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RESEARCH ARTICLE

The stimulatory effect of the TLR4-mediated adjuvant glucopyranosyl lipid A is well preserved in old age

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Abstract Many subunit vaccines require adjuvants to improve their limited immunogenicity. Various adjuvant candidates targeting toll-like receptors (TLRs) are currently under development including the synthetic TLR4 agonist glucopyranosyl lipid A (GLA). GLA has been investigated in the context of influenza vaccine, which is of particular importance for the elderly population. This study investigates the effect of GLA on antigen-presenting cells from young (median age 29 years, range 26–33 years) and older (median age 72 years, range 61–78 years) adults. Treatment with GLA efficiently increases the expression of co-stimulatory molecules on human monocyte-derived dendritic cells (DC) as well as on ex vivo myeloid DC. Expression of co-stimulatory molecules is less pronounced on ex vivo monocytes. Production of pro-inflammatory cytokines (IL-6, TNF-α, IL-12) as well as of the anti-inflammatory cytokine IL-10 is induced in monocyte-derived DC. In PBMC cultures myeloid DC and to an even greater extent monocytes produce TNF-α and IL-6 after stimulation with GLA. Production of IL-12 can also be observed in these cultures. There are no age-related differences in the capacity of GLA to induce expression of co-stimulatory molecules or production of cytokines by human antigen-presenting cells. Therefore, TLR4 agonists like GLA are particularly promising candidates as adjuvants of vaccines designed for elderly individuals.

Keywords TLR 4 · Adjuvant · Antigen-presenting cells · Ageing · Elderly

Introduction

Many subunit vaccines require adjuvants to improve their limited immunogenicity, as they frequently lack microbial structures that act as natural immune stimulators, which are present in killed whole-organism vaccines and live-attenuated vaccines. Whereas subunit or protein vaccines adjuvanted with aluminum salts potently induce Th_2-biased and antibody responses (Grun and Maurer 1989; Marrack et al. 2009), other adjuvants are needed to effectively stimulate Th_1 and $CD8^+$ T cell responses, which are of particular importance for pathogens that require not only humoral, but also cellular protection.

Until recently the only adjuvants available for human use were aluminum phosphate and aluminum hydroxide. The oil-in-water emulsion MF59, consisting of squalene, Tween80 and Span 85 (Ott et al. 1995) was licensed in 1997 for use with seasonal influenza vaccines in Europe. Over the last few years several

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other adjuvants such as AS03 (oil-in water emulsion containing squalene, Tween80 and α-tocopherol) (Walker and Faust 2010), AS04 (aluminum hydroxide and monophosphoryl lipid A) (Thoelen et al. 1998; Alderson et al. 2006), as well as virosomal formulations of antigens (Huckriede et al. 2005) have been introduced in combination with various antigens, such as seasonal and pandemic influenza, hepatitis A and B and human papilloma virus (HPV). Many more adjuvants are currently investigated in clinical trials. One class of adjuvants that has been studied extensively are Toll-like receptor (TLR) agonists. In humans 10 TLRs have been described, which are localized at the cell membrane (TLR1, 2, 4, 5, 6, 10) or intracellularly (TLR3, 7, 8, 9). TLRs recognize different ligands derived from bacteria, fungi, viruses or damaged host cells [reviewed in (Lee et al. 2012; Lim and Staudt 2013)]. One of the TLRs targeted by novel adjuvants is TLR4, which recognizes lipopolysaccharide (LPS) of gram-negative bacteria. The licensed adjuvant AS04 contains monophosphoryl lipid A (MPL), which is derived from LPS but shows markedly reduced endotoxicity (Cluff et al. 2005). MPL has been shown to successfully enhance murine and human T cell responses against viruses and bacteria (Masihi et al. 1986; Persing et al. 2002) and to induce predominantly Th1 responses (Alderson et al. 2006). The synthetic TLR4 agonist glucopyranosyl lipid A (GLA) efficiently binds the human myeloid differentiation factor 2 (MD-2) molecule, which is part of the TLR4-complex (Coler et al. 2011). MPL, which is naturally derived from LPS of *Salmonella minnesota*, contains multiple structurally distinct pharmacophores (Alderson et al. 2006) with four to seven acyl chains. In contrast, GLA is a synthetic lipid A mimetic, which consists of a single, defined, hexa-acylated molecule (Coler et al. 2011). Several studies have tested GLA as an adjuvant in human and murine vaccination and found GLA to be effective in inducing immunity to the adjuvanted antigen by stimulating DC (Coler et al. 2010, 2011; Arias et al. 2012; Pantel et al. 2012; Schneider et al. 2012). One of the antigens tested in combination with GLA is influenza. As the burden of disease is particularly high in the elderly, for whom existing vaccines show unsatisfactory efficacy, improved vaccines, e.g. by adjuvantation are highly desirable. It has recently been shown that GLA

enhances T cell responses to influenza vaccine in old adults (Behzad et al. 2012).

With increasing age the innate and adaptive immune system undergoes characteristic changes, termed immunosenescence (Weinberger et al. 2008). For the TLR4 agonist GLA, age-related changes in innate immunity are of particular relevance. In mice, aged macrophages were found to express lower levels of TLRs 1–9 and to secrete less IL-6 and TNF-α upon TLR stimulation (Renshaw et al. 2002). In mouse and man the activation of monocytes/macrophages as well as their capacity to present antigen and stimulate T cells seems to be impaired (McLachlan et al. 1995; Pawelec 1999; Castle 2000; Herrero et al. 2002; Plowden et al. 2004). Age-related changes of DC are discussed controversially reporting increased, unchanged, or decreased function with age [reviewed in (Wong and Goldstein 2013)]. Comparison of different studies is difficult as various sources of DC have been used. Each DC subset is likely to be differently affected by aging and is also influenced by the local microenvironment. In addition, the health status of the blood donors might greatly influence the outcome (Castle et al. 1999; Uyemura et al. 2002). Many studies focused on monocyte-derived DC (moDC), which are a popular source of in vitro generated DC. However, there are clear differences between moDC and DC that are analyzed directly ex vivo.

The aim of this study was to characterize the effect of GLA on human antigen-presenting cells. Cytokine-production and expression of maturation markers was evaluated ex vivo in monocytes and myeloid DC (mDC) as well as in moDC from young and older adults in order to evaluate the suitability of GLA as an adjuvant in vaccines for the elderly.

Materials and methods

Blood collection

Venous blood was collected from healthy young (median age 29 years, range 26–33 years) and elderly (median age 72 years, range 61–78 years) volunteers. The study was approved by the ethics committee of the Innsbruck Medical University and all participants

gave their written informed consent. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Ficoll–Hypaque (GE Health Care, Germany).

Generation of moDC

moDC were generated by seeding 0.67×10^6 PBMC per square centimeter into cell culture flasks. After 2 h at 37 °C the supernatant was removed and adherent cells were washed once with RPMI 1640 (Lonza, Belgium) containing 1 % kanamycin (Gibco, USA). Cells were cultured for 7 days in RPMI 1640 supplemented with 1 % kanamycin and 10 % fetal calf serum (Sigma-Aldrich, USA) in the presence of 500 IU/ml IL-4 and 800 IU/ml granulocyte macrophage colony-stimulating factor (GM-CSF) (both ImmunoTools, Germany). Cytokines were re-supplemented after 4 days.

Stimulation with GLA

GLA (molecular weight 1763.5) was prepared as aqueous suspension as previously described (Anderson et al. 2010). moDC or PBMC were seeded in 24-well plates at a density of 0.5×10^6 moDC/well or in six-well plates at a density of 4×10^6 PBMC/well, respectively. Cells were cultivated in the presence of different concentrations of GLA or LPS (*Escherichia coli* serotype 026:B6; Sigma-Aldrich, USA) for 6–24 h depending on the analysis performed. For intracellular analysis of cytokines by flow cytometry 10 µg/ml Brefeldin A was added for the last 6 h of culture.

Enzyme-linked immunosorbent assays (ELISA)

Detection of Interferon-α (IFN-α), IL-1β, IL-6, IL-10, IL-12(p40) and TNF-α in the supernatants of moDC cultures was performed using commercially available ELISA-Kits (Mabtech, Sweden) following manufacturer's instructions. Protein standards for quantification were purchased from Sigma-Aldrich, USA (IL-1β, IL-6 and IL-10) or from Mabtech, Sweden (all others). Supernatants were diluted 1:1–1:15 and were measured in duplicates. For samples with concentrations below the detection limit of the assay a value of $0.5\times$ detection limit was set.

Flow cytometry

PBMC were washed with PBS and stained with anti-CD3-APC-Cy7, anti-CD11c-APC, anti-CD16-APC-Cy7, anti-CD19-APC-Cy7, anti-CD80-PE, anti-CD86-FITC, anti-HLA-DR-PerCP (all BD, USA), anti-TLR4-PE (eBiosience, USA) and anti-CD14-PE-Cy7 (Biolegend, USA) antibodies for 25 min at 4 °C. For intracellular staining cells were washed in PBS and resuspended in 300 µl Cytofix/Cytoperm Fixation and Permeabilization Solution (BD, USA). After 20 min at 4 °C cells were washed and resuspended with Perm/Wash Solution (BD, USA; diluted 1:10 in a. dest.). Antibodies for intracellular staining (anti-IL-6-PE, anti-TNF-α-FITC, both BD, USA) were added for 30 min at 4 °C and cells were washed with Perm/Wash Solution. Flow cytometric analysis was performed using a FACS Canto II cytometer and FACSDiva software (BD, USA).

Statistical analysis

Statistical analysis of the data was performed using SPSS version 11 (SPSS Inc., Chicago, US). Wilcoxon signed-rank test was used to compare stimulated versus unstimulated samples. Comparisons between age-groups were performed using Mann–Whitney U test and p values <0.05 were considered significant.

Results

GLA induces maturation of moDC

Monocytes were enriched from human PBMC and cultured in the presence of IL-4 and GM-CSF to generate moDC, which were stimulated with GLA at varying concentrations for 24 h. LPS was used as a positive control. Figure 1a shows representative examples of baseline expression of CD86, CD80 and HLA-DR on the surface of unstimulated moDC from one young and one old donor. Stimulation with TLR4 agonists induced a dose-dependent upregulation of CD86 (Fig. 1b) and CD80 (Fig. 1c), which indicates maturation of immature DC. There were no differences between young and elderly donors. In addition, there was as slight, but not significant upregulation of MHC class II molecules as measured by flow cytometric analysis of HLA-DR (data not shown).

Fig. 1 Expression of surface markers on moDC. **a** Expression of CD86, CD80 and HLA-DR on the surface of unstimulated moDC (gated on HLA-DR$^+$ CD11c$^+$ cells) for one young (*white*) and one old (*grey*) donor. **b** and **c** Mean fluorescent intensities (MFI) of CD86 (**b**) and CD80 (**c**) expression on the surface of moDCs (gated on HLA-DR+ CD11c+ cells) from young (*black circles*) and elderly (*white squares*) donors after 24 h of stimulation with GLA or LPS at the indicated concentrations. Mean values of MFI ± SEM are given in arbitrary units (AU) for young (n = 5) and elderly (n = 5). No statistically significant differences could be detected between young and elderly donors (Mann–Whitney *U* test). Significant differences (p < 0.05) between unstimulated and stimulated moDC are indicated by *asterisk* (*) for young and by # for elderly donors (Wilcoxon signed-rank test)
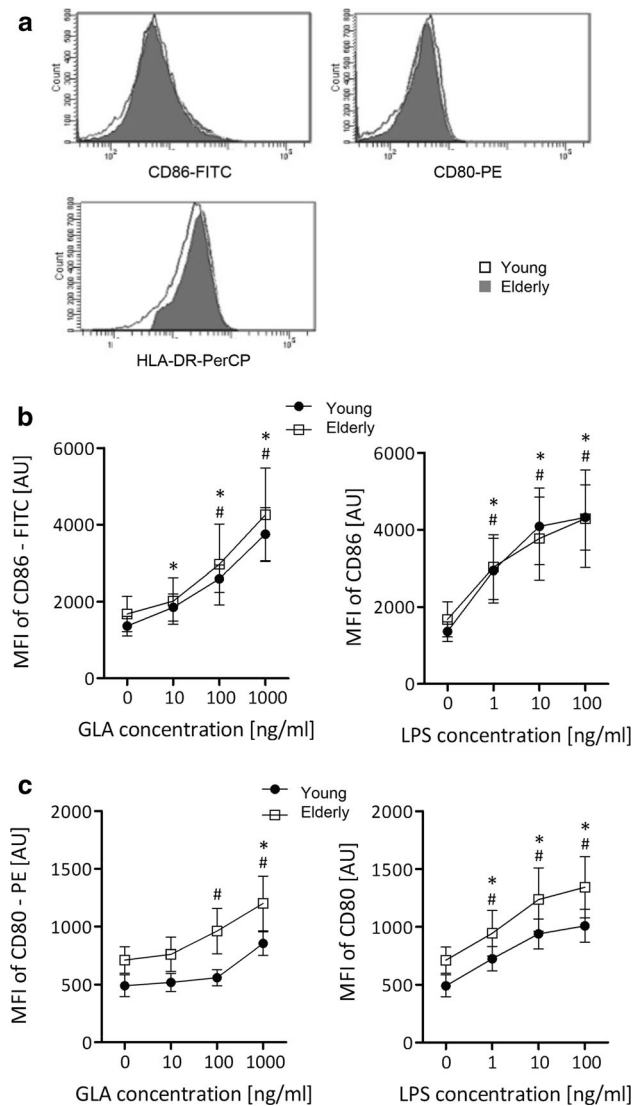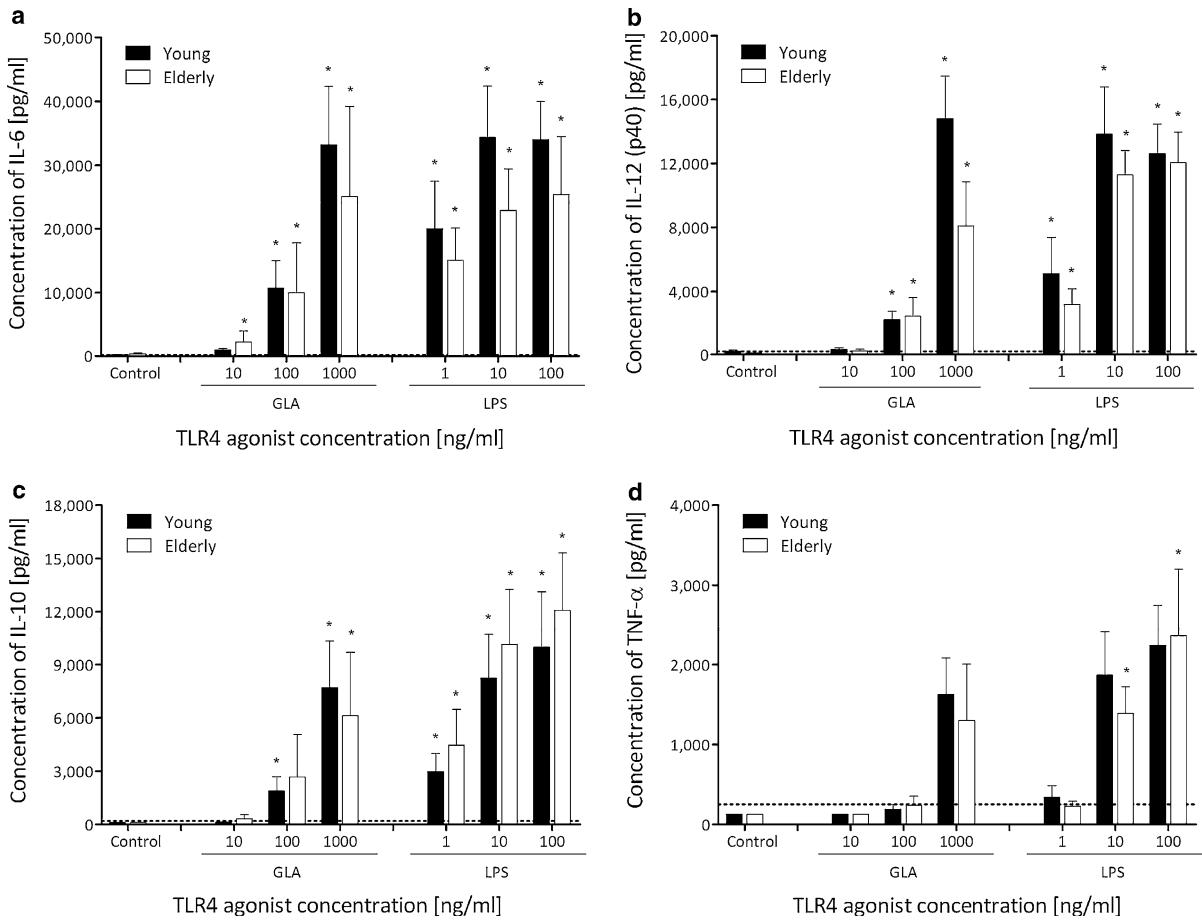

GLA induces cytokine production by moDC

In the absence of TLR4 stimulation, IL-6, IL-12p40, IL-10 and TNF-α concentrations were low in the supernatants of moDC from young as well as elderly donors. Upon treatment with GLA or LPS moDC produced large quantities of IL-6 and IL-12p40. Maximum IL-6 and IL12p40 secretion, which was seen after stimulation with 10 ng/ml LPS, was induced by 1000 ng/ml GLA (Fig. 2a, b). Secretion of TNF-α was only induced by high concentrations of GLA (Fig. 2d). In addition to pro-inflammatory cytokines stimulation of moDC with TLR4 agonists also induced the anti-inflammatory cytokine IL-10 (Fig. 2c). No differences were detected between moDC from young versus elderly donors.

GLA induces maturation of mDC ex vivo

The expression of TLR4 was analyzed on freshly isolated human PBMC. Flow cytometry was used to define mDC (CD3$^-$CD16$^-$CD19$^-$CD14$^-$HLA-DR^{+-} CD11c$^+$) and classical monocytes (CD3$^-$CD16$^-$ CD19$^-$CD14$^+$HLA-DR$^+$CD11c$^-$). TLR4 expression was significantly higher on mDC from elderly donors

Fig. 2 Cytokine production by moDC. Levels of **a** IL-6, **b** IL-12p40, **c** IL-10 and **d** TNF-α in the supernatant of moDC cultures from young (n = 5; *black bars*) and elderly (n = 5; *white bars*) donors after 24 h of stimulation with GLA or LPS at the given concentrations. *Bars* represent mean ± SEM. The detection limits of the ELISAs (250 pg/ml for TNF-α, 200 pg/ml for the other cytokines) are indicated as *dashed lines*. No statistically significant differences could be detected between young and elderly donors using Mann–Whitney *U* test. Significant differences (p < 0.05) between unstimulated and stimulated moDC are indicated by *asterisk* (*) for young and by # for elderly donors (Wilcoxon signed-rank test)

(MFI 65.5 ± 10.5) than from young donors (MFI 36.5 ± 3.4; p = 0.004), whereas no age-related differences were found for TLR4 expression on monocytes (MFI 102.5 ± 18.4 or MFI 111.7 ± 18.4, respectively). Figure 3 shows representative examples of TLR4 expression on mDC and monocytes from one young and one old donor.

PBMC were stimulated for 24 h with GLA in varying concentrations ranging from 10 to 1000 ng/ml. Monocytes as well as mDC showed a dose-dependent upregulation of the co-stimulatory molecule CD80 with the maximal expression observed for 300 ng/ml GLA. Higher concentrations of GLA did not increase the expression of CD80. In contrast, expression of CD86 was highly expressed on mDC after TLR4 stimulation with a dose-dependency similar to CD80, but was not upregulated on monocytes. Figure 4 shows results from six young and six old donors after stimulation with the optimal GLA concentration (300 ng/ml). Stimulation with 100 ng/ml LPS served as a positive control. No age-related differences were observed for either mDC or monocytes indicating that the stimulatory effects of GLA are well preserved in old age.

myeloid DC

monocytes
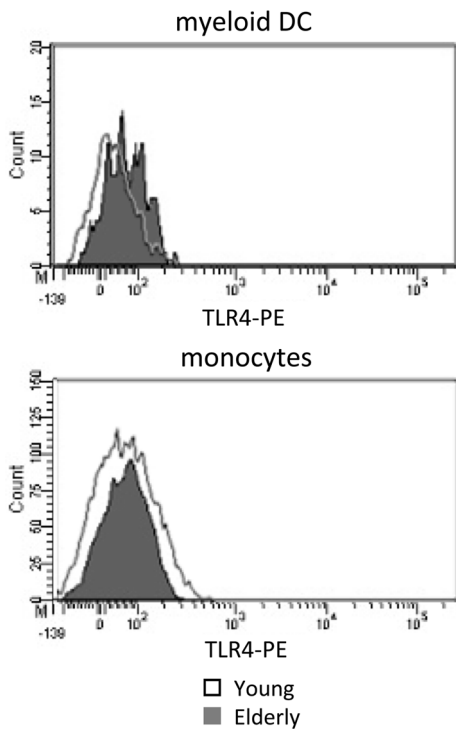
□ Young
■ Elderly

Fig. 3 Expression of TLR on antigen-presenting cells. Expression of TLR4 on the surface of unstimulated mDC and monocytes [gating strategies are shown for one young (*white*) and one old (*grey*) donor]

GLA induces cytokine production in monocytes and mDC ex vivo

After 6 h of stimulation with 300 ng/ml GLA or 100 ng/ml LPS, mDC and monocytes from young and elderly persons showed a significantly increased proportion of TNF-α-producing cells and IL-6 producing cells in comparison to respective controls (p < 0.05). Figure 5 shows percentages of mDC (Fig. 5a) and monocytes (Fig. 5b) producing either TNF-α or IL-6 alone, as well as double-positive cells producing both cytokines. No age-related differences were detected. Cytokine-production was also analyzed after 24 h of stimulation. At this time point, TNF-α production was substantially lower in mDC, whereas IL-6 production could still be observed. Monocytes had TNF-α production downregulated to baseline levels after 24 h, but also continued to produce IL-6 at that time point. Overall these differences in kinetics led to the presence of a larger number of IL-6 single-positive cells after 24 h of stimulation. Production of

IL-12 was determined in supernatants of PBMC stimulated with 300 ng/ml GLA or 100 ng/ml LPS. PBMC from young and old donors significantly upregulated IL-12 production after stimulation with TLR4 agonists. Interestingly, IL-12 concentrations were very heterogeneous within the older age group. Whereas IL-12 concentrations were similar to the levels observed with young adults for four elderly donors, two older individuals showed substantially higher IL-12 concentrations.

Discussion

TLR agonists are widely explored as novel adjuvants in combination with various antigens. The MPL-containing adjuvant AS04, which targets TLR4, is licensed and used in combination with Hepatitis B virus and HPV antigens and has been shown to successfully enhance T cell responses. The current study focused on the TLR4-mediated adjuvant candidate GLA, which has been shown to enhance the immunogenicity of co-administered antigens in mice and non-human primates (Coler et al. 2010, 2011; Arias et al. 2012) without causing adverse side effects (Coler et al. 2010). The compound is able to enhance the capacity of murine and human DC (Coler et al. 2011; Schneider et al. 2012). In mice, GLA induced a Th$_1$-polarized immune response in vivo (Pantel et al. 2012). GLA has been successfully tested in the context of influenza vaccination (Coler et al. 2010; Behzad et al. 2012), which is of particular importance for the elderly. Therefore, this study aimed to investigate the stimulatory capacity of GLA on innate immune cells from aged individuals. The impact of age on human innate immune cells, particularly on DC has been controversially discussed. It has been demonstrated that there are differences in DC function depending on the source of DC i.e., blood-derived versus moDC (Osugi et al. 2002; Radford et al. 2006; Robbins et al. 2008) and the mode of cell preparation (Jiang et al. 2009). Therefore, the effect of GLA on human moDC, blood-derived monocytes and blood-derived mDC was analyzed in order to address the question whether professional antigen-presenting cells from elderly persons are functionally comparable to cells from young persons after stimulation with TLR4 agonists.

Expression of co-stimulatory molecules, such as CD80 and CD86, by antigen-presenting cells is a
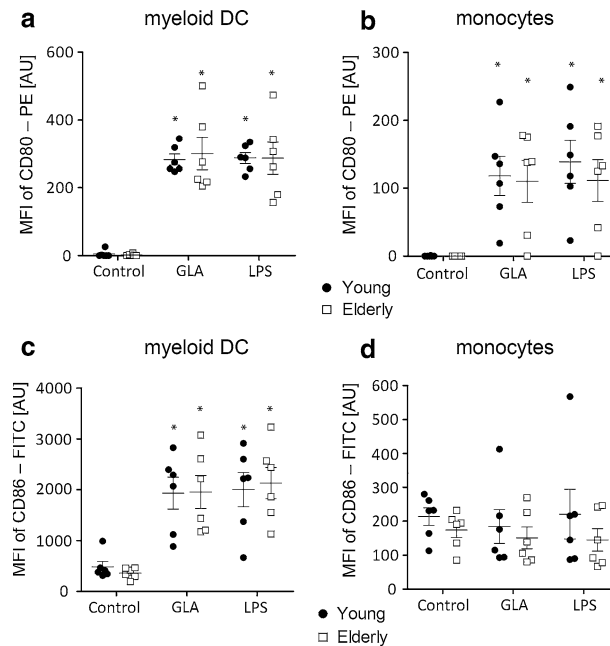
Fig. 4 Expression of surface markers on mDC and monocytes. Mean fluorescence intensities (MFIs) of CD80 and CD86 on mDC and on monocytes from young and elderly donors after 24 h of stimulation with TLR4 agonists. PBMCs of six young (*black*) and six elderly (*white*) donors were cultured in the presence of medium (control), 300 ng/ml GLA or 100 ng/ml LPS. Expression of CD80 (**a**) and CD86 (**c**) on mDC from both young and elderly donors is shown as assessed by flow cytometry. MFI expression levels of CD80 (**b**) and CD86 (**d**) on monocytes from young and elderly donors. No age-related differences in expression of CD86 and CD80 on mDC and monocytes were observed (Mann–Whitney U test). *Asterisk* (*) indicates p value of <0.05 comparing the expression of either CD80 or CD86 on mDC or monocytes stimulated with a TLR4 agonist to the respective control (Wilcoxon signed-rank test). MFI values are given in arbitrary units (AU) as mean ± SEM

prerequisite for efficient T cell activation. Unstimulated monocyte-derived and blood-derived DC have been shown to progressively up-regulate CD80 and CD86 in culture (Lung et al. 2000; Ho et al. 2002). Indeed, moDC did readily express CD80 and CD86 after 7 days of in vitro differentiation in the absence of TLR4 agonists (Fig. 1b, c, controls). In contrast, unstimulated monocytes and mDC were found to express CD86, but not CD80 ex vivo (Fig. 4, control), which has also been reported for freshly isolated mDC (Ju et al. 2010). After stimulation with at least 100 ng/ml GLA or 10 ng/ml LPS, the expression of both CD86 and CD80 was significantly increased on the surfaces of moDC and mDC from young and elderly donors (Figs. 1, 4) without significant differences between the two age groups. Our data are in concordance with previous findings showing intact responses of moDC from old donors to different stimuli (Saurwein-Teissl et al. 1998, 2000; Lung et al. 2000) and of murine conventional DC stimulated with LPS

(Pereira et al. 2011; Tan et al. 2012). While Lim et al. (Lim et al. 2002) found a down-regulation of CD86 on human monocytes after LPS stimulation in vitro, CD86 expression on monocytes from young and elderly donors was not affected by TLR4 stimulation in this study (Fig. 4a, b). Monocytes from both age groups significantly up-regulated CD80 expression after 24 h in response to GLA or LPS, but no differences between age groups were observed. Up-regulation of CD80 on human monocytes from young donors after TLR stimulation has been reported previously (van Duin et al. 2007). In contrast to our results the up-regulation of CD80 was decreased on elderly monocytes stimulated with various TLR ligands, including 1000 ng/ml LPS in this study. Expression of CD80 and CD86 was generally higher in mDC and moDC than in monocytes, both with or without TLR stimulation, highlighting the importance of DC in providing efficient co-stimulation for T cells. However, neither baseline expression nor

a

b

Young Elderly
■ □ TNF-α/IL-6 double positive
▨ ▨ TNF-α single positive
▨ ▨ IL-6 single positive

c
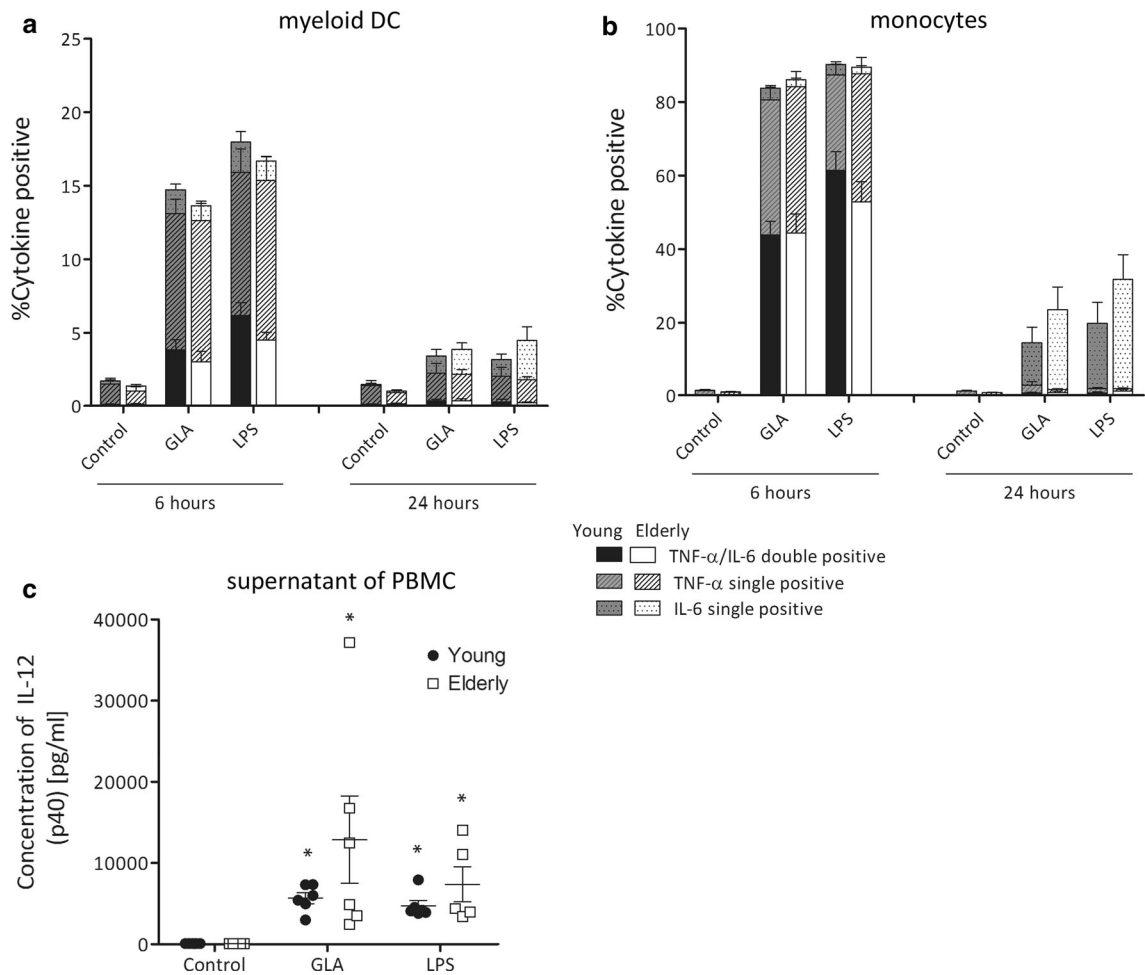
Fig. 5 Cytokine production in PBMC culture. **a** and **b** Percent IL-6 positive and TNF-α positive mDC (**a**) and monocytes (**b**) from young and elderly donors after 6 and 24 h of stimulation with TLR4 agonists. PBCM of 6 young and 6 elderly donors were cultured in the presence of 300 ng/ml GLA or 100 ng/ml LPS. Mean percentages ± SEM of cytokine-positive mDC and monocytes are shown as assessed by flow cytometry. No significant differences between age groups were found (Mann–Whitney U test). **c** Levels of IL-12p40 in the supernatant of PBMC cultures from young (n = 6) and elderly (n = 6) donors after 6 h of stimulation with GLA (300 ng/ml) or LPS (100 ng/ml). IL-12 production did not differ between age groups (Mann–Whitney U test). *Asterisk* (*) indicates p value of <0.05 comparing IL-12 concentrations in the supernatants after stimulation with a TLR4 agonist to the respective control (Wilcoxon signed-rank test)

upregulation after TLR4-stimulation was impaired in old age. Expression of TLR4 was not assessed on moDC in the present study, but was reported to be unaltered on moDC from elderly persons (Agrawal et al. 2007). Expression of TLR4 was slightly higher on mDC from elderly persons compared to young adults. Previous reports demonstrated intact TLR4 expression in old age on human conventional DC and murine splenic DC (Jing et al. 2009; Wong et al. 2010), whereas macrophages of elderly mice were found to express lower levels of TLR4 and other TLRs

on their surfaces than young mice (Renshaw et al. 2002).

Cytokine production is an important effector function of monocytes and DC. The production of TNF-α and IL-6 mRNA and protein by moDC stimulated with 1000 ng/ml GLA has already been shown (Coler et al. 2011). Neither moDC, nor monocytes or mDC showed age-related differences in the secretion or production of IL-6 and TNF-α after 24 or 6 h of stimulation with GLA. In contrast to the findings on human monocytes (Fig. 5b), splenic and peritoneal macrophages of aged

mice were found to secrete significantly lower amounts of TNF-α and IL-6 upon stimulation with LPS and other TLR agonists in comparison to macrophages from young mice (Renshaw et al. 2002). The dynamics of TNF-α and IL-6 production by monocytes and mDC differed as the proportion of IL-6 single positive (SP) cells was very low for both cell types and age groups after 6 h, but markedly increased after 24 h of stimulation with GLA or LPS due to a faster cessation of TNF-α production (Fig. 5). Generally, cytokine production by monocytes was far more pronounced than by mDC, demonstrating the important role of monocytes and macrophages in promoting the recruitment of effector cells to the site of infection or vaccine injection. Efficient induction of Th$_1$-responses requires IL-12, which is provided by DC. It has been shown that GLA induces the production of IL-12 by human moDC (Coler et al. 2011). moDC from both young and elderly donors secreted IL-12(p40) after 24 h of stimulation with GLA at similar levels (Fig. 2). In addition, IL-12(p40) was also secreted in PBMC cultures after 6 h of stimulation with TLR4 agonists. IL-12 concentrations were very heterogeneous within the older age group, but were at least as high as in the younger group. Two of the six elderly donors showed substantially higher IL-12 concentrations after stimulation with GLA compared to young adults. In summary, these results indicate that the ability of antigen-presenting cells to prime T cell responses by the secretion of IL-12 is unimpaired with age after stimulation of TLR4. In contrast, a decreased production of IL-12(p40) was shown for mDC from elderly persons stimulated with other TLR agonists in comparison to young adults (Panda et al. 2010).

Anti-inflammatory cytokines, such as IL-10 inhibit the activation of various immune cells, and are also critical for the termination and regulation of immune responses. It has been shown that stimulation of human PBMC with GLA or LPS induces IL-10 production (Coler et al. 2011). moDC from young and elderly donors secrete considerable amounts of IL-10, when being stimulated with GLA for 24 h (Fig. 2c). Similar to other adjuvants, such as e.g. MF59 (Mosca et al. 2008) GLA induces pro-inflammatory as well as regulatory factors. It can be hypothesized that this regulation and the ability to not only stimulate but also control and terminate

innate immune responses is a prerequisite for safe adjuvants.

In conclusion, no age-related differences in the expression of the co-stimulatory molecules CD80 and CD86 as well as in the cytokine production after stimulation with GLA were found, irrespective of the source of DC. The response of monocytes to stimulation with GLA was also well preserved in old age. These findings strongly argue against a decreased responsiveness to TLR4-agonists with age in contrast to impaired responses to agonists of other TLRs (van Duin et al. 2007; Panda et al. 2010). Moreover, in the absence of stimulation the expression of TLR 4 was even increased on mDC from elderly persons compared to young adults.

Therefore, TLR4 agonists like GLA are particularly promising candidates as adjuvants of vaccines designed for elderly individuals.

Compliance with ethical standards The study was approved by the ethics committee of the Innsbruck Medical University and all participants gave their written informed consent.

Conflict of interest BW, CJ, BGL and RC have no conflicts of interest to declare. SGR is a founder of, and holds an equity interest in, Immune Design Corp., a licensee of certain rights associated with GLA.

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