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Mushroom immunomodulation and germplasm: variation between and within mushroom species and perspectives for application.

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ABSTRACT

The fact that mushrooms modulate the immune system is well documented. Few studies compared the efficacy of different mushrooms species to modulate the immune system, whereas, an intraspecies comparison of strains has hardly been described. Our **aim** was to make this comparison between seven species and within the species *Agaricus bisporus*. The *in vitro* effect on dendritic cells (DCs) as key antigen presenting cells and on cocultures of DCs and T cells was assessed. Thereto, freeze dried pulverized mushrooms were added to *ex vivo* DCs and DC-T cell cocultures. In addition the effect of five *A. bisporus* strains on the development of allergy was assessed *in* vivo. Mice were fed a diet containing 5% freeze dried *A. bisporus* followed by a sensitization and finally a challenge with egg allergen. In both experiments the effect of cytokine secretion by immune cells was measured. In the *in vivo* experiment the acute allergen-specific skin response was measured as well as serum IgE and mast cell protease. The **results** revealed a large difference in cytokine secretion by immune cells as induced by different mushrooms species. Similar results were obtained when comparing different strains of *A. bisporus*. In the *in vivo* experiment it was shown that some *A. bisporus* strains alleviated the onset of egg allergy. This was shown using the acute allergen-specific skin response as well as through the measurement of various immune parameters using isolated immune cells. Taken together it was **concluded** that different mushroom species as well as different strains of one species differentially modulate the immune system. Some *A. bisporus* strains are promising candidates to alleviate the onset of allergy.

Keywords: immunomodulation, *Agaricus bisporus*

INTRODUCTION

The incidence of hypersensitive disorders, i.e. allergies, autoimmune disorders and chronic inflammations, in industrialized countries has dramatically increased over the past decades. Currently 40-50% of the population suffers from at least one of these disorders varying from a mild hay fever to a chronic inflammatory bowel disorder requiring hospitalization. Mushrooms have been shown to modulate the immune system and to remedy infectious diseases, cancer, allergies, autoimmune and inflammatory disorders (Chan, Chan et al. 2009,

Rop, Mlcek et al. 2009). The role of mushroom polysaccharides, notably β-glucans, as immunomodulators has been well documented although other macromolecules and low molecular weight compounds may also play a role (Borchers, Krishnamurthy et al. 2008). In immunomodulation, mushroom macromolecules function as pathogen associated molecular patterns (PAMPs) to be recognized by pattern recognition receptors (PRRs). The PRRs reside on innate immune cells that interact with cells of the adaptive immune system. Although edible mushrooms are non-invasive fungi, mushroom PAMPs are recognized by dendritic cells (DCs), notably in the gut, and may be activated and mature. The mature DCs may stimulate the adaptive immune system through interaction with T cells. Although, stimulation of DCs by mushroom compounds has been described, little comparative data do exist on the immunomodulatory capacity of different mushrooms species or of varieties of the same mushroom species. Here we describe the effect of seven mushroom species on DCs alone and in co-culture with T-cells. Because genetic diversity within a species may also lead to differences in immune modulation we also compared the effect of 16 *Agaricus bisporus* strains on DCs and in DC-T cell cocultures. Five strains were chosen and tested *in vivo* to assess their effect on the development of egg allergy in a mouse model.

Results & Discussion

To assess the effect of mushrooms on dendritic cells (DCs) and on DCs cocultured with T-cells we lyophilized mushrooms and ground them to a fine powder. The powder was used as such or was extracted in boiling water with a subsequent precipitation of the extract with alcohol. Next, 100 µl (500 µg/ml) of the pulverized mushrooms were added to 100 µl murine DCs (1-5x10^5 cells/ml). After 24 hrs the cell culture medium was harvested and cytokines measured or T cells (5x10^5 cells/ml) were added. In the latter case the cells were cultured for another seven days where after cell culture medium was harvested and cytokines measured. In figures 1 and 2 the results of these experiments are displayed. It was revealed that secretion of tumor necrosis factor α (TNF-α) a proinflammatory cytokine was induced by all mushroom species and all strains of *Agaricus bisporus* (figures 1A and 2A). However, the level to which TNF-α was secreted differed between mushroom species and strains. Also, the complete homogenates (CH) and alcohol precipitates (AP) differed in the levels to which they induced TNF-α secretion. Alcohol precipitation usually leads to an enrichment of the β-glucan fraction. Although β-glucans are appreciated for their immunomodulatory properties, the combination with other compounds present in CH preparations may still induce stronger cytokine responses as is seen for several mushroom species. Similar results were observed for interleukin (IL)-6 and IL-12 although the response for most mushroom species lagged behind that of *Agaricus subrufescence* and *Grifola frondosa* (figures 1C and D). The anti-inflammatory cytokine IL-10 was only induced by *Agaricus subrufescence, Grifola frondosa* and, to a minor extent, by *Pleurotus ostreatus* (Figure 1C). For the *A. bisporus* strains a differential pattern of cytokine secretion was observed for TNF-α, IL-6 and -10 (Figure 2A-C).

In the DC-T cell coculture experiments it was observed that only the AP and CH preparations of *A. subrufescence* induced both interferon (IFN)-γ and IL-17A. The CH of three other mushrooms did induce the secretion of IFN-γ albeit to a low level (figures 1 E and F). For the

A. bisporus strains it was observed that six of the 16 strains stimulated the secretion of both IFN-γ and IL-17A. The cytokines IFN-γ and IL-17A are the signature cytokines for respectively a T helper (Th)1 and Th17 response. A Th1 response is usually induced upon an infection with intracellular pathogens (viruses, bacteria) whereas a Th17 is induced by extracellular pathogens (bacteria, fungi). However, this is never a black and white situation and often both responses are to some extent simultaneously induced.

The induction of Th1 and Th17 responses by six *A. bisporus* strains urged us to investigate their effect on the development of allergy *in vivo*. Thereto five strains were selected, two good, two medium and one none inducer of IFN-γ/IL17A in the *in vitro* experiment. Mice were fed a diet containing 5% freeze dried *A. bisporus* for two weeks followed by a sensitization and finally a challenge with egg allergen. The effect on cytokine secretion by immune cells ,the acute allergen-specific skin response , as well as serum IgE and mast cell protease were assessed. Two of the five selected *A. bisporus* strains alleviated the onset of egg allergy revealed by the skin response. IL-10 secretion by immune cells from the sensitized mice corroborated these results.

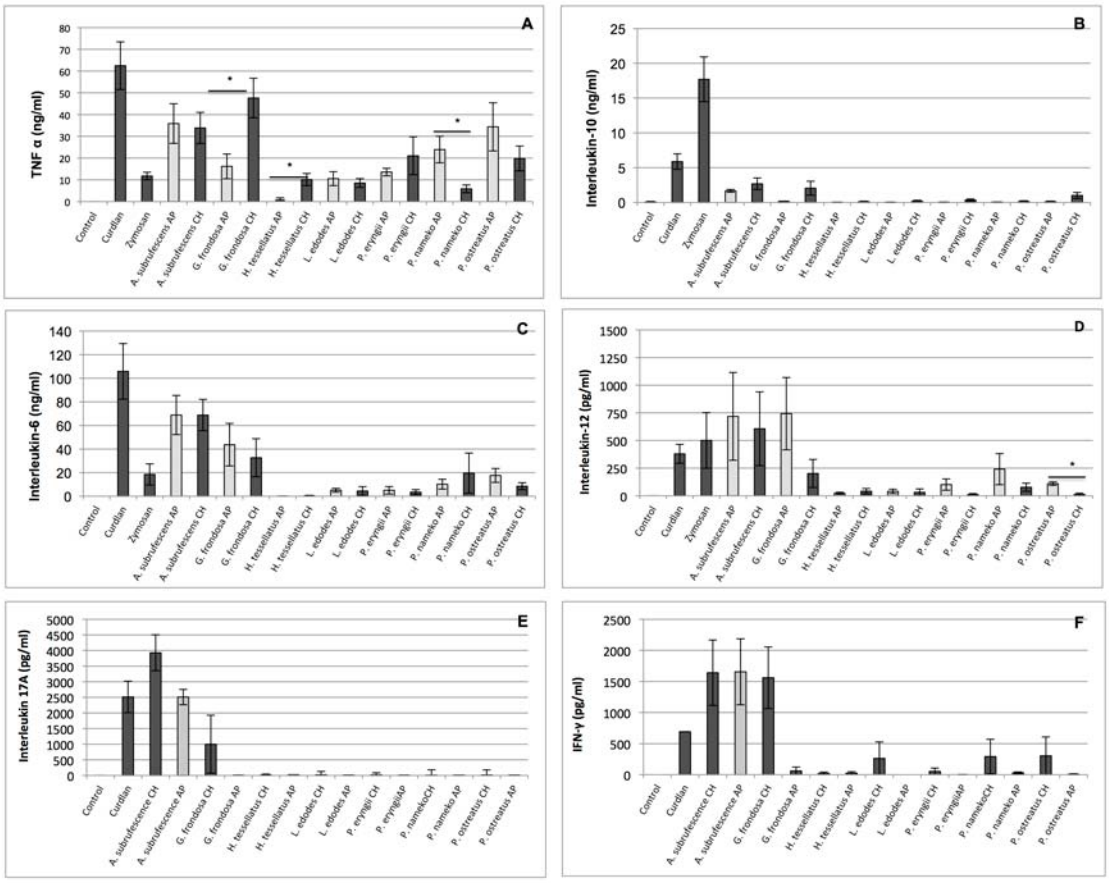

Figure 1. The effect of seven mushroom species on dendritic cells (DCs) (panels A-D) and DC-T cell cocultures (panels E &F). Both complete homogenates (CH) as well as alcohol precipitates (AP) comprising predominantly the β-glucan fraction were evaluated. **A-D**. The results obtained for the cytokines TNF-α, interleukin (IL)-10, IL-6 and IL-12. **E&F**. The results obtained for the cytokines IL-17A and interferon-γ.

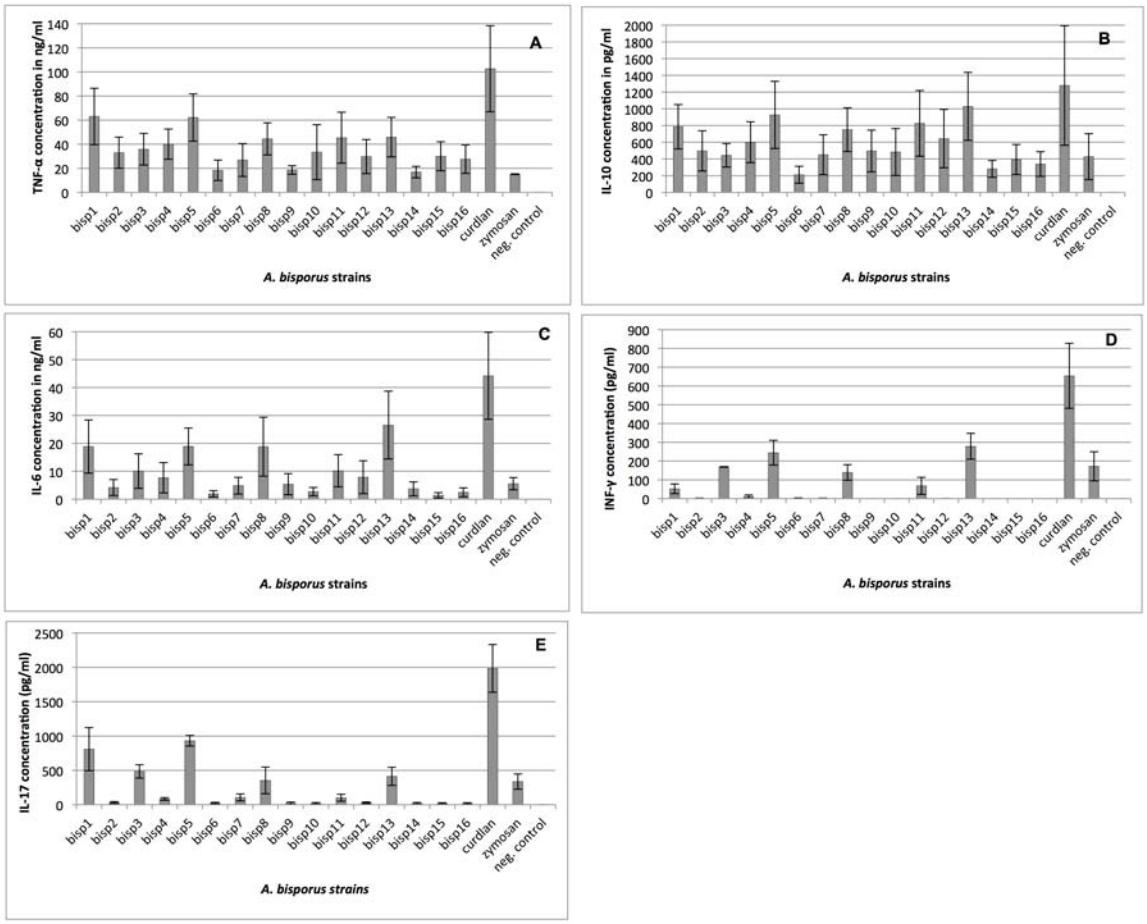

Figure 2. The effect of 16 strains of Agaricus bisporus on dendritic cells (DCs) (panels A-C) and DC-T cell cocultures (panels D & E). Complete homogenates of lyophilized mushrooms were added to DCs and DC-T cell cocultures. The effect on the secretion of TNF-α, IL-6 and IL-10 by DCs (panels A-C) and IFN-γ and IL-17 by T cells (panels D and E) is shown.

In conclusion, both different mushrooms species as well as strains from one species differentially modulate the immune system. In a mouse egg allergy model it was demonstrated that some *A. bisporus* strains are promising candidates to alleviate the onset of allergy.

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