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Coronary atherosclerosis severity is closely associated with decreased GLP-1R positivity among CD16^{+} pro-inflammatory and patrolling monocyte subsets

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ABSTRACT

Background and aims: Glucagon Like Peptide-1 Receptor (GLP-1R) activation reduces pro-inflammatory responses of human monocytes, their accumulation in the vascular wall and foam cell formation inhibiting atherosclerogenesis. This suggests that reduction of circulating GLP-1-1R positive monocytes may have pro-atherogenic effects. It is unknown whether different CD14/CD16 monocytes subsets display GLP-1R and whether their relative proportions correlate with atherosclerosis severity. We evaluated the association between GLP-1R positivity in different CD14/CD16 monocyte subsets and coronary atherosclerosis severity.

Methods: Relative amounts of classical (CD14+/CD16-), intermediate pro-inflammatory (CD14+/CD16+) and non-classical patrolling (CD14-/CD16+) subsets of total circulating monocytes and the proportions of GLP-1R positive monocytes in these subsets were determined in 13 control subjects and 10 dyslipidemic ischemic heart disease (IHD) patients with severe angiographic proven coronary atherosclerosis using flow cytometry analysis. Atherosclerosis severity was calculated by SYNTAX score.

Results: In univariable analysis, severe atherosclerosis was associated with decreased proportion of classical monocytes and two fold increased CD16^{+} pro-inflammatory and patrolling subsets as compared with controls ($p = 0.01$, $p = 0.02$ and $p = 0.01$, respectively). Frequency of GLP-1R positive monocytes was decreased in both CD16^{+} subsets ($p = 0.02$ and $p = 0.05$, respectively) and negatively correlated with atherosclerosis severity ($r = -0.65$, $p = 0.005$ and $r = -0.44$, $p = 0.05$, respectively).

Conclusions: Increased skewing of the classical monocyte population toward CD16^{+} pro-inflammatory and patrolling subsets accompanied by decreased in GLP-1R positivity are associated with coronary atherosclerosis severity in IHD patients with dyslipidemia. Although the effect of potential confounders cannot be ruled out, our data suggest that failure of GLP-1R-dependent anti-inflammatory/anti-atherogenic control results in innate immune system dysfunction and can promote atherosclerogenesis.

Introduction

The inflammation underlying atherosclerosis is strongly related to monocyte-related actions [1]. Monocytes are a main component of innate immunity and heterogeneous in terms of phenotype and

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function. Subset identification of human monocytes is based on their relative expression of CD14 co-receptor for toll-like receptor 4 (TLR4) which mediates lipopolysaccharide (LPS) signaling and CD16, the FcγRIIIa immunoglobulin receptor [2]. $CD14^{+}CD16^{-}$classical monocytes (CM) involved in phagocytosis, innate sensing/immune responses and migration represent up to 90% of blood monocytes. Remaining monocytes are equally represented by $CD14^{+}CD16^{+}$ inflammatory intermediate monocytes (IMM) and patrolling $CD14^{-}CD16^{+}$ non-classical monocytes (NCM) [3,4]. CM convert into NCM through an IMM subset [5]. A crucial step in atherogenesis is the infiltration of the sub-endothelial space of large arteries by monocytes, which subsequently differentiate into macrophages transformed into lipid-loaded foam cells [6].

CM were assumed to predict cardiovascular events [7], but subsequent work showed that pro-inflammatory IMM, are the key cell type in the development of atherosclerosis [8]. The highest levels of IMM associated with increased serum pro-inflammatory cytokine TNF-α were demonstrated in patients with coronary atherosclerosis [9] and a pivotal role of elevated IMM was confirmed in the formation, growth, and stability of atherosclerotic lesions [8,10–13]. In addition, patrolling anti-inflammatory NCM [14,15], may also possess pro-inflammatory functions depending on disease-specific context [14,16,17]. Only one of the four NCM subphenotypes is more prevalent in patients with coronary artery disease (CAD) [18]. Thus, both IMM and NCM $CD16^{+}$ subsets may play a significant role in the development of atherosclerosis.

Glucagon Like Peptide-1 (GLP-1) hormone is known as immunomodulator of atherosclerosis [19]. GLP-1 receptor (GLP-1R) agonists may halt atherosclerosis through anti-inflammatory effects of monocytes/macrophages [20–22]. An impaired anti-inflammatory GLP-1R signaling in monocytes treated *in vitro* by oxidized low-density lipoprotein (ox-LDL) in obese patients was demonstrated [23]. Ox-LDL's induce pro-inflammatory, pro-atherogenic effects via epigenetic reprogramming of monocytes *in vivo* [24] and pro-inflammatory phenotype of circulating monocytes is attribute of symptomatic patients with atherosclerosis [25]. Thus, GLP-R 1R deficiency and dyslipidemia may be implicated in monocyte reprogramming toward pro-inflammatory and pro-atherogenic phenotypes accelerating atherosclerosis severity. There are currently no data on GLP-1R expression among different CD14/CD16 monocyte subsets in humans associated with atherosclerosis severity. The aims of our study were to determine the proportions of CD14/CD16 GLP-1R positive monocytes subsets and their association with atherosclerosis severity.

Methods

Subjects

In this case control study 10 IHD patients, mean age 64.5 ± 13.01 (range 46–75 years), 80% male, with angiographically proven 3 vessel severe coronary atherosclerosis disease were recruited. Thirteen adults (38.5% male), mean age 58.0 ± 8.11 (range 44–70 years) were used as controls. Severity of coronary atherosclerosis was calculated according to SYNTAX score [26]. Exclusion criteria were malignancy, chronic dialysis treatment and refusal to participate and/or inability to sign an informed consent. This study was approved by the institutional Helsinki committee. All patients signed an informed consent before enrollment.

Blood samples collection, preparation and FACS analysis

Whole blood samples were collected into K2-EDTA tubes (BD-Plymouth, PL6 7BP, UK) by vein puncture and immediately prepared for FACS analysis. Briefly, blood was treated with human Fc-Receptor blocking solution (Biolegend, San Diego, CA), and subsequently stained with detection antibodies: anti human CD14 (Biogems, Westlake Village, CA); anti human CD16 (Biolegend, San Diego, CA); and either anti GLP-1R or mouse IgG2b isotype control (R&D Systems Minneapolis, MN). After erythrocytes lysis, cells were centrifuged, re-suspended in cold PBS and analyzed by flow cytometer (Navios, Beckman Coulter, Indianapolis, IN) using Flowing Software 2.5.1 (Turku Center for Biotechnology, Finland). Proportions of CD14/CD16 monocyte subsets were determined within the monocyte-enriched population based on forward and side-scatter gating. GLP-1R expression was assessed as the percentage of cells with AlexaFluor 488 signal greater than the isotype control.

Statistical analysis

Data were analyzed using the BMDP Statistical Software [27]. Pearson's chi-squared test, or Fisher's exact test, Student's *t*-test and Spearman's correlations analysis were used. A p value of ≤ 0.05 was considered significant.

Results

Table 1 demonstrates that in comparison with control subjects, patients with severe atherosclerosis had significantly higher prevalence of hypertension ($p = 0.04$), hypercholesterolemia ($p = 0.001$), and pharmacological treatment ($p = 0.0001$, $p = 0.005$, respectively) and lower HDL ($p = 0.001$) but comparable LDL levels.

WBC and total monocyte counts were comparable in the 2 groups. However, severe atherosclerosis group demonstrated significantly decreased proportion of $CD14^{+}$ CM and increased $CD16^{+}$ (both IMM and NCM) ($p < 0.01$; $p < 0.02$ and $p < 0.01$, respectively, Fig. 1A). Severe atherosclerosis group demonstrated non-significant decreased frequency of GLP-1R positive cells in $CD14^{+}$ CM ($p = 0.23$) but these frequencies were significantly decreased among $CD16^{+}$ IMM ($p < 0.03$) and NCM ($p < 0.05$)) subsets as compared to healthy controls (Fig. 1B). The decreased GLP-1R positivity in $CD16^{+}$ subsets negatively correlated with atherosclerosis severity estimated by SYNTAX score ($r = -0.65$, $p = 0.005$ and $r = -0.44$, $p = 0.05$, respectively).

Discussion

Our novel results show that severe atherosclerosis in IHD patients is characterized by redistribution of the proportions of different CD14/CD16 monocyte subsets from $CD14^{+}$ CM towards $CD16^{+}$ IMM and NCM both subsets. CM are not associated with a vulnerable plaque phenotype, and do not predict secondary events in severe atherosclerotic patients [28]. However, CM are involved in the progression of early stages of atherosclerogenesis through initial transformation into an inflammatory phenotype [29] termed pro-inflammatory IMM**,** having a key role in the development of atherosclerosis [5,8]. IMM further, convert into patrolling NCM (5). It was suggested that an increase in the one of four NCM subphenotypes is a compensatory attempt to resolve the atherosclerotic plaque and prevent progression of coronary atherosclerosis [18]. This is in accordance with our data that **suggest** an increase of both $CD16^{+}$ IMM and NCM subsets in severe atherosclerosis.

Furthermore, our results show GLP-1R positivity among different CD14/CD16 monocyte subsets having various attributes, suggesting for the first time that GLP-1R dependent signaling can directly modulate diverse monocyte functions. We also show decreased GLP-1R positivity among all CD14/CD16 monocytes of patients with severe atherosclerosis. In addition, the significant GLP-1Rreduction among $CD16^{+}$ subsets both pro-inflammatory IMM and patrolling NCM was negatively correlated with

Table 1
Clinical characteristics of patients with severe atherosclerosis and control group.

	Severe atherosclerosis patients (n = 10)	Control subjects (n = 13)	*p-value*
Age, yrs.	64.9 ± 13.66	58.2 ± 8.41	*0.19*
Sex, male/female	8/10	5/13	-
Clinical background			
HTN	6(60)	3(23)	***0.04***
Anti-HTN therapy	9(90)	2(15)	***0.0001***
Hypercholesterolemia	8(80)	2(15)	***0.001***
Statin use	7(70)	1(8)	***0.005***
Current Smoker	2(20)	1(8)	*0.44*
T2D	3(30)	1(8)	*0.21*
BMI	28.3 ± 5.71	26.3 ± 2.56	*0.31*
IHD status			
s/p CABG	7 (70)	–	-
s/p PCI	4 (40)	–	-
s/p STEMI	1 (10)	–	-
SYNTAX score,(range)	27.1 ± 7.43,(20–34)	–	-
Laboratory parameters			
WBC, 10^3/μl	7.4 ± 1.02	7.6 ± 0.62	*0.89*
Monocytes, 10^3/μl	0.58 ± 0.09	0.55 ± 0.04	*0.67*
CM,10^3/μl	0.48 ± 0.08	0.50 ± 0.03	*0.86*
CM GLP-1R+,10^3/μl	0.01 ± 0.004	0.02 ± 0.002	*0.23*
IMM,10^3/μl	0.04 ± 0.01	0.02 ± 0.01	*0.07*
IMM GLP-1R+,10^3/μl	0.003 ± 0.001	0.002 ± 0.0004	*0.62*
NCM,10^3/μl	0.05 ± 0.01	0.03 ± 0.004	***0.05***
NCM GLP-1R+,10^3/μl	0.003 ± 0.001	0.003 ± 0.001	*1.00*
CRP, mg/L	54.5 ± 36.83	8.8 ± 3.25	*0.15*
Cholesterol, mg/dL	178.9 ± 71.42	174.2 ± 72.65	*0.88*
LDL-cholesterol, mg/dL	92.3 ± 32.09	108.5 ± 36.02	*0.31*
HDL-cholesterol, mg/dL	25.6 ± 7.80	45.4 ± 14.30	***0.001***
TG, mg/dL	164.3 ± 76.08	130.7 ± 62.77	*0.34*
Creatinine, mg/dl	1.5 ± 1.85	0.7 ± 0.16	*0.25*

Data are expressed as mean ± SD or n (%). Continuous variables were compared using Student's t-test.
T2D: type 2 diabetes mellitus; **HTN:** hypertension**; CABG**: coronary artery bypass surgery; **PCI**: percutaneous coronary intervention; **STEMI**: ST-elevation myocardial infarction; **SYNTAX score**: an angiographic grading of coronary artery disease severity; **BMI**: body mass index; **WBC:** white blood cells; **CM**: classical monocytes; **IMM:** intermediate monocytes**; NCM**: non-classical monocytes; **GLP-1R**+: Glucagon Like Peptide-1 Receptor positivity; **CRP**: C-reactive protein; **LDL**: low-density lipoprotein; **HDL** high-density lipoprotein; **TG**: triglycerides.

atherosclerosis severity. Therefore, the prominent down-regulation of GLP-1R-dependent signaling in $CD16^+$ monocytes subsets is a characteristic sign of atherosclerosis severity.

It was previously suggested that an impaired anti-inflammatory GLP-1R signaling in monocytes of morbidly obese patients (BMI >40) may be involved in atherosclerosis development [23].

However, obesity associated impaired GLP-1R signaling are less relevant to our study as our patients with severe atherosclerosis were overweight, but not obese. Moreover, most of our patients with severe atherosclerosis and reduced monocytes' GLP-1 R positivity had hypercholesterolemia, significantly decreased HDL levels accompanied by a six-fold elevated C-reactive protein (CRP) compared with control subjects. This suggests that during atherosclerosis progression down regulated anti-inflammatory GLP-1R signals in monocytes are associated with dyslipidemia rather than with obesity.

Complex mechanisms underlie the association between dyslipidemia, hypercholesterolemia and reduced monocyte's GLP-1R. Hypercholesterolemia is associated with increased systemic levels of ox- LDL known to be involved in endothelial cell dysfunction [30] increasing the number of monocytes recruited to atherosclerotic plaques. These monocytes differentiate into macrophages that interact with ox- LDL resulting in the formation of foam cells, which in turn secrete pro-inflammatory cytokines that amplify the inflammatory response [31]. GLP-1 agonists prevent ox-LDL-induced adhesion of monocytes to human endothelial cells [32] and ox-LDL uptake by macrophages [33]. In addition, HDL is also important for the physiological detoxification of ox-LDL [34] but HDL of patients with CAD lacks this ability [35]. Taken together, this suggests that decreased GLP-1R signals in concert with reduced/aberrant HDL may jointly down-regulate the anti-inflammatory effects of monocytes in severe atherosclerosis.

Conceivably, dyslipidemia and decrease of anti-inflammatory GLP-1R signals initially stimulate reprogramming of the circulating CM subset towards pro-inflammatory IMM subset increasing their numbers. Further, significant loss of GLP-1R dependent anti-inflammatory signals in these pro-inflammatory monocytes enhances their migration into the inflamed vessel wall [10] which closely correlates with plaque vulnerability in asymptomatic CAD patients [36]. The elevated pro-inflammatory IMM ultimately result in an increased proportion of NCM patrolling subset which normally differentiate into anti-inflammatory resident “M2-like” macrophage populations that suppress inflammatory conditions in atherosclerotic plaques [37,38]. Since GLP-1 induces human macrophage polarization toward the anti-inflammatory M2 phenotype [39], GLP-1R deficiency in the patrolling NCM may shift macrophage polarization toward inflammatory M1 phenotype, promoting atherogenesis by generation inflammatory foam cells [6].

Other inflammatory mediators in addition to dyslipidemia may also intensify atherosclerogenesis. It was estimated that as many 40% of new atherosclerosis cases may result from common chronic infections [40] and a low level of LPS derived endotoxemia constitutes a strong risk factor for the development of atherosclerosis [41]. In addition, both ox-LDL and LPS endotoxin bind to $CD14^+$ co-receptor of TLR4 activating TLR4 mediated monocytes inflammatory pathways [42–45]. Furthermore, ox-LDL and low doses of LPS cooperatively activate macrophages to express higher levels of pro-inflammatory cytokines [46] and are implicated epigenetic and metabolic reprogramming of monocytes and trained immunity underlying the pathophysiology of atherosclerosis [47]. This suggests that decreased anti-inflammatory GLP-1R signals in monocytes of dyslipidemic patients with subclinical endotoxemia may be

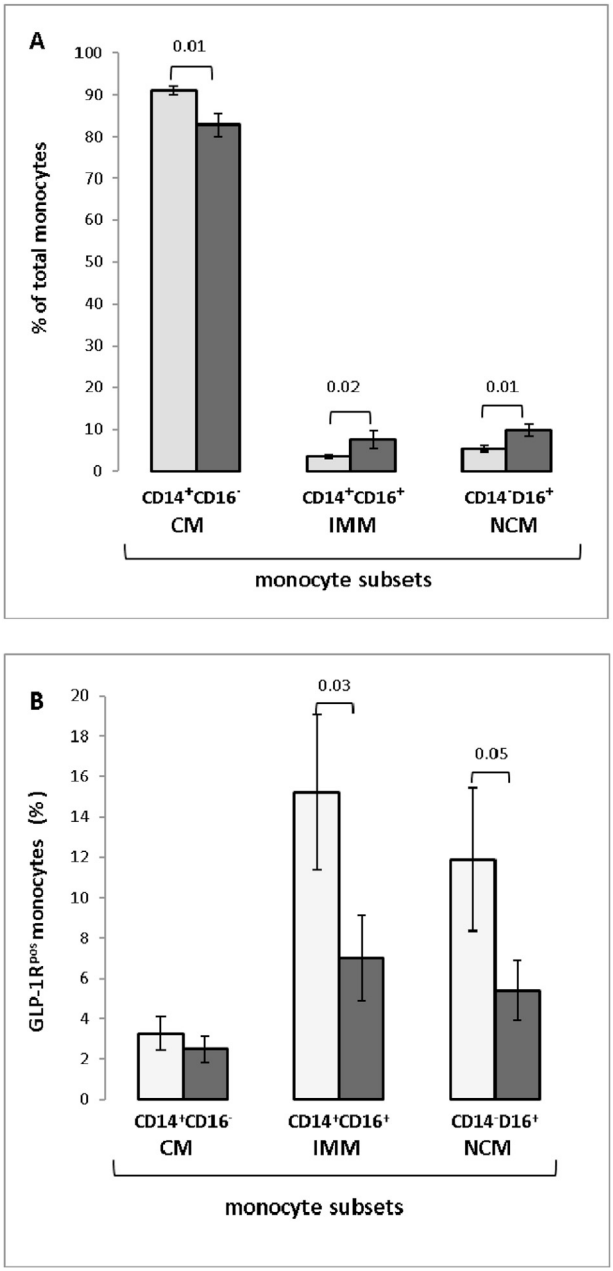

Fig. 1. Severe atherosclerosis is associated with increased proportions of intermediate (CD14+/CD16+ IMM) and non-classical (CD14-/CD16+ NC) monocytes and decreased frequency of GLP-1R positivity in these subsets.
(A) Proportions of classical (CD14+/CD16+ CM), intermediate (CD14+/CD16+ IMM) and non-classical (CD14-/CD16 + NCM) subsets in total circulating monocyte amount of healthy controls (grey bars, n = 13) and patients with severe atherosclerosis (black bars, n = 10). (**B**) Frequency of monocytes expressing GLP-1R among different monocyte subsets in healthy controls (grey bars, n = 13) and patients with severe atherosclerosis (black bars, n = 10). Data are means ± SE.

implicated in programming monocytes into a non-resolving inflammatory state aggravating atherosclerosis.

Several limitations restrict the interpretation of our findings. The small size of our cohort did not allow a multivariable analysis. Therefore, our unadjusted results should be considered as indicative, until confirmed in a larger sample. Additionally, functional *in vitro* experiments with GLP-1 treated monocytes were not performed due to the insufficient number of these cells in the obtained limited whole blood samples. Monocytes' subsets distribution and its association with GLP-1R positivity were also not estimated during atherosclerosis progression in patients with extreme obesity and low-grade endotoxemia. In addition, the SYNTAX revascularization oriented score including lesions >50% stenosis rather than atherosclerotic burden score, may underestimate the coronary atherosclerosis severity.

In conclusion, although the effect of potential confounders cannot be ruled out, our novel results indicate that patients with severe atherosclerosis have significantly increased proportions of both $CD16^{+}$ pro-inflammatory & patrolling subsets accompanied by significantly decreased GLP-1R positivity. The negative correlation between $CD16^{+}$ monocyte expressing GLP-1R and atherosclerosis severity suggests that the modulated function of these monocyte subsets plays an important role in atherosclerosis progression.

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CRediT authorship contributions statement

OB, AB and **MJR** developed the concept of this study. **OB, MYA, MG, GBY** and **MJR** designed the experiments, interpreted the data, **OB** and **MJR** wrote the manuscript, **MYA, GBY** and **OB** performed flow cytometry analysis assays, **OB** and **HAK** performed the statistical analysis. **AB, DC** and **IL** selected suitable patients, provided clinical data and blood sampling. **MJR** supervised the project. **MJR** is the guarantor of this work.

Declaration of competing interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Idzkowska E, Eljaszewicz A, Miklasz P, Musial WJ, Tycinska AM, Moniuszko M. The role of different monocyte subsets in the pathogenesis of atherosclerosis and acute coronary syndromes. Scand J Immunol 2015;82:163–73. https://doi.org/10.1111/sji.12314.
[2] Ziegler-Heitbrock L, Ancuta P, Crowe S, Dalod M, Grau V, Hart DN, Leenen PJM, Liu Y-J, MacPherson G, Randolph GJ, Scherberich J, Schmitz J, Shortman K, Sozzani S, Strobl H, Zembala M, Austyn JM, Lutz MB. Nomenclature of monocytes and dendritic cells in blood. Blood 2010;116:e74–80. https://doi.org/10.1182/blood-2010-02-258558.
[3] Wong KL, Tai JJ-Y, Wong WC, Han H, Sem X, Yeap WH, Kourilsky P, Wong S-C. Gene expression profiling reveals the defining features of the classical, intermediate, and nonclassical human monocyte subsets. Blood 2011;118:e16–31. https://doi.org/10.1182/blood-010-12-326355.
[4] Gren ST, Rasmussen TB, Janciauskiene S, Håkansson K, Gerwien JG, Grip O. A single-cell gene-expression profile reveals inter-cellular heterogeneity within human monocyte subsets. PLoS One 2015;10:e014435. https://doi.org/10.1371/journal.pone.0144351.
[5] Korkosz M, Bukowska-Strakova K, Sadis S, Grodzicki T, Siedla M. Monoclonal antibodies against macrophage colony-stimulating factor diminish the number of circulating intermediate and nonclassical (CD14(++)CD16(+)/CD14(+)CD16(++)) monocytes in rheumatoid arthritis patient. Blood 2012;119:5329–30. https://doi.org/10.1182/blood-2012-02-412551.
[6] Chinetti-Gbaguidi G, Baron M, Bouhlel MA, Vanhoutte J, Copin C, Sebti Y, Derudas B, Mayi T, Bories G, Tailleux A, Haulon S, Zawadzki C, Jude B, Staels B. Human atherosclerotic plaque alternative macrophages display low cholesterol handling but high phagocytosis because of distinct activities of the PPARγ and LXRα pathways. Circ Res 2011;108:985–95. https://doi.org/10.1161/CIRCRESAHA.110.233775.
[7] Berg KE, Ljungcrantz I, Andersson L, Bryngelsson C, Hedblad B, Fredrikson GN, Nilsson J, Björkbacka H. Elevated CD14++CD16− monocytes predict cardiovascular events. Circ. Cardiovasc. Genet 2012;5:122–31. https://doi.org/10.1161/CIRCGENETICS.111.960385.
[8] Rogacev KS, Cremers B, Zawada AM, Seiler S, Binder N, Ege P, Große-Dunker G, Heisel I, Hornof F, Jeken J, Rebling NM, Ulrich C, Scheller B, Böhm M, Fliser D, Heine GH. CD14++CD16+ monocytes independently predict cardiovascular events: a cohort study of 951 patients referred for elective coronary

angiography. J Am Coll Cardiol 2012;60:1512–20. https://doi.org/10.1016/j.jacc.2012.07.019.
[9] Belge K-U, Dayyani F, Horelt A, Siedlar M, Frankenberger M, Frankenberger B, Espevik T, Ziegler-Heitbrock L. The proinflammatory CD14+CD16+DR++ monocytes are a major source of TNF. J Immunol 2002;168:3536–42. https://doi.org/10.4049/jimmunol.168.7.3536.
[10] Schlitt A, Heine GH, Blankenberg S, Espinola-Klein C, Dopheide JF, Bickel C, Lackner KJ, Iz M, Meyer J, Darius H, Rupprecht HJ. CD14+CD16+ monocytes in coronary artery disease and their relationship to serum TNF-α levels. Thromb Haemostasis 2004;92:419–24. https://doi.org/10.1160/TH04-02-0095.
[11] Kashiwagi M, Imanishi T, Tsujioka H, Ikejima H, Kuroi A, Ozaki Y, Ishibashi K, Komukai K, Tanimoto T, Ino Y, Kitabata H, Hirata K, Akasaka T. Association of monocyte subsets with vulnerability characteristics of coronary plaques as assessed by 64-slice multidetector computed tomography in patients with stable angina pectoris. Atherosclerosis 2010;212:171–6. https://doi.org/10.1016/j.atherosclerosis.2010.05.004.
[12] Cappellari R, D'Anna M, Bonora BM, Rigato M, Cignarella A, Avogaro A, PaoloFadini G. Shift of monocyte subsets along their continuum predicts cardiovascular outcomes. Atherosclerosis 2017;266:95–102. https://doi.org/10.1016/j.atherosclerosis.2017.09.032,04.
[13] Ozaki Y, Imanishi T, Hosokawa S, Nishiguchi T, Taruya A, Tanimoto T, Kuroi A, Yamano T, Matsuo Y, Ino Y, Kitabata H, Kubo T, Tanaka A, Akasaka T. Association of toll-like receptor 4 on human monocyte subsets and vulnerability characteristics of coronary plaque as assessed by 64- slice multidetector computed tomography. Circ J 2017;81:837–45. https://doi.org/10.1253/circj.CJ-16-0688.
[14] Thomas G, Tacke R, Hedrick CC, Hanna RN. Nonclassical patrolling monocyte function in the vasculature. Arterioscler Thromb Vasc Biol 2015;35:1306–16. https://doi.org/10.1161/ATVBAHA.114.304650.
[15] Cros J, Cagnard N, Woollard K, Patey N, Zhang SY, Senechal B, Puel A, Biswas SK, Moshous D, Picard C, Jais J-P, D'Cruz D, Casanova J-L, Trouillet C, Geissmann F. Human CD14dim monocytes patrol and sense nucleic acids and viruses via TLR7 and TLR8 receptors. Immunity 2010;33:375–86. https://doi.org/10.1016/j.immuni.2010.08.012.
[16] Aguilar-Ruiz SR, Torres-Aguilar H, González-Domínguez É, Narváez J, González-Pérez G, Vargas-Ayala G, Meraz-Ríos MA, García-Zepeda EA, Sánchez-Torres C. Human CD16+ and CD16- mono-cyte subsets display unique effector properties in inflammatory conditions in vivo. J Leukoc Biol 2011;90:1119–31. https://doi.org/10.1189/jlb.0111022.
[17] Thaler B, Hohensinner PJ, Krychtiuk KA, Matzneller P, Koller L, Brekalo M, Maurer G, Huber K, Zeitlinger M, Jilma B, Wojta J, Speidl WS. Differential in vivo activation of monocyte subsets during low-grade inflammation through experimental endotoxemia in humans. Sci Rep 2016;6:30162. https://doi.org/10.1038/srep3016.
[18] Hamers AAJ, Dinh HQ, Thomas GD, Marcovecchio P, Blatchley A, Nakao CS, Kim C, McSkimming C, Taylor AM, Nguyen AT, McNamara CA, Hedrick CC. Human monocyte heterogeneity as revealed by highdimensional mass cytometry. ArterioscIerThrombVasc Biol 2019;39:25–36. https://doi.org/10.1161/ATVBAHA.118.311022.
[19] Alonso N, Julián MT, Puig-Domingo M, Vives-Pi M. Incretin hormones as immunomodulators of atherosclerosis. Front Endocrinol 2012;3:112. https://doi.org/10.3389/fendo.2012.00112.
[20] Mita T, Watada H. Glucagon like peptide-1 and atherosclerosis, : cardiovasc. Hematol. Agents. Med Chem 2012;10:309–18. https://doi.org/10.2174/187152512803530388.
[21] Rakipovski GG, Rolin B, Nøhr J, Klewe I, Frederiksen KS, Augustin R, Hecksher-Sørensen J, Ingvorsen C, Polex-Wolf J, Knudsen LB. The GLP-1 analogs liraglutide and semaglutide reduce atherosclerosis in ApoE-/- and LDLr-/- mice by a mechanism that includes inflammatory pathways. JACC Basic Transl Sci 2018;3:844–57. https://doi.org/10.1016/j.jacbts.2018.09.004.
[22] Arakawa M, Mita T, Azuma K, Ebato C, Goto H, Nomiyama T, Fujitani Y, Hirose T, Kawamori R, Watada H. Inhibition of monocyte adhesion to endothelial cells and attenuation of atherosclerotic lesion by a glucagon-like peptide-1 receptor agonist, exendin-4. Diabetes 2010;59:1030–7. https://doi.org/10.2337/db09-1694.
[23] Tanaka M, Matsuo Y, Yamakage H, Masuda S, Terada Y, Muranaka K, Wada H, Hasegawa K, Shimatsu A, Satoh-Asahara N. Differential effects of GLP-1 receptor agonist on foam cell formation in monocytes between non-obese and obese subjects. Metabolism 2016;65:1–11. https://doi.org/10.1016/j.metabol.2015.10.009.
[24] Bekkering S, Quintin J, Joosten LAB, van der Meer JWM, Netea MG, Riksen NP. Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. Arterioscler Thromb Vasc Biol 2014;34:1731–8. https://doi.org/10.1161/ATVBAHA.114.303887.
[25] Bekkering S, van den Munckhof I, Nielen T, Lamfers E, Dinarello C, Rutten J, de Graaf J, Joosten LAB, Netea MG, Gomes MER, Riksen NP. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. Atherosclerosis 2016;254:228e–236. https://doi.org/10.1016/j.atherosclerosis.2016.10.019.
[26] Sianos G, Morel M-A, Kappetein AP, Morice M-C, Colombo A, Dawkins K, Marcel van den Brand, Van Dyck N, Russell ME, Mohr FW, Serruys PW. The SYNTAX Score: an angiographic tool grading the complexity of coronary artery disease. EuroIntervention 2005;1:219–27.
[27] Dixon WJ, editor. BMDP statistical software. chief ed. Los Angeles, CA, USA: University of California Press; 1993.
[28] Meeuwsen JAL, de Vries JJ, van Duijvenvoorde A, van der Velden S, van der Laan SW, van Koeverden ID, van de Weg SM, de Borst GJ, de Winther MPJ, Kuiper J, Pasterkamp G, Hoefer IE. Circulating CD14+CD16− classical monocytes do not associate with a vulnerable plaque phenotype, and do not predict secondary events in severe atherosclerotic patients. J Mol Cell Cardiol 2019;127:260–9. https://doi.org/10.1016/j.yjmcc.2019.01.002.
[29] Dai Y, Wang X, Ding Z, Dai D, Mehta JL. DPP-4 inhibitors repress foam cell formation by inhibiting scavenger receptors through protein kinase C pathway. Acta Diabetol 2014;51:471–8. https://doi.org/10.1007/s00592-013-0541-3.
[30] Duarte MM, Rocha JB, Moresco RN, Duarte T, Da Cruz IB, Loro VL, Schetinger MR. Association between ischemia-modified albumin, lipids and inflammation biomarkers in patients with hypercholesterolemia. Clin Biochem 2009;42:666–71. https://doi.org/10.1016/j.clinbiochem.2009.01.010.
[31] Moore K, Sheedy F, Fisher E. Macrophages in atherosclerosis: a dynamic balance. Nat Rev Immunol 2013;13:709–21. https://doi.org/10.1038/nri3520.
[32] Chang W, Zhua F, Zhenga H, Zhoua Z, Miaoa P, Zhaoa L, Maoa Z. Glucagon-like peptide-1 receptor agonist dulaglutide prevents ox-LDLinduced adhesion of monocytes to human endothelial cells: an implication in the treatment of atherosclerosis. Mol Immunol 2019;116:73–9. https://doi.org/10.1016/j.molimm.2019.09.021.
[33] Dai Y, Dai D, Wang X, Ding Z, Li C, Mehta JL. GLP-1 agonists inhibit ox- LDL uptake in macrophages by activation Protein Kinase. J Cardiovasc Pharmacol 2014;64:47–52. https://doi.org/10.1097/FJC.0000000000000087.
[34] Garner B, Waldeck AR, Witting PK, Rye KA, Stocker R. Oxidation of high density lipoproteins. II. Evidence for direct reduction of lipid hydroperoxides by methionine residues of apolipoproteins AI and AII. J Biol Chem 1998;273:6088–95. https://doi.org/10.1074/jbc.273.11.6088.
[35] Navab M, Hama SY, Anantharamaiah GM, Hassan K, Hough GP, Watson AD, Reddy ST, Sevanian A, Fonarow GC, Fogelman AM. Normal high density lipoprotein inhibits three steps in the formation of mildly oxidized low density lipoprotein: steps 2 and 3. J Lipid Res 2000;41:1495–508. https://doi.org/10.1016/S0022-2275(20)33462-3.
[36] Yoshida N, Yamamoto H, Shinke T, Otake H, Kuroda M, Terashita D, Takahashi H, Sakaguchi K, Hirota Y, Emoto T, Amin HZ, Mizoguchi T, Hayashi T, Sasaki N, Yamashita T, Ogawa W, Hirata K. Impact of CD14++CD16+ monocytes on plaque vulnerability in diabetic and non-diabetic patients with asymptomatic coronary artery disease: a cross-sectional study, Cardiovasc. Diabetol 2017;16:96. https://doi.org/10.1186/s12933-017-0577-8.
[37] Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol 2010;7:77–86. https://doi.org/10.1038/nrcardio.2009.228.
[38] Ley K, Miller YI, Hedrick CC. Monocyte and macrophage dynamics during atherogenesis. Arterioscler Thromb Vasc Biol 2011;31:1506–16. https://doi.org/10.1161/ATVBAHA.110.221127.
[39] Shiraishi D, Fujiwara Y, Komohara Y, Mizuta H, Takeya M. Glucagon-like peptide-1 (GLP-1) induces M2 polarization of human macrophages via STAT3 activation. Biochem Biophys Res Commun 2012;425:304–8. https://doi.org/10.1016/j.bbrc.2012.07.086.
[40] Chatzidimitriou D, Kirmizis D, Gavriilaki E, Chatzidimitriou M, Malisiovas N. Atherosclerosis and infection: is the jury still not in? Future Microbiol 2012;7:1217–30. https://doi.org/10.2217/fmb.12.87.
[41] Wiedermann CJ, Kiechl S, Dunzendorfer P Schratzberger F, Egger G, Oberhollenzer F, Willeit J. Association of endotoxaemia with carotid atherosclerosis and cardiovascular disease: prospective results from the Bruneck Study. J Am Coll Cardiol 1998;34:1975. https://doi.org/10.1016/s0735-1097(99)00448-9. – 81.
[42] Arroyo-Espliguero R, Avanzas P, Jeffery S, Kaski JC. CD14 and toll-like receptor 4: a link between infection and acute coronary events? Heart 2004;90:983–8. https://doi.org/10.1136/hrt.2002.001297.
[43] Falck-Hansen M, Kassiteridi C, Monaco C. Toll-like receptors in atherosclerosis. Int J Mol Sci 2013;14:14008–23. https://doi.org/10.3390/ijms140714008.
[44] Miller YI, Choi SH, Wiesner P, Fang L, Harkewicz R, Hartvigsen K, Boullier A, Gonen A, Diehl CJ, Que X, Montano E, Shaw PX, Tsimikas S, Binder CJ, Witztum JL. Oxidation-specific epitopes are danger-associated molecular patterns recognized by pattern recognition receptors of innate immunity. Circ Res 2011;108:235–48. https://doi.org/10.1161/CIRCRESAHA.110.223875.
[45] Wick MC, Mayerl C, Backovic A, van der Zee R, Jaschke W, Dietrich H, Wick G. In vivo imaging of the effect of LPS on arterial endothelial cells: molecular imaging of heat shock protein 60 expression. Cell Stress Chaperones 2008;13:275–85. https://doi.org/10.1007/s12192-008-0044-2.
[46] Wiesner P, Choi S-H, Almazan F, Benner C, Huang W, Diehl CJ, Gonen A, Butler S, Witztum JL, Glass CK, Miller YI. Low doses of LPS and minimally oxidized LDL cooperatively activate macrophages via NF-kappaB and AP-1: possible mechanism for acceleration of atherosclerosis by subclinical endotoxemia. Circ Res 2010;107:56–65. https://doi.org/10.1161/CIRCRESAHA.110.218420.
[47] van Tuijl J, Joosten LAB, Netea MG, Bekkering S, Riksen NP. Immunometabolism orchestrates training of innate immunity in atherosclerosis. Cardiovasc Res 2019;115:1416–24. https://doi.org/10.1093/cvr/cvz107.