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Interleukin 10 Induces the Maturation of Putative Tolerogenic Monocyte-derived Dendritic Cells as Revealed by GeneArray Analysis

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Abstract: IL-10 is a multifunctional cytokine with known inhibitory effect on DC maturation. In the present study, we show that the presence of IL-10 during the first 6 days of maturation of Mo-DC has severe effects on the phenotype with IL-10 treated Mo-DC retaining a high expression of factors involved in antigen uptake and having low expression of costimulatory factors and MHC II. The effect of IL-10 on DC maturation is further characterized by a comprehensive investigation of the expression profile of 12500 genes using GeneArrays and comparing the data for IL-10 treated and untreated Mo-DC at day 6 and 8 with monocytes and macrophages. The data is confirmed with semi-quantitative RT-PCR. We find that the transcriptional pattern of IL-10 treated Mo-DC at day 6 and day 8 is distinct from both untreated Mo-DC and macrophages. IL-10 induces the expression of genes coding for CCL18, ILT3, ILT4 and TGFβ while inhibiting the expression of IL-12 p40 mRNA, and genes coding for CCL17, CCL19 and CCL22 suggesting a likely effect of IL-10 on Mo-DC maturation to be the generation of tolerogenic Mo-DC.

Key words: monocytes-derived, dendritic cells, macrophages, IL-10, microarray

INTRODUCTION

IL-10, previously known as cytokine synthesis inhibiting factor, is a multifunctional cytokine that has diverse effects on most hematopoietic cells. IL-10 is expressed by a variety of cells including macrophages, T cells and B cells. The general function of IL-10 is the control of the proinflammatory response by limiting and ultimately terminating the immune response to pathogens. In addition, IL-10 regulates the growth and/or differentiation of many different cell types including dendritic cells [1]. Furthermore, IL-10 has dual functions and also exerts immunostimulatory effects on NK, B cells and cytotoxic T cells [2,3].

Dendritic cells are the most potent APC of the immune system and induce the primary immune response. Immature DC residing in peripheral tissues have high capacity for antigen uptake and processing. Upon maturation, mature DC migrate to secondary lymphoid tissues where DC present antigen to and stimulate activation of T cells. These processes involve changes in the expression of costimulatory molecules as well as chemokines and chemokine receptors on dendritic cells [4,5]. Experiments have shown that IL-10 downregulates the expression of MHC II and costimulatory molecules on in vitro generated DC and inhibits their T cell stimulatory capacity [4,6,7,8,9]. Furthermore, IL-10 prevents the migration of DC from peripheral tissue by upregulating CCR5 and downregulating CCR7, the chemokine receptor important for migration to secondary lymphoid tissues [10]. It has been suggested that the effect of IL-10 on in vitro maturation of monocytes to DC is the redirection of monocytes into macrophages [11] or into tolerogenic DC driving a Th2 response [7,6].

In an effort to characterize more clearly the effect of IL-10 on DC maturation, we compared the phenotype of IL-10 treated and untreated monocytes-derived DC (Mo-DC) and performed an analysis of the gene expression profiles of monocytes, macrophages; IL-10 treated and untreated Mo-DC. Selected gene expression profiles were confirmed using semi-quantitative RT-PCR.

MATERIALS AND METHODS

Culture medium: RPMI 1640 supplemented with 100 μg/ml streptomycin, 100 IU/ml penicillin, 2 mM L-glutamine and either 1 % or 10 % heat-inactivated human AB-serum (pooled from at least 10 normal donors).

Cytokines and growth factors: GM-CSF was Leucomax, clinical grade from Novartis/Schering-Plough (Basel, Switzerland). The recombinant human cytokines IL-4, IL-10 and M-CSF were purchased from R&D systems (Abingdon, UK), and LPS (E.Coli O26:B6) from Sigma (Copenhagen, Denmark).

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Purification of cells: Peripheral blood was obtained as standard buffy coat preparations and was from healthy donors from the Department of Clinical Immunology, Odense University Hospital. Peripheral blood monocytes were isolated by Lymphoprep density centrifugation in Blood-Sep-Filter tubes (CM-lab, Vordingborg, Denmark) followed by isolation on a discontinuous Percoll gradient (42.5 %/50 %). For cell culturing, PBMC were plated at a concentration of 1×10^6 cells/ml in 250 ml culture flasks (Nunc, Copenhagen, Denmark) and allowed to adhere for 2 hours at 37°C, 5 % CO2. The non-adherent cells were removed by washing in ice-cold PBS. The final population consisted of at least 80 % of monocytes as demonstrated by flow cytometry of anti-CD14 stained isolates. For total RNA extraction, monocytes were further purified by cell sorting of CD14+CD45+ cells using a FACSVantage (Becton Dickinson, Copenhagen, Denmark). The sorted populations had a purity of ≥99 %. To avoid donor variation, total RNA from monocytes of 5 different donors were used for one GeneArray analysis.

Cell culture: Mo-DC were generated using medium containing 1 % human AB-serum. At day 0 and 3, GM-CSF and IL-4 was added to a concentration of 20 ng/ml and 10 ng/ml, respectively. At day 6, cells were replated at a density of 3.3×10^5 cells/ml and further incubated with 1-5 ng/ml LPS (E.Coli O26:B6, Sigma, Copenhagen, Denmark) for 2 additional days. For generation of IL-10 Mo-DC, hIL-10 was included at 10 ng/ml at day 0-6. For further analysis, Mo-DC and IL-10 Mo-DC were harvested at day 6 and 8. Macrophages were generated by culturing monocytes in medium containing 10 % human AB-serum. M-CSF was added to a concentration of 100 U/ml at day 0 and 3. Macrophages were harvested at day 7.

Antibodies and flow cytometry: The following antibodies were used: FITC-conjugated anti-CD14 (18D11, Diatec, Oslo, Norway), PE-conjugated anti-CD45 (E01, Diatec, Oslo, Normay), PE-conjugated anti-CD83 (HB15a, Immunotech, Krefeld, Germany), biotin-conjugated anti-CD86 (2331, Pharmingen, Copenhagen, Denmark), PE-conjugated anti-MMR (19, Pharmingen, Copenhagen, Denmark), FITC-conjugated anti-CD32 (FLI8.26, Pharmingen, Copenhagen, Denmark), biotin-conjugated anti-HLA-DR (L243, Becton Dickinson, Copenhagen, Denmark). Binding of biotin-conjugated anti-HLA-DR and CD86 was revealed in a second step using streptavidin-PerCP (Becton Dickinson, Copenhagen, Denmark). Stained cells were analyzed using a FACScan (Becton Dickinson, Copenhagen, Denmark). Mo-DC were gated according to FSC and SSC. The results were analyzed using FlowJo (Tree Star, Ashland, California, US).

Total RNA extraction, hybridization and microarrays analysis: For gene expression profiles total RNA was extracted from $>2\times10^6$ cells of either monocytes, macrophages, immature and mature Mo-DC and IL-10 treated Mo-DC using QiagenAmp Total RNA kit as described by the manufacturer. Preparations of cRNA and hybridisation were performed according to the protocols from Affymetrix. Briefly, using total RNA, synthesis of double-stranded cDNA was performed using an oligo dT primer with the T7 RNA polymerase recognition sequence appended, biotinylated nucleotides were incorporated by in vitro transcription using T7 RNA polymerase, and the resulting cRNA transcripts were fragmented before hybridisation. The human genome U95Av2 chip was used, and The Affymetrix Test Array was used for verifying the integrity of the RNA. GeneChips were scanned using a laser scanner according to instructions from Affymetrix. (For a detailed description of the technology please see: www.affymetrix.com). Data were entered into Filemaker Pro and further analyzed.

Quantitative RT-PCR: A two-step real time RT-PCR technique was used to determine the relative mRNA levels of the desired genes (for primers, see table I). mRNA was isolated from a total of 50.000 cells of each population. Cells were disrupted in lysis-binding buffer and extracts were incubated for 15 min with RNAse-free DNAse (Qiagen, Copenhagen, Denmark) before mRNA was purified as described by the supplier using the Dynabeads mRNA DIRECT kit (Dynal, Oslo, Norway) and eluted in 40 µl dH_2O. Synthesis of cDNA was performed in 1X PCR buffer II, 5 mM $MgCl_2$, 0.6 U/µl RNnase inhibitor, 0.25 mM dNTP mix, 2.5 mM hexamer primer, 2.5 U/µl MulV reverse transcriptase (all from Applied Biosystems; Stockholm, Sweden) and 20 µl of mRNA in a total volume of 100 µl. The reaction mixtures were incubated at 22°C for 12 min, at 42°C for 22 min, and at 99°C for 7 min. To perform PCR, either 3 µl or 8.8 µl synthesized cDNA was added to 1X SYBR green master mix (Applied Biosystems, Stockholm, Sweden), 0.33 pmol/µl of each primer and 0.015 U/µl AmpErase UNG in a total volume of 15 µl or 25 µl, respectively. The reaction, 48°C for 2 min and 95°C for 10 min and 50 cycles of 95°C for 15 sec. and 60°C for 1 min, and detection were carried out on an ABI 7700 (Applied Biosystems, Stockholm, Sweden). The C_t value (threshold cycle) was set according to the recommendations. We were not able to find a house keeping gene that was constant upon maturation (unpublished result). Instead, the relative mRNA levels were calculated by $2^{-\Delta(Cts-Ctc)}$ (with Cts being the Ct value set for the sample and Ctc for monocytes) and compared for an equal number of cells as suggested by [12]. Similar methods have been used in [13,14].

Table 1: primer sequences.

Gene	Forward primer	Reverse primer
CCL5	CCTCATTGCTACTGCCCTCTG	AGCAGCAGGGTGTGGTGTC
CCL7	CAGAAGGACCACCAGTAGCCA	GGGTCAGCACAGATCTCCTTGT
CCL8	TGCTCATGGCAGCCACTTT	AGCAGGTGATTGGAATGGAAA
CCL13	AGCAGCTTTCAACCCCCAG	AAATGTGAAGCAGCAAGTAGATGG
CCL18	CCTCCTTGTCCTCGTCTGCA	TATAGACGAGGCAGCAGAGCTCT
MARCO	ATCTACCTGATCCTGCTCACCG	AGCCGCGCCTGCAGA
IL-10	TGAGAACAGCTGCACCCACTT	TGTCCAGCTGATCCTTCATTTG
IL-12 p35	CTTCACCACTCCCAAAACC	AATCTCTTCAGAAGTGCAAGG
IL-12 p40	TGCTGGTGGCTGACGACAATC	AGAGTGTAGCAGCTCCGCACG

Mixed Leukocyte Reaction: At day 8, IL-10 treated or untreated Mo-DC and macrophages were harvested, irradiated and incubated at graded doses with allogeneic leukocytes in 96-well round-bottomed plates. Allospecific proliferation was measured after 5 days of incubation. 2.5 μCi [3H]-thymidine per well was added 20 hours prior to measurements.

RESULTS

The phenotype of IL-10 treated Mo-DC is distinct from untreated Mo-DC: Mo-DC were maturated in the presence or absence of human IL-10 for the first 6 days as described in Materials and Methods. Upon maturation in LPS for additional 2 days, Mo-DC were harvested and their phenotype was determined by flow cytometry (see Fig. 1). We find that the expression of the monocyte marker CD14 on untreated Mo-DC is almost absent whereas IL-10 treated Mo-DC have a relatively high expression of CD14. However, the expression of CD14 on IL-10 treated Mo-DC is lower than the expression found on in vitro generated macrophages (data not shown). Furthermore, the expression level of macrophages mannose receptor MMR and CD32, both involved in antigen uptake, is significantly higher on IL-10 treated Mo-DC than on untreated Mo-DC. In contrast, expression of the dendritic cell marker CD83, HLA-DR and the costimulatory factors CD80 and CD86 is significantly higher on untreated cells compared to IL-10 treated cells. This suggest that addition of IL-10 for the first 6 day of differentiation inhibit the full maturation of Mo-DC and induce maturation of cells with high capacity for antigen uptake like macrophages.

The expression profiles of selected genes vary significantly between macrophages and IL-10 untreated and treated Mo-DC: For a comprehensive characterization of IL-10 treated Mo-DC, total RNA was extracted from IL-10 treated and untreated Mo-DC and the RNA transcription levels for different genes were determined using Affymetrix microarrays containing probes of around 12.500 different genes and

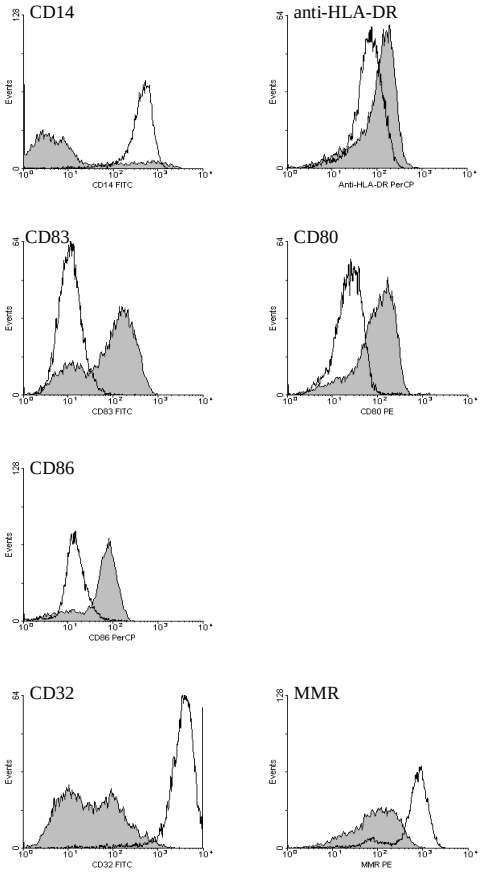

Fig. 1: The phenotype of IL-10 treated Mo-DC is distinct from untreated Mo-DC. Mo-DC were generated in the presence (shaded area) or absence (black line) of IL-10 for the first 6 days. Upon maturation in LPS for two additional days cells were harvested and analyzed by flow cytometry. Mo-DC were gated according to FSC-SSC. The data illustrated is one experiment representative of three.

compared with expression profiles of monocytes and in vitro generated macrophages. The in vitro generated macrophages were shown to have a phenotype characteristic of macrophages by flow cytometry (data not shown). The signal strengths of selected genes important for the function of Mo-DC and macrophages were compared (table 2). Untreated Mo-DC at day 6 have the characteristics of immature DC with high RNA transcription levels of receptors like the CD1 family and Fc receptors involved in antigen uptake and CCR2 and CCR5 involved in homing to peripheral tissue. Upon maturation with LPS, the RNA transcription levels of these factors decreased and instead we observed an increased expression level of CCL5, CCL19 and CCL22 known to be expressed in mature DC and being chemotaxis to DC and activated T cells [15,16,4]. The expression of these factors is significantly lower in IL-10 treated Mo-DC at day 8. In contrast we observed high expression of genes coding for CCL18, CXCL2, CXCL3, CXCL5 and IL-8 in IL-10 treated Mo-DC at day 8. Comparison of the RNA transcript levels for macrophages and IL-10 treated day

Table 2: The expression profile of selected genes**.** Total RNA was extracted from the six populations, processed and hybridized to GeneArrays. The values given in the table is the detected signal strength**.**

Descriptions	Day 0 Monocytes	Day 7 Macrophages	Day 6 Mo-DC	Day 6 IL-10	Day 8 Mo-DC	Day 8 IL-10	GeneBank Acc. No.
Chemokines and receptors							
CCL5/RANTES	7,1	1,6	2,6	8,1	221,4	358,6	M21121
CCL7/MCP-3	23,5	101,3	33,4	811,8	34,6	476,4	X72308
CCL8/MCP-2	2	2,7	91,3	1429,1	65,6	440,8	Y16645
CCL13/MCP-4	7,4	6,8	2315,7	3509,2	29,2	196,5	AJ001634
CCL17/TARC	9,7	2,8	4613,1	182,2	3996,2	46,5	D43767
CCL18/AMAC1	2,3	9,1	82	5380,6	35,5	4810,5	Y13710
CCL19/EBI1-ligand chemokine	3,1	3,2	9,3	4,1	335	5,7	AB000887
CCL22/MDC	72,8	2120,8	4423,5	2767,2	6305,2	1709,2	U83171
CXCL2/GRO–β	509,8	14,1	6,2	20,3	48,9	533,2	M36820
CXCL3/GRO-γ	83,4	9,8	4	28,9	47	315,5	M36821
CXCL5/ENA-78	27,3	31,5	8,3	15,1	78,4	1619,2	X78686
CCR2, CCR5 and CCR6	2,6	58,7	109,5	59,7	33,2	42,6	U95626
Growth factors and receptors							
Transforming growth factor alpha (TGFα)	6,9	17,3	89,8	118,5	31,7	48,4	X70340
Transforming growth factor-beta (TGF-β)	17,1	11	8,8	24,2	25,4	118,1	M60315
Vascular endothelial growth factor (VEGF)	828	79,9	174,8	156,2	226,7	720,9	AF022375
G-CSFR-1	684,8	82,7	46,4	86,1	72,3	172,1	M59818
GM-CSF receptor	37,3	10,9	73,5	55,8	340,1	73,2	M73832
Interleukin and receptors							
Interleukin 1 alpha (IL 1)	16	5,9	0,6	8,6	11,4	13,2	M28983
Interleukin 8 (IL-8)/CXCL8	4709,6	17,4	10,5	22,9	753	3548	M28130
Interleukin 15 (IL15)	23,5	5,1	12,2	0,6	42,2	11,9	U14407
Interleukin 15 precursor (IL-15)	96,7	19,3	30,8	1,6	169,7	14,1	AF031167
Interleukin 2 receptor gamma chain	49,2	285,9	293	130	574,2	239,6	D11086
Interleukin-2 receptor	1,8	3,8	9,3	6,3	33,7	31,4	X01057
Interleukin 8 receptor beta (IL8RB)	1,6	0,9	23,7	0,9	6,8	0,8	L19593
Interleukin 1 receptor antagonist, type III	0,9	15,3	11,2	5,7	2,2	10,2	AJ005835
Antigen uptake							
CD1b	4,4	51,1	1488,2	71,2	102,7	13,8	M28826
CD1c	107,6	73,1	2792,4	187,2	198,4	45,4	M28827
CD1d	449,4	53,2	142,8	16,7	58,5	26,7	L38820
CD1e	11,7	25,3	1785,7	22	612,8	9,2	X14975
FcERI	37	4,4	1060,3	82,7	24,1	23,7	X06948
IgE receptor	9,1	8,4	891,4	719,7	62	67,8	M15059
beta-Fc-gamma-RII	183,9	753,4	630,8	3089,9	183,2	812,9	M28696
Fc alpha receptor, (CD89)	75	10	4,8	10	4,2	54,7	U43774
IgG Fc receptor I	185,5	130,4	7	165,7	108,4	223,6	M63835
Macrophage mannose receptor (MMR)	9,6	139,5	2170,6	2432,5	453,1	1852,4	M93221
CD14	1932,1	836,9	96,2	2874,4	2343,1	5116,5	X06882
Transcription and nuclear factors							
Nuclear orphan receptor LXR-alpha	6,6	755,3	36,2	219,6	305,1	977,3	U22662
NOTCH3	5,3	32,6	6,2	5,5	41,5	89,1	U97669
Nuclear factor kappa-B2 (NF-KB2)	5,7	24,2	29	15,4	162,6	96,6	U20816
RANK	2,9	17,2	78,8	33,4	75,4	3,5	AF018253
PPARγ	4,3	62,5	176,8	103,1	51,6	27,6	L40904
STAT4	13,2	64,2	30,9	45,6	58,4	211,8	L78440
Immunoglobulin like transcript							
ILT1/LIR-7	647,6	405,7	428,2	111,9	108,6	71	AF025531
Immunoglobulin-like transcript 1b	107,2	121,1	150,9	17,6	23	4,3	U82277
Immunoglobulin-like transcript 3 (ILT3)	335,1	1046,2	1252,1	1935,8	940,3	3905,9	AF072099
ILT4	177,4	29,8	12,3	65,4	16,1	105,6	AF004231
Lipid metabolism							
15-lipoxygenase	16,8	36,3	2439,1	1248,1	107	14	M23892
15S-lipoxygenase	6,5	86	41,3	54,6	260,6	73,9	U78294
Lipoprotein lipase (LPL)	22,1	2698,1	810,4	609,3	788	289,8	M15856
Apolipoprotein E	8,1	4786,6	773,5	1091,2	1660,1	2085,5	M12529
Cyclooxygenase-2 (hCox-2)	63,7	10,4	3,6	1,8	20,5	40,5	U04636
PA-FABP	22,4	493	433,2	148,2	168,8	110,9	M944856
Others							
DEC-205	63	189,2	562,5	92,8	860,2	197,1	AF011333
4-1BB	2	77	19,8	1,7	66,2	13	U03397
LFA-1 alpha subunit	183,7	91,3	63,6	15,3	123,4	51,3	Y00796
Macrophage receptor MARCO	99,4	52,1	29,6	780,8	84,5	1100,4	AF035819

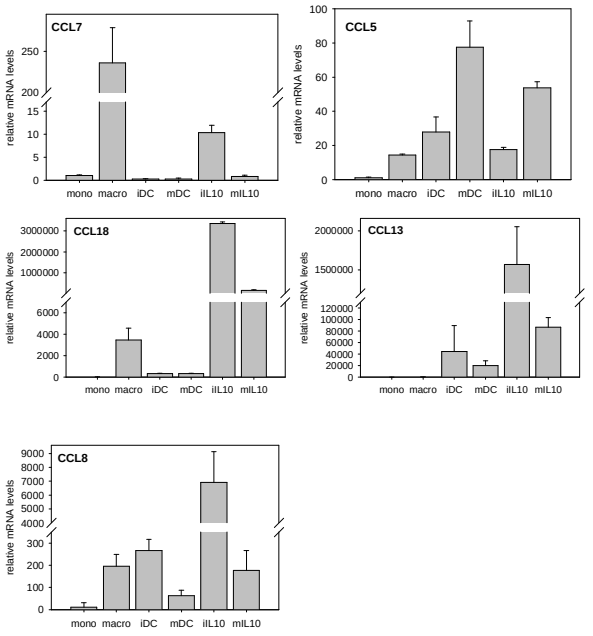

Fig. 2: The gene expression profile is distinct for the six populations**.** mRNA was extracted from a total of 50.000 cells. Quantitative RT-PCR was performed using SYBR green (Applied Biosystems) and detection was carried out on an ABI 7700. Ct value was set according to the recommendations and the relative level of mRNA in the samples was determined as described in Materials and Methods. Each reaction was carried out in triplicates and repeated three times with mRNA from populations generated from three different donors. Data was expressed as the mean +/- s.d. Mono: for monocytes; macro: macrophages. iIL-10 and mIL-10 is IL-10 treated Mo-DC at day 6 and 8. iMo-DC and mMo-DC is untreated Mo-DC at day 6 and 8.

6 Mo-DC show significant differences (table 2). The expression of genes coding for CCL13, CCL17 and CCL18 are high in IL-10 treated immature Mo-DC whereas the expression is almost absent in macrophages. Furthermore, the RNA expression pattern of immunoglobulin like transcripts also varies with higher expression of ILT1 in macrophages but lower expression of ILT3 and ILT4 compared to IL-10 treated immature Mo-DC. Finally, macrophages have significantly higher expression of genes coding for lipoprotein lipase and Apolipoprotein E than day 6 IL-10 treated Mo-DC. These data suggest that maturation of Mo-DC in the presence of IL-10 generates cells distinct from both Mo-DC and macrophages.

Semi-quantitative RT-PCR show variances in the expression profiles: The expression pattern of 12 genes encoding cytokines was reanalyzed with semi-quantitative RT-PCR to validate the data obtained with the GeneArray analysis. The analysis was repeated on three different donors that were all distinct from the person used for the GeneArray assay. We found that expression patterns of genes coding for CCL5, CCL7, CCL8, CCL13, CCL17, CCL18, and CCL19 in the 6 cell populations shown by semi-quantitative RT-PCR were similar to the patterns identified by the GeneArray analysis (see Fig. 2 and Fig. 3) thus confirming the results. In contrast, the expression of CCL22 changed between donors and no clear pattern could be observed (data not shown) Furthermore, a donor-dependent variance in the expression profiles was observed for macrophages with clear difference in the expression of macrophage markers like MARCO and of the factors like TGFβ, ILT3 and ILT4 (see table 2, Fig. 2 and Fig. 3). This may reflect differences in maturation state of the in vitro generated macrophages. We find that the expression level of CCL7 mRNA is high in macrophages whereas the expression of CCL8, CCL13 and CCL18 mRNA is high in day 6 IL-10 treated Mo-DC. Furthermore, expression profiles of IL-10, IL-12 p35 and p40 mRNA were also determined by semi-quantitative RT-PCR. The expression of IL-12 p35 mRNA was low in macrophages and untreated Mo-DC but almost absent in IL-10 treated Mo-DC (see Fig. 4). In contrast, high expression of IL-12 p40 mRNA was observed in untreated Mo-DC at day 8 whereas high expression of IL-10 mRNA was found in macrophages and day 6 IL-10 treated Mo-DC.

The stimulatory activity of Mo-DC is significantly reduced by IL-10: The stimulatory function of IL-10 treated or untreated Mo-DC at day 8 was analyzed in an allogeneic T cell stimulatory assay and compared with the activity of macrophages (see Fig. 5). Untreated Mo-DC induce high proliferation of allogeneic T cells, whereas both IL-10 treated Mo-DC and macrophages were poor stimulators.

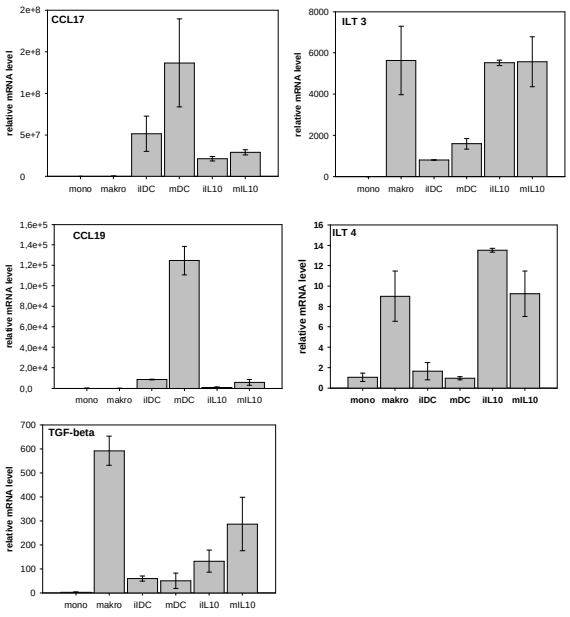

Fig. 3: IL-10 treated Mo-DC have high expression of negative regulators**.** Experiments were performed as described in the legends to Fig. 2.

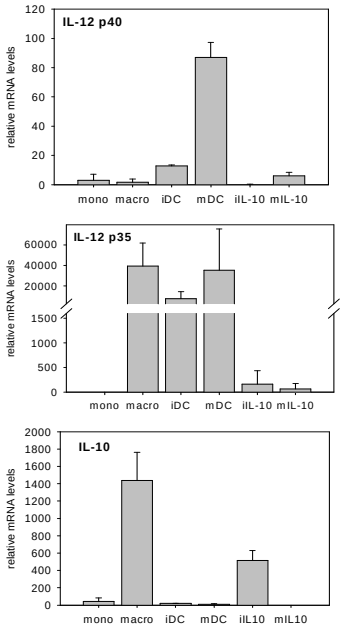

Fig. 4: The six populations have distinct expression pattern of IL-10, IL-12 p35 and IL-12 p40 mRNA. Experiments were performed as described in the legends to Fig. 2.

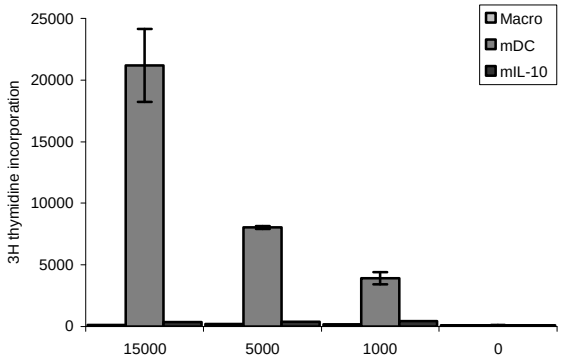

Fig. 5: hIL-10 inhibts the stimulatory activity of DC. DC were maturated in the absence or presence of hIL-10 for day 0 to day 6 and recultured in LPS for additional 2 days. The allogenic stimulatory activities of day 8 DC and macrophages were assessed by culturing 5×10^4 allogeneic T-lymphocytes with graded numbers of the different cell types. The proliferative response was determined by 3H-thymidine incorporation at day 5. Each reaction were carried out in triplicates and repeated at least three times. The data is represented as the mean +/- s.e. Macro: macrophages; mDC: untreated Mo-DC at day 8; mIL-10: IL-10 treated Mo-DC at day 8.

DISCUSSION

We show here that the phenotype and gene expression profile of Mo-DC is dramatically affected by treatment with IL-10. The observed gene expression profile of untreated Mo-DC resembles previously published data from human and mice [17,18]. Untreated Mo-DC at day 6 have the characteristic previously described for immature DC with high expression of factors involved in antigen uptake and homing receptors for the peripheral tissue. Upon maturation in LPS, Mo-DC resembling mature DC with expression of factors involved in antigen presentation and chemotaxis and activation of T cells are generated. This pattern is significantly altered by IL-10. At day 6, IL-10 treated Mo-DC like untreated Mo-DC have high expression of factors involved in antigen uptake like CD32, MMR and genes coding for several others Fc receptors. However, when IL-10 treated Mo-DC are maturated with LPS, these receptors and genes are not downregulated as seen in untreated Mo-DC. Likewise, the expression of costimulatory factors, HLA-DR, and genes coding for IL-12 p40 and chemokines involved in T cell chemotaxis are not increased. Instead, IL-10 treated Mo-DC have high expression of genes coding for CCL18, IL-8, CXCL2, CXCL3 and CXL5. Furthermore, the stimulatory activity of day 8 Mo-DC is significantly reduced by treatment with IL-10.

It has previously been suggested that the treatment of Mo-DC with IL-10 drives the maturation of macrophages [11]. However, when the signal strength of selected genes are compared we find that macrophages and day 6 IL-10 treated Mo-DC have very different gene expression profiles of especially genes coding for chemokines, several cell surface markers involved in antigen uptake and factors involved in lipid metabolism. These differences become even more significant upon LPS stimulation of the IL-10 treated Mo-DC. Therefore, we suggest that the maturation of monocytes in the presence of IL-10 is not simply redirecting the cells into macrophages. It can be argued that the RNA used for the GeneArrays may not be representative of fully mature macrophages as we observed a clear donor variation of factors like MARCO, ILT3, ILT4 and TGFβ. However, using flow cytometry we find that the phenotype of the cells used resembles macrophages (data not shown) and the gene expression profile is similar to what has previously been observed for macrophages generated in M-CSF [19].

Previous experiments suggest that the treatment of Mo-DC with IL-10 stimulates the maturation of tolerogenic DC that induce anergy in T cells [6,20,21]. Our data on gene expression profiles of IL-10 treated Mo-DC at day 8 support this hypothesis. At day 8, IL-10 treated Mo-DC have significantly higher expression of genes coding for among others CCL18, TGFβ, ILT3 and ILT4 than untreated Mo-DC. CCL18 has been suggested to direct the generation of T regulatory cells by promoting the colocalization of DC and naïve T cells in IL-10-dominated environment [22,23]. ILT3 and ILT4 are general features of tolerogenic DC [24,25], whereas TGFβ is a negative regulator of the primary immune response induced by Th1 [26]. Furthermore, IL-10 treated Mo-DC at day 8 have no expression of IL-12 p40 mRNA and only low expression of genes coding for CCL17 and CCL19 known to be involved in chemotaxis and activation of T

cells [15,16,4]. Indeed, we find that IL-10 treated Mo-DC are incapable of inducing an allogeneic T cell response. In contrast, we find that IL-10 treated Mo-DC have high expression of genes coding for CXCL2, CXCL3, CXCL5, CXCL8, all active on neutrophils [27] suggesting that IL-10 treated Mo-DC stimulate the activity of neutrophils. However, this has to be further investigated.

In conclusion, based on the different gene expression profiles our data suggest that the addition of IL-10 during differentiation of monocytes into Mo-DC directs the maturation of tolerogenic Mo-DC distinct from both ordinary Mo-DC and macrophages.

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