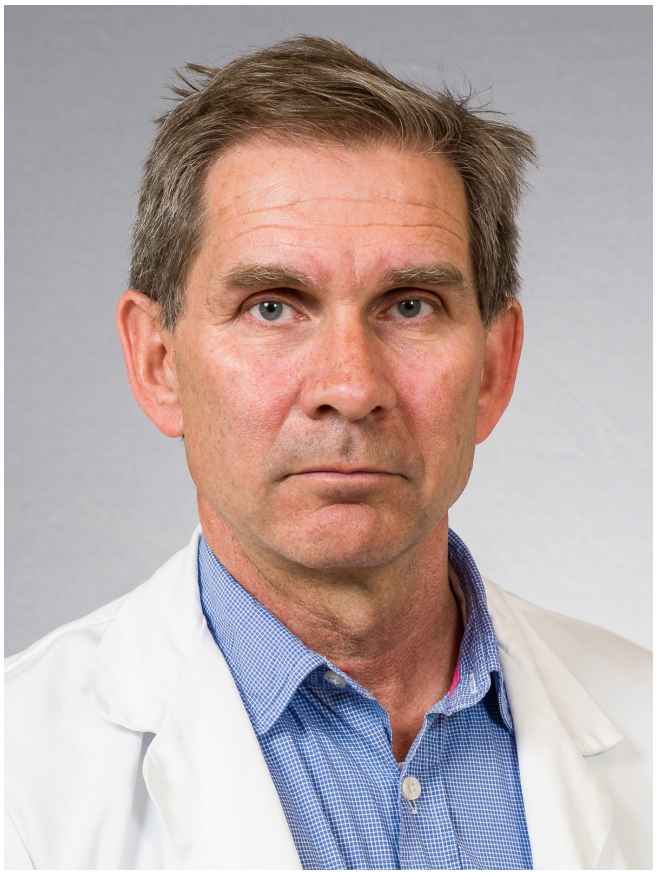
An extract* of the medicinal mushroom *Agaricus blazei* Murill can protect against allergy

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Immunology Dept.
Oslo University Hospital
Oslo, Norway

Presentation at Immunology Summit-2014, Baltimore



Disclosure: ImmunoPharma, Norway, producer of *Andosan™

Agaricus bM - origin and use as health food

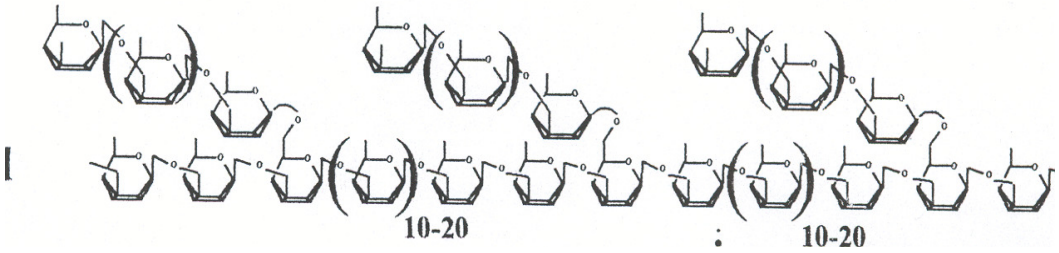


Agaricus blazei cultivated in Piedade, Brazil. Photo G Hetland 2012

- ***Agaricus blazei* Murill (AbM)** of the family, *Basidiomycetes*, grows naturally in coastal rain forest (Piedade) near São Paulo, Brazil
- **Edible mushroom** - a relative to champignon (*Agaricus bisporus*): cultivated as health food and used as remedy against cancer, chronic hepatitis, diabetes, etc.

Agaricus bM – immunomodulation

- **AbM is rich in** immunomodulating β-Glucans – make up cell wall cytoskeleton in yeast (figure), fungi and mushrooms



- **β-Glucans from mushrooms**, yeast and fungi have anticancer and anti-infection effects i.p. in mouse models (Takeyama 1987, Franek 1992, Hetland 2000, Ohno 2001)

- **Adjuvant effect of AbM in** HBV and F&MD DNA **vaccines** in mice – ↑ humoral and cellular responses (Chen et al Int Immunopharmacol 2004, Vet Immunol Immunopathol 2006)

Anti-tumor effects of *Agaricus* bM

Tumor/ -activity	In vivo effects of *Agaricus* bM	References
Leukemia *	Inhibition	Xiaohui 1994
Fibrosarcoma Sarcoma	Inhibition of tumor size and neoangiogenesis	Itoh 1997, Fujimiya 1998, Ohno 2001, Kimura 2004,
Gynecological cancer *	Increased NK cell activity and QOL	Ahn 2004
Ovarian cancer	Inhibition of metastasis	Kobayashi 2005
Lung cancer	Inhibition of metastasis	Kimura 2004, Kobayashi 2005
Myeloma	AbM + squid phospholipid: Inhibition of tumor size	Murakawa 2007
Carcinogenity	No	Lee 2008
Prostate cancer	Reduced tumor growth &neoangiogenesis *No significant effect on PSA values*	Yu 2009 Yoshimura 2010
Colon cancer	Reduced tumor cell growth in SCID mice Reduced tumor load in mouse FAP model	Wu 2011 Hetland et al, unpublished

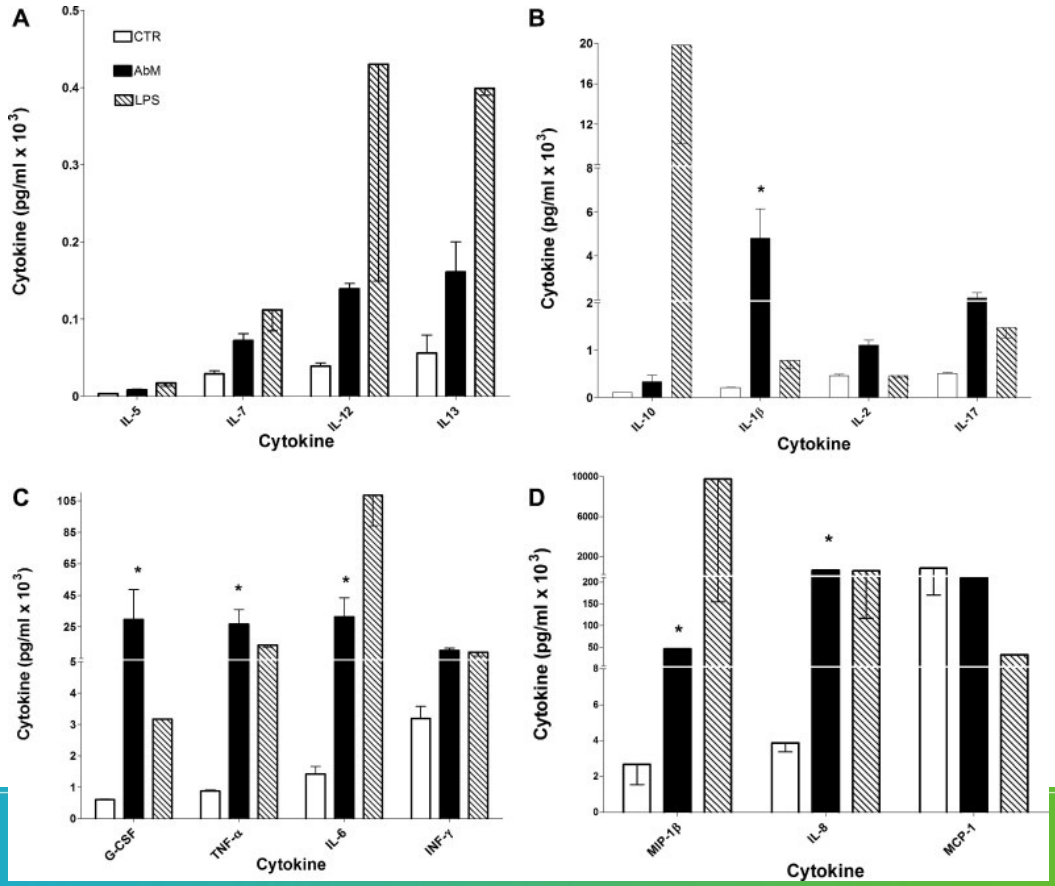
* **Human studies, otherwise mouse studies,** ***low values, few patients**

Cellular effects of *Agaricus* bM in vitro - cytokines

- NF-κB translocation in monocytic THP-1 cells induced via TLR2 (Tryggestad et al, 2013)
  - probably also stim. via Dectin-1 and CD11b/18 (CR3)
- IL-6, IL-8, TNF… produced in Mø (Sorimachi et al, Cell Struct Funct 2001) , EC (Bernardshaw et al, Inflamm 2005)
- Cytokines in MDDC stim. with Andosan AbM-extract (10%,1d):

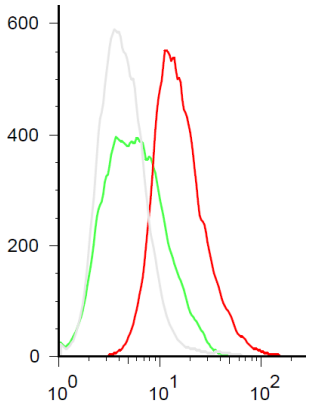
  LPS (0.5 μg/ml, 1d), CTR=10% PBS

  (Førland et al, Cytokine 2010)

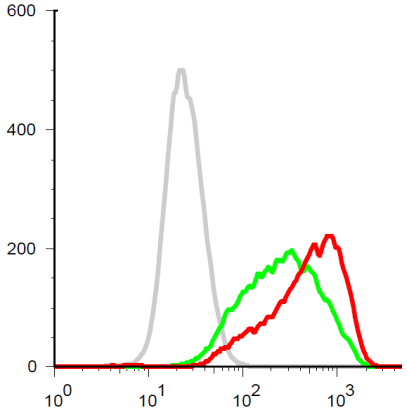


Cellular effects of AbM – membrane markers

- **DC activation**: Bone marrow DC -↑ CD40, CD80, MHC I Ag, Th1 cell development (Kawamura et al Immunol 2005)
- Monocyte derived DC (MDDC):
  also ↑ CD40, CD80, CD83 (Tangen et al, Appl Sci Rep 2014)

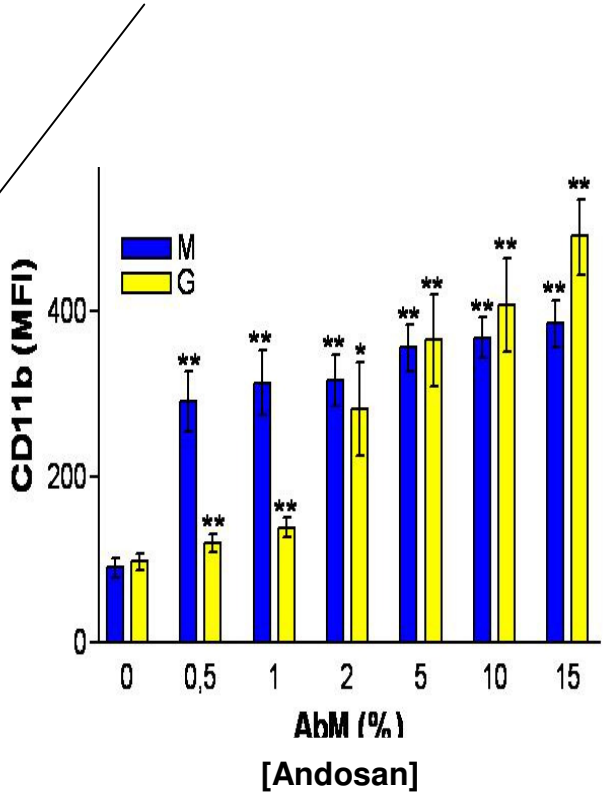


CD69



CD86





- Up-regulation of adhesion molecules in blood:
  - also for CD62L (Bernardshaw et al, APMIS 2007)

Effects of Andosan AbM extract on human genes

- THP-1 cells in vitro: ↑ expression of genes related to immune function (e.g. IL1A, CXCL1-3)

  (Ellertsen et al, Int Immunopharmacol 2006) [Method: Global 29 k oligo-based, single-channel microarray]

- In patients w/ HCV* infection: ↑ gene expression after 7d mostly related to G-protein-R-signalling, cell cycling and transcriptional regulation

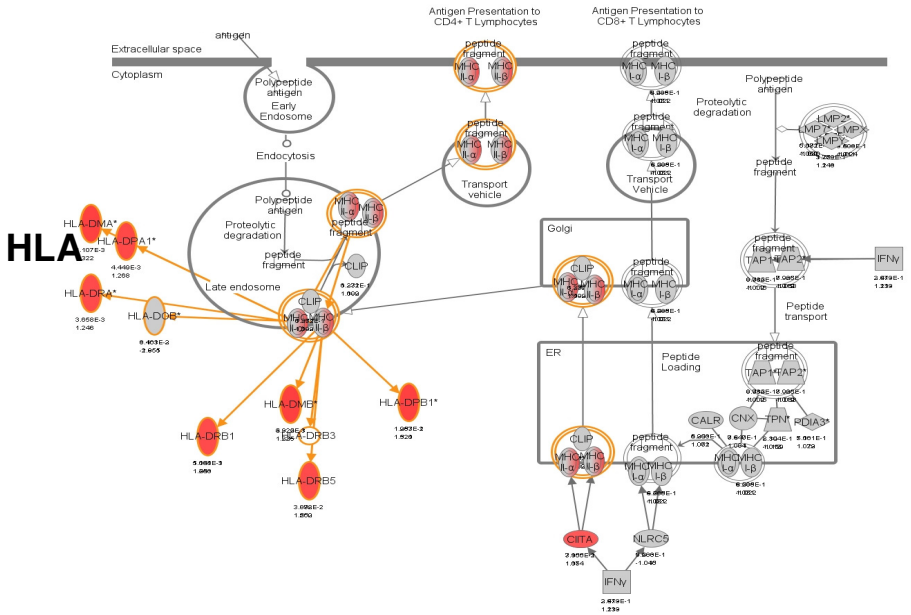
  (Grinde et al, Int Immunopharmacol 2006)

* Insignificant reduction of HCV load in blood

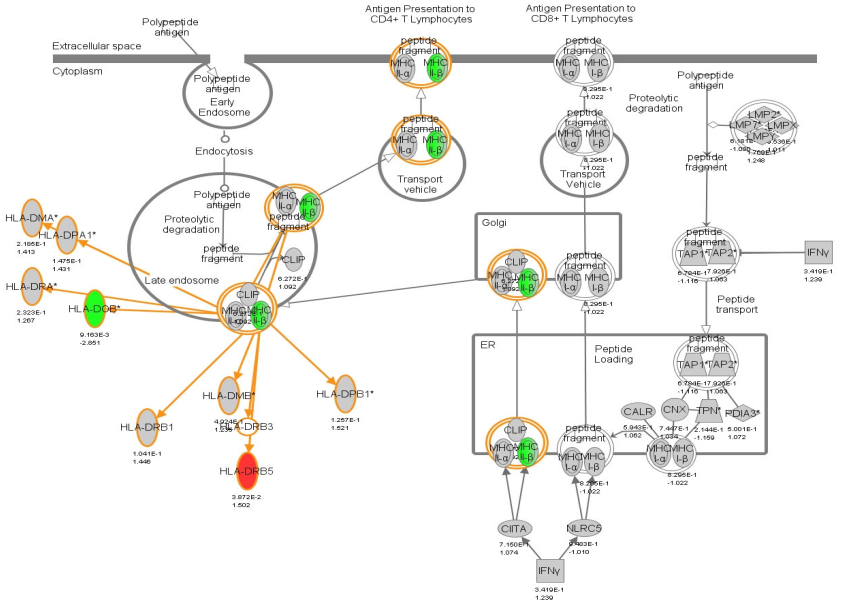
Effect on genes related to Ag presentation

Affymetrix microarray platform

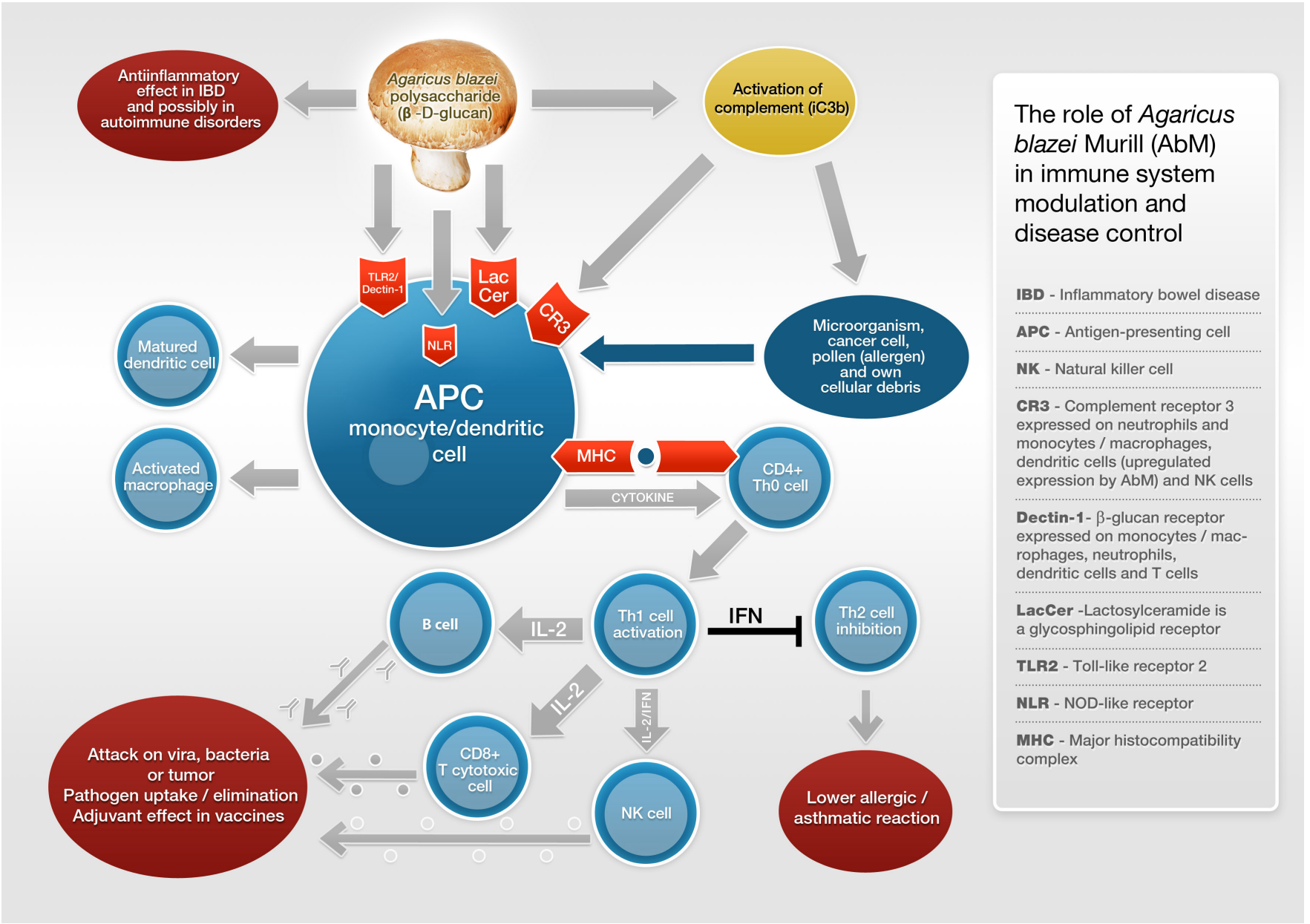
Andosan



Placebo



Andosan add-on treatment for multiple myeloma patients given high-dose chemo, Tangen et al, submitted



Hetland et al, Avd Pharmacol Sci 2011

What is Andosan?

- a mixed *Basidiomycetes* mushroom water extract of the mycelium,

- heat-sterilized, GMP-certified

Andosan™ (ImmunoPharma) produced in Gifu-ken, Japan, and GMP-certified by Meiji Co, contains:



***Agaricus blazei* Murill (82%)** – a.k.a. *A brasiliensis*, *A. subrufescens*, *A.rufotegulis*

("Almond or God's mushroom")



***Hericium erinaceus* (15%)** – edible, used in Chinese medicine, antitumor effects (Kim et al, J Agric Food Chem 2011)

("Lion's mane mushroom")



***Grifola frondosa* (3%)** – edible, immunomodulating & antitumor effects (Suzuki et al, J Pharmacobiodyn 1984, Masuda et al, Int J Cancer 2013)

("Hen of the woods")



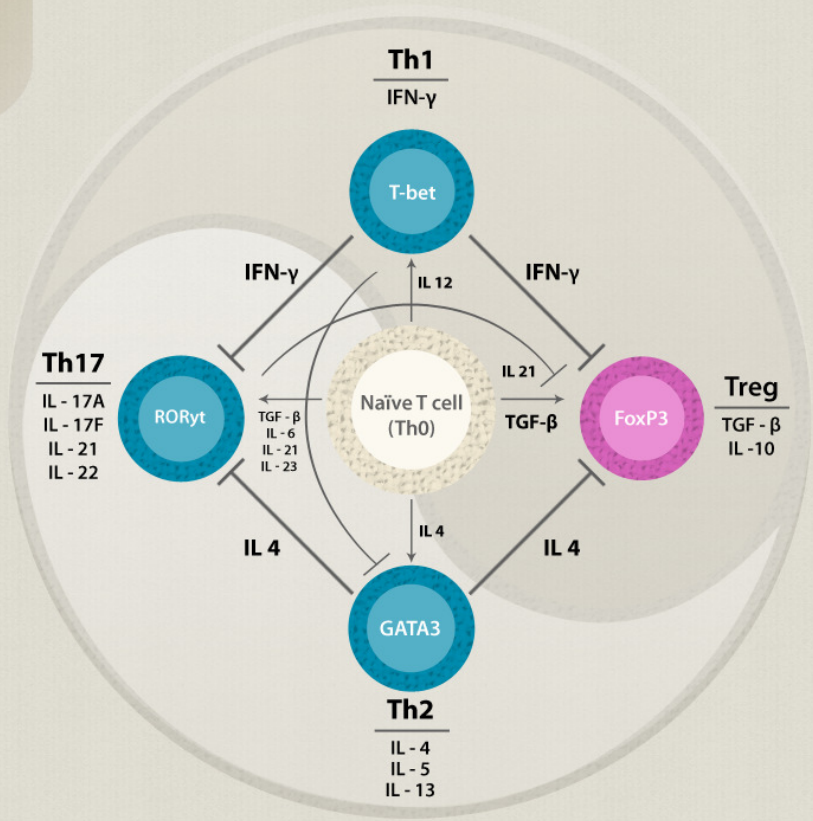
*The Ying and Yang of **Th**-cell differentiation and outcome in adaptive immunity.*

Transcription factors specific for the commited cells:

***FOXP3** - forkhead box P3*
***GATA3** - GATA-binding protein 3*
***ROR** - retinoic-acid-receptor-related orphan receptor*
***T-bet** - T box expressed in T cells*

Control of intracellurar pathogens (eg. vira, bacteria) and antitumor action.

Autoimmunity (eg. multiple sclerosis, arthritis, Crohn's disease), delayed-type hypersensitivity.



Control of extracellular pathogens (eg. fungi, bacteria).

Autoimmunity (eg. Crohn's disease, arthritis).

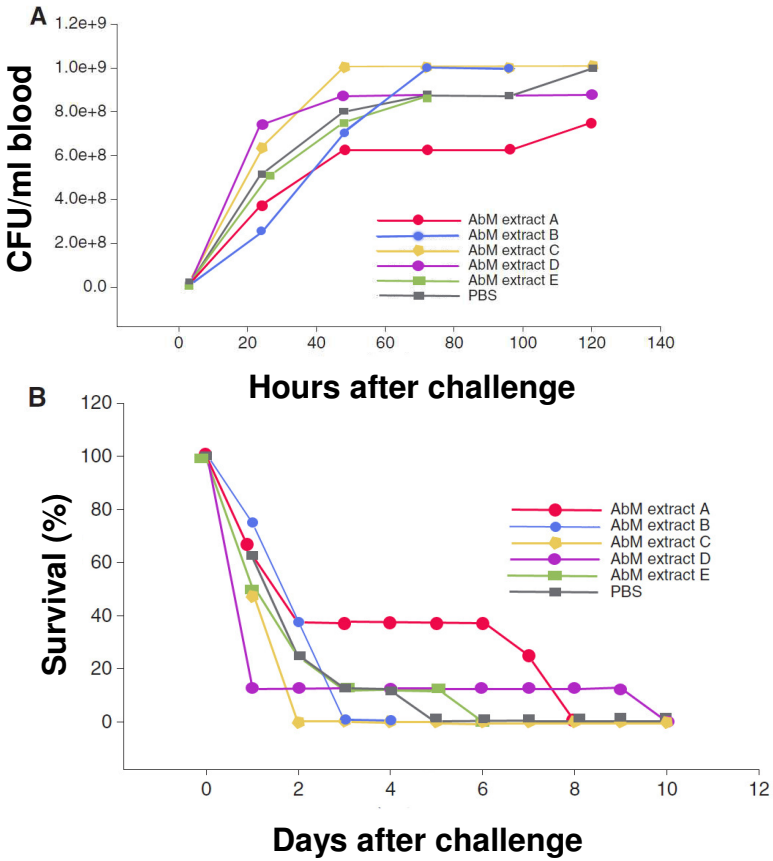
Immunosuppression, Tolerance

Control of extracellular pathogens (eg. parasites, bacteria).

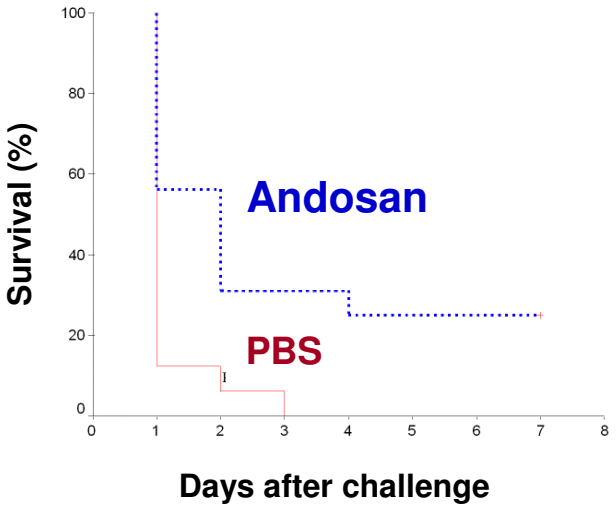
Allergy, atopy, asthma, ulcerative colitis.

Bacterial sepsis in mice – comparison of AbM extracts

Bacteremia (A) and **Survival** (B) of mice challenged with *S. pneumoniae* 2h after oral administration of different AbM extracts



Andosan/PBS p.o. 24h before faecal sepsis



Bernardshaw et al, Shock 2006

Only extract A (Andosan) gave ↓ bacteremia and ↑ survival vs PBS ($p<0.05$)

Hetland et al, Scand J Immunol 2008

Animal model for allergy :

–

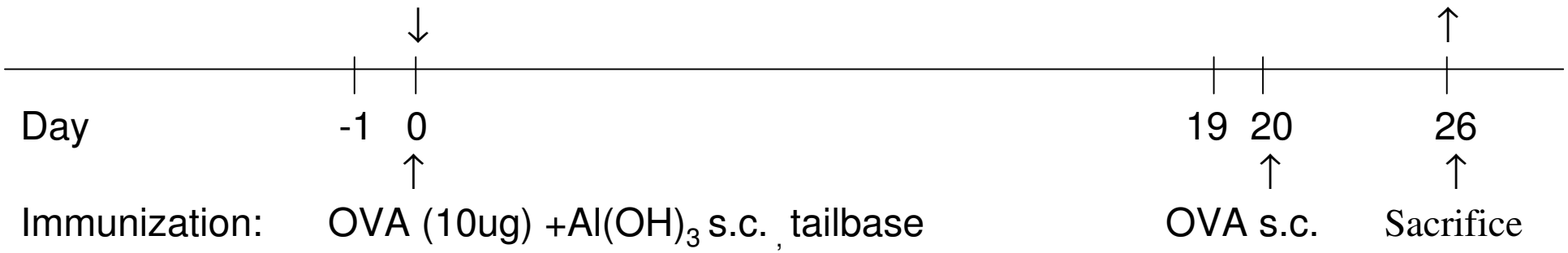
Mice sensitized with model allergen, ovalbumin

Allergy model in mice

- **AndoSan™ or PBS** given p.o. 1 day before or 3 wks after immunization s.c. with model allergen, ovalbumin
- **After 26 days: mice sacrificed**, bled for serum and spleen removed and its cells cultivated 1 day further with AndoSan or PBS
- **ELISA** analyses: **specific** anti-ovalbumin **IgE** (Th2 response) or control IgG2a (Th1 response) antibodies **in serum**, and IL-4 and IL-5 **Th2 type cytokines**, and IL-2 and IFNγ **Th1 type cytokines in spleen cell culture** supernatants

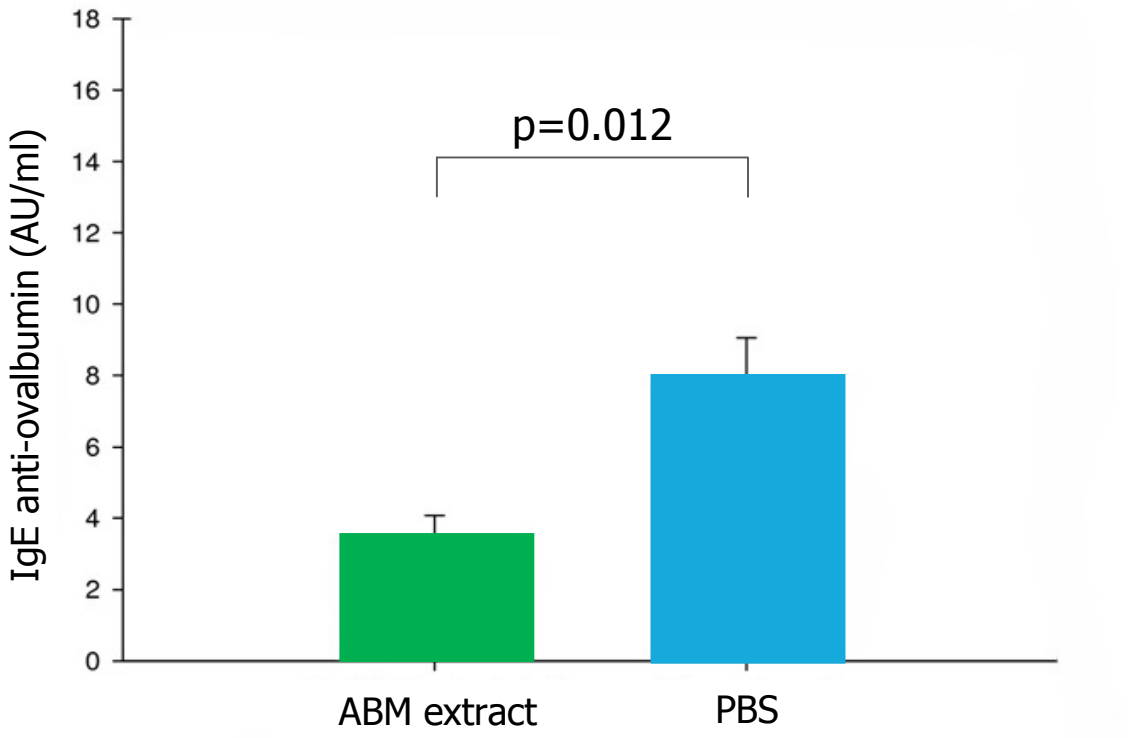
Table 1. Scheme for experimental design in murine allergy model

Exp #	Mice, strain #/group	Treatment before and/or after OVA immunization	Harvest
Exp 1:	16 NIH/Ola	AndoSan™ or PBS p.o. (200 µl)	serum,spleen
Exp 2:	16 NIH/OlaHsd	AndoSan™/PBS p.o.	serum,spleen
Exp 3:	8 C57Bl/6	AndoSan™/PBS p.o. and/or AndoSan™/PBS p.o.	serum,spleen
Exp 4:	8 Balb/c	AndoSan™/PBS s.c., foot pad (20 µl)	serum, PLN



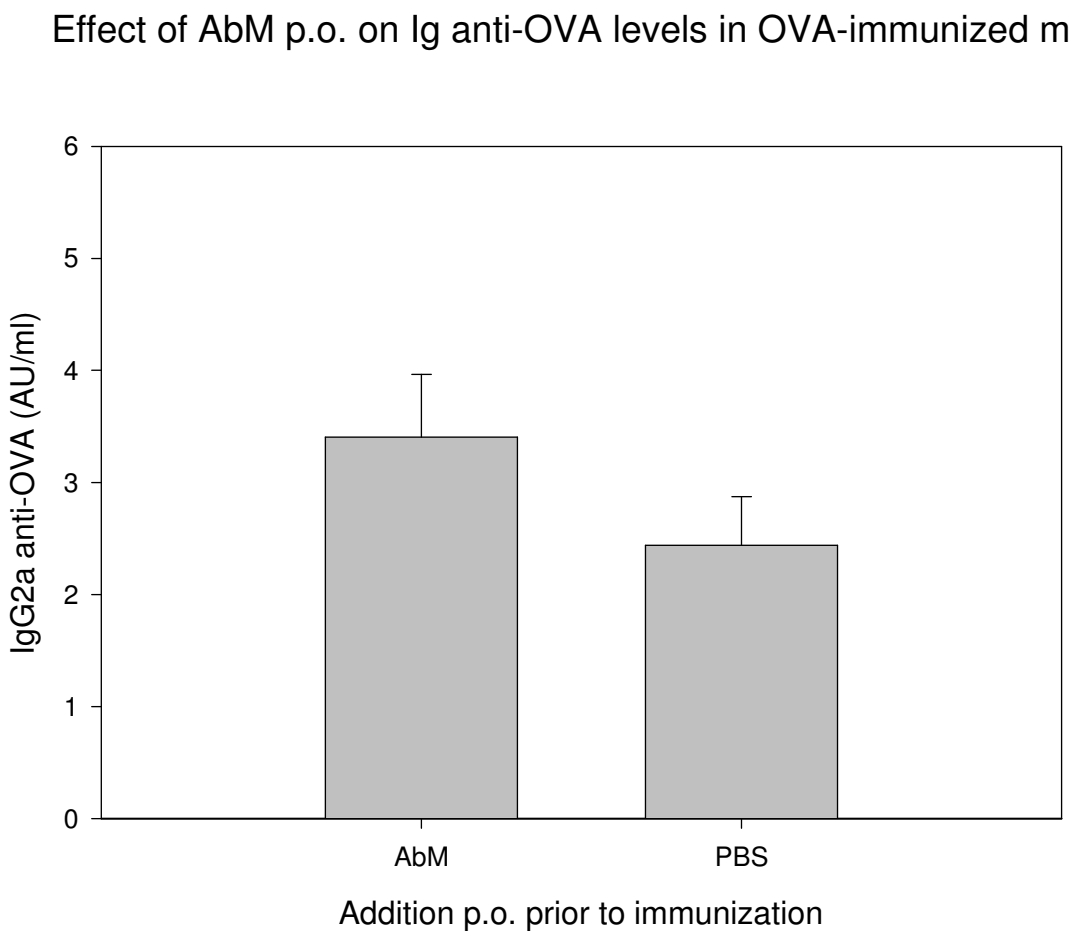
Results: Allergy I

- **AndoSan™ ABM-based extract** protects against allergy in mice
- **Less IgE antibodies** against model allergen, ovalbumin, in mice given AndoSan™ **before** ovalbumin **immunization**

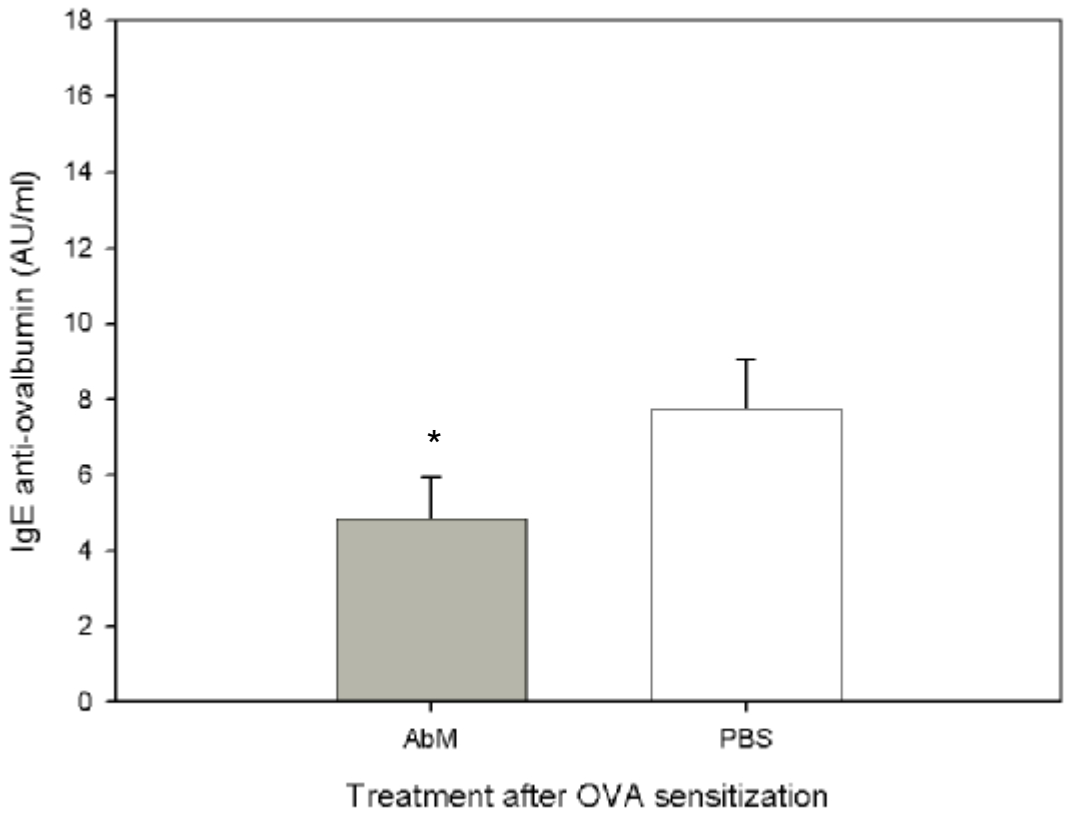


(Ellertsen & Hetland, Clin Mol Allergy 2009 May 5;7:6)

- Tendency to **more control IgG2a antibodies** against ovalbumin in mice given AndoSan™ before ovalbumin immunization

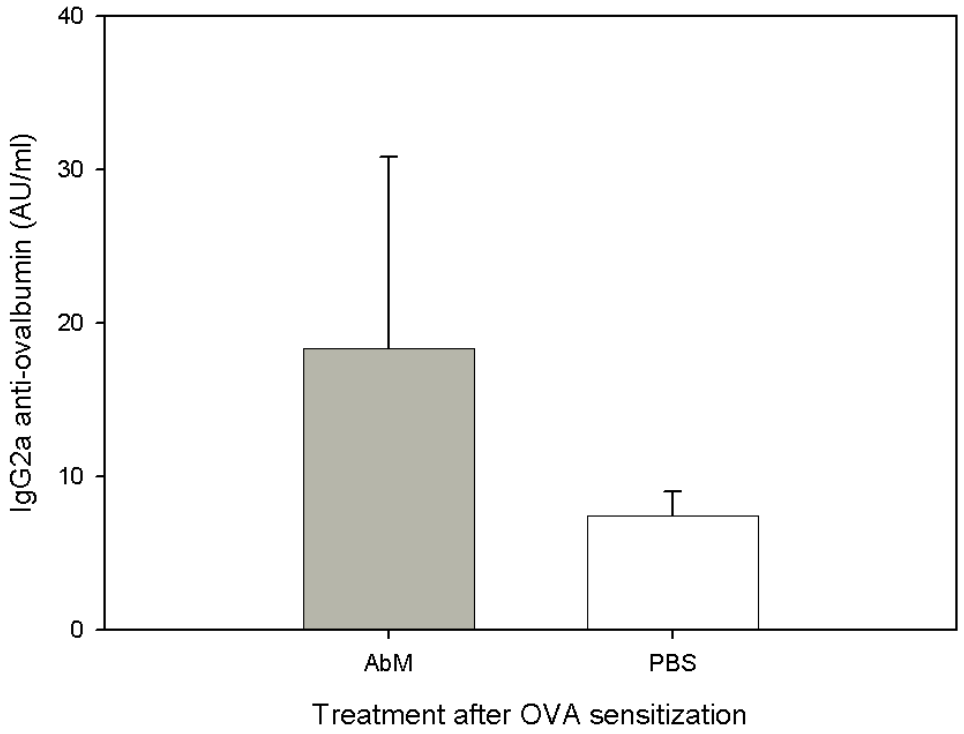


- **Less IgE antibodies** against model allergen, ovalbumin, in mice given AndoSan™ **after** ovalbumin **immunization**



*p=0.048

- Tendency to **more control IgG2a antibodies** against ovalbumin in mice given AndoSan™ also after ovalbumin immunization



Results: Allergy II

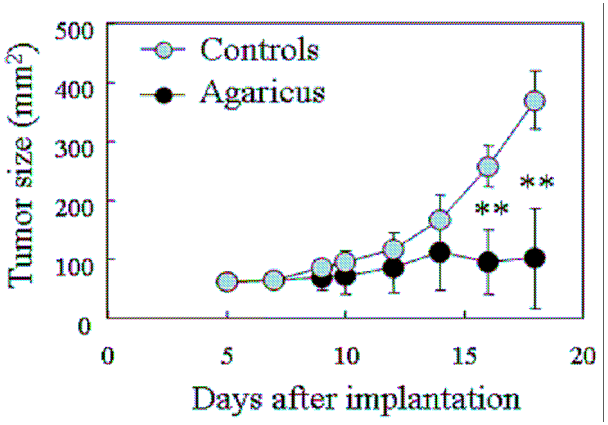
- **AndoSan effect in spleen cell cultures:**

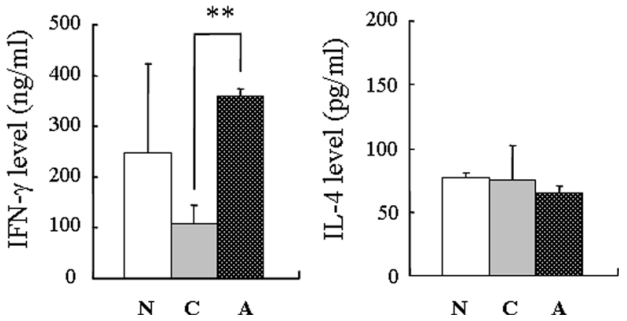
- **Less Th2 cytokines (IL-4 and IL-5) relative to Th1 cytokines (IL-2 and IFNγ) in spleen cell cultures from ovalbumin-sensitized mice that were given AndoSan™ AbM-based extract either before or after the ovalbumin immunization** ($p = 0.026$)

(Ellertsen & Hetland, Clin Mol Allergy 2009 May 5;7:6)

AbM-mediated amelioration of skewed Th1/Th2 balance also in asthma and cancer

In fibrosarcoma-bearing and asthma-induced mice by oral administration of *Agaricus blazei* extracts (Takimoto et al, Immunopharm Immunotox 2008)



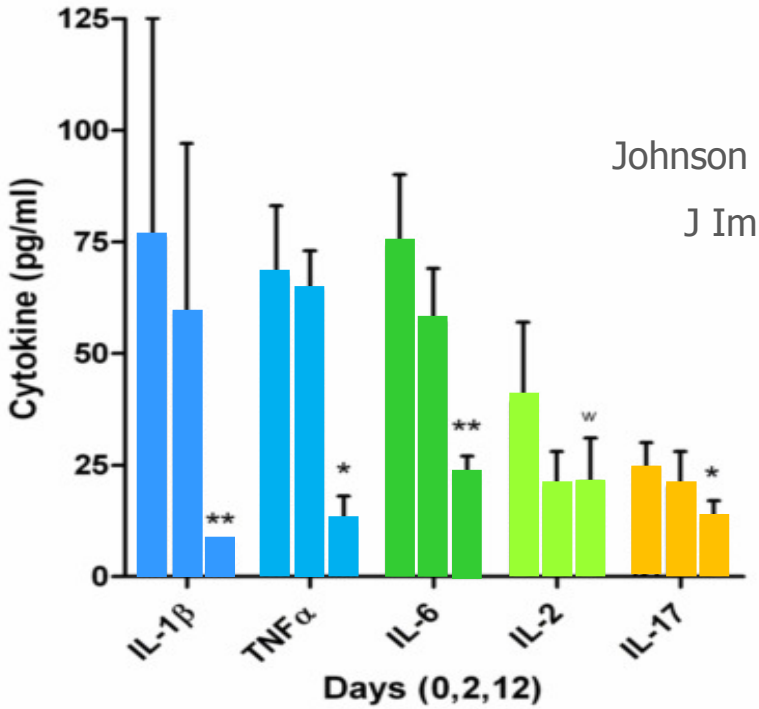


Other Anti-inflammatory effects of Andosan

Phase I study in 15 healthy volunteers who drank Andosan™ Agaricus extract for 12 days (Oslo Univ. Hospital)

- **No pathological effects** in blood tests for liver, pancreas or kidney function
- **Anti-inflammatory effect** on cytokines in serum (lower levels at day 12)
- **No clinical side-effects**

Clinically relevant interactions unlikely between Agaricus bM and cytochrome P-450 metabolism (Engdal & Nilsen, Phytother Res 2009)



Johnson et al, Scand J Immunol 2009

Clinical trials – inflammatory bowel disease (IBD)

Pilot study at Oslo Univ. Hospital, Ulleval:

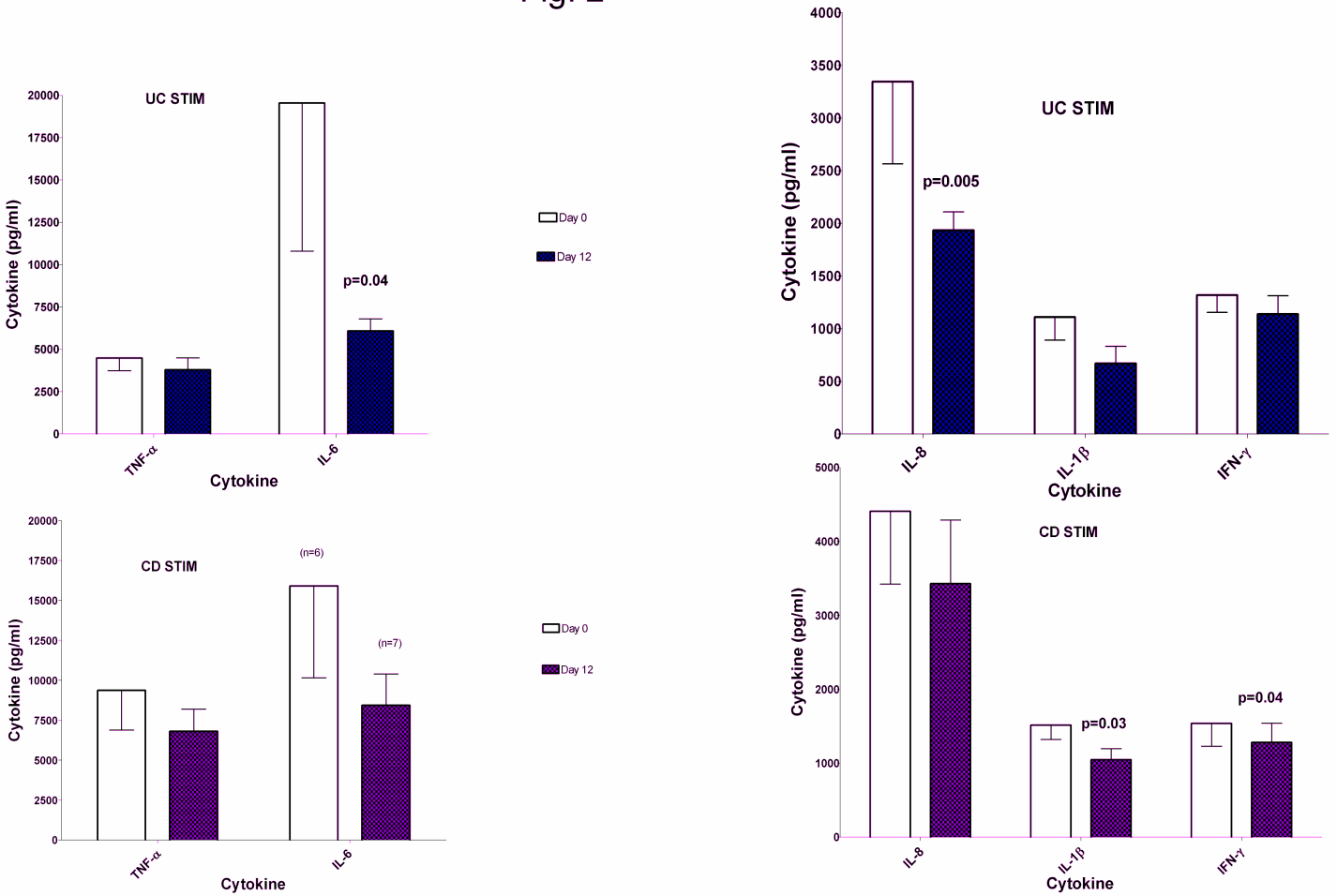
- **IBD patients:** 10 ulcerous colitis (UC)
  10 Crohn's disease (CD)
- **AndoSan™ (6 cl/d)** for 12 days at OUH: **No side effects**
- **UC:** ↓ inflammation markers in feces (calprotectin) and serum + ↓ diarrea and blood in feces (spontaneous feed back from patients)
- **DC:** ↓ inflammation markers in serum and ↓ symptoms from joint

(Førland et al, Scand J Immunol, 2011)

- **Placebo-controlled, double-blinded trial** in 100 IBD patients (50 UC and 50 DC), at OUH, Ulleval 2014 (Therkelsen/Johnson), preliminary results: Less clinical symptoms in Andosan groups

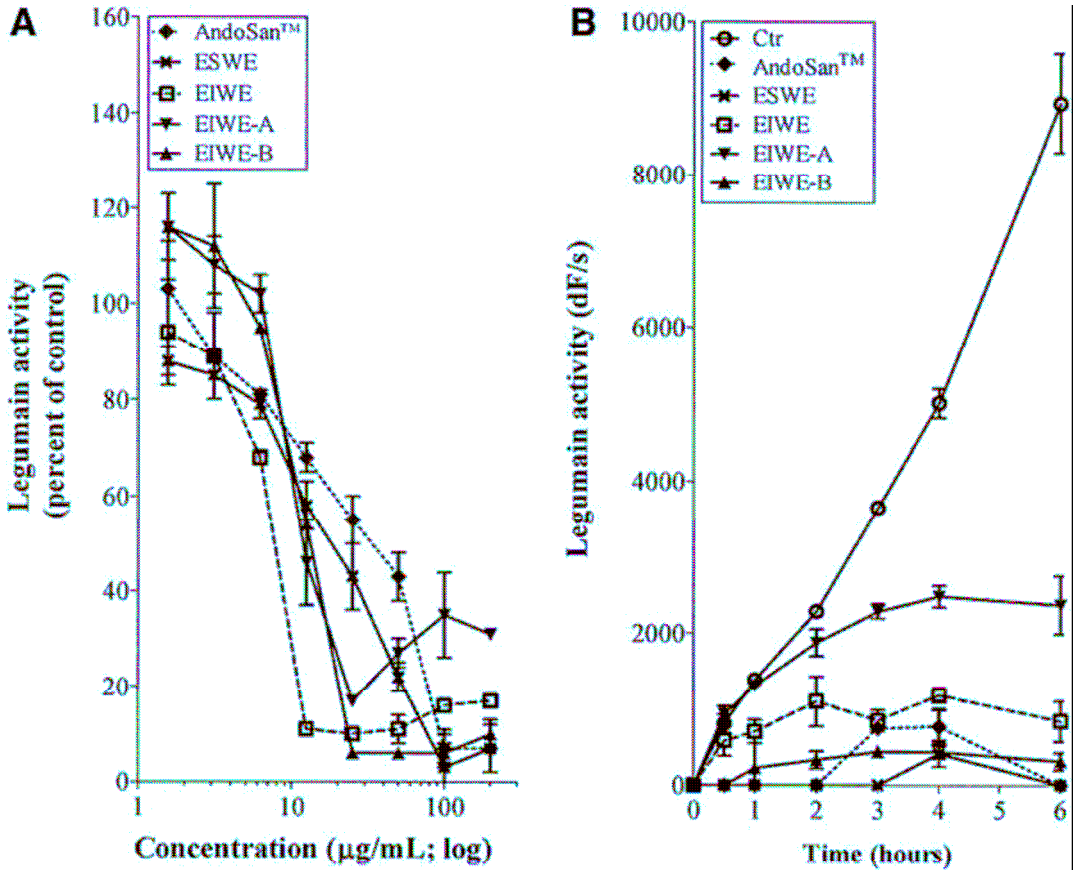
Decline in pro-inflammatory cytokines IL-1β, IL-6, IL-8, TNFα, and in IFNγ, from day 0 to day 12 in LPS stimulated blood ex vivo from UC and CD-patients

Fig. 2



(Førland et al, Scand J Immunol, 2011)

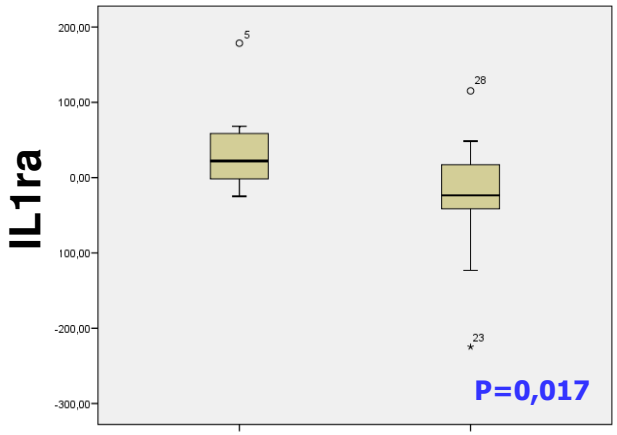
Andosan reduces activity of tumor-associated proinflammatory protease, Legumain, in Mø

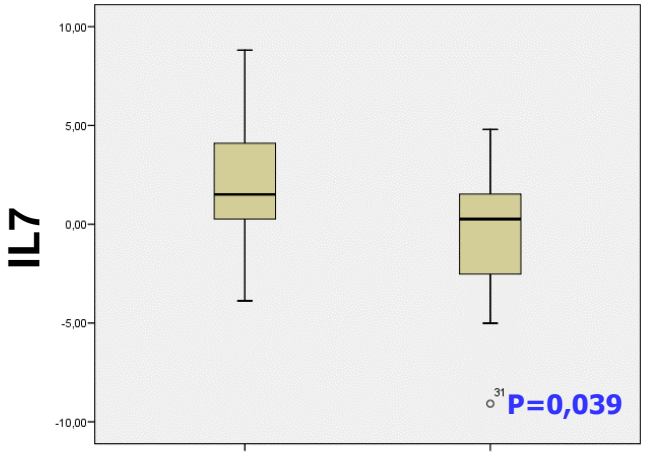


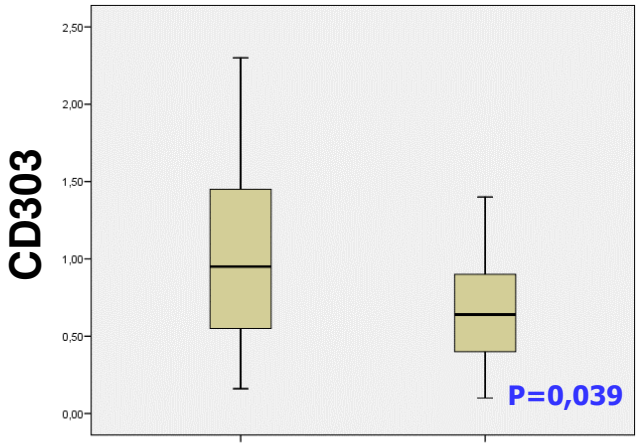
ESWE etc are isolated fractions of Andosan

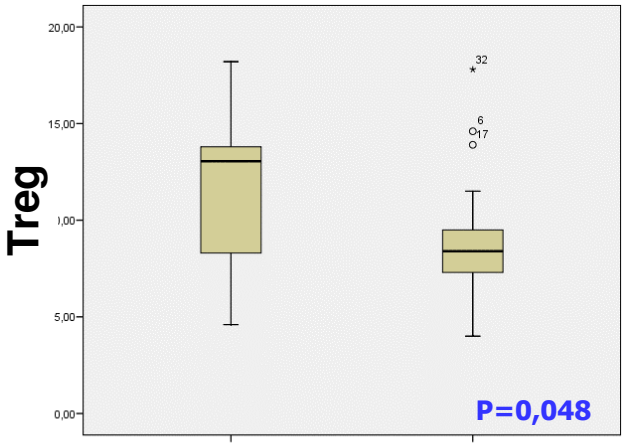
Berven et al,
J Med Food 2014

Andosan effect on plasma Cytokines, pDC and Tregs ($CD4^+$,$CD127^{dim}$, $CD25^+$) in HSC product









BioPlex hu 27-plex group I assay from BioRad

Multiple myeloma patients, Tangen submitted

Conclusions

- The *Agaricus* bM mushroom extract, Andosan™, has protective effect against allergy in a mouse model
- **Andosan™** may also have **therapeutic effect on established allergy**
- The mushroom extract has **anti-inflammatory effect in patients** with IBD or multiple myeloma

Collaborators on Andosan studies

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Rafal Biedron

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Meiji Co., Gifu-ken

Sponsor: ImmunoPharma AS, Oslo,
- producer of Andosan

Thanks!

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