EXTENDED REPORT

Inhibition of TNFα during maturation of dendritic cells results in the development of semi-mature cells: a potential mechanism for the beneficial effects of TNFα blockade in rheumatoid arthritis

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Background: Dendritic cells orchestrate pivotal immunological processes mediated by the production of cytokines and chemokines.
Objective: To assess whether neutralisation of tumour necrosis factor α (TNFα) during maturation of dendritic cells affects their phenotype and behaviour, which might explain the beneficial effects of TNFα neutralisation in rheumatoid arthritis.
Methods: Immature and fully matured dendritic cells were cultured from blood monocytes from patients with rheumatoid arthritis and healthy controls following standardised protocols. TNFα was neutralised by addition of the p55 soluble TNFα receptor, PEGsTNFRI. The effect of TNFα neutralisation on the phenotype (CD14, CD16, CD32, CD64, CD80, CD83, CD86, and MHC) of dendritic cells was investigated by flow cytometry. Expression of chemokines (CCL17, CCL18, CCL19, CCL22, CCL3, and CXCL8) and production of IL1β and IL6 during dendritic cell differentiation and maturation were examined.
Results: Neutralisation of TNFα during the differentiation and maturation of dendritic cells did not result in an altered dendritic cell phenotype in the rheumatoid patients or the healthy controls. In contrast, the expression of CCL17, CCL18, CCL19, CCL22, CCL3, and CXCL8 by dendritic cells was significantly reduced when TNFα activity was inhibited during lipopolysaccharide triggered dendritic cell maturation. The production of IL1β and IL6 by mature dendritic cells was inhibited by PEGsTNFRI.
Conclusions: Inhibition of TNFα activity during dendritic cell maturation leads to the development of semi-mature cells. These data suggest a novel pathway by which the neutralisation of TNFα might exert its therapeutic effects.

Rheumatoid arthritis is a chronic autoimmune disease characterised by a symmetrical polyarthritis of the synovial joints leading to destruction of the cartilage and underlying bone. Although the exact mechanisms of disease pathogenesis are still unclear, there is a large body of evidence suggesting a critical role for inflammatory mediators such as cytokines and chemokines.[1–3]

Proinflammatory and anti-inflammatory cytokines—and more importantly, the balance between these opposing groups—are likely to play a crucial role in the onset and perpetuation of rheumatoid arthritis.[4–6] In this disease, interleukin 1β (IL1β), interleukin 6 (IL6), and tumour necrosis factor α (TNFα) are thought to be the key mediators, as concentrations of these cytokines are raised in the peripheral blood and the synovial compartment of patients with rheumatoid arthritis, and blockade of these mediators has proved to be a highly successful form of treatment.[7–9]

Chemokines are members of a superfamily of proteins that ensure that cell trafficking occurs in a proper temporal and spatial fashion.[10] There is evidence for an important role for the chemokines interleukin 8 (IL8; CXCL8), macrophage inflammatory protein 1α (MIP1α; CCL3), and fractalkine (CX3CL1) in rheumatoid arthritis.[11–13] The fact that the blocking of chemokine receptor 1 in patients with rheumatoid arthritis results in a significant reduction in synovial cellularity further substantiates the important role of chemokines in synovial inflammation.[14]

Previous work from our group has shown that dendritic cells from patients with rheumatoid arthritis express significantly higher levels of the chemokines CCL17, CCL18, CCL19, CCL22, CCL3, and CXCL8 than those from healthy donors.[15] In particular, chemokines CCL17, CCL18, and CCL19 were expressed at high levels, which is intriguing as these chemokines largely orchestrate the attraction of T cell subsets and might thus play an important role in rheumatoid pathology.

In rheumatoid arthritis, both the synovial tissue and synovial fluid contain large numbers of inflammatory cells, including T cells, fibroblasts, monocytes, macrophages, and dendritic cells.[16–18] Dendritic cells are professional antigen presenting cells (APC) that are crucial in directing tolerance and immunity as well as in the initiation of innate and adaptive immune responses.[19 20] Immature dendritic cells reside in the periphery and are specialised in uptake and processing of antigens. Maturation of these cells is triggered by a multitude of proinflammatory stimuli, and coincides with major changes in their phenotype and a loss of their ability to capture antigens.[21 22] To date, a large body of evidence points to an important role for dendritic cells in synovial inflammation.[22–26]

Abbreviations: APC, antigen presenting cells; CDC, dendritic cells from healthy controls; DAS28, 28 joint disease activity score; FACS, fluorescence activated cell sorting; IL, interleukin; LPS, lipopolysaccharide; MHC, major histocompatibility complex; MIP, macrophage inflammatory protein; RADC, dendritic cells from patients with rheumatoid arthritis; TNF, tumour necrosis factor

For optimal dendritic cell function, the phenotypic characteristics (signals 1 and 2) are crucial and are obligatory for T cell activation,[27–29] while the capacity of mature dendritic cells to prime naive T cells and promote their differentiation is attributed to their cytokine secretion pattern (signal 3).[30 31] While immature dendritic cells induce tolerance in the steady state, mature cells induce antigen specific immunity.[27 31 32] A disturbed balance of proinflammatory cytokines, which is undoubtedly present in rheumatoid arthritis, might therefore contribute to enhanced dendritic cell maturation, culminating in a disturbed balance between tolerance and (auto)-immunity in rheumatoid arthritis.

The potential role of TNFα in dendritic cell biology, along with the clinical effects of TNFα blockade in rheumatoid arthritis, prompted us to investigate the effects of TNFα blockade on dendritic cell development. We hypothesised that inhibition of TNFα during this process would interfere with dendritic cell maturation and reduce the production of inflammatory mediators, so leading to restoration of immunological balance. We show here that neutralisation of TNFα during dendritic cell maturation leads to the development of semi-mature dendritic cells.

METHODS

Patients

Eleven patients with active rheumatoid arthritis and 10 healthy volunteer controls were enrolled in the study. All patients fulfilled the American College of Rheumatology (ACR) criteria for rheumatoid arthritis and gave informed consent for the study.[33] We excluded patients who had been treated with systemic steroids or biological agents. All patients were on disease modifying anti-rheumatic drugs alone or in combination with non-steroidal anti-inflammatory agents. The DAS28 score was used to assess disease activity.[34]

The medical ethics committee of the University Medical Centre Nijmegen approved the study protocol.

Dendritic cell cultures

For cell cultures, 50 ml blood samples were taken from the rheumatoid patients and the controls into 10 ml heparinised Vacutainer tubes. Dendritic cells were cultured from peripheral blood monocytes using standardised protocols, essentially as previously described.[35] In brief, the procedures were as follows. Peripheral blood mononuclear cells were isolated by means of Ficoll gradient centrifugation (Ficoll Paque, Amersham Biosciences, Uppsala, Sweden). After several stringent washing steps, cells were incubated in six-well plates (Costar, Badhoevedorp, Netherlands) for one hour at 37°C to allow adherence of monocytes. Thereafter, adherent cells were cultured in medium (RPMI-1640 (Dutch modification), Life Technologies, Grand Island, New York, USA) supplemented with glutamine, antibiotics/antimycotics (both from Life Technologies), and 10% fetal calf serum (FCS) for six days in the presence of interleukin 4 (IL4) (500 U/ml) and granulocyte macrophage colony stimulating factor (GM-CSF) (800 U/ml) (Schering-Plough, Amstelveen, Netherlands). On day 6, a proportion of the immature dendritic cells was harvested for analysis of the immature state. To obtain full maturation, the remaining cells were cultured for two more consecutive days in the presence of 2 μg/ml *E coli* lipopolysaccharide (LPS) (Sigma-Aldrich, St Louis, Missouri, USA). On day 8, fully mature dendritic cells were harvested and studied.

Effective blockade of TNFα activity in dendritic cell cultures

For TNFα blockade, we used a p55 soluble TNFα receptor (PEGsTNFRI), kindly given to us by AMGEN®. To assess the amount of PEGsTNFRI needed for full inhibition of TNFα activity during the culture of dendritic cells, we determined the maximum production of TNFα by mature cells grown in one of our previous experiments.[34] To achieve efficient neutralisation of TNFα we added a 1000-fold excess of PEGsTNFRI to the cultures. On days 0, 3, and 6, PEGsTNFRI was added to the culture medium to examine its effect on the differentiation and maturation. As a control, carrier fluid provided by AMGEN® was added to the cultures where appropriate.

To check whether a 1000-fold excess of PEGsTNFRI was sufficient for maximum inhibition of TNFα, we carried out a bioassay using a 3T3 luciferase reporter cell line that is highly sensitive for mediators that trigger NFκB signalling, including TNFα and IL1β.[36] As an initial step, we carried out a dose–response curve analysis to test whether the PEGsTNFRI was capable of neutralising TNFα, and to study the value of our bioassay for this purpose. Second, the supernatant obtained from the dendritic cells cultured with or without PEGsTNFRI was added to the 3T3 cells and the luciferase response was studied. Another 1000-fold excess of PEGsTNFRI was added to the system to test whether maximum inhibition of TNFα had been achieved in our experimental setup.

Table 1 Primers and Taqman™ probes used for real time quantitative polymerase chain reaction (PCR) analysis[40]

	Primer		Probe	
Chemokine	5′ end	3′ end	5′ end	3′ end
DC-CK1 (CCL18)	CCTGGAGGCCACCTCTTCTAA TGCAGCTCAACAATAGAAATCAATT		AGTCCCATCTGCTATGCCCAGCCAC	
ELC (CCL19)	CAGAGGACCTCAGCCAAGATG TTCACAATGCTTGACTCGGACT		CCTATGACCGTGCAGAGGGAGCCC	
IL-8 (CXCL8)	AGAAGTTTTTGAAGAGGGCTGAGA CAGACCCACACAATACATGAAGTG		TCCAAGAATCAGTGAAGATGCCAGTGAAACTT	
MDC (CCL22)	GTCCTGTTCCCATCAGCGAT CAGGCTGGAGACAGAGATGGA		CCATGACTCCCCACTGCCCTAAGCT	
MIP-1α (CCL3)	TGTGTTTGTGATTGTTTGCTCTGA TGGTGCCATGACTGCCTACA		CCTTCCCTCACACCGCGTCTGG	
TARC (CCL17)	GCAAAGCCTTGAGAGGTCTTGA CGGTGGAGGTCCCAGGTAGT		CCTCCTCACCCCAGACTCCTGACTGTC	
GAPDH	GAAGGTGAAGGTCGGAGT AGATGGTGATGGGATTTC		CAAGCTTCCCGTTCTCAGCC	
PBGD	GGCAATGCGGCTGCAA GGGTACCCACGCGAATCAC		CTCATCTTTGGGCTGTTTTCTTCCGCC	

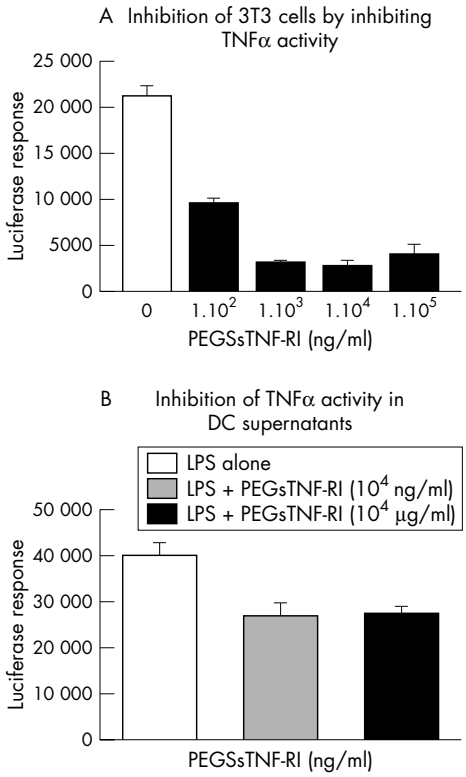

Figure 1 Inhibition of tumour necrosis α (TNFα) activity by the addition of the p55 soluble TNFα receptor PEGsTNFRI. (A) Inhibition of 3T3 cells (luciferase response) stimulated by TNFα (1 ng/ml) following the addition of various concentrations of PEGsTNFRI. (B) Neutralisation of TNFα activity in the supernatant of mature dendritic cells by addition of PEGsTNFRI. A 1000-fold excess of PEGsTNFRI was sufficient to provide maximum inhibition of TNFα activity, as a further 1000-fold excess did not cause any additional decrease in luciferase. DC, dendritic cell.

Determination of dendritic cells phenotype using FACS techniques

The phenotype of dendritic cells was characterised using flow cytometry techniques (fluorescence activated cell sorting, FACS) (FACSCalibur®, Becton-Dickinson, San Jose, California, USA). For this, dendritic cells were harvested and collected by centrifugation and further processed on melting ice. Cells were diluted in buffer solution (phosphate buffered saline (PBS) with 1% bovine albumin, pH 7.4) in a concentration of 1×10^6 cells/ml and plated in V shaped 96-well plates (1×10^5 cells per plate). Cells were labelled with monoclonal mouse antihuman antibodies against the monocyte markers CD14, FcγRI (CD64), FcγRII (CD32), and FcγRIII (CD16) (all from Dako, Glostrup, Denmark), DC-SIGN,[37] the co-stimulatory molecules CD80 (Becton Dickinson) and CD86 (PharMingen, San Diego, California, USA), the mature dendritic cell marker CD83 (Beckman Coulter, Mijdrecht, Netherlands), MHC-I (clone W6/32), and MHC-II (clone q1513), with mouse isotype control, and incubated at 4℃ for 45 minutes. Cells were then washed and labelled with goat antimouse FITC (Zymed Laboratories, South San Francisco, California, USA) as a secondary antibody. After another 30 minutes' incubation at 4℃, cells were again washed, diluted in buffer solution, and transferred into FACS tubes. Cell phenotype was then analysed using FACS.

Radioimmunoassay for IL1β production levels

When the dendritic cells were harvested, the supernatants were collected and stored for the measurement of cytokines. Polyclonal antibodies for IL1β were kindly provided by Sclavo (Siena, Italy). Human recombinant IL1β was radiolabelled using the chloramine-T method.[38] Briefly, all samples and standards were prepared and mixed with a standard buffer which contains 13 mM NA_2 EDTA, 0.02% sodium azide, 0.25% bovine serum albumin (Boehring, Marburg, Germany), and the inactivating agent aprotinine, pH 7.4 (Bayer, Leverkussen, Germany). For measurement of IL1β in supernatants, 10 µl of sample or standard were added to the buffer. The mixture was incubated for one day at room temperature. After the addition of tracer (≈7000 dpm/100 µl) the incubation was continued for two more days. Separation of bound and free tracer was achieved by the addition of 100 µl of a separation agent containing sheep antirabbit immunoglobulin G and 0.01% rabbit immunoglobulin G (Sigma). After incubation for 30 minutes, the antibody complex was completely precipitated by the addition of 1 ml of 7.5% polyethylene glycol 6000 (Merck, Darmstadt, Germany). The range of the standard curve was 20 to 3000 pg/ml, with a sensitivity of 40 pg/ml. To minimise interassay variation, all samples from the same experiments were analysed in the same run in duplicate. The inter-assay variation of our RIA is estimated at ⩽15%, whereas the intra-assay variation is ⩽10%.

Table 2 Phenotypical characteristics after lipopolysaccharide mediated maturation with or without the p55 soluble tumour necrosis α receptor PEGsTNFRI

	Healthy controls				Rheumatoid patients			
	LPS alone		LPS + PEGsTNFRI		LPS alone		LPS + PEGsTNFRI	
Marker	% Positive cells	MFI	% Positive cells	MFI	% Positive cells	MFI	% Positive cells	MFI
CD14	10 (4)	11 (3)	11 (5)	12 (4)	10 (3)	13 (4)	13 (4)	15 (6)
FcγRI	8 (2)	13 (4)	12 (2)	14 (2)	10 (3)	15 (4)	11 (4)	16 (4)
FcγRII	22 (4)	43 (8)	24 (7)	47 (8)	53 (6)*	83 (8)*	51 (9)*	84 (7)*
FcγRIII	12 (4)	17 (7)	14 (5)	16 (5)	26 (6)*	19 (4)	27 (5)*	18 (7)
DC-SIGN	71 (10)	29 (5)	71 (11)	27 (3)	76 (10)	26 (3)	79 (9)	29 (7)
CD80	97 (1)	62 (8)	94 (2)	53 (10)	95 (3)	81 (15)	95 (2)	63 (14)
CD83	87 (4)	30 (4)	77 (5)	25 (4)	87 (4)	38 (7)	77 (7)	32 (5)
CD86	97 (1)	113 (19)	94 (3)	99 (15)	97 (1)	108 (16)	89 (5)	102 (16)
MHC-I	96 (2)	112 (29)	95 (2)	92 (21)	96 (2)	106 (26)	94 (3)	149 (61)
MHC-II†	98 (1)	312 (118)	99 (1)	331 (98)	98 (1)	343 (112)	97 (2)	388 (123)

Values are mean (SD).
†MHC-II resembles HLA-DR/DP.
*Significant difference between dendritic cells from healthy donors and from rheumatoid patients, $p<0.05$.
LPS, lipopolysaccharide; MFI, mean fluorescence intensity.

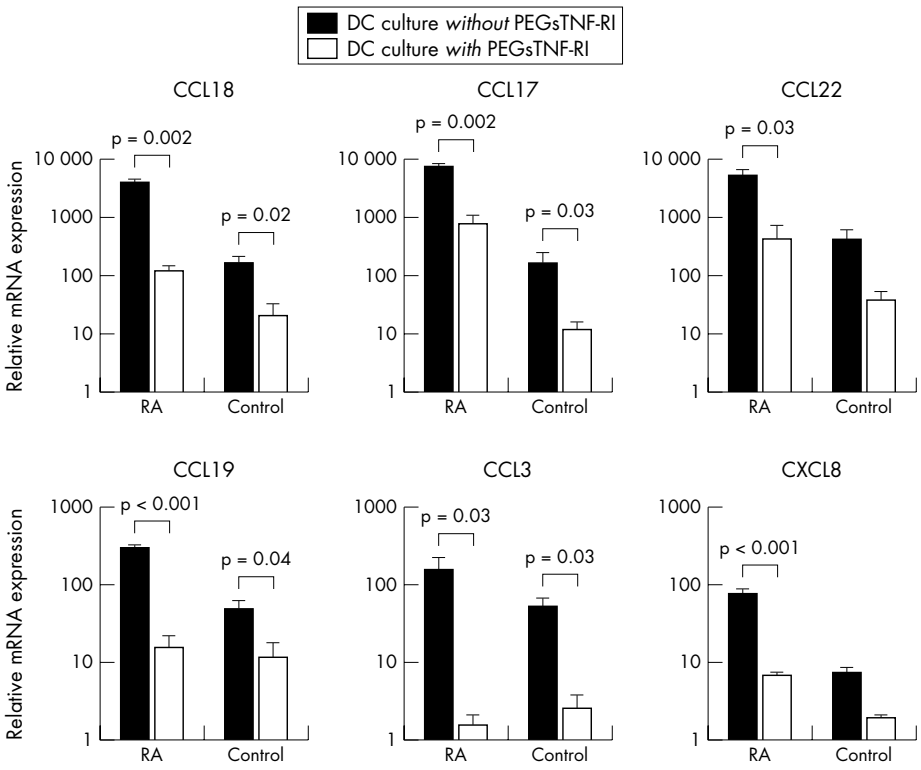

Figure 2 Decrease in chemokine expression by mature dendritic cells when tumour necrosis α (TNFα) activity was inhibited. Expression of the chemokines CCL17, CCL18, CCL19, CCL22, CCL3, and CXCL8 by lipopolysaccharide matured dendritic cells was measured using real time polymerase chain reaction techniques. A clear decrease in chemokine expression was observed when TNFα activity was inhibited during the maturation process in both rheumatoid patients and healthy controls.

Enzyme linked immunosorbent assay for IL6 production levels

IL6 was measured using a commercially available enzyme linked immunosorbent assay (ELISA) (Pelikine Compact™ human IL6 ELISA kit, CLB, Amsterdam, Netherlands) according to the manufacturer's instructions.[39]

Primers and probes for chemokine production measurements

The sequences of the primers (Life Technologies) and Taqman™ probes (PE Biosystems, Branchburg, New Jersey, USA) used in this study are given in table 1. The chemokine specific probes were labelled at the 5′ end with a FAM fluorescent group and at the 3′ end with a TAMRA quencher group. The probes specific for the housekeeping genes glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and porphobilinogen deaminase (PBGD) were labelled with a VIC fluorescent group at the 5′ end.

Real time polymerase chain reaction for chemokine production levels

Expression levels of CCL17, CCL18, CCL19, CCL3, CCL22, and CXCL8 were measured in six patients and six controls using real time polymerase chain reaction (PCR) techniques. After isolation of the dendritic cells, the cells were centrifuged and the pellet was dissolved in the Trizol reagent and stored at −70°C. Thereafter, RNA was extracted using reverse transcriptase polymerase chain reaction (RT-PCR) with 1 μg of RNA. OligoT primers were used for reversed transcription of mRNA. For PCR amplification, 5% of the cDNA was used. Our standard PCR buffer contained 200 μM dNTPs, 0.1 μM forward and reverse primer, and 1 unit Taq polymerase. We followed Taqman™ assay instructions for PCR procedures, with an end concentration of 175 nM probe and 600 nM primers. All PCR amplifications were done on an ABI/PRISM 7700 sequence detector system. This system produces a real time amplification plot based upon the normalised fluorescence signal. The expression levels of the chemokines were related to the expression level of PBGD, a housekeeping gene with intermediate expression levels. Another housekeeping gene, GAPDH, was used as an internal control for the amount of cDNA in each individual.

Statistical analysis

Comparisons between groups were assessed using paired *t* tests or Wilcoxon's signed rank test as appropriate. For statistical analysis, the GraphPad Prism® version 4 statistical program was used. Probability (p) values <0.05 were considered significant.

RESULTS

TNFα activity inhibition

The maximum production of TNFα by mature dendritic cells was around 5000 pg/ml, in line with a previous study.[35] To determine the concentration of PEGsTNFRI needed for optimal TNFα activity inhibition, we added different concentrations of the soluble TNFα receptor to 3T3 reporter cells which were stimulated with 1 ng/ml TNFα (fig 1A). A PEGsTNFRI concentration of 100 ng/ml decreased the luciferase response by 56% ($p<0.001$), while maximum (86%) inhibition of TNFα activity was achieved by 1000 ng/ml PEGsTNFR (a 1000-fold excess). A further increase in PEGsTNFRI did not result in greater inhibition of the luciferase response. In our supernatants obtained from fully mature dendritic cells, there was a 33% inhibition of the luciferase response after the addition of a 1000-fold excess (10^4 ng/ml) of PEGsTNFRI (fig 1B). A further 1000-fold increase in PEGsTNFRI did not result in a further decrease in

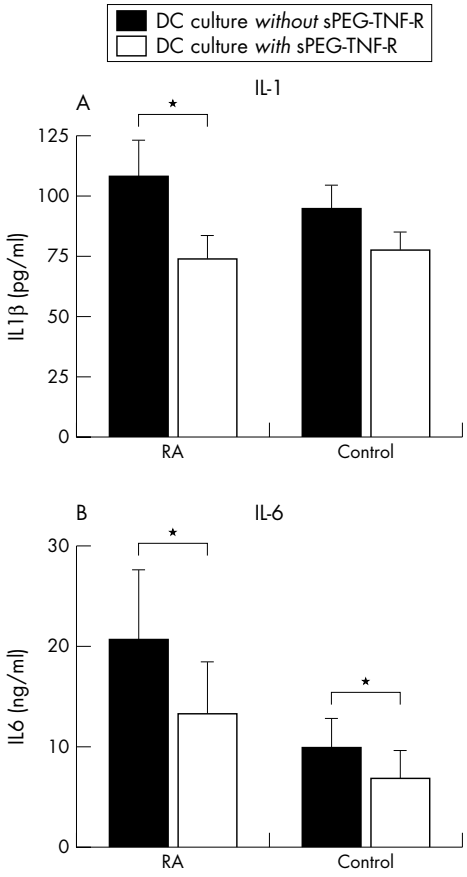

Figure 3 Inhibition of tumour necrosis factor α (TNFα) by PEGsTNFRI during dendritic cell maturation resulted in decreased production of interleukin 1β (IL1β) and interleukin 6 (IL-6). (A) Production of IL1β. (B) IL6 secretion. Both proinflammatory mediators were significantly reduced when TNFα was neutralised during dendritic cell maturation triggered by the addition of lipopolysaccharide. Although the production of both IL1β and IL6 by dendritic cells from rheumatoid patients was significantly greater than that from controls, the decreased production because by blockade of TNFα was similar in the two groups. DC, dendritic cells; RA, rheumatoid arthritis.

the luciferase response, indicating that maximum neutralisation of TNFα in the supernatant had been achieved. The remaining luciferase response probably resulted from other nFκB activating cytokines in the supernatant.

Effect of TNFα blockade on dendritic cell differentiation and maturation

We next investigated the effect of TNFα neutralisation during monocyte differentiation into immature dendritic cells. We added PEGsTNFRI (1000-fold excess) to the monocytes on day 0 and day 3 during dendritic cell development and compared the phenotype of the resulting immature dendritic cells with cells cultured under the same conditions but in the absence of PEGsTNFRI. Both cell cultures expressed high levels of FcγRI, II, and III, intermediate levels of the markers CD80, CD86, and MHC-I and II, and low levels of CD14 and CD83—typical phenotypical features of the immature dendritic cell.[41] In line with previous observations, expression of FcγRII was increased in immature dendritic cells from patients with rheumatoid arthritis (henceforth referred to as RADC) compared with the healthy controls (CDC) (table 2). However, no differences in dendritic cell phenotype were detected between cells cultured with and without PEGsTNFRI, either in the rheumatoid group or the control group, nor did the addition of PEGsTNFRI affect the phenotype of dendritic cells after LPS mediated maturation in either group. In contrast, a clearly increased expression of costimulatory molecules and MHC was observed in both groups, whereas expression of FcγRs was downregulated, resulting in a mature dendritic cell phenotype (table 2) even when TNFα was inhibited to the maximum extent.

Decreased expression of CCL17, CCL18, CCL19, CCL3, CCL22, and CXCL8 after TNFα neutralisation

As we found previously that RADC expressed higher levels of chemokines and proinflammatory cytokines than dendritic cells from healthy controls, we examined whether this process was TNFα driven. To do this, we investigated the effect of TNFα neutralisation on the expression of several dendritic cell specific and non-specific chemokines during LPS triggered dendritic cell maturation. Fully matured RADC expressed significantly higher levels of the chemokines CCL17, CCL18, CCL19, CCL22, CCL3, and CXCL8 than CDC (fig 2). Intriguingly, the neutralisation of TNFα during maturation of RADC resulted in decreased production of CCL18 (mean (SEM), pg/ml: 3514 (581) *v* 114 (27), $p = 0.002$), CCL17 (7261 (935) *v* 424 (178), $p = 0.002$), CCL22 (4636 (1278) *v* 405 (268), $p = 0.03$), CCL19 (281 (35) *v* 15 (6), $p<0.001$), CCL3 (147 (66) *v* 1.5 (1.0), $p = 0.03$), and CXCL8 (76 (10) *v* 7 (1), $p<0.001$). A similar trend was observed on chemokine expression by dendritic cells from healthy donors, although this effect failed to reach statistical significance for CCL22 ($p = 0.09$) or CXCL8 ($p = 0.06$). Whereas inhibition of TNFα activity in dendritic cell cultures from rheumatoid patients led to a significant decrease in chemokine expression, expression of CCL17, CCL18, CCL19, CXCL8, and CCL22 remained increased compared with healthy controls. This suggests that chemokine expression is not driven by TNFα alone.

Decreased production of IL1β and IL6 by mature dendritic cells after TNFα inhibition

As IL1 and IL6 are defined as key inflammatory mediators in rheumatoid arthritis, we investigated whether inhibition TNFα during dendritic cell culture influenced the secretion of these mediators. As with the expression of chemokines, the secretion of both IL1 (mean (SEM): 108 (16) *v* 74 (10) pg/ml, $p = 0.02$) and IL6 (21 282 (6543) *v* 13 482 (5037) pg/ml, $p = 0.007$) decreased significantly when TNFα was neutralised during maturation of RADC (fig 3). Similarly, the addition of PEGsTNFRI during maturation of CDC also resulted in a decreased production of IL6 (10 040 (3200) *v* 6970 (2100) pg/ml, $p = 0.005$), although the decrease in IL1 secretion was not significant (94 (10) *v* 78 (8) pg/ml, $p = 0.08$).

DISCUSSION

Our results show that the neutralisation of TNFα during dendritic cell maturation does not inhibit the maturation process as expressed by the phenotype but does lead to decreased production of inflammatory mediators. This reflects the development of the so called ''semi-mature'' dendritic cells. The presence of these semi-mature cells might at least partly explain the therapeutic effects of neutralising TNFα in vivo.

Autoimmunity is characterised by a loss of tolerance to the body's own constituents that results in a destructive process directed to a specific organ. The major goal in the treatment of autoimmune diseases would be the inhibition of antigen presenting cell function and the generation of tolerogenic dendritic cells.[32] As rheumatoid arthritis is an autoimmune disease, dendritic cells are an attractive therapeutic target. Their importance in synovial inflammation was suggested by the fact that mature dendritic cells are present in synovial

tissue of rheumatoid patients and are located strategically in well organised structures.[22 23 26] Furthermore, dendritic cells are able to trigger and abrogate experimental arthritis, depending on the time of administration and the experimental setup.[24 25] Modulation of dendritic cells in rheumatoid arthritis might therefore be used to combat autoimmunity, as has already been achieved successfully in other autoimmune diseases.[42]

Dendritic cells are professional antigen presenting cells that play a critical role in the fine tuning of the balance between immunity and tolerance. While immature dendritic cells are perfectly adapted for antigen uptake and processing, their maturation leads to functional changes that enhance their ability to attract and activate T cells.[31 43] However, recent evidence has challenged this oversimplified model of immature and mature dendritic cells.[44 45] Lutz and coworkers suggested that cytokines produced by fully matured dendritic cells are crucial for T cell immunity and proposed the theory of so called semi-mature dendritic cells, which resemble the mature cells in terms of their phenotype but produce low levels inflammatory mediators, and are critical for induction of tolerance.[45] However, the level of T cell stimulation remains strongly dependent on the signals mediated through various molecules expressed on the dendritic cell surface. The first signal accounts for the specificity of the immune response and involves the engagement of T cell receptors by an appropriate peptide–MHC complex. Second, interaction between costimulatory molecules (signal 2) is required and determines the quality and fate of the immune response. Finally, proinflammatory mediators are thought to function as additional signals (third signal), which direct T cells in harmony with signals received through the T cell receptors and costimulatory molecules.[30] The type and quantity of these signals is likely to determine the fate of the T cells. It is therefore tempting to speculate that these so called semi-mature dendritic cells drive the immune response toward the induction of T cell anergy.

We have recently shown that dendritic cells obtained from rheumatoid patients produce higher levels of proinflammatory mediators than those from healthy controls.[35] This suggests that in rheumatoid arthritis, dendritic cells possess a lower threshold for activation, which might lead to an altered balance between tolerance and (auto)immunity. The fact that the production of inflammatory mediators by dendritic cells is at least partly inhibited by the neutralisation of TNFα during dendritic cell maturation suggests that this might be one explanation for the clinical success of TNFα blockade. However, the production of inflammatory mediators by dendritic cells in rheumatoid arthritis remains increased despite the inhibition of TNFα, which suggests the involvement of other pathways.

The exact mechanisms whereby TNFα inhibition has its beneficial effects have not been defined. Nevertheless, the critical role of TNFα in dendritic cell development was recently shown in experimental models of arthritis and indicated that TNFα has potentially opposing effects depending on the maturational stage of the dendritic cells.[24 46] Another effect of TNFα blockade in rheumatoid arthritis might be the control of the chemoattraction of inflammatory cells into the synovial compartment. The decreased expression of chemokines by dendritic cells after the inhibition of TNFα in vitro suggests an important role for TNFα in the control of cell influx in vivo. In fact, a critical role for TNFα in the fine tuning of cell influx was demonstrated recently in synovial sections of patients after the administration of anti-TNFα.[47]

Besides the development of semi-mature dendritic cells and a diminished production of chemokines, our current study shows that other mechanisms might explain the beneficial effects of TNFα inhibition. One of these is the deceased production of IL6, high levels of which are known to block the suppressive effect of CD4+CD25+ T regulatory cells and skew the differentiation of monocytes towards macrophages.[48 49] The inhibition of IL6 secretion may thus potentially restore the function of these regulatory T cells and lead to the development of immature dendritic cells designed to restore tolerance. The beneficial results derived from clinical trials of IL6 blockade emphasise its potential role in rheumatoid arthritis.[9]

Although inhibition of TNFα has sparked a revolution in the treatment of rheumatoid arthritis, full inhibition is accompanied by side effects such as serious infections,[50] SLE-like disease,[51] and symptoms resembling multiple sclerosis.[40] Detailed information on the actions of TNFα and its inhibition in the modulation of the immune system would provide increase our understanding of these issues.

Conclusions

We provide evidence for potential new mechanisms whereby neutralisation of TNFα may achieve its beneficial effects in clinical practice. These mechanisms include the development of semi-mature dendritic cells and decreased chemoattraction following inhibition of chemokine production. Further research into the precise mechanisms of TNFα on dendritic cell mediated T cell polarisation and chemokine production is warranted.

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