IFN-γ Enhances T_H1 Polarisation of Monocyte-derived Dendritic Cells Matured with Clinical-grade Cytokines Using Serum-free Conditions

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Abstract. *Using serum-free conditions, human monocyte-derived dendritic cells (MoDCs) tend to mature insufficiently in a T_H1-polarizing direction under approved and standardized clinical conditions. However, for the initiation of an efficient tumour antigen-specific cytotoxic T-cell response, the induction of a distinct T_H1 response is favourable. Therefore, to improve T_H1 polarisation, the influence of interferon-gamma (IFN-γ) on the maturation of MoDCs was investigated with clinical-grade cytokines or lipopolysaccharide (LPS) in serum-free medium focusing on the viability, phenotypic characteristics, cytokine profile and restimulating capacities. As in previous research, we confirmed that in respect of viability and phenotypic characteristics, cytokine cocktails consisting of tumour necrosis factor-alpha (TNF-α), interleukin (IL)-1β, IL-6 and prostaglandin (PG) E_2, mature MoDCs most efficiently. However, these cytokine-matured MoDCs secreted relatively high levels of IL-10 and only low levels of IL-12p70. Remarkably, if IFN-γ was added, significantly lower levels of IL-10 concomitant with higher levels of IL-12p70 could be detected. Pretreatment with IFN-γ did not improve the phenotypic characteristics nor the T_H1 polarisation of MoDCs. Nevertheless, MoDCs matured with clinical-grade cytokines and IFN-γ could be re-stimulated most effectively with IFN-γ. In conclusion, our work demonstrates that addition of INF-γ to clinical-grade cytokine cocktails readily matures MoDCs and enhances their T_H1 polarisation efficiently under serum-free conditions.*

Dendritic cells (DCs) are most potent antigen-presenting cells playing a pivotal role in the induction of the immune response (1, 2). Fully matured DCs are effective activators of naive T-cells and are therefore regarded as important initiators of primary T-cell immune responses. In recent years, several methods have been established to generate DCs *in vitro* from monocytes isolated from the peripheral blood of volunteers or cancer patients, the so-called monocyte-derived DCs (MoDCs) (3, 4). Loaded with tumour antigen, MoDCs are increasingly applied as vaccines for cancer patients to induce a tumour antigen-specific cytotoxic T-cell (CTL) response (5-7). Nevertheless, an efficient induction of tumour antigen-specific antitumoural CTL response requires both breaking antigen-specific tolerance, as well as changing an immune-suppressive tumour-associated environment (8). As shown in preclinical studies, only fully matured MoDCs characterized by high expression levels of costimulatory molecules (8-10), high migratory activity (11), as well as the induction of a distinct T_H1-response (12-15) were able to induce an efficient tumour antigen-specific CTL response *in vivo*. Unfortunately,

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Abbrevations: CCR, chemokine receptor; CD, cluster of differentiation; CTL, cytotoxic T lymphocytes; DCs, dendritic cells; moDCs, monocyte-derived dendritic cells; DC_1, TH1 polarised dendritic cells; ELISPOT, enzyme-linked immunosorbent spot; FITC: fluorescein-5-isothiocyanat; GM-CSF, granulocyte-macrophage colony stimulating factor; HLA, human leucocyte antigen; INF-γ, interferon-gamma; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; PBMC, peripheral blood mononuclear cells; PBS, phosphate-buffered saline; PE, phycoerythrin; PGE_2, prostaglandin E_2; TH-response, T-helper cell response; TLR, toll-like receptors; TNF, tumour necrosis factor.

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most clinically approved maturation protocols (with serum-free medium) tend to mature MoDCs insufficiently in a T_H1 polarizing direction (DC_1), partly explaining the low antitumoral efficacy of DC-based cancer vaccines (16-18). For this reason, improved DC_1 maturation protocols for clinical use are required. In accordance with other groups, we found that the addition of INF-γ to TLR agonists can effectively improve the maturation status as well as the T_H1 polarisation of MoDCs (16, 17). Nevertheless, most TLR agonists are not clinically approved and therefore are not yet allowed to be used in clinical trails.

In this work, we tested the influence of IFN-γ (Imukin®) on the viability, maturation status, migratory activity and T_H1 polarisation of MoDCs, in order to improve the maturation protocol using exclusively clinical-grade cytokines and serum-free medium.

Materials and Methods

The preclinical investigations with blood from healthy volunteers and patients with advanced tumours were approved by the local Ethics Committee.

Generation of immature MoDCs. Peripheral blood mononuclear cells (PBMNCs) were obtained from the peripheral blood of healthy volunteers and patients with advanced tumours by plastic adherence or elutriation. For plastic adherence, the PBMNCs were isolated on density (1.077) gradients with Biocoll Separating Solution (Biochrom AG, Berlin, Germany) and cultured for adherence of monocytes for 1.5 h at 5% CO_2, 37˚C. The non-adherent cells were carefully removed. As a high enrichment of monocytes from leukapheresis products, the cells were elutriated using the cell separator ELUTRA (Gambro BCT, Lakewood, CO, USA). In both cases, the cell viability was more than 95% as determined with propidium iodide staining solution by FACS analysis. The purity of CD14+ cells was also determined by FACS analysis. The purity of the elutriated CD14+ cells was 2.5 to 3.0-fold higher than those obtained by plastic adherence.

For the generation of immature MoDCs, the cells were always cultured in serum-free CellGro DC Medium (CellGenix, Freiburg, Germany) plus 800 U/ml rhIL4 (R&D Systems, MN, USA / CellGenix) and 1.000 U/ml rhGM-CSF (R&D Systems / Leukine Liquid, Berlex, NJ, USA) for six days at 5% CO_2, 37˚C.

Maturation of MoDCs. The harvested immature MoDCs were recultured at a density of $1.0x10^6$/ml using serum-free CellGro DC Medium and again incubated at 5% CO_2, 37˚C for different time intervals. Different options for the maturation were chosen: the cytokine cocktail rhTNF-α 1.100U/ml, rhIL1β 1.900U/ml, rhIL6 1.000U/ml (all R&D Systems) and prostaglandin (PG)E_2 1 μg/ml (Minprostin®, Pharmacia – Pfizer, Erlangen, Germany) or LPS Re from *Salmonella minnesota* R595 1 μg/ml (Professor C. Galanos, Max Planck Institute for Immunobiology, Freiburg, Germany). All options were tested in the presence or absence of INF-γ (Immukin®, Boehringer Ingelheim, Germany). To analyse the stability of maturation, MoDCs were recultured in serum-free CellGro DC Medium without any cytokines. The effect of INF-γ alone was also investigated.

Immunophenotyping by flow cytometry. A FACS flow cytometer (Becton Dickinson, Heidelberg, Germany) was used to analyse the surface markers after labeling the mature DC cell culture with specific antibodies. The staining was performed after the standard protocol: washing the cells twice, labeling with antibodies for 30 min at 4˚C, washing the cells three times, resuspension with buffer for analysis. In this case the staining was performed with anti-human monoclonal antibodies CD86 Fluorescein-5-isothiocyanat (FITC), CD80 Phycoerythrin (PE), CD83 (PE), CD3 (FITC), CD8 (PE), CD4 (FITC) and CD14 (FITC). To exclude any non-specific reaction, the anti-human monoclonal IgG isotype control provided a negative sample. Directly before measurement, propidium iodide staining solution was added to exclude the dead cells. (all antibodies: BD/BD Pharmingen, Heidelberg, Germany). For staining the migration receptor CCR7 three steps were needed. Firstly the cells were labeled with the anti human CCR7 (CD197) or IgG isotype control, secondly a biotin rat anti-mouse polyclonal antibody (both NatuTec, Frankfurt, Germany) was used and briefly a streptavidin-phycoerythrin conjugate (BD Pharmingen) was used for fluorenscence measurement.

Cytokine analysis. For the evaluation of cytokine production, the supernatant of the matured MoDCs cellcultures were collected at different time points. Independently of the maturation protocol the concentrations of IL-6, -10 and -12p70 were measured using an enzyme-linked immunabsorbent assay (ELISA) (all: Quantikine Immunoassay; Wiesbaden, Germany) according to the manufacturer's protocol.

ELISPOT assay. IL-10- and IL-12p70-producing MoDCs were quantified by ELISPOT assays (Diaclone Research, France) based on sandwich immunoenzyme technology. Ethanol-treated PVDF-bottomed 96-well microtiter plates were coated overnight at 4˚C with 100 μl capture antibody. After washing with phosphate-buffered saline (PBS), unoccupied sites were blocked with the corresponding blocking buffer for 2 h at room temperature and the plates were then washed. MoDCs were resuspended in CellGro medium + 1% autologous serum and transfered to the precoated ELISPOT plates at a final density of $2.5x10^4$ (for IL-10) or $5x10^4$ (for IL-12p70) MoDCs per well. Unstimulated MoDCs or cells stimulated with 500 U/ml IFN-γ or 1 μg/ml LPS + 500 U/ml IFN-γ, locally produced cytokines which were then captured by monoclonal antibodies. The cells were removed after 20 h, the plates were washed with PBS + 0.1% Tween 20 and a specific biotinylated detection antibody (BD/BD Pharmingen) was added. After washing and incubation with streptavidin-alkaline phosphatase, a buffered substrate (BCIP/NBT) solution (Sigma, Saint Louis, MO, USA) was added to each and the plate developed at room temperature. After final washing with distilled water, the dark violet spots were counted automatically (C.T.L. Europe, Aalen, Germany) and the results are expressed as number of spots per $2.5x10^4$ cells.

Results

Phenotypic characterisation of MoDCs. In accordance with Jonuleit *et al*. (19) we found that the cytokine combination of TNF-α (1.100 U/ml), IL-1β (1.000 U/ml), IL-6 (800 U/ml) and PGE_2 (1 μg/ml) most efficiently up-regulated the

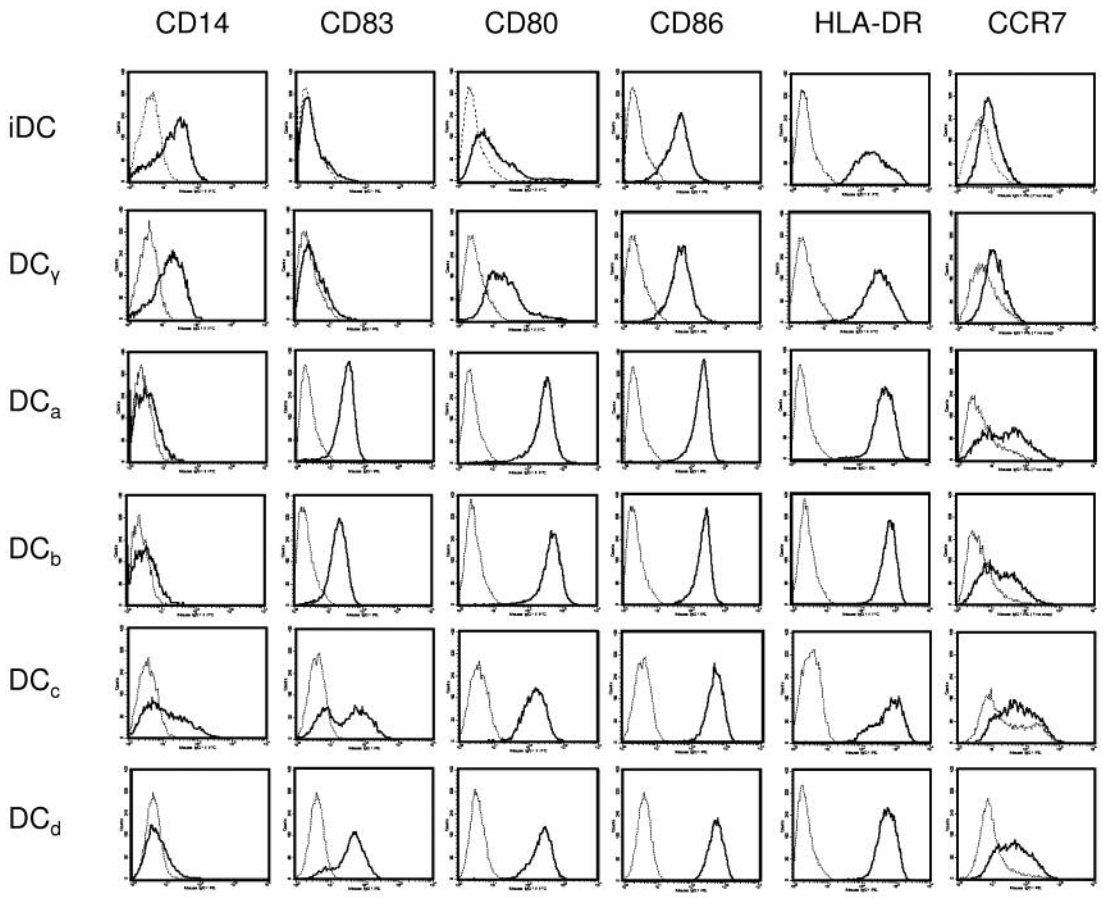

Figure 1. *Phenotypic characterisation of MoDCs from one representative donor matured for 24 h with different cytokines or LPS +/– IFN-γ (Table I) analysed by FACS.*

characteristic maturation markers of MoDCs such as CD83, CD80, CD86 and HLA-DR, as well as the migration marker CCR7, indicating that the majority of matured MoDCs had migratory potential to lymph nodes. The addition of IFN-γ resulted in an even more enhanced up-regulation of the B7 molecules and major histocompatibility complex class I, as well as in a minor inhibition of the expression of CD83 and CCR7. Figure 1 shows the variety of expression markers before and after maturation of MoDCs with the improved clinical-grade cytokine cocktails or the LPS control introduced in Table I. Interestingly, only marginal differences could be detected regarding the phenotypic characteristics of immature MoDCs generated from monocytes separated by adherence or by elutriation.

Viability of matured MoDCs, based on total numbers of seeded cells and starting numbers of CD14-positive monocytes, were lower for MoDCs matured with LPS- and/or IFN-γ (data not shown) due to cell loss through stronger plastic adherence of MoDCs. DC viability ranged from 78.2% to 98.4%, with the lowest value for DC_c and DC_d cells. The monocyte expression marker CD14 virtually disappeared within 24 h of maturation while the maturation marker CD83 increased up to 60-85% within 24 h and stabilised after 48 h, thereby demonstrating that

Table I. *Cytokine cocktail compositions used for MoDC maturation.*

DC population	Cytokines	TLR4-ligand	Comments
iDC	-	--	Immature MoDC
DC_γ	[2]IFN-γ	--	
DC_a	TNF-α, PGE_2, IL-1β, IL-6	--	[1]Jonuleit cocktail
DC_b	TNF-α, PGE_2, IL-1β, IL-6, [2]IFN-γ	--	
DC_c	-	[3]LPS	Positive control
DC_d	[2]IFN-γ	[3]LPS	

[1]Cytokine cocktail in accordance with Jonuleit: TNF-α (1100 U/ml), PGE_2 (1 μg/ml), IL-1β (1900 U/ml), IL-6 (1000 U/ml); [2]IFN-γ (500 U/ml); [3]LPS R595 from *Salmonella minnesota* (1 μg/ml).

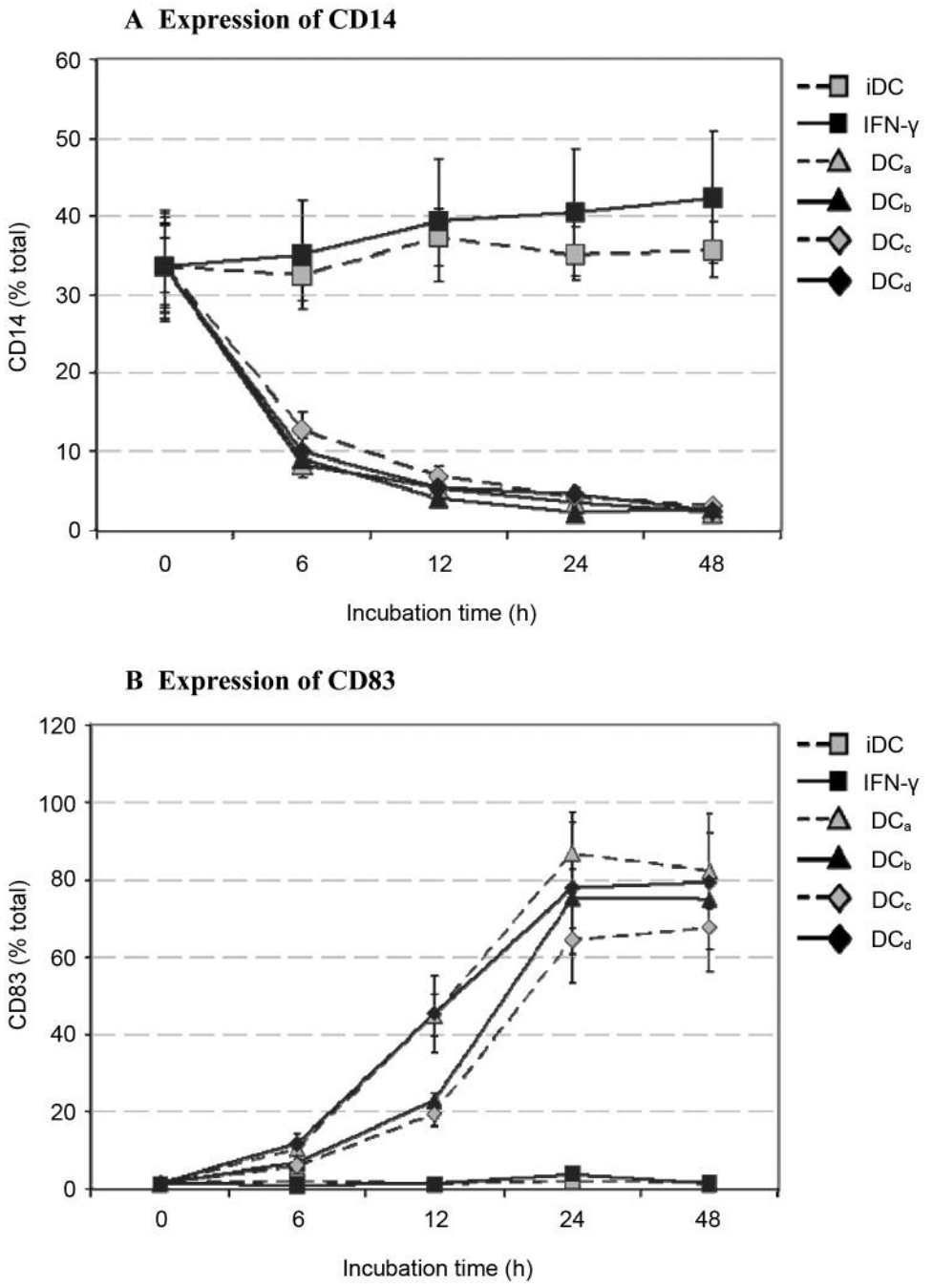

Figure 2. *Expression of CD14 (A) and CD83 (B) of MoDCs matured as shown in Table I with TNF-α/PGE_2/IL-1β/IL-6 or LPS in the presence or absence of IFN-γ (500 U/ml) analysed by FACS. Mean values and standard deviations of three independent experiments are shown.*

both the tested cytokine cocktails as well as the LPS control fully matured MoDCs in serum-free medium (Figure 2).

To compare the capacity of the clinical-grade cytokine cocktails with LPS to mature MoDCs in the presence or absence of IFN-γ, we determined the kinetics of CD14 and CD83 expression over a period of 48 h (Figure 2). Moreover, we examined the stability of differentiation after 24 h of maturation followed by re-culturing of $MoDC_{a-d}$ in serum-free medium without any cytokines (data not shown). In all cases only little reversal of differentiation was observed since all MoDCs remained CD14-negative and retained high levels of CD83, CD80, CD86, HLA-DR and CCR7. Thus, both the selected cytokine cocktail and the LPS control induced

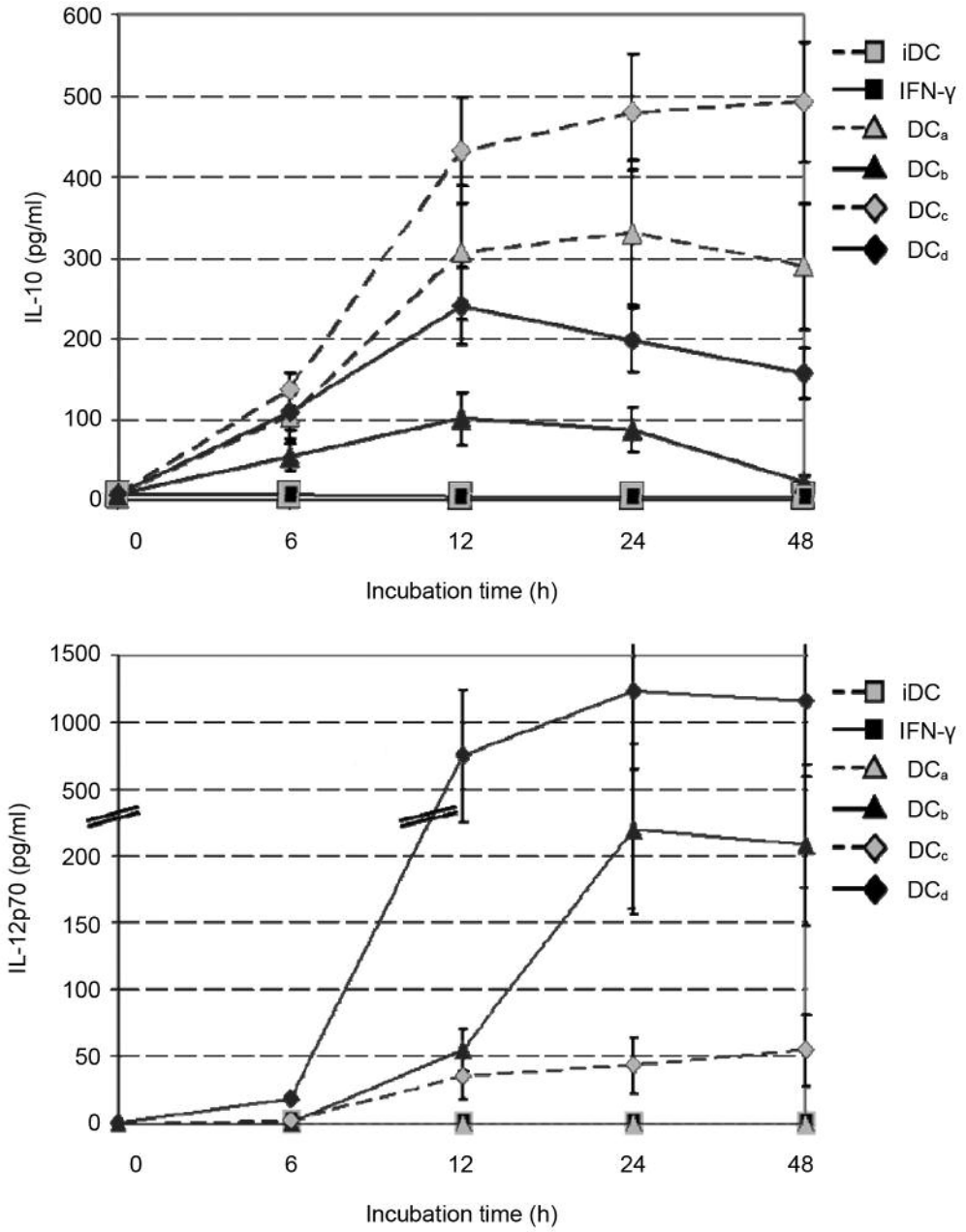

Figure 3. *Levels of IL-10 (A) and IL-12p70 (B) in cultures of MoDCs (DC_a-DC_d) matured for 0-48 h with TNF-α/PGE_2/IL-1β/IL-6 or LPS in the presence or absence of IFN-γ (500 U/ml) measured by ELISA. Mean values and standard deviations of three independent experiments are shown.*

comparable stable maturation of MoDCs independently of the addition of IFN-γ.

Cytokine release. As previously reported by other groups and shown for the LPS in Figures 2 and 3, the inclusion of IFN-γ in the maturation cocktail containing TLR agonists results in the development of stable T_H1-polarized $MoDC_1$ in serum-free medium, characterized by a high ability to produce IL-12p70 upon subsequent stimulation (16, 17). However, most TLR agonists are not approved for clinical use. Therefore, we tested the capacity of MoDCs to secret IL-10 and IL-12p70 after maturation in serum-free medium exclusively with clinically approved cytokines, with or without IFN-γ.

As shown in Figure 3, DC_a and DC_c produced relatively high levels of IL-10 and no biologically active IL-12p70, whereas DC_b and DC_d cells secreted significantly less IL-10 but much more IL-12p70. As IL-10 counteracts the

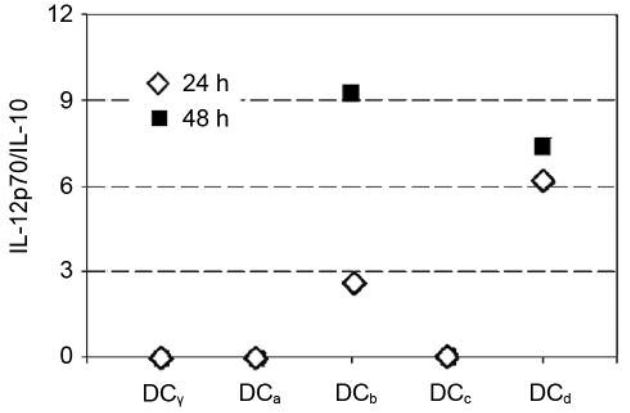

Figure 4. *IL-12p70/IL-10 ratios in cultures after maturation of MoDCs (DC_a-DC_d) with TNF-α/PGE_2/IL-1β/IL-6 or LPS for 0-48 h in the presence or absence of IFN-γ (500 U/ml) as measured by ELISA.*

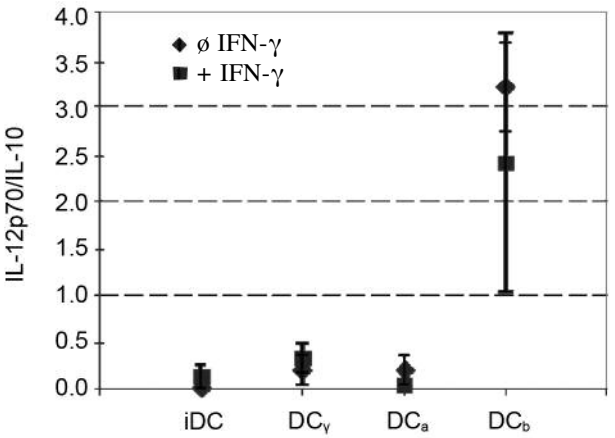

Figure 5. *IL-12p70/IL-10 ratios in cultures of MoDCs matured over a period of 24 h with TNF-α/PGE_2/IL-1β/IL-6 in the presence or absence of IFN-γ (500 U/ml). The cytokine secreting MoDCs were detected by ELISPOT. Mean values and standard deviations of three independent experiments, each with MoDCs from patients or from healthy volunteers, are shown.*

efficiency of IL-12p70, we additionally calculated the ratio of IL-12p70/IL-10 for all maturation protocols (Figure 4).

DC_a and DC_c cells had the lowest ratio, whereas DC_b and DC_d populations showed higher ratios after 24 and 48 h of maturation respectively. Remarkably, after 48 h of maturation, the IL-12p70/IL-10 ratio was highest for DC_b. However, although MoDCs generated from different donors showed considerable differences concerning the extent of cytokine production, MoDCs matured with IFN-γ were generally superior with respect to their IL-12p70/IL-10 ratio.

To monitor the frequency of IL-10- and IL-12p70-secreting MoDCs matured over a period of 24 h with clinical-grade cytokines with or without IFN-γ and IL-10 and IL-12p70 ELISPOT was used (Figure 5). In accordance with the results presented above, IFN-γ increased the ratio of IL-12p70/IL-10 producing MoDCs. Remarkably, comparing DC_a and DC_b generated from patients and healthy donors, our results showed no significant differences in regard to their IL-12p70- or IL-10-producing capacities.

Maturation of MoDCs with different concentrations of IFN-γ. To further optimise the maturation of MoDCs (DC_a), we simultaneously added different concentrations of IFN-γ (200-4000 U/ml) to the clinical-grade cytokines under serum-free conditions. Focusing on phenotypic characterisation, no significant difference in the expression of the classical maturation markers such as CD83, CD80, CD86 and HLA-DR, or the migration marker CCR7, could be detected (data not shown). However, despite considerable variations of the IL-10 and IL-12p70 levels between different donors, our results indicate an optimal IL-12p70/IL-10 ratio after addition of 500-1.000 U/ml IFN-γ to the cytokine maturation protocol (Figure 6).

Comparable results were obtained when looking for the influence of different concentrations of IFN-γ on the maturation of MoDCs with TLR agonists (data not shown).

Pretreatment of immature MoDCs with IFN-γ. In pre-clinical and clinical studies, we were able to show that pretreatment with IFN-γ can enhance both endotoxin-induced cytokine production as well as the activity of immunological effector and regulator cells (20-23). Therefore, we expected that pretreatment with IFN-γ could enhance both the expression of maturation markers as well as the activity and T_H1 polarisation of human MoDCs *in vitro*. Comparable to the simultaneous stimulation with different concentrations of IFN-γ, concentrations of 500-1.000 U/ml seem to be optimal for pretreatment of MoDCs with IFN-γ (data not shown). In Figure 7, the expression of CD14 and CD83 as well as the cytokine production of DC_a pre-treated with IFN-γ for periods of 0-48 h is shown.

Surprisingly, pretreatment with IFN-γ (500 U/ml) did not lead to an enhanced production of IL12p70 compared to the simultaneous addition of IFN-γ. However, for all measured time intervals, the secretion of IL-10 decreased considerably, possibly reflecting the T_H1 polarisation of the MoDCs. Interestingly, pretreament with IFN-γ showed a time-dependent influence on the down-regulation of CD14 and up-regulation of CD83. Pretreatment for ≥3 h and ≤48 h led to a down-regulation of the CD83, with a minimum after 6 h, and an increase of CD14 expression respectively. No differences of the expression level could be observed between MoDCs matured simultaneously with clinical-grade cytokines plus IFN-γ (DC_b) and MoDCs (DC_a) pretreated with IFN-γ before being matured with PGE_2, IL-1β, IL-6 and TNF-α.

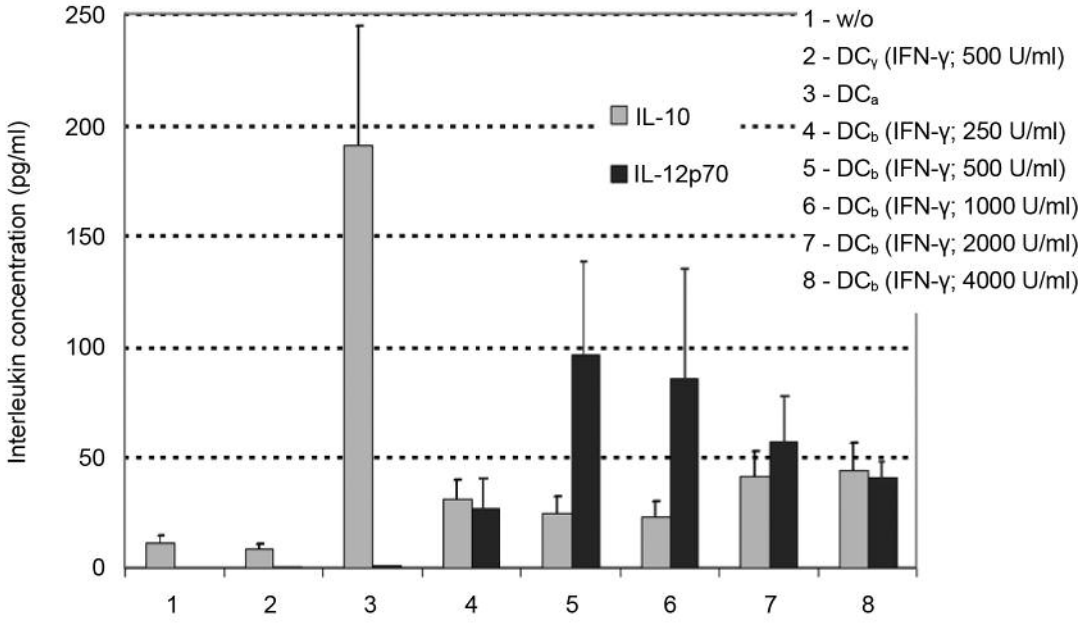

Figure 6. *IL-10 and IL-12p70 levels of supernatants in cultures of $MoDC_a$ matured in the presence or absence of IFN-γ in different concentrations measured by ELISA. Mean values and standard deviations of three independent experiments are shown.*

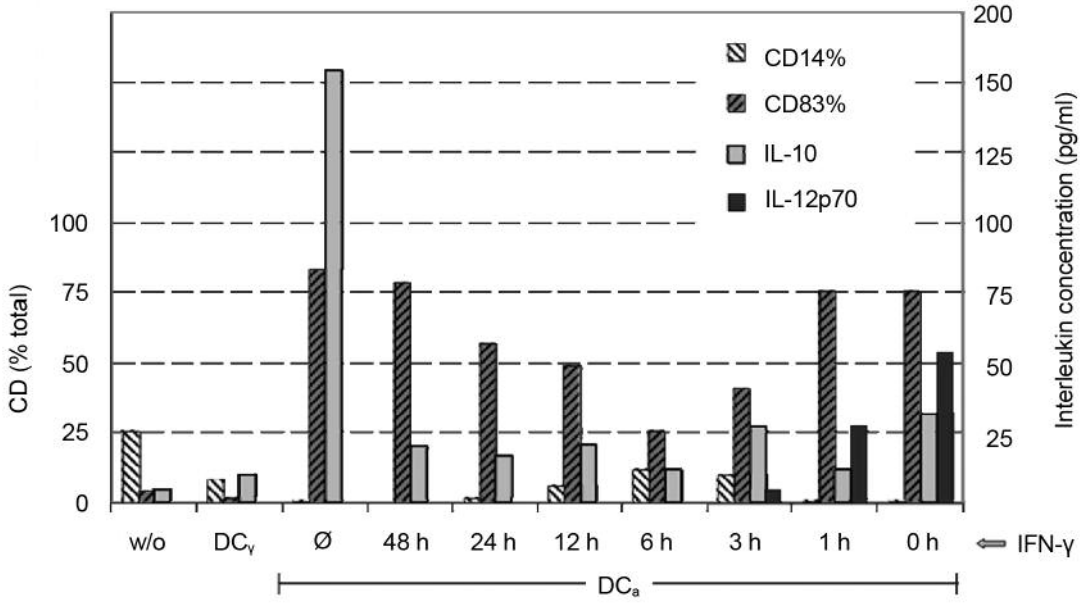

Figure 7. *Phenotypic characterisation and cytokine levels in cultures of DC_a pretreated with IFN-γ (500 U/ml) for 1-48 h. One representative experiment of three is shown. The absolute quantities of cytokines for matured MoDCs varied between different donors, however, the response pattern observed for the analysed cytokines was very similar.*

Restimulation of matured MoDCs with IFN-γ. To test the influence of IFN-γ on matured DC_a and DC_b, we restimulated them with IFN-γ after 24 or 48 h of maturation and assessed the IL-10- or IL-12p70-producing MoDCs using a standard ELISPOT assay. Whereas, after 48 h only a few IL-10-producing MoDCs could be detected (data not shown), the frequency of IL-12p70- secreting MoDCs was clearly enhanced after 24 h of maturation for both DC_a and DC_b (Figure 8). Simultaneously, the number of IL-10-secreting MoDCs diminished. In accordance with the results

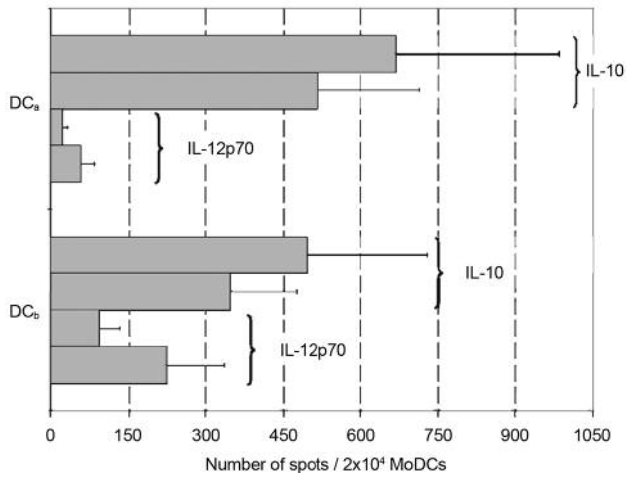

Figure 8. *Number of IL-10 and IL12p70 spots per 2x10^4 MoDCs after restimulation of DC$_a$ or DC$_b$ with IFN-γ (500 U/ml). The cytokine secreting MoDCs were detected by ELISPOT. Mean values and standard deviations of nine independent experiments are shown. *Restimulation of the MoDCs with IFN-γ.*

achieved from the maturation experiments, DC_b showed higher IL-12p70/IL-10 ratios than did DC_a.

Discussion

Cancer therapy based on MoDCs pulsed with tumour-specific antigens has been proven to be safe with minimal side-effects and some clinical efficacy. However, compared to cytotoxic approaches, the anticancer response is relatively low. As shown in preclinical studies, only fully matured MoDCs characterised by high expression levels of costimulatory molecules, high migratory activity, as well as the induction of a distinct T_H1 response were able to induce an efficient tumour antigen-specific CTL response *in vivo*. Therefore, one main reason for the low clinical efficacy of DC based anticancer therapies to date could be due to DC clinical-grade cytokine cocktails maturing MoDCs insufficiently in a T_H1-polarizing direction. Thus, improved $MoDC_1$ maturation protocols for clinical use are required.

In this context, we evaluated several clinical-grade cytokines such as TNF-α, IL-1β, IL-6, PGE_2, IFN-α and IFN-β for their capacity to readily mature $MoDC_1$ from healthy volunteer and cancer patients reliably to T_H1-polarized matured MoDCs in serum-free medium.

In accordance with Jonuleit *et al.*, we found that only cytokine cocktails composed of TNF-α, IL-1β, IL-6 and PGE_2 reliably induced the phenotypic characteristics of readily matured MoDCs expressing high levels of CD83, MHC, co-stimulatory molecules and CCR7 (Figure 1) (19). However, characterisation of their cytokine secretion profiles revealed high levels of IL-10 but only low levels of IL-12p70, suggesting a preference for TH2 polarisation and partly explaining the relatively low efficacy of cancer vaccines.

In order to improve T_H1 polarisation of the MoDCs matured with clinical-grade cytokines, we tested the influence of IFN-γ (Imukin®) on (i) the viability of MoDCs, (ii) the expression levels of CD83, MHC, co-stimulatory molecules and CCR7, as well as on (iii) the cytokine secretion profile. Due to stronger adherence of MoDCs additionally matured with IFN-γ, we observed only slightly lower viabilities after simultaneous addition and considerably lower viabilities after pretreatment with IFN-γ. Focusing on their expression levels, the simultaneous addition of IFN-γ resulted in an enhanced up-regulation of B7 molecules and major histocompatibility complex class I as well a minor inhibition of CD83 and CCR7 expression. Moreover, the presence of IFN-γ induced the production of considerably lower levels of IL-10 and higher levels of IL-12p70, resulting in a significantly higher ratio of IL-12p70/IL-10, clearly demonstrating the T_H1-polarizing capacity of IFN-γ when added to the tested clinical-grade cytokines. Remarkably, after 48 h of maturation (not after 24 h) the IL-12p70/IL-10 ratio was higher for MoDCs (DC_b) exclusively matured with TNF-α, IL-1β, IL-6, PGE_2 and IFN-γ than for MoDCs (DC_d) matured with LPS and IFN-γ, possibly reflecting a longer continuing maturation process and later exhausted status with respect to cytokine production (24, 25).

To further improve the T_H1 polarisation of cytokine-matured MoDCs, we tested the influence of different IFN-γ concentrations on the maturation process. According to previous investigations using TLR agonists and IFN-γ, best T_H1 polarisation and phenotypic characteristics of matured MoDCs could be achieved when 500 to 1.000 U/ml of IFN-γ were added to the cytokine maturation cocktail.

To further enhance the maturation status as well as the T_H1 polarisation, we pretreated the MoDCs with IFN-γ (500 U/ml). Unexpectedly, our data showed a decline of the IL-12p70/IL-10 ratio compared to the simultaneous addition of IFN-γ to the maturation cytokine cocktail. Therefore, in contrast to our expectations, pretreatment with IFN-γ could not improve the T_H1 polarisation of cytokine-matured MoDCs in serum-free medium.

Previously, we vaccinated several HLA-A2 positive patients suffering from hormone refractory prostate carcinoma with MoDCs matured with clinical-grade cytokines and pulsed with defined prostate specific antigen (PSA)-peptides (16). Before the intracutaneous application of the PSA-peptide-loaded MoDCs, we subcutaneously administered IFN-γ in the same area to restimulate the matured MoDCs at the site of injection. To verify this concept, we compared the restimulation capacity of DC_a and DC_b, both matured for 24 and 48 h. Whereas the number of MoDCs secreting IL-12p70 increased considerably after

short-time maturation (≤24 h), most MoDCs seemed to be increasingly exhausted when matured longer than 24 h. As expected, DC_b showed considerably higher levels of IL-12p70 and lower levels of IL-10 than DC_a when restimulated with IFN-γ.

In summary, our results revealed that MoDCs (DC_b) matured with the clinical-grade cytokines PGE_2, IL-1β, IL-6, TNF-α and IFN-γ induced both the phenotypic characteristics of completely matured MoDCs as well as the highest IL-12p70/IL-10 ratios. Moreover, DC_b could be re-stimulated most efficiently with IFN-γ to produce IL-12p70. Only the expression of the maturation marker CD83 and the migration marker CCR7 were slightly reduced compared to DC_a. In this context, it is also worth mentioning that pretreatment of immature MoDCs with IFN-γ improved neither the phenotypic characteristics nor the T_H1 polarisation of DC_a compared to DC_b.

Conclusion

In this work, we investigated the influence of IFN-γ on the maturation of human MoDCs with the clinical-grade cytokines TNF-α, IL-1β, IL-6, PGE_2 or LPS under serum-free conditions focusing on the viability, phenotypic characteristics, cytokine profile and restimulating capacities. All cytokine-matured MoDCs showed good harvesting characteristics and high viabilities. Their generation could be easily adapted for compliance with good manufacturing practices. Our preferred MoDC population for use as an anticancer vaccine is the DC_b population, which combines optimal phenotypic characteristics, highest IL-12p70/IL-10 ratios as well as an efficient restimulation capacity. DC_b prepared from cancer patients seems to be suitable for use in addition with peptides, for example as a source of tumour-associated antigens.

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