

ORIGINAL PAPER

Differential expression of monocyte CD163 in single – and dual-asthmatic responders during allergen-induced bronchoconstriction

K. Kowal*, H. J. Møller†, L. M. DuBuske‡, S. K. Moestrup†§ and A. Bodzenta-Lukaszyk*

*Department of Allergology and Internal Medicine, Medical University of Białystok, Białystok, Poland, †Department of Clinical Biochemistry, NBG Aarhus University Hospital, Aarhus C, Denmark, ‡Immunology Research Institute of New England, Gardner, MA USA and §Department of Medical Biochemistry, University of Aarhus, Aarhus C, Denmark

Clinical and
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Allergy

Summary

Background Mononuclear phagocytes play an important role in modulating inflammatory reactions in response to antigen challenge.

Objective To investigate the regulation of CD163, a marker of anti-inflammatory macrophages, during *Dermatophagoides pteronyssinus* (Dp)-induced bronchoconstriction.

Methods Among 110 Dp-sensitive patients who underwent bronchial challenge with Dp, there were 51 dual responders (DR), 32 single responders (SR) and 27 non-responders (NR).

Monocyte expression of CD14 and CD163 was evaluated by flow cytometry. Exhaled NO (eNO) concentration was determined on-line using a chemiluminescence analyser. In 21 DR, nine SR and 13 NR-soluble CD163 in plasma was measured by ELISA before, 1, 8 and 24 h after the challenge.

Results In DR, the baseline expression of monocyte CD163 and eNO were significantly greater than in SR and NR. A pattern of (1) decreased monocyte CD163 expression, (2) unchanged sCD163 and (3) increased eNO in DR was contrasted by a pattern of (1) increased CD163 expression, (2) increased sCD163 and (3) unchanged eNO in SR. During allergen challenge, the changes in monocyte CD163 expression and sCD163 inversely correlated with the changes in eNO.

Conclusion Both pro-inflammatory and anti-inflammatory responses to allergen challenge are uniquely expressed among SR and DR.

Keywords asthma, bronchial reactivity, cd163

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Correspondence:

Krzysztof Kowal, Department of Allergology and Internal Medicine, Medical University of Białystok, Skłodowskiej-Curie 24a, 15-276 Białystok, Poland.

E-mail: Krzysztof.9327125@pharmanet.com.pl

Introduction

In healthy subjects, alveolar macrophages (AMs) and recruited peripheral blood monocytes (PBM) play a crucial role in the down-regulation of the inflammatory reaction in the lungs in response to antigen challenge [1–5]. In asthmatic patients, AMs have decreased phagocytic function, and have a decreased inhibitory effect on T cell proliferation [2–5]. Furthermore, experimental asthma models support a role for AM in the development of asthma and airway hyper-reactivity [6, 7].

Allergic asthma patients exposed to a relevant allergen develop immediate bronchoconstriction [early asthmatic reaction (EAR)]. In some patients, this is followed 3–8 h later by prolonged narrowing of the airways referred to as the late asthmatic reaction (LAR) [8]. The appearance of an LAR is associated with infiltration of the bronchial wall by inflammatory cells, including monocytes [9–10].

The histological changes in the bronchial wall during the LAR resemble those seen in patients with persistent asthma [10, 11].

A subpopulation of monocytes/macrophages expressing an anti-inflammatory phenotype are thought to play a crucial role during the resolution of inflammation [12, 13]. A surface marker molecule for this phenotype has recently been identified as the haptoglobin-haemoglobin scavenger receptor CD163, which is a monocytic/macrophage lineage-specific glycoprotein [14]. The expression of CD163 is induced by glucocorticoids, IL-10 and IL-6 and suppressed by IFN- γ , IL-4, TNF- α or lipopolysaccharide [15, 16]. CD163⁺ cells are expanded in chronic inflammation and during the healing phase of the acute inflammatory reaction [14–18]. In arthritis, these CD163⁺ cells seem to be involved in the down-regulation of T lymphocyte function [19, 20]. Moreover, a soluble form of CD163 (sCD163), which is shed from the

cell membrane upon inflammatory stimuli, has also been reported to possess anti-inflammatory activity [21–23]. Soluble CD163 effectively inhibits phorbol ester-induced T cell proliferation and activation [21–23]. sCD163 is present in normal human plasma; however, the levels increase significantly upon activation or proliferation of mononuclear phagocytes [20, 24–27].

The purpose of the present study was to investigate the regulation of CD163 on PBM and sCD163 in the plasma of patients who develop EAR and LAR in response to allergen challenge.

Methods

Patients

Two hundred non-smoking allergic rhinitis patients, mean age 26.7 ± 8.6 years, referred to our allergy clinic due to a recent onset, within 12 months, of episodes of dyspnoea, wheezing and cough upon dust exposure, were screened for this study. All patients had a baseline forced expiratory volume in 1 s (FEV₁) above 70% of the predictive value. In 185 patients (92.5%), positive skin prick test results with *Dermatophagoides pteronyssinus* (Dp) and *Dermatophagoides farinae* (Df) extracts were demonstrated. Based on a detailed medical history, 143 patients were selected for the study. The selected patients had never been treated for asthma before the study except for episodic use of inhaled short-acting β_2 -agonists. Patients who had already been treated with allergen immunotherapy or any systemic medications as well as patients having upper respiratory tract infections within 3 months of the study were not included. One hundred and ten of 143 selected patients (76.9%), mean age 25.3 ± 7.2 years, agreed to participate. The remaining 33 patients declined to participate in the study for non-medical reasons. Nineteen non-smoking healthy non-atopic subjects, mean age 24.7 ± 6.8 years, were included as a control group (HC). Bronchial challenges were not performed in HC for ethical reasons. All subjects signed an informed consent. The study was approved by the local Ethics Committee.

Skin prick tests

All persons were skin tested using prick methodology with a screening panel of aeroallergens (Allergopharma, Joachim Ganzer, Reinbeck, Germany) as described elsewhere [28].

Bronchial challenge

Histamine and allergen bronchial challenge tests were performed as described elsewhere [28]. All patients inhaled doubling concentrations of histamine starting from a concentration of 0.062 mg/mL. The procedure was continued until either at least a 20% fall of FEV₁ or a

histamine concentration of 32 mg/mL was reached. The FEV₁ results obtained after inhalation of 0.9% solution of NaCl were used as reference values.

Increasing doses (0.8, 4, 20, 100, 500 and 2500 standardized biological unit (SBE)) of aqueous Dp extract (Allergopharma) were administered until either at least a 20% fall of FEV₁ or a cumulative dose 5000 SBE was reached. Forced expiratory manoeuvres were performed 15 min after inhalation of each dose of the allergen extract. The FEV₁ was measured every 15 min during the first hour, every 60 min during the next 11 h and after 24 h. The EAR was evaluated as a dose of allergen causing a 20% decline of FEV₁.

Exhaled nitric oxide measurements

The concentration of nitric oxide (NO) in the expired air was evaluated 'online' using a chemiluminescence analyser NOATM 280i (Sievers, Boulder, CO, USA). The measurements were performed according to American Thoracic Society (ATS) recommendations [29]. Briefly, each patient exhaled over a 30-s period against the fixed expiratory resistance of 16 cmH₂O, which resulted in a constant flow of 50 mL/s. Both NO concentration and flow rate were displayed on the screen. A plateau of NO concentration in the exhaled air at the selected exhalation rate was automatically selected by the computer software according to the ATS recommendations. The NO measurements were performed immediately before lung function test (T₀) and 24 h after allergen challenge (T₂₄). At each time-point, measurements were repeated three times and the mean value was used for analysis.

Flow cytometry analysis

Samples of venous blood for flow cytometry analysis were collected from each patient at T₀ and T₂₄. Flow cytometry analysis was performed on the whole-blood samples using the following monoclonal antibodies labelled with fluorochromes: anti-CD14 FITC with anti-CD163 PE (BD Biosciences, Pharmingen, San Diego, CA, USA) as described before [28]. Matched labelled anti-idiotypic antibodies were used as negative controls. After 30 min of incubation at room temperature, erythrocytes were lysed using a set of lysing reagents (Beckman Coulter Co., Miami, FL, USA). The remaining white cells were analysed by flow cytometry (Coulter Epics, Beckman Coulter Co., Miami, IL, USA). Monocytes were selected on the basis of their shape and size [side scatter(SS) and forward scatter(FS)] and on the basis of high expression of CD14. The expression of individual receptors is presented in fluorescence units (FU), which represents a ratio between the intensity of staining with an individual antibody in comparison with the intensity of staining using appropriate isotype control antibody.

Immune assays

Concentrations of serum total IgE (tIgE) and specific anti-*Dp* IgE (sIgE) were evaluated using UniCap (Pharmacia, Uppsala, Sweden).

In 43 patients, four consecutive plasma samples were available. They were collected at T_0 , and then 60 min (T_{EAR}), 8 h (T_{LAR}) and 24 h (T_{24}) after administration of the last allergen dose. In these patients, the plasma concentration of sCD163 was evaluated. The plasma level of sCD163 was evaluated using ELISA as described elsewhere [25, 30]. Briefly, polyclonal rabbit anti-CD163 bound to the microtitre wells were incubated with plasma samples for 60 min. After being washed, monoclonal anti-CD163 antibody (GHI/61) was added and incubated for 60 min. After another washing, peroxidase-labelled goat anti-mouse antibody was added (DAKO P447, Carpinteria, CA, USA) and incubated for 60 min. Subsequently, the wells were washed and substrate solution was added. After 15 min, the reaction was stopped with 50 μ L of 1 M H_2SO_4 and the plates were read at 492/620 nm. Control samples and standards of purified CD163 were co-analysed in each run.

Study protocol

All patients were admitted to the hospital. On the first day, histamine bronchial challenge was performed between 07:00 and 9:00 hours. The patients were observed overnight and on the second day, between 07:00 and 08:00 hours, allergen bronchial challenge was performed and

lung function tests were monitored as described above. In those having seasonal allergy, bronchial challenge tests were performed out of their allergy season.

Statistical analysis

Statistical analysis was performed using the non-parametric Wilcoxon's test. The results are expressed as median with 95% confidence interval for the median. For comparison of three or more unpaired groups, the Kruskal–Wallis test was used. Analysis of repeated measurements was performed using Friedman's ANOVA test. A probability value of $P < 0.05$ was taken to indicate statistical significance. The Spearman correlation coefficient was used to examine the relationship between quantitative parameters. Categorical data were analysed using the χ^2 test.

Results

Significant bronchoconstriction to allergen challenge was demonstrated in 83 of 110 patients (75.5%). In 32 patients, only an EAR was demonstrated [single responders (SR)] while 51 developed also an LAR [dual responders (DR)]. Significant bronchoconstriction to histamine challenge was demonstrated in all 83 responders ($PC_{20} = 2.0$ mg/mL; 95% CI 1.1–2.7 mg/mL). In SR, the median PC_{20} was significantly greater than in DR ($P = 0.0026$; Table 1). Patients who did not respond to histamine provocation and to allergen challenge ($n = 21$) were considered non-responders (NR) and were used for comparison. Six patients who responded

Table 1. Baseline characteristics of patients studied [median (95% confidence intervals (CI))]

	Patients			
	DR ($n = 51$)	SR ($n = 32$)	NR ($n = 21$)	HC ($n = 19$)
FEV ₁ (% predicted)	83 (80–87)* [†]	89 (86–95)*	97 (89–105)	95 (92–102)
PC ₂₀ (mg/mL)	1.2 (0.79–2.0)* [†]	3.7 (2.2–9.8)*	> 32	ND
PD ₂₀ (SBE)	459 (341–750)* [†]	1800 (1300–2240)*	> 5000	ND
Total IgE (U/mL)	734 (375–913)* [†]	223 (97.0–442)	149 (59–271)	ND
Anti <i>Dp</i> IgE (U/mL)	13.1 (5.40–24.1)* [†]	2.99 (1.75–4.82)*	1.76 (0.84–4.79)	ND
Eosinophils (mm ³)	360 (315–425) [‡] *	275 (239–385) [‡] *	210 (160–342) [‡]	150 (130–188)
Monocytes (mm ³)	362 (308–391)	280 (205–394)	330 (290–410)	320 (272–378)
Exhaled NO (p.p.b.)	137 (117–165) [‡] *	89.5 (70.8–106) [‡] *	52.0 (28.0–84.9) [‡]	8.0 (6.8–9.5)
CD 163 (% of mono)	52.0 (47.9–54.9) [‡] * [†]	37.5 (31.5–42.1) [‡]	31.5 (30.0–36.6) [‡]	26.0 (19.7–31.8)
CD163 (FU)	1.99 (1.95–2.04) [‡] * [†]	1.88 (1.82–1.99) [‡] *	1.89 (1.78–1.92) [‡]	1.72 (1.60–1.87)
CD14 (% of mono)	87.0 (84.3–89.5)	86.7 (85.4–89.1)	87.0 (86.0–93.9)	91.0 (87.0–96.0)
CD14 (FU)	5.6 (4.7–6.0) [‡]	5.4 (4.0–6.5) [‡]	5.1 (4.6–6.9) [‡]	6.7 (5.1–8.8)
	DR ($n = 21$)	SR ($n = 9$)	NR ($n = 13$)	
sCD163 (mg/L)	2.51 (1.92–3.07)	2.38 (1.48–3.52)	1.99 (1.42–2.64)	

*significantly different from NR.

[†]significantly different from SR.

[‡]significantly different from HC.

SBE, standardized biological unit; NO, nitric oxide; FU, fluorescence units; DR, dual responders; SR, single responders; NR, non-responders; HC, healthy controls.

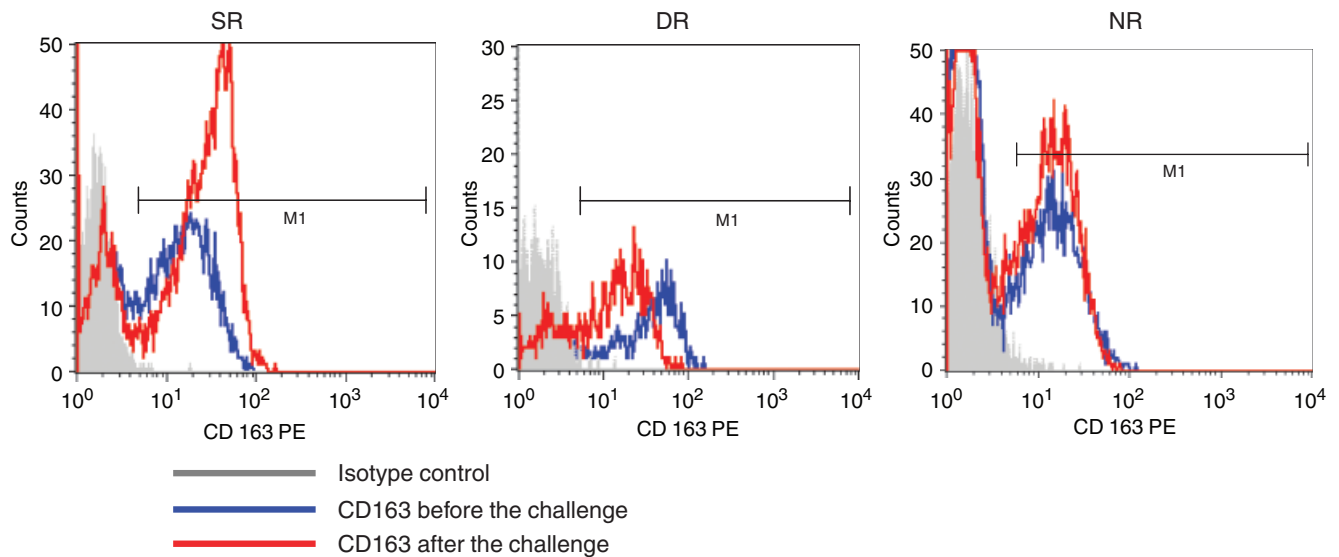


Fig. 1. Comparison of representative flow cytometry analysis of blood samples from a single responder (SR) (left) and a dual responder (DR) (middle) non-responder (NR) (right). Expression of CD163 on peripheral blood monocytes was evaluated before (blue) and after (red) allergen challenge. Staining with anti-isotype antibodies is depicted in grey.

to histamine challenge ($PC_{20} < 32$ mg/mL) but did not respond to allergen challenge were not included in the analysis. Significantly greater bronchial responsiveness to allergen challenge, evaluated as median PD_{20} , was found in DR than in SR ($P < 0.0001$; Table 1).

Expression of CD163

No difference in the number of circulating monocytes was found between DR, SR, NR and HC (Table 1). At T_0 , the percentage of PBM that expressed CD163 was the greatest in DR, being significantly greater than in SR ($P = 0.0002$), NR ($P < 0.0001$) or HC ($P < 0.0001$). Similarly, the density of CD163 on PBM expressed in FU was the greatest in DR, being significantly greater than in SR ($P = 0.0072$), NR ($P < 0.001$) or HC ($P < 0.001$; Table 1). The median percentages of PBM expressing CD14 were not statistically different among DR, SR, NR and HC (Table 1). The median density of CD14 on PBM did not statistically differ between DR, SR and NR but the median density of CD14 in HC was significantly greater than that in any of the allergic patient groups (Table 1). No significant difference was found in the total number of PBM before and after allergen challenge in DR, SR or NR. In SR, the median number of CD163 expressing monocytes increased from 37.5% to 53.0% (95% CI 49.0–59.0%; $P < 0.0001$; Figs 1 and 2) after allergen challenge, and the median density of CD163 expression on PBM increased from 1.88 FU to 2.24 (95% CI 2.01–2.46 FU; $P < 0.0001$), while in DR the median number of CD163 expressing monocytes decreased from 52.0% to 42.0% (95% CI 39.7–43.0; $P < 0.0011$; Figs 1 and 2) and the median density of CD163 expression on PBM decreased from 1.99 to 1.8 FU

(95% CI 1.77–1.87; $P < 0.0001$). Neither the median number of CD163-expressing monocytes nor the median density of CD163 on PBM changed significantly in NR after the allergen challenge. An increase in monocyte CD163 expression was demonstrated in 27 of 32 SR (84.4%), 10 of 21 NR (47.6%) and in only eight of 51 DR (15.7%; $P < 0.0005$). No change in expression of CD14 after the allergen challenge was observed in any of the groups (Fig. 2).

The concentration of sCD163 in plasma was determined in nine SR, 21 DR and 13 NR. In these patients, the baseline plasma concentrations of sCD163 were within the reference limits determined in studies of healthy subjects [30]. The highest plasma concentrations of sCD163 at T_0 was found in DR but this was not significantly different from SR ($P = 0.717$) or NR ($P = 0.123$) (Table 1). Serial measurements of sCD163 in plasma revealed no significant change in median plasma concentrations of sCD163 in NR ($P = 0.989$) or DR ($P = 0.297$) during the 24 h observation period. In SR a significant increase in the median sCD163 concentration was seen at T_{LAR} (2.55; 95% CI 1.67–3.97 mg/L; $P < 0.01$) and at T_{24} (2.78; 95% CI 1.55–3.97; $P < 0.01$; Fig. 3). The increase in plasma sCD163 concentration was seen in all SR (100%), in seven of 12 NR (58.3%) and in only eight of 21 DR (38.1%; $P = 0.007$).

CD163 expression and mediators of airway inflammation

The median baseline NO concentrations in exhaled air of DR, SR and NR were significantly greater than in HC ($P < 0.01$ for each studied group; Table 1). No significant changes in the concentration of NO in exhaled air were

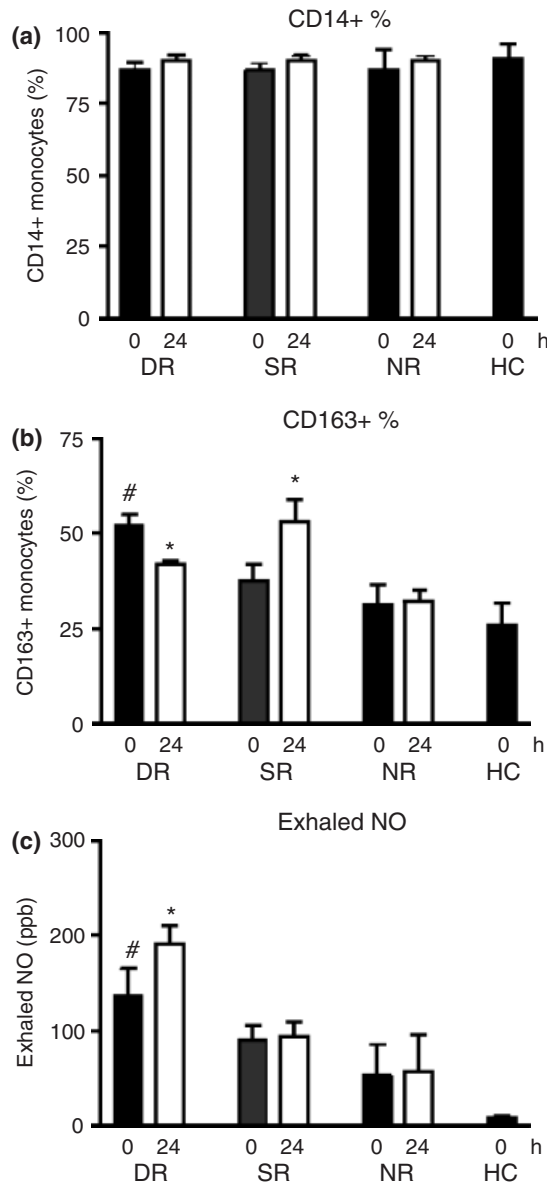


Fig. 2. Frequency of peripheral blood monocytes expressing CD14 (a) or CD163 (b) and the mean concentration of nitric oxide (NO) (c) in the exhaled air before (0) and 24 h after allergen challenge [24]. Data are presented as median with 95% confidence intervals. DR, dual responders; SR, single responders; NR, non-responders; HC, healthy controls, #significantly different from SR; *significantly different than at T_0 .

seen either in SR or in NR at T_{24} . In DR, however, a significant increase in the median NO concentration was seen from 137 p.p.b. at T_0 to 192 p.p.b. (95% CI 166–211 p.p.b.) at T_{24} ($P < 0.0001$; Fig. 2). In neither SR nor in DR was there a direct correlation between exhaled NO concentration and expression of CD163 on PBM or the sCD163 plasma concentration. Significant inverse correlations, however, were found between the change in expression of CD163 on PBM and the change in exhaled NO concentration ($n = 83$, $r = -0.3987$, $P = 0.0002$) and between the change in plasma concentration of sCD163

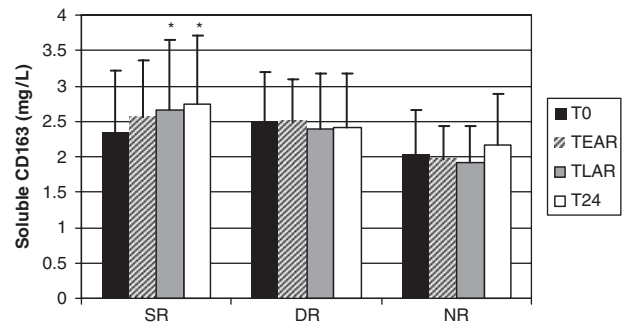


Fig. 3. Changes in the plasma concentration of sCD163 during allergen challenge. Data are presented as median with 95% confidence intervals, before (0), 60 min (T_{EAR}), 8 h (T_{LAR}) and 24 h (T_{24}) after the challenge. DR, dual responders; SR, single responders; NR, non-responders; HC, healthy controls. #significantly different from SR; *significantly different than at T_0 .

and the change in NO concentration ($n = 30$, $r = -0.4251$, $P = 0.03$) during allergen challenge. An increase in monocyte CD163 expression was seen in 26 of 30 (87%) patients who did not respond to the allergen challenge with an increase in exhaled NO concentration and only in 14 of 53 (26%) patients, who demonstrated an increase in exhaled NO concentration after the challenge ($P < 0.0001$). Similarly, plasma sCD163 concentration increased in 13 of 16 (81%) patients who did not respond with an increase in exhaled NO concentration to the allergen challenge and only in 10 of 24 (42%) patients who demonstrated an increase in exhaled NO concentration after the challenge ($P = 0.03$) (Fig 4).

Discussion

This study demonstrates a specific pattern of monocyte activation in patients who respond with bronchoconstriction to intrabronchial allergen challenge. The monocyte/macrophage-specific scavenger receptor CD163 is a marker of macrophages that possess anti-inflammatory functions, and increased expression of CD163 on mononuclear phagocytes is associated with down-regulation of inflammatory responses [12–14, 20, 24–27]. As AMs play a crucial role in dampening of the inflammatory reaction in the lungs, it was of interest to investigate the regulation of CD163 in response to allergen challenge, and especially to investigate observed changes in patients who develop a prolonged inflammatory response (DR). At baseline, there was a higher number of CD163⁺ monocytes in asthma patients than in control subjects that was paralleled by an increased mean fluorescence intensity. Moreover, significant opposite changes in the number of CD163⁺ cells were observed in DR and SR during allergen challenge that were not accompanied by any change in CD14 expression. The changes in CD163 expression appear not to be related to increased shedding of sCD163 in DR or decreased shedding in SR as the levels of sCD163 remained constant

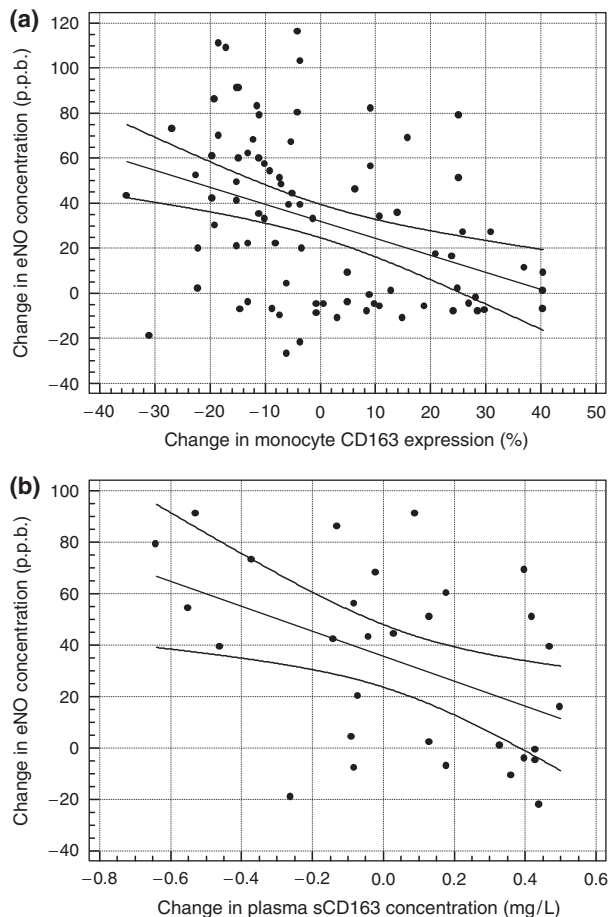


Fig. 4. Correlation between change in exhaled nitric oxide (NO) concentration and (upper panel) change in monocyte CD163 expression or (lower panel) change in sCD163 plasma concentration during allergen challenge.

in DR, and increased in SR. It cannot, however, be excluded that the fall in CD163 expression in DR was due to an increased recruitment of CD163⁺ cells to the lungs. Independent of the reason, however, the fall of CD163 expression in circulating monocytes seen in DR indicates that the effects of pro-inflammatory mediators or 'utilization' of CD163⁺ monocytes are not balanced by anti-inflammatory mechanisms leading to increased CD163 expression. The changes therefore probably reflect true alterations in the activation of PBM, and in SR might suggest a general shift in monocyte activation towards an anti-inflammatory state, whereas the ability to raise a reinforced anti-inflammatory response in connection with antigen challenge seems disturbed in DR.

The anti-inflammatory cytokine IL-10 is a strong inducer of CD163 expression and in an experiment with application of gene-chip technology, CD163 displayed the strongest response to IL-10 among the 19 up-regulated genes [31]. Under the influence of IL-10, monocytes and dendritic cells develop an anti-inflammatory phenotype. Interestingly, the increase in the number of circulating

IL-10-producing CD4⁺ cells in SR observed during allergen challenge by other investigators mirrors the changes in CD163⁺ monocytes seen in the current study [32]. This suggests that Tcell-monocyte interaction within circulation may play a role in regulation of the inflammatory response induced by bronchial allergen challenge. *In vitro*, monocytes induced by lactic acid bacteria to secrete IL-10 inhibited production of T-helper type 2 (Th2) cytokines by lymphocytes [33]. The effect was not linked to an increase in IFN- γ or IL-12 secretion, indicating that these 'anti-inflammatory' monocytes may divert the immune system in a regulatory or tolerant mode [33]. Moreover, the induction of CD163 itself appears to be involved in the anti-inflammatory actions of mononuclear phagocytes. Binding of CD163 by monoclonal antibodies or its ligand induces intracellular signalling that leads to increased IL-10 production by macrophages [13]. Even sCD163 present in plasma has attributed anti-inflammatory actions [21–23]. Soluble CD163 effectively inhibits phorbol ester-induced, protein kinase C (PKC)-dependent, T cells' activation and proliferation [21–23]. As activation of PKC is crucial for proliferation of Th2 cells, it can be argued that sCD163 cannot inhibit only Th1 cells shifting the immune response towards Th2-type [34]. The inhibition of T cell activation and proliferation by sCD163 should rather result in a tolerant state. The T cell inhibitory effect of sCD163 was achieved *in vitro* at a concentration of 0.5 mcg/mL, which is much below the plasma concentration of sCD163 [24, 25]. Moreover, in SR during the 24 h period, the median increase in plasma sCD163 concentration was 0.4 mcg/mL, being in the range of biologically active concentrations. A negative correlation between sCD163 and the number of activated T cells has been described in arthritic joints [20]. Soluble CD163 is present in broncho-alveolar fluids (H. J. M. unpublished results); however, a possible role for sCD163 in the local regulation of inflammation in the lungs needs further investigation. Finally, binding of haemoglobin-haptoglobin complex by CD163 fuels haeme oxygenase-1 (HO-1), resulting in the production of carbon monoxide (CO), which exerts strong anti-inflammatory and anti-proliferative actions [35].

The change in exhaled NO during allergen challenge was different in DR and SR, and contrasted the changes seen in CD163 assessments. In DR, the decrease in CD163 expression was accompanied by increases in exhaled NO and, in SR, the increase in CD163 expression was accompanied by unchanged concentrations of NO in the exhaled air. These relations were further emphasized by the inverse correlations between CD163 expression/sCD163 and change in exhaled NO concentration.

General alterations in monocyte phenotype and function during intrabronchial allergen challenge have been described [36, 37]. Unfortunately, no association between the pattern of monocyte activation and specific

types of bronchial response has been reported. Recent observations in humans and mice emphasize a possible association between mononuclear phagocytes and the resolution of the inflammatory response in asthma. It was demonstrated in an allergen-induced animal asthma model that allergen-loaded mononuclear phagocytes could induce an indirect, allergen-specific, long-lasting, immunosuppressive response [38]. The effect was associated with migration of these allergen-loaded mononuclear phagocytes to the spleen [38]. Recently, in asthmatic patients increased concentrations of another macrophage scavenger receptor (90K protein) were detected in plasma and bronchoalveolar lavage fluid. The protein was shown to inhibit the production of IL-4, IL-5 and IL-13 [39]. Whether CD163 or sCD163 plays a direct role in the down-regulation of the inflammatory responses in asthma, perhaps by inhibition of T lymphocytes, or is merely a marker of anti-inflammatory responses, cannot be concluded from the present study.

Taken together, our findings support the hypothesis of a combined up-regulation of both pro-inflammatory and anti-inflammatory responses in asthma, and indicate that both pro- and anti-inflammatory responses to allergen challenge are differentially regulated in SR and DR. In DR, pro-inflammatory mechanisms seem to dominate, whereas SR seem able to mount a dominant anti-inflammatory response to allergen provocation. Moreover, a tight association between local inflammation in the airways and systemic response of mononuclear phagocytes was demonstrated.

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References

- Holt PG, Oliver J, Bilyk N *et al.* Downregulation of the antigen presenting cell function(s) of pulmonary dendritic cells *in vivo* by resident alveolar macrophages. *J Exp Med* 1993; **177**:397-407.
- Alexis NE, Soukup J, Nierkens S, Becker S. Association between airway hyperreactivity and bronchial dysfunction in individuals with mild asthma. *Am J Physiol Cell Mol Physiol* 2001; **280**:L369-75.
- Aubas P, Cosso B, Godard P, Michel FB, Clot J. Decreased suppressor cell activity of alveolar macrophages in bronchial asthma. *Am Rev Respir Dis* 1984; **130**:875-8.
- Gant V, Cluzel M, Shakoor Z, Rees PJ, Lee TH, Hamblin AS. Alveolar macrophage accessory cell function in bronchial asthma. *Am Rev Respir Dis* 1992; **148**:900-4.
- Tang C, Rolland JM, Ward C *et al.* Differential regulation of allergen-specific TH2- but not TH1-type responses by alveolar macrophages in atopic asthma. *J Allergy Clin Immunol* 1998; **102**:368-75.
- Sirois J, Bissonnette EY. Alveolar macrophages of allergic resistant and susceptible strains of rats show distinct cytokine profiles. *Clin Exp Immunol* 2001; **126**:9-15.
- Careau E, Bissonnette EY. Adoptive transfer of alveolar macrophages abrogates bronchial hyperresponsiveness. *Am J Respir Cell Mol Biol* 2004; **31**:22-7.
- Pepys J, Hutchcroft BJ. Bronchial provocation tests in etiologic diagnosis and analysis of asthma. *Am Rev Respir Dis* 1975; **112**:829-59.
- Robinson DS, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased Th-2 type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation in atopic asthmatics. *J Allergy Clin Immunol* 1993; **92**:313-24.
- Haselden BM, Kay AB, Larche M. Immunoglobulin E-independent major histocompatibility complex-restricted T cell peptide epitope-induced late asthmatic reaction. *J Exp Med* 1999; **189**:1885-94.
- Robinson DS, Ying S, Bentley AM *et al.* Relationships among number of bronchoalveolar lavage cells expressing messenger ribonucleic acid for cytokines, asthma symptoms and airway metacholine responsiveness in atopic asthma. *J Allergy Clin Immunol* 1993; **92**:397-403.
- Mosser DM. The many faces of macrophage activation. *J Leukocyte Biol* 2003; **73**:209-12.
- Philippidis P, Mason JC, Evans BJ *et al.* Hemoglobin scavenger receptor CD163 mediates interleukin-10 release and heme oxygenase-1 synthesis: antiinflammatory monocyte-macrophage responses *in vitro*, in resolving skin blisters *in vivo*, and after cardiopulmonary bypass surgery. *Circ Res* 2004; **94**:119-26.
- Kristiansen M, Graversen JH, Jacobsen C *et al.* Identification of the haemoglobin scavenger receptor. *Nature* 2001; **409**:198-201.
- Buechler C, Ritter M, Orso E, Langmann T, Klucken J, Schmitz G. Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. *J Leukocyte Biol* 2000; **67**:97-103.
- Van den Heuvel, Tansen CP, van As JH *et al.* Regulation of CD163 on human macrophages: cross-linking of CD163 induces signaling and activation. *J Leukocyte Biol* 1999; **66**:858-66.
- Djemadjji-Oudjil N, Goerdt S, Kodelja V, Schmuth M, Orfanos CE. Immunohistochemical identification of type II alternatively activated dendritic macrophages (RM 3/1+3, MS-1+/-, 25F9-) in psoriatic dermis. *Arch Dermatol Res* 1996; **288**:757-64.
- Zwadlo G, Voegeli R, Osthoff KS, Sorg C. A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the downregulatory phase of the inflammatory process. *Exp Cell Biol* 1987; **55**:295-304.
- Fonseca JE, Edwards JCW, Blades S, Goulding NJ. Macrophage subpopulations in rheumatoid synovium. Reduced CD163 expression in CD4+ T lymphocyte-rich microenvironments. *Arthritis Rheum* 2002; **46**:1210-6.
- Baeten D, Møller HJ, Delanghe J, Veys EM, Moestrup SK, De Keyser F. Association of CD163+ macrophages and local production of soluble CD163 with decreased lymphocyte activation in spondylarthropathy synovitis. *Arthritis Rheum* 2004; **50**:1611-23.

- 21 Hogger P, Sorg C. Soluble CD163 inhibits phorbol ester-induced lymphocyte proliferation. *Biochem Biophys Res Commun* 2001; **288**:841–3.
- 22 Frings W, Dreier J, Sorg C. Only the soluble form of the scavenger receptor CD163 acts inhibitory on phorbol ester-activated T lymphocytes, whereas membrane-bound protein has no effect. *FEBS Lett* 2002; **523**:93–6.
- 23 Timmermann M, Buck F, Sorg C, Hogger P. Interaction of soluble CD163 with activated T lymphocytes involves its association with non-muscle myosin heavy chain type A. *Immunol Cell Biol* 2004; **82**:479–87.
- 24 Møller HJ, de Fost M, Aerts H, Hollak C, Moestrup SK. Plasma level of the macrophage-derived soluble CD163 is increased and positively correlates with severity in Gaucher's disease. *Eur J Haematol* 2004; **72**:135–9.
- 25 Møller HJ, Peterslund NA, Graversen JH, Moestrup SK. Identification of the hemoglobin scavenger receptor/CD163 as a natural soluble protein in plasma. *Blood* 2002; **99**:378–80.
- 26 Schaer DJ, Schleiffenbaum B, Bächli E *et al*. Soluble hemoglobin-haptoglobin scavenger receptor CD163 as a lineage specific marker of macrophage activation in the hemophagocytic syndrome. *Eur J Haematol* 2005; **74**:6–10.
- 27 Hintz KA, Rassias AJ, Wardwell K *et al*. Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. *J Leukocyte Biol* 2002; **72**:711–7.
- 28 Kowal K, Osada J, Zukowski S, Dabrowska M, DuBuske LM, Bodzenta-Lukaszyk A. Expression of interleukin 4 receptors in bronchial asthma patients who underwent specific immunotherapy. *Ann Allergy Asthma Im* 2004; **93**:68–75.
- 29 Recommendations for the standardized procedures for the online and offline measurements of exhaled lower respiratory nitric oxide and nasal nitric oxide in adults and children. *Am J Respi Crit Care* 1999; **160**:2104–17.
- 30 Møller HJ, Hald K, Moestrup S. Characterisation of an enzyme linked immunosorbent assay for soluble CD163. *Scand J Clin Lab Invest* 2002; **62**:293–300.
- 31 Williams L, Jarai G, Smith A, Finan P. IL-10 expression profiling in human monocytes. *J Leukocyte Biol* 2002; **72**:800–9.
- 32 Matsumoto K, Gauvreau GM, Rerecich T, Watson RM, Wood LJ, O'Byrne PM. IL-10 production in circulating T cells differ between allergen-induced isolated early and dual asthmatic responders. *J Allergy Clin Immunol* 2002; **109**:281–6.
- 33 Niers LEM, Timmerman HM, Rijkers GT *et al*. Identification of strong interleukin-10 inducing lactic acid bacteria which down-regulate T helper type 2 cytokines. *Clin Exp Allergy* 2005; **35**:1481.
- 34 Salek-Ardakani S, So T, Halteman BS, Altman A, Croft M. Differential regulation of Th2 and Th1 lung inflammatory responses by protein kinase C θ . *J Immunol* 2004; **173**:6440–7.
- 35 Slebos DJ, Ryter SW, Choi AMK. Heme oxygenase-1 and carbon monoxide in pulmonary medicine. *Respir Res* 2003; **4**:1–13.
- 36 Durham SR, Carroll M, Walsh GM, Kay AB. Leukocyte activation in allergen induced late-phase asthmatic reactions. *N Engl J Med* 1984; **311**:1398–402.
- 37 Carrol MP, Durham SR, Walsh G, Kay AB. Activation of neutrophils and monocytes after allergen- and histamine-induced bronchoconstriction. *J Allergy Clin Immunol* 1985; **75**:290–6.
- 38 Vissers JLM, van Esch BCAM, Hofman GA, van Oosterhout AJM. Macrophages induce an allergen-specific and long-term suppression in a mouse asthma model. *Eur J Respir Dis* 2005; **26**:1040–6.
- 39 Kalayci O, Birben E, Tinari N, Oguma T, Iacobelli S, Lilly CM. Role of 90K protein in asthma and TH2-type cytokine expression. *Ann Allergy Asthma Immunol* 2004; **93**:485–92.