Finnish Institute of Occupational Health

Activation of inflammatory response by fungal cell wall components and toxins

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1) Mycotoxins and β-glucans in the activation of inflammatory response in human macrophages

2) Proteomics and transcriptomics to study β-glucan-induced innate immune response in a global manner

Microbes in moisture-damaged buildings

Respiratory, inflammatory, and neurologic symptoms

Only 3-5 % of symptoms are due to IgE-mediated allergy

The role of innate immunity?

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Pattern recognition receptors (PRRs) activate innate immune response

PRRs detect the presence of pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs)

Inflammatory response is activated

Innate immunity has a central role in the development and maintenance of inflammatory and autoimmune diseases

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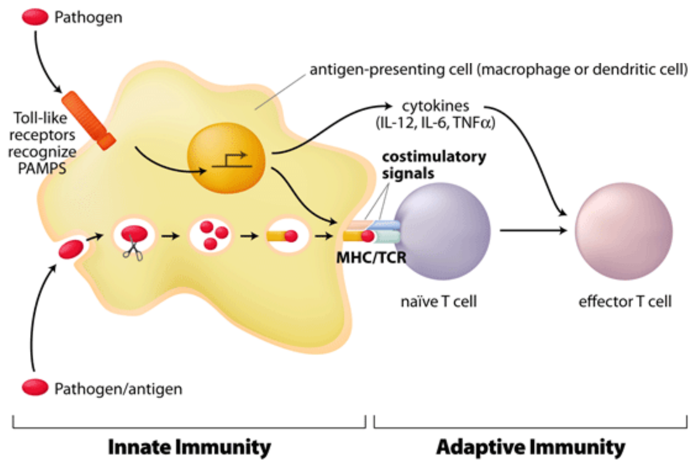
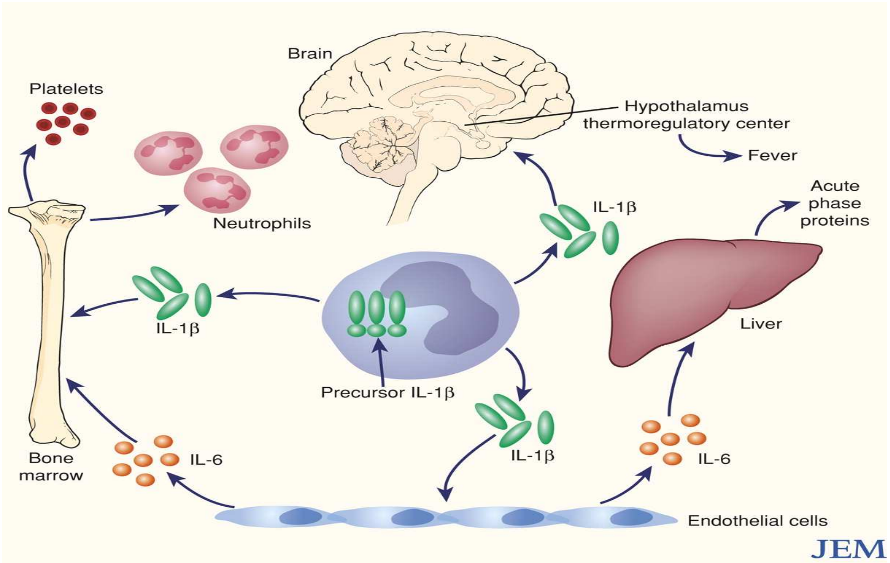
Innate immunity is critical to adaptive immune response



Hours Days Time after infection

Interleukin-1β (IL-1β) is the key inflammatory cytokine



Moisture damage and microbial growth

Microbial spores and cellular components of microbes are released to indoor air

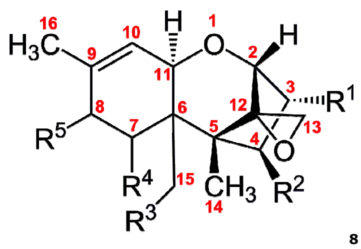
1) Microbial toxins

2) Cell wall components including β-glucans and lipopolysaccharide (LPS)

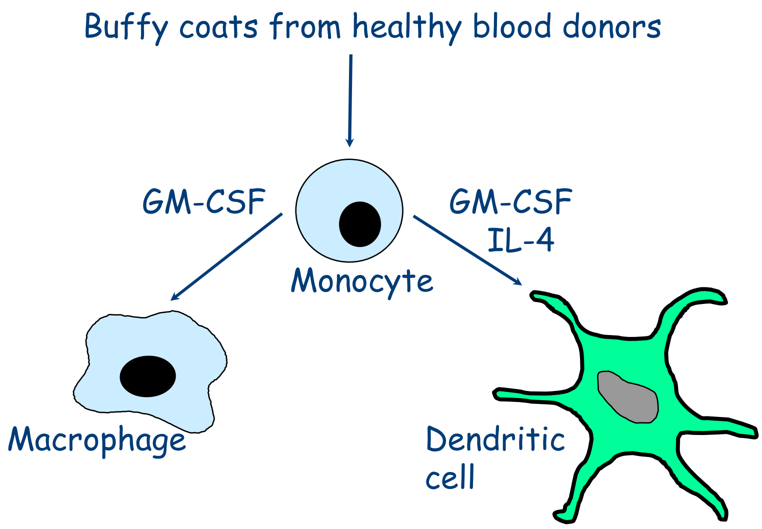
Stachybotrys Chartarum is a tertiary colonizers of moisture-damaged buildings

S. chartarum is a greenish-black mould that is commonly found outdoors and grows also in moisture-damaged buildings

S. chartarum produces **trichothecene mycotoxins** including satratoxin, roridin, and verrucarin



Cell models to study inflammatory response



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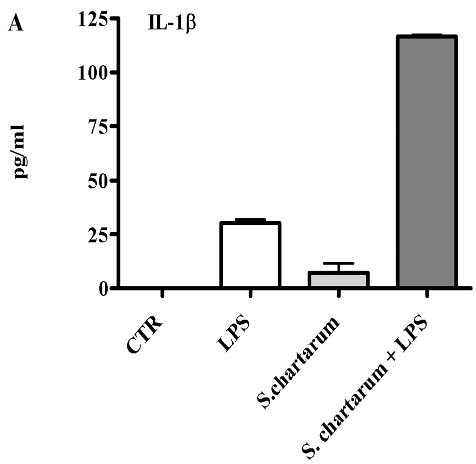
S. chartarum and LPS activate synergistically secretion of IL-1β in human macrophages



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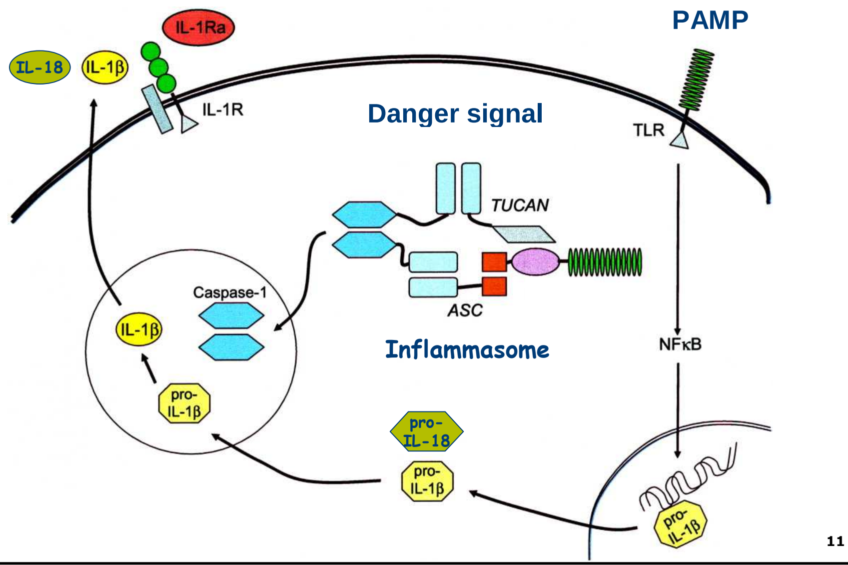
Inflammasome is involved in proteolytic processing of proIL-1β and proIL-18



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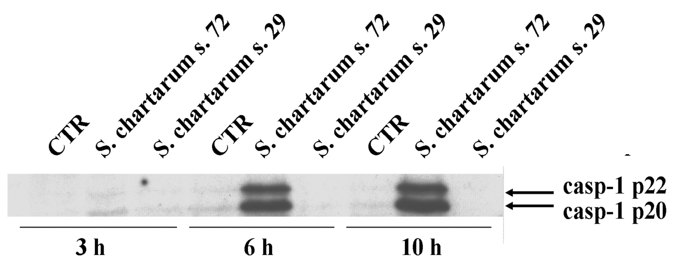
Toxin-positive strain of *S. chartarum* activates Inflammasome-associated caspase-1



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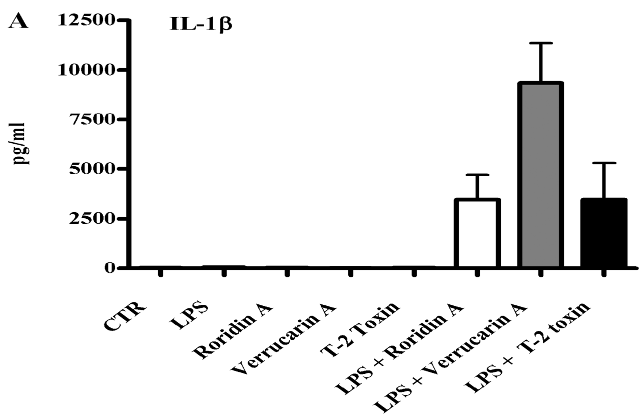
Mycotoxins activate IL-1β secretion in LPS-primed macrophages



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Conclusions

Mycotoxins are microbial danger signals that activate inflammasome resulting in secretion pro-inflammatory cytokine IL-1β

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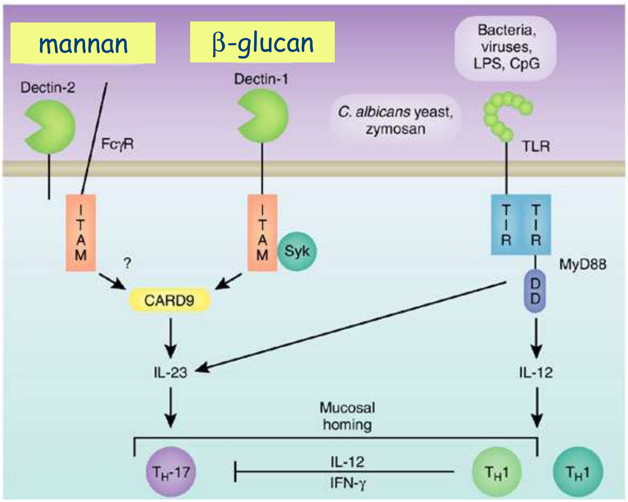
β-glucans, the major component of fungal cell walls, are recognized by C-type lectin receptor Dectin-1



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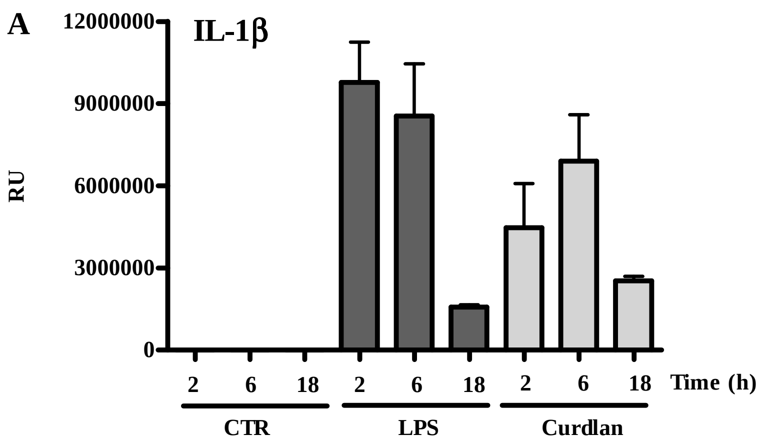
Both β-glucans and LPS activate transcription of IL-1β gene in human macrophages



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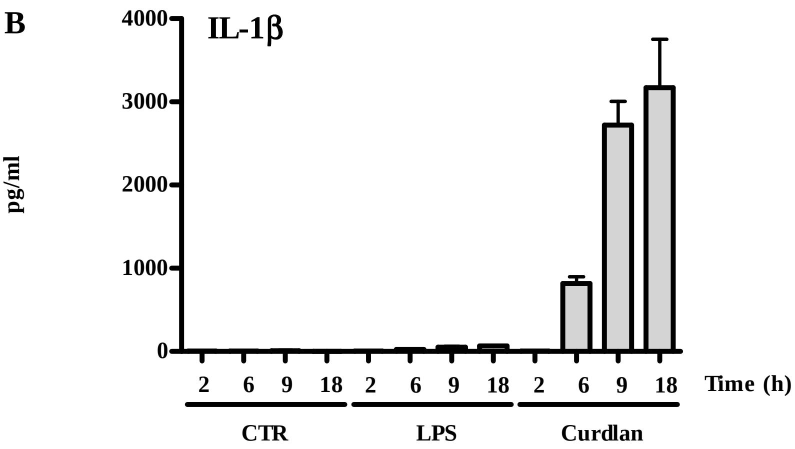
β-glucan, curdlan, activates IL-1β secretion



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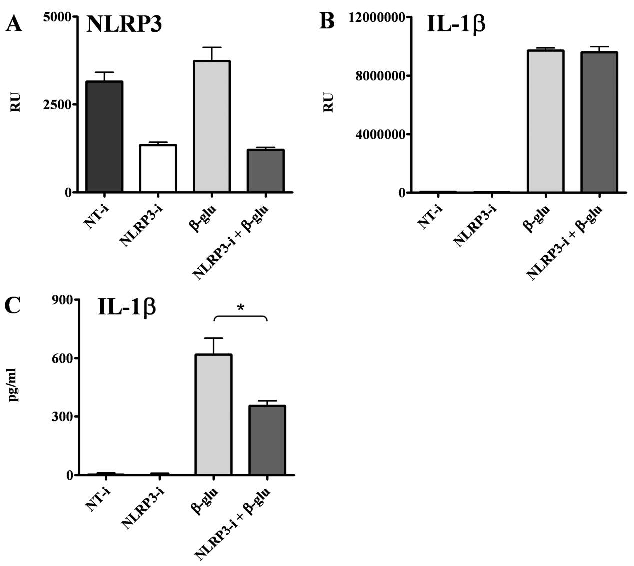
β-glucans are novel activators of NLRP3 Inflammasome



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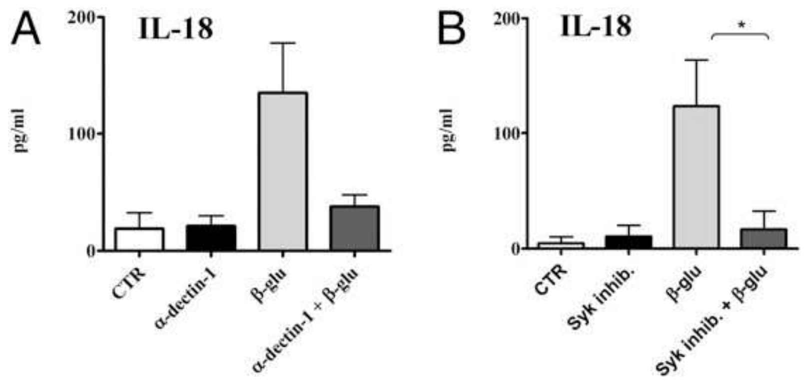
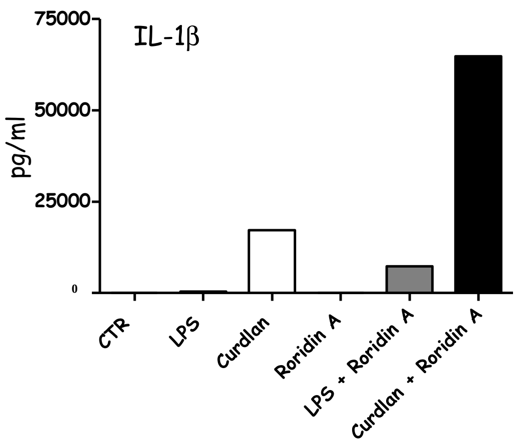
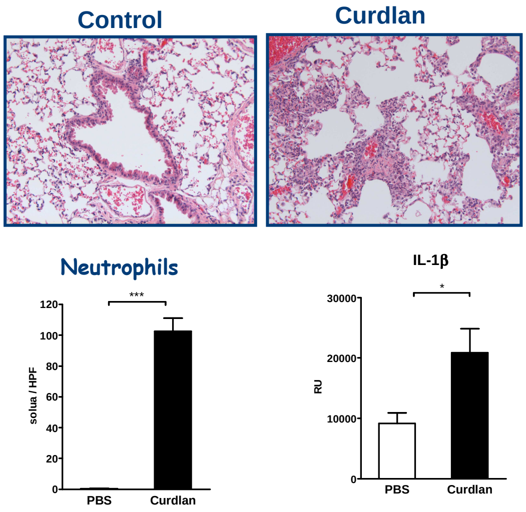
β-glucan-induced inflammasome activation is dependent on Dectin-1/Syk signalling



Microbial cell wall components and mycotoxins synergistically activate IL-1β secretion



Intranasal exposure of mice to β-glucans induces lung inflammation



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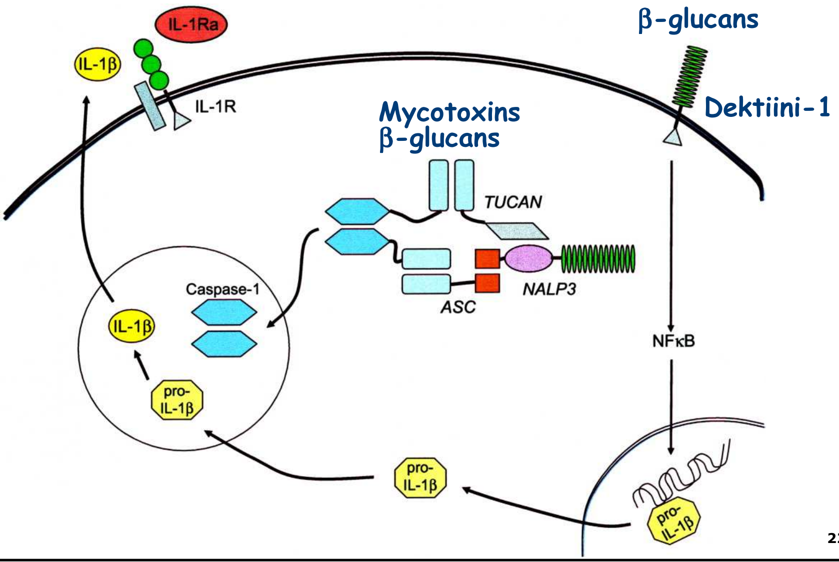
Conclusions



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There is high interindividual variation in responses to β-glucans and mycotoxins

Genes that regulate inflammatory response are under high evolutionary pressure

→ Enhanced polymorphism

The immunologic and metabolic status of people is different

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NLRP3 Inflammasome is a sensor of infection and metabolic danger

Disease	Inflammasome activator
Alzheimer disease	β-amyloid fibers
Atherosclerosis	Crystalline cholesterol
Diabetes	Glucose, Islet amyloid polypeptide
Gout	Crystalline uric acid

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Systemsbiology to study activation of innate immunity

Transcriptomics:
Analysis of mRNA expression in a global manner in human macrophages in response to LPS and β-glucan stimulation

Quantitative high-throughput proteomics:
Secretome, the global pattern of secreted proteins, in macrophages in response to β-glucan stimulation

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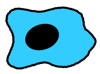
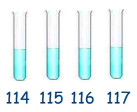
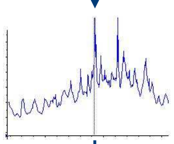
Quantitative proteomics to characterize macrophage secretome



Human macrophages: Control and β-glucan-exposed cells
Proteins from cell culture supernatants are purified.



Protein digestion and iTRAQ-labelling.
Labelled peptides are fractionated with cation exchange chromatography.



LC-MS/MS analysis.

Annexin A1 - Homo sapiens
Annexin A11 - Homo sapiens
Annexin A11 variant (Fragment) -
Annexin A2 - Homo sapiens

Protein identification and quantification.

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Macrophage secretome

Fingerprint for β-glucan exposure

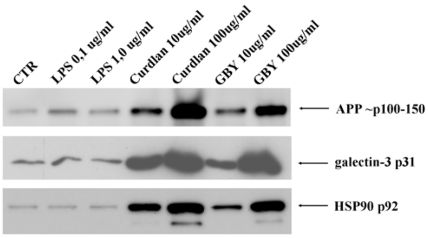
Exposure	Secreted proteins
β-glucan (Curdlan)	1537
β-glucan (GBY)	1557

β-glucans activate secretion of DAMPs

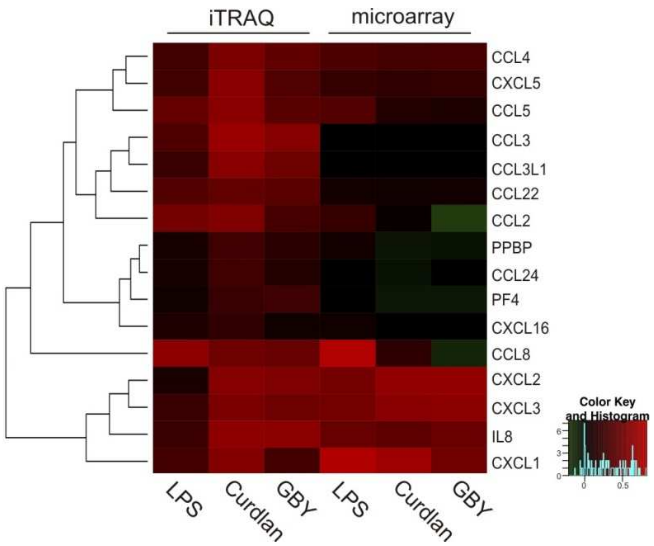
A

	LPS	Curdlan	GBY
Amyloid beta A4	1.2	2.9	2.8
Galectin-1	1.1	2.6	4.4
Galectin-3	1.1	2.5	4.2
Galectin-9	1.1	3.4	4.1
HSP 90-alpha	1.1	3.3	4.0
HSP 90-beta	1.0	2.6	3.6
Heat shock protein beta-1	1.2	2.8	4.3
Heat shock 70 kDa protein-1	1.1	2.7	4.6
Heat shock protein 105 kDa	1.1	3.1	4.7
HMG protein B1	0.9	1.9	4.1
Protein S100 A4	1.0	2.3	4.0
Protein S100 A6	1.0	1.9	3.1
Protein S100 A8	1.1	1.9	2.4
Protein S100 A9	1.1	1.8	2.5
Protein S100 A10	1.2	3.0	3.9
Protein S100 A11	1.0	2.2	4.2
Protein S100 A12	1.1	1.7	2.2
Protein S100 A13	1.2	2.1	3.9

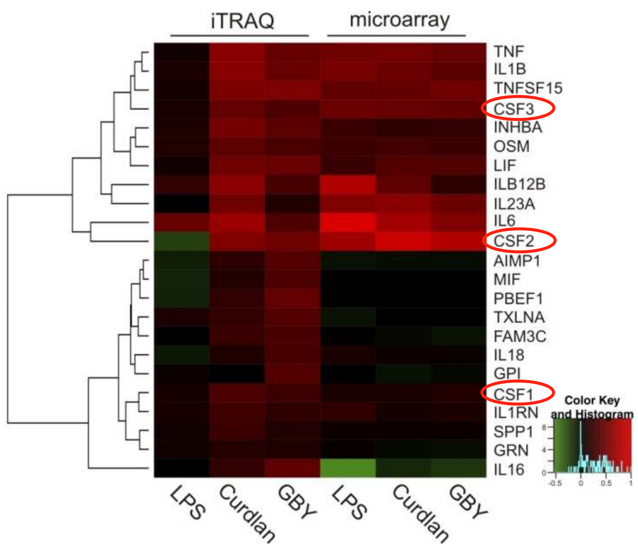
B



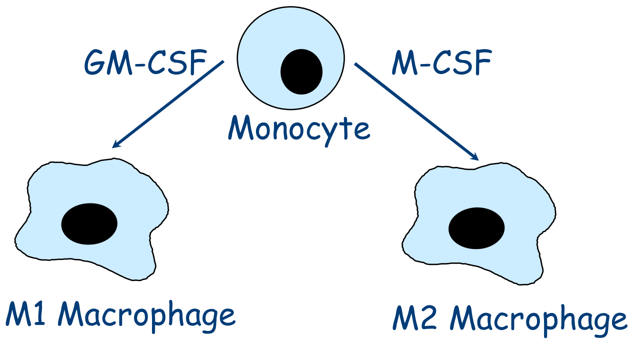
β-glucan stimulation of human macrophages activates robust secretion of chemokines



β-glucan-induced cytokine secretion and mRNA expression in human macrophages



Differentiation of monocytes to macrophages



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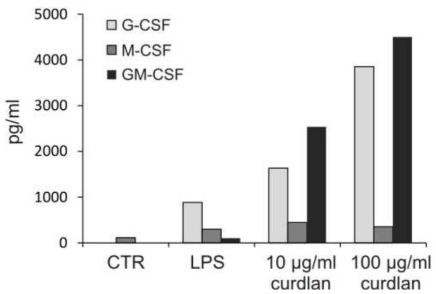
β-glucans induce secretion of GM-CSF and activation of STAT5 in human monocytes

A)



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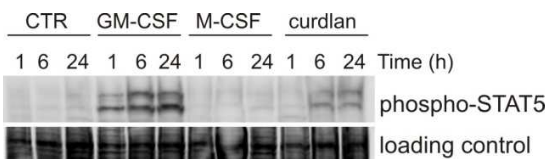

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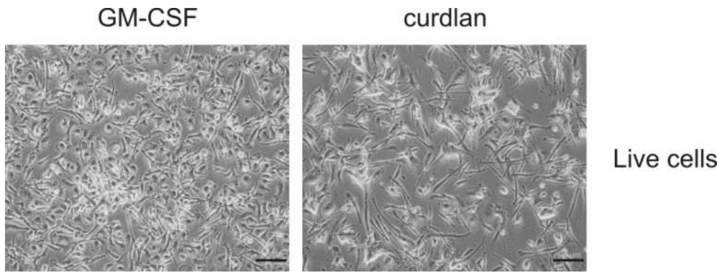
β-glucans are able to induce monocyte-macrophage differentiation



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Acknowledgements

Finnish Institute of Occupational Health

Päivi Kankkunen
Laura Teirilä
Johanna Rintahaka
Elina Välimäki
Alina Poltajainen
Henrik Wolff
Sampsa Matikainen

Institute of Biotechnology, University of Helsinki

Tiina Öhman
Tuula Nyman

Biomedicum Helsinki, University of Helsinki

Anna-Mari Lahesmaa
Sampsa Hautaniemi

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