Lene N. Johannessen

Fungal products and inflammatory responses in human monocytes and epithelial cells

Thesis for the degree of philosophiae doctor

Trondheim, August 2008

Norwegian University of Science and Technology
Faculty of Medicine
Department of Cancer Research and Molecular Medicine

NTNU
Norwegian University of
Science and Technology

NTNU
Norwegian University of Science and Technology

Thesis for the degree of philosophiae doctor

Faculty of Medicine
Department of Cancer Research and Molecular Medicine

© Lene N. Johannessen

ISBN 978-82-471-1167-3 (printed ver.)
ISBN 978-82-471-1168-0 (electronic ver.)
ISSN 1503-8181

Doctoral theses at NTNU, 2008:238

Printed by NTNU-trykk

Sammendrag
Tittel: Fungal products and inflammatory responses in human monocytes and epithelial cells

Norsk tittel:
Virkningen av muggsoppkomponenter på immunforsvaret hos mennesker – reaksjoner fra lunge- og blodceller

Muggsopp i innemiljø kan forårsake flere ulike symptomer hos mennesker som eksponeres. De vanligste reaksjonene ligner betennelsesreaksjoner i slimhinner, særlig i øvre luftveier, men også andre symptomer slik som hodepine, kvalme, unormal trøtthet, konsentrasjonsvansker (”tung i hodet”) og eksem kan være vanlige. Personer med astma er særlig utsatt for inneklimarelaterte plager, men det er hittil ukjent hvorfor disse personene er særlig utsatt. Til tross for mye fokus på allergiske reaksjoner på grunn av eksponering for muggsopp, er det sjeldent at mennesker får allergiske reaksjoner pga muggsopp sett i fra et immunologisk perspektiv. Det er imidlertid grunn til å tro at andre typer reaksjoner fra immunforsvaret (ikke-allergiske) kan være mer aktuell. Det er holdepunkter for at stoffer/komponenter som muggsopp produserer er viktige bidragsytere for reaksjoner.

I tillegg til en rekke andre komponenter som muggsopp produserer, er muggsoppgifter (mykotoksiner) særlig aktuelle. Doktorgradsarbeidet har i hovedsak fokusert på hvordan celler i immunforsvaret reagerer når de blir utsatt for særlig små doser av disse giftene, noe som kan være situasjonen ved forurensing av muggsopp innendørs. Det er i hovedsak vist at muggsoppgifter bidrar til å forstyrre cellenes produksjon av ikke-allergiske signalstoffer (inflammatoriske cytokiner). På denne måten kan det oppstå en ubalanse i immunforsvaret slik at sjansen for betennelsesreaksjoner kan øke. De påviste cellereaksjonene kan muligens ha betydning for noen av de opplevde symptomene som relateres til muggsopp i innemiljø. Det er også vist i et delarbeid at komponenter fra muggsopp kan bidra til å frembringe spesielle reaksjonsmønster hos astmatikere sammenliknet med ikke-astmatikere, og dette kan indikere/dokumentere at immunresponsen hos astmatikere faktisk er forskjellig sammenliknet med reaksjonen hos ikke-astmatikere. Dette delarbeidet kan være begynnelsen på den videre dokumentasjonen av helseplager som astmatikere kan oppleve på grunn av muggsopp i innemiljø.

Navn kandidat: Lene N. Johannessen
Institutt:Kreftforskning og Molekylærmedisin(IKM)
Veileder(e):Martinus Løvik (Folkehelseinstituttet (FHI) og IKM),
Asbjørn Nilsen (IKM)
Finansieringskilde:
Stiftelsen Helse og Rehabilitering (HR) via Norges Astma og Allergi forbund (NAAF) og i samarbeid med NTNU (Trondheim), St.Olavs Hospital (Trondheim) og FHI (Oslo)

Ovennevnte avhandling er funnet verdig til å forsvares offentlig for graden PhD i molekylærmedisin. Disputas finner sted i Seminarrom, 1902-bygget. Tirsdag 23.september 2008 , kl 12.15.

Acknowledgements

This works was performed at the Department of Cancer Research and Molecular Medicine, Faculty of Medicine, the Norwegian University of Science and Technology (NTNU) in Trondheim. My supervisors have been Martinus Løvik at the Norwegian Institute of Public Health (FHI) and Asbjørn Nilsen at NTNU. The work was financed by NTNU, FHI, and the Norwegian Foundation for Health and Rehabilitation (HR) through the Norwegian Asthma and Allergy Association (NAAF).

I am grateful to my supervisors for the scientific and moral support given to me through these past years. I have been given great freedom of choosing which paths to pursuit and I have been encouraged to discuss new and good, and not so good, ideas. This has helped me to develop the scientific way of thinking.

Stian Lydersen (NTNU) and Sigurd Steinshamn (St. Olavs Hospital) have contributed greatly in this thesis, and through their participation as co-authors of papers they have given glance into the fascinating worlds of statistics and lung medicine, respectively.

I greatly appreciate the technical assistance throughout various parts of this work given by Liv Ryan and Tenna Nørkov (both NTNU), Else-Carin Groeng (FHI), Birgit Pedersen, Anne-Stine Fossum and Inger Lise Bjerkan (all St. Olavs Hospital).

I also want to thank Terje Espevik in particular for his positive attitude when various questions needed an answer, and also thanks to all my other colleagues at the Department.

Special thanks to Tore Syversen (NTNU), Lars Bevanger and Kåre Bergh at the Department of Medical Microbiology (St. Olavs Hospital) for giving professional inspirations in the early days of this project. Also, special thanks to Bjørn Hilt and colleagues at the Department of Occupational Medicine (St. Olavs Hospital) for providing office facilities in times when I had none and for professional inspirations.

Also thanks to my friends and colleagues at Thelma AS, and especially to Arvid Påsche and to Vibeke Nossum, for their patience during my years at the University. Now, I am looking forward to continue working on the issues of microbial exposure.

Last, but not least, thanks to my friends and family, and especially to my father Jarle, my brother Stig and his family, and to my dear Hans Jørgen who have taught me to stay focused on what's important and to always believe that things will sort out right. And finally, to my daughters Tuva (7 yrs) and Hedda (3 yrs) who with their lovely personalities and great humour have shown me that there are still far more important things in life than scientific papers.

Lene N. Johannessen
Trondheim, *May 16, 2008.*

List of papers

PAPER I
Johannessen LN, Nilsen AM, Løvik M (2005). The mycotoxins gliotoxin and citrinin differentially affect production of the pro-inflammatory cytokines TNF-α and IL-6, and the anti-inflammatory cytokine IL-10. Clin Exp Allergy **35:** 782-789.

PAPER II
Johannessen LN, Nilsen AM, Løvik M (2007). Mycotoxin-induced depletion of intracellular glutathione and altered cytokine production in the human alveolar epithelial cell line A549. Toxicol Lett **168:**103-112.

PAPER III
Johannessen LN, Løvik M, Lydersen S, Nilsen AM (2008). Combined cell wall polysaccharide, mycotoxin and bacterial lipopolysaccharide exposure and inflammatory cytokine responses. Submitted.

PAPER IV
Johannessen LN, Løvik M, Steinshamn S, Nilsen AM (2008). Reduced mannan-induced monocyte TNF-α production in mild asthma: A role for mannan-binding lectin (MBL)? Submitted.

Contents

Abbreviations

ATP	Adenosine-triphosphate
BAL	Bronchoalveolar lavage
BRS	Building related syndrome
DNA	Deoxyribonucleic acid
EPS	Extracellular polysaccharides/ extracellular polymeric substances
EU	Endotoxin unit
GP	Glutathione peroxidase
GR	Glutathione reductase
GSH	γ-glutamyl-L-cysteinyl glycin
GSSG	γ -glutamyl-L-cysteinyl glycin disulfide
GST	Glutathione transferase
IAQ	Indoor air quality
IgE	Immunoglobulin E
IL	Interleukin
LPS	Lipopolysaccharide
MBL	Mannan-binding lectin
MRP	Multidrug resistance protein
NADPH	Nicotinamide adenine dinucleotide phosphate
PAMP	Pathogen-associated molecular pattern
PRR	Pathogen recognition receptor
ROS	Reactive oxygen species
SBS	Sick building syndrome
sp.	Species
TGF-β	Transforming growth factor- β
TLR	Toll-like receptor
TNF-α	Tumour necrosis factor- α

1 Introduction

1.1 Environmental microbes and associations to negative health effects

Negative health effects due to environmental microbes have been described and known to people for centuries. In the 1833, collections and descriptions of environmental microbes were shown in Ehrenberg's Illustrations of Dust collected by Charles Darwin. Later, Pasteur and co-workers developed theories which associated many airborne environmental microbes directly to the development of human disease. This led to systematic research on various microbes present in food, water and in the air and the human health effects of this microbial exposure (Lacey and West, 2006).

Throughout the last century, an increasing number of publications have reported symptoms experienced by individuals living or working in modern buildings with poor indoor air quality (IAQ). In the early 1980's the concept of sick building syndrome (SBS) emerged (Finningan *et al.,* 1984). SBS, or in recent years called building-related symptoms (BRS), has been characterised as symptoms related to environmental exposure (sometimes of unknown origin) in a building that is experienced by otherwise healthy individuals. The symptoms can be associated with effects on multiple organ systems and includes respiratory symptoms, headaches, nausea, fatigue, concentration problems and dizziness (Hardin *et al.*, 2003; Terr, 2004; Shoemaker *et al*., 2006). In view of the complexity of chemical, microbial and physical exposure agents in a modern building, the evidence that microbes are the (only) cause of SBS is missing (Kolstad *et al.*, 2002).

The association between damp houses and SBS seems to be more established than the link between SBS and microbial exposure. However, the damp indoor environment may harbour a wide range of microorganisms including several kinds of environmental bacteria, such as gram-negative species and their products such as endotoxins as well as mycobacteria, moulds and other fungi which together create the microbial exposure (Anderson *et al.*, 1997). The health effects associated to damp buildings generally consist of upper respiratory tract complaints of inflammatory nature such as rhinitis, sinusitis, irritation of the mucous membrane, cough and cold-like symptoms (Mahmoudi *et al.*, 2000; Fisk *et al.*, 2007). Also, neurological associated symptoms such as headache, concentrations problems, fatigue and nausea have been reported (Pirhonen *et al.,* 1996; Shoemaker and House, 2006). Reports of eczema (Bornehag *et al.*, 2005) and fever (Pirhonen *et al.,* 1996) are less common. Further, several reports of an increased prevalence of asthma symptoms in damp buildings with mould problems have been published (Taskinen *et al*., 1997; Taskinen *et al*., 1999; Engvall *et al*., 2001; Trout *et al.*, 2001). In occupational settings, such as in the composting and waste-handling industry, in agricultural settings and during building demolition, exposure to high concentrations of airborne moulds is well known to induce negative health effects (Brun, 2007).

Inhalation of airborne mould spores has in particular been associated with the aggravation of respiratory symptoms and asthma (Fisk *et al.,* 2007; Hirvonen *et al.,* 2005; Hope and Simon, 2007; Nevalainen and Seuri, 2005). Asthma is a chronic hypersensitivity condition with inflammatory reactions and bronchial constriction. Asthma is traditionally classified as IgE-mediated (extrinsic asthma or allergic/atopic asthma), non-IgE-mediated (intrinsic asthma or non-allergic/non-atopic asthma) and in recent years also as a mixed form of the two types. Clinical symptoms in non-allergic asthma resemble those in allergic asthma, but the immune response

mechanisms are partially different (Comi *et al.,* 2007). In general, however, fungi seem to cause symptoms of inflammation in both allergic and non-allergic asthma patients.

Although, moulds possess potentially harmful properties, infections due to mould exposure are relatively rare. An increasing number of cases have been reported, however, primarily in hospitalised and critically ill patients with severe underlying illness (Khasawneh *et al.,* 2006). In immune suppressed patients, fungal infections may be life threatening. Moulds such as *Aspergillus*, *Fusarium*, *Scedosporium* and *Penicillium,* and yeasts such as *Candida* and *Cryptococcus* are most frequently isolated from fungal infections (Enoch *et al.,* 2006).

A large amount of studies have associated mould exposure to various (non-allergic) symptoms of inflammation. Therefore, it seems warranted to investigate some of the cellular responses that may be involved in the symptoms experienced by individuals living or working in damp and mouldy buildings.

1.2 The Kingdom Fungi

Mycology, the study of fungi, has originated from botany as fungi earlier were considered members of the plant kingdom. Classification has now placed fungi in one of five eukaryotic kingdoms, the others being animals, plants, chromista and protozoa. Indeed, many fungi resemble plants in that they develop fruiting bodies and produce spores which disperse by air currents. The fruiting body of filamentous fungi is composed of long branching threads (hyphae) of multicellular filaments which are individually divided into smaller compartments and typically contain several nuclei. These compartments are fungal cells. The spores, or conidia, are often attached to specialised cells arising from the hyphae. Many filamentous fungi produce large networks of hyphae (when visible called mycel) and vast amount of spores.

The largest group of filamentous microscopic fungi (microfungi) is known as moulds. Moulds are ubiquitous in nature and are frequently found on decaying plants and in soil where they function as important organisms to regulate the ecosystem. Moulds are saprophytes that decompose and metabolise almost any kind of organic material. Also, moulds are closely associated with environmental bacteria and yeasts and compete with them for nutrition. In this setting, mycology is considered a branch of microbiology.

Metabolites that are produced and secreted by many types of fungi, both moulds and yeasts, are often used in the food and brewing industry because fungi are easily grown in pure cultures with relatively high growth rates. Fungi may be used as sources to produce catalytic enzymes used in fermentation and in other biochemical processes (Baker, 2006). Many features of fungi have also made them attractive in research on fundamental biological processes such as the study of cellular energy metabolisms, genome studies, protein synthesis and expression, and in evolutionary and mutation research. In recent years, fungi have been frequently used as model organisms to study the structure and function of genes and gene products (Matsumoto and Ledbetter, 1999).

Fungal cells produce a variety of metabolites due to primary and secondary metabolic processes (Carlile *et al.,* 2001). Primary metabolites are produced as a result of cellular processes essential to maintain survival of the fungal cell, i.e. maintenance of the cell wall or energy metabolism. Secondary metabolites are largely not essential for the survival of fungal cells, although production of some secondary metabolites, such as toxins, may offer evolutionary advantages in the competition with other environmental microbes.

1.2.1 Cell composition of fungi and virulence factors

Fungi are eukaryotic cells with intracellular compartments including endoplasmatic reticulum, Golgi apparatus, mitochondria and vacuoles used for transport and storage of nutrients. Fungal DNA is organised into a number of chromosomes. Several of the *Aspergills sp*. typically contains 8 chromosomes (Baker, 2006). The phospholipid bilayer and proteins in the cell membrane is covered by a rigid, but also elastic and dynamic cell wall consisting mainly of carbohydrates (polysaccharides) and structure proteins. Other proteins embedded in the cell wall are transport proteins and enzymes used in primary and secondary metabolism, and pigment molecules such as melanin (Fog-Nielsen, 2003; Horner *et al.,* 1995; Bruneau *et al.,* 2001; Jahn *et al.,* 1997). Some of these proteins may be allergens. Generally, fungi produce a vast amount of metabolites that potentially may enhance their inflammagenic, allergenic and toxic properties.

Fungi have through evolution adapted to handle almost any type of changing environment. Although survival is easy for most environmental fungi, the ideal condition for growth is a moderate temperature (20-30°C), a relatively high moisture environment and a high carbohydrate content of the growth substrate. Moulds such as *Penicillium* and *Aspergillus* are often found in small amounts in outdoor air and in a balanced mixture with other fungal genera such as *Cladosporium*, *Mucor, Acremonium, Paecilomyces* and many others. Some species, especially within the *Aspergillus* and *Paecilomyces* genera, have developed thermotolerant or thermophilic properties and are able to grow at 37°C and above. This feature is one of many virulence factors that could facilitate mould invasion in human tissues (Aufauvre-Brown *et al.,* 1998).

Molecules of the carbohydrate rich cell wall of fungi are thought to be involved in induction of inflammatory responses. The cell wall of fungi consists of several polysaccharide layers, mannosylated and glycosylated structure proteins, and small amounts of lipids (such as ergosterol) that together form a rigid skeleton providing strong physical properties (Carlile *et al.,* 2001). The inner polysaccharide layers consist mainly of glucose polymers (glucan), which occurs with α- and/or β- linkages (α-and β-glucans) and also relative small amount of chitin (Bernard and Latgé, 2001). Generally, glucans may appear with a relatively wide molecular weight range (from 7 to 500 kDa for some well characterised molecules) and with various degrees of branching (Barreto-Bergter *et al.,* 1983). The biologically most active form of glucan found in fungi has a β-1, 3-D-glucan backbone. Various forms of soluble and particulate β-glucans induce pro-inflammatory cytokines in human immune cells, such as IL-1, IL-2 and TNF-α, both *in vitro* and *in vivo* (Rylander and Lin, 2000; Hohl *et al.,* 2005; Taylor *et al.,* 2007; Beijer *et al.,* 2003; Sherwood *et al.,* 1987, Sigsgaard *et al.,* 2000).

Glucans may be liberated to the environment from the cell wall of decomposed, fragmented or structurally damaged fungal cells and also during germination and growth when cell walls are recomposing (Hohl et al., 2005). The content of β-glucans in dust has been assessed (Giovannangelo et al., 2007) and the glucan content in samples of airborne or settled dust has been used as a surrogate measurement of moulds in the indoor environment (Schram-Bijkerk et al., 2005; Douwes et al., 1999).

Although β-glucans have been a main subject of investigation in relation to symptoms experienced in mouldy buildings, most intact fungal cells and resting airborne conidia do not display large amounts of β- glucans on the outer surface (Gantner *et al.,* 2005; Hohl *et al.,* 2005). Other soluble extracellular polysaccharides (EPS) are present in the outer cell wall layer of i.e. *A.*

fumigatus, such as α-mannose polymers (mannan) supplemented with side chains of galactose or β-galactofuranose (called galactomannan) (Bernard *et al.,* 2001; Latge, 2005). Important moulds found in the indoor environment such as species within the *Aspergillus, Penicillium, Cladosporium, Stachybotrys and Trichophyton* genera have largely similar composition of the cell wall structure (Leal *et al.,* 1992; Ikuta *et al.,* 1997; François *et al.,* 2006; Latge, 1988). Galactomannan polysaccharides (Fig. 1) are covalently bound to mannosylated proteins on the cell wall surface (Fontaine *et al.,* 2000; Bernard *et al*., 2001). Earlier, this mannan coat has been thought to be biologically inert and mask the immune stimulating properties of the glucan layer (Artursson *et al.,* 1987). However, recently it has become evident that mannan polysaccharides are recognised by cell surface receptors on monocytes and macrophages and induce inflammatory cytokine responses such as TNF-α production (Netea *et al.,* 2006; Netea *et al.,* 2006). This understanding has opened up for the possibility that intact and non-sporulating fungal conidia may induce inflammatory responses without fungal growth in the host.

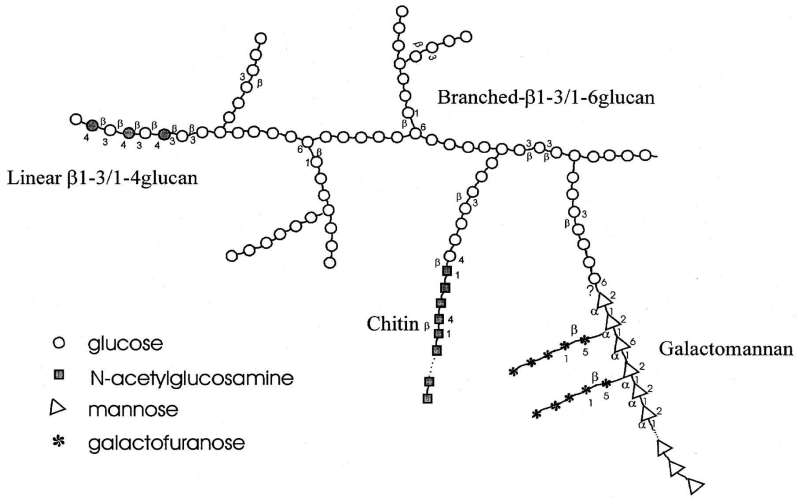


Fig 1. A tentative scheme of the organization of the β-1, 3-glucan and its covalently associated polymers found in the cell wall of *A. fumigatus.* The polysaccharide network is formed by (i) biosynthesis of the individual polysaccharides (β-1, 3-glucan, β-1, 3/1, 4 glucan, chitin, and galactomannan), (ii) branching of β-1, 3-glucan and increasing the number of acceptor sites, and (iii) covalent addition of chitin, galactomannan and β-1, 3/1, 4-glucan to glucan branches (Fontaine *et al.,* 2000).

1.3 Mycotoxins

Secondary metabolites produced by fungi include the mycotoxins. Mycotoxins are a large group of several hundred (over 400 are described) chemically distinct organic compounds synthesised through secondary metabolic pathways in the fungi. Some mycotoxins are amongst the most toxic biological compounds known.

Health effects of mycotoxins are dependent of the time and dose of exposure, the route of administration (oral/dermal/inhalation), the toxic effects on specific cells/organs, the health status of the individual and possibly the co-effects with other chemicals to which the individual is exposed (Bennett and Klick, 2003). Exposure to high concentrations of mycotoxins may cause a

wide range of health effects, from short-term and immediate toxic ones to long-term mutagenic and carcinogenic ones (Peraica *et al.,* 1999).

In relation to poisoning and carcinogenicity the oral route of mycotoxin exposure is the most common, but also dermal routes may be involved. The toxic effects of mycotoxins have been recognised since the medieval times. Ergotamine (ergot alkaloids) produced by the mould *Claviceps purpurea* on contaminated rye caused thousands of deaths in both animals and humans due to ergot poisoning, known as St. Anthony's fire. In 1777 in France, 8,000 people died of gangrenous ergotism. Even today, aflatoxin, produced by *Aspergillus flavus* on many agricultural products, constitutes a major problem because of contamination of food and feed, especially in developing countries. In recent years, however, quantitative standards and guidelines to avoid negative health effects from consumption of contaminated foods and feeds have been established throughout the world for the most potent mycotoxins such as aflatoxin, ochratoxin, trichothecenes and fumonisins.

Although mycotoxins are of concern to humans and animals, many beneficial effects have been appreciated. Possibly the most important benefits of mycotoxins were established through the discovery of penicillin in the 1930's. This was a breakthrough for medical research and in the treatment of infections. Later, many types of mycotoxins have been investigated and used for e.g. their antibiotic effects.

Mycotoxins are produced during the late log-phase of fungal growth and probably when they may be beneficial for the fungi in order to compete with other microbes in the environment (Calvo *et al.,* 2002). Mycotoxin production is often associated with certain fungal species, but several mycotoxins may be produced by one single species and one single mycotoxin may be produced by several different species (Table 1). Also, different strains within the same species

have shown to produce various types of mycotoxins (Fog-Nielsen, 2003) suggesting that toxin production is dependent on environmental growth conditions such as the carbon and nitrogen source, temperature, light and pH (Bennett and Ciegler, 1983).

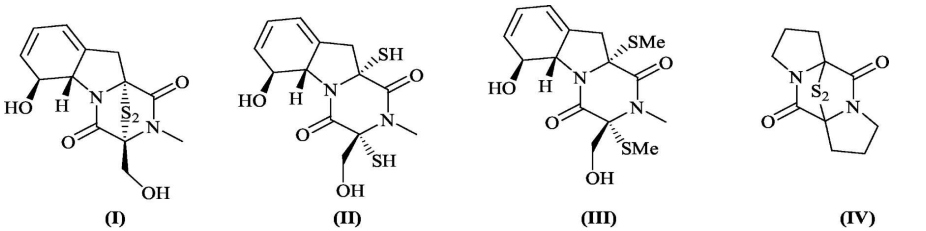
Table 1. Indoor air fungi and associated mycotoxins (modified from Abbott, 2002).

Acremonium	citrinin
Alternaria	altenuene, altenusin, alternariol, altertoxin, tenuazonic acid
Arthrinium	nitropropionic acid
Aspergillus	aflatoxin, austin, citrinin, cytochalasin, fumitoxin, nidulotoxin, ochratoxin, patulin, sterigmatocystin, gliotoxin, fumitremorgen, penitrem, territrem, verruculogen, viomellein, vioxanthin, xanthomegnin
Bipolaris	cytochalasin, sporidesmin, sterigmatocystin
Chaetomium	chaetoglobosin, chetomin, chaetochromin, chaetosin, cochliodinol, sterigmatocystin
Cladosporium	cladosporic acid
Fusarium	fumonisin, fusaric acid, fusarin, fusarochromanone, moniliformin, trichothecenes (deoxynivalinol, T2 toxin), zearlenol, zearalenone
Gliocladium	gliotoxin
Paecilomyces	patulin, viriditoxin
Penicillium	citrinin, citreoviridin, citromycetin, erythroskyrin, ochratoxin, gliotoxin, griseofulvin, luteoskyrin, oxaline, patulin, penicillic acid, roquefortine, rubratoxin, rugulosin, rugulovasine, penitrem, territrem, verruculogen, verrucosidin, viomellein, viridicatin, xanthomegnin
Phoma	brefeldin, cytochalasin, secalonic acid, tenuazonic acid
Rhizopus	rhizonin
Sclerotinia	furanocoumarins
Stachybotrys	griseofulvin, trichothecenes (isosatratoxin, roridin, satratoxin, trichodermol, trichoverrol)
Trichoderma	gliotoxin, koninginin, trichodermin
Trichothecium	roseotoxin, trichothecenes (trichothecin)
Wallemia	walleminol
Zygosporium	cytochalasin

In the indoor environment, mycotoxins have been isolated from spores, hyphal fragments and fungal particulates as well as in dust, bioaerosols and in building materials (Fog-Nielsen, 2003; Tuomi *et al.,* 2000). Some recent experimental studies suggest that mycotoxins may adversely affect biological processes and in particular modulate immune responses, e.g. the production of cytokines involved in inflammation (Chung *et al.,* 2003; Pestka *et al.,* 2006; Theumer *et al*., 2003; Islam and Pestka, 2006). Although pulmonary mycotoxicosis in humans due to inhalation of toxic mould spores has been reported in a few case studies, e.g. during the cleaning of silos (Perry *et al.,* 1998), it has not been described in an ordinary indoor air context. Nevertheless, it is a general problem that safe exposure limits with regard to inhaled mould spores and mycotoxins have not been established. This has raised concerns among individuals living or working in mouldy environments.

1.3.1 Gliotoxin

Gliotoxin is an epipolythiodioxopiperazine (ETP) secondary metabolite of about 326 Da traditionally known to be cytotoxic and to induce dose-dependent apoptosis or necrosis in prokaryotes and eukaryotes (Kamei and Watanabe, 2005). Gliotoxin has been isolated from fungi such as *Aspergillus* sp., *Penicillium* sp., *Gliocladium* sp. and *Trichoderma* sp. (Table 1). The biologically active form of gliotoxin is oxidised with an intact disulfide bridge (Fig.2).



di-sulfide form (oxidised) di-thiol form (reduced) bis-metyl-thioeter form di-prolyl ETP

Fig 2. Chemical structures of gliotoxin (Bernardo *et al.,* 2003)

Uptake of gliotoxin in target cells initiates an intracellular red-ox reaction that reduces the di-sulfide form (oxidised) of the molecule to the inactive di-thiol form (reduced) by a mechanism that is dependent on the intracellular antioxidant glutathione (Bernardo *et al.,* 2003). This suggests that gliotoxin may be involved in cellular oxidative stress mechanisms. Gliotoxin in cytotoxic doses have been studied, and in this setting the mycotoxin has been regarded as an immunosuppressive compound that reduces inflammation by inducing cellular apoptosis (López-Franco *et al.,* 2002). However, the cellular effects of non-cytotoxic doses of gliotoxin in relation to inflammation have not been well investigated.

1.3.2 Citrinin

Citrinin was first isolated from *Penicillium citrinum* (Hetherington and Raistrick, 1931) and the chemical structure was proposed (Fig. 3). The mycotoxin is a 250 Da organic compound produced by fungal genera including *Aspergillus, Penicillium* and *Acremonium.* It is known to be nephrotoxic by inducing dose-dependent apoptosis or necrosis in kidney tissue (Clark *et al.*, 2006; Bennett and Klich, 2003). The cellular mechanisms that induce cell death upon citrinin

exposure are not fully known. The electron transport systems in mitochondria have been suggested as a target of the toxic action of citrinin (Da Lozzo *et al.,* 1998). However, recent investigations using DNA microarray technology have also suggested that induction of oxidative stress genes and interference with the antioxidant glutathione in the target cell also may be involved (Iwahashi *et al.,* 2007). *In vivo* studies have suggested that citrinin may contribute to inflammatory responses after oral consumption (Hanika *et al*., 1986). However, effects of low concentrations, or non-toxic doses, in relation to inflammatory responses in immune cells or cells of the respiratory mucosa have not been investigated.

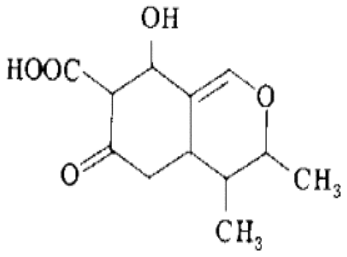


Fig. 3 Chemical structure of citrinin (Bennett and Klich, 2003)

1.4 Bacterial endotoxin

Endotoxins are compounds produced by many environmental as well as pathogenic gram-negative bacteria. The endotoxins consist mainly of immune stimulating molecules of lipopolysaccharide (LPS) in which the lipid component is well known to induce inflammatory responses (Su *et al*., 1995). The host responds to LPS exposure by inducing an efficient inflammatory response with the production of pro-inflammatory mediators. Inhalation of elevated

concentrations endotoxin in the environment cause inflammatory cytokine production by cells in the bronchial and alveolar mucosa (Thorn, 2001).

Clinical symptoms of endotoxin-induced inflammation in the lungs include bronchial hyperreactivity and fever. Exposure to endotoxin has also been associated with the aggravation of chronic inflammatory lung diseases, including asthma and chronic bronchitis (Michel *et al.,* 1992; Vernooy *et al.,* 2002).

In some indoor air environments, increased concentrations of endotoxins have been associated with a number of household factors, such as pets, floor cover, the amount of people living in the home and the efficiency of ventilation (Giovannangelo *et al*., 2007). Also, an elevated concentration of endotoxin-producing bacteria has been found in indoor environments with a general enhanced microbial flora (Schram *et al.,* 2005).

1.5 Important cells and mediators of the innate immune system

1.5.1 Cells

The innate immune system in mammalians is crucial for survival. The cellular immune response to invading microbes is primarily to kill the microbe and clear inflammation, alternatively to halt further invasion of microbes until an adaptive immune response with the production of antibodies can develop. Fig. 4 shows an overview of the time courses of innate and adaptive cellular responses to invading microbes.

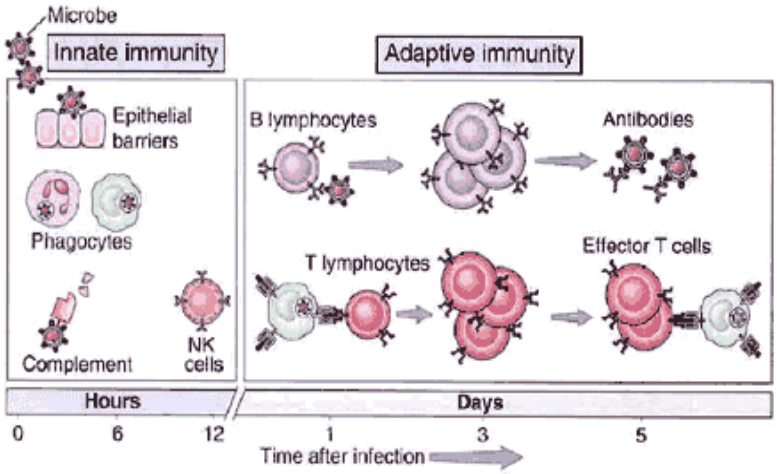


Fig. 4

Cells and mediators involved in an innate and adaptive immune response to invading microbes (Adapted from www.actagainstallergy.com).

The epithelial layer represents the first cellular barrier that prevents microbes from invading the host. Epithelial surfaces are important physical barriers, but the cells are also involved in defence mechanisms with a small production of inflammatory chemokines and cytokines (Takizawa, 1998). Monocytes, macrophages, granulocytes and dendritic cells are the most important cells in innate immune defence for the recognition and uptake (phagocytosis) of pathogens. Receptors on monocytes and macrophages are specialised to recognise characteristics of microbial surfaces, such as evolutionally preserved pathogen-associated molecular patterns (PAMP). The specialised pathogen recognition receptors (PRR) include the Toll-like receptors (TLR) and members of the C-Lectin super family. When receptors recognise microbes, cell activation and a subsequent release of inflammatory mediators, such as cytokines, may proceed. Cytokines produced by activated monocytes and macrophages further affect the immune response in many ways, both locally and systemically.

1.5.2 Inflammatory cytokines

A number of important cytokines and chemokines are produced by activated innate immune cells in response to receptor binding of a trigger agent. The following cytokines are of special interest in the mediation of inflammatory responses and is dealt with in this thesis.

The most abundant pro-inflammatory cytokine produced in early stages of an inflammatory response is tumour necrosis factor-α (TNF-α) discussed elsewhere in this thesis. Further, IL-12p70 have dual roles in both being pro-inflammatory and in skewing the innate immune response towards an adaptive response with the subsequent production of antibodies, e.g. IL-12p70 modulate an IgE-mediated immune response to various allergens (Kim *et al*., 1997). Also, the pleiotrophic cytokine IL-6 is mainly known to be involved in pro-inflammatory responses of the innate immune system, but also as a connector to the adaptive immune system by being involved in cell proliferation of lymphocytes and antibody production. The chemokine IL-8 has a primary role of being a granulocyte attractant to recruit other inflammatory cells to the site of an infection.

The anti-inflammatory cytokines transforming growth factor-β (TGF-β) and IL-10 are important mediators in order to terminate or halt an inappropriate pro-inflammatory cytokine response initiated by e.g. TNF-α (Chung, 2001; Saxena *et al.*, 2008). Although less is known about anti-inflammatory regulation within the cell, the beneficial effects of regulative cytokines have been proposed, e.g. TGF-β has been suggested in treatment of autoimmune diseases (Prud'homme *et al.,* 2000). However, the fact that high TGF-β levels has a role in tumour progression (Medicherla *et al*., 2007), development of organ damage (Saxena *et al.,* 2008) and

also in the aggravation of asthma (Duvernelle *et al*., 2003) suggest that increased TGF-β levels could be responsible for several negative health effects.

1.5.3 Inflammatory cytokines and apoptosis

Inflammatory cytokines play major roles in the regulation of apoptotic cell death. Apoptosis is a useful process for the healthy organism in order to eliminate highly activated or damaged cells and to resolve inflammatory processes in order to seal off damaged tissues and limit prolonged inflammation (Savill *et al.,* 1997). The cytokines dealt with in this thesis are all connected to apoptotic processes within the cell.

Briefly, TNF-α initiates cell death through TNF-receptor mediated apoptosis (Van der Meide *et al.,* 1996; Chen *et al.,* 2002). The pleiotropic cytokine IL-6 has been shown to play a role in apoptosis especially by participating in the regulation of inflammation (Hodge *et al.,* 2002). TGF-β production has been shown increase just prior to a TGF-β dependent apoptotic process (Hodge *et al.,* 2002; Freire-de-Lima *et al.,* 2006). Finally, increased IL-10 production has been shown to subsequently inhibit apoptotic processes (Zhou *et al*., 2001; Eslick *et al.,* 2004).

1.5.4 Mannan-binding lectin (MBL)

The involvement of many types of serum/plasma proteins is of vital importance in the innate host defence. Acute phase proteins are associated with inflammatory responses and several are able to bind to microbes in order to facilitate recognition by receptors on phagocytic cells. One important acute-phase protein is the mannan-binding lectin (MBL) which recognises specific PAMP's such as mannose residues on a wide range of microbes including viruses, bacteria, fungi

and protozoa (Janeway *et al.,* 2005; Turner *et al.,* 1998). MBL-microbe complexes activate the complement system through the MBL pathway (Fig. 5).

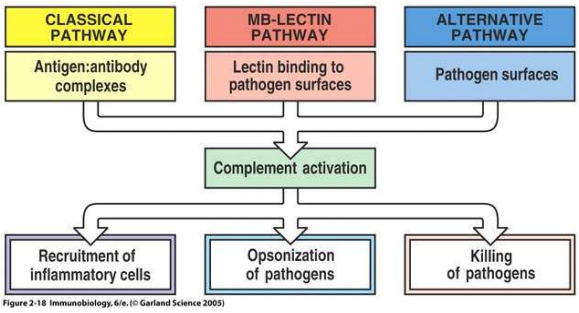


Fig 5
Complement pathways in innate immune responses (Janeway *et al.*, 2005).

MBL deficiency in humans mainly results from one or several mutations in MBL genes and is primarily associated with increased susceptibility to infections and some autoimmune diseases (Garred *et al.,* 2001). Recently, a link between high serum MBL concentrations and certain inflammatory diseases, such as childhood asthma, has been proposed (Uguz *et al.,* 2005). However, the physiological role of high MBL concentrations in asthma is not well understood. MBL has also been investigated in relation to pro-inflammatory cytokine production and it has been suggested that microbes with highly mannosylated surfaces induce lower levels of pro-inflammatory cytokines from monocytes in the presence of high MBL concentrations, while pro-inflammatory cytokine production is enhanced in the presence of low MBL concentrations (Jack *et al*., 2001). Further, some authors report that the production of regulatory and anti-inflammatory cytokines, such as IL-10, is enhanced in the presence of high MBL concentrations (Fraser *et al.,* 2006). This suggests that circulating MBL may be involved in modulation of

inflammatory cytokine responses and possibly plays an important role in the progression and severity of inflammatory diseases.

1.6 Oxidative stress and glutathione

Adverse cell damage caused by the exposure to oxidative agents, e.g. reactive oxygen species (ROS), is commonly referred to as oxidative stress. Many oxidising compounds may be harmful to the cell as they bind to negatively charged intracellular molecules such as DNA and proteins. Binding of oxidants to DNA may induce mutagenic or carcinogenic effects in the cell, while proteins or lipids may alter their functions when bound to or influenced by oxidative agents.

Host defence mechanisms against oxidative agents involve the action of intracellular antioxidants present in all healthy cells. One important antioxidant is glutathione. Glutathione is typically used as a term to refer to tripeptide L-gamma-glutamyl-L-cysteinyl glycine in both its reduced and oxidised form (López-Mirabal and Winther, 2007).

The reduced form of glutathione is N-N-L-gamma-glutamyl-L-cysteinyl glycine (GSH) while the oxidised form of glutathione is L-gamma-glutamyl-L-cysteinyl glycine disulfide (GSSG). GSH is involved in many catalytic and metabolic processes in the cell, in signal transduction, gene expression and regulation of apoptosis (Anderson, 1998; Higuchi, 2004; Franco *et al*., 2007). In the normal situation of low cellular oxidative stress the GSH/GSSG ratio is close to 1. The enzyme glutathione reductase (GR), together with the co-factor nicotinamide adenine dinucleotide phosphate (NADPH), maintains glutathione in its reduced form (Griffith, 1999). The reduction, or neutralisation, of oxidative agents by GSH is mainly catalysed by the enzyme glutathione peroxidase (GP). During this process the unstable and highly reactive

oxidised glutathione, GSSG, is formed. GSSG is then reduced to GSH by GR in order to maintain the high GSH pool in the cell (Anderson, 1998).

1.6.1 The link between glutathione and inflammatory cytokines

Production of pro-inflammatory cytokines is a redox-sensitive process and the red-ox equilibrium is influenced by cytokine production and vice versa (Haddad *et al.,* 2002; Haddad and Land, 2002). For example, GSH depletion activates inflammatory signals in the cell and the subsequent release of pro-inflammatory cytokines such as IL-1, IL-6 and TNF-α from alveolar epithelial cells (Haddad, 2000). Also, increased GSH levels have shown to inhibit pro-inflammatory cytokine production *in vitro* and in some clinical trials (Pena *et al.,* 1999). Less is known about the effect of GSH on anti-inflammatory cytokines, such as TGF-β and IL-10. Some studies have indicated that TGF-β may inhibit GSH synthesis (Arsalene *et al*., 1997; Jardine *et al*., 2002) suggesting that regulatory cytokines also may also influence, or be influenced by, the cellular red-ox state.

1.6.2 The role for glutathione in host defence against mycotoxins

The role for GSH is primarily to neutralise oxidative compounds by providing reducing equivalents, but GSH is also known to form temporary conjugates with oxidative agents. This mechanism has been suggested for some mycotoxins, i.e. depletion of the intracellular GSH pool is presumably caused by the formation of conjugates between GSH and the mycotoxin (Pfeiffer *et al.,* 2005; Dai *et al.,* 2002). Although this process is not well understood, it is thought that glutathione-transferases (GST), a family of multifunctional enzymes, catalyse this conjugation resulting in GSH-conjugates which are transported out of the cell. The export of GSH-conjugates

from cells is an ATP-dependent process mediated by proteins belonging to the multidrug-resistance protein family (MRP). Proteins of the MRP family are also known as GSH-conjugate pumps (Cole *et al*., 2006).

Whether mycotoxins contribute to inflammatory responses through their interaction with GSH is poorly investigated. Mycotoxins often do not contribute directly to increase pro-inflammatory cytokine production, but rather modulate or decrease cytokine production induced by other inflammatory compounds such as LPS **(**Chung *et al.,* 2003). However, the involvement of mycotoxins in both oxidative stress mechanisms and in the modulation of inflammatory cytokine production seems plausible as summarised in Fig. 6, and discussed in this thesis.

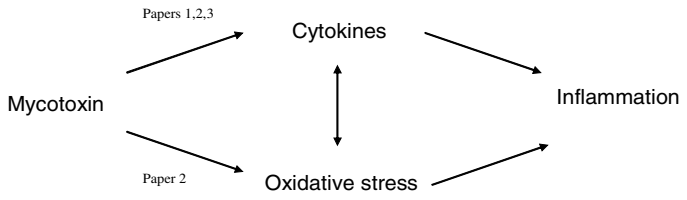


Fig. 6 Possible roles for a mycotoxin as a mediator of inflammation

2 Aims of the study

In view of the literature describing adverse health effects in humans as a result of fungal exposure and the lack of known responsible molecular and immunological mechanisms, it is important to elucidate some of the cellular responses that could be involved. The role of fungal compounds in relation to inflammatory responses is of particular interest.

The objectives of this thesis were as follows:

- Determine time- and dose-effects of the mycotoxins citrinin and gliotoxin on inflammatory cytokine production in monocytes (paper I).
- Elucidate the ability of the mycotoxins citrinin and gliotoxin to affect cellular oxidative stress mechanisms and to modulate inflammatory cytokine production in alveolar epithelial cells (paper II).
- Determine the effects of mixed microbial compound exposure and detect possible interactions between the compounds that could influence inflammatory cytokine production by monocytes (paper III).
- Measure inflammatory cytokine production by monocytes from asthmatic individuals after *in vitro* exposure to fungal cell wall mannan polysaccharides and relate the cytokine production to autologous plasma mannan-binding lectin concentrations (paper IV).

3 Summary of papers

Paper I

Mycotoxins are present on airborne fungal spores and fragments and may possibly play a role in adverse health effects. In **paper I** we measured inflammatory cytokine production from a monocytic cell line (MM6) and elucidated time-and dose-effects of the mycotoxins citrinin or gliotoxin. Non-toxic mycotoxin concentrations reduced LPS-induced production of the regulatory and anti-inflammatory cytokine IL-10 while TNF-α production was not reduced. The reduced IL-10 production was not caused by a general cytotoxic effect, as cell death by apoptosis did not increase. Reduced IL-10 production by the mycotoxins could support an inappropriate inflammatory response driven by TNF-α. LPS-induced IL-6 production was slightly influenced by the mycotoxins, but not to the same extent as IL-10 production. We conclude that the mycotoxins citrinin or gliotoxin cause an imbalance in inflammatory cytokine production *in vitro*. We propose from this study that exposure to the investigated mycotoxins may influence or enhance an inflammatory response.

Paper II

Production of inflammatory cytokines and induction of cellular oxidative stress mechanisms could be influenced by mycotoxins. We showed in **paper II** that LPS-induced production of the regulatory and anti-inflammatory cytokine TGF-β by an alveolar epithelial cell line (A549) was somewhat reduced when exposed to relatively low concentrations of the mycotoxins citrinin or gliotoxin. TGF-β production increased when cells were exposed to higher mycotoxin concentrations. Production of the pleiotropic cytokine IL-6 and the chemokine IL-8

was not affected. Levels of the antioxidant glutathione were reduced in A549 with increasing, but non-toxic concentrations of citrinin after 48 or 72 hrs. Dose-and time effects of mycotoxin exposure were also indicated by a tendency to an opposite relationship between TGFβ production and glutathione levels. We conclude that the investigated mycotoxins may induce cellular oxidative stress responses by reducing intracellular levels of the antioxidant glutathione and also modulate production of the regulatory and anti-inflammatory cytokine TGF-β.

Paper III

Exposure to combinations of microbial compounds (bacterial LPS, fungal polysaccharides and mycotoxin) may occur simultaneously in environmental exposure situations, but the cellular responses to such are poorly studied and not well understood. With different exposure regimes we showed in **paper III** that the fungal polysaccharides mannan or β-glucan induced TNF-α production, but not detectable IL-10 production in normal human monocytes. The mycotoxin citrinin reduced LPS-induced IL-10 production, but not the TNF-α production, thus confirming earlier observations from paper I.

We statistically estimated interactions between the bacterial and fungal compounds and the influence on TNF-α production by monocytes. By using a multiple regression model (linear mixed model) we found that monocyte exposure to combinations of the fungal polysaccharides, β-glucan in particular, and LPS reduced TNF-α production compared to single compound exposures. We conclude that TNF-α production by monocytes due to combined microbial compound exposure may be different from that deducted from exposure to the single compounds.

Paper IV

Individuals with asthma are at risk of bronchial symptoms when exposed to airborne fungi. It seems that increased levels of the plasma protein mannan-binding lectin (MBL) play a

role in severe asthmatic disease, although the role for MBL in mild and untreated adult asthma is poorly investigated. Also, MBL has been shown to be involved in modulation of inflammatory cytokine production.

We conducted a pilot study and report in **paper IV** that *in vitro* exposure of monocytes to the fungal cell wall polysaccharide mannan, in the presence of autologous plasma, induced lower TNF-α production in cells isolated from adults with mild and untreated asthma compared to healthy controls. A tendency to increased plasma MBL levels in asthmatics was observed, although not statistically significant, suggesting that MBL levels may be associated to the severity of asthma. Results from this small study call for further investigations on the role of MBL in asthma and in particular on the role of MBL in modulation of non-allergic inflammatory cytokine production induced by the fungal polysaccharide mannan.

4 General discussion

4.1 Methodological considerations

4.1.1 *In vitro* assays and the extrapolation to human exposure

Environmental exposures are most often highly complex and involve a variety of agents, i.e. microbes, microbial products, particles and chemicals. Time and route of the exposure may vary and there is also variability of individual responses and subjective symptoms. Hence, *in vitro* investigations of exposure effects may sometimes be of limited relevance to describe actual biological effects of single or multiple agents in the environment. Also, *in vitro* experimental studies, commonly using single types of cells, have limited relevance in describing complex local and systemic responses seen in tissues and whole organisms. Observed modulations and alterations of cellular responses due to defined exposures are sometimes difficult to relate to specific clinical symptoms. In order to obtain a more detailed understanding of biological effects due to factors present in the environment, however, *in vitro* assays are essential. With the increasing availability of techniques used to explore complex processes in cell biology, the *in vitro* approach is invaluable to help us understand processes in the individual cell. New emerging genomics and proteomics techniques will probably be of great importance for large scale screening of single and multiple effects of exposure agents, thus, mechanisms in host responses can be studied more thoroughly. Once a more complete picture of biological mechanisms is known, understanding of physiological processes can emerge.

4.1.2 Soluble substances versus particles

The route of microbial exposure in an indoor air setting is through inhalation and it is commonly thought that inhalation of particles, such as fungal spores and other airborne fragments, leads to the distribution of inflammatory compounds into the lungs. Thus, *in vitro* studies using particles coated with inflammatory compounds of interest, such as LPS or fungal polysaccharides, may be useful in order to simulate the inflammatory potential of bacteria or fungal spores, respectively. The initiation of an inflammatory response may proceed through different membrane receptors used by soluble or particulate forms of inflammatory substances (Flo *et al.,* 2000). Also, an increased inflammatory cytokine response to particles, probably because of adjuvant effects, is often observed (Berntzen *et al.,* 1998). However, dose-effects or interactions between various microbial compounds may be difficult to observe using particles, and moreover the inflammatory compounds are not always linked to particles/microbes in the environment, but rather the compounds may be airborne and are inhaled i.e. through aerosols (Wouters *et al.,* 2006). Thus, it is meaningful to study the inflammatory potential of defined soluble compounds and in particular when the vehicles for the various compounds are not well defined as in the case when the compounds are separated from spores, fragments, dust particles or whole cell microbes.

4.1.3 Cell lines versus primary cells

Cell-specific differences in the production of inflammatory cytokines are essential for the innate immune response and also for disease pathogenesis. *In vitro* cell line systems offer highly

reproducible cellular responses and minimal loss of cell viability, which often is a problem with normal immune cells especially when prolonged exposures are required.

However, cell lines may also differ considerably in the ability to produce inflammatory cytokines probably because of different cell specific signalling through activated receptors (Zughaier *et al*., 2005). The alveolar epithelial cancer cell line A549 (paper II) has shown to respond somewhat different from normal epithelial cells during e.g. virus infections (Wansley *et al.,* 2005), although inflammatory cytokine production after environmental LPS exposure of normal lung epithelial cells or A549 is similar (Palmberg *et al*., 1998).

Some commonly used monocytic cell lines, such as THP1, have shown not to respond with inflammatory cytokine production when exposed to various sources of endotoxins (LPS) compared to normal blood monocytes (Yamamoto *et al.,* 2003). The fact that cell line responses may not always be representative for normal cell responses makes it necessary to additionally test exposure effects in normal cell systems. The monocytic cell line MM6 (paper I) showed similar cytokine responses to LPS stimulation as primary blood monocytes did (papers III and IV).

Some recent *in vitro* studies have shown interactions between different types of cells, i.e. lung epithelial cells and alveolar macrophages, and some of the complicated cellular processes and interactions that exists are suggested (Rubovitch *et al.,* 2007; Elizur *et al*., 2008). However, the primary limitation of all *in vitro* studies will still be that the results obtained are only relevant for certain experimental settings, and thus they are difficult to extrapolate directly to *in vivo* situations.

4.1.4 Human immune and epithelial cells as exposure targets in vitro

Alveolar epithelial cells are relevant to study because the exposure route of indoor microbial compounds is through inhalation. Although alveolar epithelial cells (paper II) are biologically plausible actors in an immune response, the presumably most important immune cells in lung tissues are the alveolar macrophages. Monocytes resemble macrophages with regard to most innate immune responses, except for the superior phagocytic capacity of the macrophages and differences in the magnitude of inflammatory cytokine production (Losa-García *et al*., 1999). We used the adherent cell fraction of blood monocytes isolated from PBMC to simulate macrophage activity with regard to cytokine production (papers III and IV).

4.2 Findings and implications

4.2.1 The ”relevant dose” question – from culture wells to damp buildings

Exposure effects to environmental microbes must be assumed to vary. Besides the concentration of inhaled microbes and their products, other factors such as the thresholds for activation of various cell types and differences in the individual susceptibility to the exposure substance are of importance.

The presence of mycotoxins in damp buildings is presumably of importance, but as the concentration of inhaled toxins is highly uncertain, human health effects are difficult to evaluate. In fact, it may not be possible to predict concentrations of inhaled mycotoxins due to lack of validated investigation methods. Perhaps the most uncertain method is to detect mycotoxin exposure by determining the presence of a fungal species known to be responsible for toxin production. Different fungi may favour certain building materials and the growth conditions for a particular fungal strain, in order to produce toxins, may differ. E.g. species of the fungal contaminant *Chaetomium* may produce the mycotoxin chaetoglobosin A at 50 µg/cm^2 on wallpapered gypsum boards, but at the same time the quantities of mycotoxins isolated from *Penicillium sp.* may be low (Nielsen *et al*., 1999). Another method used to determine airborne mycotoxin exposure is to extract mycotoxins from fungal spores and fragments. This may, however, not be representative for the actual environmental toxin concentration if (i) the fungal strain is grown on standardised nutrition media as toxin production may be depleted at laboratory conditions, and (ii) the yield of toxin after extraction from the fungal strain may be low. In recent years, however, the methods used to detect mycotoxins from indoor environments, predominantly produced by *Aspergillus* sp. and *Stachybotrys* sp., have improved (Bloom *et al*., 2007).

On the basis of existing research on the topic, it cannot be ruled out that doses of mycotoxins that could affect inflammatory responses may be encountered in certain indoor exposure situations. Fungal spores and fragments may be effectively released from microbial growth on modern building materials, and especially during agitation or handling of the infected materials (Madsen *et al.,* 2006). Moreover, for the mycotoxins investigated in this thesis, it has been shown that *A. fumigatus* grown on wallpapered gypsum boards can produce gliotoxin in concentrations of about 20 ng/cm^2 (Nieminen *et al.,* 2002), that would equal 2 µg mycotoxin/100 cm^2. Thus, it can be presumed that high enough mycotoxin doses to influence inflammatory responses can be encountered from relatively small areas with heavy mould growth on indoor materials, provided that the dose is quantitatively released and subsequently inhaled.

Citrinin is a mycotoxin that has been isolated both from food, e.g. cheese (Kokkonen *et al.,* 2005), and from indoor materials (Jarvis and Miller, 2005; Tuomi *et al*., 2000). Possibly, citrinin production is favoured on materials with high sucrose content, in contrast to cellulose content (El-Magraby and El-Maraghy, 1988). Thus, the production of citrinin on many cellulose based indoor materials may be restricted. However, it has been suggested that concentrations of citrinin in indoor environments may be up to 20 µg /g fungal biomass (Tuomi *et al*., 2000) suggesting that high enough doses to influence inflammatory responses could be relevant in certain settings.

The water soluble extracellular polysaccharides, often called extracellular polymeric substances (EPS), are major components of most microbial cell walls including all kinds of fungi. The saccharides may contain monomers of different sugars such as mannose, fucose, glucose, galactose or arabinose and the production of the different sugars is highly dependent of the growth conditions of the microbe (Cho *et al.,* 2001). EPS extracted from *Aspergillus* and

Penicillium isolates (EPS-Asp/Pen) have been used in immunoassays in order to detect environmental EPS as surrogates for fungal exposure (Chew *et al*., 2001; Douwes *et al.*, 1999). As mentioned, there may be several different saccharides and the amount of mannose polymers (as parts of the EPS) has not been reported.

High concentrations of EPS in indoor environments have been suggested to correlate with increased building-related respiratory symptoms (Douwes *et al*., 1999). However, it has also been pointed out that increased EPS concentrations in indoor environments of e.g. farm children may be inversely related to asthma development (Ege *et al*., 2007). The conflicting reports may be due to lack of characterisation of the exposure agent or different methods used in the investigations. The latter study showed high variability of exposure effects between different countries, suggesting that confounding factors may be involved.

A high variability in detected EPS concentrations has been observed particularly in damp buildings. Some authors have suggested that relative concentrations of EPS in settled house dust could be in the range between 25,000 and 50,000 U/g (Chew *et al*., 2001; Douwes *et al.,* 1999; Douwes *et al*., 2006). It must be assumed that a variability of EPS concentrations in damp buildings is caused by various amounts of microbes present, especially fungi, but also the lack of standardised and validated methods for analysis and characterisation of these polysaccharides (and their biological activity) may lead to a high uncertainty with regard to the interpretation of findings.

Glucan content of indoor settled house dust has been detected in various concentrations, e.g. about 22 mg/g (Gehring *et al*., 2001), about 1 mg/g (Chew *et al*., 2001) and about 0.6 mg/g (Douwes *et al*., 2000). Concentrations up to 300 µg glucan/mg dust have been detected in an occupational waste handling industry (Wouters *et al*., 2006). None of these studies provided

information about the molecular forms of the glucans detected, which presumably are highly different depending on environmental conditions (Sletmoen and Stokke, 2008).

Only a few studies have addressed the glucan concentrations in bronchoalveolar lavage (BAL) fluids from individuals exposed to airborne fungi. Although increased glucan concentrations in BAL fluids (in the range from 200 to 2,500 pg/mL) have been measured along with enhanced pro-inflammatory cytokine release, this has only been investigated in patients diagnosed with the inflammatory lung disease farmer's lung or in patients with acute eosinophilic pneumonia (described as a variant of summer-type hypersensitivity pneumonitis) (Ashitani *et al.,* 2008; Kawayama *et al.,* 2003). However, these findings suggest that increased concentrations of glucan in BAL fluids can be associated with the development of inflammatory lung diseases.

It should be emphasised that the ability of different glucans to exert biological activity, i.e. modulate cytokine production, is dependent on that a sufficient amount of glucan molecules bind to cell surface receptors, and therefore on the administrated dose, molecular weight and branching, and conformation of the molecule (Okazaki *et al*., 1995; Sletmoen and Stokke, 2008; Falch *et al*., 2000). In order to identify the active component that presumably gives a particular biological effect, the sample should be well characterised and the number of interfering substances that may affect cellular responses should be minimised. These requirements are challenging when working with fungal glucan extracts, since small differences in the fungal genetics as well as environmental factors such as growth substrate, temperature and moisture could lead to differences in the sample composition. Also, the fact that glucans may interact with 'guest' molecules, i.e. bind other molecules in the environment, complicates characterisation and the measurement of actual biological activity of the glucan sample. Hence, the diversity of glucans found in nature is a result of the diversity of structurally distinct molecules and

impurities. When used in research projects, this could lead to different conclusions when comparing results from different research groups (Brown and Gordon, 2003).

Health effects of endotoxin exposure in indoor environments, and in particular in damp buildings, have been reported and as for the fungal products there are variable concentrations measured, e.g. in house dust samples in a range between 30,000 EU/g dust (Heinrich *et al*., 2003) and 60,000 EU/g dust (Douwes *et al*., 2006; Hines *et al*., 2000). In occupational settings, such as waste handling industries, bioaerosol concentration has shown to exceed conventional exposure limits (Dutch) (50-200 EU/m^3 for inhaled endotoxin) (Wouters *et al*., 2006). Building-related symptoms associated with endotoxin exposure are overall similar to those of fungal exposure, especially upper and lower respiratory symptoms and headache (Park *et al*., 2006).

Reports of modulation of inflammatory symptoms due to exposure to mixed microbial compounds such as environmental endotoxin (LPS) and glucan are few. However, it has been reported in an epidemiologically study that exposure to endotoxin may change the effect of fungal exposure (and vice versa), more specifically that lower respiratory symptoms are enhanced by a mixed endotoxin and fungal exposure (Park *et al.,* 2006). This suggests that symptoms due to mixed exposure could be different, and may be enhanced, compared to symptoms from single compound exposure. However, the molecular mechanisms of glucans as biological response modifiers are not well understood, but may involve both activating and inhibitory pathways. Some authors have demonstrated inhibitory effects of mixed LPS and glucan exposure with regard to pro-inflammatory cytokine production (Soltys *et al*., 1999), thus supporting the findings in paper III. Also, it has been suggested that individuals exposed to organic dust when living at farms, presumably involving combined exposure of endotoxin and fungal glucan, could be protected against adult asthma (Douwes *et al*., 2007). On the other hand, another report concludes

that exposure to a farming environment is not always beneficial, but rather that e.g. pig-keeping farms constitute so-called "protective factors" (Ege *et al*., 2007). Further, and on a more general basis, *in vivo* observations indicate that microbial exposure effects and susceptibility for asthma development may be strongly regulated by genetic factors, such as the individual expression of TLR genes (Eder *et al*., 2004; Ege *et al*., 2006). Also, another recent study emphasise that genetic variations in the innate immunity gene expression could modulate the individual response to microbial agents and the susceptibility and development of atopy and asthma (Smit *et al*., 2007). In this context, it is tempting to suggest that the results presented in paper IV could reflect that reduced pro-inflammatory cytokine production in asthmatics may partly be regulated by the levels of circulating MBL that is presumably regulated by MBL genes. The possible associations between fungal exposure, individual MBL levels and asthma development remain to be elucidated.

4.2.2 What do our findings tell us about the symptoms experienced in damp buildings?

Findings in this thesis can partly explain some of the experienced non-allergic inflammatory symptoms in damp buildings. More specifically, results reported in paper I, II and III suggest that the mycotoxins citrinin or gliotoxin may act indirectly to support a pro-inflammatory cytokine production and reduce anti-inflammatory cytokine production induced by LPS. Further, the mycotoxin citrinin in particular, increased oxidative stress mechanisms and this may support an inflammatory state of the cells.

Reports of symptoms including irritation of mucous membranes and cough may be explained by a general sensitivity to particulate substances, including spores, and it seems plausible that inflammagenic compounds present on the inhaled bacteria, spores, fungal

fragments or other carrier substances would further enhance such irritation. Other symptoms of inflammatory processes, including headache, fever, nausea and fatigue, may be initiated by production of TNF-α in particular (Oshiro and Morris, 1997; Sanchez-Del-Rio and Reuter, 2004). Yet, other symptoms are more difficult to relate directly to known specific or non-specific cellular immune responses. Respiratory symptoms reported by means of damp buildings, especially by asthmatics, but the cellular mechanisms responsible for these symptoms are still inconclusive. Several factors of the innate and/or adaptive immune system may be involved (Eisenbarth *et al*., 2004). However, aggravation of inflammatory respiratory symptoms is likely to be related to pro-inflammatory cytokine production, both in normal and asthmatics.

4.2.3 The role for mannose-binding lectin (MBL) in asthma and the asthmatic response to fungal exposure

Although a strong correlation between fungal exposure and severity and aggravation of asthmatic disease has been reported (Denning *et al*., 2006; Zureik *et al*., 2002; O'Driscoll *et al*., 2005), the molecular mechanisms underlying the clinical symptoms in asthma due to fungal exposure are still not clarified.

Among the genetically influenced immune modulating proteins of the innate immune system, MBL has emerged (Turner and Hamvas, 2000). Little is known about the role for MBL in immune responses to fungal exposure, but several aspects of MBL may be of importance. First, high levels of MBL have been implicated in the development and maintenance of airway hyper responsiveness (Hogaboam *et al.,* 2004). Further, MBL is

capable of binding to fungi such as *Candida albicans*, *Cryptococcus neoformans* and *A. fumigatus* (Neth *et al.,* 2000). Environmental bacteria are also recognised by MBL (Niedoszytko *et al.,* 2007). Also, a role for MBL has been shown in the modulation of inflammatory cytokine production in monocytes, as discussed in paper IV. It seems that high physiological levels of MBL may inhibit or reduce pro-inflammatory cytokine production from monocytes/macrophages (Jack *et al.,* 2001). However, the role for MBL in asthma, in the context of inhibition of TNF-α production, brings up conflicting theories. The fact that clinical symptoms of inflammation in both normal and asthmatic subjects is largely characterised by high systemic TNF-α production (Gaga *et al*., 2007), does not support a role for MBL in reducing TNF-α production. In allergic asthma, however, where the cytokine profile is skewed towards high IL-4, IL-5 and IL-13 production, mast cells seem to be an important source of TNF-α production (Nakae *et al*., 2007). This suggests that MBL may have a role in reducing TNF-α production from monocytes, but not from mast cells, and that the influence of MBL may be restricted to professional phagocytes.

4.2.4 Implications for future practice and future research

The cellular inflammatory response mechanisms responsible for symptoms in asthmatics due to fungal exposure need to be further elucidated. It is of particular interest to investigate the non-allergic inflammatory responses to fungal exposure in relation to MBL production. The fact that MBL may be an important modulator of inflammatory diseases could induce the hypothesis that certain individuals may be particularly susceptible for the development of asthmatic disease. Moreover, it may be of particular interest to explore if and

how specific fungal cell wall polysaccharides bind to MBL and whether this could affect the inflammatory response in asthma.

In a more practical setting, it is of particular interest to further elucidate the role for mycotoxins in a fungal exposure situation. The fact that only a limited amount of reports have suggested that mycotoxins may be present in sufficient concentrations to affects immune responses in exposed individuals calls for further research on this topic.

The most important issue, however, is to develop and improve methods used for the measurement of airborne mycotoxins and other fungal products. When increased knowledge of actual and relevant exposure doses is established, symptoms reported from exposed individuals in damp and mouldy buildings may better be related to cellular responses.

4.3 Overall conclusions

The mycotoxins studied may promote inflammatory responses by inhibiting production of anti-inflammatory cytokines at doses which do not inhibit pro-inflammatory cytokine production (paper I).

The mycotoxin citrinin in particular reduces levels of the antioxidant glutathione which may enhance inflammatory responses related to oxidative stress in alveolar epithelial cells. Also, the mycotoxins studied may interfere with the production of the anti-inflammatory cytokine TGF- β while not affecting pro-inflammatory cytokine/chemokine production (paper II).

Interactions between microbial products (LPS and glucan in particular) can modulate TNF-α production from monocytes. These interactions cannot be predicted by the exposure to one substance at a time. These results may possibly explain some of the conflicting reports of combinatory exposures in damp buildings (paper III).

Asthmatics produce lower levels of the pro-inflammatory cytokine TNF-α after fungal cell wall mannan exposure of monocytes. The autologous plasma concentration of mannan-binding lectin (MBL) may be relevant for the pro-inflammatory cytokine production in asthma (paper IV).

5 References

Abbott (2002). "Mycotoxins and Indoor Molds." Indoor Environment Connections **3**: 14-24.

Anderson (1998). "Glutathione: an overview of biosynthesis and modulation." Chem Biol Interact. **111-112**: 1-14.

Andersson MA, N. M., Koljalg U, Andersson MC, Rainey F, Reijula K, Hintikka EL, Salkinoja-Salonen M (1997). "Bacteria, molds, and toxins in water-damaged building materials." Appl Environ Microbiol. **63**: 387-393.

Arsalane K, D. C., Muanza T, Bégin R, Boudreau F, Asselin C, Cantin AM. (1997). "Transforming growth factor-beta1 is a potent inhibitor of glutathione synthesis in the lung epithelial cell line A549: transcriptional effect on the GSH rate-limiting enzyme gamma-glutamylcysteine synthetase." Am J Respir Cell Mol Biol **17**: 599-607.

Artursson P, A. E., Edman P, Ericsson JL, Sjöholm I (1987). "Biodegradable microspheres. V: Stimulation of macrophages with microparticles made of various polysaccharides." J Pharm Sci. **76**: 127-133.

Aufauvre-Brown A, B. J., Holden DW. (1998). "Comparison of virulence between clinical and environmental isolates of Aspergillus fumigatus." Eur J Clin Microbiol Infect Dis. **17**: 778-780.

Baker (2006). "Aspergillus niger genomics: past, present and into the future." Med Mycol **44**: 17-21.

Barreto-Bergter E, G. P. (1983). "Structural chemistry of polysaccharides from fungi and lichens." Adv Carbohydr Chem Biochem **41**: 67–101.

Beijer L, T. J., Rylander R (2003). "Mould exposure at home relates to inflammatory markers in blood." Eur Respir J. **21**: 317-322.

Bennett, J. C., A (1983). Secondary Metabolism and Differentiation in Fungi. New York, Marcel Dekker.

Bennett JW, K. M. (2003). "Mycotoxins." Clin Microbiol Rev **16**: 497-516.

Bernard M, L. J. (2001). "Aspergillus fumigatus cell wall: composition and biosynthesis." Med Mycology **39**: 9-17.

Bernardo PH, B. N., Chai CL, Waring P. (2003). "A novel redox mechanism for the glutathione-dependent reversible uptake of a fungal toxin in cells." J Biol. Chem **278**: 46549-46555.

Bernton (1930). "Asthma due to a Mold - Aspergillus fumigatus." JAMA **95**: 189-192.

Berntzen G, F. T., Medvedev A, Kilaas L, Skjåk-Braek G, Sundan A, Espevik T. (1998). "The tumor necrosis factor-inducing potency of lipopolysaccharide and uronic acid polymers is increased when they are covalently linked to particles." Clin Diagn Lab Immunol. **5**: 355-361.

Bloom E, B. K., Nyman E, Must A, Larsson L. (2007). "Mass spectrometry-based strategy for direct detection and quantification of some mycotoxins produced by Stachybotrys and Aspergillus spp. in indoor environments." Appl Environ Microbiol. **73**: 4211-4217.

Bornehag CG, S. J., Hagerhed-Engman L, Sigsggard T, Janson S, Aberg N; DBH Study Group. (2005). "'Dampness' at home and its association with airway, nose, and skin symptoms among 10,851 preschool children in Sweden: a cross-sectional study. ." Indoor Air. **15**: 48-55.

Bortner CD, S. M., Cidlowski JA (2007). "New approaches for determining apoptotic volume decrease in cells. ." Methods Enzymol. **428**: 161-181

Brown GD, G. S. (2003). "Fungal beta-glucans and mammalian immunity. ." Immunity. **19**: 311-315.

Brun (2007). EUROPEAN RISK OBSERVATORY REPORT. Luxembourg, Office for Official Publications of the European Communities.

Bruneau JM, M. T., Tagat E, Legrand R, Bernard M, Diaquin M, Fudali C, Latgé JP (2001). "Proteome analysis of Aspergillus fumigatus identifies glycosylphosphatidylinositol-anchored proteins associated to the cell wall biosynthesis." Electrophoresis. **22**: 2812-2823.

Calvo A, W. R., Bok J, Keller N. (2002). "Relationship between secondary metabolism and fungal development." Mol Microbiol Rev. **66**: 447–459.

Carlile Michael J, W. S. C., Gooday Graham W (2001). The Fungi. London, Academic Press.

Chen W, P. G., Tang A, Wang K, Zhou M, Wang L. (2002). "Study on the expression of IkappaB-alpha protein in TNF-alpha induced apoptosis of U937 cells." Zhonghua Xue Ye Xue Za Zhi. **23**: 353-355.

Chew GL, D. J., Doekes G, Higgins KM, van Strien R, Spithoven J, Brunekreef B (2001). "Fungal extracellular polysaccharides, beta (1-->3)-glucans and culturable fungi in repeated sampling of house dust." Indoor Air. **11**: 171-178.

Cho DH, C. H., Kim EY (2001). "Synthesis and characterization of a novel extracellular polysaccharide by Rhodotorula glutinis." Appl Biochem Biotechnol. **95**: 183-193.

Chung (2001). "Anti-inflammatory cytokines in asthma and allergy: interleukin-10, interleukin-12, interferon-gamma." Mediators Inflamm. **10**: 51-59

Chung YJ, J. B., Pestka J. (2003). "Modulation of lipopolysaccharide-induced proinflammatory cytokine production by satratoxins and other macrocyclic trichothecenes in the murine macrophage. ." J Toxicol Environ Health A. **66**: 379-391.
Clark BR, C. R., Lacey E, Tennant S, Gill JH. (2006). "Citrinin revisited: from monomers to dimers and beyond." Org Biomol Chem. **4**: 1520-1528.
Cole SP, D. R. (2006). "Transport of glutathione and glutathione conjugates by MRP1." Trends Pharmacol Sci. **27**: 438-446.
Comi, A. L., A. Tedeschi, et al. (2007). "Novel clinical and serological aspects in non-allergic asthma." Respiratory Medicine **101**(12): 2526-2533.
Cooley (1998). "Correlation between the prevalence of certain fungi and sick building syndrome." Occup Environ Med **55**: 579-584.
Da Lozzo EJ, O. M., Carnieri EG (1998). "Citrinin-induced mitochondrial permeability transition." J Biochem Mol Toxicol **12**: 291-297.
Dai J, P. G., Wright MW, Adams M, Akman SA, Manderville RA. (2002). "Detection and Characterization of a Glutathione Conjugate of Ochratoxin A." Chem Res Toxicol. **15**: 1581 -1588.
Denning DW, O. D. B., Hogaboam CM, Bowyer P, Niven RM. (2006). "The link between fungi and severe asthma: a summary of the evidence. ." Eur Respir J. **27**: 615-626.
Douwes J, S. R., Wouters I, Doekes G, Fitzharris P, Crane J. (2006). "Endotoxin, (1 --> 3)-beta-D-glucans and fungal extra-cellular polysaccharides in New Zealand homes: a pilot study." Ann Agric Environ Med. **13**: 361-365
Douwes J, T. N., Huang K, Cheng S, McKenzie J, Le Gros G, von Mutius E, Pearce N. (2007). "Lifelong farm exposure may strongly reduce the risk of asthma in adults." Allergy. **62**: 1158-1165
Douwes J, v. d. S. B., Doekes G, van Leusden F, Wijnands L, van Strien R, Verhoeff A, Brunekreef B (1999). "Fungal extracellular polysaccharides in house dust as a marker for exposure to fungi: relations with culturable fungi, reported home dampness, and respiratory symptoms." J Allergy Clin Immunol. **103**: 494-500. .
Douwes J, Z. A., Doekes G, van der Zee SC, Wouters I, Boezen MH, Brunekreef B. (2000). "(1-->3)-beta-D-glucan and endotoxin in house dust and peak flow variability in children." Am J Respir Crit Care Med. **162**: 1348-1354
Duvernelle C, F. V., Frossard N. (2003). "Transforming growth factor-beta and its role in asthma." Pulm Pharmacol Ther. **16**: 181-196.
Eder W, K. W., Yu L, von Mutius E, Riedler J, Braun-Fahrländer C, Nowak D, Martinez FD; ALEX Study Team. (2004). "Toll-like receptor 2 as a major gene for asthma in children of European farmers. ." J Allergy Clin Immunol. **113**: 482-488.
Ege MJ, B. C., Frei R, van Strien RT, Riedler J, Ublagger E, Schram-Bijkerk D, Brunekreef B, van Hage M, Scheynius A, Pershagen G, Benz MR, Lauener R, von Mutius E, Braun-Fahrländer C; Parsifal Study team. (2006). "Prenatal farm exposure is related to the expression of receptors of the innate immunity and to atopic sensitization in school-age children. ." J Allergy Clin Immunol. **117**: 817-823.
Ege MJ, F. R., Bieli C, Schram-Bijkerk D, Waser M, Benz MR, Weiss G, Nyberg F, van Hage M, Pershagen G, Brunekreef B, Riedler J, Lauener R, Braun-Fahrländer C, von Mutius E; PARSIFAL Study team (2007). "Not all farming environments protect against the development of asthma and wheeze in children. ." J Allergy Clin Immunol. **119**: 1140-1147. .
Eisenbarth SC, C. S., Bottomly K. (2004). "Understanding asthma pathogenesis: linking innate and adaptive immunity." Curr Opin Pediatr. **16**: 659-66.
Elizur A, A.-K. T., Kelley DG, Griffin GL, Demello DE, Senior RM. (2008). "Tumor necrosis factor-alpha from macrophages enhances LPS-induced clara cell expression of keratinocyte-derived chemokine." Am J Respir Cell Mol Biol. **38**: 8-15.
El-Magraby OM, e.-M. S. (1988). "Mycoflora and mycotoxins of peanut (Arachis hypogaea L.) seeds in Egypt. III. Cellulose-decomposing and mycotoxin-producing fungi. ." Mycopathologia. **104**: 19-24.
Engvall K, N. C., Norback D. (2001). "Asthma symptoms in relation to building dampness and odour in older multifamily houses in Stockholm. ." Int J Tuberc Lung Dis. **5**: 468-477.
Enoch DA, L. H., Brown NM. (2006). "Invasive fungal infections: a review of epidemiology and management options." J Med Microbiol **55**: 809-818.
Eslick J, S. J., Albee L, Bickel E, Bradley K, Perlman H. (2004). "IL-4 and IL-10 Inhibition of Spontaneous Monocyte Apoptosis is Associated with Flip Upregulation." Inflammation. **28**: 139-145.

Falch BH, E. T., Ryan L, Stokke BT. (2000). "The cytokine stimulating activity of (1-->3)-beta-D-glucans is dependent on the triple helix conformation. ." Carbohydr Res. **329**: 587-596.
Finningan MS, P. C., Burge PS (1984). "The sick building syndrome: prevalence studies." BMJ **289**: 1573-1575.
Fisk, W. J. L.-G., Q.Mendell, M. J. (2007). "Meta-analyses of the associations of respiratory health effects with dampness and mold in homes." Indoor Air **17**(4): 284-296.
Flemming HC, W. J. (2001). "Relevance of microbial extracellular polymeric substances (EPSs)--Part I: Structural and ecological aspects." Water Sci Technol. **43**: 1-8.
Flo TH, R. L., Kilaas L, Skjâk-Braek G, Ingalls RR, Sundan A, Golenbock DT, Espevik T (2000). "Involvement of CD14 and beta2-integrins in activating cells with soluble and particulate lipopolysaccharides and mannuronic acid polymers." Infect Immun. **68**: 6770-6776.
Fog-Nielsen (2003). "Mycotoxin production by indoor molds." Fungal Genet Biol **39**: 103-117.
Fontaine T, S. C., Dubreucq G, Adam O, Delepierre M, Lemoine J, Vorgias CE, Diaquin M, Latgé JP. (2000). "Molecular organization of the alkali-insoluble fraction of Aspergillus fumigatus cell wall." J Biol Chem. **275**: 27594-27607.
Franco R, S. O., Pappa A, Panayiotidis MI. (2007). "The central role of glutathione in the pathophysiology of human diseases." Arch Physiol Biochem. **113**: 234-258.
François (2006). "A simple method for quantitative determination of polysaccharides in fungal cell walls." Nat Protoc **1**: 2995-3000.
Fraser DA, B. S., Jasinskiene N, Rawal N, Palmarini G, Ruiz S, Rochford R, Tenner AJ (2006). "C1q and MBL, components of the innate immune system, influence monocyte cytokine expression." J Leukoc Biol. **80**: 107-116.
Freire-de-Lima CG, X. Y., Gardai SJ, Bratton DL, Schiemann WP, Henson PM. (2006). "Apoptotic cells, through transforming growth factor-beta, coordinately induce anti-inflammatory and suppress pro-inflammatory eicosanoid and NO synthesis in murine macrophages." J Biol. Chem. **281**: 38376-38384.
Gaga M, Z. E., Grivas S, Castro M, Chanez P (2007). "Evaluation and management of severe asthma." Curr Med Chem. **14**: 1049-1059.
Gantner BN, S. R., Underhill DM. (2005). "Dectin-1 mediates macrophage recognition of Candida albicans yeast but not filaments." EMBO J. **24**: 1277-1286.
Garred P, V. A., Madsen HO, Junker P. (2001). "Association of mannose-binding lectin gene variation with disease severity and infections in a population-based cohort of systemic lupus erythematosus patients." Genes Immun. **2**: 442-450.
Garrett MH, R. P., Hooper MA, Abramson MJ, Hooper BM (1998). "Indoor airborne fungal spores, house dampness and associations with environmental factors and respiratory health in children. ." Clin Exp Allergy. **28**: 459-467.
Gehring U, D. J., Doekes G, Koch A, Bischof W, Fahlbusch B, Richter K, Wichmann HE, Heinrich J; INGA Study Group (2001). "Indoor Factors and Genetics in Asthma. Beta(1-->3)-glucan in house dust of German homes: housing characteristics, occupant behavior, and relations with endotoxins, allergens, and molds." Environ Health Perspect. **109**: 139-144
Giovannangelo M, G. U., Nordling E, Oldenwening M, Terpstra G, Bellander T, Hoek G, Heinrich J, Brunekreef B (2007). "Determinants of house dust endotoxin in three European countries - the AIRALLERG study. ." Indoor Air **17**: 70-79.
Giovannangelo ME, G. U., Nordling E, Oldenwening M, van Rijswijk K, de Wind S, Hoek G, Heinrich J, Bellander T, Brunekreef B. (2007). "Levels and determinants of beta (1-->3)-glucans and fungal extracellular polysaccharides in house dust of (pre-)school children in three European countries. ." Environ Int. **33**: 9-16.
Griffith (1999). "Biologic and pharmacologic regulation of mammalian glutathione synthesis." Free Radic Biol Med. **27**: 922-935.
Gutteridge, B. H. a. J. M. C. (1989). Free radicals in biology and medicine. Oxford (UK), Clarendon Press.
Haddad (2000). "Glutathione depletion is associated with augmenting a proinflammatory signal: evidence for an antioxidant/pro-oxidant mechanism regulating cytokines in the alveolar epithelium." Cytokines Cell Mol Ther. **6**: 177-187.
Haddad JJ, L. S. (2002). "Redox signaling-mediated regulation of lipopolysaccharide-induced proinflammatory cytokine biosynthesis in alveolar epithelial cells." Antioxid Redox Signal. **4**: 179-193.
Haddad JJ, S.-G. B., Saadé NE, Lauterbach R. (2002). "Inhibition of glutathione-related enzymes augments LPS-mediated cytokine biosynthesis: involvement of an IkappaB/NF-kappaB-sensitive pathway in the alveolar epithelium." Int Immunopharmacol **2**: 1567-1583.

Hanika C, C. W., Hinsman EJ, Tuite J (1986). "Citrinin mycotoxicosis in the rabbit: ultrastructural alterations." Vet Pathol. **23**: 245-253.
Hardin BD, K. B., Saxon A (2003). "Adverse human health effects associated with molds in the indoor environment." J Occup Environ Med. **45**: 470-478.
Heinrich J, G. U., Douwes J, Koch A, Fahlbusch B, Bischof W, Wichmann HE; INGA-Study Group. (2001). "Pets and vermin are associated with high endotoxin levels in house dust." Clin Exp Allergy. **31**: 1839-1845.
Heinrich J, H. B., Douwes J, Richter K, Koch A, Bischof W, Fahlbusch B, Kinne RW, Wichmann HE; INGA Study Group. (2003). "Reproducibility of allergen, endotoxin and fungi measurements in the indoor environment." J Expo Anal Environ Epidemiol. **13**: 152-160.
Hetherington AC, R. H. (1931). "Studies in the biochemistry of micro-organisms. On the production and chemical constitution of a new yellow colouring matter, citrinin, produced from glucose by Penicillium citrinum Thom. ." Phil. Trans. Roy. Soc. Ser. B. **220**: 269-297.
Higuchi (2004). "Glutathione depletion-induced chromosomal DNA fragmentation associated with apoptosis and necrosis." J Cell Mol Med. **8**: 455-464.
Hines CJ, M. D., Larsson L, Petersen MR, Fisk WJ, Mendell MJ. (2000). "Characterization and variability of endotoxin and 3-hydroxy fatty acids in an office building during a particle intervention study. ." Indoor Air. **10**: 2-12.
Hirvonen MR, H. K., Roponen M (2005). "Bacterial strains from moldy buildings are highly potent inducers of inflammatory and cytotoxic effects. ." Indoor Air **15**: 65-70.
Hodge S, H. G., Flower R, Reynolds PN, Scicchitano R, Holmes M. (2002). "Up-regulation of production of TGF-beta and IL-4 and down-regulation of IL-6 by apoptotic human bronchial epithelial cells." Immunol Cell Biol. **80**: 537-543.
Hogaboam CM, B. K., Schuh JM (2003). "Cytokines and chemokines in allergic bronchopulmonary aspergillosis (ABPA) and experimental Aspergillus-induced allergic airway or asthmatic disease." Front Biosci. **8**: 147-156.
Hogaboam CM, T. K., Ezekowitz RA, Kunkel SL, Schuh JM (2004). "Mannose-binding lectin deficiency alters the development of fungal asthma: effects on airway response, inflammation, and cytokine profile." J Leukoc Biol. **75**: 805-814.
Hohl TM, V. E. H., Rivera A, Morgan LA, Chen PL, Feldmesser M, Pamer EG (2005). "Aspergillus fumigatus triggers inflammatory responses by stage-specific beta-glucan display." PLoS Pathog. **1**.
Hope AP, S. R. (2007). "Excess dampness and mold growth in homes: an evidence-based review of the aeroirritant effect and its potential causes." Allergy Asthma Proc. **28**: 262-270.
Horner WE, H. A., Salvaggio JE, Lehrer SB. (1995). "Fungal allergens." Clin Microbiol Rev. **8**: 161-179.
Ikuta K, S. N., Blake JS, Dahl MV, Nelson RD, Hisamichi K, Kobayashi H, Suzuki S, Okawa Y. (1997). "NMR study of the galactomannans of Trichophyton mentagrophytes and Trichophyton rubrum." Biochem J. **323**: 297-305.
Islam Z, P. J. (2006). "LPS priming potentiates and prolongs proinflammatory cytokine response to the trichothecene deoxynivalenol in the mouse." Toxicol Appl Pharmacol **211**: 53-63.
Iwahashi H, K. E., Suzuki Y, Ueda Y, Ishizawa YH, Nobumasa H, Kuboki Y, Hosoda H, Iwahashi Y. (2007). "Evaluation of toxicity of the mycotoxin citrinin using yeast ORF DNA microarray and Oligo DNA microarray." BMC Genomics. **8**.
Jack DL, R. R., Tenner AJ, Frosch M, Turner MW, Klein NJ (2001). "Mannose-binding lectin regulates the inflammatory response of human professional phagocytes to Neisseria meningitidis serogroup B." J Infect Dis. **184**: 1152-1162.
Jahn B, K. A., Schmidt A, Wanner G, Gehringer H, Bhakdi S, Brakhage AA. (1997). "Isolation and characterization of a pigmentless-conidium mutant of Aspergillus fumigatus with altered conidial surface and reduced virulence." Infect. Immun **65**: 5110–5117.
Janeway, C., Travers, P, Walport, M, Shlomchik, MJ. (2005). Immunobiology: The Immune System in Health and Disease. New York, NY, Garland Science Publishing.
Jardine H, M. W., Donaldson K, Rahman I. (2002). "Molecular mechanism of transforming growth factor (TGF)-beta1-induced glutathione depletion in alveolar epithelial cells. Involvement of AP-1/ARE and Fra-1." J Biol Chem **277**: 21158-21166.
Jarvis BB, M. J. (2005). "Mycotoxins as harmful indoor air contaminants." Appl Microbiol Biotechnol. **66**: 367-372.

Jun-ichi Ashitani, Y. K., Shigehisa Yanagi, Nobuhiro Matsumoto, Masamitsu Nakazato (2008). "Elevated Levels of -D-Glucan in Bronchoalveolar Lavage Fluid in Patients with Farmer's Lung in Miyazaki, Japan." Respiration **75**: 182-188

Kamei K, W. A. (2005). "Aspergillus mycotoxins and their effect on the host. ." Med Mycol. **43**: 95-99.

Kawayama T, F. R., Honda J, Rikimaru T, Aizawa H. (2003). "High concentration of (1-->3)-beta-D-glucan in BAL fluid in patients with acute eosinophilic pneumonia." Chest. **123**: 1302-1307.

Khasawneh F, M. T., Moughrabieh MK, Lai Z, Ager J, Soubani AO. (2006). "Isolation of Aspergillus in critically ill patients: a potential marker of poor outcome." J Crit Care. **21**: 322-327.

Kilpatrick (2003). "Introduction to mannan-binding lectin." Biochem Soc Trans. **31**: 745-747.

Kim TS, D. R., Rupper R, Maecker HT, Levy S, Umetsu DT. (1997). "An ovalbumin-IL-12 fusion protein is more effective than ovalbumin plus free recombinant IL-12 in inducing a T helper cell type 1-dominated immune response and inhibiting antigen-specific IgE production." J Immunol **158**: 4137-4144.

Kokkonen M, J. M., Rizzo A (2005). "The effect of substrate on mycotoxin production of selected Penicillium strains." Int J Food Microbiol. **99**: 207-214.

Kolstad HA, B. C., Iversen M, Sigsgaard T, Mikkelsen S. (2002). "Do Indoor Molds in Nonindustrial Environments Threaten Workers' Health? A Review of the Epidemiologic Evidence." Epidemiol Rev. **24**: 203-217.

Lacey, W. (2006). The Air Spora. Dordrecht, The Netherlands, Springer.

Latgé (2001). "The pathobiology of Aspergillus fumigatus." Trends Microbiol **9**: 382-389.

Latgé JP, B. H., Diaquin M. (1988). "Ultrastructure and composition of the conidial wall of Cladosporium cladosporioides." Can J Microbiol. **34**: 1325-1329.

Leal JA, G. C., Gómez-Miranda B, Prieto A, Bernabé M. (1992). "Chemical and structural similarities in wall polysaccharides of some Penicillium, Eupenicillium and Aspergillus species." FEMS Microbiol Lett. **69**: 165-168.

López-Franco O, S. Y., Sanjuán G, Blanco J, Hernández-Vargas P, Yo Y, Kopp J, Egido J, Gómez-Guerrero C (2002). "Nuclear factor-kappa B inhibitors as potential novel anti-inflammatory agents for the treatment of immune glomerulonephritis. ." Am J Pathol. **161**: 1497-1505.

López-Mirabal HR, W. J. (2007). "Redox characteristics of the eukaryotic cytosol. ." Biochim Biophys Acta.

Losa García JE, R. F., Martín de Cabo MR, García Salgado MJ, Losada JP, Villarón LG, López AJ, Arellano JL. (1999). "Evaluation of inflammatory cytokine secretion by human alveolar macrophages." Mediators Inflamm. **8**: 43-51.

Madsen AM, K. P., Schneider T (2006). "Characterization of microbial particle release from biomass and building material surfaces for inhalation exposure risk assessment." Ann Occup Hyg. **50**: 175-187.

Mahmoudi M, G. M. (2000). "Sick building syndrome. III. Stachybotrys chartarum." J Asthma. **37**: 191-198.

Matsumoto N, L. D. (1999). "Molecular cloning and characterization of the human NUDC gene. ." Hum Genet **104**: 498-504.

McGrath JJ, W. W., Cooley JD, Straus DC. (1999). "Continually measured fungal profiles in sick building syndrome. ." Curr Microbiol. **38**: 33-36.

Medicherla S, L. L., Ma JY, Kapoun AM, Gaspar NJ, Liu YW, Mangadu R, O'Young G, Protter AA, Schreiner GF, Wong DH, Higgins LS. (2007). "Antitumor activity of TGF-beta inhibitor is dependent on the microenvironment." Anticancer Res. **27**: 4149-4157.

Michel O, G. R., Le Bon B, Content J, Duchateau J, Sergysels R. (1992). "Inflammatory response to acute inhalation of endotoxin in asthmatic patients." Am Rev Respir Dis. **146**: 352-357.

Nakae S, H. L., Yu M, Monteforte R, Iikura M, Suto H, Galli SJ. (2007). "Mast cell-derived TNF contributes to airway hyperreactivity, inflammation, and TH2 cytokine production in an asthma model in mice." J Allergy Clin Immunol. **120**: 48-55.

Netea, M. G., G. Ferwerda, et al. (2006). "Recognition of Fungal Pathogens by Toll-Like Receptors." Current Pharmaceutical Design **12**: 4195-4201.

Netea MG, G. N., Munro CA, Bates S, Collins C, Ferwerda G, Hobson RP, Bertram G, Hughes HB, Jansen T, Jacobs L, Buurman ET, Gijzen K, Williams DL, Torensma R, McKinnon A, MacCallum DM, Odds FC, Van der Meer JW, Brown AJ, Kullberg BJ. (2006). "Immune sensing of Candida albicans requires cooperative recognition of mannans and glucans by lectin and Toll-like receptors." J Clin Invest. **116**: 1642-1650.

Neth O, J. D., Dodds AW, Holzel H, Klein NJ, Turner MW (2000). "Mannose-binding lectin binds to a range of clinically relevant microorganisms and promotes complement deposition." Infect. Immun **68**: 688–693.

Nevalainen A, S. M. (2005). "Of microbes and men." Indoor Air. **15**: 58-64.

Niedoszytko M, C. M., Jassem E, Czestochowska E (2007). "Association between sensitization to Aureobasidium pullulans (Pullularia sp) and severity of asthma. ." Ann Allergy Asthma Immunol. **98**: 153-156.
Nielsen KF, G. S., Nielsen PA, Andersen B, Thrane U, Frisvad JC. (1999). "Production of mycotoxins on artificially and naturally infested building materials." Mycopathologia. **145**: 43-56.
Nieminen SM, K. R., Auriola S, Toivola M, Laatsch H, Laatikainen R, Hyvärinen A, Von Wright A (2002). "Isolation and identification of Aspergillus fumigatus mycotoxins on growth medium and some building materials. ." Appl Environ Microbiol. **68**: 4871-4875.
O'Driscoll BR, H. L., Denning DW (2005). "Mold sensitization is common amongst patients with severe asthma requiring multiple hospital admissions." BMC Pulm Med. **5**.
Okazaki M, A. Y., Ohno N, Yadomae T. (1995). "Structure-activity relationship of (1-->3)-beta-D-glucans in the induction of cytokine production from macrophages, in vitro. ." Biol Pharm Bull. **18**: 1320-1327.
Oshiro Y, M. D. (1997). "TNF-alpha release from human peripheral blood mononuclear cells to predict the proinflammatory activity of cytokines and growth factors." J Pharmacol Toxicol Methods. **37**: 55-59.
Palaniyar N, N. J., Reid KB (2002). "Pulmonary innate immune proteins and receptors that interact with gram-positive bacterial ligands." Immunobiology. **205**: 575-594.
Palmberg L, L. B., Malmberg P, Larsson K (1998). "Induction of IL-8 production in human alveolar macrophages and human bronchial epithelial cells in vitro by swine dust." Thorax **53**: 260-264.
Park JH, C.-G. J., Rao C, Kreiss K. (2006). "Fungal and endotoxin measurements in dust associated with respiratory symptoms in a water-damaged office building. ." Indoor Air. **16**: 192-203.
Park JH, S. P., Attfield MD, Cox-Ganser JM, Kreiss K (2004). "Building-related respiratory symptoms can be predicted with semi-quantitative indices of exposure to dampness and mold." Indoor Air. **14**: 425-433
Pena LR, H. D., McClain CJ. (1999). "Treatment with glutathione precursor decreases cytokine activity." JPEN J Parenter Enteral Nutr. **23**: 1-6.
Peraica M, R. B., Luciæ A, Pavloviæ M. (1999). "Toxic effects of mycotoxins in humans." Bull World Health Organ. **77**: 754-766.
Perry LP, I. M., Tazelaar HD, Colby TV, Yousem SA. (1998). "Pulmonary mycotoxicosis: a clinicopathologic study of three cases." Mod Pathol. **11**: 432-436.
Pestka J, Z. H. (2006). "Toll-like receptor priming sensitizes macrophages to proinflammatory cytokine gene induction by deoxynivalenol and other toxicants." Toxicol Sci. **92**: 445-455.
Pfeiffer E, D. T., Metzler M (2005). "Patulin reduces glutathione level and enzyme activities in rat liver slices." Mol Nutr Food Res **49**: 329–336.
Pirhonen I, N. A., Husman T, Pekkanen J (1996). "Home dampness, moulds and their influence on respiratory infections and symptoms in adults in Finland. ." Eur Respir J. **9**: 2618-2622.
Platt SD, M. C., Hunt SM, Lewis CW (1989). "Damp housing, mould growth, and symptomatic health state." Br Med J **298**: 1673-1678.
Prud'homme (2007). "Pathobiology of transforming growth factor beta in cancer, fibrosis and immunologic disease, and therapeutic considerations." Lab Invest. **87**: 1077-1091.
Rath J, M. R., Kosma P, Altmann F, März L, Kubicek CP. (1995). "The alpha-D-mannan core of a complex cell-wall heteroglycan of Trichoderma reesei is responsible for beta-glucosidase activation." Arch Microbiol. **164**: 414-419.
Rubovitch V, G. S., Kalina M (2007). "Lung epithelial cells modulate the inflammatory response of alveolar macrophages." Inflammation. **30**: 236-243.
Rylander, R. and R.-H. Lin (2000). "(1-->3)-[beta]--glucan -- relationship to indoor air-related symptoms, allergy and asthma." Toxicology **152**(1-3): 47-52.
Sánchez-del-Rio M, R. U. (2004). "Migraine aura: new information on underlying mechanisms." Curr Opin Neurol. **17**: 289-293.
Savill (1997). "Apoptosis in resolution of inflammation." J Leukoc Biol. **61**: 375-380.
Saxena V, L. D., Zhou M, Bommireddy R, Azhar M, Doetschman T, Singh RR. (2008). "Dual roles of immunoregulatory cytokine TGF-beta in the pathogenesis of autoimmunity-mediated organ damage." J. Immunol **180**: 1903-1912
Schram D, D. G., Boeve M, Douwes J, Riedler J, Ublagger E, von Mutius E, Budde J, Pershagen G, Nyberg F, Alm J, Braun-Fahrländer C, Waser M, Brunekreef B; PARSIFAL Study Group. (2005). "Bacterial and fungal components in house dust of farm children, Rudolf Steiner school children and reference children--the PARSIFAL Study." Allergy **60**: 611-618

Schram-Bijkerk D, D. G., Douwes J, Boeve M, Riedler J, Ublagger E, et al. (2005). "Bacterial and fungal agents in house dust and wheeze in children: the PARSIFAL study." Clin Exp Allergy. **35**: 1272–1278.
Sherwood, E. R., D. L. Williams, et al. (1987). "Enhancement of interleukin-1 and interleukin-2 production by soluble glucan." International Journal of Immunopharmacology **9**(3): 261-267.
Shoemaker RC, H. D. (2006). "Sick building syndrome (SBS) and exposure to water-damaged buildings: time series study, clinical trial and mechanisms." Neurotoxicol Teratol **28**: 573-588.
Sigsgaard T, B.-J. E., Kjaergaard SK, Mamas S, Pedersen OF. (2000). "Cytokine release from the nasal mucosa and whole blood after experimental exposures to organic dusts." Eur Respir J. **16**: 140-145.
Sletmoen M, S. B. (2008). "Higher order structure of (1,3)-beta-D-glucans and its influence on their biological activities and complexation abilities." Biopolymers. **89**: 310-321.
Smit LA, B. S., Ruven HJ, Rijkers GT, Wouters IM, Heederik D, Omland Ø, Sigsgaard T. (2007). "Atopy and new-onset asthma in young Danish farmers and CD14, TLR2, and TLR4 genetic polymorphisms: a nested case-control study." Clin Exp Allergy. **37**: 1602-1608.
Soltys J, Q. M. (1999). "Modulation of endotoxin- and enterotoxin-induced cytokine release by in vivo treatment with beta-(1,6)-branched beta-(1,3)-glucan." Infect Immun. **67**: 244-252
Su GL, S. R., Wang SC (1995). "Lipopolysaccharide binding protein participation in cellular activation by LPS. ." Crit Rev Immunol. **15**: 201-214.
Takizawa (1998). "Airway epithelial cells as regulators of airway inflammation." Int J Mol Med. **1**: 367-378.
Taskinen T, H. A., Meklin T (1999). "Asthma and repiratory infections in school children with special reference to moisture and mold problems in the school. ." Acta Paediatr. **88**: 1373-1379.
Taskinen T, M. T., Nousiainen M, Husman T, Nevalainen A, Korppi M. (1997). "Moisture and mold problems in schools and respiratory manifestations in school children: clinical and skin test findings." Acta Paediatr. **86**: 1153-1154.
Taylor PR, T. S., Willment JA, Dennehy KM, Rosas M, Findon H, Haynes K, Steele C, Botto M, Gordon S, Brown GD. (2007). "Dectin-1 is required for beta-glucan recognition and control of fungal infection." Nat Immunol. **8**: 31-38.
Terr (2004). "Are indoor molds causing a new disease?" J Allergy Clin Immunol. **113**: 221-226.
Theumer, M. G., A. G. López, et al. (2003). "Immunobiological effects of AFB1 and AFB1-FB1 mixture in experimental subchronic mycotoxicoses in rats." Toxicology **186**(1-2): 159-170.
Thorn (2001). "The inflammatory response in humans after inhalation of bacterial endotoxin: a review." Inflamm Res. **50**: 254-261.
Trout D, B. J., Martinez K, Biagini R, Wallingford K (2001). "Bioaerosol lung damage in a worker with repeated exposure to fungi in a water- damaged building." Environ Health Perspect. **109**: 641-644.
Tuomi T, R. K., Johnsson T, Hemminki K, Hintikka EL, Lindroos O, Kalso S, Koukila-Kähkölä P, Mussalo-Rauhamaa H, Haahtela T. (2000). "Mycotoxins in crude building materials from water-damaged buildings." Appl Environ Microbiol. **66**: 1899-1904.
Turner (1998). "Mannose-binding lectin (MBL) in health and disease." Immunobiology **199**: 327-339.
Turner MW, H. R. (2000). "Mannose-binding lectin: structure, function, genetics and disease associations." Rev Immunogenet **2**: 305–322.
Uguz A, B. Z., Coskun M, Halide Akbas S, Yegin O. (2005). "Mannose-binding lectin levels in children with asthma." Pediatr Allergy Immunol. **16**: 231-235.
Valdimarsson (2003). "Infusion of plasma-derived mannan-binding lectin (MBL) into MBL-deficient humans." Biochem Soc Trans. **31**: 768-769.
Van der Meide PH, S. H. (1996). "Cytokines and the immune response. ." Biotherapy. **8**: 243-249.
Vernooy JH, D. M., van Suylen RJ, Buurman WA, Wouters EF. (2002). "Long-term intratracheal lipopolysaccharide exposure in mice results in chronic lung inflammation and persistent pathology." Am. J. Respir. Cell Mol. Biol. **26**: 152–159.
Wansley EK, D. P., Gainey MD, Tam J, Cramer SD, Parks GD. (2005). "Growth sensitivity of a recombinant simian virus 5 P/V mutant to type I interferon differs between tumor cell lines and normal primary cells. ." Virology. **335**: 131-144.
West, M. E. L. a. J. S. (2006). The Air Spora. The Netherlands., Springer Dordrecht.
Wouters IM, S. S., Douwes J, Doekes G, Heederik D. (2006). "Overview of personal occupational exposure levels to inhalable dust, endotoxin, beta(1-->3)-glucan and fungal extracellular polysaccharides in the waste management chain." Ann Occup Hyg. **50**: 39-53.

Yamamoto A, S. T., Ochiai M, Kamachi K, Kataoka M, Toyoizumi H, Horiuchi Y (2003). "A cell line assay system for predicting the response of human blood to endotoxin." Jpn J Infect Dis. **56**: 93-100.
Zhou JH, B. S., Strle K, Freund GG, Johnson RW, Dantzer R, Kelley KW (2001). "IL-10 Inhibits Apoptosis of Promyeloid Cells by Activating Insulin Receptor Substrate-2 and Phosphatidylinositol 3'-Kinase." J Immunol. **167**: 4436-4442.
Zock JP, J. D., Luczynska C, Sunyer J, Burney P. (2002). "Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey." J Allergy Clinical Immunol **110**: 285-292.
Zughaier SM, Z. S., Datta A, Carlson RW, Stephens DS (2005). "Differential induction of the toll-like receptor 4-MyD88-dependent and -independent signaling pathways by endotoxins." Infect Immun. **73**: 2940-2950.
Zureik M, N. C., Leynaert B, Liard R, Bousquet J, Neukirch F; European Community Respiratory Health Survey (2002). "Sensitisation to airborne moulds and severity of asthma: cross sectional study from European Community respiratory health survey." BMJ. **325**: 411-414

PAPER I

Is not included due to copyright

PAPER II

Available online at www.sciencedirect.com
ScienceDirect
Toxicology Letters 168 (2007) 103–112
Toxicology Letters
www.elsevier.com/locate/toxlet

Mycotoxin-induced depletion of intracellular glutathione and altered cytokine production in the human alveolar epithelial cell line A549☆

L.N. Johannessen[a,*], A.M. Nilsen[a], M. Løvik[a,b]

[a] *Department of Cancer Research and Molecular Medicine, Faculty of Medicine, Norwegian University of Science and Technology, N-7489 Trondheim, Norway*
[b] *Division of Environmental Medicine, Norwegian Institute of Public Health, Oslo, Norway*

Received 16 July 2006; received in revised form 30 October 2006; accepted 2 November 2006
Available online 15 November 2006

Abstract

Mould exposure has been associated with asthma and other inflammatory airway conditions. However, cellular effects of inhaled mould components are not well understood. We hypothesised that host defence mechanisms, such as production of cytokines (TGFβ1, IL-6 and IL-8) and the intracellular antioxidant glutathione (GSH), could be adversely affected by different concentrations of mycotoxins. We studied the effects of citrinin and gliotoxin on lipopolysaccharide (LPS)-stimulated alveolar epithelial cells (A549). Cytokines in cell culture supernatants were analysed by ELISA and levels of GSH were measured by colorimetric (absorbance) determination. We found that GSH decreased in a dose- and time-dependent manner when cells were exposed to citrinin in particular. TGFβ1 was moderately reduced at low mycotoxin concentrations but elevated at higher sub-toxic concentrations. A tendency for an inverse relationship between TGFβ1 and GSH levels was observed. IL-6 and IL-8 were not significantly reduced at non-toxic mycotoxin concentrations. Thus, reduced epithelial GSH and TGFβ1 levels combined with elevated IL-6 and IL-8 levels may result in increased pro-inflammatory activity during mycotoxin exposure. We suggest that this mechanism can contribute to inflammation in mould exposure.
© 2006 Elsevier Ireland Ltd. All rights reserved.

Keywords: Indoor moulds; Mycotoxins; Inflammation; Oxidative stress; Cytokines

1. Introduction

An association between indoor mould exposure and inflammatory airway responses such as asthma has frequently been reported (Douwes and Pearce, 2003; Norbäck et al., 1999; Peat et al., 1998; Zock et al., 2002). The exposure situation is complicated by the presence of several different compounds produced by moulds, along with the presence of bacterial endotoxins (Thorn, 2001). Indoor moulds may be a source of potentially inflammagenic, allergenic and toxic compounds, e.g. cell wall polysaccharides such as β-glucans, enzymes such as glucoamylases, allergenic proteins, and also several volatile organic compounds (MVOC's) that could act additively or synergistically to induce and increase inflammation and immune responses (Beijer et al., 2003; Kreja and Seidel, 2002; Mari et al., 2003; Rylander and Rong-Hwa, 2000). Some studies indicate that adverse health

☆ This work was done at the Department of Cancer Research and Molecular Medicine.

* Corresponding author. Tel.: +47 73 59 89 28; fax: +47 73 59 88 01.
E-mail address: Lene.Johannessen@ntnu.no (L.N. Johannessen).

0378-4274/$ – see front matter © 2006 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.toxlet.2006.11.002

effects in mouldy buildings could in part be related to toxic metabolites (mycotoxins) produced by moulds (Bunger et al., 2005; Fischer and Dott, 2003; Jarvis and Miller, 2005). Several mycotoxins have been associated with health effects seen in humans due to mould exposure (Bennett and Klich, 2003). Common mould genera such as *Aspergillus* and *Penicillium* are frequently isolated from water-damaged buildings and, depending on growth conditions, they may produce mycotoxins. One mycotoxin that is often produced by *A. fumigatus* on water damaged building materials is the immunosuppressive compound gliotoxin (Nieminen et al., 2002; Schulz et al., 2004; Watanabe et al., 2004). Citrinin, which is known to be nephrotoxic, has been isolated from mouldy building materials with growth of *Penicillium* sp. and *Aspergillus* sp. among other mould species (Tuomi et al., 2000).

Host defence mechanisms against inhaled microbes include production of cytokines and chemokines by several different cell types including epithelial cells in the airway mucosa. The normal regulation of an immune reaction is dependent on the balance between pro-inflammatory mediators, such as IL-1, TNF-α, IL-6, IL-8 and IL-12, and regulatory cytokines such as IL-10 and TGFβ1. In this study, we explored the effects of mycotoxins on production of IL-6, IL-8 and TGFβ1. IL-6 is a pleiotropic pro- and anti-inflammatory cytokine involved in cell proliferation, antibody production and acute-phase protein formation. Depletion of IL-6 may also be important during pro-apoptotic processes (Hodge et al., 2002). IL-8 is a chemokine generally important in chemotaxis during inflammation. Production of transforming growth factor beta 1 (TGFβ1) is important in the development of several chronic lung diseases, including asthma (Howell and McAnulty, 2006; Redington et al., 1997; Tillie-Leblond et al., 1999). Altered TGFβ1 production has also been associated with chronic inflammatory pulmonary diseases with the induction of oxidative stress in alveolar epithelial cells (Mastruzzo et al., 2002). Normal host defence mechanisms include the constant production of relatively high amounts of the antioxidant glutathione (GSH). GSH is present in all mammalian cells and provides reducing equivalents for the redox-reaction catalysed by the reducing enzyme glutathione peroxidase. During this process GSH becomes oxidised glutathione (GSSG). The GSSG is then recycled into GSH by glutathione reductase and 2-nicotinamide adenine dinucleotide phosphate (NADPH). It has been shown that adequate production of GSH controls and regulates inflammatory processes in the lung (Rahman and MacNee, 2000). Hence, alteration in alveolar GSH production is recognised as a central feature of progressive inflammatory lung diseases, such as asthma.

We hypothesised that mycotoxins may interfere with the normal levels of GSH and the balance of cytokines produced during inflammatory responses triggered by LPS. The aim of this study was to explore whether mycotoxins affect the production of some of the cytokines involved in lung inflammation (IL-6, IL-8 and TGFβ1) and also whether oxidative stress occurred in alveolar epithelial cells when they were exposed to mycotoxins.

2. Materials and methods

2.1. Experimental design

The human alveolar epithelial carcinoma cell line A549 (Lieber et al., 1976) was cultured with or without lipopolysaccharide (LPS) to induce production of IL-6, IL-8 and TGFβ1. After exposure to citrinin or gliotoxin, cytokine levels were analysed in cell culture supernatants. Further, cell viability was measured to provide information on whether or not LPS and mycotoxins had cytotoxic effects in the concentrations used. To investigate oxidative stress in cells when exposed to mycotoxins, intracellular reduced glutathione levels (GSH) and oxidised glutathione levels (GSSG) were determined.

2.2. Culture of alveolar epithelial cells

The cell line A549 (ATCC CCL-185) was obtained from the American Type Culture Collection (Manassas, VA, USA) and cultured at 37 °C and 5% CO_2 in Ham's F12K growth medium with L-glutamine (Gibco Life Tech., Eggenstein, Germany) supplemented with 10% heat-inactivated foetal bovine serum (FBS) (EuroClone, Milano, Italy) and 0.5% gentamicin (Gibco).

2.3. Mycotoxins

Citrinin from *Penicillium citrinum* and gliotoxin from *Gliocladium virens* (Sigma–Aldrich, Steinheim, Germany) were dissolved in 96% ethanol and serially diluted in cell culture medium as described below. The final concentration of ethanol in the cell culture experiments was maximum 1% (v/v) for the highest mycotoxin concentrations.

2.4. Cell culture assay

A549 cells were adjusted to 10^5 cells/mL and 400 μL was added into three separate 24-well culture plates (Costar, Corning, NY, USA) for each of the mycotoxins. Cells were allowed to rest overnight at 37 °C and 5% CO_2. Then, 50 μL lipopolysaccharide (LPS) from *Pseudomonas aeruginosa* (Sigma) was added to a final concentration of 10 μg/mL. The mycotoxins were added in concentrations ranging from 0 to 50 μg/mL citrinin and 0 to 100 ng/mL gliotoxin in dilution

steps of 1:2. The exposed and non-exposed cells were incubated for 0 h, 1 h, 6 h, 24 h, 48 h and 72 h (for GSH and viability analysis) or 48 h and 72 h (for cytokine and viability analyses). All experiments were repeated on two (GSH) or three (cytokine) different occasions.

2.5. Analysis of cytokines

A quantitative sandwich ELISA cytoset (BioSource Europe, Nivelles, Belgium) was used for measurement of the cytokines IL-6, IL-8 and TGFβ1 in cell culture supernatants and the analysis was performed according to the manufacturer's recommendations. Briefly, 96-well ELISA plates (NUNC, Roskilde, Denmark) were coated with murine monoclonal anti-human IL-6 antibody, anti-human IL-8 antibody or anti-multispecies TGFβ1 antibody. After addition of the cell culture supernatants, incubation and washing, biotinylated murine anti-IL-6 antibody, anti-IL-8 antibody or anti-TGFβ1 antibody was added. Recombinant human IL-6, IL-8 and multispecies TGFβ1 proteins were used as standards. After incubation, HRP-streptavidine enzyme was added, followed by substrate solution containing tetramethylbenzidine (Sigma). The optical density was read in a microplate reader (BioRad, Hercules, CA, USA) at 450 nm. Concentrations of cytokines were calculated from standards ranging from 1000 to 7.8 pg/mL (IL-6), 800 to 7.8 pg/mL (IL-8) or 4000 to 7.8 pg/mL (TGFβ1) in dilution steps of 1:2. The results are presented as mean and standard deviation (S.D.) of duplicate ELISA samples (two samples from each of three separate culture plates in each of three independent experiments).

2.6. Cell viability testing

To investigate the time- and dose-dependent effects of the mycotoxins on A549 cells, Alamar Blue dye (BioSource) was used as recommended by the manufacturer. Cells were incubated with increasing concentrations of mycotoxins (with or without LPS) for 0 h, 1 h, 6 h, 24 h, 48 h and 72 h (GSH analysis) or 48 h and 72 h (cytokine analysis). Cells in the culture plates were spun down, and the cells were provided with fresh culture media before 10% Alamar Blue dye was added and the cultures were incubated further for 3 h at 37 °C and 5% CO_2. Change in cell metabolic activity was registered as a colour change in the wells. Aliquots of 100 μL were taken from cell culture supernatants and added into 96-well plates (Costar), and fluorometric determinations were made in a Fluoroscan II plate reader (Labsystems, Stockholm, Sweden) at emission wavelength 590 nm and excitation wavelength 544 nm. Cell viability after mycotoxin/LPS exposure was calculated as percent of controls (cells with no mycotoxin added).

2.7. GSH/GSSG assay

Analysis of GSH and GSSG levels was performed according to a modified form of the Tietze method (Tietze, 1969) by using the Bioxytech GSH/GSSG-412 kit (Oxis Research, Portland, OR, USA) which utilises the change in colour development during the reaction. The reaction rate is proportional to the GSH or GSSG concentrations in each sample. Experiments were performed according to the manufacturer's recommendations, with slight modifications. Briefly, cells were exposed to mycotoxins (with or without LPS) as described above. After 0 h, 1 h, 6 h, 24 h, 48 h or 72 h incubation, cell culture plates were centrifuged at 500 × *g* for 5 min and supernatants were gently removed without removing unattached cells in the cell culture. Then, 100 μL ice cold PBS was added to the wells, and additionally 10 μL of scavenger reagent (M2VP) was added to the GSSG samples to scavenge GSH and prevent oxidation of GSH and hence an overestimation of GSSG in the samples. Cell culture plates were then immediately frozen at −80 °C until GSH/GSSG analysis. Before analysis, samples were thawed for 2 min, and 300 μL ice cold 5% metaphosphoric acid (MPA) (Sigma) was added. Then, the samples were sonicated in the cell culture plates for at least 15 min keeping the water in the sonicator cold by adding ice. The lysed cells were mixed gently and the culture plates were centrifuged for 10 min at 1000 × *g* and 4 °C before adding 50 μL of MPA extract (cell culture supernatant) to 700 μL of ice-cold GSSG or GSH buffer provided with the kit. Final dilution of samples was 1:60. The samples were kept on ice until final preparation and analysis of absorbance at 412 nm in a Hitachi U-2000 spectrophotometer (Hitachi, Tokyo, Japan).

2.8. Data presentation and statistical analysis

Analysis of cytokines, GSH/GSSG and viability are presented as means and standard deviation (S.D.) from two (GSH/GSSG with viability testing) or three (cytokine with viability testing) separate experiments. Statistical analysis was performed using unpaired two-tailed Student's *t*-test to compare the exposed cultures (LPS/mycotoxin) with the non-exposed cultures (no mycotoxin). For comparison of relative TGFβ1 and GSH curves, a two-way analysis of variance of means (ANOVA) was used. A probability value of 0.05 or less was considered statistically significant in all experiments.

3. Results

3.1. Cell viability

Cell viability after mycotoxin exposure was determined by the Alamar Blue dye method. The mycotoxins generally reduced cell viability in a dose- and time-dependent manner both for citrinin and gliotoxin, as seen in Figs. 1 and 2. Citrinin at 50 μg/mL reduced cell viability to 80% of control values after 6 h incubation (Fig. 1C) and after 24 h incubation, viability was reduced to approximately 40% of control values (Fig. 1D). The majority of cells did not survive 50 μg/mL citrinin after 72 h incubation as cell viability dropped to approximately 20% of control values (Fig. 1F). Cell viability

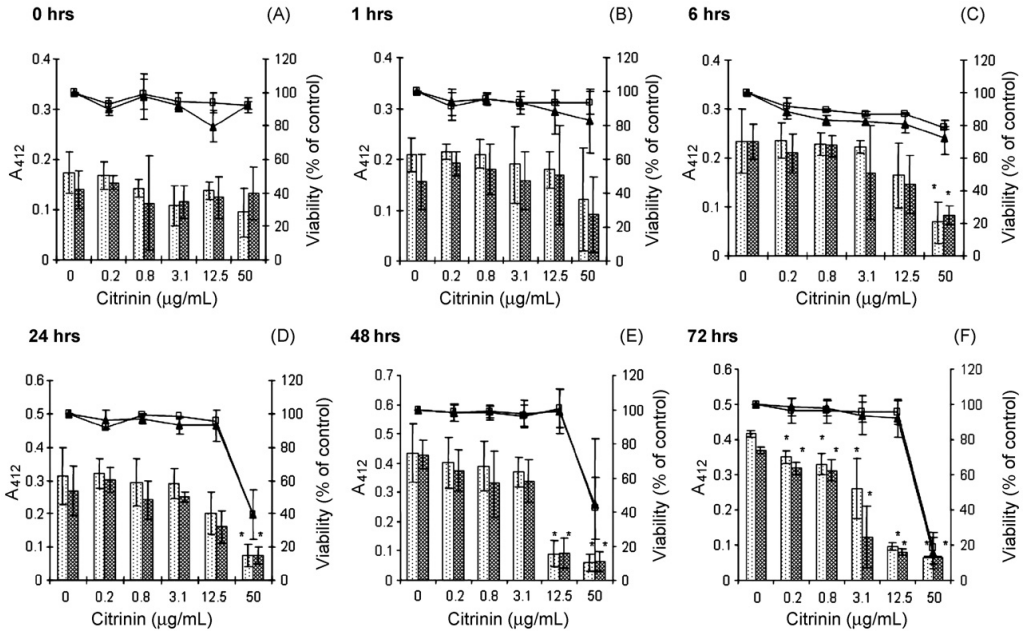


Fig. 1. GSH levels (A_{412}) and cell viability of A549 cells after incubation for 0 h (A), 1 h (B), 6 h (C), 24 h (D), 48 h (E) and 72 h (F) with or without *Pseudomonas aeruginosa* LPS (10 µg/mL) and different concentrations of citrinin. GSH values are given as net absorbance (subtraction of blanks) at 412 nm and are the means ± S.D. of two separate experiments. (*) Significant decreased GSH levels compared to control ($P < 0.05$). ( ) GSH (without LPS); ( ) GSH (with LPS); (▲) viability (without LPS); (□) viability (with LPS).

was reduced to approximately 40% after 6 h (Fig. 2C) and to approximately 20% after 24 h and 48 h when cells were incubated with 100 ng/mL gliotoxin (Fig. 2D and E). In contrast, cell viability was not significantly reduced after incubation with 100 ng/mL gliotoxin for 72 h (Fig. 2F). Exposure to mycotoxin with LPS in the cell culture medium did not reduce cell viability to a greater extent than mycotoxin without LPS. Ethanol (as solvent for the mycotoxins) in the concentrations used (maximum 1% (v/v)) did not exert any significant effect on cell viability (results not shown).

3.2. GSH production and cell viability

A time-dependent increase in relative GSH levels in non-exposed control cultures was observed (Figs. 1 and 2). With increasing doses of mycotoxins, GSH levels were reduced. Notably, for citrinin, a significant reduction of GSH occurred at concentrations below toxic doses at incubation times 48 h and 72 h (Fig. 1E and F), while this was not seen for gliotoxin (Fig. 2E and F). GSH levels were not significantly affected by LPS alone. The oxidised form of glutathione (GSSG) was not increased with decreasing GSH levels, and GSSG levels were not elevated above baseline levels at any mycotoxin concentrations (results not shown). Ethanol (as solvent for the mycotoxins) in the concentrations used (maximum 1% (v/v) and maximum 72 h incubation) did not exert any significant effect on GSH levels (results not shown).

3.3. Cytokine production and cell viability

Production of IL-6, IL-8 and TGFβ1 from A549 cells was induced by the presence of *P. aeruginosa* LPS (Figs. 3 and 4). In contrast, LPS from *Escherichia coli* (serotype 026:B6) did not induce detectable amounts of IL-6, IL-8 or TGFβ1 (results not shown). None of the LPS preparations induced detectable production of TNF-α, IL-12, IL-10 or IL-4 from A549 cells (results not shown). Mycotoxin exposure without LPS did not induce cytokine production above baseline levels at the concentrations used (Fig. 3; results not shown for IL-6/IL-8 analysis in Fig. 4).

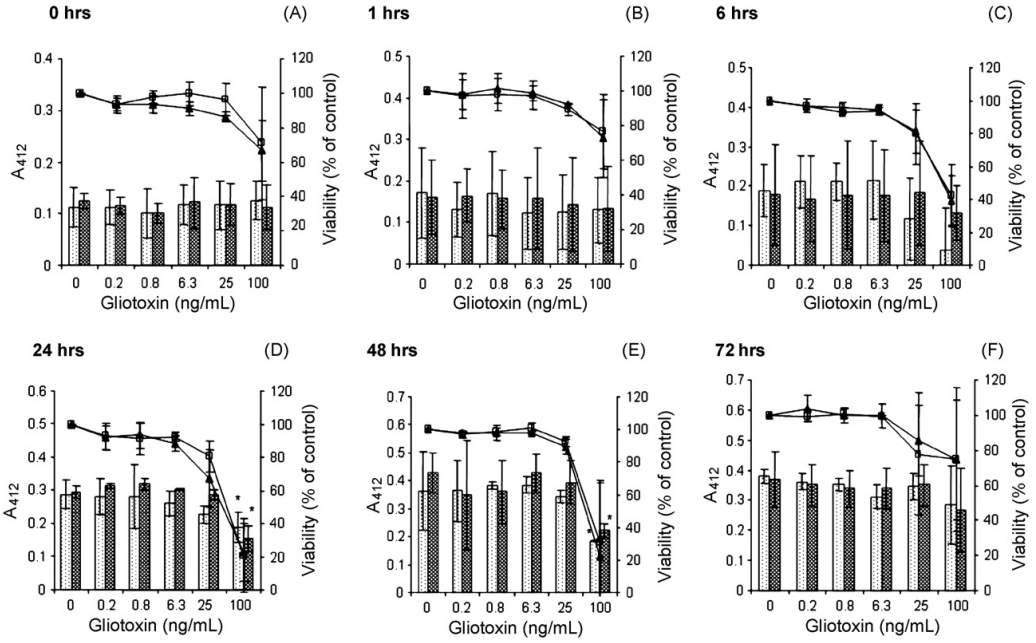


Fig. 2. GSH levels (A_{412}) and cell viability of A549 cells after incubation for 0 h (A), 1 h (B), 6 h (C), 24 h (D), 48 h (E) and 72 h (F) with or without *P. aeruginosa* LPS (10 μg/mL) and different concentrations of gliotoxin. GSH values are given as net absorbance (subtraction of blanks) at 412 nm and are the means ± S.D. of two separate experiments. (*) Significant decreased GSH levels compared to control ($P < 0.05$). ( ) GSH (without LPS); ( ) GSH (with LPS); (▲) viability (without LPS); (□) viability (with LPS).

3.3.1. TGFβ1

Although not consistently statistically significant, TGFβ1 levels seemed to be overall reduced at the lower mycotoxin concentration range (below 0.8 μg/mL citrinin and below 1.6 ng/mL gliotoxin) as shown in Fig. 3. A tendency to increased TGFβ1 levels was consistently observed at higher mycotoxin concentrations (1.6–12.5 μg/mL citrinin and 3.1–50 ng/mL gliotoxin) in the presence of LPS. Citrinin at 25 μg/mL (with and without LPS) and 48 h incubation reduced TGFβ1 abruptly along with a small reduction of cell viability to approximately 90% of control values. Citrinin at 50 μg/mL significantly reduced TGFβ1 levels both after 48 h and 72 h along with reduction of cell viability to about 40% and 20% of control values. The highest concentration of gliotoxin (100 ng/mL) did not reduce TGFβ1, in contrast to the findings with citrinin.

3.3.2. IL-6 and IL-8

The concentration of IL-6 was reduced after 48 h incubation when cells were exposed to 25 μg/mL citrinin in the presence of LPS while cell viability was reduced to about 90% (Fig. 4A). The highest concentration of citrinin (50 μg/mL) reduced IL-6 levels with a simultaneous reduction of cell viability to approximately 40%. A similar tendency was observed at 72 h incubation, although not statistically significant. Gliotoxin at 50 ng/mL and 48 h incubation reduced IL-6 levels significantly (Fig. 4B), while gliotoxin at the highest concentration after 72 h incubation did not reduce IL-6 levels significantly. IL-8 was significantly reduced after 72 h incubation with 25 μg/mL citrinin, with a simultaneous reduction of cell viability to approximately 80% (Fig. 4A), while 50 μg/mL citrinin reduced IL-8 along with loss of cell viability to about 40% of control values after 48 h and to about 20% after 72 h incubation. Gliotoxin reduced IL-8 production significantly at 100 ng/mL after 48 h and caused reduction in cell viability to approximately 40% of control values (Fig. 4B). IL-8 was not reduced after 72 h incubation with any of the gliotoxin concentrations, and also cell culture viability was maintained after 72 h.

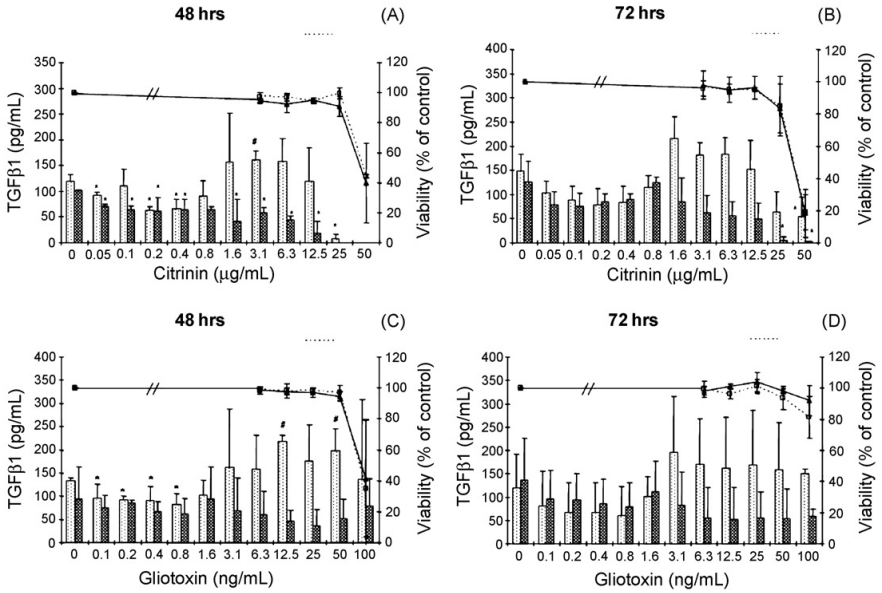


Fig. 3. TGFβ1 production and cell viability of A549 cells after incubation for 48 h or 72 h with or without *P. aeruginosa* LPS (10 μg/mL) and different concentrations of citrinin (A and B) or gliotoxin (C and D). Values are the means ± S.D. of three separate experiments. (*) Significantly decreased TGFβ1 levels compared to control ($P < 0.05$). (#) Significantly increased TGFβ1 levels compared to control ($P < 0.05$). ( ) TGFβ1 (with LPS); ( ) TGFβ1 (without LPS); (▲) viability (with LPS); (□) viability (without LPS).

3.4. Inverse co-variation between TGFβ1 and GSH

Fig. 5 shows the relative (compared to control) levels of GSH and TGFβ1 after 48 h or 72 h incubation of A549 cells with mycotoxin and LPS. A tendency to an inverse co-variation between GSH and TGFβ1 can be observed. Comparison of GSH curves with TGFβ1 curves at citrinin concentration steps identical to those used in the GSH assay, shows a significant difference between the curves at concentrations from 0.8 to 12.5 μg/mL (Fig. 5A and B). In the lower concentration range (below 0.8 μg/mL citrinin), relative GSH levels were higher than relative amounts of TGFβ1. The highest dose of citrinin (50 μg/mL) reduced both GSH and TGFβ1 corresponding to decreased cell viability. At 0.8–3.1 μg/mL citrinin, TGFβ1 levels increased while GSH levels were decreased. Gliotoxin did not affect GSH and TGFβ1 levels in the same manner as citrinin. However, concentrations from 0.8 to 100 ng/mL gliotoxin seemed to increase TGFβ1 slightly (Fig. 5C and D), while GSH was not significantly affected by gliotoxin in this concentration range.

4. Discussion

The mycotoxins gliotoxin and citrinin are produced by common indoor moulds such as *Aspergillus* and *Penicillium*. These toxins have been shown to exhibit diverse cellular effects, including cytotoxicity. The aim of this study was to investigate the dose- and time-dependent effects of citrinin and gliotoxin on the alveolar epithelial cell line A549. Both citrinin and gliotoxin are known to be toxic to various types of cells, and the cytotoxicity is known to be due to apoptosis (Johannessen et al., 2005; Waring et al., 1988; Yu et al., 2006; Zhou et al., 2000). Apoptosis is an efficient cellular mechanism to remove damaged cells without causing sustained organ inflammation. However, very high doses of these mycotoxins will kill A549 cells directly by necrosis (unpublished observations). Our hypothesis was that non-cytotoxic concentrations of mycotoxins would affect intracellular oxidant/antioxidant balance mechanisms and/or interfere with inflammatory cytokine responses triggered by LPS.

We found that the mycotoxins, in a dose- and time-dependent manner, decreased cell viability of A549 cells.

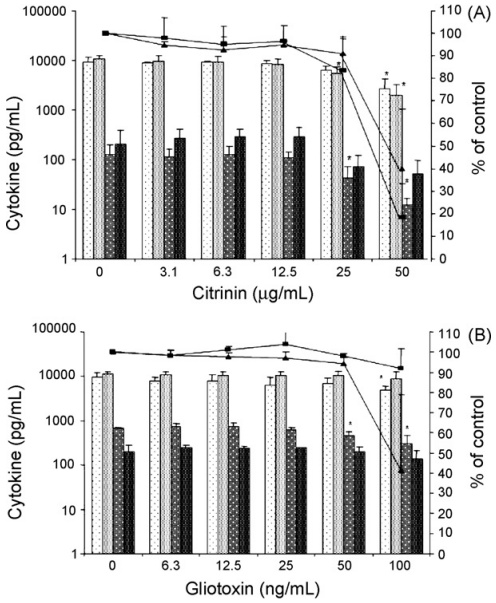


Fig. 4. IL-6 and IL-8 production and cell viability of A549 cells after incubation for 48 h or 72 h with *P. aeruginosa* LPS (10 μg/mL) and different concentrations of citrinin (A) or gliotoxin (B). Values are the means ± S.D. of three separate experiments. (*) Significant reduction compared to control ($P < 0.05$). ( ) IL-8 (48 h); ( ) IL-8 (72 h); ( ) IL-6 (48 h); ( ) IL-6 (72 h); (▲) viability (48 h); (■) viability (72 h).

After 6 h incubation, viability was reduced to 80% (citrinin) and to approximately 40% (gliotoxin) when the highest mycotoxin concentrations were used (50 μg/mL citrinin or 100 ng/mL gliotoxin). After 72 h incubation, 50 μg/mL citrinin reduced cell viability to about 20% of control values. Gliotoxin at 100 ng/mL seemed not to be completely cytotoxic to A549 cells as we observed a population of surviving cells after 72 h incubation. Doubling time of A549 cells is about 22 h (Lieber et al., 1976) and we suggest that some cells after 72 h may have proliferated, hence the maintenance of cell culture viability. Possibly, this indicates some heterogeneity of the cell line, with selection of gliotoxin-resistant variants.

We now tested if oxidative stress could be induced by sub-toxic doses of the mycotoxins. The levels of reduced glutathione (GSH) and oxidised glutathione (GSSG) were measured in relation to dose and time of exposure for both mycotoxins. Under normal conditions, cellular redox- and defence-mechanisms include action of regulatory enzymes keeping the oxidised form of glutathione (GSSG) at low intracellular levels, while the reduced form of glutathione (GSH) is produced in high amounts (Rahman and MacNee, 2000). GSH depletion is in some cases associated with GSSG elevation and this is known to occur in response to some oxidative stimuli, such as H_2O_2, while GSH depletion in parallel with unchanged and low GSSG levels have been described as a response to other oxidative stimuli, such as cigarette smoke condensate (CSC) (Rahman et al., 1995). First, we observed a time-dependent increase of GSH levels in the non-exposed control cultures, suggesting a time-dependent increase of cell density. Then, we found that citrinin, in a dose- and time-dependent manner, reduced GSH levels before any extensive loss of cell viability, while GSH reduction by gliotoxin was minimal before loss of cell viability. Neither of the mycotoxins caused elevated GSSG levels above baseline. Reactive oxygen species (ROS) may cause direct oxidising of GSH and form GSSG, while other types of oxidative agents may form conjugates with GSH, and temporarily prevent re-synthesis of GSH rather than elevating the intracellular GSSG levels (Rahman et al., 1995). We speculate that GSH may counteract the damaging effects of citrinin by forming GSH-citrinin conjugates, although this has to be confirmed by further studies. Such formation of GSH-citrinin conjugates has to our knowledge not been described in the literature. Recently, it has been suggested that apoptosis in human promyelocytic leukemia cells after citrinin exposure is not associated with formation of cellular oxidative stress (Yu et al., 2006). Here, we report that citrinin significantly reduce the antioxidant glutathione at sub-toxic doses, therefore we suggest that induction of oxidative stress may be part of citrinin cytotoxicity in alveolar epithelial cells.

Gliotoxin exists naturally in an oxidised form and cellular uptake is a glutathione-dependent mechanism, as shown in a murine tumour cell line (Bernardo et al., 2003). The toxin is rapidly reduced by GSH after cellular internalisation. However, induction of reactive oxygen species (ROS) followed by apoptosis have been observed in many cell types, including renal cells at a relatively high dose of gliotoxin (1200 ng/mL) (Zhou et al., 2000). We observed no decrease in GSH levels at concentrations below 100 ng/mL gliotoxin, indicating that no extensive oxidative stress occurs in gliotoxin-exposed alveolar epithelial cells below toxic concentrations.

We wanted to test if the mycotoxins affected the release of cytokines from epithelial cells triggered by LPS. First, we tested the ability of LPS from *E. coli* (serotype 026:B6) to trigger cytokine release, and then LPS from *P. aeruginosa*. No cytokine production was detected with LPS from *E. coli*. We found that LPS from *P. aeruginosa* at a relatively high dose (10 μg/mL) stimulated A549 cells to release IL-6, IL-8 and TGFβ1, but not

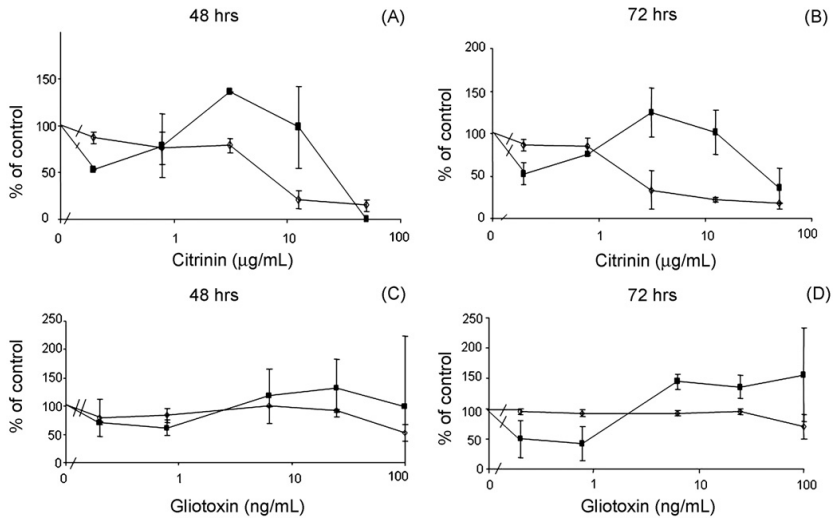


Fig. 5. Relative GSH (◊) and TGFβ1 (■) production from A549 cells after incubation for 48 h or 72 h with *P. aeruginosa* LPS (10 μg/mL) and different concentrations of citrinin (A and B) or gliotoxin (C and D). Statistical calculations were performed by two-way ANOVA.

TNF-α, IL-12, IL-10 or IL-4. Previously, similar results on the ability of different LPS preparations to stimulate A549 cells have been reported (Koyama et al., 2000). It is likely that different bacterial LPS have somewhat different potentials to cause inflammatory reactions in various cells and organs. Thus, *P. aeruginosa*, being a pathogen of the lung, may be more likely than *E. coli* to induce LPS-induced inflammatory reactions in alveolar epithelial cells.

We found that the pleiotropic cytokine TGFβ1 and the pro-inflammatory cytokines IL-6 and IL-8 were affected by increasing mycotoxin doses, although IL-6 and IL-8 largely remained elevated at non-toxic concentrations. IL-6 was slightly reduced at sub-toxic doses of 25 μg/mL citrinin and at 50 ng/mL gliotoxin after 48 h incubation, while IL-8 remained elevated until the highest mycotoxin concentrations. Although not consistently significant, we found that TGFβ1 was slightly reduced at the lower mycotoxin concentration range, while a tendency to increased levels was observed at higher mycotoxin doses. Asthma, and other inflammatory diseases, is characterised by high levels of bio-active TGFβ1 *in vivo* (Redington et al., 1997), but also by even higher levels of pro-inflammatory cytokines (Tillie-Leblond et al., 1999).

Regulation of TGFβ1 expression in the cell still remains largely unclear. Epithelial TGFβ1 production may not depend on the induction of pro-inflammatory mediators such as TNF-α, IL-1β and IL-8, but rather TGFβ1 production may involve pro-inflammation independent pathways (Kwong et al., 2004). Interestingly, we found that there seems to be an inverse co-variation between levels of GSH and production of TGFβ1 at sub-toxic mycotoxin concentrations. This effect was most clearly seen with citrinin. At low citrinin concentrations (<0.8 μg/mL) GSH levels were relatively higher than TGFβ1. At higher citrinin concentrations (from 3.1 to 12.5 μg/mL citrinin) TGFβ1 was elevated along with a corresponding decrease in GSH.

Previously, it has been reported that TGFβ1 is a potent inhibitor of GSH, and that GSH depletion in A549 is executed by TGFβ1 by transcriptional down-regulation of the enzyme responsible for GSH formation, γ-glutamylcysteine synthetase (Arsalene et al., 1997; Jardine et al., 2002). Although it seems that citrinin reduces GSH in parallel to elevated TGFβ1 production in our experiments, it cannot be ruled out that TGFβ1 may be the true inhibitor of GSH. In this context, elevated TGFβl may be the response to sub-toxic doses of citrinin, while depletion of GSH will be the indirect consequence. However, this cannot explain the situation after gliotoxin exposure as non-toxic doses did not deplete GSH levels significantly, in spite of elevated TGFβ1 production.

Our experiments showed that increased TGFβ1 production occurred at relatively high mycotoxin concentrations, although well below concentrations causing extensive cell death. Previously, it has been shown that

human bronchial epithelial cells that underwent apoptosis up-regulated TGFβ1, while IL-6 was down-regulated (Hodge et al., 2002). Cytotoxicity in A549 cells is likely to proceed by apoptosis and the elevated TGFβ1 levels and the decreased IL-6 observed may have been pro-apoptotic effects of the mycotoxins. However, at prolonged incubation times, the tested sub-toxic exposure doses did not cause further reduction of cell viability, as would have been expected with an induction of apoptosis, but rather cell viability remained constant after 48 h and 72 h incubation.

The mechanisms behind health effects caused by inhalation of toxigenic mould spores are yet to be further investigated. In this study we demonstrated that citrinin, but not gliotoxin, reduced glutathione levels in A549 cells at non-toxic concentrations. An inverse co-variation between TGFβ1 and GSH levels was also demonstrated. Further, the levels of IL-6 and IL-8 were largely maintained in the non-toxic concentration range.

The levels of airborne mycotoxins that readily may be inhaled in mouldy environments must be assumed to vary depending on the amount of toxigenic moulds present. However, in a case of favourable growth of toxigenic moulds, we speculate that the observations in this study may reflect an ability of gliotoxin and citrinin in particular, to render human alveolar epithelial cells susceptible to inflammatory trigger agents in the environment, such as other mould components and bacterial LPS. Also, a susceptibility to environmental oxidative agents may develop. We suggest that this may facilitate asthma and other inflammatory lung diseases experienced by individuals in mouldy environments.

Acknowledgements

This project was supported by a grant from the Norwegian Foundation for Health and Rehabilitation, and the Norwegian Asthma and Allergy Association.

References

Arsalene, K., Dubois, C., Muanza, T., Begin, R., Boudreau, F., Asselin, C., Cantin, A., 1997. Transforming growth factor-β is a potent inhibitor of glutathione synthesis in the lung epithelial cell line A549: transcriptional effect on the GSH rate-limiting enzyme γ-glutamylcysteine synthetase. Am. J. Respir. Cell Mol. Biol. 17, 599–607.

Beijer, L., Thorn, J., Rylander, R., 2003. Mould exposure at home relates to inflammatory markers in blood. Eur. Respir. J. 21, 317–322.

Bennett, J.W., Klich, M., 2003. Mycotoxins. Clin. Microbiol. Rev. 16, 497–516.

Bernardo, P., Brasch, N., Chai, C., Waring, P., 2003. A novel redox mechanism for the glutathione-dependent reversible uptake of a fungal toxin in cells. J. Biol. Chem. 278, 46549–46555.

Bunger, J., Westpal, G., Monnich, A., Hinnendahl, B., Hallier, E., Muller, M., 2005. Cytotoxicity of occupationally and environmentally relevant mycotoxins. Toxicology 202, 199–211.

Douwes, J., Pearce, N., 2003. Invited commentary: is indoor mold exposure a risk factor for asthma? Am. J. Epidemiol. 158, 203–206.

Fischer, G., Dott, W., 2003. Relevance of airborne fungi and their secondary metabolites for environmental, occupational and indoor hygiene. Arch. Microbiol. 179, 75–82.

Hodge, S., Hodge, G., Flower, R., Reynolds, P., Scicchitano, R., Holmes, M., 2002. Up-regulation of production of TGF-β and IL-4 and down-regulation of IL-6 by apoptotic human bronchial epithelial cells. Immunol. Cell Biol. 80, 537–543.

Howell, J.E., McAnulty, R.J., 2006. TGF-beta: its role in asthma and therapeutic potential. Curr. Drug Targets 7, 547–565.

Jardine, H., MacNee, W., Donaldson, K., Rahman, I., 2002. Molecular mechanism of transforming growth factor (TGF)-beta1-induced glutathione depletion in alveolar epithelial cells. Involvement of AP-1/ARE and Fra-1. J. Biol. Chem. 277, 21158–21166.

Jarvis, B.B., Miller, J.D., 2005. Mycotoxins as harmful indoor air contaminants. Appl. Microbiol. Biotechnol. 66, 367–372.

Johannessen, L.N., Nilsen, A.M., Løvik, M., 2005. The mycotoxins citrinin and gliotoxin differentially affect production of the pro-inflammatory cytokines tumour necrosis factor-α and interleukin-6, and the anti-inflammatory cytokine interleukin-10. Clin. Exp. Allergy 35, 782–789.

Koyama, S., Sato, E., Nomura, H., Kubo, K., Miura, M., Yamashita, T., Nagai, S., Izumi, T., 2000. The potential of various lipopolysaccharides to release IL-8 and G-CSF. Am. J. Physiol. Lung Cell. Mol. Physiol. 278, 658–666.

Kreja, L., Seidel, H.J., 2002. Evaluation of the genotoxic potential of some microbial volatile organic compounds (MVOC) with the comet assay, the micronucleus assay and the HPRT gene mutation assay. Mutat. Res. 513, 143–150.

Kwong, K., Literat, A., Zhu, N., Huang, H., Li, C., Jones, C., Minoo, P., 2004. Expression of transforming growth factor beta (TGF-β1) in human epithelial alveolar cells: a pro-inflammatory mediator independent pathway. Life Sci. 74, 2941–2957.

Lieber, M., Smith, B., Szakal, A., Nelson-Rees, W., Todaro, G., 1976. A continuous tumor-cell line from a human lung carcinoma with properties of type II alveolar epithelial cells. Int. J. Cancer 17, 62–70.

Mari, A., Schneider, P., Wally, V., Breitenbach, M., Simon-Nobbe, B., 2003. Sensitization to fungi: epidemiology, comparative skin tests, and IgE reactivity of fungal extracts. Clin. Exp. Allergy 33, 1429–1438.

Mastruzzo, C., Crimi, N., Vancheri, C., 2002. Role of oxidative stress in pulmonary fibrosis. Monaldi. Arch. Chest Dis. 57, 173–176.

Nieminen, S.M., Karki, R., Auriola, S., Toivola, M., Laatsch, H., Laatikainen, R., Hyvarinen, A., Von Wright, A., 2002. Isolation and identification of *Aspergillus fumigatus* mycotoxins on growth medium and some building materials. Appl. Environ. Microbiol. 68, 4871–4875.

Norbäck, D., Björnsson, E., Janson, C., Palmgren, U., Boman, G., 1999. Current asthma and biochemical signs of inflammation in relation to building dampness in dwellings. Int. J. Tubercul. Lung Dis. 3, 368–376.

Peat, J.K., Dickerson, J., Li, J., 1998. Effects of damp and mould in the home on respiratory health: a review of the literature. Allergy 53, 120–128.

Rahman, I., Li, X.Y., Donaldson, K., Harrison, D.J., MacNee, W., 1995. Glutathione homeostasis in alveolar epithelial cell in vitro and lung in vivo under oxidative stress. Am. J. Physiol. 269, 285–292.

Rahman, I., MacNee, W., 2000. Oxidative stress and regulation of glutathione in lung inflammation. Eur. Respir. J. 16, 534–554.

Redington, A.E., Madden, J., Frew, A.J., Djukanovic, R., Roche, W.R., Holgate, S.T., Howard, P.H., 1997. Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid. Am. J. Respir. Crit. Care Med. 156, 642–647.

Rylander, R., Rong-Hwa, L., 2000. (1 → 3)-β-D-glucan—relationship to indoor air-related symptoms, allergy and asthma. Toxicology 152, 47–52.

Schulz, T., Senkpiel, K., Ohgke, H., 2004. Comparison of the toxicity of reference mycotoxins and spore extracts of common indoor moulds. Int. J. Hyg. Environ. Health 207, 267–277.

Thorn, J., 2001. The inflammatory response in humans after inhalation of bacterial endotoxin: a review. Inflamm. Res. 50, 254–261.

Tietze, F., 1969. Enzymic method for quantitative determination of nanogram amounts of total and oxidized glutathione: applications to mammalian blood and other tissues. Anal. Chem. 27, 502–522.

Tillie-Leblond, I., Pugin, J., Marquette, C., Lamblin, C., Saulnier, F., Brichet, A., Wallaert, B., Tonnel, A., Gosset, P., 1999. Balance between proinflammatory cytokines and their inhibitors in bronchial lavage from patients with status asthmaticus. Am. J. Respir. Crit. Care Med. 159, 487–494.

Tuomi, T., Reijula, K., Johnsson, T., Hemminki, K., Hintikka, E.L., Lindroos, O., Kalso, S., Koukila-Kahkola, P., Mussalo-Rauhamaa, H., Haahtela, T., 2000. Mycotoxins in crude building materials from water-damaged buildings. Appl. Environ. Microbiol. 66, 1899–1904.

Waring, P., Eichner, R.D., Mullbacher, A., Sjaarda, A., 1988. Gliotoxin induces apoptosis in macrophages unrelated to its antiphagocytic properties. J. Biol. Chem. 263, 18493–18499.

Watanabe, A., Kamei, K., Sekine, T., Waku, M., Nishimura, K., Miyaji, M., Tatsumi, K., Kuriyama, T., 2004. Effect of aeration on gliotoxin production by *Aspergillus fumigatus* in its culture filtrate. Mycopathologia 157, 245–254.

Yu, F., Liao, Y., Chang, C., Liu, B., 2006. Citrinin induces apoptosis in HL-60 cells via activation of the mitochondrial pathway. Toxicol. Lett. 161, 143–151.

Zhou, X., Zhao, A., Goping, G., Hirszel, P., 2000. Gliotoxin-induced cytotoxicity proceeds via apoptosis and is mediated by caspases and reactive oxygen species in LLC-PK1 cells. Toxicol. Sci. 54, 194–202.

Zock, J.P., Jarvis, D., Luczynska, C., Sunyer, J., Burney, P., 2002. Housing characteristics, reported mold exposure, and asthma in the European Community Respiratory Health Survey. J. Allergy Clin. Immunol. 110, 285–292.

PAPER III

Is not included due to copyright

PAPER IV

Is not included due to copyright

Dissertations at the Faculty of Medicine, NTNU

1977
1. Knut Joachim Berg: EFFECT OF ACETYLSALICYLIC ACID ON RENAL FUNCTION
2. Karl Erik Viken and Arne Ødegaard: STUDIES ON HUMAN MONOCYTES CULTURED *IN VITRO*

1978
3. Karel Bjørn Cyvin: CONGENITAL DISLOCATION OF THE HIP JOINT.
4. Alf O. Brubakk: METHODS FOR STUDYING FLOW DYNAMICS IN THE LEFT VENTRICLE AND THE AORTA IN MAN.

1979
5. Geirmund Unsgaard: CYTOSTATIC AND IMMUNOREGULATORY ABILITIES OF HUMAN BLOOD MONOCYTES CULTURED IN VITRO

1980
6. Størker Jørstad: URAEMIC TOXINS
7. Arne Olav Jenssen: SOME RHEOLOGICAL, CHEMICAL AND STRUCTURAL PROPERTIES OF MUCOID SPUTUM FROM PATIENTS WITH CHRONIC OBSTRUCTIVE BRONCHITIS

1981
8. Jens Hammerstrøm: CYTOSTATIC AND CYTOLYTIC ACTIVITY OF HUMAN MONOCYTES AND EFFUSION MACROPHAGES AGAINST TUMOR CELLS *IN VITRO*

1983
9. Tore Syversen: EFFECTS OF METHYLMERCURY ON RAT BRAIN PROTEIN.
10. Torbjørn Iversen: SQUAMOUS CELL CARCINOMA OF THE VULVA.

1984
11. Tor-Erik Widerøe: ASPECTS OF CONTINUOUS AMBULATORY PERITONEAL DIALYSIS.
12. Anton Hole: ALTERATIONS OF MONOCYTE AND LYMPHOCYTE FUNCTIONS IN REALTION TO SURGERY UNDER EPIDURAL OR GENERAL ANAESTHESIA.
13. Terje Terjesen: FRACTURE HEALING AN STRESS-PROTECTION AFTER METAL PLATE FIXATION AND EXTERNAL FIXATION.
14. Carsten Saunte: CLUSTER HEADACHE SYNDROME.
15. Inggard Lereim: TRAFFIC ACCIDENTS AND THEIR CONSEQUENCES.
16. Bjørn Magne Eggen: STUDIES IN CYTOTOXICITY IN HUMAN ADHERENT MONONUCLEAR BLOOD CELLS.
17. Trond Haug: FACTORS REGULATING BEHAVIORAL EFFECTS OG DRUGS.

1985
18. Sven Erik Gisvold: RESUSCITATION AFTER COMPLETE GLOBAL BRAIN ISCHEMIA.
19. Terje Espevik: THE CYTOSKELETON OF HUMAN MONOCYTES.
20. Lars Bevanger: STUDIES OF THE Ibc (c) PROTEIN ANTIGENS OF GROUP B STREPTOCOCCI.
21. Ole-Jan Iversen: RETROVIRUS-LIKE PARTICLES IN THE PATHOGENESIS OF PSORIASIS.
22. Lasse Eriksen: EVALUATION AND TREATMENT OF ALCOHOL DEPENDENT BEHAVIOUR.
23. Per I. Lundmo: ANDROGEN METABOLISM IN THE PROSTATE.

1986
24. Dagfinn Berntzen: ANALYSIS AND MANAGEMENT OF EXPERIMENTAL AND CLINICAL PAIN.
25. Odd Arnold Kildahl-Andersen: PRODUCTION AND CHARACTERIZATION OF MONOCYTE-DERIVED CYTOTOXIN AND ITS ROLE IN MONOCYTE-MEDIATED CYTOTOXICITY.
26. Ola Dale: VOLATILE ANAESTHETICS.

1987
27. Per Martin Kleveland: STUDIES ON GASTRIN.
28. Audun N. Øksendal: THE CALCIUM PARADOX AND THE HEART.
29. Vilhjalmur R. Finsen: HIP FRACTURES

1988
30. Rigmor Austgulen: TUMOR NECROSIS FACTOR: A MONOCYTE-DERIVED REGULATOR OF CELLULAR GROWTH.
31. Tom-Harald Edna: HEAD INJURIES ADMITTED TO HOSPITAL.

32. Joseph D. Borsi: NEW ASPECTS OF THE CLINICAL PHARMACOKINETICS OF METHOTREXATE.
33. Olav F. M. Sellevold: GLUCOCORTICOIDS IN MYOCARDIAL PROTECTION.
34. Terje Skjærpe: NONINVASIVE QUANTITATION OF GLOBAL PARAMETERS ON LEFT VENTRICULAR FUNCTION: THE SYSTOLIC PULMONARY ARTERY PRESSURE AND CARDIAC OUTPUT.
35. Eyvind Rødahl: STUDIES OF IMMUNE COMPLEXES AND RETROVIRUS-LIKE ANTIGENS IN PATIENTS WITH ANKYLOSING SPONDYLITIS.
36. Ketil Thorstensen: STUDIES ON THE MECHANISMS OF CELLULAR UPTAKE OF IRON FROM TRANSFERRIN.
37. Anna Midelfart: STUDIES OF THE MECHANISMS OF ION AND FLUID TRANSPORT IN THE BOVINE CORNEA.
38. Eirik Helseth: GROWTH AND PLASMINOGEN ACTIVATOR ACTIVITY OF HUMAN GLIOMAS AND BRAIN METASTASES - WITH SPECIAL REFERENCE TO TRANSFORMING GROWTH FACTOR BETA AND THE EPIDERMAL GROWTH FACTOR RECEPTOR.
39. Petter C. Borchgrevink: MAGNESIUM AND THE ISCHEMIC HEART.
40. Kjell-Arne Rein: THE EFFECT OF EXTRACORPOREAL CIRCULATION ON SUBCUTANEOUS TRANSCAPILLARY FLUID BALANCE.
41. Arne Kristian Sandvik: RAT GASTRIC HISTAMINE.
42. Carl Bredo Dahl: ANIMAL MODELS IN PSYCHIATRY.

1989

43. Torbjørn A. Fredriksen: CERVICOGENIC HEADACHE.
44. Rolf A. Walstad: CEFTAZIDIME.
45. Rolf Salvesen: THE PUPIL IN CLUSTER HEADACHE.
46. Nils Petter Jørgensen: DRUG EXPOSURE IN EARLY PREGNANCY.
47. Johan C. Ræder: PREMEDICATION AND GENERAL ANAESTHESIA IN OUTPATIENT GYNECOLOGICAL SURGERY.
48. M. R. Shalaby: IMMUNOREGULATORY PROPERTIES OF TNF-α AND THE RELATED CYTOKINES.
49. Anders Waage: THE COMPLEX PATTERN OF CYTOKINES IN SEPTIC SHOCK.
50. Bjarne Christian Eriksen: ELECTROSTIMULATION OF THE PELVIC FLOOR IN FEMALE URINARY INCONTINENCE.
51. Tore B. Halvorsen: PROGNOSTIC FACTORS IN COLORECTAL CANCER.

1990

52. Asbjørn Nordby: CELLULAR TOXICITY OF ROENTGEN CONTRAST MEDIA.
53. Kåre E. Tvedt: X-RAY MICROANALYSIS OF BIOLOGICAL MATERIAL.
54. Tore C. Stiles: COGNITIVE VULNERABILITY FACTORS IN THE DEVELOPMENT AND MAINTENANCE OF DEPRESSION.
55. Eva Hofsli: TUMOR NECROSIS FACTOR AND MULTIDRUG RESISTANCE.
56. Helge S. Haarstad: TROPHIC EFFECTS OF CHOLECYSTOKININ AND SECRETIN ON THE RAT PANCREAS.
57. Lars Engebretsen: TREATMENT OF ACUTE ANTERIOR CRUCIATE LIGAMENT INJURIES.
58. Tarjei Rygnestad: DELIBERATE SELF-POISONING IN TRONDHEIM.
59. Arne Z. Henriksen: STUDIES ON CONSERVED ANTIGENIC DOMAINS ON MAJOR OUTER MEMBRANE PROTEINS FROM ENTEROBACTERIA.
60. Steinar Westin: UNEMPLOYMENT AND HEALTH: Medical and social consequences of a factory closure in a ten-year controlled follow-up study.
61. Ylva Sahlin: INJURY REGISTRATION, a tool for accident preventive work.
62. Helge Bjørnstad Pettersen: BIOSYNTHESIS OF COMPLEMENT BY HUMAN ALVEOLAR MACROPHAGES WITH SPECIAL REFERENCE TO SARCOIDOSIS.
63. Berit Schei: TRAPPED IN PAINFUL LOVE.
64. Lars J. Vatten: PROSPECTIVE STUDIES OF THE RISK OF BREAST CANCER IN A COHORT OF NORWEGIAN WOMAN.

1991

65. Kåre Bergh: APPLICATIONS OF ANTI-C5a SPECIFIC MONOCLONAL ANTIBODIES FOR THE ASSESSMENT OF COMPLEMENT ACTIVATION.
66. Svein Svenningsen: THE CLINICAL SIGNIFICANCE OF INCREASED FEMORAL ANTEVERSION.

67. Olbjørn Klepp: NONSEMINOMATOUS GERM CELL TESTIS CANCER: THERAPEUTIC OUTCOME AND PROGNOSTIC FACTORS.
68. Trond Sand: THE EFFECTS OF CLICK POLARITY ON BRAINSTEM AUDITORY EVOKED POTENTIALS AMPLITUDE, DISPERSION, AND LATENCY VARIABLES.
69. Kjetil B. Åsbakk: STUDIES OF A PROTEIN FROM PSORIATIC SCALE, PSO P27, WITH RESPECT TO ITS POTENTIAL ROLE IN IMMUNE REACTIONS IN PSORIASIS.
70. Arnulf Hestnes: STUDIES ON DOWN´S SYNDROME.
71. Randi Nygaard: LONG-TERM SURVIVAL IN CHILDHOOD LEUKEMIA.
72. Bjørn Hagen: THIO-TEPA.
73. Svein Anda: EVALUATION OF THE HIP JOINT BY COMPUTED TOMOGRAMPHY AND ULTRASONOGRAPHY.

1992

74. Martin Svartberg: AN INVESTIGATION OF PROCESS AND OUTCOME OF SHORT-TERM PSYCHODYNAMIC PSYCHOTHERAPY.
75. Stig Arild Slørdahl: AORTIC REGURGITATION.
76. Harold C Sexton: STUDIES RELATING TO THE TREATMENT OF SYMPTOMATIC NON-PSYCHOTIC PATIENTS.
77. Maurice B. Vincent: VASOACTIVE PEPTIDES IN THE OCULAR/FOREHEAD AREA.
78. Terje Johannessen: CONTROLLED TRIALS IN SINGLE SUBJECTS.
79. Turid Nilsen: PYROPHOSPHATE IN HEPATOCYTE IRON METABOLISM.
80. Olav Haraldseth: NMR SPECTROSCOPY OF CEREBRAL ISCHEMIA AND REPERFUSION IN RAT.
81. Eiliv Brenna: REGULATION OF FUNCTION AND GROWTH OF THE OXYNTIC MUCOSA.

1993

82. Gunnar Bovim: CERVICOGENIC HEADACHE.
83. Jarl Arne Kahn: ASSISTED PROCREATION.
84. Bjørn Naume: IMMUNOREGULATORY EFFECTS OF CYTOKINES ON NK CELLS.
85. Rune Wiseth: AORTIC VALVE REPLACEMENT.
86. Jie Ming Shen: BLOOD FLOW VELOCITY AND RESPIRATORY STUDIES.
87. Piotr Kruszewski: SUNCT SYNDROME WITH SPECIAL REFERENCE TO THE AUTONOMIC NERVOUS SYSTEM.
88. Mette Haase Moen: ENDOMETRIOSIS.
89. Anne Vik: VASCULAR GAS EMBOLISM DURING AIR INFUSION AND AFTER DECOMPRESSION IN PIGS.
90. Lars Jacob Stovner: THE CHIARI TYPE I MALFORMATION.
91. Kjell Å. Salvesen: ROUTINE ULTRASONOGRAPHY IN UTERO AND DEVELOPMENT IN CHILDHOOD.

1994

92. Nina-Beate Liabakk: DEVELOPMENT OF IMMUNOASSAYS FOR TNF AND ITS SOLUBLE RECEPTORS.
93. Sverre Helge Torp: *erb*B ONCOGENES IN HUMAN GLIOMAS AND MENINGIOMAS.
94. Olav M. Linaker: MENTAL RETARDATION AND PSYCHIATRY. Past and present.
95. Per Oscar Feet: INCREASED ANTIDEPRESSANT AND ANTIPANIC EFFECT IN COMBINED TREATMENT WITH DIXYRAZINE AND TRICYCLIC ANTIDEPRESSANTS.
96. Stein Olav Samstad: CROSS SECTIONAL FLOW VELOCITY PROFILES FROM TWO-DIMENSIONAL DOPPLER ULTRASOUND: Studies on early mitral blood flow.
97. Bjørn Backe: STUDIES IN ANTENATAL CARE.
98. Gerd Inger Ringdal: QUALITY OF LIFE IN CANCER PATIENTS.
99. Torvid Kiserud: THE DUCTUS VENOSUS IN THE HUMAN FETUS.
100. Hans E. Fjøsne: HORMONAL REGULATION OF PROSTATIC METABOLISM.
101. Eylert Brodtkorb: CLINICAL ASPECTS OF EPILEPSY IN THE MENTALLY RETARDED.
102. Roar Juul: PEPTIDERGIC MECHANISMS IN HUMAN SUBARACHNOID HEMORRHAGE.
103. Unni Syversen: CHROMOGRANIN A. Phsysiological and Clinical Role.

1995

104. Odd Gunnar Brakstad: THERMOSTABLE NUCLEASE AND THE *nuc* GENE IN THE DIAGNOSIS OF *Staphylococcus aureus* INFECTIONS.
105. Terje Engan: NUCLEAR MAGNETIC RESONANCE (NMR) SPECTROSCOPY OF PLASMA IN MALIGNANT DISEASE.
106. Kirsten Rasmussen: VIOLENCE IN THE MENTALLY DISORDERED.
107. Finn Egil Skjeldestad: INDUCED ABORTION: Timetrends and Determinants.

108. Roar Stenseth: THORACIC EPIDURAL ANALGESIA IN AORTOCORONARY BYPASS SURGERY.
109. Arild Faxvaag: STUDIES OF IMMUNE CELL FUNCTION *in mice infected with* MURINE RETROVIRUS.

1996

110. Svend Aakhus: NONINVASIVE COMPUTERIZED ASSESSMENT OF LEFT VENTRICULAR FUNCTION AND SYSTEMIC ARTERIAL PROPERTIES. Methodology and some clinical applications.
111. Klaus-Dieter Bolz: INTRAVASCULAR ULTRASONOGRAPHY.
112. Petter Aadahl: CARDIOVASCULAR EFFECTS OF THORACIC AORTIC CROSS-CLAMPING.
113. Sigurd Steinshamn: CYTOKINE MEDIATORS DURING GRANULOCYTOPENIC INFECTIONS.
114. Hans Stifoss-Hanssen: SEEKING MEANING OR HAPPINESS?
115. Anne Kvikstad: LIFE CHANGE EVENTS AND MARITAL STATUS IN RELATION TO RISK AND PROGNOSIS OF CANSER.
116. Torbjørn Grøntvedt: TREATMENT OF ACUTE AND CHRONIC ANTERIOR CRUCIATE LIGAMENT INJURIES. A clinical and biomechanical study.
117. Sigrid Hørven Wigers: CLINICAL STUDIES OF FIBROMYALGIA WITH FOCUS ON ETIOLOGY, TREATMENT AND OUTCOME.
118. Jan Schjøtt: MYOCARDIAL PROTECTION: Functional and Metabolic Characteristics of Two Endogenous Protective Principles.
119. Marit Martinussen: STUDIES OF INTESTINAL BLOOD FLOW AND ITS RELATION TO TRANSITIONAL CIRCULATORY ADAPATION IN NEWBORN INFANTS.
120. Tomm B. Müller: MAGNETIC RESONANCE IMAGING IN FOCAL CEREBRAL ISCHEMIA.
121. Rune Haaverstad: OEDEMA FORMATION OF THE LOWER EXTREMITIES.
122. Magne Børset: THE ROLE OF CYTOKINES IN MULTIPLE MYELOMA, WITH SPECIAL REFERENCE TO HEPATOCYTE GROWTH FACTOR.
123. Geir Smedslund: A THEORETICAL AND EMPIRICAL INVESTIGATION OF SMOKING, STRESS AND DISEASE: RESULTS FROM A POPULATION SURVEY.

1997

124. Torstein Vik: GROWTH, MORBIDITY, AND PSYCHOMOTOR DEVELOPMENT IN INFANTS WHO WERE GROWTH RETARDED *IN UTERO*.
125. Siri Forsmo: ASPECTS AND CONSEQUENCES OF OPPORTUNISTIC SCREENING FOR CERVICAL CANCER. Results based on data from three Norwegian counties.
126. Jon S. Skranes: CEREBRAL MRI AND NEURODEVELOPMENTAL OUTCOME IN VERY LOW BIRTH WEIGHT (VLBW) CHILDREN. A follow-up study of a geographically based year cohort of VLBW children at ages one and six years.
127. Knut Bjørnstad: COMPUTERIZED ECHOCARDIOGRAPHY FOR EVALUTION OF CORONARY ARTERY DISEASE.
128. Grethe Elisabeth Borchgrevink: DIAGNOSIS AND TREATMENT OF WHIPLASH/NECK SPRAIN INJURIES CAUSED BY CAR ACCIDENTS.
129. Tor Elsås: NEUROPEPTIDES AND NITRIC OXIDE SYNTHASE IN OCULAR AUTONOMIC AND SENSORY NERVES.
130. Rolf W. Gråwe: EPIDEMIOLOGICAL AND NEUROPSYCHOLOGICAL PERSPECTIVES ON SCHIZOPHRENIA.
131. Tonje Strømholm: CEREBRAL HAEMODYNAMICS DURING THORACIC AORTIC CROSSCLAMPING. An experimental study in pigs.

1998

132. Martinus Bråten: STUDIES ON SOME PROBLEMS REALTED TO INTRAMEDULLARY NAILING OF FEMORAL FRACTURES.
133. Ståle Nordgård: PROLIFERATIVE ACTIVITY AND DNA CONTENT AS PROGNOSTIC INDICATORS IN ADENOID CYSTIC CARCINOMA OF THE HEAD AND NECK.
134. Egil Lien: SOLUBLE RECEPTORS FOR **TNF** AND **LPS**: RELEASE PATTERN AND POSSIBLE SIGNIFICANCE IN DISEASE.
135. Marit Bjørgaas: HYPOGLYCAEMIA IN CHILDREN WITH DIABETES MELLITUS
136. Frank Skorpen: GENETIC AND FUNCTIONAL ANALYSES OF DNA REPAIR IN HUMAN CELLS.
137. Juan A. Pareja: SUNCT SYNDROME. ON THE CLINICAL PICTURE. ITS DISTINCTION FROM OTHER, SIMILAR HEADACHES.

138. Anders Angelsen: NEUROENDOCRINE CELLS IN HUMAN PROSTATIC CARCINOMAS AND THE PROSTATIC COMPLEX OF RAT, GUINEA PIG, CAT AND DOG.
139. Fabio Antonaci: CHRONIC PAROXYSMAL HEMICRANIA AND HEMICRANIA CONTINUA: TWO DIFFERENT ENTITIES?
140. Sven M. Carlsen: ENDOCRINE AND METABOLIC EFFECTS OF METFORMIN WITH SPECIAL EMPHASIS ON CARDIOVASCULAR RISK FACTORES.

1999

141. Terje A. Murberg: DEPRESSIVE SYMPTOMS AND COPING AMONG PATIENTS WITH CONGESTIVE HEART FAILURE.
142. Harm-Gerd Karl Blaas: THE EMBRYONIC EXAMINATION. Ultrasound studies on the development of the human embryo.
143. Noèmi Becser Andersen:THE CEPHALIC SENSORY NERVES IN UNILATERAL HEADACHES. Anatomical background and neurophysiological evaluation.
144. Eli-Janne Fiskerstrand: LASER TREATMENT OF PORT WINE STAINS. A study of the efficacy and limitations of the pulsed dye laser. Clinical and morfological analyses aimed at improving the therapeutic outcome.
145. Bård Kulseng: A STUDY OF ALGINATE CAPSULE PROPERTIES AND CYTOKINES IN RELATION TO INSULIN DEPENDENT DIABETES MELLITUS.
146. Terje Haug: STRUCTURE AND REGULATION OF THE HUMAN UNG GENE ENCODING URACIL-DNA GLYCOSYLASE.
147. Heidi Brurok: MANGANESE AND THE HEART. A Magic Metal with Diagnostic and Therapeutic Possibilites.
148. Agnes Kathrine Lie: DIAGNOSIS AND PREVALENCE OF HUMAN PAPILLOMAVIRUS INFECTION IN CERVICAL INTRAEPITELIAL NEOPLASIA. Relationship to Cell Cycle Regulatory Proteins and HLA DQBI Genes.
149. Ronald Mårvik: PHARMACOLOGICAL, PHYSIOLOGICAL AND PATHOPHYSIOLOGICAL STUDIES ON ISOLATED STOMACS.
150. Ketil Jarl Holen: THE ROLE OF ULTRASONOGRAPHY IN THE DIAGNOSIS AND TREATMENT OF HIP DYSPLASIA IN NEWBORNS.
151. Irene Hetlevik: THE ROLE OF CLINICAL GUIDELINES IN CARDIOVASCULAR RISK INTERVENTION IN GENERAL PRACTICE.
152. Katarina Tunòn: ULTRASOUND AND PREDICTION OF GESTATIONAL AGE.
153. Johannes Soma: INTERACTION BETWEEN THE LEFT VENTRICLE AND THE SYSTEMIC ARTERIES.
154. Arild Aamodt: DEVELOPMENT AND PRE-CLINICAL EVALUATION OF A CUSTOM-MADE FEMORAL STEM.
155. Agnar Tegnander: DIAGNOSIS AND FOLLOW-UP OF CHILDREN WITH SUSPECTED OR KNOWN HIP DYSPLASIA.
156. Bent Indredavik: STROKE UNIT TREATMENT: SHORT AND LONG-TERM EFFECTS
157. Jolanta Vanagaite Vingen: PHOTOPHOBIA AND PHONOPHOBIA IN PRIMARY HEADACHES

2000

158. Ola Dalsegg Sæther: PATHOPHYSIOLOGY DURING PROXIMAL AORTIC CROSS-CLAMPING CLINICAL AND EXPERIMENTAL STUDIES
159. xxxxxxxxx (blind number)
160. Christina Vogt Isaksen: PRENATAL ULTRASOUND AND POSTMORTEM FINDINGS – A TEN YEAR CORRELATIVE STUDY OF FETUSES AND INFANTS WITH DEVELOPMENTAL ANOMALIES.
161. Holger Seidel: HIGH-DOSE METHOTREXATE THERAPY IN CHILDREN WITH ACUTE LYMPHOCYTIC LEUKEMIA: DOSE, CONCENTRATION, AND EFFECT CONSIDERATIONS.
162. Stein Hallan: IMPLEMENTATION OF MODERN MEDICAL DECISION ANALYSIS INTO CLINICAL DIAGNOSIS AND TREATMENT.
163. Malcolm Sue-Chu: INVASIVE AND NON-INVASIVE STUDIES IN CROSS-COUNTRY SKIERS WITH ASTHMA-LIKE SYMPTOMS.
164. Ole-Lars Brekke: EFFECTS OF ANTIOXIDANTS AND FATTY ACIDS ON TUMOR NECROSIS FACTOR-INDUCED CYTOTOXICITY.
165. Jan Lundbom: AORTOCORONARY BYPASS SURGERY: CLINICAL ASPECTS, COST CONSIDERATIONS AND WORKING ABILITY.

166. John-Anker Zwart: LUMBAR NERVE ROOT COMPRESSION, BIOCHEMICAL AND NEUROPHYSIOLOGICAL ASPECTS.
167. Geir Falck: HYPEROSMOLALITY AND THE HEART.
168. Eirik Skogvoll: CARDIAC ARREST Incidence, Intervention and Outcome.
169. Dalius Bansevicius: SHOULDER-NECK REGION IN CERTAIN HEADACHES AND CHRONIC PAIN SYNDROMES.
170. Bettina Kinge: REFRACTIVE ERRORS AND BIOMETRIC CHANGES AMONG UNIVERSITY STUDENTS IN NORWAY.
171. Gunnar Qvigstad: CONSEQUENCES OF HYPERGASTRINEMIA IN MAN
172. Hanne Ellekjær: EPIDEMIOLOGICAL STUDIES OF STROKE IN A NORWEGIAN POPULATION. INCIDENCE, RISK FACTORS AND PROGNOSIS
173. Hilde Grimstad: VIOLENCE AGAINST WOMEN AND PREGNANCY OUTCOME.
174. Astrid Hjelde: SURFACE TENSION AND COMPLEMENT ACTIVATION: Factors influencing bubble formation and bubble effects after decompression.
175. Kjell A. Kvistad: MR IN BREAST CANCER – A CLINICAL STUDY.
176. Ivar Rossvoll: ELECTIVE ORTHOPAEDIC SURGERY IN A DEFINED POPULATION. Studies on demand, waiting time for treatment and incapacity for work.
177. Carina Seidel: PROGNOSTIC VALUE AND BIOLOGICAL EFFECTS OF HEPATOCYTE GROWTH FACTOR AND SYNDECAN-1 IN MULTIPLE MYELOMA.

2001

178. Alexander Wahba: THE INFLUENCE OF CARDIOPULMONARY BYPASS ON PLATELET FUNCTION AND BLOOD COAGULATION – DETERMINANTS AND CLINICAL CONSEQUENSES
179. Marcus Schmitt-Egenolf: THE RELEVANCE OF THE MAJOR hISTOCOMPATIBILITY COMPLEX FOR THE GENETICS OF PSORIASIS
180. Odrun Arna Gederaas: BIOLOGICAL MECHANISMS INVOLVED IN 5-AMINOLEVULINIC ACID BASED PHOTODYNAMIC THERAPY
181. Pål Richard Romundstad: CANCER INCIDENCE AMONG NORWEGIAN ALUMINIUM WORKERS
182. Henrik Hjorth-Hansen: NOVEL CYTOKINES IN GROWTH CONTROL AND BONE DISEASE OF MULTIPLE MYELOMA
183. Gunnar Morken: SEASONAL VARIATION OF HUMAN MOOD AND BEHAVIOUR
184. Bjørn Olav Haugen: MEASUREMENT OF CARDIAC OUTPUT AND STUDIES OF VELOCITY PROFILES IN AORTIC AND MITRAL FLOW USING TWO- AND THREE-DIMENSIONAL COLOUR FLOW IMAGING
185. Geir Bråthen: THE CLASSIFICATION AND CLINICAL DIAGNOSIS OF ALCOHOL-RELATED SEIZURES
186. Knut Ivar Aasarød: RENAL INVOLVEMENT IN INFLAMMATORY RHEUMATIC DISEASE. A Study of Renal Disease in Wegener's Granulomatosis and in Primary Sjögren's Syndrome
187. Trude Helen Flo: RESEPTORS INVOLVED IN CELL ACTIVATION BY DEFINED URONIC ACID POLYMERS AND BACTERIAL COMPONENTS
188. Bodil Kavli: HUMAN URACIL-DNA GLYCOSYLASES FROM THE UNG GENE: STRUCTRUAL BASIS FOR SUBSTRATE SPECIFICITY AND REPAIR
189. Liv Thommesen: MOLECULAR MECHANISMS INVOLVED IN TNF- AND GASTRIN-MEDIATED GENE REGULATION
190. Turid Lingaas Holmen: SMOKING AND HEALTH IN ADOLESCENCE; THE NORD-TRØNDELAG HEALTH STUDY, 1995-97
191. Øyvind Hjertner: MULTIPLE MYELOMA: INTERACTIONS BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MICROENVIRONMENT
192. Asbjørn Støylen: STRAIN RATE IMAGING OF THE LEFT VENTRICLE BY ULTRASOUND. FEASIBILITY, CLINICAL VALIDATION AND PHYSIOLOGICAL ASPECTS
193. Kristian Midthjell: DIABETES IN ADULTS IN NORD-TRØNDELAG. PUBLIC HEALTH ASPECTS OF DIABETES MELLITUS IN A LARGE, NON-SELECTED NORWEGIAN POPULATION.
194. Guanglin Cui: FUNCTIONAL ASPECTS OF THE ECL CELL IN RODENTS
195. Ulrik Wisløff: CARDIAC EFFECTS OF AEROBIC ENDURANCE TRAINING: HYPERTROPHY, CONTRACTILITY AND CALCUIM HANDLING IN NORMAL AND FAILING HEART
196. Øyvind Halaas: MECHANISMS OF IMMUNOMODULATION AND CELL-MEDIATED CYTOTOXICITY INDUCED BY BACTERIAL PRODUCTS

197. Tore Amundsen: PERFUSION MR IMAGING IN THE DIAGNOSIS OF PULMONARY EMBOLISM
198. Nanna Kurtze: THE SIGNIFICANCE OF ANXIETY AND DEPRESSION IN FATIQUE AND PATTERNS OF PAIN AMONG INDIVIDUALS DIAGNOSED WITH FIBROMYALGIA: RELATIONS WITH QUALITY OF LIFE, FUNCTIONAL DISABILITY, LIFESTYLE, EMPLOYMENT STATUS, CO-MORBIDITY AND GENDER
199. Tom Ivar Lund Nilsen: PROSPECTIVE STUDIES OF CANCER RISK IN NORD-TRØNDELAG: THE HUNT STUDY. Associations with anthropometric, socioeconomic, and lifestyle risk factors
200. Asta Kristine Håberg: A NEW APPROACH TO THE STUDY OF MIDDLE CEREBRAL ARTERY OCCLUSION IN THE RAT USING MAGNETIC RESONANCE TECHNIQUES

2002

201. Knut Jørgen Arntzen: PREGNANCY AND CYTOKINES
202. Henrik Døllner: INFLAMMATORY MEDIATORS IN PERINATAL INFECTIONS
203. Asta Bye: LOW FAT, LOW LACTOSE DIET USED AS PROPHYLACTIC TREATMENT OF ACUTE INTESTINAL REACTIONS DURING PELVIC RADIOTHERAPY. A PROSPECTIVE RANDOMISED STUDY.
204. Sylvester Moyo: STUDIES ON STREPTOCOCCUS AGALACTIAE (GROUP B STREPTOCOCCUS) SURFACE-ANCHORED MARKERS WITH EMPHASIS ON STRAINS AND HUMAN SERA FROM ZIMBABWE.
205. Knut Hagen: HEAD-HUNT: THE EPIDEMIOLOGY OF HEADACHE IN NORD-TRØNDELAG
206. Li Lixin: ON THE REGULATION AND ROLE OF UNCOUPLING PROTEIN-2 IN INSULIN PRODUCING ß-CELLS
207. Anne Hildur Henriksen: SYMPTOMS OF ALLERGY AND ASTHMA VERSUS MARKERS OF LOWER AIRWAY INFLAMMATION AMONG ADOLESCENTS
208. Egil Andreas Fors: NON-MALIGNANT PAIN IN RELATION TO PSYCHOLOGICAL AND ENVIRONTENTAL FACTORS. EXPERIENTAL AND CLINICAL STUDES OF PAIN WITH FOCUS ON FIBROMYALGIA
209. Pål Klepstad: MORPHINE FOR CANCER PAIN
210. Ingunn Bakke: MECHANISMS AND CONSEQUENCES OF PEROXISOME PROLIFERATOR-INDUCED HYPERFUNCTION OF THE RAT GASTRIN PRODUCING CELL
211. Ingrid Susann Gribbestad: MAGNETIC RESONANCE IMAGING AND SPECTROSCOPY OF BREAST CANCER
212. Rønnaug Astri Ødegård: PREECLAMPSIA – MATERNAL RISK FACTORS AND FETAL GROWTH
213. Johan Haux: STUDIES ON CYTOTOXICITY INDUCED BY HUMAN NATURAL KILLER CELLS AND DIGITOXIN
214. Turid Suzanne Berg-Nielsen: PARENTING PRACTICES AND MENTALLY DISORDERED ADOLESCENTS
215. Astrid Rydning: BLOOD FLOW AS A PROTECTIVE FACTOR FOR THE STOMACH MUCOSA. AN EXPERIMENTAL STUDY ON THE ROLE OF MAST CELLS AND SENSORY AFFERENT NEURONS

2003

216. Jan Pål Loennechen: HEART FAILURE AFTER MYOCARDIAL INFARCTION. Regional Differences, Myocyte Function, Gene Expression, and Response to Cariporide, Losartan, and Exercise Training.
217. Elisabeth Qvigstad: EFFECTS OF FATTY ACIDS AND OVER-STIMULATION ON INSULIN SECRETION IN MAN
218. Arne Åsberg: EPIDEMIOLOGICAL STUDIES IN HEREDITARY HEMOCHROMATOSIS: PREVALENCE, MORBIDITY AND BENEFIT OF SCREENING.
219. Johan Fredrik Skomsvoll: REPRODUCTIVE OUTCOME IN WOMEN WITH RHEUMATIC DISEASE. A population registry based study of the effects of inflammatory rheumatic disease and connective tissue disease on reproductive outcome in Norwegian women in 1967-1995.
220. Siv Mørkved: URINARY INCONTINENCE DURING PREGNANCY AND AFTER DELIVERY: EFFECT OF PELVIC FLOOR MUSCLE TRAINING IN PREVENTION AND TREATMENT
221. Marit S. Jordhøy: THE IMPACT OF COMPREHENSIVE PALLIATIVE CARE
222. Tom Christian Martinsen: HYPERGASTRINEMIA AND HYPOACIDITY IN RODENTS – CAUSES AND CONSEQUENCES

223. Solveig Tingulstad: CENTRALIZATION OF PRIMARY SURGERY FOR OVARAIN CANCER. FEASIBILITY AND IMPACT ON SURVIVAL
224. Haytham Eloqayli: METABOLIC CHANGES IN THE BRAIN CAUSED BY EPILEPTIC SEIZURES
225. Torunn Bruland: STUDIES OF EARLY RETROVIRUS-HOST INTERACTIONS – VIRAL DETERMINANTS FOR PATHOGENESIS AND THE INFLUENCE OF SEX ON THE SUSCEPTIBILITY TO FRIEND MURINE LEUKAEMIA VIRUS INFECTION
226. Torstein Hole: DOPPLER ECHOCARDIOGRAPHIC EVALUATION OF LEFT VENTRICULAR FUNCTION IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION
227. Vibeke Nossum: THE EFFECT OF VASCULAR BUBBLES ON ENDOTHELIAL FUNCTION
228. Sigurd Fasting: ROUTINE BASED RECORDING OF ADVERSE EVENTS DURING ANAESTHESIA – APPLICATION IN QUALITY IMPROVEMENT AND SAFETY
229. Solfrid Romundstad: EPIDEMIOLOGICAL STUDIES OF MICROALBUMINURIA. THE NORD-TRØNDELAG HEALTH STUDY 1995-97 (HUNT 2)
230. Geir Torheim: PROCESSING OF DYNAMIC DATA SETS IN MAGNETIC RESONANCE IMAGING
231. Catrine Ahlén: SKIN INFECTIONS IN OCCUPATIONAL SATURATION DIVERS IN THE NORTH SEA AND THE IMPACT OF THE ENVIRONMENT
232. Arnulf Langhammer: RESPIRATORY SYMPTOMS, LUNG FUNCTION AND BONE MINERAL DENSITY IN A COMPREHENSIVE POPULATION SURVEY. THE NORD-TRØNDELAG HEALTH STUDY 1995-97. THE BRONCHIAL OBSTRUCTION IN NORD-TRØNDELAG STUDY
233. Einar Kjelsås: EATING DISORDERS AND PHYSICAL ACTIVITY IN NON-CLINICAL SAMPLES
234. Arne Wibe: RECTAL CANCER TREATMENT IN NORWAY – STANDARDISATION OF SURGERY AND QUALITY ASSURANCE

2004

235. Eivind Witsø: BONE GRAFT AS AN ANTIBIOTIC CARRIER
236. Anne Mari Sund: DEVELOPMENT OF DEPRESSIVE SYMPTOMS IN EARLY ADOLESCENCE
237. Hallvard Lærum: EVALUATION OF ELECTRONIC MEDICAL RECORDS – A CLINICAL TASK PERSPECTIVE
238. Gustav Mikkelsen: ACCESSIBILITY OF INFORMATION IN ELECTRONIC PATIENT RECORDS; AN EVALUATION OF THE ROLE OF DATA QUALITY
239. Steinar Krokstad: SOCIOECONOMIC INEQUALITIES IN HEALTH AND DISABILITY. SOCIAL EPIDEMIOLOGY IN THE NORD-TRØNDELAG HEALTH STUDY (HUNT), NORWAY
240. Arne Kristian Myhre: NORMAL VARIATION IN ANOGENITAL ANATOMY AND MICROBIOLOGY IN NON-ABUSED PRESCHOOL CHILDREN
241. Ingunn Dybedal: NEGATIVE REGULATORS OF HEMATOPOIETEC STEM AND PROGENITOR CELLS
242. Beate Sitter: TISSUE CHARACTERIZATION BY HIGH RESOLUTION MAGIC ANGLE SPINNING MR SPECTROSCOPY
243. Per Arne Aas: MACROMOLECULAR MAINTENANCE IN HUMAN CELLS – REPAIR OF URACIL IN DNA AND METHYLATIONS IN DNA AND RNA
244. Anna Bofin: FINE NEEDLE ASPIRATION CYTOLOGY IN THE PRIMARY INVESTIGATION OF BREAST TUMOURS AND IN THE DETERMINATION OF TREATMENT STRATEGIES
245. Jim Aage Nøttestad: DEINSTITUTIONALIZATION AND MENTAL HEALTH CHANGES AMONG PEOPLE WITH MENTAL RETARDATION
246. Reidar Fossmark: GASTRIC CANCER IN JAPANESE COTTON RATS
247. Wibeke Nordhøy: MANGANESE AND THE HEART, INTRACELLULAR MR RELAXATION AND WATER EXCHANGE ACROSS THE CARDIAC CELL MEMBRANE

2005

248. Sturla Molden: QUANTITATIVE ANALYSES OF SINGLE UNITS RECORDED FROM THE HIPPOCAMPUS AND ENTORHINAL CORTEX OF BEHAVING RATS
249. Wenche Brenne Drøyvold: EPIDEMIOLOGICAL STUDIES ON WEIGHT CHANGE AND HEALTH IN A LARGE POPULATION. THE NORD-TRØNDELAG HEALTH STUDY (HUNT)

250. Ragnhild Støen: ENDOTHELIUM-DEPENDENT VASODILATION IN THE FEMORAL ARTERY OF DEVELOPING PIGLETS
251. Aslak Steinsbekk: HOMEOPATHY IN THE PREVENTION OF UPPER RESPIRATORY TRACT INFECTIONS IN CHILDREN
252. Hill-Aina Steffenach: MEMORY IN HIPPOCAMPAL AND CORTICO-HIPPOCAMPAL CIRCUITS
253. Eystein Stordal: ASPECTS OF THE EPIDEMIOLOGY OF DEPRESSIONS BASED ON SELF-RATING IN A LARGE GENERAL HEALTH STUDY (THE HUNT-2 STUDY)
254. Viggo Pettersen: FROM MUSCLES TO SINGING: THE ACTIVITY OF ACCESSORY BREATHING MUSCLES AND THORAX MOVEMENT IN CLASSICAL SINGING
255. Marianne Fyhn: SPATIAL MAPS IN THE HIPPOCAMPUS AND ENTORHINAL CORTEX
256. Robert Valderhaug: OBSESSIVE-COMPULSIVE DISORDER AMONG CHILDREN AND ADOLESCENTS: CHARACTERISTICS AND PSYCHOLOGICAL MANAGEMENT OF PATIENTS IN OUTPATIENT PSYCHIATRIC CLINICS
257. Erik Skaaheim Haug: INFRARENAL ABDOMINAL AORTIC ANEURYSMS – COMORBIDITY AND RESULTS FOLLOWING OPEN SURGERY
258. Daniel Kondziella: GLIAL-NEURONAL INTERACTIONS IN EXPERIMENTAL BRAIN DISORDERS
259. Vegard Heimly Brun: ROUTES TO SPATIAL MEMORY IN HIPPOCAMPAL PLACE CELLS
260. Kenneth McMillan: PHYSIOLOGICAL ASSESSMENT AND TRAINING OF ENDURANCE AND STRENGTH IN PROFESSIONAL YOUTH SOCCER PLAYERS
261. Marit Sæbø Indredavik: MENTAL HEALTH AND CEREBRAL MAGNETIC RESONANCE IMAGING IN ADOLESCENTS WITH LOW BIRTH WEIGHT
262. Ole Johan Kemi: ON THE CELLULAR BASIS OF AEROBIC FITNESS, INTENSITY-DEPENDENCE AND TIME-COURSE OF CARDIOMYOCYTE AND ENDOTHELIAL ADAPTATIONS TO EXERCISE TRAINING
263. Eszter Vanky: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN PREGNANCY
264. Hild Fjærtoft: EXTENDED STROKE UNIT SERVICE AND EARLY SUPPORTED DISCHARGE. SHORT AND LONG-TERM EFFECTS
265. Grete Dyb: POSTTRAUMATIC STRESS REACTIONS IN CHILDREN AND ADOLESCENTS
266. Vidar Fykse: SOMATOSTATIN AND THE STOMACH
267. Kirsti Berg: OXIDATIVE STRESS AND THE ISCHEMIC HEART: A STUDY IN PATIENTS UNDERGOING CORONARY REVASCULARIZATION
268. Björn Inge Gustafsson: THE SEROTONIN PRODUCING ENTEROCHROMAFFIN CELL, AND EFFECTS OF HYPERSEROTONINEMIA ON HEART AND BONE

2006

269. Torstein Baade Rø: EFFECTS OF BONE MORPHOGENETIC PROTEINS, HEPATOCYTE GROWTH FACTOR AND INTERLEUKIN-21 IN MULTIPLE MYELOMA
270. May-Britt Tessem: METABOLIC EFFECTS OF ULTRAVIOLET RADIATION ON THE ANTERIOR PART OF THE EYE
271. Anne-Sofie Helvik: COPING AND EVERYDAY LIFE IN A POPULATION OF ADULTS WITH HEARING IMPAIRMENT
272. Therese Standal: MULTIPLE MYELOMA: THE INTERPLAY BETWEEN MALIGNANT PLASMA CELLS AND THE BONE MARROW MICROENVIRONMENT
273. Ingvild Saltvedt: TREATMENT OF ACUTELY SICK, FRAIL ELDERLY PATIENTS IN A GERIATRIC EVALUATION AND MANAGEMENT UNIT – RESULTS FROM A PROSPECTIVE RANDOMISED TRIAL
274. Birger Henning Endreseth: STRATEGIES IN RECTAL CANCER TREATMENT – FOCUS ON EARLY RECTAL CANCER AND THE INFLUENCE OF AGE ON PROGNOSIS
275. Anne Mari Aukan Rokstad: ALGINATE CAPSULES AS BIOREACTORS FOR CELL THERAPY
276. Mansour Akbari: HUMAN BASE EXCISION REPAIR FOR PRESERVATION OF GENOMIC STABILITY
277. Stein Sundstrøm: IMPROVING TREATMENT IN PATIENTS WITH LUNG CANCER – RESULTS FROM TWO MULITCENTRE RANDOMISED STUDIES
278. Hilde Pleym: BLEEDING AFTER CORONARY ARTERY BYPASS SURGERY - STUDIES ON HEMOSTATIC MECHANISMS, PROPHYLACTIC DRUG TREATMENT AND EFFECTS OF AUTOTRANSFUSION

279. Line Merethe Oldervoll: PHYSICAL ACTIVITY AND EXERCISE INTERVENTIONS IN CANCER PATIENTS
280. Boye Welde: THE SIGNIFICANCE OF ENDURANCE TRAINING, RESISTANCE TRAINING AND MOTIVATIONAL STYLES IN ATHLETIC PERFORMANCE AMONG ELITE JUNIOR CROSS-COUNTRY SKIERS
281. Per Olav Vandvik: IRRITABLE BOWEL SYNDROME IN NORWAY, STUDIES OF PREVALENCE, DIAGNOSIS AND CHARACTERISTICS IN GENERAL PRACTICE AND IN THE POPULATION
282. Idar Kirkeby-Garstad: CLINICAL PHYSIOLOGY OF EARLY MOBILIZATION AFTER CARDIAC SURGERY
283. Linn Getz: SUSTAINABLE AND RESPONSIBLE PREVENTIVE MEDICINE. CONCEPTUALISING ETHICAL DILEMMAS ARISING FROM CLINICAL IMPLEMENTATION OF ADVANCING MEDICAL TECHNOLOGY
284. Eva Tegnander: DETECTION OF CONGENITAL HEART DEFECTS IN A NON-SELECTED POPULATION OF 42,381 FETUSES
285. Kristin Gabestad Nørsett: GENE EXPRESSION STUDIES IN GASTROINTESTINAL PATHOPHYSIOLOGY AND NEOPLASIA
286. Per Magnus Haram: GENETIC VS. AQUIRED FITNESS: METABOLIC, VASCULAR AND CARDIOMYOCYTE ADAPTATIONS
287. Agneta Johansson: GENERAL RISK FACTORS FOR GAMBLING PROBLEMS AND THE PREVALENCE OG PATHOLOGICAL GAMBLING IN NORWAY
288. Svein Artur Jensen: THE PREVALENCE OF SYMPTOMATIC ARTERIAL DISEASE OF THE LOWER LIMB
289. Charlotte Björk Ingul: QUANITIFICATION OF REGIONAL MYOCARDIAL FUNCTION BY STRAIN RATE AND STRAIN FOR EVALUATION OF CORONARY ARTERY DISEASE. AUTOMATED VERSUS MANUAL ANALYSIS DURING ACUTE MYOCARDIAL INFARCTION AND DOBUTAMINE STRESS ECHOCARDIOGRAPHY
290. Jakob Nakling: RESULTS AND CONSEQUENCES OF ROUTINE ULTRASOUND SCREENING IN PREGNANCY – A GEOGRAPHIC BASED POPULATION STUDY
291. Anne Engum: DEPRESSION AND ANXIETY – THEIR RELATIONS TO THYROID DYSFUNCTION AND DIABETES IN A LARGE EPIDEMIOLOGICAL STUDY
292. Ottar Bjerkeset: ANXIETY AND DEPRESSION IN THE GENERAL POPULATION: RISK FACTORS, INTERVENTION AND OUTCOME – THE NORD-TRØNDELAG HEALTH STUDY (HUNT)
293. Jon Olav Drogset: RESULTS AFTER SURGICAL TREATMENT OF ANTERIOR CRUCIATE LIGAMENT INJURIES – A CLINICAL STUDY
294. Lars Fosse: MECHANICAL BEHAVIOUR OF COMPACTED MORSELLISED BONE – AN EXPERIMENTAL IN VITRO STUDY
295. Gunilla Klensmeden Fosse: MENTAL HEALTH OF PSYCHIATRIC OUTPATIENTS BULLIED IN CHILDHOOD
296. Paul Jarle Mork: MUSCLE ACTIVITY IN WORK AND LEISURE AND ITS ASSOCIATION TO MUSCULOSKELETAL PAIN
297. Björn Stenström: LESSONS FROM RODENTS: I: MECHANISMS OF OBESITY SURGERY – ROLE OF STOMACH. II: CARCINOGENIC EFFECTS OF *HELICOBACTER PYLORI* AND SNUS IN THE STOMACH

2007

298. Haakon R. Skogseth: INVASIVE PROPERTIES OF CANCER – A TREATMENT TARGET ? IN VITRO STUDIES IN HUMAN PROSTATE CANCER CELL LINES
299. Janniche Hammer: GLUTAMATE METABOLISM AND CYCLING IN MESIAL TEMPORAL LOBE EPILEPSY
300. May Britt Drugli: YOUNG CHILDREN TREATED BECAUSE OF ODD/CD: CONDUCT PROBLEMS AND SOCIAL COMPETENCIES IN DAY-CARE AND SCHOOL SETTINGS
301. Arne Skjold: MAGNETIC RESONANCE KINETICS OF MANGANESE DIPYRIDOXYL DIPHOSPHATE (MnDPDP) IN HUMAN MYOCARDIUM. STUDIES IN HEALTHY VOLUNTEERS AND IN PATIENTS WITH RECENT MYOCARDIAL INFARCTION
302. Siri Malm: LEFT VENTRICULAR SYSTOLIC FUNCTION AND MYOCARDIAL PERFUSION ASSESSED BY CONTRAST ECHOCARDIOGRAPHY
303. Valentina Maria do Rosario Cabral Iversen: MENTAL HEALTH AND PSYCHOLOGICAL ADAPTATION OF CLINICAL AND NON-CLINICAL MIGRANT GROUPS

304. Lasse Løvstakken: SIGNAL PROCESSING IN DIAGNOSTIC ULTRASOUND: ALGORITHMS FOR REAL-TIME ESTIMATION AND VISUALIZATION OF BLOOD FLOW VELOCITY
305. Elisabeth Olstad: GLUTAMATE AND GABA: MAJOR PLAYERS IN NEURONAL METABOLISM
306. Lilian Leistad: THE ROLE OF CYTOKINES AND PHOSPHOLIPASE A_2S IN ARTICULAR CARTILAGE CHONDROCYTES IN RHEUMATOID ARTHRITIS AND OSTEOARTHRITIS
307. Arne Vaaler: EFFECTS OF PSYCHIATRIC INTENSIVE CARE UNIT IN AN ACUTE PSYCIATHRIC WARD
308. Mathias Toft: GENETIC STUDIES OF LRRK2 AND PINK1 IN PARKINSON'S DISEASE
309. Ingrid Løvold Mostad: IMPACT OF DIETARY FAT QUANTITY AND QUALITY IN TYPE 2 DIABETES WITH EMPHASIS ON MARINE N-3 FATTY ACIDS
310. Torill Eidhammer Sjøbakk: MR DETERMINED BRAIN METABOLIC PATTERN IN PATIENTS WITH BRAIN METASTASES AND ADOLESCENTS WITH LOW BIRTH WEIGHT
311. Vidar Beisvåg: PHYSIOLOGICAL GENOMICS OF HEART FAILURE: FROM TECHNOLOGY TO PHYSIOLOGY
312. Olav Magnus Søndenå Fredheim: HEALTH RELATED QUALITY OF LIFE ASSESSMENT AND ASPECTS OF THE CLINICAL PHARMACOLOGY OF METHADONE IN PATIENTS WITH CHRONIC NON-MALIGNANT PAIN
313. Anne Brantberg: FETAL AND PERINATAL IMPLICATIONS OF ANOMALIES IN THE GASTROINTESTINAL TRACT AND THE ABDOMINAL WALL
314. Erik Solligård: GUT LUMINAL MICRODIALYSIS
315. Elin Tollefsen: RESPIRATORY SYMPTOMS IN A COMPREHENSIVE POPULATION BASED STUDY AMONG ADOLESCENTS 13-19 YEARS. YOUNG-HUNT 1995-97 AND 2000-01; THE NORD-TRØNDELAG HEALTH STUDIES (HUNT)
316. Anne-Tove Brenne: GROWTH REGULATION OF MYELOMA CELLS
317. Heidi Knobel: FATIGUE IN CANCER TREATMENT – ASSESSMENT, COURSE AND ETIOLOGY
318. Torbjørn Dahl: CAROTID ARTERY STENOSIS. DIAGNOSTIC AND THERAPEUTIC ASPECTS
319. Inge-Andre Rasmussen jr.: FUNCTIONAL AND DIFFUSION TENSOR MAGNETIC RESONANCE IMAGING IN NEUROSURGICAL PATIENTS
320. Grete Helen Bratberg: PUBERTAL TIMING – ANTECEDENT TO RISK OR RESILIENCE ? EPIDEMIOLOGICAL STUDIES ON GROWTH, MATURATION AND HEALTH RISK BEHAVIOURS; THE YOUNG HUNT STUDY, NORD-TRØNDELAG, NORWAY
321. Sveinung Sørhaug: THE PULMONARY NEUROENDOCRINE SYSTEM. PHYSIOLOGICAL, PATHOLOGICAL AND TUMOURIGENIC ASPECTS
322. Olav Sande Eftedal: ULTRASONIC DETECTION OF DECOMPRESSION INDUCED VASCULAR MICROBUBBLES
323. Rune Bang Leistad: PAIN, AUTONOMIC ACTIVATION AND MUSCULAR ACTIVITY RELATED TO EXPERIMENTALLY-INDUCED COGNITIVE STRESS IN HEADACHE PATIENTS
324. Svein Brekke: TECHNIQUES FOR ENHANCEMENT OF TEMPORAL RESOLUTION IN THREE-DIMENSIONAL ECHOCARDIOGRAPHY
325. Kristian Bernhard Nilsen: AUTONOMIC ACTIVATION AND MUSCLE ACTIVITY IN RELATION TO MUSCULOSKELETAL PAIN
326. Anne Irene Hagen: HEREDITARY BREAST CANCER IN NORWAY. DETECTION AND PROGNOSIS OF BREAST CANCER IN FAMILIES WITH *BRCA1*GENE MUTATION
327. Ingebjørg S. Juel : INTESTINAL INJURY AND RECOVERY AFTER ISCHEMIA. AN EXPERIMENTAL STUDY ON RESTITUTION OF THE SURFACE EPITHELIUM, INTESTINAL PERMEABILITY, AND RELEASE OF BIOMARKERS FROM THE MUCOSA
328. Runa Heimstad: POST-TERM PREGNANCY
329. Jan Egil Afset: ROLE OF ENTEROPATHOGENIC *ESCHERICHIA COLI* IN CHILDHOOD DIARRHOEA IN NORWAY
330. Bent Håvard Hellum: *IN VITRO* INTERACTIONS BETWEEN MEDICINAL DRUGS AND HERBS ON CYTOCHROME P-450 METABOLISM AND P-GLYCOPROTEIN TRANSPORT
331. Morten André Høydal: CARDIAC DYSFUNCTION AND MAXIMAL OXYGEN UPTAKE MYOCARDIAL ADAPTATION TO ENDURANCE TRAINING

2008

332. Andreas Møllerløkken: REDUCTION OF VASCULAR BUBBLES: METHODS TO PREVENT THE ADVERSE EFFECTS OF DECOMPRESSION
333. Anne Hege Aamodt: COMORBIDITY OF HEADACHE AND MIGRAINE IN THE NORD-TRØNDELAG HEALTH STUDY 1995-97
334. Brage Høyem Amundsen: MYOCARDIAL FUNCTION QUANTIFIED BY SPECKLE TRACKING AND TISSUE DOPPLER ECHOCARDIOGRAPHY – VALIDATION AND APPLICATION IN EXERCISE TESTING AND TRAINING
335. Inger Anne Næss: INCIDENCE, MORTALITY AND RISK FACTORS OF FIRST VENOUS THROMBOSIS IN A GENERAL POPULATION. RESULTS FROM THE SECOND NORD-TRØNDELAG HEALTH STUDY (HUNT2)
336. Vegard Bugten: EFFECTS OF POSTOPERATIVE MEASURES AFTER FUNCTIONAL ENDOSCOPIC SINUS SURGERY
337. Morten Bruvold: MANGANESE AND WATER IN CARDIAC MAGNETIC RESONANCE IMAGING
338. Miroslav Fris: THE EFFECT OF SINGLE AND REPEATED ULTRAVIOLET RADIATION ON THE ANTERIOR SEGMENT OF THE RABBIT EYE
339. Svein Arne Aase: METHODS FOR IMPROVING QUALITY AND EFFICIENCY IN QUANTITATIVE ECHOCARDIOGRAPHY – ASPECTS OF USING HIGH FRAME RATE
340. Roger Almvik: ASSESSING THE RISK OF VIOLENCE: DEVELOPMENT AND VALIDATION OF THE BRØSET VIOLENCE CHECKLIST
341. Ottar Sundheim: STRUCTURE-FUNCTION ANALYSIS OF HUMAN ENZYMES INITIATING NUCLEOBASE REPAIR IN DNA AND RNA
342. Anne Mari Undheim: SHORT AND LONG-TERM OUTCOME OF EMOTIONAL AND BEHAVIOURAL PROBLEMS IN YOUNG ADOLESCENTS WITH AND WITHOUT READING DIFFICULTIES
343. Helge Garåsen: THE TRONDHEIM MODEL. IMPROVING THE PROFESSIONAL COMMUNICATION BETWEEN THE VARIOUS LEVELS OF HEALTH CARE SERVICES AND IMPLEMENTATION OF INTERMEDIATE CARE AT A COMMUNITY HOSPITAL COULD PROVIDE BETTER CARE FOR OLDER PATIENTS. SHORT AND LONG TERM EFFECTS
344. Olav A. Foss: "THE ROTATION RATIOS METHOD". A METHOD TO DESCRIBE ALTERED SPATIAL ORIENTATION IN SEQUENTIAL RADIOGRAPHS FROM ONE PELVIS
345. Bjørn Olav Åsvold: THYROID FUNCTION AND CARDIOVASCULAR HEALTH
346. Torun Margareta Melø: NEURONAL GLIAL INTERACTIONS IN EPILEPSY
347. Irina Poliakova Eide: FETAL GROWTH RESTRICTION AND PRE-ECLAMPSIA: SOME CHARACTERISTICS OF FETO-MATERNAL INTERACTIONS IN DECIDUA BASALIS
348. Torunn Askim: RECOVERY AFTER STROKE. ASSESSMENT AND TREATMENT; WITH FOCUS ON MOTOR FUNCTION
349. Ann Elisabeth Åsberg: NEUTROPHIL ACTIVATION IN A ROLLER PUMP MODEL OF CARDIOPULMONARY BYPASS. INFLUENCE ON BIOMATERIAL, PLATELETS AND COMPLEMENT
350. Lars Hagen: REGULATION OF DNA BASE EXCISION REPAIR BY PROTEIN INTERACTIONS AND POST TRANSLATIONAL MODIFICATIONS
351. Sigrun Beate Kjøtrød: POLYCYSTIC OVARY SYNDROME – METFORMIN TREATMENT IN ASSISTED REPRODUCTION
352. Steven Keita Nishiyama: PERSPECTIVES ON LIMB-VASCULAR HETEROGENEITY: IMPLICATIONS FOR HUMAN AGING, SEX, AND EXERCISE
353. Sven Peter Näsholm: ULTRASOUND BEAMS FOR ENHANCED IMAGE QUALITY
354. Jon Ståle Ritland: PRIMARY OPEN-ANGLE GLAUCOMA & EXFOLIATIVE GLAUCOMA. SURVIVAL, COMORBIDITY AND GENETICS
355. Sigrid Botne Sando: ALZHEIMER'S DISEASE IN CENTRAL NORWAY. GENETIC AND EDUCATIONAL ASPECTS
356. Parvinder Kaur: CELLULAR AND MOLECULAR MECHANISMS BEHIND METHYLMERCURY-INDUCED NEUROTOXICITY
357. Ismail Cüneyt Güzey: DOPAMINE AND SEROTONIN RECEPTOR AND TRANSPORTER GENE POLYMORPHISMS AND EXTRAPYRAMIDAL SYMPTOMS. STUDIES IN PARKINSON'S DISEASE AND IN PATIENTS TREATED WITH ANTIPSYCHOTIC OR ANTIDEPRESSANT DRUGS

358. Brit Dybdahl: EXTRA-CELLULAR INDUCIBLE HEAT-SHOCK PROTEIN 70 (Hsp70) – A ROLE IN THE INFLAMMATORY RESPONSE ?
359. Kristoffer Haugarvoll: IDENTIFYING GENETIC CAUSES OF PARKINSON'S DISEASE IN NORWAY
360. Nadra Nilsen: TOLL.LIKE RECEPTOR – EXPRESSION, REGULATION AND SIGNALING
361. Johan Håkon Bjørngaard: PATIENT SATISFACTION WITH OUTPATIENT MENTAL HEALTH SERVICES – THE INFLUENCE OF ORGANIZATIONAL FACTORS.
362. Kjetil Høydal : EFFECTS OF HIGH INTENSITY AEROBIC TRAINING IN HEALTHY SUBJECTS AND CORONARY ARTERY DISEASE PATIENTS; THE IMPORTANCE OF INTENSITY,, DURATION AND FREQUENCY OF TRAINING.
363. Trine Karlsen: TRAINING IS MEDICINE: ENDURANCE3 AND STRANGTH TRAINING IN CORONARY ARTERY DISESE AND HEALTH.
364. Marte Thuen: MANGANASE-ENHANCED AND DIFFUSION TENSOR MR IMAGING OF THE NORMAL, INJURED AND REGENERATING RAT VISUAL PATHWAY
365. Cathrine Broberg Vågbø: DIRECT REPAIR OF ALKYLATION DAMAGE IN DNA AND RNA BY 2-OXOGLUTARATE- AND IRON-DEPENDENT DIOXYGENASES
366. Arnt Erik Tjønna: AEROBIC EXERCISE AND CARDIOVASCULAR RISK FACTORS IN OVERWEIGHT AND OBESE ADOLESCENTS AND ADULTS
367. Marianne W. Furnes: FEEDING BEHAVIOR AND BODY WEIGHT DEVELOPMENT: LESSONS FROM RATS
368. Lene N. Johannessen: FUNGAL PRODUCTS AND INFLAMMATORY RESPONSES IN HUMAN MONOCYTES AND EPITHELIAL CELLS