). The macrophage membrane has receptors for antibody subclasses and for complement components which can bind to antigen, and thereby act as opsonins. Opsonization enhances the phagocytosis more rapidly. The alveolar macrophages are exposed to a large variety of particles in the inhaled air and are thereby contributing to the first line of defence (Gordon et al., 1995).

The white blood cells of the immune system are derived from precursors in the bone marrow. All blood cells arise from hematopoietic stem cells (HSC) that differentiate into other cell types, are self re-newing and maintain their population level by cell division. Particular growth factors and cytokines direct the development into red blood cells, monocytes, granulocytes, lymphocytes or platelets (Golde et al., 1991).

Monocytes circulate in the blood stream for approximately 8 hours after which they differentiate into macrophages (Golde et al., 1991). Lymphocytes constitute 20-40% of the human white blood cells and 99% of the cells in the lymph. The lymphocytes can be divided into three subgroups: T cells, B cells and Natural killer (NK) cells. T cells can be further sub-divided into T helper cells (T_H) and cytotoxic T cells (T_C). T lymphocytes recognise processed antigens together with the MHC complex. T_H cells ($CD4^+$) bind to MHC class II, while cytotoxic T cells ($CD8^+$) bind to MHC class I (Braciale et al., 1987). The main reason for binding to the MHC complex is to recognise and kill infected host cells and to activate other cells of the immune system. $CD4^+$ T cells can further be functionally divided into Th_1 or Th_2 cells (Mosmann et al., 1989) and this may be directed by different cytokines (Reiner et al., 1995). Upon activation Th_1 cells produce IL-2, IFN-γ, TNF–β while characteristic cytokines of Th_2 cells are IL-4, IL-5, IL-6 (Mosmann et al., 1990; Mosmann et al., 1991).

B cells contribute to the immune system mainly by production of antibodies but is also acting as antigen presenting cells. (Henderson et al., 1995)

NK cells play an important role in host defence against tumour cells but also against virus infected cells (Sen et al., 1993; Biron et al., 1995).

Granulocytic cells are divided into neutrophils, eosinophils and basophils. The neutrophils are circulating in the peripheral blood for 7-10 hours before migrating into the tissue, where they have a life span of only a few days (Gallin et al., 1988). The bone marrow releases neutrophils as a response to infection and the neutrophils are the first cells arriving to the site of infection (Rosales et al., 1993). Eosinophils are involved in the defence against parasites and are together with basophils capable of releasing biologically active substances, which play an important role in the allergic response.

Mastcells are large cells containing cytoplasmatic granules. The granules are containing chemical mediators such as histamine and other pharmacologically active substances. Mastcells can be activated to release their granules when

antigens cross link antibodies bound to Fc receptors specific for IgE (FcεR1) and IgG (FcγRIII) (Sutton et al., 1993; Beaven et al., 1993; Schwartz et al., 1994).

Cytokines in the inflammatory response

Cytokines are small proteins released from nucleated cells upon activation. They can exist as cell surface molecules but also as soluble proteins. The following cytokines are described due to participation in the innate inflammatory response although not all of these cytokines are studied in this thesis.

Interleukine-1 is a pro-inflammatory cytokine mainly produced by monocytes, macrophages, B cells, dendritic cells and endothelial cells (Auronet al., 1984). IL-1 activates the vascular endothelium to express the intercellular adhesion molecule (ICAM-1) and has a co-stimulatory activating effect on T-lymphocytes. It also induces fever and increases the production of IL-6 (Acres et al., 1987; Akira et al., 1990).

Naive T cells re-enters the cell cycle upon activation and divide rapidly. The proliferation and differentiation is driven by interleukine-2 (IL-2) a protein growth factor, produced by the activated T cell itself (Jain et al., 1995; Robertson et al., 1990). IL-2 therefore has an autocrine as well as a paracrine function, which supports long term growth and enhances T cell activity. Co-stimulatory signals are required for synthesis and secretion of IL-2. The CD28 molecule, on the T cell, has to bind to its ligand CD80/CD86 on antigen presenting cells (Fraser et al., 1991; Allison et al., 1994). When the co-stimulatory signal CD28- CD80/CD86 fails the T cell becomes anergic which results in an attenuated production of cytokines particularly IL-2 (Fields et al., 1996).

Interleukine-4 is mainly produced by Th_2 cells (Bendelac et al., 1997) but also by mastcells (Schwartz et al., 1994) and the target cells are B cells (Banchereau et al., 1994). It has the capacity to up-regulate class II MHC expression on resting B cells. IL-4 is capable of down regulating Th_1 activity and to promote Th_2 development and direct class switch IgG_1 into IgE (Lorenz et al., 1995; Stavnezer et al., 1996).

Interleukine-6 (IL-6) is produced mainly by monocytes, macrophages and T cells and stimulates lymphocyte activation and antibody production. IL-6 also induces formation of acute phase protein in the liver and is responsible for induction of fever (Gauldie et al., 1987; Muraguchi et al., 1988; Ritchie et al., 1983; Nijsten et al., 1987).

Interleukine-8 (IL-8) is produced by phagocytic cells such as monocytes, neutrophils and endothelial cells and functions as a chemotactic factor for neutrophils (Cassatella et al., 1992; Streiter et al., 1992; Fujishima et al., 1993).

Interleukine-12 (IL-12) is mostly produced by monocytes and macrophages (D´Andrea et al., 1992). IL-12 is also known as natural killer cell stimulatory factor (NKSF) (Kobayashi et al., 1989) and it is a pleiotropic cytokine, that activates NK cells and induces differentiation of CD4 Th_0 cells into Th_1 cells (Germann et al., 1993).

Tumour necrosis factor-alpha (TNF-α) is produced by monocytes, macrophages, mast cells and T cells. It activates the vascular endothelium and

increases vascular permeability, which leads to increased fluid drainage and the entry of IgG, complement and leukocytes to the tissue (Amitage et al., 1994; Beutler et al., 1990). TNF-α, like IL-1 and IL-6, induce fever (Beutler et al., 1990) and may, when released into the blood stream in high concentrations, induce circulatory shock (Baumann et al., 1994).

Interferon-gamma (IFN-γ) is a potent multi-functional interferon secreted by activated NK cells and T cells (DeMaeyer et al., 1988). Human IFN-γ is a 35 kD protein and contains 143 amino acid residues (Sen et al 1993). IFN-γ has antiviral effects and it has the capacity to up-regulate a number of cell functions of macrophages, NK cells and neutrophils such as anti-microbial and anti-tumour responses (Sen et al., 1993; DeMaeyer et al., 1988). IFN-γ can also exert strong regulatory influence on the proliferation and differentiation of effector responses of B cell and T cell subsets and it has Th_1 promoting effect (Pernis et al., 1995). These influences can involve IFN-γ´s capacity to increase the expression of MHC class I and MHC class II present on antigen presenting cells (APC) but it has also a direct effect on NK cells and T cells. IFN-γ also up-regulate ICAM-1 expression on endothelial cells (DeMaeyer et al., 1988; Arai et al., 1990).

Lymphocyte activation markers

CD69, the earliest activation molecule also known as Activation Inducer Molecule (AIM) or Early Activation Antigen (EAA) is expressed by T, B NK cells and eosinophils (López-Cabrera et al., 1993). CD69 is involved in early events of lymphocyte, monocyte and platelet activation and contributes to T cell activation, by increased Ca^{2+} influx, and in the synthesis of different cytokines, and their receptors (Testi et al., 1989; Testi et al., 1989).

The expression of CD25 (IL-2 receptor) is increased on T cells following activation (Corrigan et al., 1992; Hemler et al., 1984). The high affinity IL-2 receptor is a three chain structure (alpha (α), beta (β) and the gamma (γ) chains) expressed only on activated T cells (Leonard et al., 1994). The β and γ chains are expressed constitutively on resting T cells. Upon activation, T cells induces synthesis of the α chain and the high affinity heterotrimeric receptor will be formed and responds strongly to very low concentrations of IL-2 (Jain et al., 1995; Minami et al., 1993). HLA-DR (human leukocyte antigen) also called transplantation antigens are present on all nucleated cells. HLA-DR is a late activation marker for T cells (Corrigan et al., 1992).

Table 3. Other surface markers CD (Cluster of Differentiation) used in this studies are :

CD2	Expressed on T cells, thymocytes and NK cells, also called E-rosette receptor, and is an adhesion molecule involved in T cell activation
CD3	Composed of a complex of five invariant polypeptide chains that associate with the T cell receptor
CD4	Co-stimulary receptor to MHC class II restricted T cells
CD8	Co-stimulary receptor to MHC class I restricted T cells
CD14	LPS receptor expressed on monocytes and macrophages.
CD16	FcγRIII expressed on macrophages, NK cells and neutrophils, involved in phagocytosis of antibody complexed antigens and in antibody-dependent cell mediated cytoxicity (ADCC).
CD19	The earliest cell surface molecule recognisable B lineage cells.
CD45	Leukocyte common antigen. CD45 RA is expressed on naive cells and CD45 RO is expressed on mature (memory) cells
CD56	NKH1 neural cell adhesion molecule (NCAM). Expressed on human NK cells and a subset of T cells.

Aims

The aims of the thesis are:

1. To investigate the participation and activation of lymphocytes in the inflammatory response to inhaled dust *in vivo*.
2. To characterise the human T cell response to the dust *in vitro*.
3. To study the role of phagocytic cells and humoral mediators in dust induced T cell activation.

Methods

Subjects

In study I, 24 healthy, non-smoking, non-allergic subjects underwent bronchoalveolar lavage (BAL) and blood was collected. In study II-IV, blood was collected from 22 healthy, non-smoking, non-allergic subjects.

Bronchoalveolar Lavage

Bronchoalveolar lavage (BAL) was performed 14 days before exposure and 24 hours after the start of exposure in a swine house (I). BAL was performed by the use of flexible fibreoptic bronchoscopy under local anaesthesia after pre-medication with morphine-scopolamine. Five aliquots of 50 ml sterile saline was instilled, and the fluid was gently aspirated and collected in a siliconized plastic bottle kept on ice. The fluid was centrifugated at 400g for 5 minutes at 4°C and cells and supernatant were separated. The BAL technique has been described in detail by (Eklund et al., 1986)

In vitro exposures

Dust, collected approximately 1.5 m above the floor in a swine confinement building, and the polyclonal activator, phytohemagglutinin (PHA), used as positive control, was dissolved in RPMI 1640, sonicated for 10 minutes, and incubated with heparinized whole blood (II, III, IV). Whole blood containing RPMI 1640, was used as negative control (study II, III, IV).

Blood was incubated with dust in RPMI, at different concentrations (10, 31.4, 100 and 314 μg/ml) (II), or 50 μg/ml (III) or 100 μg/ml (IV). In parallel blood was incubated with PHA at (3.14, 10, 31.4 or 100 μg/ml) (II), and 10 μg/ml (III, IV). RPMI 1640 only was used as negative control (II, III, IV). The blood sample tubes were closed and incubated at 37°C in a shaking water bath for 4, 24, 48 and 72 hours, (II) and incubated for 24 hours (III) or 1 and 22 hours (IV).

Cell Preparation Tubes was used for cell gradient separation (III). Peripheral blood mononuclear cells (PBMC) was incubated with plasma received from whole blood previously incubated with 50 μg/ml swine dust, i.e. “swine dust conditioned plasma” or "10 μg/ml PHA conditioned plasma" or "RPMI conditioned plasma" for 24 hours (III). PBMC was also resuspended in autologous plasma and incubated with 50μg/ml swine dust, 10 μg/ml PHA or RPMI for 24 hours (III).

Phagocytic cells were removed from whole blood through incubation with carbonyl 5μm iron particles, 4 mg/ml (III). Blood and carbonyl iron particles were incubated for 30 min at 37°C and mixed occasionally. After incubation the blood sample tubes were placed on one of the pools of a magnet for 10 min at 4°C. The non-iron containing i.e. the non-phagocytic cells were removed (with the tube still standing on the magnet) and transferred to a second plastic tube. Cells were

resettled on the magnet for further 10 min at 4°C and the procedure was repeated. The non-phagocytic cells were incubated with swine dust (50 μg/ml), PHA (10 μg /ml), or with RPMI for 24 hours (III).

Whole blood was also activated in the presence of brefeldin A and swine dust (100μg/ml), PHA (10μg/ml) or RPMI 1640 (control) for 1 hour, or incubated 12 hours with swine dust (100μg/ml), PHA (10μg/ml) or RPMI 1640 (control) and additional 10 hours in the presence of brefeldin A (IV). Whole blood was pre-incubated with control and blocking antibodies against the human IL-12 receptor for 1 hour. Thereafter the blood was incubated for 12 hours with swine dust (100μg/ml), PHA (10μg/ml) or RPMI 1640 (control) and additional 10 hours in the presence of brefeldin A. All incubations in study IV were performed at 37°C in the presence of 5 % CO_2 .

Intracellular staining procedure

Whole blood was diluted 1:1 with the culture medium RPMI 1640 containing 1% L-glutamate in the presence of brefeldin A, and incubated for 1 or 10 hours with dust or PHA. Brefeldin A blocks the intracellular protein transport process. This results in the accumulation of secreted proteins in the Golgi complex and enhances detection of normally secreted protein (Jung et al., 1993). Red blood cells were lysed with lysing solution. A combination of fixation with paraformaldehyd and permeabilization with the detergent saponin was used. For optimal intracellular staining of cytokines, fixation and permeabilization is necessary before staining intracellular antigens with fluorochrome conjugated antibodies and to maintain cells in a permeabilized state during the operating procedure according to the manufacturer (IV).

Flow cytometry

Flow cytometry was used for analysis and separation of cells stained with fluorochrome labelled antibodies. In flow cytometery, a laser beam is used and a light detector counts and registers the characteristics of intact cells in suspension. When a single cell passes the laser beam the laser light is deflected from the detector, and the interruption of the laser signal is recorded. The cells are detected by size, translated by the laser beam as forward scatter and by complexity translated as sidescatter. Fluorochrome conjugated monoclonal antibodies bound to the cell surface of a certain cell type are excited by the laser beam and emit light that is recorded by a second detector located at a right angel to the laser beam. The instrument counts each cell as it passes the laser beam and records the levels of fluorescence emitted by the cell. An attached computer generates plots of cell number of cells and their fluorescence intensity (I, II, III, IV).

ELISA technique

A solid phase sandwich ELISA (Enzyme Linked Immuno Sorbent Assay) was used. Monoclonal antibodies specific for human cytokine of interest were coated

on a 96 well plate. Known standards and samples were added to the wells and the present cytokine binds to the specific antiboby. After washing the plate, a mixture of biotinylated anti-human cytokine- antibody and avidin-horseradish peroxidase was added (antibody-antigen-antibody "sandwich"). After a second wash, the substrate (hydrogen peroxide/ tetramethylbenzidine TMB) solution was added, and a blue colour was developed which is direct proportional to the amount of the cytokine present in the initial sample. A stop solution (phosphoric acid, 1M) changes the colour from blue to yellow, and the wells were read at 450 nm in a spectrophotometer (II, III).

Statistics

In all studies, results are presented as median values and 25^{th}-75^{th} percentiles. Wilcoxon´s signed rank test was used for paired comparisons (I).

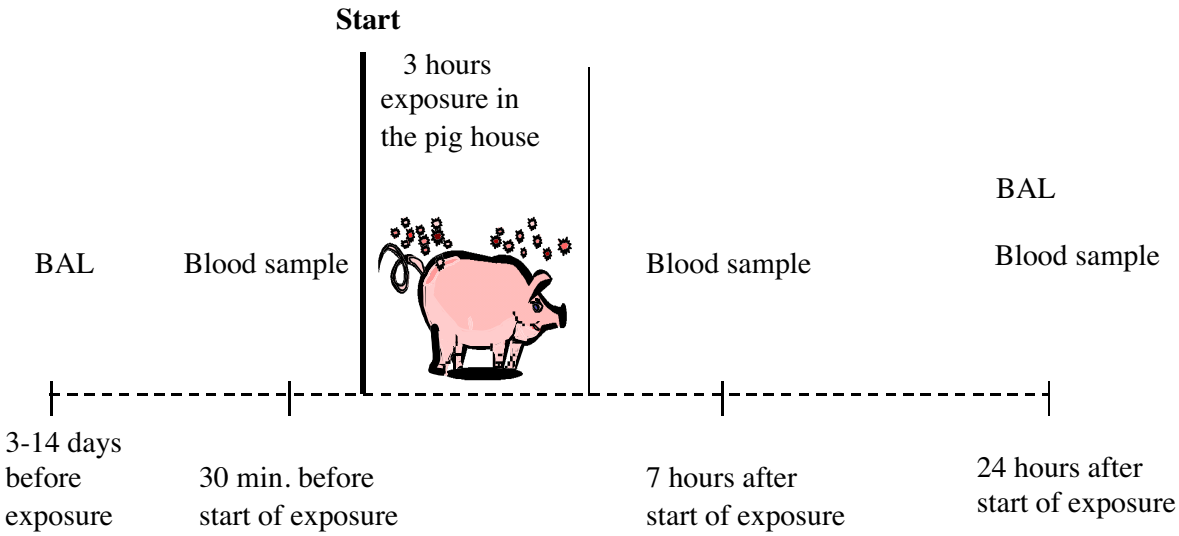
In vitro comparisons were performed by ANOVA with post hoc Fischer´s PLSD (II-IV). A p-value < 0.05 was considered significant.

Results and discussion

In study I, healthy subjects were exposed to dust in a swine confinement building during 3 hours, while weighing swines prior to slaughter. The air in the confinement buildings contained a high concentration of inhalable dust 20-23 mg/m^3 and the respirable fraction, i.e. particles less than 5μm, was 0.7-1.0 μg/m^3. The study design is shown in Figure 1.

Symptoms as fever, headache, malaise, shivering and muscle pain was registered. The exposure induced an increase of IL-6, IL-8 and TNF-α in BAL fluid (Wang et al., 1997).

Figure 1.Study design



An increased number of neutrophils in peripheral blood were observed 7 and 24 hours after the start of exposure, whereas the number of lymphocytes decreased (Figure 2).

Swine dust inhalation induced > 50 fold increase in the number of granulocytes in BAL fluid (Figure 2). The concentration of alveolar macrophages in BAL was approximately doubled (90 (68 - 106) x 10^6 cells/L to 190 (136 - 307) x 10^6 cells/L) and the lymphocytes in BAL fluid increased three times (Figure 2).

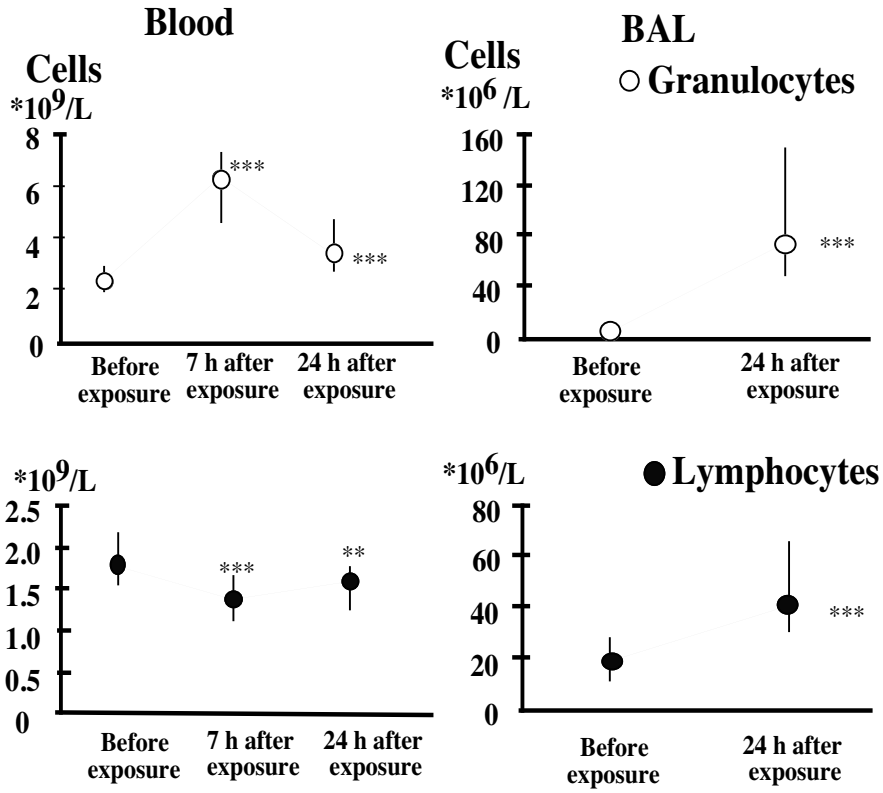


Figure 2. Granulocyte and lymphocyte numbers in peripheral blood and BAL fluid before and after exposure to swine dust. The results are presented as medians and interquartile ranges. ** p< 0.01, *** p< 0.001 compared to pre-exposure values.

Not only the number of BAL lymphocytes increased following exposure in the swine confinement house, but also the expression of activation markers (CD69, CD25 and HLA-DR) on T cells increased (Figure 3).

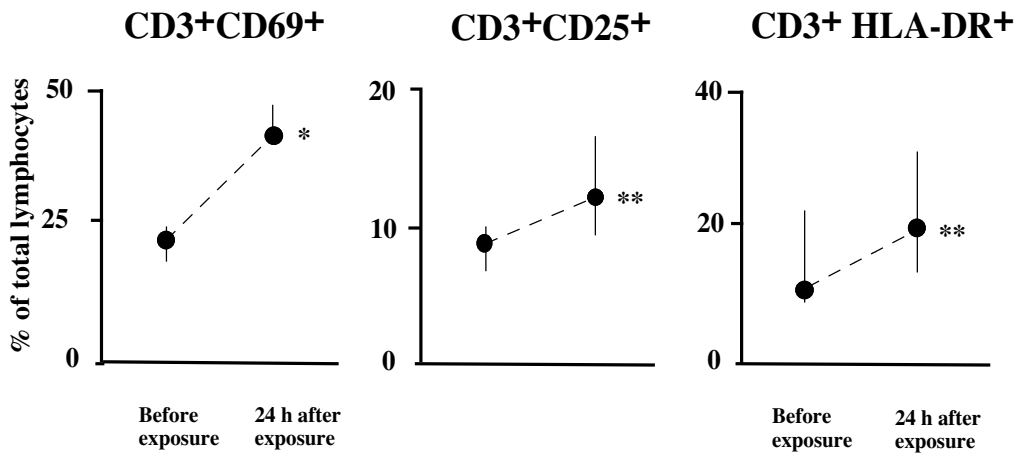


Figure 3. Activation markers in BAL fluid before and after exposure to swine dust. The results are presented as medians and interquartile ranges. * p<0.05, ** p< 0.01 compared to pre-exposure values.

In study II, we used a logarithmic titration concentration of dust to obtain a wide range in order to find a time and dose dependent response of the activation markers CD69, CD25 and HLA-DR. After incubation *in vitro* with swine dust, PHA (positive control) or RPMI-1640 (negative control) the expression of CD69 and CD25 reached its maximum after 24 hours. HLA-DR expressed on CD3 positive cells increased from 1.5 % to 13 % after 72 hours incubation with high concentrations of dust. We followed the activation for 72 hours, and it is possible that the HLA-DR expression had not reached its maximum at that time point (Figure 4).

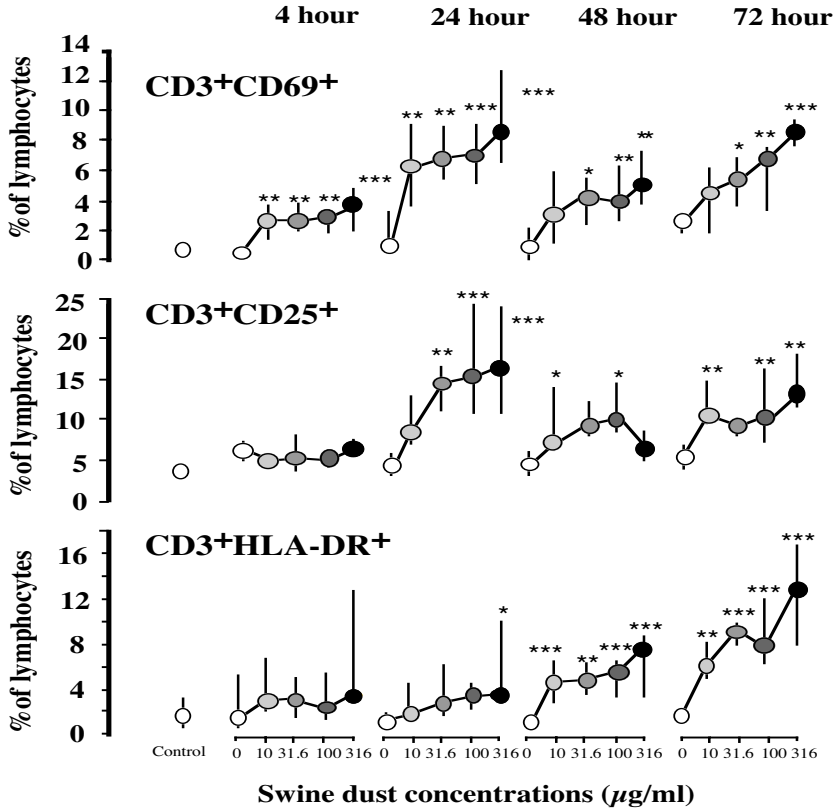


Figure 4. The activation markers CD69, CD25 and HLA-DR expressed on T-lymphocytes (CD3+) in peripheral blood incubated with swine dust in a dose-response manner for 4, 24, 48 and 72 h. Results are presented as medians and interquartile ranges. * p< 0.05, **p<0.01, ***p<0.001 compared to unstimulated blood (time-point 0) at each incubation time-point. Control = unstimulated blood, pre-exposure value.

The expression of CD69 and CD25 decreased after 48 hours incubation compared with 24 and 72 hour whereas HLA-DR did not (Figure 4). We hypothesised that a shedding of the IL-2 receptor might explain the reduced expression of CD25. Therefore the soluble IL-2 receptor-α (IL-2sRα) in plasma, obtained from blood samples incubated with swine dust or PHA, was investigated. We found a continuous time and dose dependent increase of the soluble IL-2sRα (II), which thus differed from the pattern observed for CD69 and CD25. Thus, we found no corresponding reduction of the IL-2sRα levels after incubation with dust for 48 hours compared to 24 hours and 72 hours.

In study III whole blood was incubated with swine dust for 24 hours, which induced 27.6 % of the CD3 positive T cells to express CD69 compared to 3.3 % of T cells in non-stimulated whole blood ($p < 0.0001$). Whole blood incubation with PHA induced CD69 expression in 71.3 % of the T cells (Figure 5).

When PBMC was separated from whole blood and incubated with dust for 24 hours no significant CD69 expression on T cells was found, while PHA induced CD69 expression in 70.9 % of the T cells (Figure 5).

Phagocytic cells were removed from whole blood through incubation with carbonyl iron, reducing the proportion of monocytes from 7 % to 1 % and of granulocytes from 58 % to 35 % (median values). In such "phagocyte reduced" whole blood, incubation with dust for 24 hours induced CD69 expression in only 4.5 % of the T cells compared to 1.5 % in non stimulated T cells (ns). Incubation with PHA as a positive control, however, induced CD69 expression on 71.3 % of the T lymphocytes (Figure 5).

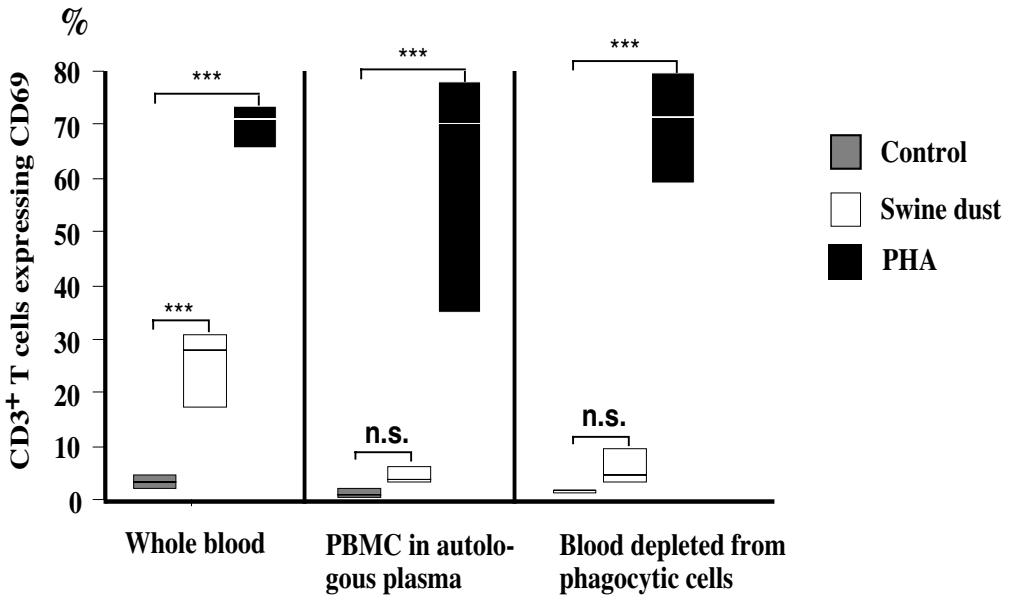


Figure 5. CD69 expression of T lymphocytes in peripheral whole blood (A), PBMC resuspended in autologous plasma (B) and peripheral blood depleted from phagocytic cells (C), following incubation with either RPMI (control), swine dust or PHA for 24 hours. Values are presented as median values and interquartile ranges. n.s.= no significant difference *** $p < 0.001$ compared to unstimulated cells.

Conditioned plasma

We transferred plasma from dust exposed whole blood, i.e. "conditioned plasma", and incubated with PBMC for 24 hours. Following this incubation, 32.4 % of the CD3 positive T cells expressed CD69 compared to 3.3 % of T cells of PBMC incubated with control plasma ($p < 0.0001$). Plasma from whole blood stimulated with PHA induced expression of CD69 to a similar level (Figure 6).

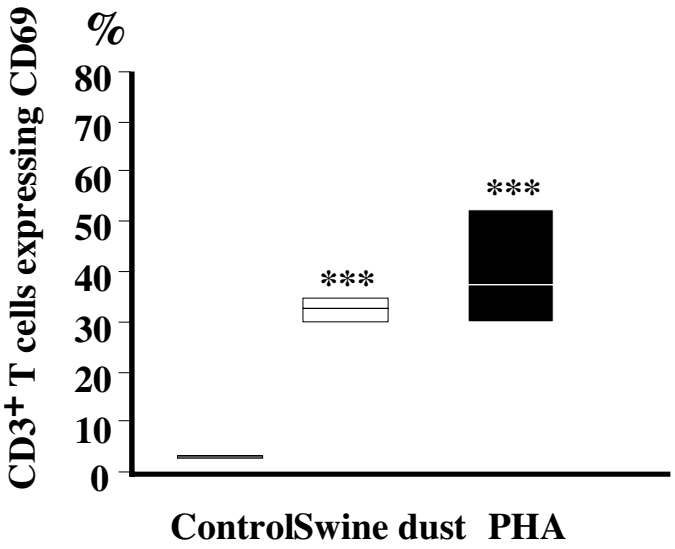


Figure 6. CD3+ T lymphocytes in PBMC expressing CD69 following 24 hours incubation with RPMI (control), plasma from swine dust (white box) or PHA (black box) conditioned plasma. Values are presented as median values and interquartile ranges. *** $p < 0.0001$ compared to control.

Soluble mediators in conditioned plasma

In an attempt to identify soluble mediators of importance for the T lymphocyte stimulation, we measured IL-12 and IFN-γ concentrations in conditioned plasma. In the dust incubated whole blood, i.e. the conditioned plasma, the IL-12 concentration was 14.1 pg/ml, while the concentration was below detection limit in control plasma. The concentration of IL-12 in PHA stimulated whole blood was 47.4 pg/ml. In plasma of PBMC stimulated with dust, the IL-12 concentration was 2.3 pg/ml. Supernatant from PHA stimulated PBMC contained 5.9 pg/ml, while negative controls had undetectable levels (Figure 7).

Interferon-γ levels in dust conditioned plasma were 2284 pg/ml compared to 4776 pg/ml in PHA stimulated plasma and 6.5 pg/ml in control plasma. The interferon-γ in PBMC plasma from swine dust incubations was 196 pg/ml, compared to 804 pg/ml in PHA stimulated PBMC and 6.0 pg/ml in control supernatant (Figure 7).

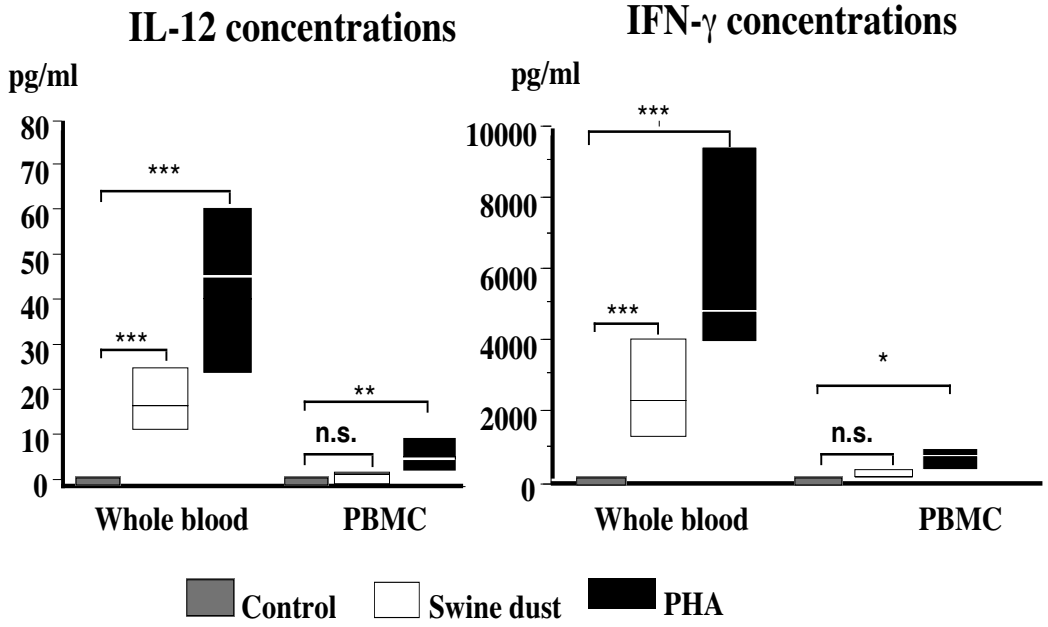


Figure 7. IL-12 and IFN γ concentrations in plasma from whole peripheral blood and PBMC, incubated with either RPMI (control) swine dust or PHA for 24 hours. Values are presented as median values and interquartile ranges. n.s.= no significant difference; * $p<0.05$; ** $p< 0.001$; *** $p< 0.0001$ compared to control.

Intracellular cytokine production

In study IV 100 μg dust/ml blood was used to stimulate cytokine production. After one hour of incubation with dust, TNF-α production was detected in 17.8 % of the monocytes defined as $CD14^+$ cells, ($p<0.0001$) compared to 0.2 % of the monocytes incubated with RPMI (control). Likewise, dust-induced IL-12 production was found in 19.1% of the monocytes exposed to dust and 0.8 % in the negative control ($p<0.0001$) (Figure 8).

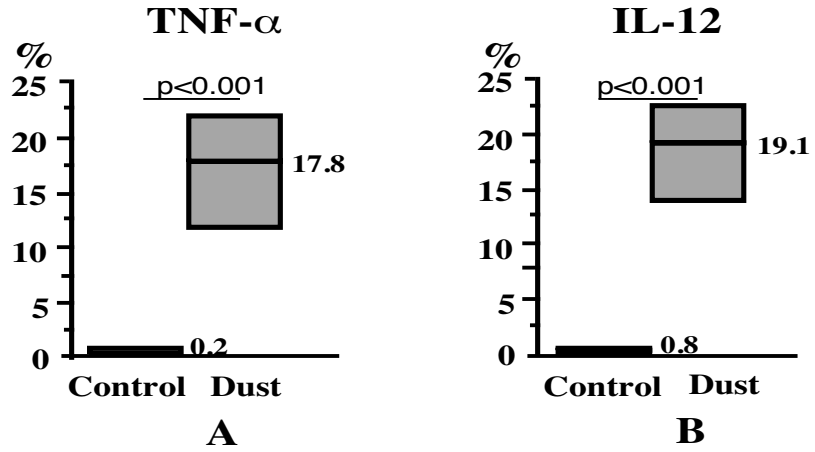


Figure 8. TNF-α and IL-12 production from monocytes (CD14+). Results are presented as medians and interquartile ranges.

No intracellular cytokine production was observed in lymphocytes after one hour of incubation with dust. However 22 hours dust incubation including brefeldin A during the last 10 hours, generated IFN-γ production in 7.8 % of the NK cells (i.e. $CD3^-$ $CD56^+CD16^+$ in the lymphocyte gate) and in 0.8 % in negative controls (p=0.0001). TNF-α was found in 3.6 % of the NK cells compared to 0.9 % in negative controls (Figure 9).

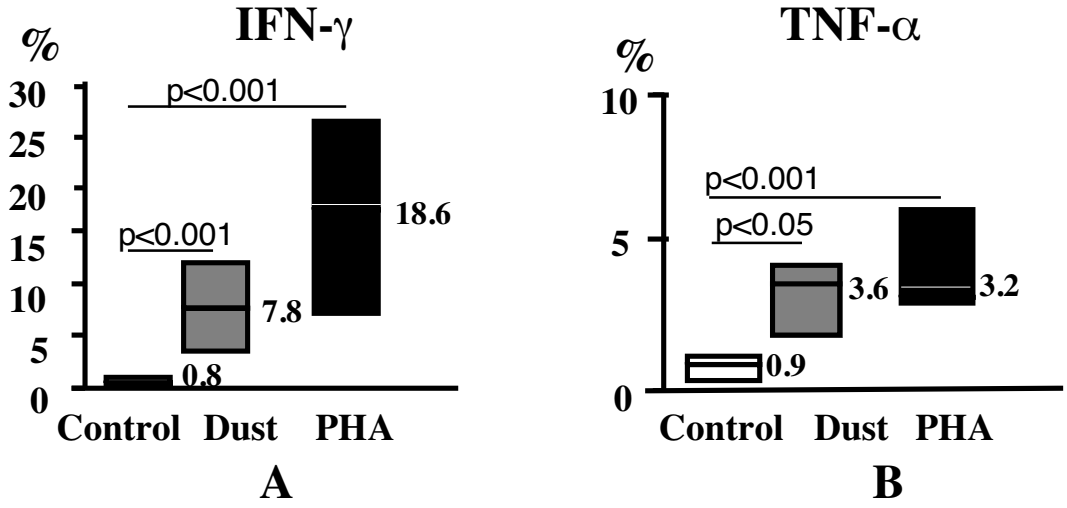


Figure 9. IFN-γ and TNF-α production by NK cells (CD3-CD56+CD16+). Results are presented as medians and interquartile ranges.

Following 22 hours of dust stimulation, 2.5 % of the $CD3^+$ T cells, 1.8 % of the $CD4^+$ T cells and 5.0 % of the $CD8^+$ cells produced IFN-γ. The levels of TNF-α and IL-4 in $CD3^+$ T cells, $CD4^+$ T cells and $CD8^+$ T cells were slightly, but not significantly increased (2-3%) compared to negative controls. We then used IL-12 receptor blocking antibodies to investigate any effect on IFN-γ production by NK cells and on the CD69 cell surface expression on T cells in whole blood stimulated with dust. We found a reduced IFN-γ production by NK cells from 7.8% to 1.8 % by IL-12 receptor blocking antibodies (Figure 10).

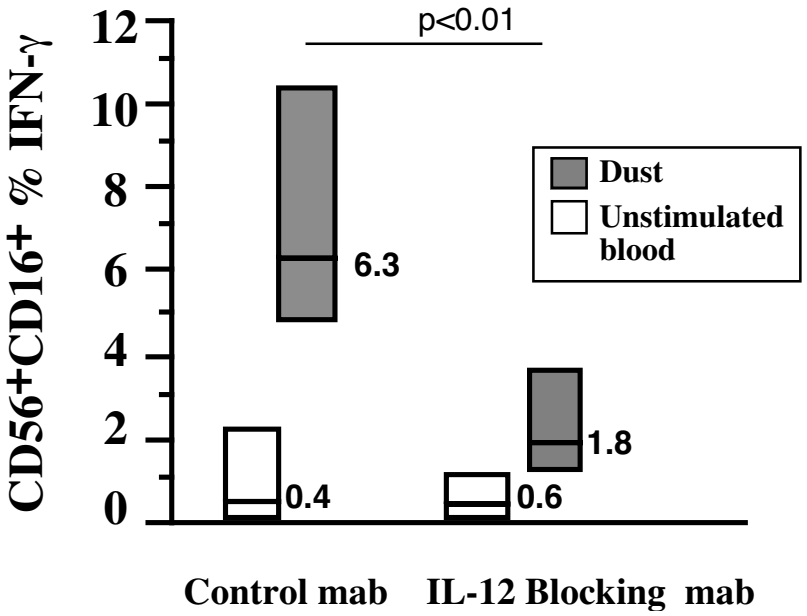


Figure 10. IFN-γ production in NK (CD56+CD16+) cells after incubation with dust. Results are presented as median values and interquartile ranges.

In four experiments the CD69 expression on $CD3^+$T cells decreased from 19.1% to 12.2% in the presence of IL-12 receptor blocking antibodies (Figure 11). No difference was found when dust was incubated with whole blood in the presence or absence of control antibodies (data not shown).

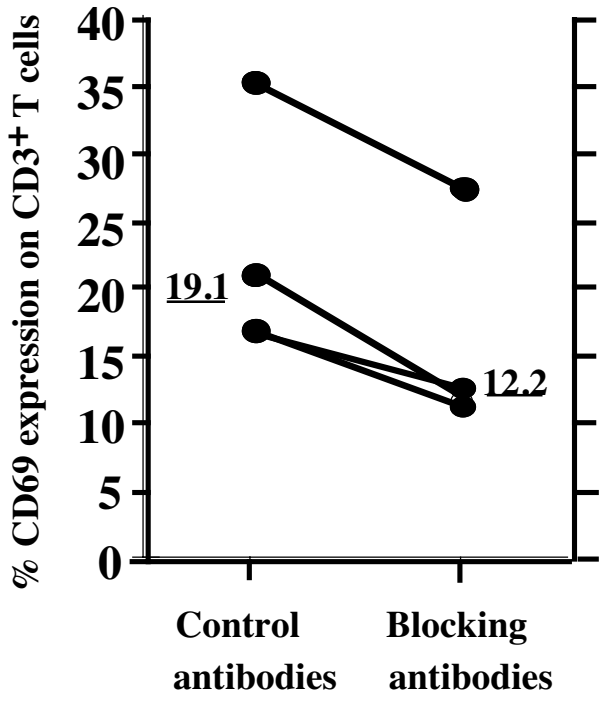


Figure 11. CD69 expression on T cells ($CD3^+$) in whole blood pre-incubated with control or blocking antibodies to the IL-12 recptor and stimulated with dust.

We have previously demonstrated that three hours´ exposure in a swine confinement facility induces a massive influx of inflammatory cells into the airways in healthy subjects. The influx was dominated of neutrophilic granulocytes, which increased more than 50 fold in BAL fluid obtained 24 hours after the start of exposure (Larsson et al., 1994). We also found a 2-3 fold increase of macrophages and T-and B cells in BAL fluid. The proportion of T cells was unaltered while the proportion of B cells was slightly increased. In blood the number of neutrophils also increased after exposure while lymphocytes decreased, probably reflecting an increased recruitment of lymphocytes to the airways (Berman et al., 1990; Dunkley et al., 1995; Nelson et al., 1990; Picker et al., 1994). The time course for this lymphocyte recruitment is not known but there are data suggesting that the lymphocyte influx commence soon after the start of exposure (Berman et al., 1990). High levels of IL-8 and IL-17 (Laan et al., 2002) which may function as chemoattractants for neutrophils and also for lymphocytes (IL-8), were found in BAL fluid following dust exposure (Bellini et al., 1993; Marini et al., 1992; Larsen et al., 1989). While IL-8 is produced mainly by monocytes and macrophages, it is assumed that IL-17 originates from activated lymphocytes (Laan et al., 2002; Yao et al., 1995; Yao et al., 1995). We also found signs of activation of BAL fluid T lymphocytes, with increased expression of CD69 after exposure. CD69 expression reaches peak levels 18 - 24 hours after stimulation *in vivo* (Testi et al., 1989; Testi et al., 1989; Testi et al., 1994) i.e. approximately at a time point when BAL lymphocytes were analysed after stimulation *in vivo* in the present study. Approximately 22 % of the lymphocyte gated BAL cells expressed CD3 and CD69 prior to exposure with an increase up to 45% after exposure. It could be expected that a large number of airways T cells normally express CD69 since cells in the airways continuously are exposed to inhaled agents that are capable of inducing cell activation. In previous studies on BAL T cells from patients with sarcoidosis and healthy subjects (Katchar et al., 2001) the CD69 expression on CD4+ and CD8+ T cells was significantly higher than in our study. Other groups have however, published data more similar to the present data (Meyer et al., 1999). These differences could partly be explained by differences in data calculation. In the present study we calculated CD3 and CD69 as percentage of the BAL lymphocyte population. In the study by Katchar et al. (2001) CD69 is calculated as percent of the $CD3^+$ population.

We found an increased expression of CD25 as well as HLA-DR on T cells both in BAL fluid *in vivo* and in peripheral blood *in vitro* following exposure. It seems likely that the IL-2 receptor expression on T cells, obtained from BAL fluid 24 hours after exposure, reflects maximal or near maximal activation, assuming that the time course for lymphocyte activation by inhaled swine dust is similar to that of antigen stimulation *in vitro*. HLA-DR molecules are maximally expressed on the T lymphocyte surface several days after antigen stimulation (Corrigan et al., 1992). Although we could detect a significant increase of the HLA-DR expression on CD3 cells *in vivo*, we have probably been able to demonstrate this only at an

early phase and it is conceivable that maximal expression would have occurred later (Corrigan et al., 1992; Corrigan et al., 1995).

In study II we analysed *in vitro* activation of dust stimulated blood as assessed by the expression of the activation markers CD69, CD25 and HLA-DR.
We showed that the activation was dose dependent and that the time course of stimulation was similar for dust and PHA.
The T cell activation assessed by expression of CD69 and CD25, tended to be biphasic with attenuation after 48 hours of exposure. This may, at least in part, be explained by antigen receptor shedding of the IL-2 receptor. The assumption of receptor shedding is supported by the finding of a dose and time dependent, continuous (non-biphasic) increase of the soluble IL-2 sRα in plasma from blood samples incubated with swine dust.

There were differences in the magnitude of the CD69 expression on T cells in study II and III. This variation may be explained by differences in optimal incubation conditions. We used closed sample tubes in study II and plastic plates in study III.

Interestingly, when incubating swine dust with PBMC separated from whole blood, T lymphocytes were not activated. In contrast to whole blood, the PBMC fraction consists mainly of lymphocytes. T lymphocytes can be activated through different mechanisms. Such as a specific activation via (antigen presenting cells) APC presenting antigenic peptides in the context of MHC molecules and recognised by the T cell receptor (TCR) or by superantigens that binds outside the MHC molecule and to the Vβ segment of the TCR. There are also unspecific activation mechanisms induced by mitogens (plant origin or LPS) e.g. PHA (which was used as positive control in study II, III and IV) or soluble mediators e.g. cytokines (Figure 12).

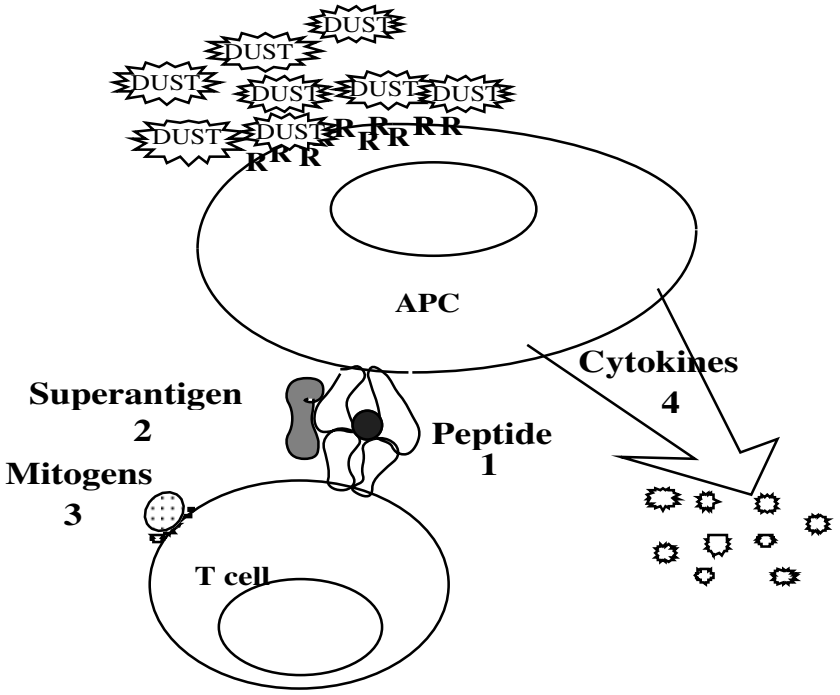


Figure 12. Possible mechanisms of lymphocyte activation.

After finishing study III, it was concluded that cytokines probably are important for the T cell activation. We also investigated whether phagocytic cells such as granulocytes and monocytes could influence the dust-mediated activation of T lymphocytes. A reduced number of phagocytes inhibited the capacity of the dust to activate T lymphocytes whereas plasma from swine dust exposed whole blood, i.e. conditioned plasma, induced lymphocyte activation in PBMC. Based on these data we concluded that T cell activation induced by the dust requires phagocytic cells. The T cell activation did not seem to be mediated by a direct cell-to-cell interaction but rather by the release of one or more mediators.

The finding of high levels of IL-12 and IFN-γ in the conditioned plasma is in line with this hypothesis. Thus phagocytic cells such as granulocytes and monocytes present in whole blood during swine dust incubation could be responsible for the release of IL-12 and IFN-γ, two cytokines that are known to be of importance in T cell activation (D´Andrea et al., 1992; Mattern et al., 1998).

To improve the understanding of the immune response following exposure to organic dust, intracellular cytokine release of swine dust activated peripheral blood was studied. In kinetic studies of whole blood stimulated with dust, we found that one hour of incubation was enough to induce monocytes to produce TNF-α and IL-12. A previous report also showed a rapid IL-12 production of monocytes after stimulation with Trypanosoma cruzi (Frosch et al., 1996) while LPS was less sufficient to induce IL-12 production by monocytes (Frosch et al., 1996). We were also unable to induce IL-12 production by monocytes after one hour of stimulation with LPS (unpublished data). One hour's incubation with dust did not result in cytokine release by NK or T cells. However after incubation with dust for 22 hours, increased levels of IFN-γ and TNF-α were produced by NK cells while T cells only produced low levels of these cytokines after dust incubation. Other groups also found NK cells to be a more efficient IFN-γ producers than T cells (Vitale et al., 2000). Compared to PMA and PHA stimulation of T cells, swine dust induces lower levels of cytokine production from T cells (Wahlström et al., 2001; Vitale et al., 2000). We also found that blocking antibodies to the IL-12 receptor had an inhibitory effect on IFN-γ production by NK cells and on CD69 expression on T cells. IFN-γ production was difficult to evaluate in T cells due to the low IFN-γ levels in those cells. An inhibitory effect on NK cell IFN-γ production by blocking the IL-12 receptor was also demonstrated by Wu et al (Wu et al., 1996). Furthermore, in that study blocking the IL-12 receptor inhibited T cell proliferation, which is in accordance with our results and also strengthens our hypothesis that IL-12 may be crucial for dust induced T cell activation (Wu et al., 1996).

It is unknown what agents in the swine dust that are responsible for the inflammatory reaction and activation of lymphocytes. However, dust in swine confinement buildings contains microorganisms, dominated by Gram-positive bacteria (Crock et al., 1991). Lipopolysacharide and peptidoglycans, used as markers for Gram-negative and Gram-positive bacteria, have been found in concentrations of approximately 4 and 6.5 μg per m^3, respectively in air sampled from swine confinement buildings (Wang et al., 1997). The measured endotoxin content was only 2.2 ng/100 μg swine dust and it has been demonstrated that

swine dust is a far more potent cell activator than LPS (Palmberg et al., 1998). In a recent report, it was found that NK ($CD3^{-}CD56^{+}$) cells proliferated and expanded in cultures in response to LPS (Goodier et al., 2000). The response was enhanced after removal of $CD14^{+}$ cells from the culture. Our studies demonstrated that depletion of $CD14^{+}$ cells inhibited the dust induced CD69 expression on T cells. If LPS would be the main component in dust responsible for the T cell activation in our studies it should also have activated lymphocytes to produce IFN-γ, but no IFN-γ was detected in plasma samples from PBMC incubations (Study III).

Gram-positive bacteria are capable of T lymphocyte activation (Fleischer et al., 1992; Dannecker et al., 1994; Herman et al., 1991) and may thus be involved in the swine dust induced lymphocyte activation. Peptidoglycans have been found to correlate with the increased number of granulocytes in BAL, following dust exposure (Larsson et al., 1997). Wang et al also found a correlation between peptidoglycan and IL-6 in serum and with granulocyte concentrations in blood (Wang et al., 1996). It has been shown that Gram + bacteria and their cell wall components stimulate cellular responses similar to LPS, i.e. by using the CD14 receptor (Gupta et al., 1996) and/or the Toll-like receptor 2 (Yoshimura et al., 1999). Medzhitov et al demonstrated that Toll-like receptor 2 depleted mice had no response to two major pathogen-associated molecular patterns: peptidoglycans and lipoproteins. Thus the Toll-like receptor 2 is important for recognition of peptidoglycans and lipoproteins (Medzhitov et al., 2000). Interestingly the Toll-like receptor 2 was recently shown to be highly expressed in human airway epithelial cells (Diamond et al., 2000) and may thus be involved in the airway response to inhaled swine dust.

In conclusion we have found that swine dust activates BAL fluid T cells *in vivo* as assessed by the expression of the activation marker CD69, CD25 and HLA-DR.

In vitro stimulation of whole blood increased expression of CD69, CD25 and HLA-DR in a time and dose dependent manner. Soluble mediators released by phagocytic cells seem to be of importance for the T cell activation since plasma pre-stimulated with dust i.e. conditioned plasma was able to induce CD69 expression on $CD3^{+}$ cells from PBMC fractions. It was also demonstrated that when the IL-12 receptor was blocked with blocking antibodies NK cells produced less IFN-γ and the CD69 expression on T cells decreased.

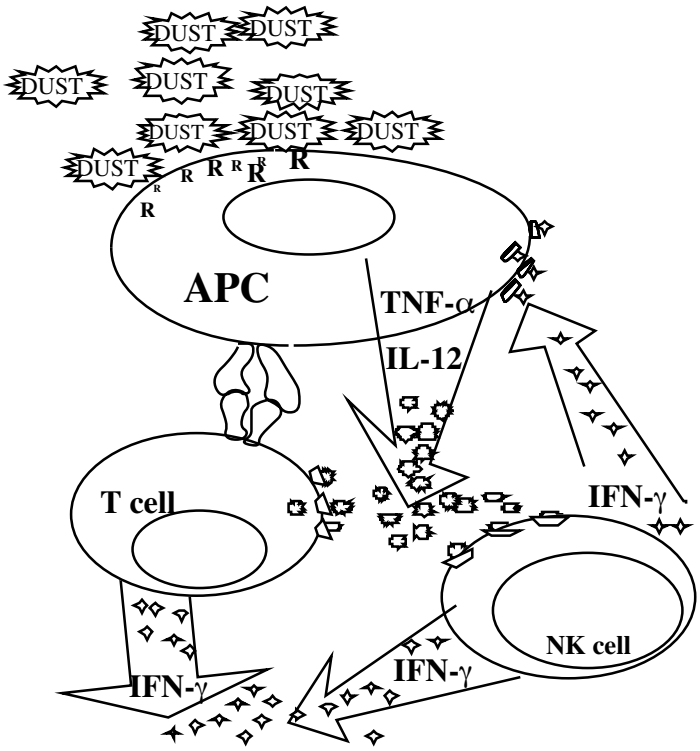


Figure 13. Cytokines released from monocytes /macrophages as a possible mechanism for lymphocyte activation.

Summary

Farmers suffer more frequently from inflammatory airway disorders than the population in general and a ongoing airway inflammation has been shown also in symptomfree healthy farmers. Exposure in a swine confinement building causes an intense airway inflammation with recruitment of inflammatory cells, predominately neutrophils, in healthy, previously, non-exposed subjects. In the present study exposure to organic dust from a swine confinement house has been used as a model to study lymphocyte reaction and activation.

We found an increased number of lymphocytes in bronchoalveolar lavage (BAL) fluid following exposure in a swine house. It was, moreover, found that BAL T lymphocytes of previously non-exposed subjects were activated as the expression of the lymphocyte activation markers CD69, CD25 and HLA-DR increased following exposure.

In vitro stimulation of whole blood with dust from swine confinement buildings also resulted in an increased expression of the activation markers CD69, CD25 and HLA-DR. The expression of the activation markers was dose and time dependent and occurred similar to phytohaemagglutinin (PHA) stimulated cells.

It was demonstrated that phagocytic cells and mediators released by phagocytic cells were of importance for lymphocyte activation following exposure to dust. In phagocyte depleted whole blood cells, and in peripheral blood mononuclear cell (PBMC) fractions, the dust did not induce CD69 expression on T cells. Neither was IL-12 nor IFN-γ found in plasma from these cell fractions. However, whole blood stimulated with swine dust, and plasma obtained from stimulated whole blood i.e. conditioned plasma, induced T cell activation and generated high concentrations of IL-12 and IFN-γ. Monocytes ($CD14^+$) rapidly produced IL-12 and TNF–α after one hour of stimulation with the dust whereas T- and NK cells produced IFN-γ only after a prolonged incubation (22 hours) with swine dust. The IFN-γ production by NK cells and the CD69 expression on T cells were reduced after pre-incubation with antibodies blocking the IL-12 receptor.

In conclusion, we have shown that swine dust induces airway influx and activation of human bronchoalveolar lavage T cells *in vivo* and *in vitro*. T cell activiation occurs in a time and dose dependent manner *in vitro*. The mechanisms of the T cell activation involve stimulation of phagocytic cells and production of cytokines such as TNF-α, IL-12 and IFN-γ. The results indicate a crucial role for IL-12 in the stimulation of T cells.

Keywords: organic dust, T cells, NK cells, cytokines, cellular activation

Sammanfattning

Sjukligheten i luftvägssjukdomar är hög hos lantbrukare jämfört med befolkningen i övrigt. Friska symtomfria lantbrukare, som undersöks med bronkoalveolärt lavage (BAL), visar ofta tecken på inflammation i luftvägarna. Exponering för damm i svinstallar orsakar en kraftig luftvägsinflammation hos friska tidigare icke exponerade försökspersoner. Det cellulära svaret domineras av neutrofila granulocyter, men även antalet makrofager och T lymfocyter ökar i bronkoalveolär lavage (BAL) vätska efter exponering. Dessutom visar BAL T lymfocyterna tecken på aktivering efter exponering för svindamm *in vivo*.

Svindamm, d.v.s. sedimenterat damm som insamlats i en svingård och som lösts i RPMI, aktiverade också T lymfocyter vid stimulering av perifera blodceller *in vitro*. En tids- och koncentrations- beroende ökning av uttryck för aktiveringsmarkörerna CD69, CD25 och HLA-DR noterades vid stimulering med damm *in vitro*.

Olika typer av celler och lösliga mediatorer har betydelse för lymfocytaktiveringen. Svindamm aktiverade inte T celler i frånvaro av fagocyterande celler såsom monocyter (CD14+) och granulocyter. Vi fann inte heller ökad produktion av IL-12 eller IFN-γ när blodceller stimulerades utan närvaro av fagocyterande celler. När vi däremot stimulerade helblod med damm, eller när T celler inkuberades med plasma från stimulerat helblod (”konditionerad plasma”) aktiverades T cellerna och uttryckte CD69. Likaså fann vi höga halter av IL-12 och IFN-γ i plasman från dessa stimuleringar. Monocyter producerade IL-12 och TNF-α redan efter en timmes stimulering med damm medan T och NK celler producerade IFN-γ och TNF-α först efter längre tids stimulering (22 timmar). Vid samtidig stimulering med svindamm och blockering av IL-12 receptorn (IL-12R) med hjälp av IL-12R specifika monoklonala antikroppar, minskade den svindammsinducerade T cells aktiveringen. Dessutom noterades en signifikant reduktion av NK cellers IFN-γ produktion. IL-12 förefaller således vara av central betydelse för dammaktivering av lymfocyter.

Sammanfattningsvis har vi i detta arbete visat att exponering i lantbruksmiljö aktiverar T lymfocyter i lungorna *in vivo*. *In vitro* stimuleringar har påvisat en tids- och koncentrationsberoende aktivering av T celler. Fagocyterande celler såsom monocyter och granulocyter är betydelsefulla för den damminduceade T cellsaktiveringen. Vidare har vi funnit att IL-12 tycks vara en cytokin av central betydelse i detta avseende.

Acknowledgements

This work was carried out at the National Institute for Working Life, during the years: IFL, IMA, Lung and Allergy, Program for Lung and Climate and the Lung Research Laboratory at the Karolinska Hospital. It has been a nice time and I would like to express my deep gratitude to all the people who have supported me during my "doktorand" studies. First of all I want to thank my two supervisors:

Professor **Kjell Larsson** for sharing his profound knowledge in all fields and for his never ending-support, his trust and encouragement. Always having new ideas and good advises and for being an excellent scientific mentor.

Docent **Johan Grunewald** introducing me into the field of immunology and the clinical aspect of it. For his never-ending engagement and for new ideas and discussions and for being an excellent scientific mentor.

Professor **Anders Eklund** to whom I am very grateful for giving me the opportunity to be a Ph D-student at the Lung Research laboratory and for all support when I needed it.

My co-author, my basic teacher and friend **Per Larsson**, without you this thesis would never been written.

Professor **Per Malmberg**, my co-author, for introducing me into the field of Lungs, and to enter the cellular level of inflammation, induced by organic dust "swine dust."

Britt-Marie Sundblad for being a real good friend and for all her care and listening during the years we were roommates. All the nice time we have spent together measuring lung function in Lund, Örnsköldsvik, Björklinge ……..

Britt-Marie Larsson for being a really god friend with all her support, which was needed for "Friskis & Svettis" and I miss it!!

Zhiping Wang for her kindness and willingness always to help, especially with the computer.

Lena Palmberg and **Fernando Acevedo** for many good advises.

Benita Dahlberg for the excellent way she introduced me into the Lung laboratory and for friendship with many nice evenings.

Kia Katchar for being a nice friend and for sharing "problems" and fun.

Anders Planck for “Lung disease lessons”

Margita Dahl and **Gunnel de Forest** for always supporting me with "material" together with a nice smile on their faces.

Ulla Sundberg and **Eva Marie Karlsson** for there help and support with many administration things.

Thanks to all my colleagues at "**Lunggruppen**" through out the years and thanks to all my new colleagues at the **Lungforskningslab**.

And finally my family**: Roland** for sharing my life and for his never ending encouragement and support; **Katrin** and **Annika** with enthusiastic encouragement and trust.

References

Acres R, Larsen A, Conlon P. IL-1 expression in a clone of human T cells. *J Immunol*. 1987;138:2132-2136.

Akira S, Hirano T, Taga T, Kishimoto T. Biology of multifunctional cytokines: IL-6 and related molecules (IL-1 and TNF-a). *FASEB*. 1990;4(8):2860-2867.

Allison J. CD28-B7 interactions in T cell activation. *Curr Opin Immunol*. 1994;6:414-419.

Amitage R. Tumo necrosis factor receptor superfamily members and their ligands. *Curr Opin Immunol*. 1994;6:407-413.

Anonymous. Request for assistance in preventing organic dust toxic syndrome. 1994;102:11.

Arai K, Lee F, Miyajima A, Miyatake S, Arai N, Yokota T. Cytokines: Coordinators of immune and inflammatory responses. *Annu. Rev. Biochem*. 1990;59:783-836.

Attwood P, Brouwer R, Ruigrwaard P, et al. A study of the relationship between airborne contaminants and environmental factors in dutch swine confinement buildings. *AM. Ind. Assoc. J*. 1987;48(8):745-751.

Auron P, Webb A, Rosenwasser L. Nucleotide sequence of human monocyte interleukine-1 precurser. *Proc Natl Acad Sci USA*. 1984;81:7907-7912.

Banchereau J, Bazan F, Blanchard D, et al. The CD40 antigen and its ligand. *Annu Rev Immunol*. 1994;12:881-922.

Baumann H, Gauldie J. The acute response. *Immunol Today*. 1994;15:74.

Beaven M, Metzger H. Signal transduction by Fc receptors: The FCeR1 case. *Immunol today*. 1993;14:222-226.

Bellini A, Yoshimura H, Vittori E, Marini M, Mattoli S. Respiratory pathophysiologic responses. Bronchial epithelial cells of patients with asthma release chemoattractant factors for T lymphocytes. *J Allergy Clin Immunol*. 1993;92:412-424.

Bendelac A, Rivera M, Parck S, Roark J. Mouse CD1 specific NK1 T cells: developement, specificity, and function. *Annu Rev Immun*. 1997;15:535-562.

Berman J, Beer D, Theodore A, Kornfeld H, Bernardo J, Center D. Lymphocyte recruitment to the lung. *Am Rev Respir Dis*. 1990;142:238-257.

Beutler B. The Tumor Necrosis Factors: Cachetin and Lymphotoxin. *Hospital practice*. 1990;February 15:45-56.

Biron CG, RT. Effects of IL-12 on immune responses to microbial infection: a key in regulating disease outcome. *Curr Opin Immunol*. 1995;7:485-496.

Braciale T, Morrison L, Swerzer M, Sambrook T, Gething M, Braciale V. Antigen presentation pathways to class I- and class I-restricted T lymphocytes. *Immunol Rev*. 1987;98:95-114.

Brouwer R, Biersteker K, Bongers P, Remijn B, Houtthuijs D. Respiratory symptoms, lung function, and IgG4 levels against pig antigens in a sample of Dutch pig farmers. *Am J Ind Med*. 1986;10:283-285.

Cassatella M, Bazzoni F, Ceska M, Ferro L, Baggiolini M, Berton G. IL-8 production by human polymorphonuclear leucocytes. *J Immunol*. 1992;148:3216-3220.

Clark C, Rylander R, Larsen L. Airborne bactria, endotoxin and fughi in dust in poultry ans swine confinement buildings. *Am Ind Hyg Assoc J*. 1983;44:537-541.

Cormier Y, Belanger J, Laviolette M, Beaudoin R, Hebert J. Abnormal bronchalveolar lavage in asymptomatic dairy farmers. Study of lymphocytes. *Am Rev Respir Dis*. 1984;130:1046-1049.

Corrigan C, Kay A. T-Lymphocytes. In: Barnes PJ RI, Thomson NC., ed. *Asthma. Basic mechanisms and clinical management*. London: Academic Press Limited; 1992:125-135.

Corrigan C, Kay A. The lymphocyte in asthma. In: Busse W.W HST, ed. *Asthma and Rhinitis*: Blackwell Scientific Publications; 1995:450-464.

Crock B, Robertson J, Travers Glass S, Botheroyd E, Lacey J, Topping M. Airborne dust, ammonia, microorganism, and antigenes in pig confinement houses and the respiratory health of exposed farm workers. *Am.Ind. Hyg. Assoc. J*. 1991;52:271-279.

Dannecker G, Mahlknecht U, Schultz H, Hoffmann M, Niethammer D. Activation of human T cells by the superantigen Staphylococcus Enterotoxin B: Analysis on a cellular level. *Immunobiol*. 1994;190:116-126.

DeMaeyer E, DeMaeyer-Guignard J. *Interferons and other regulatory cytokines* New York: John Wiley & Sons; 1988.

Diamond G, Legarda D, Ryan L. The innate immune response of the respiratory epithelium. *Immunological Reviews*. 2000;173:27-38.

Donham K, Yeggy J, Dague R. Chemical and physical parameters of liquid manue from swine confinement facilities: Health implication for workers, swine and environment. *Agric Wastes*. 1985;14:97-113.

Donham K, Scallon L, Popendorf W, Treuhaft M, Roberts R. Characterization of dusts collected from swine confinement buildings. *Am. Ind. Hyg. Assoc J*. 1986;47(7):404-410.

Donham K, Haglind P, Peterson Y, Rylander R. Environmental and health studies in swine confinement buildings. *American Journal of Industrial Medicine*. 1986;10:289-293.

Donham K. Hazardous agent in agricultural dust and method of evluation. *Am J Ind Med*. 1986;10:205-220.

Donham K, Popendorf W, Palmgren U, Larsson L. Characterization of dusts collected from swine confinement buildings. *Am. J. of Ind.Med*. 1986;10:294-297.

Donham K, Haglind P, Peterson Y, Rylander R, Belin L. Environmental and health studies in farm workers in swedish swine confinement buildings. *British Journal if Industrial Medicine*. 1989;46:31-37.

Donham K. Health effects from work in swine confinement buildings. *Am J Ind Med*. 1990;17:17-25.

doPico G, Reddan W, Tsiatis A, Peters M, Rankin J. Epidemiologic study of clinical and physiological parameters in grain handlersin northern united states. *Am Rev Respir Dis*. 1984;130:759-765.

Dunkley M, Pabst R, Cripps A. An important role for intestinally derived T cells in respiratory defence. *Immunology Today*. 1995;16(5):231-236.

D´Andrea A, Rengaraju M, Valiante N, et al. Production of natural killer cell stimulatory factor (Interleukin 12) by peripheral blood mononuclear cells. *J Exp Med*. 1992;176:1387-1398.

Eklund A, Blaschke E. Relationship between changed alveolar-capillary permeability and angiotensin converting enzyme activity in serum in sarcoidosis. *Thorax*. 1986;41:629-634.

Fields P, Gajewski T, Frank W. Blocked ras activation in anergic CD4+ T cells. *Science*. 1996;273:1276-1278.

Fleischer B. Superantigens. *Current Opinion in Immunology*. 1992;4:392-395.

Fraser D, Irving B, Crabtree G, Weiss A. Regulation of the interleukin-2 gene enhancer activity by the T cell accessory molecule CD28. *Science*. 1991;251:313-316.

Frosch S, Kraus S, Fleischer B. Trypanosoma cruzi is a potent inducer of interleukin-12 production in macrophages. *Med Microbiol Immunol*. 1996;185:189-193.

Fujishima S, Hoffman A, Vu T. Regulation of neutrophil interleukine 8 gene expression and protein secretion by LPS, TNF-alpha, and IL-1 beta. *J Cell Physiol*. 1993;154:478-485.

Gallin J. The Neutrophil. In: Samer M, Talmage T, Frank M, Austen K, Claman H, eds. *Immunological diseases*. 4th ed. Boston: Little Brown &Co; 1988:737-788.

Gauldie J, Richards C, Harnish D, Lansdorp P, Baumann H. Interferon b2/B cell stimulatory factor type 2 shares identity with monocyte-derieved hepatocyte-stimulating factor and regulates the major acute phase protein response in liver cells. *Proc Natl Acad Sci USA*. 1987;84:7251-7255.

Germann T, Gately M, Schoenhaut D. Interleukin12/T cell stimulating factor, a cytokine with multiple effects on T helper type1 (Th1) but not on Th2 cells. *Eur J Immunol*. 1993;23:1762-1770.

Golde D. The stem cell. *Sci. Am*. 1991;255((Dec)):86.

Goodier M, Londei M. Lipopolysaccharide stimulates the prolifiration of human CD56+CD3- NK cell: A regulatory role of monocytes and IL-10. *J Immunology*. 2000;165:139-147.

Gordon S, Clark S, Greaves D, Doyle A. Molecular immunobiology of macrophages: recent progress. *Curr. Opin. Immunol*. 1995;7:24-33.

Gupta D, Kirkland T, Viriyakosol, Dziarski R. CD14 is a cell-activating receptor for bacterial peptidoglycan. *J Biol Chem*. 1996;271(38):2310-2316.

Heederik D, Brouwer R, Biersteker K, Boleij J. Relationship of airborne endotoxin and bacteria levels in pig farms wiyh the lung function and respiratory symptoms of farmers. *Int Arch Occup Environ Health*. 1991;62:595-601.

Hemler M, Brenner M, McLean J, Strominger J. Antigenic stimulation regultes the level of expression of interleukin 2 receptor on human T cells. *Proc Nat Acad Sci USA*. 1984;81:2172-2176.

Henderson H, Calame K. Transcriptional regulation during B cell development. *Annu. Rev. Immunol*. 1995;16:163.

Herman A, Kappler J, Marrack P, Pullen A. Superantigens: Mechanism of T-cell stimmulation and role in immune responses. *Annu. Rev. Immunol*. 1991;9(9):745-772.

Iversen M, Dahl R, Korsgaard J, Hallas T, Jensen E. Respiratory symptoms in danish farmers: an epidemiological study of risk factors. *Thorax*. 1988;43:872-877.

Jain J, Loh C, Rao A. Transcriptional regulation of the IL-2 gene. *Current Opinion in Immunology*. 1995;7:333-342.

Jung T, Schauer U, Heusser C, Neumann C, Rieger C. Detection of intracellular cytokines by flow cytometry. *J Immunol Methods*. 1993;159:197-207.

Katchar K, Wahlström J, Eklund A, Grunewald J. Highly activated T-cell receptor AV2S3+CD4+ lung T-ceell expansions in pulmonary sarcoidosis. *Am J Respir Crit Care Med*. 2001;163:1540-1545.

Kobayashi M, Fitz L, Ryan M. Identification and purification of natural killer cell stimulatory factor (NKSF) a cytokine with multiple biological effects on human lymphocytes. *J Exp Med*. 1989;170:827-845.

Laan M, Palmberg L, Larsson K, Linden A. Free, soluble Interleukin-17 rotein during severe inflammation in human airways. *Eur Respir J*. 2002;19:534-537.

Larsen C, Anderson A, Appella E, Oppenheim J, Matsushima K. The neutrophil-activating protein NAP-1) is also chemotactic for T lymphocytes. *Science*. 1989;243:1464-1466.

Larsson K, Malmberg P, Eklund A, Belin L, Blaschke E. Exposure to micoorganisms, airway inflammatory changes and immune reactions in asymptomatic dairy farmers. *Int Arch Allergy Appl Immunol*. 1988;87:127-133.

Larsson K, Eklund A, Malmberg P, Belin L. Alterations in bronchoalveolar lavage fluid but not in lung function and bronchial responsiveness in swine confinement workers. *Chest*. 1992;101(3):767-74.

Larsson K, Eklund A, Hansson L, Isaksson B, Malmberg P. Swine dust cause intense airways inflammation in healthy subjects. *Am J Respir Crit Care Med*. 1994;150:973-977.

Larsson B-M, Palmberg L, Malmberg P, Larsson K. Effect of exposure to swine dust on levels of IL-8 in airway lavage fluid. *Thorax*. 1997;52(7):638-642.

Leonard W, Naguchi M, Russell S, McBride O. The molecular basis of x-linked severe-combined immunodeficiency:the role of the interleukin-2 receptor g chain as a common g chain. *Immunol Rev*. 1994;138:61-86.

Lorenz M, Jung S, Radbruch A. Switch transcript s in immunoglobulin class switching. *Science*. 1995;267:1825-1828.

López-Cabrera M, Santis AG, Fernandez-Ruiz E, et al. Molecular cloning, expression, and chromosomal localzation of the human earliest lymphocyte activation antigen AIM/CD69, a new member of the C-type animal lectin superfamily of signal-transmitting receptors. *J Exp Med*. 1993;178:537-547.

Malmberg P, Rask-Andersson A, Höglund S, Kolmodin-Hedman B, Read-Guernsey J. Incidence of organic dust toxic syndrome and allergic alveolitis in swedish farmers. *Int Arch Allergy Appl Immunol*. 1988;87:47-54.

Malmberg P, Rask-Andersson A. Natural and adaptive immune reactions to inhaled microorganisms in the lungs of farmers. *Scand J Work Environ Health*. 1988;14(1):68-71.

Malmberg P, Larsson K, Eklund A, et al. *Inflammatoriska förändringar i lungerna och immunstimmulering hos svingårdsarbetare*. Vol. 31; 1991.

Malmberg P, Rask-Andersson A. Organic dust toxic syndrome. *Seminars in Respiatory Medicine*. 1993;14(1):38-48.

Marini M, Vittori E, Hollemburg J, Mattoli S. Expression of the potent inflammatory cytokines, granulocyte-macrophage-colony-stimulating factor and interleukin-6 and interleukin-8, in bronchial epithelial cells of patients with asthma. *J Allergy Clin Immunol*. 1992;89:1001-1009.

Mattern T, Flad HD, Brade L, Rietschel E, Ulmer AJ. Stimulation of human T lymphocytes by LPS is unrectricted but strongly dependent on B7 interactions. *J Immunology*. 1998;160:3412-3418.

May J, Marvel L, Pratt D, Coppolo D. Organic dust toxic syndrome: A follow-up study. *Am J Ind Med*. 1990;17(1):111-113.

Medzhitov R, Janeway C. Innate Immunity. *The new England Journal of Medicine*. 2000;343(5):338-344.

Meyer K, Soergel P. Variation of bronchoalveolar lavage lymphocyte phenotype with age in the physiologically normal human lung. *Thorax*. 1999;54:697-700.

Minami Y, Kono T, Miyazaki T, Taniguchi T. The IL-2 receptor complex: its structure, function and target genes. *Annu. Rev. Immunol*. 1993;11:245-267.

Mosmann T, Coffman R. Th1 and TH2 cells: Differnt patterns of lymphokine secretion to different functional properties. *Ann Rev Immunol*. 1989;7:145.

Mosmann T, Schuhmacher J, Fiorentino D. Isolation of monoclonal antibodies specific for IL-4, IL-5, IL-6 and a new Th2 specific cytokine (IL-10), cytokine synthesis inhibitory factor, by using a solid phase radioimmunoabsorbentassay. *J Immunol*. 1990;145:2928.

Mosmann T, Moore K. The role of IL-10 in cross regulationof Th1 and Th2 responses. *Immunol Today*. 1991;12(A):A49.

Muraguchi A, Hirano T, Tang B. The essential role of B cell stimulatory facttor (BSF-2/IL-6) for the terminal differntiation of B cells. *J Exp Med*. 1988;167:332.

Nelson D, Strickland D, Holt P. Selective attrition of non-circulating T cells during normal passage through the lung vascular bed. *Immunology*. 1990;69:476-481.

Nijsten M, de Groot E, ten Duis H, Hack C, Arden L. Serum levels of interleukin-6 on acute phase responses (letter). *Lancet*. 1987;2:921.

Palmberg L, Larsson B-M. Induction of IL-8 production in human alveolar macrophages and human bronchal epithelial cells in vitro by swine dust. *Thorax*. 1998;53(260-264).

Parker J, Petson E, Weber S. Hypersensivity pneumonitis and organic dust toxic syndrome. *Immunology and Allergy Clinics of North America*. 1992;12(2):279-290.

Pedersen B, Iversen M, Bundgaard Larsen B, Dahl R. Pig farmers have signs of bronchial inflammation and increased numbers of lymphocytes and neutrophils in BAL fluid. *Eur Respir J*. 1996;9(3):524-530.

Pernis A. Th1 and Th2 cells differ in expression of IFN-g recptor b. *Science*. 1995;269:245.

Picker L. Control of lymphocyte homing. *Current opinion in Immunology*. 1994;6:394-406.

Randon K, Danuser B, Iversen M, et al. Respiratory symptoms in european animal farmers. *Eur Respir J*. 2001;17(4):747-754.

Reiner S, Seder R. T helper cell differentiation in immune response. *Curr. Opin. Immunol*. 1995;7(3):360-366.

Ritchie D, Fuller G. Hepatocyte-stimulating factor a monocyte dirived acute phase regulatory protein. *Ann NY Acad Sci*. 1983;408:490-502.

Robertson R, Ritz J. Biology and clinical relevance of human natural killer cells. *Blood*. 1990;76:2421-2438.

Rosales C, Brown E. *Neutrophil receptors and modulation of the immune response* New York:; 1993. (Abrahamson JS WJ, ed.

Russell D. Of microbes and macrophages: entry, survival and persistance. *Curr Opin Immunol*. 1995;7(4):479-484.

Rylander R, Donham K, Hjort C, Brower R, Heederik D. Effects of exposure to dust in swine confinement buildings- a work group report. *Scand J Work Environ Health*. 1989;15:309-312.

Schollmeyer K. Immunologic and pathophysiologic role of tumour necrosis factor. *Am J Respir Cell Mol Biol*. 1990;3:11-12.

Schwartz LB. Mast cells: function and contents. *Curr Opin Immunol*. 1994;6:91-97.

Sen G, Ransohoff R. Interferon induced antiviral actions and their regulation. *Adv Virus Res*. 1993;42:57-102.

Stavnezer J. Immunoglobulin class swiching. *Curr Opin Immunol*. 1996;8:199-205.

Streiter R, Kashahara K, Standiford T, Rolfe M, Chensue S, Kunkel S. Cytokine induced neutrophil derived interleukine-8. *Am J Pathol*. 1992;141:397-407.

Sutton B, Gould H. The human IgE network. *Nature*. 1993;366:421-328.

Testi R, Phillips J, Lanier L. Leu 23 induction as an early marker of functional CD3/T cell antigen receptor triggering. *J Immunology*. 1989;142(6):1854-1860.

Testi R, Phillips J, Lanier L. T cell activation via Leu-23 (CD69). *J Immunology*. 1989;143(4):1123-1128.

Testi R, D´Ambrosio D, De Maria R, Santoni A. The CD69 receptor: a multipurpose cell-surface trigger for hematopoietic cells. *Immunology Today*. 1994;15(10):479-483.

Travers S, Crook B, Lacey J. Micro-organismsand dust in pighouses in aberdeenshire. In: Griffiths WD, ed. Aerosol Society. London; 1988: 139-145.

Vitale M, Caruso A, Licenziati S, et al. Differential production of IFN- g, analysed at the single level, by specific subsets of human NK and T cells from healthy and HIV+ subjects. *Cytometry*. 2000;39:189-194.

Von Essen S, Robbins R, Thompson A, Rennard S. Organic dust toxic syndrome: an acute febrile reaction to organic dust exposure distinct from hypersensivity pneumonitis. *Clin Toxicol*. 1990;38:389-420.

von Essen S, Donham K. Illness and injury in animal confinement workers. *Occop Med*. 1999;14(2):337-350.

Wahlström J, Katchar K, Wigzell H, Olerup O, Eklund A, Grunewald J. Analysis of intracellular cytokinesin CD4+ and CD8+ Lung and blood T cells in sarcoidosis. *Am J Respir Crit Care Med*. 2001;163:115-121.

Wang Z, Malmberg P, Larsson B, Larsson K, Larsson L, Saraf A. Exposure to bacteria in swine house dust and acute inflmmatory reactions in humans. *Am J Respir Crit Care Med*. 1996;154:1261-1266.

Wang Z, Malmberg P, Larsson P, Larsson B-M, Larsson K. Time course of IL-6 and TNF-alpha in serum following inhalation of swine dust. *Am J Respir Crit Care Med*. 1996;153:147-152.

Wang Z, Larsson K, Palmberg L, Malmberg P, Larsson P, Larsson L. Inhalation of swine dust induces cytokine release in the upper and lower airways. *Eur Respir J*. 1997;10:381-387.

Wu C, Warrier R, Carvajal D, et al. Biologicalfunction and distribution of human interleukin-12 receptor beta chain. *Eur J Immunol*. 1996;26:345-350.

Yao Zea. IL-17. *J Immunol*. 1995;155:5483.

Yao Zea. IL-17. *Immunity*. 1995;3:811.

Yoshimura A, Lien E, Ingalls R, Tuomanen E, Dziarski R, Golenbock D. Cutting edge: Recognition of Gram-positive bacterial cell wall components by the innate immune system occurs via Toll-like receptor 2. *J immunol*. 1999;163:1-5.

Zejda J, Dosman A. Respiratory disorders in agriculture. *Tubercle Lung Dis*. 1993;74:74-86.