J Mol Med
DOI 10.1007/s00109-014-1200-z

JMolMed

ORIGINAL ARTICLE

Decreased expression of HLA-DQ and HLA-DR on cells of the monocytic lineage in cystic fibrosis

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Received: 27 March 2014 / Revised: 15 July 2014 / Accepted: 29 July 2014
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Abstract
We studied HLA class II molecules on blood monocyte subsets, blood dendritic cells, sputum macrophages, and monocyte-derived macrophages at the protein (flow cytometry) and mRNA level (RT-PCR) in adult patients with cystic fibrosis (CF) and healthy control subjects as putative contributors to the CF phenotype. In healthy donors, we found a high average HLA-DQ expression of 4.35 mean specific fluorescence intensity units (ΔMnI) on classical blood monocytes. In F508del homozygous CF patients, the average ΔMnI was low (1.80). Patients were divided into two groups, in which 14 of these patients had HLA-DQ expression above 2 ΔMnI (average 3.25 ΔMnI, CF-DQ^{group1}) and 36 below (average 1.24 ΔMnI, CF-DQ^{group2}). Also, the CD16-positive monocyte subset and blood dendritic cells showed much lower levels of HLA-DQ for the CF-DQ^{group2} patients compared with healthy controls. In macrophages from sputum and derived from monocytes, in vitro HLA-DQ expression was dramatically decreased to background levels in CF-DQ^{group2}. MHC class II transcripts were reduced in CF with a sevenfold decrease in HLA-DQβ1 for CF-DQ^{group2} patients. Higher levels of the inflammation marker CRP were associated with low HLA-DQ protein expression, and in vitro treatment with the inflammatory molecule lipopolysaccharide reduced HLA-DQ expression. Interferon γ (IFNγ) could overcome this effect in healthy donor cells while, in CF, the IFNγ-induced activation was impaired. Our data demonstrate a pronounced reduction of HLA-DQ expression in CF, which is associated with inflammation and a reduced response to IFNγ.

Key message

- CF patients show a reduced expression of MHCII molecules in monocytes and macrophages.
- HLA-DQ and HLA-DR transcript levels are also reduced in CF patients.
- CF patient C-reactive protein levels correlate with low HLA-DQ expression.
- Reduced expression of MHC class II molecules appears to be linked to inflammation.
- CF patients exhibit an impaired response to IFNgamma.

Keywords Cystic fibrosis · HLA-DQ · HLA-DR · Inflammation · Monocytes, macrophages and dendritic cells

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Published online: 23 August 2014

Introduction

Cystic fibrosis (CF) is an autosomal recessive disease caused by mutations in the *cystic fibrosis transmembrane conductance regulator* gene (*CFTR*). This gene encodes for a transmembrane protein, which is a chloride ion channel that regulates salt and fluid transport in cells in various organs including lungs, intestine, reproductive tract, and glands.

The F508del mutation accounts for approximately 70 % of all CF alleles and is found in up to 90 % of CF patients. The mutation results in a loss of the amino acid phenylalanine at position 508 and leads to a functionally reduced protein that is not transported to the apical plasma membrane [1].

Regarding the lungs and following the airway surface liquid volume theory, this leads to dehydration of the airway surface [2–4], which impairs mucociliary clearance, innate and adaptive immune function, and other defense mechanisms [5]. This contributes to an environment that supports microbial colonization and infection [6].

A central step of the adaptive immune response is antigen presentation to CD4 T cells, and this is efficiently performed by MHC (major histocompatibility complex) class II-positive monocytes and macrophages. Monocytes are phagocytes that circulate in blood for 2–3 days and, from there, can go into tissue. They are subdivided into classical CD16-negative cells and the CD16-positive monocytes. The latter cells show a pro-inflammatory cytokine profile, and they have a higher level of MHC class II expression compared with the classical monocytes [7]. Macrophages are more mature cells of that lineage, and they reside in the various tissues including the lungs, and here, they form an early line of defense against pathogens. A separate lineage of MHC class II-positive antigen-presenting cells is formed by the dendritic cells (DCs), which can be found in blood and in tissue. In human blood, they are subdivided into three subsets, i.e., the CD1c+ myeloid DCs, the CD141+ myeloid DCs, and the CD303+ plasmacytoid DCs [8].

All of these cells are capable of phagocytosis of microbes and of processing the microbial proteins into small peptides [9]. These peptides are then loaded into a cleft of the MHC class II molecule, and this complex then travels to the surface of the antigen-presenting cell. Here, the complex of class II and peptide is recognized by the T cell receptor of CD4 helper cells [10]. The CD4 cells produce various cytokines and thereby orchestrate a specific immune response. Among the MHC class II molecules of man, the molecules HLA (human leukocyte antigen)-DR and HLA-DQ are most prominent [11].

HLA class II molecules are discussed as possible modifiers in CF, because polymorphisms in DR4 and DR7 alleles and the DR7/DQA*0201 haplotype contribute to the CF-associated pulmonary phenotype [12]. In addition to polymorphisms, the level of cell surface expression of MHC class II molecules can impact on the efficiency of antigen presentation and of the subsequent immune response.

Therefore, we herein have investigated the expression of MHC class II molecules on monocytes, macrophages, and DCs, and we show that a subgroup of CF patients exhibit reduced expression which is most pronounced for HLA-DQ, both at the mRNA and protein levels.

Materials and methods

Donors

EDTA-blood was drawn, and induced sputum was collected from healthy human volunteers and from 50 CF patients aged between 20 and 50 (32.74±7.83 years) and homozygous for F508del. All patients with CF were recruited at the outpatient clinic for adult cystic fibrosis patients (Medizinische Klinik Innenstadt, Ludwig-Maximilians University, Munich). The patients were monitored on a regular basis at least twice a year; clinical data were retrieved from the doctors' reports including health status, clinical chemistry, medication, lung function test.

Written informed consent was obtained from each individual. The protocol was approved by the Ethics Committee of the Medical School of the Ludwigs-Maximilians University (Munich, Germany).

Isolation of PBMC, enrichment of monocytes and generation of monocyte-derived macrophages (MDM)

From whole blood, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Lymphoprep (Axis-Shield plc). For lipopolysaccharide (LPS) treatment of PBMC, LPS from *Salmonella enterica Minnesota* (Sigma-Aldrich) was used (10 ng/ml). For enrichment of monocytes, PBMC were plated on a 24-well plate (Costar) in supplemented and LPS-free RPMI 1640 medium [13]. Cells were cultured for 2 days with 100 ng/ml M-CSF (rhM-CSF, kindly provided by Genetics Institute, USA). On day 2, medium and non-adherent cells were resuspended and discarded. After rinsing the wells with phosphate buffered saline (PBS) twice, fresh medium supplemented with 100 ng/ml M-CSF was added, and incubation was continued for three more days. On day 5, the cells were rinsed with PBS twice, and adherent MDM were harvested using a cell scraper. Maturation of MDM was monitored by increase in size measured by flow cytometry.

Enrichment of CD14++ monocytes

For enrichment of CD14++ monocytes, the MACS magnetic separation technique was used (Miltenyi Biotec). The detailed procedure is described in Eder et al. [13]. In short, PBMC were first depleted of CD16-positive cells, and then CD14++ cells were positively selected. Purity of the CD14++ monocytes was determined by flow cytometry, and cells were used for mRNA expression analysis or were treated for 48 h with LPS (10 ng/ml, *S. enterica Minnesota*, Sigma-Aldrich) and IFNγ (1,000 U/ml, Imukin, Boehringer Ingelheim).

Sputum induction and processing

Sputum macrophages were collected from healthy human volunteers and from adult CF patients described previously [14]. Briefly, the subjects were instructed in stepwise inhalation of increasing concentrations of saline solutions (concentrations from 0.9 % up to 3 % in CF and to 5 % in healthy controls) and to cough into Petri dishes. Sputum samples were then processed, and purity was analyzed by flow cytometry.

Flow cytometry

Flow cytometery analysis of HLA-DQ and HLA-DR expression on blood monocytes and blood dendritic cells was performed in erythrocyte-lysed whole blood. MDM and sputum macrophages were stained and analyzed in phosphate buffer. Cells were stained with HLA-DQ-FITC, Isotype IgG1-FITC (BD) and HLA-DR-FITC and Isotype IgG1-FITC (Coulter). To distinguish between CD14++ (classical) and CD16-positive blood monocyte subsets [8], samples were additionally stained with and gated on CD14-PC5 (Coulter) and CD16-PE (BD) [15]. Sputum macrophages were stained with HLA-DQ-FITC or HLA-DR-FITC (or their isotype controls) and CD14-PC5 (Coulter) and gated on CD14 to exclude contamination of granulocytes. CD1c+ and CD141+ myeloid and CD303+ plasmacytoid blood dendritic cell subpopulations were stained with CD1c-PE (Miltenyi) and CD19-APC (Pharmingen) to exclude B-lymphocytes, CD141-PE (Miltenyi), and CD303-PE (Miltenyi), respectively.

An EPICS XL (Coulter) and a FACSCalibur (Becton Dickinson) flow cytometer were used. Delta mean intensity (ΔMnI) was calculated by subtraction of MnI of the isotype control from the MnI of the specific antibody staining.

Total RNA isolation and RT-PCR

Total RNA was extracted from cells by using TRI Reagent (Sigma) according to the manufacturer's instruction. RNA was reverse-transcribed with oligo(dT) as primer. Using the LightCycler system (Roche Diagnostics, Mannheim, Germany) according to the manufacturer's instruction, semi-quantitative PCR was performed with the following primers:

DQα1	fwd: CGTTGCCTCTTGTGGTGTAAAC
	rev: GTCACGGGAGACTTGGAAAACA
DQβ1	fwd: ACTTCACCAACGGGACAGAG
	rev: AACCACCGGACTTTGATCTG
DRβ1	fwd: TGGTTTCTATCCAGGCAGCA
	rev: CATTCCACTGTGAGAGGGCT
DPα1	fwd: GAGGACTTAGGAGAGATCTG
	rev: AGGGTATTCTCGGGAAGGTG
CIITA	fwd: CCTGCTGTTCGGGACCTAAAG
	rev: GGATCCGCACCAGTTTGGGG
α-enolase	fwd: GTTAGCAAGAAACTGAACGTCACA
	rev: TGAAGGACTTGTACAGGTCAG

All samples are processed in the Light-Cycler software with the same settings (e.g., same thresholds). Cycle number of the target gene was subtracted from the corresponding α-enolase housekeeping gene, and its absolute value was subsequently calculated to the power of 2. Genes with a higher cycle number than the corresponding housekeeping gene were plotted to the negative scale.

Statistical analysis

For statistical analysis, we used the Mann–Whitney U test and Spearman's correlation. For sample sizes≥50, Student's $t$test was used. Results were considered significant when $p<0.05$.

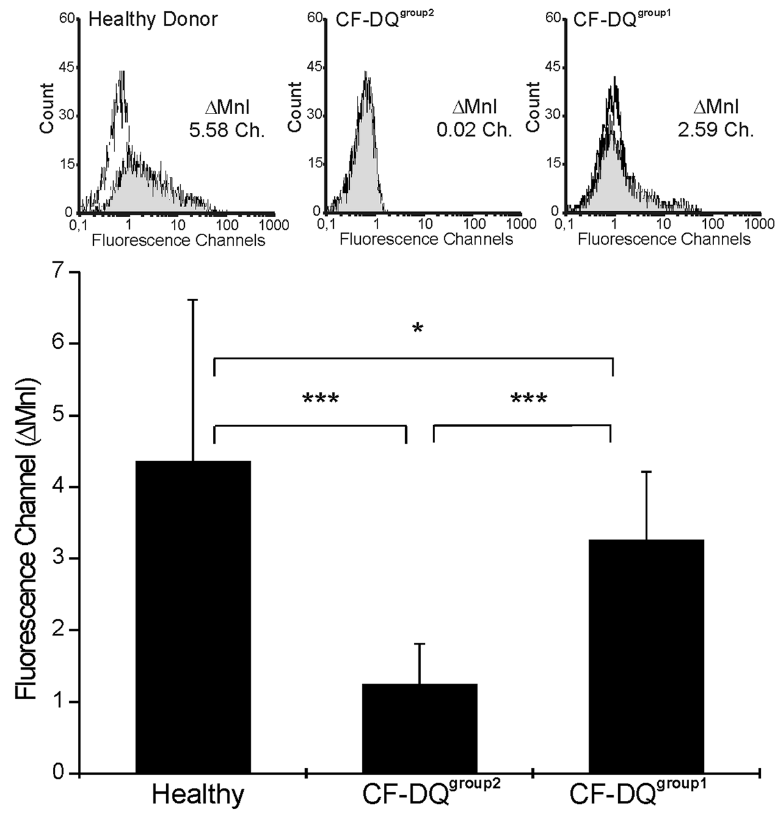
Results

HLA-DQ and HLA-DR protein expression on blood monocytes

Whole blood from 20 healthy controls and 50 patients homozygous for the F508del mutation of the *CFTR* gene was stained with specific antibodies against HLA-DQ and HLA-DR followed by flow cytometry analysis of their cell surface expression on blood monocytes.

Healthy donors show a strong cell surface expression of HLA-DQ. A typical example in Fig. 1a (upper panel) shows a mean specific fluorescence intensity channels (ΔMnI) of 5.58. On average for 20 healthy control donors, the ΔMnI was 4.35±2.27 (Fig. 1a lower panel, range 2.09 to 10.18 ΔMnI). In CF patients, monocytes show much lower levels of HLA-DQ with an average of 1.8±1.15 ΔMnI (range, 0 to 4.95 ΔMnI).

Since all healthy donor samples show HLA-DQ levels above 2, we used the 2 ΔMnI value as a cut-off to define a group with a level above 2 (CF-DQ^{group1}) and a group with a level below 2 (CF-DQ^{group2}). Examples for the expression of


a
HLA-DQ on blood monocytes
Healthy Donor
CF-DQ group2
CF-DQ group1
Count
ΔMnI
5.58 Ch.
0.02 Ch.
2.59 Ch.
Fluorescence Channels
Fluorescence Channel (ΔMnI)
*
***
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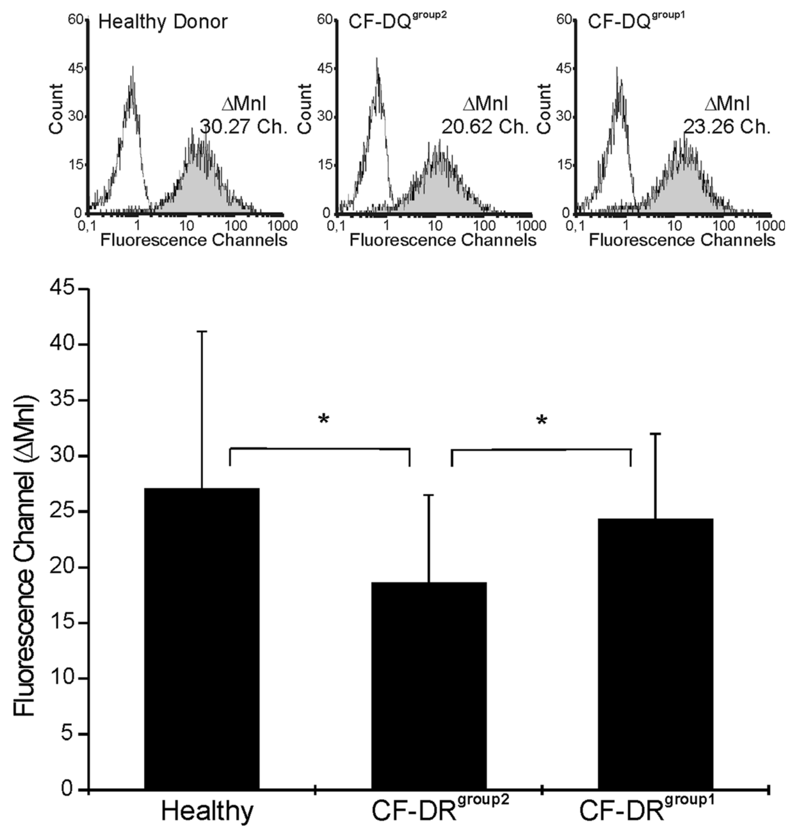
Healthy
CF-DQ group2
CF-DQ group1


b
HLA-DR on blood monocytes
Healthy Donor
CF-DQ group2
CF-DQ group1
Count
ΔMnI
30.27 Ch.
20.62 Ch.
23.26 Ch.
Fluorescence Channels
Fluorescence Channel (ΔMnI)
*
*
Healthy
CF-DR group2
CF-DR group1

◀ **Fig. 1** Cell surface protein expression of HLA-DQ (**a**) and HLA-DR (**b**) on human blood monocytes. **a** The *upper panel* shows typical histograms with isotype control (*white histogram*) and specific antibody (*grey histogram*) for a healthy donor, a CF patient with low DQ expression (CF-DQ^{group2}) and a CF patient with nearly normal level of DQ (CF-DQ^{group1}). The mean intensity channels (ΔMnI) are given on the *right side within the histograms*. The *lower panel* shows results from 20 healthy donors and 50 CF patients. CF patients are grouped into CF-DQ^{group2} (n=36) and CF-DQ^{group1} (n=14) individuals with a threshold of two fluorescence channels (mean±SD). In **b** the HLA-DR, expression is shown for the same individuals accordingly (*p<0.05, ***p<0.001)

HLA-DQ for CF-DQ^{group1} with a ΔMnI of 2.59 and for CF-DQ^{group2} with a ΔMnI of 0.02 are shown in Fig. 1a. Thirty-six out of 50 CF patients showed DQ values lower than 2 with an average of 1.24±0.57. Statistical analysis comparing DQ levels for this CF-DQ^{group2} group and the healthy controls showed a highly significant difference (p<0.001). In contrast to this, the remaining 14 patients which showed DQ levels above 2 had an average of 3.25±0.96 ΔMnI (p=0.032 compared with controls; p<0.001 compared with CF-DQ^{group2}).

The expression level for HLA-DR in healthy donors is much higher compared with DQ as can be seen in a side by side comparison in Fig. 1b, where the ΔMnI for DR is 30.27 in this example. For all 20 controls, the expression level for HLA-DR on classical monocytes was 27.03±14.16 ΔMnI (Fig. 1b). DR expression on monocytes from the CF-DQ^{group2} patients was 20.62 ΔMnI in a representative example. Although the average expression level for the 36 CF-DQ^{group2} patients at 18.62±7.78 ΔMnI was only moderately decreased, it was significant at p=0.011. DR expression in a representative CF patient from DQ^{group1} was 23.26 ΔMnI. On average, 14 patients from CF-DQ^{group1} had DR expression of 24.27±7.75 ΔMnI (p=0.015 compared with CF-DQ^{group2}; n.s. CF-DQ^{group1} compared with controls).

Since inflammation has been shown to downregulate HLA-DR expression [16], we asked whether an increase of the inflammatory marker CRP might be linked to lower HLA-DQ expression levels. In fact, patients with elevated CRP values (≥0.5 mg/dl) show a significantly lower expression of HLA-DQ (1.44±0.92 ΔMnI vs. 2.05±1.24 ΔMnI in normal CRP group, p=0.024) and a lower expression of HLA-DR (16.23±5.7 ΔMnI vs. 23.07±8.56 ΔMnI in normal CRP group, p<0.001) on blood monocytes (Fig. 2).

The peripheral blood monocytes can be divided into two subsets, which are represented by classical CD14++ CD16– monocytes (this means monocytes which are highly positive in CD14 (CD14++) and negative in CD16 (CD16-)) and CD16-positive monocytes. Compared with classical monocytes, CD16-positive monocytes from healthy controls show a high expression of HLA-DQ (in average 10.39±8.61 ΔMnI), and again, CD16-positive monocytes from the CF-DQ^{group2} group showed a pronounced decrease of HLA-DQ expression (in average 2.0±1.6 ΔMnI, p=0.002) while CF-DQ^{group1} patients were lower than but not significantly different from controls (in average 7.12±2.23 ΔMnI; Fig. 3).

HLA-DQ protein expression on macrophages

On sputum macrophages, HLA-DQ expression was high in healthy donors (Fig. 4a left, 28.80 ΔMnI) and CF patients

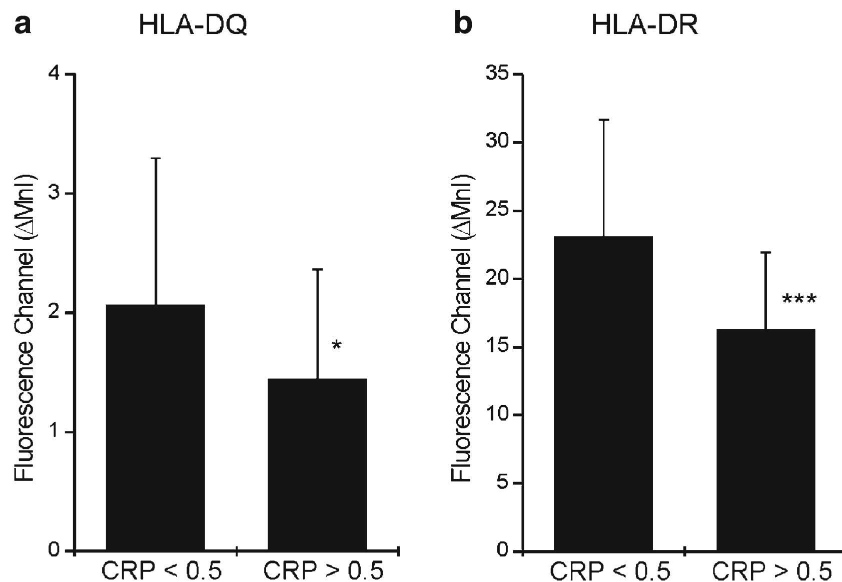


Fig. 2 Correlation between C-reactive protein (CRP) values and HLA-DQ (**a**) and HLA-DR (**b**) expression on monocytes. CF patients with pathological levels of CRP≥0.5 mg/dl (n=21) show a significant lower surface expression of **a** HLA-DQ (p=0.024) and **b** HLA-DR (p<0.001) on blood monocytes compared with patients with non-pathological levels of CRP<0.5 mg/dl (n=29). In the group of patients who exhibited elevated CRP levels above 0.5 mg/dl, only two patients out of 21 were high in HLA-DQ expression on monocytes (CF-DQ^{group1}). In the group of patients with CRP levels below 0.5 mg/dl, 12 out of 29 patients were high in HLA-DQ (CF-DQ^{group1}) (*p<0.05, ***p<0.001)

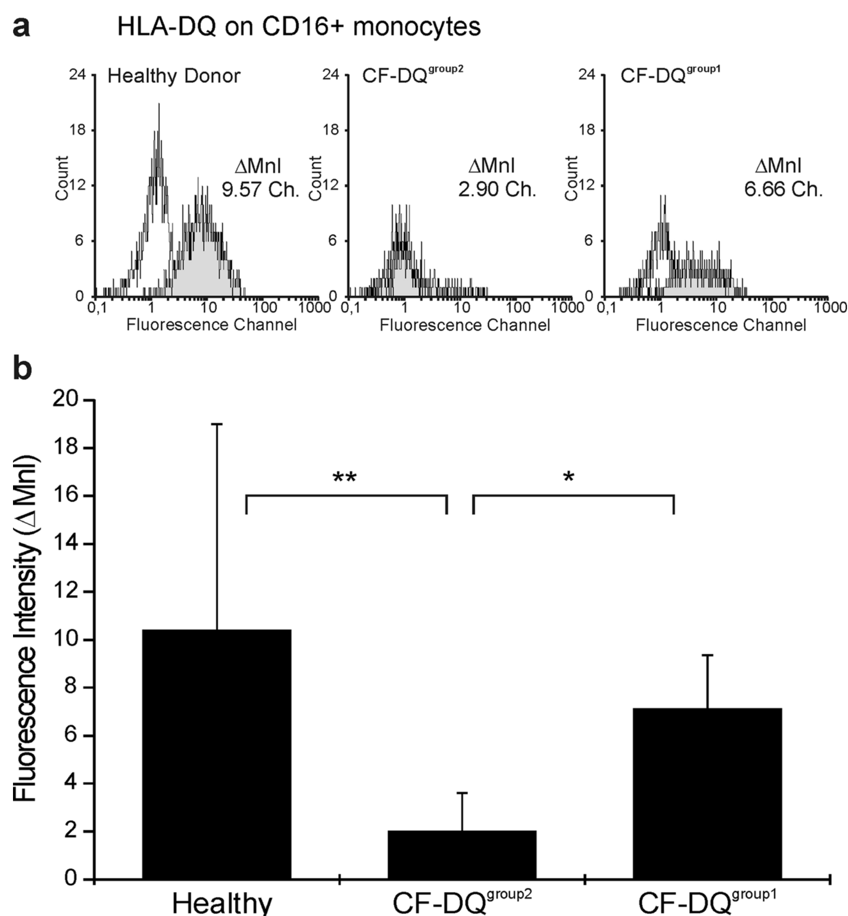


Fig. 3 Cell surface expression of HLA-DQ on CD16-positive blood monocytes in healthy donors and CF patients. **a** Typical histograms are shown; the mean intensity channels (ΔMnI) are given in the *right side within the histograms* (the graph of the isotype control in the central histogram is hidden behind the HLA-DQ signal). **b** CF-DQ^{group2} patients (n=6) show a significantly lower HLA-DQ surface expression compared with healthy subjects (n=23) while CF-DQ^{group1} patients (n=3) were not different from controls (mean ±SD, *$p<0.05$, **$p<0.01$)

from the CF-DQ^{group1} group (Fig. 4a right, 19.05 ΔMnI). Consistent with our observations in monocytes, CF-DQ^{group2} group patients showed strongly reduced DQ surface expression on sputum macrophages (Fig. 4a middle, 0.01 ΔMnI). On average, cell surface DQ expression on sputum macrophages from healthy and CF-DQ^{group1} donors was 12.72±7.59 and 12.08±6.11 ΔMnI, respectively. In contrast, DQ expression on sputum macrophages from CF-DQ^{group2} patients was at 0.44±0.66 ΔMnI, and this was significantly different from healthy controls (p=0.013) and the CF-DQ^{group1} cohort (p=0.022) (Fig. 4b).

When looking at in vitro-generated MDM, we found HLA-DQ clearly expressed in healthy controls with a level of 13.26 ΔMnI for the example in Fig. 5a. MDMs from a patient from the CF-DQ^{group1} group showed similar HLA-DQ expression (16.80 ΔMnI) compared with the healthy subject. In contrast to this, we found that DQ expression on MDMs from CF-DQ^{group2} was essentially absent (0.04 ΔMnI). On average, HLA-DQ expression on MDMs from healthy controls was 10.93±2.19 ΔMnI; for CF-DQ^{group2} patients, it was 0.09±0.10 ΔMnI ($p<0.05$), while for the CF-DQ^{group1} patients, expression levels were even higher compared with controls (19.64±11.26 ΔMnI, $p<0.05$, Fig. 5b).

Looking at blood dendritic cells, HLA-DQ expression was also decreased on CD1c, CD141, and CD303 DCs in CF-DQ^{group2} patients (Fig. 6). While for CD1c-positive DC, HLA-DQ protein expression was 104.9±60.2 ΔMnI for healthy subjects and 131.7±54.9 ΔMnI for CF-DQ^{group1} patients, in CF-DQ^{group2} patients, the expression was significantly lower (27.6±54.9 ΔMnI, p=0.014). On CD141-positive DC of healthy subjects, the HLA-DQ expression was 83.8±48.8 ΔMnI and 121.1±8.6 ΔMnI for CF-DQ^{group1} patients, and again, CF-DQ^{group2} patients were significantly lower at 27.4±36.6 ΔMnI (p=0.038). This pattern was also found for CD303-postitive DC with 38.7±18.1 ΔMnI for healthy subjects, 27.7±9.6 ΔMnI for CF-DQ^{group1} patients, and significantly lower expression of 7.9±7.9 ΔMnI for CF-DQ^{group2} patients ($p<0.01$).

Expression of HLA-DQ, HLA-DR, and HLA-DP at the transcript level

Transcript levels for HLA-DQα1, HLA−DQβ1, DRβ1, and DPα1 mRNAs were studied in classical CD14++ monocytes (Table 1). For HLA-DQα1, the average mRNA expression in

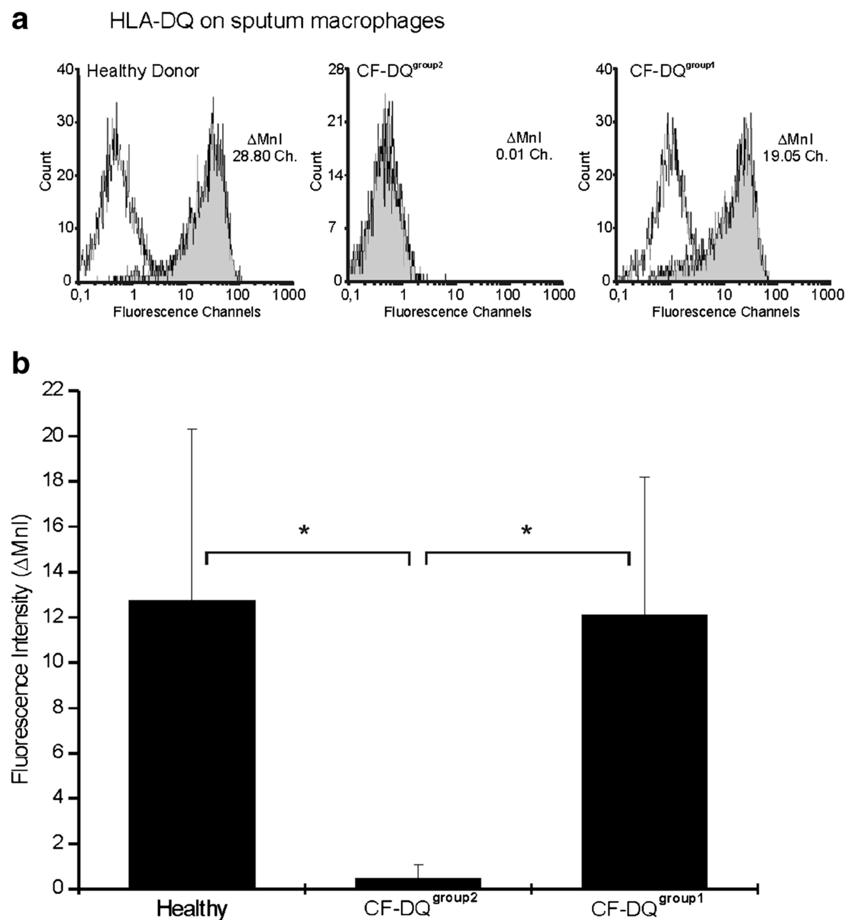


Fig. 4 Cell surface protein expression of HLA-DQ on sputum macrophages. **a** Shown are typical histograms from a healthy donor and CF patients. CF patients were grouped into CF-DQ^{group2} and CF-DQ^{group1} according to their DQ protein expression levels as determined previously on blood classical monocytes. The mean intensity channels (ΔMnI) values for sputum macrophages are given in channels in the upper *right corner of the histograms*. **b** Cell surface HLA-DQ expression on human sputum macrophages of healthy donors (n=4), and CF patients classified as CF-DQ^{group2} (n=5), and CF-DQ^{group1} (n=3) based on the DQ expression on blood monocytes (mean±SD, *p<0.05)

healthy controls was −14.53±33.33, calculated relative to the expression of α-enolase housekeeping gene. In CF-DQ^{group2} patients, these values were almost threefold lower at −39.24±50.86 (p<0.012). The DQ protein is a heterodimer consisting of DQα1 and DQβ1, so we also studied the transcript levels for DQβ1, since the amount of protein expressed on the cell surface will be determined by any one of the two partners that is lowest. DQβ1 transcript levels in monocytes from healthy controls were at −0.54±2.28 while, in CF-DQ^{group2} patients, they were almost seven times lower at −3.61±3.19 (p=0.007). These data suggest that the decreased expression of DQ protein on the cells surface in CF-DQ^{group2} patients is due to a decreased level of both contributing transcripts.

In MDM, we found no clear difference for the DQ transcripts levels in controls compared with CF patient cells (data not shown).

Analysis of DR and DP transcripts in the classical monocytes of the CF-DQ^{group2} patients also showed lower levels for DRβ1 (p<0.01) and DPα1 (p<0.001), with a twofold difference for these genes. This indicates that transcripts of the other class II molecules are also affected in CF-DQ^{group2} patients, but the most pronounced effect is seen for HLA-DQ.

Effect of IFNγ- and LPS on HLA-DQ expression in classical CD14++ monocyte-derived macrophages

Since MHC class II expression can be influenced by inflammation, we studied the regulation of HLA-DQ expression in CF in 48 h monocyte-derived macrophages from healthy donors, DQ^{group1}, and DQ^{group2} patients with and without addition of IFNγ and LPS. As shown in Fig. 7, again, the CF-DQ^{group2} macrophages had a significantly lower level of HLA-DQ in untreated cells (10.5±16.8 ΔMnI p=0.042) compared with healthy donors at 68.5±40.5 ΔMnI. IFNγ-treatment (1,000 U/ml) led to an increase in controls to more than 120 channels (122.7±36.2 ΔMnI). In CF-DQ^{group2} samples, it is evident that IFNγ was unable to recover the deficient HLA-DQ expression in these patients with an increase to only 19.9±24.4 ΔMnI. We also treated the cells with LPS (10 ng/ml), which is known to downregulate HLA-DR expression on monocytes and macrophages. In fact, in controls, LPS led to strong decrease in HLA-DQ expression to 5.9±3.7 ΔMnI (p=0.021 compared with untreated healthy donor samples). Also in DQ^{group2} samples, the low level of DQ expression was further decreased by LPS to 0.3±0.6 ΔMnI. We then asked whether IFNγ-treatment is able to overcome the LPS-induced

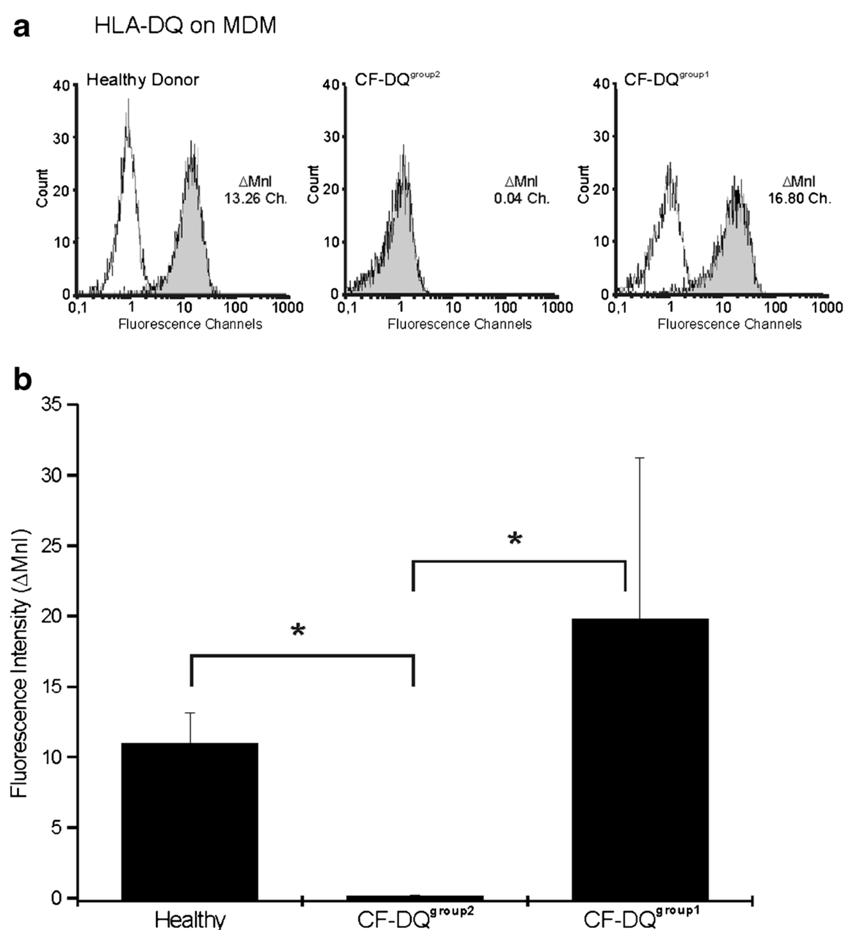


Fig. 5 Cell surface protein expression of HLA-DQ on monocyte-derived macrophages (MDM). **a** Shown are typical histograms from healthy donors and CF patients. CF patients were grouped into CF-DQ^{group2} and CF-DQ^{group1} according to their DQ protein expression levels as determined previously on blood classical monocytes. The mean intensity channel (ΔMnI) values for monocyte-derived macrophages (MDM) are given in channels in the *upper right corner of the histograms*. **b** Given is cell surface HLA-DQ expression on MDM of healthy donors, CF-DQ^{group2} and CF-DQ^{group1} CF patients classified based on the DQ expression on blood monocytes (n=3 each, mean± SD, *p<0.05)

suppression of HLA-DQ. For healthy donor samples, this was the case in that HLA-DQ increased to 34±25.6 ΔMnI in IFNγ+LPS-treated macrophages. In DQ^{group2} samples, there was, however, no activity of IFNγ on LPS-treated cells in that HLA-DQ expression was only 1.4±1.8 ΔMnI. Results for DQ^{group1} cells showed a pattern similar to healthy control samples; only the level of DQ in IFNγ+LPS-treated cells was lower compared with the respective healthy donor cells (Fig. 7). These data indicate that DQ^{group2} monocytes/macrophages have a defect in their IFNγ regulation of HLA-DQ expression.

Discussion

In this study, we found decreased HLA-DQ protein on the surface of blood monocytes in 36 out of 50 CF patients. In addition to classical monocytes, the CD16-positive monocytes and blood DC also showed a reduced level of HLA-DQ in CF. In sputum macrophages of these CF-DQ^{group2} patients, HLA-DQ was essentially absent, and the same was true for the model system of MDMs. This observation leads to the hypothesis that impaired MHC class II protein receptor expression may interfere with host defence mechanisms and may lead to elevated microbial colonization of the lungs of CF patients. This concept is supported by several other studies on the role of MHC class II molecules in CF, e.g., in Aron et al. [12] where they found higher levels of IgE and more frequent *Psuedomonas aeruginosa* colonization in CF patients with DR7/DQA*0201 haplotype [12] or in Laki et al. where they found lower frequency of bacterial colonization in CF with the 8.1 ancestral MHC haplotype [17].

For inflammatory diseases like sepsis and after surgical trauma, a decrease of HLA-DR has been demonstrated [16, 18, 19], and this went along with reduced antigen-presenting capacity. We therefore asked whether the downregulation of HLA-DQ in monocytes might be due to inflammation. For this, we analyzed the expression of HLA-DQ after in vitro exposure to IFNγ, LPS, and the combination of IFNγ and LPS. We found that IFNγ-induced upregulation of HLA-DQ is impaired in CF patients compared with healthy subjects. LPS, a potent inflammatory stimulus, showed a pronounced downregulation of HLA-DQ in CD14++ monocytes-derived macrophages, which is more pronounced in CF patients compared with healthy controls (Fig. 7). Evidence that inflammation may also be linked to reduced HLA-DQ

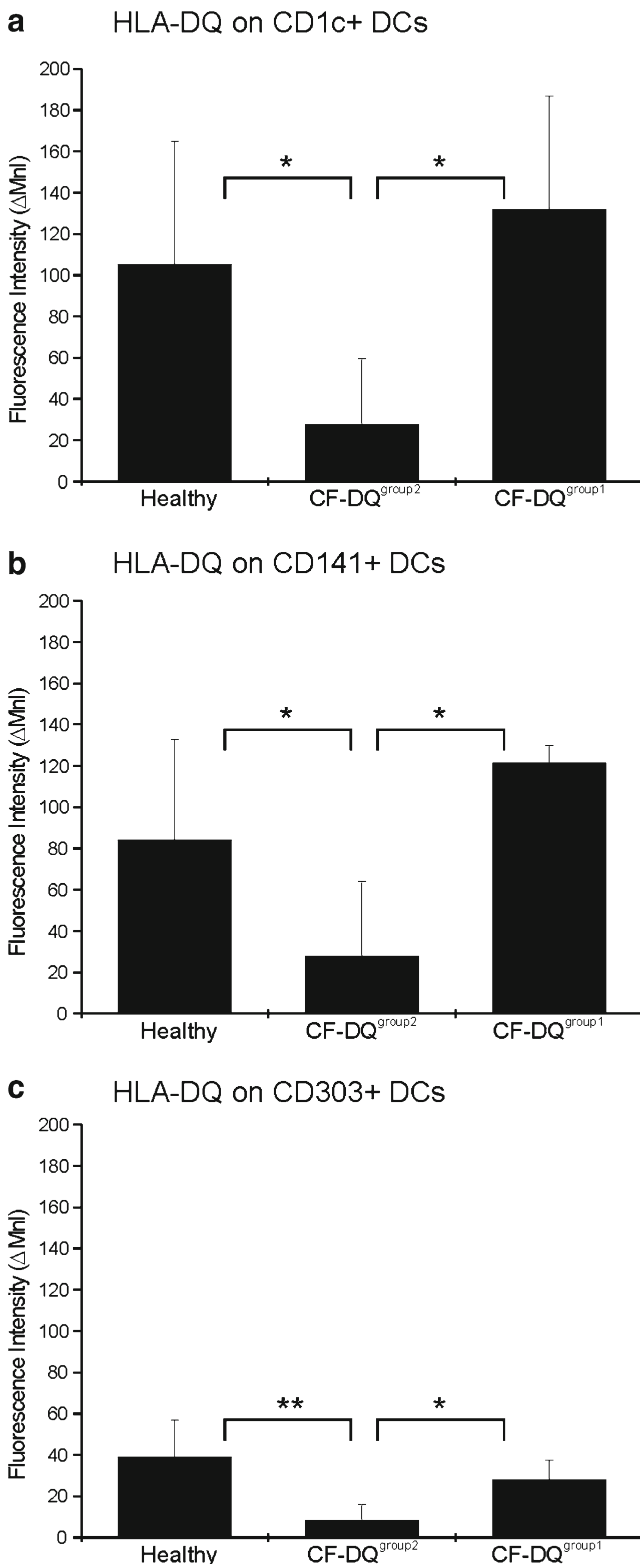


Fig. 6 Cell surface protein expression of HLA-DQ on blood dendritic cell (DC) subpopulations of **a** CD1c-positive myeloid DC, **b** CD141-positive myeloid DC, and **c** CD303-positive plasmacytoid DC of healthy donors (n=7), and CF patients classified as CF-DQ^{group2} (n=4), and CF-DQ^{group1} (n=3) based on the DQ expression on blood monocytes (mean± SD, *p<0.05)

expression in vivo comes from our observation that patients with increased CRP have lower HLA-DQ (see Fig. 2).

When we consider that, in monocytes and in tissue macrophages in our study, the HLA-DQ levels in CF-DQ^{group2} patients are extremely low, then it is conceivable that presentation of exogenous antigen through this pathway is blocked in these patients. In myeloid and plasmacytoid blood DC subsets of CF-DQ^{group2} patients, a similar pattern was seen. Therefore, it is likely that this downregulation may contribute to a reduced immune defence and an increased inflammatory condition in the lungs. This is supported by Hampton and Stanton demonstrating in a meta-analytical approach in that the F508del mutation in CFTR downregulates the antigen presentation pathway [20]. Furthermore, Knight et al. described that, in late-stage CF patients, macrophages obtained by lavage from explanted lungs during lung transplantation surgery have defective antigen presentation in contrast to the blood monocytes of the same patient [21]. This is strong evidence that antigen-presenting capacity is further decreasing during maturation of monocytes into lung macrophages.

In our study, we found lower mRNA expression levels of HLA-DQα1 and DQβ1 in CD14++ classical monocytes of CF-DQ^{group2} patients compared with healthy control group (Table 1). These data indicate that much of the downregulation of the HLA-DQ protein on monocytes in the CF-DQ^{group2} group is due to lower transcript levels. This may be due to reduced activity of the CIITA transcriptional activator in monocytes of CF patients as described by del Fresno et al. [22]. These authors discuss LPS tolerance of monocytes as a possible explanation for the downregulation of class II transcripts.

In our study, we investigated CIITA transcript levels in resting and LPS-/IFNγ-treated CD14++ monocyte-derived macrophages of CF patients compared with healthy subjects. IFNγ acts via induction of the transcription factor CIITA, which in turn binds to MHC class II promoters and induces class II transcripts. We therefore asked whether there might be a defect in CIITA transcript induction in CF. For this, we performed a limited series of experiments on this transcription factor at time point 48 h in CD14++ monocyte-derived macrophages (Fig. 8). Upon IFNγ-treatment, CIITA transcripts increased 4.5-fold in cells from healthy controls while, in CF patients, the increase was only 1.55-fold (p<0.05 compared with healthy donor cells). Also, IFNγ+LPS-treated cells showed a significantly lower CIITA transcript expression in CF patients (0.23±0.29 rel. mRNA conc.) compared with healthy subjects (1.35±0.65 rel. mRNA conc.). These data indicate that the deficient HLA-DQ expression in CF patients can be explained in part by a reduced transcriptional response of the CIITA gene to signals like IFNγ.

Additionally, del Campo et al. report of high LPS levels which were found in plasma of CF patients and were

Table 1 mRNA expression of HLA-DQα1, DQβ1, DRβ1, and DPα1 in CD14++ classical monocytes from healthy subjects and CF-DQ^{group2} CF patients

Relative amount of MHC class II transcript

		DQα1 CD14++	DQβ1 CD14++	DRβ1 CD14++	DPα1 CD14++
Healthy	Mean	−14.53	−0.54	25.50	4.10
	SD	33.33	2.28	18.63	1.24
	N	18	18	18	18
CF-DQ^{group2}	Mean	−39.24	−3.61	11.84	2.30
	SD	50.86	3.19	7.66	0.91
	N	11	11	11	11
Healthy vs. CF-DQ^{group2}	*P* value	0.012	0.007	0.007	0.0008

The amount of transcripts for the MHC class II genes is given relative to the expression level for the α-enolase housekeeping gene. Cycle number of the target gene was subtracted from the corresponding housekeeping gene, and its absolute value was subsequently calculated to the power of 2. Genes with a higher cycle number than the corresponding housekeeping gene were plotted to the negative scale (mean±SD)

suggested to contribute to endotoxin tolerance in their circulation monocytes [23]. Tolerance may contribute to our findings as well. However, our data on blood monocytes in adult CF patients show a selective decrease of DQ protein while DR expression levels are high in all CF patients.

Decreased protein expression in CF-DQ^{group2} versus healthy subjects may in addition be influenced by post-transcriptional processing of the HLA proteins and other regulatory mechanisms. This may be specifically true for macrophages, e.g., sputum macrophages from the lung or in vitro generated macrophages. This is because for these cell types the differences at the protein level were very strong with almost absent DQ cell surface protein for macrophages obtained from patients with low DQ expression on blood monocytes, while the mRNA levels in the macrophages were only slightly reduced and not significant (data not shown).

Inflammatory processes are tightly coupled to mutations in the *CFTR* gene of CF patients (such as F508del). *CFTR*--deficient macrophages retained the ability to perform phagocytosis but exhibited defective killing of the internalized bacteria through impaired acidification of the phagosomal compartment [24]. Other studies have shown that in macrophages of CF patients, cell surface MARCO scavenger receptor expression in the predominant population of small sputum macrophages is reduced which may lead to impaired clearance of inhaled particles with increased inflammation and damage to

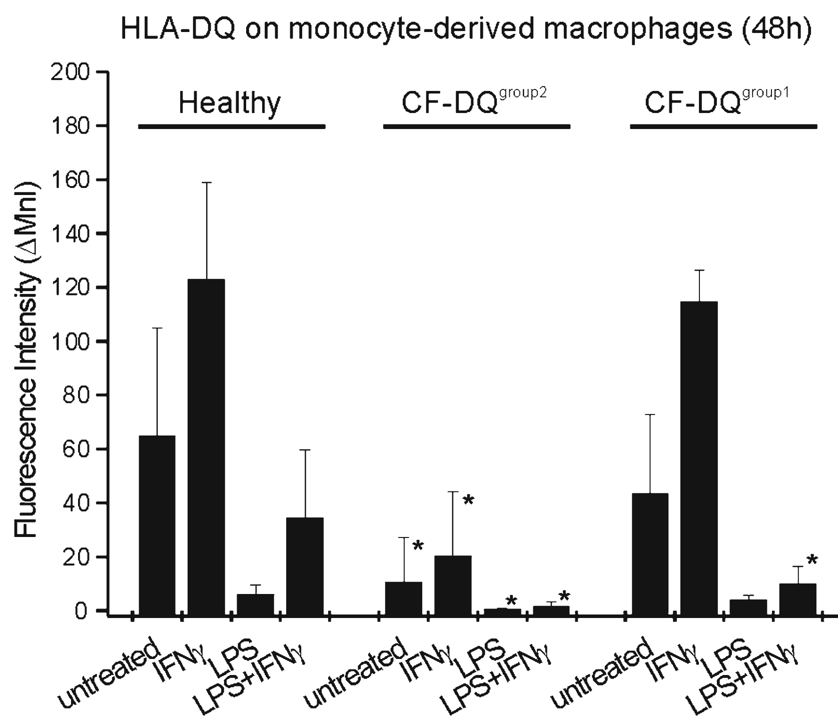


Fig. 7 Cell surface protein expression of HLA-DQ on CD14++ blood monocytes after in vitro culture for 48 h. Cells were left untreated or treated with IFNγ (1,000 U/ml), LPS (10 ng/ml), or the combination of IFNγ and LPS. Healthy donors (n=3), and CF patients classified as CF-DQ^{group2} (n=4), and CF-DQ^{group1} (n=3) based on the DQ expression on blood monocytes (mean±SD, *p<0.05). *Asterisks* indicate significant differences between healthy controls and the two groups of CF patients

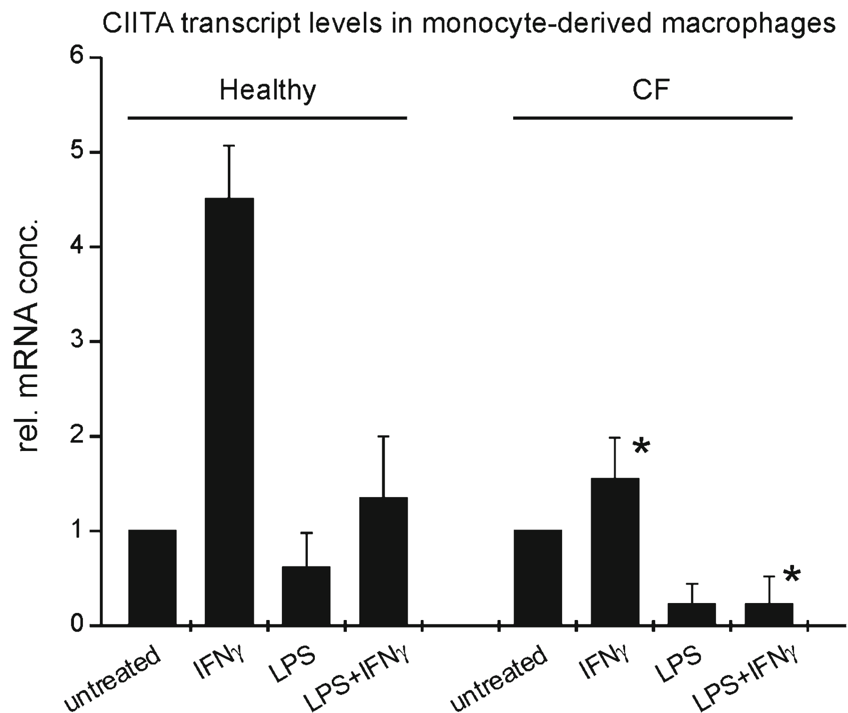


Fig. 8 CIITA transcript expression in CD14++ blood monocytes after in vitro culture for 48 h. Cells were left untreated or treated with IFNγ (1,000 U/ml), LPS (10 ng/ml), or the combination of IFNγ and LPS. Expression levels are calculated relative to untreated cells and significant differences between healthy controls, and CF patients are indicated by asterisks (mean±SD, *$p<0.05$ compared with healthy control values; healthy donors $n=3$, and CF patients $n=7$)

the CF lung [9]. Also, the CFTR cell surface protein itself was described to act as a receptor for endocytosis of *P. aeruginosa* in epithelial cells [25]. Taken together, mutations in *CFTR* (such as F508del) can lead to defects in bacterial internalization and subsequent removal.

On the other hand, patients with CF have substantial variability in pulmonary involvement that does not appear to correlate with the type of *CFTR* mutation [26]. Hence, confounding genes and/or gene pathways may modulate the severity of bronchopulmonary disease [26, 27]. HLA-DQ may be such a gene because of its role in presentation of antigen to T cells as a crucial step in immune defense against infection. While DQ expression was found low on blood monocytes and blood DCs in many patients with CF, expression on macrophages in tissue in the same patients was essentially absent. Without HLA-DQ on the cell surface, such macrophages will be unable to present antigen through this pathway such that microbial infection may prevail. In this context, the importance of a functional antigen-presenting system is demonstrated regarding the genetic disorder TAP (transporter-associated with antigen presentation) which is characterized by a defective MHC class I antigen presentation and subsequent chronic lung infection and opportunistic *Staphylococcus aureus* and *P. aeruginosa* infection [28, 29].

When comparing in regression analysis, the DQ level with all relevant clinical parameters including infections, medication, other diseases, lung, and blood parameters, we found no significant association except for the association to blood CRP levels (Fig. 2).

In summary, we report on a strong decrease in HLA-DQ protein expression on monocytes, macrophages, and blood dendritic cells and a reduction of HLA-DR on monocytes of CF patients. Given the role of class II molecules in immune defenses, we hypothesize that this defect may contribute to the pathology of the disease. Further investigations are necessary to assess the relationship between low HLA-DQ expression on monocytes and macrophages of CF patients and its role in susceptibility to infection and inflammation.

Acknowledgments We thank Karl Staples, Clinical & Experimental Sciences, University of Southampton, UK, for his valuable support on cDNA microarray analysis. Furthermore, we thank Kerstin Skokann for excellent technical assistance.

Disclosure The authors declare that they have no conflict of interest related to the publication of this manuscript.

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