Clinical Rheumatology (2019) 38:1715–1719
https://doi.org/10.1007/s10067-018-04408-x

BRIEF REPORT

Evidence for M2 macrophage activation in patients with enthesitis-related arthritis category of juvenile idiopathic arthritis

Shruti Bhattacharya[1] · Akhilesh Yadav[1] · Amita Aggarwal[1]

Received: 28 November 2018 / Revised: 10 December 2018 / Accepted: 18 December 2018 / Published online: 8 February 2019

Abstract

Recently, an increase in $CD163^+$ macrophages in ileal biopsies from ankylosing spondylitis patients and an increase in intermediate monocytes in enthesitis-related arthritis (ERA) have been reported. Thus, we studied sCD163 levels as M2 macrophage marker in serum and synovial fluid (SF) of ERA children and CD163 expression on monocyte subsets. Serum samples from ERA patients and healthy controls (HC) were assayed for sCD163 (ELISA). Serum and SF from ERA patients were analyzed when available from same patient (paired samples). In 10 patients, the CD163 expression level was analyzed on monocyte subsets by flow cytometry. Results are expressed as median (interquartile range (IQR)). Sera from 85 patients, SF from 32 ERA patients, and serum from 46 HC were analyzed. The average age at inclusion was 16 ± 3.24 years and age at onset was 11.2 ± 2.79 years. Seventy-nine of them were boys and HLA-B27 was positive in 64/80 patients. The median serum sCD163 levels were higher in patients [1080 (1305.2) ng/ml] than HC [780 (812.5) ng/ml; $p < 0.001$]. The SF levels [9000 (1250) ng/ml] were much higher than serum [3800 (3287.66) ng/ml; $p < 0.001$]. Disease activity data was available in 56 patients. Mean tender joint count was 2 (3), swollen joint count was 2 (2), ESR was 70 (65) mm and CRP was 7.1 (8.9) mg/dl. Serum sCD163 levels correlated with SF but not with disease activity. Intermediate monocytes ($CD14^+CD16^+$) from ERA patients had higher CD163 expression than HC. Elevated sCD163 levels in ERA patient's sera and even higher levels in paired SF suggest towards activation of alternatively activated macrophages in ERA. Lack of correlation with activity may suggest that they have an immune-regulatory role in ERA.

Keywords Alternatively activated macrophages · Innate immune system · Juvenile spondyloarthropathy

Introduction

Spondyloarthropathies (SpA) are a group of inflammatory arthritides that are characterized by male predominance, enthesitis, sacroiliitis, inflammatory back pain, and a strong association with human leukocyte antigen-B27 (HLA-B27). While SpA occurs most often in adulthood, 13–15% of them can have onset in childhood. Most children with SpA belong to enthesitis-related arthritis (ERA) category of juvenile idiopathic arthritis (JIA) as defined by the International League of Association of Rheumatology (ILAR).

In contrast to rheumatoid arthritis (RA), inflammation in SpA is mainly driven by the innate immune system as these patients lack autoantibodies and autoreactive T cells. Studies have shown that monocytes/macrophages, innate lymphoid cells (ILC), and γδ T cells are the major players responsible for fueling the inflammation [1, 2].

Patients with SpA have increased levels of monocyte-derived pro-inflammatory cytokines [3]. In recent years, macrophages have been classified into two subsets: M1 (classically activated) subset has a pro-inflammatory phenotype while the M2 subset (alternatively activated) helps towards resolution of inflammation, repair, and remodeling of tissues. CD163 is one of the receptors expressed on M2 macrophages.

CD163 is a glucocorticoid-inducible member of the group B scavenger receptor cysteine-rich family (SRCR). It is exclusively present on monocyte lineage cells in advanced maturation stages and peripheral blood (PB) monocytes. It is also highly expressed from freshly isolated intermediate monocyte subset [4]. CD163 is present both on the cell surface as well as in the intracellular compartments. It is also secreted as a soluble

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s10067-018-04408-x) contains supplementary material, which is available to authorized users.

✉ Amita Aggarwal
aa.amita@gmail.com

[1] Department of Clinical Immunology and Rheumatology, Sanjay Gandhi Postgraduate Institute of Medical Sciences, Lucknow 226014, India

protein and circulates in plasma [5]. Its expression is enhanced in response to IL-10 cytokine [6]. Cross-linking of CD163 protein with monoclonal antibody directed against CD163 results in production of inflammatory mediators like IL-6, nitric oxide, tumor necrosis factor-α, IL-1β, and IL-10 [7].

Increased levels of CD163 has been detected in the inflamed ileal specimens of the colon in SpA patients displaying chronic gut inflammation [8]. This is associated with activation and proliferation of monocytes in response to inflammation or infection [9]. Increased numbers of $CD163^+$ macrophages in the synovial sub-lining of SpA patients has shown correlation with the swollen joint count, erythrocyte sedimentation rate (ESR), and C-reactive protein (CRP). These macrophages also produced increased levels of pro-inflammatory cytokine, TNF-α responsible for mediating joint inflammation [10]. Synovial fluid (SF) from patients with SpA causes more M2 polarization of PB monocytes than SF from patients with RA.

Thus, we studied the levels of soluble CD163 (sCD163) in serum and SF to see evidence of M2 macrophage activation in children with ERA. In addition, as we had previously shown that children with ERA have increased frequency of intermediate monocytes [11] and CD163 is highly expressed on intermediate monocytes, so we analyzed the expression of CD163 on intermediate monocytes to see if there is any difference in patients and controls.

Material and methods

Patients

Eighty-five ERA patients fulfilling the JIA-ILAR classification criteria [2] were included as study subjects. Forty-six young adult males were included as healthy controls (HC). The demographic data and baseline disease activity of patients are given in Table 1.

Peripheral blood was collected from all subjects, whereas SF was collected from only those patients who required intra-articular steroid injection as a part of their treatment. Cell-free synovial fluid (CFSF) and serum samples were collected and stored at − 80 °C until further analysis. ESR was done by the Westergren method and CRP via nephelometry; HLA B27 was done by polymerase chain reaction (PCR).

Clinical assessments like swollen joint count (SJC), tender joint count (TJC), and duration of the disease were assessed by the treating rheumatologist. Written informed consent was taken from all subjects. The study was approved by the institutional ethics committee (EMR/2015/000834).

Measurement of soluble CD163 levels

The levels of sCD163 in serum and SF was measured via human CD163 quantikine ELISA Kit (R & D systems, MN, USA) as per the manufacturer's instructions. The minimum detection limit for the kit is 1.6 ng/ml.

Table 1 Clinical and demographic details of ERA patients

Features	Values
Mean age (years)	16 ± 3.24
Age at onset	11.2 ± 2.79
Boys:girls	79:6
Presence of hla-b27	64/80 (80%)
Tender joint count	2 (3)
Swollen joint count	2 (2)
ESR (mm)	70 (65)
CRP (mg/dl)	7.1 (8.965)
Drug usage (number)	
NSAIDs	41
Methotrexate	4
Steroids	3

Mean age and age at onset are represented as mean ± SD. Forty-six young adult males were included in the study as HC (17 ± 6.5) years. Tender joint count, swollen joint count, ESR, and CRP are represented as median (IQR). *HC* healthy controls, *ESR* erythrocyte sedimentation rate, *CRP* C-reactive protein

Assessment of CD163 expression on monocyte subsets

In ten patients, peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation using Histopaque 1077 (Sigma, USA). PBMC's were surface stained with anti-CD14-FITC, anti-CD16-PECy5, and anti-CD163-PE (BD Biosciences, CA, USA) monoclonal antibody. 10^5 cells were acquired in a flow cytometer (Beckman Coulter, CA USA). The monocytes were gated in the FSC vs SSC plot. $CD14^+CD16^+$ cells were considered as intermediate monocytes, $CD14^-CD16^{++}$ cells were considered as non-classical monocytes and $CD14^{++}CD16^-$ cells as classical monocytes. CD163 mean fluorescence intensity (MFI) was measured in all three monocyte subsets. The data was analyzed using Flo-jo (trial version) (Gating strategy shown in supplementary figure 1).

Statistical analysis

The frequency of cytokine-producing monocytes and cytokine levels are reported as median [interquartile range (IQR)]. Intergroup comparison was done using non-parametric tests. Correlation with disease activity and duration was assessed using Spearman's correlation and values are expressed as r (95% confidence intervals). p value < 0.05 was taken as significant. Graph pad prism 7 (trial version) was used for all statistical analysis.

Results

Patients

The study included 85 ERA patients. SF was available from 32 patients with ERA. The median disease duration was 48 (66) months. Forty-six male HC were included in the study (Table 1).

Soluble CD163 level

The median level of sCD163 was observed to be elevated in ERA patients as compared to HC [ERA 1080 (1305.2) ng/ml, HC 780 (812.5) ng/ml] ($p < 0.01$) (Fig. 1a). Soluble CD163 levels were further elevated in the SF compared to the serum in paired samples [serum 3800 (3287.66) ng/ml SF 9000 (1250) ng/ml] ($p < 0.01$) (Fig. 1b). There was a positive correlation between serum and SF levels of sCD163 (r value = 0.76 $p < 0.01$) (Fig. 1d). SF sCD163 levels did not correlate with number of swollen or tender joints.

No significant difference was observed in the level of sCD163 on comparing the HLA B27 positive and negative patients [positive 1082.55 (1273.2) ng/ml, negative 1364.04 (1764.46) ng/ml] (Fig. 1c). Also, no correlation was observed between the sCD163 levels in serum and SJC, TJC, ESR, or CRP.

Monocyte subset frequency

Intermediate monocytes ($CD14^{+}CD16^{+}$) showed an elevated frequency in ERA patients as compared to healthy volunteers [ERA 36.6(17.5), HC 5.08(4.12)] ($p < 0.01$).

Though the frequency of classical ($CD14^{++}CD16^{-}$) and non-classical monocyte ($CD14^{-}CD16^{+}$) subsets in ERA patients was lower than HC, it did not reach statistical significance [classical monocytes: ERA 53.1 (28.45), HC 67.95 (19.4); non-classical monocytes: ERA 3.65 (4.7), HC 8 (5.5)] (Fig. 2).

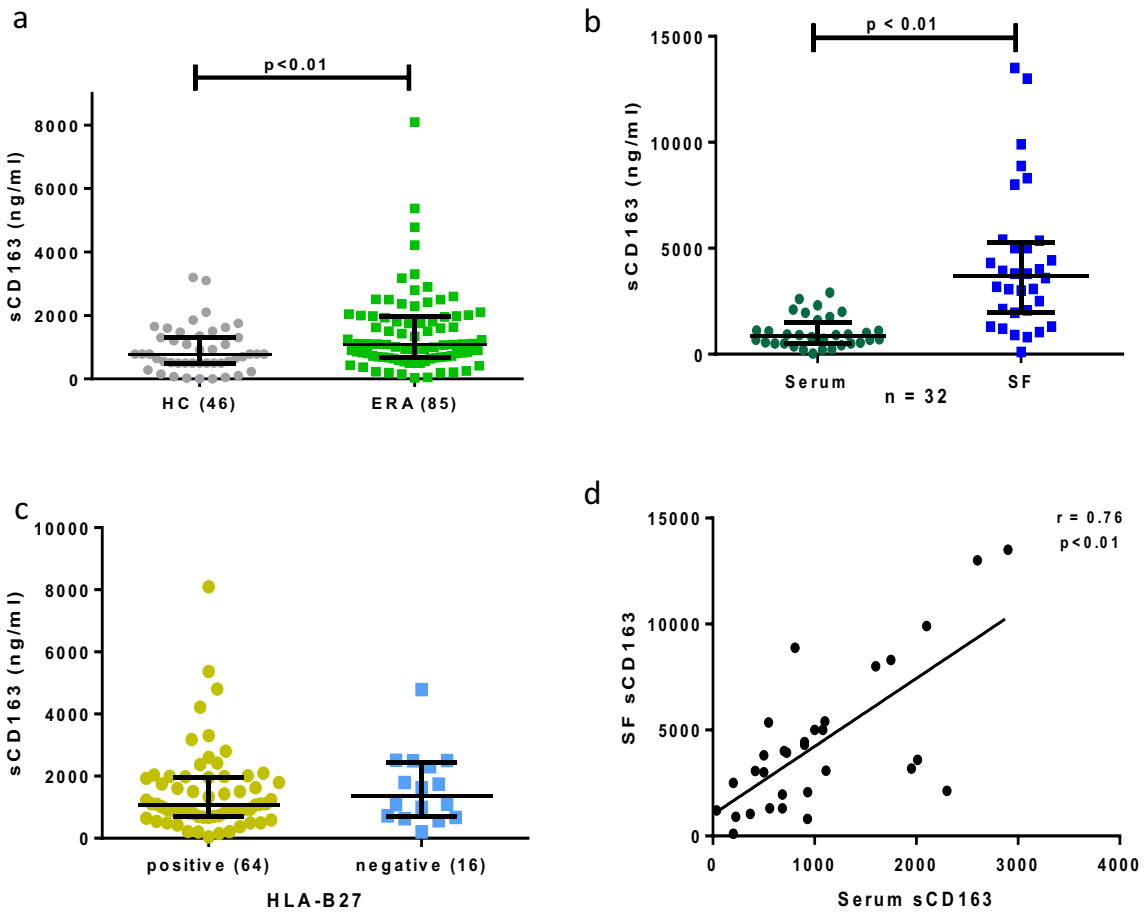

Fig. 1 Scatter plots representing the levels of soluble CD163, intermediate monocytes, and CF163 MFI. Each dot represents an individual sample. The horizontal line represents the median. **a** Level of sCD163 in the serum of HC and ERA patients. **b** Level of sCD163 in serum and SF of ERA patients. **c** Level of sCD163 in the serum of HLA-B27 positive and negative ERA patients. **d** Correlation of sCD163 levels in serum with SF in ERA patients ($n = 32$). Each dot represents an individual sample. Horizontal line represents median. The p values are indicated on the graph. *NCM* non classical monocytes, *IM* intermediate monocytes, *CM* classical monocytes

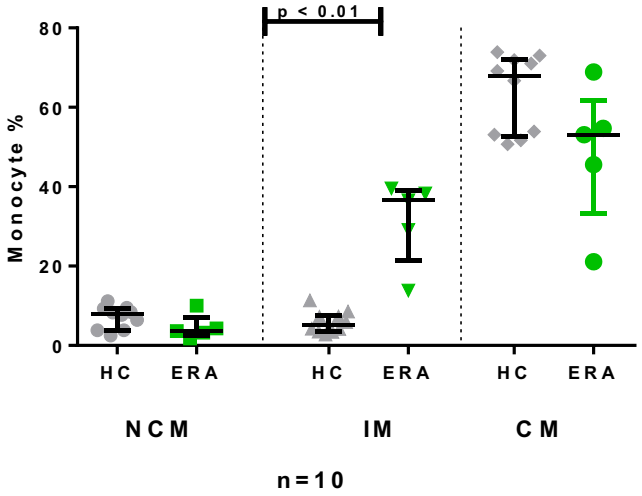

Fig. 2 Scatter plot representing the frequency of NCM, IM, and CM in ERA patients (10) and controls (10). Each dot represents an individual sample. Horizontal line represents median. The *p* values are indicated on the graph. *NCM* non classical monocytes, *IM* intermediate monocytes, *CM* classical monocytes

CD163 mean fluorescence intensity

The CD163 MFI was higher in patients compared to HC on intermediate [ERA 6214 (1133), HC 3189.5 (1671)] and classical [ERA 4278 (1511.5), HC 4055 (1703.5)] monocyte subsets ($p < 0.01$). No significant difference was observed in the case of non-classical monocytes [ERA 5086 (606.5), HC 2676 (899.8)] (Fig. 3).

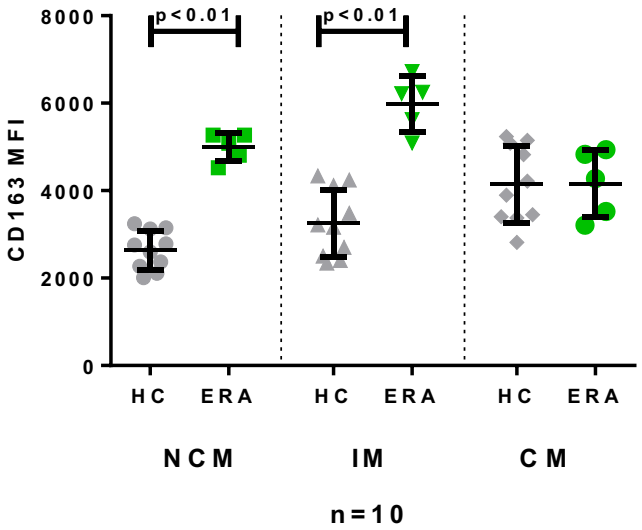

Fig. 3 Scatter plot representing the mean fluorescent intensity (MFI) of CD163 in ERA patients (10) and controls (10). Each dot represents an individual sample. Horizontal line represents median. The *p* values are indicated on the graph. *NCM* non classical monocytes, *IM* intermediate monocytes, *CM* classical monocytes

Discussion

Patients with ERA have higher serum levels of sCD163 as compared to HC and in paired samples, the levels are further higher in SF. However, the sCD163 levels do not correlate with disease activity. Intermediate monocyte frequency is increased in children with ERA and they show higher expression of CD163 as compared to healthy subjects.

The scavenger receptor CD163 is selectively expressed on mononuclear phagocytes, specifically macrophages, and monocytes. Higher frequency of $CD163^+$ macrophages has been observed in the peripheral blood of (AS) patients. These monocytes skew the naïve macrophages towards the $CD163^+$ phenotype in co-culture experiments [12, 13]. The increase in the level of sCD163 in the serum of ERA patients compared to HC is in line with previous data in adult SpA patients [14]. This may be due to the shedding of CD163 from the cell membrane by matrix metalloproteinase in response to endotoxins from the gut bacteria [15].

Higher levels of sCD163 at the local site (SF) of inflammation as compared to serum is in line with a previous study done in RA patients as well as in adult SpA [16, 17]. Elevated levels of $CD163^+$ macrophages in synovium and high CD163 expression in synovium intimal lining has been observed in adult SpA as compared to RA patients [17, 18]. Also, in ERA patients CD163 mRNA levels were 6.5-fold higher in SF mononuclear cells as compared to the PBMC [19]. This suggests that sCD163 in the SF is derived from local synovial tissue macrophages.

Studies done in adult SpA have shown a correlation between elevated levels of SF sCD163 with $CD163^+$ macrophages in the synovial sub-lining as well as with global inflammation [17]. However, in our study, we did not find a correlation of SF levels of sCD163 with swollen or tender joint count. This may be related to differences in synovial pathology between adult and juvenile SpA and there is data to suggest that patients with juvenile SpA patients have lower number of $CD163^+$ macrophages in synovial tissues as compared to adult SpA [20]. Lack of correlation between serum levels of sCD163 with disease activity (CRP) has also been seen in adult SpA [17].

sCD163 decreases the activation as well as the proliferation of T lymphocytes on phorbol myristate acetate stimulation by reducing CD69 expression [21]. $CD163^+$ macrophages may be involved in the mediation of anti-inflammatory response by the production of immunoregulatory cytokines. Indeed, the intimal lining layer macrophages in SpA show co-expression of CD163 and IL-10 [18].

We reconfirmed our previous observation that intermediate monocytes are increased in ERA patients [11]. Further, these intermediate monocytes had a higher CD163 expression in patients as compared to HC. Macrophages having a high expression of CD163 may be involved in downregulation of the

late phases of the acute inflammatory response. This anti-inflammatory response of CD163 macrophages might be due to CD163 mediated delivery of hemoglobin to macrophage as heme metabolites have been observed to have potent anti-inflammatory effects or to the production of IL-10 [22, 23].

The strength of this study is a large sample size and the availability of a substantial number of paired SF samples. However, we have not analyzed CD163 expression in the synovial tissue which is probably the best site to study [10]. Synovial tissue is generally available at the time of joint replacement surgery which is usually 10–20 years after disease onset. Thus, our data suggest that M2 macrophages may have a role in immune-pathogenesis of ERA.

Acknowledgments We thank all the residents of our department for their help in the collection of patient samples.

Funding This work was supported by a grant from the Department of Science and Technology to AA (grant no. EMR/2015/000834). SB was supported by Senior Research Fellowship grant from Department of Biotechnology, New Delhi.

Compliance with ethical standards

Written informed consent was taken from all subjects. The study was approved by the institutional ethics committee (EMR/2015/000834).

Disclosures None.

References

1. Ranganathan V, Gracey E, Brown MA, Inman RD, Haroon N (2017) Pathogenesis of ankylosing spondylitis - recent advances and future directions. Nat Rev Rheumatol 13:359–367
2. Shikhagaie MM, Germar K, Bal SM, Ros XR, Spits H (2017) Innate lymphoid cells in autoimmunity: emerging regulators in rheumatic diseases. Nat Rev Rheumatol 13:164–173
3. Conrad K, Wu P, Sieper J, Syrbe U (2015) In vivo pre-activation of monocytes in patients with axial spondyloarthritis. Arthritis Res Ther 17:179
4. Buechler C, Ritter M, Orso E, Langmann T, Klucken J, Schmitz G (2000) Regulation of scavenger receptor CD163 expression in human monocytes and macrophages by pro- and antiinflammatory stimuli. J Leukoc Biol 67:97–103
5. Etzerodt A, Moestrup SK (2013) CD163 and inflammation: biological, diagnostic, and therapeutic aspects. Antioxid Redox Signal 18: 2352–2363
6. Sulahian TH, Hogger P, Wahner AE Wardwell K, Goulding NJ, Sorg C, Droste A, Stehling M, Wallace PK, Morganelli PM, Guyre PM (2000) Human monocytes express CD163, which is upregulated by IL-10 and identical to p155. Cytokine 12:1312–1321
7. Fabriek BO, van Bruggen R, Deng DM Ligtenberg AJ, Nazmi K, Schornagel K, Vloet RP, Dijkstra CD, van den Berg TK (2009) The macrophage scavenger receptor CD163 functions as an innate immune sensor for bacteria. Blood 113:887–892
8. Ciccia F, Alessandro R, Rizzo A, Accardo-Palumbo A, Raimondo S, Raiata F, Guggino G, Giardina A, De Leo G, Sireci G, Triolo G (2014) Macrophage phenotype in the subclinical gut inflammation of patients with ankylosing spondylitis. Rheumatology (Oxford) 53:104–113
9. Moller HJ (2012) Soluble CD163. Scand J Clin Lab Invest 72:1–13
10. Baeten D, Demetter P, Cuvelier CA Kruithof E, Van Damme N, De Vos M, Veys EM, De Keyser F (2002) Macrophages expressing the scavenger receptor CD163: a link between immune alterations of the gut and synovial inflammation in spondyloarthropathy. J Pathol 196:343–350
11. Gaur P, Myles A, Misra R, Aggarwal A (2017) Intermediate monocytes are increased in enthesitis-related arthritis, a category of juvenile idiopathic arthritis. Clin Exp Immunol 187:234–241
12. Zhao J, Yuan W, Tao C, Sun P, Yang Z, Xu W (2017) M2 polarization of monocytes in ankylosing spondylitis and relationship with inflammation and structural damage. APMIS 125:1070–1075
13. Vandooren B, Noordenbos T, Ambarus C, Krausz S, Cantaert T, Yeremenko N, Boumans M, Lutter R, Tak PP, Baeten D (2009) Absence of a classically activated macrophage cytokine signature in peripheral spondylarthritis, including psoriatic arthritis. Arthritis Rheum 60:966–975
14. Tošovský M, Bradna P, Andrýs C, Andrýsova K, Čermáková E, Soukup T, Kubínová K, Krejsek J (2014) AB0166 the levels of soluble CD163 in patients with ankylosing spondylitis. Ann Rheum Dis 73:857–858
15. Hintz KA, Rassias AJ, Wardwell K, Moss ML, Morganelli PM, Pioli PA, Givan AL, Wallace PK, Yeager MP, Guyre PM (2002) Endotoxin induces rapid metalloproteinase-mediated shedding followed by up-regulation of the monocyte hemoglobin scavenger receptor CD163. J Leukoc Biol 72:711–717
16. Matsushita N, Kashiwagi M, Wait R, Nagayoshi R, Nakamura M, Matsuda T, Hogger P, Guyre PM, Nagase H, Matsuyama T (2002) Elevated levels of soluble CD163 in sera and fluids from rheumatoid arthritis patients and inhibition of the shedding of CD163 by TIMP-3. Clin Exp Immunol 130(1):156–161
17. Baeten D, Moller HJ, Delanghe J, Veys EM, Moestrup SK, De Keyser F (2004) Association of CD163+ macrophages and local production of soluble CD163 with decreased lymphocyte activation in spondylarthropathy synovitis. Arthritis Rheum 50:1611–1623
18. Ambarus CA, Noordenbos T, de Hair MJ, Tak PP, Baeten DL (2012) Intimal lining layer macrophages but not synovial sublining macrophages display an IL-10 polarized-like phenotype in chronic synovitis. Arthritis Res Ther 14:R74
19. Myles A, Tuteja A, Aggarwal A (2012) Synovial fluid mononuclear cell gene expression profiling suggests dysregulation of innate immune genes in enthesitis-related arthritis patients. Rheumatology 51:1785–1789
20. Kruithof E, Van den Bossche V, De Rycke L, Vandooren B, Joos R, Cañete JD, Tak PP, Boots AM, Veys EM, Baeten D (2006) Distinct synovial immunopathologic characteristics of juvenile-onset spondylarthritis and other forms of juvenile idiopathic arthritis. Arthritis Rheum 54:2594–2604
21. 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
22. Moestrup SK, Moller HJ (2004) CD163: a regulated hemoglobin scavenger receptor with a role in the anti-inflammatory response. Ann Med 36:347–354
23. Kristiansen M, Graversen JH, Jacobsen C, Sonne O, Hoffman HJ, Law SK, Moestrup SK (2001) Identification of the haemoglobin scavenger receptor. Nature 409:198–201