Immunobiology 224 (2019) 497–501

Contents lists available at ScienceDirect

Immunobiology

journal homepage: www.elsevier.com/locate/imbio

Synergistic effect of *Dermatophagoides pteronyssinus* allergens and dexamethasone on the expression of CD163 by peripheral blood mononuclear cells

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ARTICLE INFO

Keywords:
CD163
Asthma
Dermatophagoides pteronyssinus
Interleukin-10
Interleukin-6

ABSTRACT

Background: The CD163 is a marker of monocyte/macrophage anti-inflammatory function. Its soluble form (sCD163) also exert anti-inflammatory activities including inhibition of T cell proliferation.
Objective: To evaluate the effect of dexamethasone (Dx) and *Dermatophagoides pteronyssinus* (Dp) on *ex vivo* production of sCD163 by peripheral blood mononuclear cells (PBMCs).
Methods: PBMCs from 26 allergic asthma patients (AAs) and 12 non-atopic healthy controls (HCs) were cultured with Dp, Dx, Dp + Dx or without any stimulation for up to 144 h (T_{144}). Concentration of sCD163, interleukin (IL) -6 and IL-10 in PMBC culture supernatants was evaluated using ELISA. The mRNA expression of CD163 by PBMCs was estimated using quantitative PCR (qPCR).
Results: At T_{144} the median concentration of CD163 in unstimulated PBMC cultures of AAs was greater than that in HCs ($p = 0.008$). Concomitant application of Dp and Dx resulted in a synergistic effect reflected by a dramatic increase of sCD163 concentration both in HCs ($p = 0.0002$) and AAs ($p < 0.0001$). Also a synergistic effect of Dp and Dx on CD163 mRNA expression was seen at T_{24} and T_{48} but not at T_6 or T_{12}. Among asthmatic patients the effect of Dx on sCD163 production was attenuated in severe in comparison to mild-to-moderate AAs ($p = 0.0007$). Moreover, Dp-induced production of IL-6 but not IL-10 was inhibited by Dx ($p < 0.0001$). Inhibition of IL-10 decreased sCD163 concentration by more than 50%.
Conclusions: Dx-triggered upregulation of anti-inflammatory CD163 expression by monocytes is synergistic with endogenous mechanisms involved in the resolution of Dp-induced inflammation. This effect is impaired in severe asthma patients.

1. Introduction

Alveolar macrophages (AMs) and recruited peripheral blood monocytes (PBMs) regulate the inflammatory response in the lungs (Pappas et al., 2013; Sabatel et al., 2017). Depending on the type of activation mononuclear phagocytes are a potent source of both pro- and anti-inflammatory mediators (Wynn et al., 2013). A subpopulation of PBMs/AMs expressing an anti-inflammatory phenotype are thought to play a crucial role during the resolution phase of the inflammatory response (Mosser, 2003; Philippidis et al., 2004).

A monocyte/macrophage lineage-specific glycoprotein CD163 was identified as a marker of their anti-inflammatory function (Zwadlo et al., 1987; Kristiansen et al., 2001). The expression of CD163 is induced by glucocorticoids, interleukin (IL)-10, IL-6 and suppressed by interferon-gamma (IFN-γ), IL-4, tumor necrosis factor alpha (TNF-α) and other pro-inflammatory mediators (Kowal et al., 2011). Moreover, a soluble form of CD163, which is shed from the cell membrane upon inflammatory stimuli, has been reported by some investigators to possess anti-inflammatory activity (Högger and Sorg, 2001; Frings et al., 2002). Its concentration is elevated in serum of patients with chronic inflammatory diseases including some asthma patients (Kowal et al., 2011, 2006).

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https://doi.org/10.1016/j.imbio.2019.05.005
Received 19 February 2019; Received in revised form 3 May 2019; Accepted 21 May 2019
Available online 22 May 2019

It has already been demonstrated that systemic administration of corticosteroids leads to upregulation of CD163 on PBMs both in healthy subjects and in asthmatic patients (Zwadlo-Klarwasser et al., 1990; Yeager et al., 2008; Moniuszko et al., 2009). On the other hand, in induced sputum expression of CD163 on the membrane (mCD163) of lung macrophages in asthmatic patients is less than that in healthy controls and is accompanied by an elevated concentration of sCD163 (Kowal et al., 2014; GINA guidlines, 2006). Moreover, therapy with inhaled corticosteroids (ICS) resulted in a dramatic increase of sCD163 concentration in induced sputum (Kowal et al., 2014).

Since CD163 is exclusively produced by monocytes/macrophages it was of interest to evaluate the effect of house dust mite (HDM) allergens and corticosteroids on the expression of CD163 by PBMs of allergic asthma patients *in vitro*.

2. Materials and methods

The study was performed on 26 nonsmoking AAs, including 16 mild-to-moderate and 10 severe asthmatics. In addition, 12 non-smoking, non-atopic HCs were included. All AAs had positive skin prick test results with Dp and *Dermatophagoides farinae* (Df) extracts. Asthma diagnosis and severity were established according to the Global Initiative for Asthma (GINA) guidelines (GINA guidlines, 2006). Patients who had already been treated with allergen immunotherapy, had any systemic diseases or had suffered from upper respiratory tract infections within 3 months of the study were not included. All subjects signed informed consent. The study was approved by the local Bioethics Committee (R-I-002/114/2014).

Allergy status was evaluated using skin prick testing with a screening panel of aeroallergens (Allergopharma, Reinbek, Germany) as described earlier (Kowal et al., 2006). In addition serum concentration of allergen-specific IgE (sIgE) was evaluated using ImmunoCap (Phadia, Uppsala, Sweden). Lung function was evaluated as previously described (Kowal et al., 2006).

3. Cell isolation and cultures

Peripheral blood mononuclear cells were isolated from the heparinized venous blood samples. Twenty milliliters of blood was diluted in 1:1 proportion with Hanks solution overlaid on Histopaque 1077 (Sigma-Aldrich, St Louis, MI) and centrifuged at 400 x g for 30 min at room temperature. The cells from the Histopaque-plasma interface were collected and after washed three times in phosphate buffered saline (PBS) were resuspended in complete RPMI1640 medium supplemented with L-glutamine, 5% fetal calf serum, penicillin (100 U/ml) and streptomycin (0.1 mg/ml) (Sigma- Aldrich, St Louis, MI) at a final density 5×10^5 cells/ml. Total cell count and cell viability were evaluated using Fuchs-Rosenthal chamber after mixing the cell suspension with trypan blue at a 9:1 ratio. All cell cultures were run in duplicates. The amount of Dp (Allergopharma, Reinbek, Germany) used for the cells cultures was calculated so the final concentration of Der p 1 and Der p 2 in the culture medium was 10 mcg/ml and 35 mcg/ml, respectively. The final concentration of Dx (Sigma-Aldrich, St Louis, MI) was 10^{-6} M. The final concentration of Dp and Dx was chosen based on previous dose-response studies in healthy subjects. Those concentrations provided the strongest effect [Suppl Fig. 1 and 2]. In PBMCs from 6 healthy subjects experiments with inhibition of endogenous IL-10 and IL-6 with monoclonal anti-IL-10 (clone B-S10, Abcam, Cambridge, UK) and anti-IL-6 (clone 3H3, InvivoGen, Toulouse, France) antibodies were performed. Each antibody was used at a final concentration 1 mcg/ml. The control cultures were treated with control isotype mouse IgG1 antibodies at the same concentration.

4. Biochemical and immunological assays

The content of major allergens group 1 and 2 in the allergen extract was performed using ELISA kits (EL-DP1 and EL-D2, respectively, Indoor Biotechnologies, Cardiff, UK) according to the manufacturer instruction. The plates were read at 405 nm. Universal Allergen Standard which allows to make a control curve ranging from 250 - 0.5 ng/ml of Der p 1 or Der p 2 was also included. The samples were run in triplicates and the mean values were calculated.

RNA isolation was performed using the RNeasy Mini Kit (Qiagen GmbH, Hilden, Germany) according to the manufacturer instruction as previously described (Bernatowicz and Kowal, 2016).

Reverse transcription with random primers was performed on 600 ng of RNA using High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Carlsbad, CA, USA) according to the manufacturer instruction.

TaqMan-based real-time PCR was used to quantitatively evaluate the expression of CD163 (Hs.504641), with detections of NM_004244.5 and NM_203416.3 variants. As a control housekeeping Beta-2-microglobulin gene (B2M, Hs.534255, NM_004048.2) was used. The reaction was performed using 384-well plates in a final volume of 10 µl per reaction using ABI Prism 7900 H T (Applied Biosystems, Carlsbad, CA, USA) with the following thermal cycle profile: initial denaturation 10 min at 95 °C, then 40 cycles each 15 s at 95 °C and 60 s at 60 °C.

Quantitative assessment of gene expression was performed using the comparative threshold cycle (C_T) method. For comparative purpose, a C_T of an individual gene is normalized to a C_T of a housekeeping gene and the result is called ΔC_T. Then the difference between unstimulated and stimulated samples ($\Delta\Delta C_T$) is calculated by subtracting ΔC_T of the former from the ΔC_T of the latter. The fold change was calculated from $2^{-\Delta\Delta CT}$ formula.

The concentration of sCD163 in cell culture supernatants was evaluated using Quantikine ELISA Kits (R&D Diagnostics, Minneapolis, MO, USA) according to the manufacturer instruction. The concentration of IL-10 and IL-6 was evaluated using High Sensitivity Quantikine ELISA Kits (R&D Diagnostics, Minneapolis, MO, USA). All assays were run in duplicate.

5. Statistical analysis methods

Data are presented as medians with 95% confidence intervals (95%CI). Normal distribution was evaluated using the Wilk-Shapiro test. Continuous variables were compared using the Wilcoxon test. For comparison of 3 or more unpaired groups the Kruskal-Wallis test was used. Analysis of repeated measurements was performed using the Friedman ANOVA test. All computations were carried out using the Statistica software.

6. Results

Characteristics of the studied subjects are presented in Table 1. There was no significant difference in age and sex distribution between the studied groups. Asthma patients were characterized by significantly

Table 1
Patients characteristics.

	HCs (n = 12)	Mild-moderate AAs (n = 16)	Severe-AAs (n = 10)	P value
Age (years)	31.3 + /-9.82	30.4 + /-10.13	35.5 + /-11.75	P = 0.475
Sex (female/ male)	6/6	7/9	4/6	P = 0.95
FEV_1 (% predicted)	102.4 + /-14.74	85.4 + /-11.51*	74.2 + /-6.09*,^	P < 0.001
ICS (mcg/day)	_____	550 + /-171#	1360 + /-207	P < 0.001

ICS – dose of fluticasone propionate from dry a powder inhaler.
* significantly less than in HCs.
^ significantly less than in mild-moderate -AAs.
# signifcantly less than in severe-AAs.

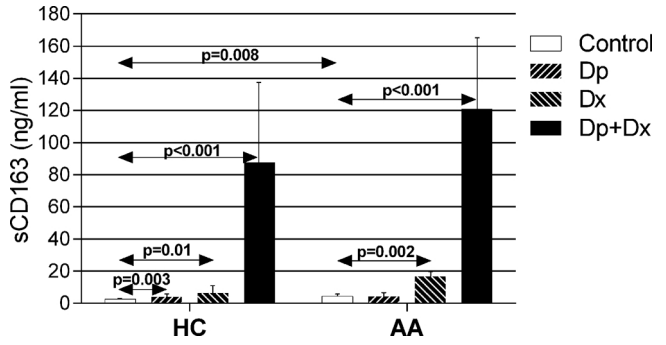

Fig. 1. The concentration of sCD163 in PBMCs culture supernatants after 144 h.

impaired lung function ($p < 0.001$). In severe asthmatics FEV_1 was significantly lower than that in mild-moderate asthmatics ($p < 0.001$).

Soluble CD163 was detectable in all supernatants of PBMCs cultured for 144 h. The median concentration of sCD163 was significantly greater in AAs (4.45; 3.8 to 6.62 ng/ml) than in HCs (2.58; 1.67 to 3.1 ng/ml; $p = 0.008$) (Fig. 1). Stimulation with Dp resulted in an increase of sCD163 concentration in HCs (to 4.0; 3.04 to 5.9 ng/ml; $p < 0.0001$) but not in AAs (to 4.3; 3.17 to 6.88 ng/ml; $p = 0.085$). Significant increase of sCD163 concentration was observed after Dx stimulation both in HCs (to 6.45; 4.8 to 10.63 ng/ml; $p = 0.0002$) and in AAs (to 16.8; 9.8 to 19.8 ng/ml; $p < 0.0001$). Concomitant application of Dp and Dx resulted in a synergistic effect leading to a dramatic increase of sCD163 concentration both in HCs (to 87.7; 25.9 to 137 ng/ml; $p < 0.0001$) and AAs (to 121; 63 to 165 ng/ml; $p < 0.0001$). Among AAs the effect was attenuated in severe AAs (51.2; 21.7 to 125 ng/ml) in comparison to mild-to-moderate AAs (156; 99 to 273 ng/ml; $p = 0.0007$) (Fig. 2). However, there was no significant difference between the median concentration of sCD163 in the unstimulated cell cultures derived from mild-moderate AAs (4,45; 3.31 to 6.62 ng/ml) and severe AAs (4.6; 3.7 to 9.1 ng/ml; $p = 0.617$). Similarly, no difference could be demonstrated between the median sCD163 concentrations in the supernatants of Dp stimulated cultures in mild-moderate and severe AAs. However, the median concentration of sCD163 in cultures stimulated with Dx was significantly greater in mild-moderate AAs (19.6; 10.3 to 22.2 ng/ml) than in severe AAs (9.3; 7.2 to 17.6 ng/ml; $p = 0.004$).

We subsequently evaluated the effect of Dp and Dx on the expression of CD163 mRNA on PBMCs derived from 10 HCs and 10 mild-moderate AAs cultured for up to 48 h (T_{48}) (Fig. 3). Stimulation of PBMCs with Dp induced a rapid decrease in CD163 mRNA level both in HCs (0.375; 0.289 to 0.684 fold change) and AAs (0.37; 0.274 to 0.557 fold change) seen at T_6 (Fig. 3). This was followed by upregulation of CD163 expression at T_{24} and T_{48} in HCs but not in AAs. Concomitant stimulation with Dp and Dx attenuated early (at T_6) downregulation of CD163 expression in HCs (0.8; 0.716 to 0.96 fold change; $p < 0.001$) and in AAs (0.76; 0.649 to 0.89 fold change $p < 0.001$). At T_{24} and T_{48}

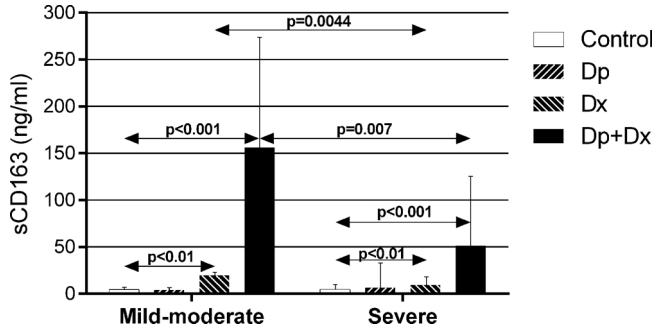

Fig. 2. The concentration of sCD163 in PBMCs culture supernatants of severe and mild-moderate asthmatic patients.

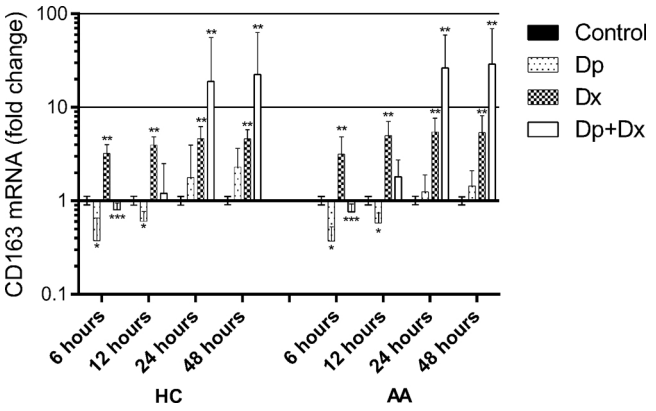

Fig. 3. Expression of CD163 mRNA in PBMCs of HCs and Aas. (* less than control, ** greater than control, *** less than control but greater than Dp; unstimulated cultures were considered as reference = 1).

simultaneous stimulation of PBMCs with Dp and Dx resulted in a synergistic upregulation of CD163 expression in both HCs and AAs (Fig. 3).

In unstimulated cultures of PBMCs from AAs concentration of IL-10 (4.1; 3.4 to 4.8 pg/ml) and IL-6 (6.2; 4.1 to 8.3 pg/ml) were significantly greater than those of HCs (2.6; 2–3.2 pg/ml; $p < 0.01$ and 3.8; 2.7 to 4.9 pg/ml; $p < 0.01$ for IL-10 and IL-6 respectively) (Figs. 4 and 5). In HCs stimulation with Dp resulted in increased concentration of IL-10 (4.0; 2.8 to 5.2 pg/ml; $p < 0.01$) and IL-6 (250; 195 to 305 pg/ml; $p < 0.001$). Similarly, in AAs Dp caused increase in the concentration of IL-10 (to 5.1; 2.7 to 7.4 pg/ml; $p < 0.01$) and IL-6 (to 368; 284 to 452 pg/ml; $p < 0.001$). In comparison, stimulation with Dp and Dx caused significantly attenuated increase of IL-6 concentration in both HCs (to 88; 39 to 137 pg/ml; $p < 0.001$) and AAs (to 177; 105 to 249 pg/ml; $p < 0.001$). No effect of Dx on Dp induced IL-10 production could be demonstrated (Fig. 4).

Inhibition of endogenous IL-10 in cultures stimulated with Dp and Dx resulted in decrease of sCD163 concentration by more than 50% (to 40.4; 28.4–52.6 pg/ml; $p < 0.001$) (Suppl Fig. 3). The mean concentration of sCD163 in Dp + Dx cultures in which endogenous IL-6 was inhibited with monoclonal antibodies (71; 56.2–85.8 pg/ml) was not significantly different than that in the control cultures ($p = 0.08$).

7. Discussion

In this study we examined the effects of corticosteroids on the Dp-induced expression of CD163 by monocytes of AAs and HCs. We have previously demonstrated that clinical improvement associated with one week of ICS therapy produced a strong increase of sputum sCD163 concentration in house dust mite allergic asthma patients (Kowal et al., 2014). CD163 is a monocyte/macrophage-specific protein, which expression is highly upregulated by corticosteroids and anti-inflammatory

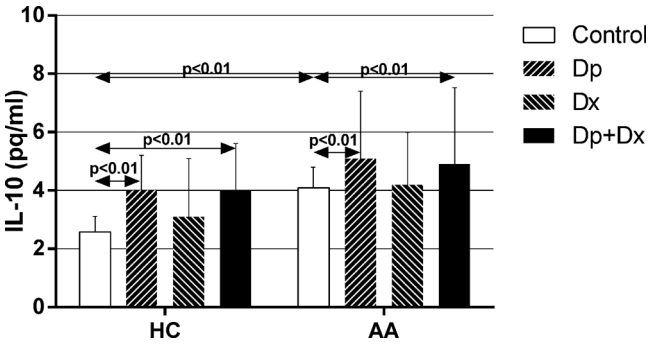

Fig. 4. The concentration of IL-10 in PBMCs of HCs and AAs.

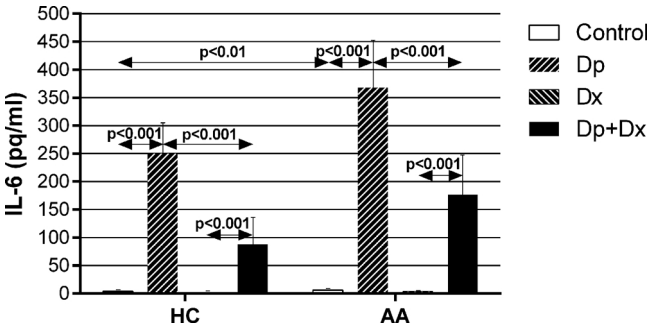

Fig. 5. The concentration of IL-6 in PBMCs of HCs and AAs.

mediators such as IL-10 (Kowal et al., 2011). Therefore evaluation of CD163 expression allows to specifically address monocyte/macrophage function using PBMC cultures. Elevated sCD163 concentration in plasma and greater expression of mCD163 on circulating monocytes was demonstrated in asthmatic patients (Kowal et al., 2006; Moniuszko et al., 2009; Periyalil et al., 2015). On the contrary, bronchoalveolar lavage and sputum macrophages of asthmatic patients express less mCD163 than those from healthy subjects (Dai et al., 2016; Staples et al., 2012). Greater shedding of CD163 from macrophage cell surface in the airways of asthmatic patients may be responsible for a decreased mCD163 expression on AMs. Therefore, concentration of sCD163 in bodily fluids including plasma and sputum may be considered a biomarker of monocyte/macrophage function in individual compartments. Unravelling mechanisms responsible for elevated sCD163 concentration in different clinical conditions may help to understand the role of monocytes/macrophages in the pathogenesis of individual diseases. In the current study we investigated mechanisms responsible for a dramatic increase in sputum sCD163 concentration seen in AAs in response to therapy with ICS. One of the mechanism is enhanced spontaneous release of sCD163 which could be demonstrated *in vitro* in AAs. This cannot explain a dramatic increase in sCD163 release when the cells are cultured in the presence of Dp and Dx. Moreover, Dp stimulation resulted in enhanced sCD163 release in HCs but not in AAs. Therefore, it is not allergen exposure itself which makes the differences in sCD163 release. This rather indicates that monocytes of AAs are characterized by impaired anti-inflammatory response to allergen challenge. The latter concept is further supported by the analysis of dynamic changes of CD163 mRNA expression. Early downregulation of CD163 mRNA by Dp-stimulated PBMCs was followed in HCs by a wave of increased CD163 mRNA expression which was absent in AAs. This indicates that in AAs transcriptional mechanisms counterbalancing inflammatory response to allergen challenge are impaired. Interestingly, this was not due to impairment of endogenous IL-10 or IL-6 production in AAs. Since both cytokines are major endogenous inducers of CD163 expression, monocytes/macrophages of AAs seem to be characterized by impaired response to endogenous anti-inflammatory stimuli. However, our study shows that increased production of IL-10 in response to Dp stimulation plays a crucial role in synergistic up-regulation of sCD163 production, at least in cells derived from healthy subjects. Interestingly, Th_2 cytokines, IL-4 and IL-13 downregulate the expression of CD163 (Van Gorp et al., 2010). Therefore, it is plausible that IL-4/IL-13 may inhibit CD163 expression after exposure of AA PBMCs to a relevant allergen. Impaired CD163 production in response to exogenous corticosteroids was demonstrated in severe in comparison to mild-to-moderate AAs. In asthmatic patients resistant to corticosteroids altered function of alveolar macrophages has been reported (Staples et al., 2012). Nonetheless, our study shows that corticosteroids synergize with naturally occurring anti-inflammatory mechanisms, which operate in monocytes/macrophages in response to allergen challenge. Those mechanisms involve transcriptional regulation of CD163 expression and endogenous IL-10 seems to be a major inducer of CD163 expression. The crucial role of IL-10 in induction of CD163 expression in different experimental settings has already been demonstrated (Lurier et al., 2017). This may explain why a strong increase of sCD163 concentration in induced sputum is observed in house dust mite AAs after one week of ICS therapy (Kowal et al., 2014). In patients with uncontrolled asthma macrophage-dependent control of inflammation is defective (Fitzpatrick et al., 2008). In the majority of asthmatics, however, ICS allow to obtain asthma control possibly due to their synergistic effects with endogenous anti-inflammatory mechanisms (Covar, 2016). This was clearly shown for allergen-induced airway inflammation (Hew et al., 2006). However, in severe asthmatics impaired response to corticosteroids was demonstrated (Kelly et al., 2000). Lung macrophages have been shown to control the inflammatory response and their function is impaired in asthma patients (Balhara and Gounni, 2012). Our results, at least partially, explain the observed *in vivo* processes which may lead to amplification of chronic inflammation in allergic asthma patients, including severe asthma. It has been already demonstrated that monocyte function can be directly affected by *Dermatophagoides pteronyssinus* allergens (Lee et al., 2008), which is consistent with showed in our study early downregulation of CD163 expression. The response reflects rapid upregulation of pro-inflammatory and downregulation of an anti-inflammatory mechanism by Dp. However, after 24 h of stimulation with Dp anti-inflammatory processes seem to dominate. The effect, however, is attenuated in house dust mite sensitive AAs in comparison with HCs, as demonstrated by the upregulation of CD163 expression in the latter but not in the former group. Lower level of mCD163 expression was demonstrated on sputum and BAL macrophages of asthmatic patients independent of inhaled corticosteroid therapy (Staples et al., 2012). This indicates that enhanced shedding of CD163 occurs in the airways of asthmatic patients leading to elevated concentration of sCD163. Interestingly, in patients with COPD increased number of alveolar macrophages expressing CD163 was demonstrated and their number correlated with severity of the disease (Kaku et al., 2014). A dramatic increase of sCD163 in sputum of AAs induced by ICS indicates that local upregulation of CD163 production by monocytes/macrophages may play a role in the anti-inflammatory action of corticosteroids (Kowal et al., 2014). This is consistent with the current study, which demonstrated *in vitro* potent upregulation of CD163 mRNA and an increase of sCD163 concentration upon stimulation of PBMCs with Dx. The increase of sCD163 concentration, which parallels strong upregulation of CD163 expression, indicates that also mechanisms involved in the shedding of CD163 are activated. Interestingly, it has been demonstrated in a mouse asthma model that CD163 plays a role in downregulation of Dp induced allergic inflammation (Dai et al., 2016). The major *Dermatophagoides pteronyssinus* allergen Der p 1 directly binds CD163 leading to inhibition of CCL24 release (Dai et al., 2016).

8. Conclusion

In summary, in this study we demonstrate that mononuclear phagocytes are an important target for both allergen and corticosteroid action. Moreover, we show that in mild-moderate asthmatic patients the anti-inflammatory effects of corticosteroids synergize with endogenous anti-inflammatory mechanisms triggered by exposure to Dp and that in severe AAs this effect is attenuated.

Acknowledgements

Intramural grant from the Medical University of BialystokN/ST/ZB/17/002/1202.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.imbio.2019.05.005.

References

Balhara, J., Gounni, A.S., 2012. The alveolar macrophages in asthma: a double-edged sword. Mucosal Immunol. 5, 605–609. https://doi.org/10.1038/mi.2012.74.

Bernatowicz, P., Kowal, K., 2016. Soluble CD163 modulates cytokine production by peripheral blood mononuclear cells stimulated by Dermatophagoides pteronyssinus allergens in vitro. Adv. Med. Sci. 61, 249–254. https://doi.org/10.1016/j.advms.2016.02.004.

Covar, R.A., 2016. Pivotal efficacy trials of inhaled corticosteroids in asthma. Ann. Allergy Asthma Immunol. 117, 582–588. https://doi.org/10.1016/j.anai.2016.07.035.

Dai, C., Yao, X., Gordon, E.M., Barochia, A., Cuento, R.A., Kaler, M., Meyer, K.S., Keeran, K.J., Nugent, G.Z., Jeffries, K.R., Qu, X., Yu, Z.X., Aponte, A., Gucek, M., Dagur, P.K., McCoy, J.P., Levine, S.J., 2016. A CCL24-dependent pathway augments eosinophilic airway inflammation in house dust mite-challenged Cd163 -/- mice. Mucosal Immunol. 9, 702–717. https://doi.org/10.1038/mi.2015.94.

Fitzpatrick, A.M., Holguin, F., Teague, W.G., Brown, L.A.S., 2008. Alveolar macrophage phagocytosis is impaired in children with poorly controlled asthma. J. Allergy Clin. Immunol. 121, 1372–1378. https://doi.org/10.1016/j.jaci.2008.03.008. e3.

Frings, W., Dreier, J., Sorg, C., 2002. 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. 526, 93–96. https://doi.org/10.1016/S0014-5793(02)03142-3.

GINA guidlines 2006. www.ginasthma.com, No Title, n.d.

Hew, M., Bhavsar, P., Torrego, A., Meah, S., Khorasani, N., Barnes, P.J., Adcock, I., Kian, F.C., 2006. Relative corticosteroid insensitivity of peripheral blood mononuclear cells in severe asthma. Am. J. Respir. Crit. Care Med. 174, 134–141. https://doi.org/10.1164/rccm.200512-1930OC.

Högger, P., Sorg, C., 2001. Soluble CD163 inhibits phorbol ester-induced lymphocyte proliferation. Biochem. Biophys. Res. Commun. 288, 841–843. https://doi.org/10.1006/bbrc.2001.5845.

Kaku, Y., Imaoka, H., Morimatsu, Y., Komohara, Y., Ohnishi, K., Oda, H., Takenaka, S., Matsuoka, M., Kawayama, T., Takeya, M., Hoshino, T., 2014. Overexpression of CD163, CD204 and CD206 on alveolar macrophages in the lungs of patients with severe chronic obstructive pulmonary disease. PLoS One 9. https://doi.org/10.1371/journal.pone.0087400.

Kelly, E.A.B., Busse, W.W., Jarjour, N.N., 2000. Inhaled budesonide decreases airway inflammatory response to allergen. Am. J. Respir. Crit. Care Med. 162, 883–890. https://doi.org/10.1164/ajrccm.162.3.9910077.

Kowal, K., Møller, H.J., DuBuske, L.M., Moestrup, S.K., Bodzenta-Lukaszyk, A., 2006. Differential expression of monocyte CD163 in single - and dual-asthmatic responders during allergen-induced bronchoconstriction. Clin. Exp. Allergy 36, 1584–1591. https://doi.org/10.1111/j.1365-2222.2006.02573.x.

Kowal, K., Silver, R., Sławińska, E., Bielecki, M., Chyczewski, L., Kowal-Bielecka, O., 2011. CD163 and its role in inflammation. Folia Histochem. Cytobiol. 49, 365–374. https://doi.org/10.5603/FHC.2011.0052.

Kowal, K., Moniuszko, M., Bodzenta-Lukaszyk, A., 2014. The effect of inhaled corticosteroids on the concentration of soluble CD163 in induced sputum of allergic asthma patients. J. Investig. Allergol. Clin. Immunol. 24, 49–55.

Kristiansen, M., Graversen, J.H., Jacobsen, C., Sonne, O., Hoffman, H.J., Law, S.K.A., Moestrup, S.K., 2001. Identification of the haemoglobin scavenger receptor. Nature 409, 198–201. https://doi.org/10.1038/35051594.

Lee, J.S., Kim, I.S., Ryu, J.S., Yun, C.Y., 2008. House dust mite, Dermatophagoides pteronissinus increases expression of MCP-1, IL-6, and IL-8 in human monocytic THP-1 cells. Cytokine 42, 365–371. https://doi.org/10.1016/j.cyto.2008.03.010.

Lurier, E.B., Dalton, D., Dampier, W., Raman, P., Nassiri, S., Ferraro, N.M., Rajagopalan, R., Sarmady, M., Spiller, K.L., 2017. Transcriptome analysis of IL-10-stimulated (M2c) macrophages by next-generation sequencing. Immunobiology 222, 847–856.

Moniuszko, M., Bodzenta-Lukaszyk, A., Kowal, K., Lenczewska, D., Dabrowska, M., 2009. Enhanced frequencies of CD14 + +CD16 +, but not CD14 +CD16 +, peripheral blood monocytes in severe asthmatic patients. Clin. Immunol. 130, 338–346. https://doi.org/10.1016/j.clim.2008.09.011.

Mosser, D.M., 2003. The many faces of macrophage activation. J. Leukoc. Biol. 73, 209–212. https://doi.org/10.1189/jlb.0602325.

Pappas, K., Papaioannou, A.I., Kostikas, K., Tzanakis, N., 2013. The role of macrophages in obstructive airways disease: chronic obstructive pulmonary disease and asthma. Cytokine 64, 613–625. https://doi.org/10.1016/j.cyto.2013.09.010.

Periyalil, H.A., Wood, L.G., Scott, H.A., Jensen, M.E., Gibson, P.G., 2015. Macrophage activation, age and sex effects of immunometabolism in obese asthma. Eur. Respir. J. 45, 388–395. https://doi.org/10.1183/09031936.00080514.

Philippidis, P., Mason, J.C., Evans, B.J., Nadra, I., Taylor, K.M., Haskard, D.O., Landis, R.C., 2004. 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. 94, 119–126. https://doi.org/10.1161/01.RES.0000109414.78907.F9.

Sabatel, C., Radermecker, C., Fievez, L., Paulissen, G., Chakarov, S., Fernandes, C., Olivier, S., Toussaint, M., Pirottin, D., Xiao, X., Quatresooz, P., Sirard, J.C., Cataldo, D., Gillet, L., Bouabe, H., Desmet, C.J., Ginhoux, F., Marichal, T., Bureau, F., 2017. Exposure to Bacterial CpG DNA Protects from Airway Allergic Inflammation by Expanding Regulatory Lung Interstitial Macrophages. Immunity. https://doi.org/10.1016/j.immuni.2017.02.016.

Staples, K.J., Hinks, T.S.C., Ward, J.A., Gunn, V., Smith, C., Djukanović, R., 2012. Phenotypic characterization of lung macrophages in asthmatic patients: over-expression of CCL17. J. Allergy Clin. Immunol. 130, 1404–1412. https://doi.org/10.1016/j.jaci.2012.07.023.

Van Gorp, H., Delputte, P.L., Nauwynck, H.J., 2010. Scavenger receptor CD163, a Jack-of-all-trades and potential target for cell-directed therapy. Mol. Immunol. 47, 1650–1660. https://doi.org/10.1016/j.molimm.2010.02.008.

Wynn, T.A., Chawla, A., Pollard, J.W., 2013. Macrophage biology in development, homeostasis and disease. Nature 496, 445–455. https://doi.org/10.1038/nature12034.

Yeager, M.P., Pioli, P.A., Wardwell, K., Beach, M.L., Martel, P., Lee, H.K., Rassias, A.J., Guyre, P.M., 2008. In vivo exposure to high or low cortisol has biphasic effects on inflammatory response pathways of human monocytes. Anesth. Analg. 107, 1726–1734. https://doi.org/10.1213/ane.0b013e3181875fb0.

Zwadlo, G., Voegeli, R., Oslhoff, K.S., Sorg, C., 1987. A monoclonal antibody to a novel differentiation antigen on human macrophages associated with the down-regulatory phase of the inflammatory process. Pathobiology 55, 295–304. https://doi.org/10.1159/000163432.

Zwadlo-Klarwasser, G., Bent, S., Haubeckh, H.D., Sorg, C., Schmutzler, W., 1990. Glucocorticoid-induced appearance of the macrophage subtype RM 3/1 in peripheral blood of man. Int. Arch. Allergy Immunol. 91, 175–180. https://doi.org/10.1159/000235111.