Monocyte and Hematopoietic Progenitor Reprogramming as Common Mechanism underlying Chronic Inflammatory and Cardiovascular Diseases

Running title: Reprogramming of monocytes and their progenitors in CID and CVD

R.M. Hoogeveen[1], M. Nahrendorf[2], N.P. Riksen[3], M.G. Netea[3], M.P.J. de Winther[4], E. Lutgens[5], B. G. Nordestgaard[6], M. Neidhart[7], E.S.G Stroes[1], A.L Catapano[8], S. Bekkering[1]

[1]Department of Vascular Medicine, Academic Medical Centre, Amsterdam, The Netherlands
[2]Center for Systems Biology and Department of Imaging, Massachusetts General Hospital and Harvard Medical School, Boston, USA
[3]Department of Internal Medicine, Radboud University Medical Center, Nijmegen, The Netherlands
[4]Department of Medical Biochemistry, Academic Medical Centre, Amsterdam, The Netherlands
[5]Institute for Cardiovascular Prevention (IPEK), Ludwig Maximilians University (LMU), Pettenkoferstraße 9, 80336, Munich, Germany
[6]The Copenhagen General Population study and Department of Clinical Biochemistry, Herlev and Gentofte Hospital, Copenhagen University Hospital, Denmark
[7]Center of Experimental Rheumatology, University Hospital Zurich, CH-8952 Schlieren, Switzerland
[8]Department of Pharmacological and Biomolecular Sciences University of Milan and IRCCS Multimedica, Milano Italy

Corresponding Authors:
Prof. Alberico L. Catapano
Department of Pharmacological and Biomolecular Sciences, University of Milan and IRCCS Multimedica
Via Balzaretti, 9 - 20133 Milan ITALY
Phone number: +39250318302
Fax number: +39250318386
e-mail: alberico.catapano@unimi.it

or

Siroon Bekkering, PhD
Department of Vascular Medicine
Academic Medical Center Amsterdam
Meibergdreef 9, 1105AZ Amsterdam, The Netherlands
Tel: +3120-5666118;
E-mail: s.bekkering@amc.nl

1

Abstract

A large number of cardiovascular events are not prevented by current therapeutic regimens. In search for additional, innovative strategies, immune cells have been recognized as key players contributing to atherosclerotic plaque progression and destabilization. Particularly the role of innate immune cells is of major interest, following the recent paradigm shift that innate immunity, long considered to be incapable of learning, does exhibit immunological memory mediated via epigenetic reprogramming. Compelling evidence shows that atherosclerotic risk factors promote immune cell migration by pre-activation of circulating innate immune cells. Innate immune cell activation via metabolic and epigenetic reprogramming perpetuates a systemic low grade inflammatory state in cardiovascular disease that is also common in other chronic inflammatory disorders. This opens a new therapeutic area in which metabolic or epigenetic modulation of innate immune cells may result in decreased systemic chronic inflammation, alleviating cardiovascular disease and its co-morbidities.

Introduction

Cardiovascular diseases (CVD) are a major health challenge for modern societies. An estimated number of 17 million people die due to CVD each year, representing ≈ 30% of all deaths worldwide. The high burden of CVD is attributable to the increasing incidence of atherosclerosis, caused amongst others by worldwide adoption of the Western lifestyle[1]. In parallel, the incidence of chronic inflammatory diseases (CID) such as rheumatoid arthritis (RA) is rising. Since CID are accompanied by a 2 to 3-fold higher CVD-risk[2], the increased CID prevalence further contributes to the overall CVD burden. Traditionally, risk factors for atherosclerosis are considered to be dyslipoproteinemia, as well as smoking, hypertension, diabetes and obesity. Subsequently, therapeutic measures have focused on lowering the most atherogenic cholesterol, predominantly low-density-lipoprotein cholesterol (LDL-C), which has successfully lowered CVD-risk by 25 to 35%[3]. This success rate, however, also discloses a large residual risk not adequately addressed by current cholesterol-lowering treatment alone.

Atherosclerosis is a chronic inflammatory disease

Atherosclerosis, formerly considered a lipid storage disease, involves a chronic, low-grade inflammatory response of the arterial wall, initiated by lipid accumulation in the intimal layer[4]. Subsequent activation and recruitment of innate immune cells contributes to plaque progression, and eventually plaque destabilization[5]. In more detail, it has been demonstrated that following accumulation of cholesterol in the arterial wall, the subendothelial lipids are modified leading to the formation of active signaling moieties. Particularly oxidized derivatives trigger a variety of inflammatory pathways, immune cells and mediators[6] that drive atherogenesis and co-morbidities of this disease. As the atherosclerotic lesion advances, the presence of immune cells in the lesion increases proportionally, creating a localized pro-inflammatory milieu within the subendothelial compartment[7]. In addition to excessive amounts of lipids, atherosclerotic lesions thus harbor all classes of immune cells and moreover, serum levels of inflammatory mediators are linked to

3

coronary heart disease[8]. Recent advances in preclinical research have established a fundamental role for cellular inflammation throughout all stages of this disease from initiation through progression and, ultimately, the thrombotic complications following plaque rupture or erosion[5, 9-11]. A key role for innate immunity is illustrated by studies showing that monocytes and macrophages are abundantly present in atherosclerotic plaques[12], and moreover, inhibiting monocyte-entry into the plaque drastically attenuates atherogenesis[13] as well as CVD-risk[7], whereas immune cell stimulation accelerates atherosclerosis[10].

In parallel, systemic monocyte production and circulating monocyte number increase progressively with advancing disease[14, 15]. These innate immune cells help drive the formation of an extensive microvascular network penetrating the more advanced atherosclerotic lesions. These leaky, immature vessels provide an easy communication and access network for both cellular as well as humoral elements[16]. The extravasated immune cells also have an intricate impact on plaque phenotype by contributing substantially to endothelial dysfunction, oxidative stress and extracellular matrix degradation[7]. From a therapeutic perspective, several randomized clinical trials are currently underway that will determine whether nonspecific anti-inflammatory therapies with for instance interleukin-1-neutralising antibodies or methotrexate can reduce the risk of cardiovascular events in patients with atherosclerosis[17-19].

To date, little is known on the mechanism(s) maintaining the chronic inflammatory state in CV patients. Recent data have moved our focus from immune cells within the atherosclerotic plaque towards a systemic pro-inflammatory state, characterized by increased production and mobilization of innate immune cells by hematopoietic organs[10, 20], lipoprotein-driven activation of innate immune cells already within the plasma compartment[21], and rapid, increased influx of immune cells into atherosclerotic plaques in patients[22], collectively driving the inflammatory state in the atherosclerotic plaque.

Whereas the contribution of immune cells to atherogenesis has been well established, it remains elusive why the strong inflammatory response in the arterial wall persists over time. In the adaptive

4

arm of the immune system, dendritic cells in the plaque promote adaptive immune responses by presenting antigens, leading to enhanced T-cell function both in experimental and clinical atherosclerosis[23]. The interplay between activated T-cells and resident macrophages perpetuates a local inflammatory cascade within the subendothelial compartment[4, 24]. In addition, increasing body of evidence directs towards a role for impaired resolution of inflammation[25-27].

From a therapeutic standpoint, however, interventions targeting the adaptive immune system directly are hampered by a markedly increased incidence of infectious complications[28, 29]. A better understanding of the role of the innate immune system in the chronic inflammatory characteristics of atherosclerosis may overcome these limitations. However, boosting the adaptive immune system using vaccination strategies has therapeutic potential and is currently being studied for atherosclerosis treatment[23, 30].

Multi-level systemic immune cell activation contributes to atherogenesis and cardiovascular risk

The challenges in targeting inflammation in any chronic disease lie in three properties that are critical for evolutionary survival: redundancy, compensation and necessity. Targeting one component may not be sufficient to attenuate a pro-inflammatory reaction. Inflammation is a carefully tuned process that has multiple feedback loops; thus, inhibition of a critical pathway may trigger a compensatory pro-inflammatory response. Finally and most importantly, the inflammatory response is critical for host defense, and even if the previous two challenges have been met, the risk-benefit ratio may still be unacceptable, exposing for example to an elevated risk of infections. Accordingly, broad immune pathway targeting is unlikely to confer therapeutic benefits. Therefore, developing anti-inflammatory therapies involves identifying suitable pathways in mice, pre-clinical testing of whether the pathway can be neutralized without causing collateral damage, translational studies identifying similarly relevant pathways in humans, and, finally, clinical trials. This challenging drug discovery path can be negotiated more efficiently with the help of immune system imaging, especially of immune cells, their subsets and their migration, production and function.

The rate of progression of atherosclerotic lesions is critically dependent on the number and characteristics of the circulating monocyte pool[31]. In view of the short half-life of innate immune cells[11], attention has shifted towards a more prominent role of bone marrow mobilization and activation of immune cell progenitors[10] and local proliferation[11]. Recently, acute events, both myocardial infarction[10] and stroke[32] were found to elicit a spike in neutrophil and monocyte production in experimental models, which subsequently accumulated in the infarcted areas. In apoE$^{-/-}$ mice with advanced atherosclerosis, acute local events were also found to augment inflammation in atherosclerotic plaques at a distance, increasing plaque size and inducing a more vulnerable lesion morphology with higher inflammatory cell content. An increased supply of innate immune cells was identified as the principal driver for this phenomenon[10]. However, the residence time of innate immune cells in ischemic tissue was estimated to be 19 hours, implying the continued need for new immune cells to maintain the high cell number in atherosclerotic lesions[33]. To support this demand, in mice after coronary ligation, increased sympathetic nervous activity released upstream precursors from bone marrow niches[10]. Markedly, both the increased monocyte count and the pro-inflammatory changes in systemic plaques persisted for several months, the mechanism for which remains yet to be established.

In humans, several lines of evidence support similar pathways. The number of circulating innate immune cells was shown to be a strong predictor of future CV-risk in patients[34]. Following an acute myocardial infarction, bone marrow activity as well as inflammatory signals in distant atherosclerotic lesions were found to be increased[20], implying a systemic cellular mobilization comparable to experimental data[10]. In advanced atherosclerotic lesions, immune cell accumulation is also markedly increased, implying a role for systemic immune cell activity in maintaining the increased inflammatory state in the arterial wall[22]. Furthermore, not only the monocyte number, but also the monocyte phenotype may be affecting atherogenesis. In vitro, several atherogenic risk factors were shown to induce a persistent activated state in monocytes[35], which can further contribute to arterial wall inflammation.

Trained innate immunity and epigenetic reprogramming: a memory for innate immune cells

In the classical immunological paradigm, activation of the innate immune cells (monocytes and macrophages) provides a rapid first line defense against infectious episodes, followed by a 'memory' response mediated by the adaptive immune system. Recently, Netea *et al*. challenged the classical dichotomy of innate versus adaptive immunity[36-38] by showing that brief exposure to microbial products induces a long-term pro-inflammatory phenotype in monocytes, which was found to be linked to metabolic and epigenetic reprogramming. After vaccination of healthy subjects with BCG, monocytes showed a profound pro-inflammatory phenotype, that persisted even three months after vaccination and could be observed even several months after the initial exposure[39, 40]. The concept that the innate immune system is incapable of mounting adaptive responses[41] has already been contradicted by studies showing that organisms lacking a specific immune system are still capable of responding adaptively to infections[42]. Epigenetic reprogramming, accompanied by markers of histone modifications such as methylation of histone 3 at lysine residue 4 (H3K4) or H3K27 acetylation, has been proposed as the molecular mechanism responsible for long-term memory of innate immunity and this process has been termed 'trained immunity'[37, 40, 43].

Epigenetic control denotes the regulation of gene transcription without altering the nucleotide sequence of the DNA by the modification of the chromatin structure. These modifications result in specific chromosomal regions becoming more or less accessible to transcription factors, leading to prolonged alterations in downstream gene-products. There are many different epigenetic modifications that are tightly regulated by a wide array of specific epigenetic writers and erasers that function in a lineage specific manner[44, 45]. Importantly, these modifications are reversible, making chromatin modifying enzymes a potentially interesting therapeutic target[46]. The drug development area of inhibitors targeting histone deacetylases (HDACs) and DNA methyltransferases (DNMTs) in various disease states is expanding rapidly[47, 48], though not yet in the cardiovascular arena. Interestingly, the epigenetic modulators suberoylanilide hydroxamic acid (SAHA, or Vorinostat) and

valproic acid (HDAC inhibitors) have already been approved by the FDA for cancer treatment and epilepsy, respectively[49, 50]. In the past years, the potential for epigenetic intervention by targeting specific histone methyltransferases has increasingly received attention[51].

In the cardiovascular domain, pro-atherogenic stimuli such as oxLDL and lipoprotein(a) [Lp(a)] have recently been shown to induce a prolonged, 'primed' or 'trained' state of the innate immune cells, already in the plasma compartment. In patients with elevated Lp(a), a major CV risk factor, monocytes showed a primed state by increased pro-inflammatory cytokine production upon ex vivo stimulation. *In vitro* priming of healthy monocytes with Lp(a) showed a training capacity: six days after first exposure to Lp(a), monocytes showed an increased pro-inflammatory phenotype. In vivo, this trained state coincided with activated monocytes which accumulated more rapidly into the arterial wall in vivo[52]. In line, oxLDL also induced trained immunity via epigenetic reprogramming, eliciting an activated monocyte phenotype. Incubation of monocytes with oxLDL was associated with increased H3K4me3[35]. The time course for the induction of these pro-inflammatory changes remains to be established. Because monocytes and macrophages have life spans in the order of hours to days, their upstream progenitors, including bone marrow hematopoietic progenitor cells (HSPCs), should also be investigated. Interestingly, recent findings by van der Valk *et al*. and Nahrendorf *et al.* did reveal a persistent inflammatory state in the arterial wall, as well as increased metabolic activity in the bone marrow more than 3 months after an acute myocardial infarction[53]. These findings, supported by experimental data, imply that epigenetic changes in short-lived monocytes are likely to be maintained by similar changes in the hematopoietic precursor cells in the bone marrow (HSPC)[53]. Detailed in vivo studies on HSPCs are, however, absent to date.

How do these pro-atherogenic stimuli modify epigenetic markers? Accumulating evidence points to a pivotal role for rewiring of intracellular metabolism in innate immune cells (Stienstra et al, Cell Metab 2017 in press). Various intermediate metabolites act as important cofactors for epigenetic enzymes, including NAD+, acetylCoA, SAM, FAD, fumarate[54-56]. Trained immunity by BCG or beta-glucan requires a shift from oxidative phosphorylation to aerobic glycolysis[57, 58]. In addition,

accumulation of fumarate due to anaplerotic replenishment of the TCA cycle by glutamine projects

onto epigenetic changes by inhibition of the lysine demethylase KDM5[59]. The relevance of these

metabolic changes for the development of atherosclerosis is illustrated by the upregulation of

glycolytic pathways in monocytes and macrophages isolated from patients with atherosclerosis[60, 61].

For a more detailed description of the role of immunometabolism in the (epigenetic) reprogramming

of innate immune cells in the context of atherosclerosis, we refer to recent excellent reviews on this

topic[62] (Stienstra et al, Cell Metab in press 2017).

In parallel, in CID, the increased inflammatory responsiveness of monocytes and macrophages

due to trained immunity is likely to play a central role[63]. The abundantly present danger associated

molecular patterns (DAMPs) in CID and particularly in RA, including biglycan[64], S100 proteins[65],

HMBG1[66], citrullinated proteins[67], heat shock proteins[68], and tenascin-C[69] might induce a trained

state of innate immune cells, leading to prolonged hyper responsiveness mediated at least partly via

histone modifications. Interestingly, synovial fibroblasts from affected joints in RA also showed

distinct global and promoter-specific changes in DNA methylation[70], collectively pointing towards a

role for epigenetic modulation being involved in the persistent pro-inflammatory state in CID.

From this perspective, the capacity of innate immunity to mount adaptive responses both

redefines the function of innate immunity and provides a potential therapeutic target in chronic

inflammation, including atherosclerosis and RA. This also echoes the highly feared recurrent CV-

events in the first months after an initial CV-event, attributed largely to persistent inflammatory

activity[71]. As active participants in arterial wall inflammation, 'trained' innate immune cells may

represent promising therapeutic targets[72-74]. Targeting innate immune cells is likely to also offer a

wider therapeutic window compared to the adaptive immune system, since patients with innate

immune deficiencies are much less prone to infectious complications compared to those with

disturbances in the adaptive immune system.

In the Horizon2020 project REPROGRAM[75], we propose that trained immunity is an important

pathway promoting an activated state of innate immune cells in the context of atherosclerotic

cardiovascular disease. Hence, modulation of the trained immunity pathway may offer an attractive strategy to effectively attenuate the chronic inflammatory state in atherosclerosis as well as other CID (figure 1).

Common immune responses in atherosclerosis and CID

Many CIDs are associated with altered monocyte phenotype and function, which may alter the potential of these cells to influence atherogenesis[76]. For example, in patients with well-controlled human immunodeficiency virus (HIV), arterial wall inflammation in the aorta is increased, associated with circulating markers of monocyte and macrophage activation[77]. Furthermore, resident macrophages and Toll-like receptor (TLR) signaling play an important role in rheumatoid arthritis[78], atherosclerotic tissue[79] and acute coronary syndromes[80]. MRP8/14, a physiological TLR4 ligand released by activated macrophages, is a prognostic biomarker in both RA[81] and acute coronary syndromes[82]. In mice, experimental osteoarthritis induced increased levels of Ly6C-high, as well as Ly6C-low monocytes, due to increased bone marrow activity[83]. It has been recently proposed that therapeutically targeting interactions between TLRs and foam cell formation may reduce adverse cardiovascular outcomes in individuals with CID[84]. These studies have contributed to uncover novel molecular mechanisms that modulate the inflammatory response in atherosclerotic lesions, and suggest that a parallel exists with chronic inflammatory diseases, such as rheumatoid arthritis (Figure 2). Particularly, a prominent role for endogenous danger signals, comprising both pathogen-associated molecular patterns (PAMPs) as well as DAMPs, in the immunological pathophysiology is emerging rapidly[85, 86]. In RA tissues and synovial fluids, multiple DAMPs are present, most of which have the capacity to act as TLR2 and/or TLR4 agonists.

Several DAMPs have now been shown to contribute to the 'memory' response in monocytes, mediated by distinct histone modifications[87]. The correlation between RA disease activity/duration and the markedly increased inflammatory activity in the arterial wall in RA patients[88] lends further support to a common underlying pathophysiology, eventually translating into a 2 to 3-fold higher CV-

10

risk in RA patients which is only partly attributable to known CV-risk factors[89]. In support of immune cell hyperactivity as a causal factor of CVD in patients with RA, Bernelot Moens et al recently observed that circulating monocytes in RA patients in remission requiring continued biological therapy are characterized by increased expression of activation/adhesion markers, which coincides with increased arterial wall inflammation in RA patients[88]. A potential contribution of epigenetic modulation to the persistent inflammatory state in RA as well as its cardiovascular co-morbidity has been put forward[90], yet the evidence for this concept remains to be delivered.

Multimodal imaging in atherosclerosis and CID

The optimal method to evaluate the presence or progression of atherosclerosis is to directly visualize the target organ for atherosclerosis: the arterial wall. In line with this, imaging modalities have been used in patient studies to measure the dimension of the arterial wall, including ultrasound[91] and magnetic resonance imaging[92]. With the new insight that inflammation is a key component dictating both the progression of atherosclerotic lesions as well as the vulnerability of advanced plaques, attention has shifted towards novel imaging modalities able to quantify the functional aspects in the arterial wall/atherosclerotic lesions, including the inflammatory activity. In this context, 18-Fluordeoxyglucose positron emission tomography with computed tomography ($_{18}$F-FDG PET/CT) imaging is increasingly applied to serve as a measure of arterial wall inflammation[93]. Although its primary target is to detect overall metabolic activity, $_{18}$F-FDG imaging in patients with atherosclerosis robustly correlates with macrophage density as measured by histology[94] and correlates to plaque macrophage content and distribution[95]. It is increasingly used to monitor inflammation in vascular beds as a function of therapy in patients, particularly in the aorta and carotid arteries[96-99].

Clinical credence for PET-based imaging emerged after several retrospective[100, 101] and small-scale prospective studies showing that PET can identify active culprit lesions[102, 103] and predict the risk of a recurrent event. In addition, the first intervention studies have been performed using changes in $_{18}$F-

11

FDG uptake PET to monitor therapeutic efficacy of novel anti-atherosclerotic strategies[99, 104]. More recently, several small ^{18}F-FDG PET/computed tomography studies in patients with AMI reported increased PET signals in ischemic myocardial regions in association with higher ^{18}F-FDG uptake in remote non-culprit atherosclerotic lesions, as well as in hematopoietic organs[105, 106], highlighting the systemic inflammation following acute ischemic events, as previously described in pre-clinical models. A retrospective trial in patients with atherosclerosis reported that increased splenic ^{18}F-FDG also predicted higher cardiovascular event rates[20]. These trials exemplify the opportunities generated by whole-body imaging, including the ability to sample more than one organ system. In order to unravel systems-wide immune actions as well as connections between cardiovascular and hematopoietic organs, imaging studies allow integration of data from cardiovascular organs, non-culprit atherosclerotic lesions, spleen, and bone marrow, which can be combined with data derived from blood and bone-marrow analyses. In this scenario, whole-body multimodal imaging can translate preclinical findings, serve as companion imaging in clinical trials, and help guide individualized therapy.

Therapeutic implications of epigenetic reprogramming of innate immune cells

CID are the most common diseases of ageing and represent one of our major health threats. These include most forms of CVD, RA, type 2 diabetes and virtually all neurodegenerative diseases. In these disease states, a non-autoimmune primary pathological process determines disease progression; for example, inflammation promotes the formation of oxidized phospholipids that may serve as DAMPs in atherosclerosis[86]. As with autoimmune diseases, inhibition of inflammation could reduce the rate of disease progression to the point of substantial clinical benefit despite not altering the underlying pathogenic process. In contrast, in primary inflammatory or autoimmune diseases there is little evidence as yet for efficacy of this approach in humans.

The increased inflammatory responsiveness of monocytes and macrophages due to trained immunity is likely to play a central role in CIDs. From this perspective, the adaptive capacity of the

12

innate immune system provides a potential therapeutic target in human diseases. It is thus essential to improve our understanding of the pathophysiology and cellular and molecular mechanisms common to chronic inflammation, starting with atherosclerosis. Moreover, it is essential to understand the cellular and molecular mechanisms that mediate trained immunity, in hopes of harnessing their therapeutic potential. An important finding in that respect is that trained immunity is characterized by a metabolic shift from oxidative phosphorylation to aerobic glycolysis, which closely interacts with the epigenetic reprogramming[57]. This opens doors for new potential therapeutic possibilities. To prevent training, this mechanism can be targeted, for example using inhibitors of glycolysis[107], or inhibitors of micro RNAs that dictate the balance between glycolysis and OXPHOS[108].

New knowledge about inflammatory signaling, particularly in the areas of endogenous homeostatic pathways and inflammation resolution also provides the promise for new therapeutic options that can adequately meet the therapeutic challenges in CID and atherosclerosis. The abundant presence of epigenetic alterations in both CID and atherosclerosis underlines the potential for clinical applications. In line with this, the potential of epigenetic alterations as molecular biomarkers are being explored for CVD risk evaluation, early detection, prognosis stratification, and treatment response prediction. On the other hand, unlike genetic mutations, epigenetic changes, including DNA methylation and histone modifications, are pharmacologically reversible, making them attractive targets in therapeutic strategies.

Novel insights that will develop by a systems biology approach of trained monocytes is highly likely to identify novel therapeutic targets to prevent or treat atherosclerosis[72]. Several preclinical studies have already provided proof-of-concept data that drugs that modulate the activity of epigenetic writers or erasers, such as HDAC inhibitors, can modulate the development of atherosclerosis[109, 110]. Furthermore, BET-inhibitors such as RVX-208, JQ1 and I-Bet that inhibit the interaction of BET proteins with acetylated histone tails, showed a repression of pathways that contribute to cardiovascular disease and inhibition of atherogenesis in mouse models[111-113]. Major

advances have been made in the field of oncology with aberrant DNA methylation profiles and alterations in histone modification being linked to specific cancers and tumor progression, some of which are already used in the clinic[114]. The discrepancy between major advances in the oncology field and scarcity of data in the cardiovascular and rheumatology field illustrates that an organized effort to address the potential of epigenetic modulation in atherosclerosis and CID is long overdue.

In conclusion, innate immune cell activation via epigenetic reprogramming perpetuates a systemic low grade inflammatory state in cardiovascular disease that is also common in other chronic inflammatory disorders. This opens a new therapeutic area in which epigenetic modulation of innate immune cells will result in a decrease of systemic chronic inflammation, alleviating cardiovascular disease and its co-morbidities.

Legend to the Figures

Figure 1. Innate immune cell activation via epigenetic reprogramming as a common pathway perpetuating the upheld inflammatory state in atherosclerosis and other chronic inflammatory disease states.

Figure 2. Different chronic inflammatory disease states and atherosclerotic DAMPs can trigger monocyte phenotype to change into a long-term activated monocyte. In the bone marrow, these PAMPs and DAMPs might even alter the phenotype of the HSCs.

References

1.   Alwan A, Armstrong T, Bettcher D. Global status report on noncommunicable diseases. World Health Organization. Geneva, Switzerland. 2011.

2.   Libby P. Inflammation in atherosclerosis. Nature 2002;420(6917):868-874.

3.   Heidenreich PA, Trogdon JG, Khavjou OA, Butler J, Dracup K, Ezekowitz MD, Finkelstein EA, Hong Y, Johnston SC, Khera A, Lloyd-Jones DM, Nelson SA, Nichol G, Orenstein D, Wilson PW, Woo YJ, American Heart Association Advocacy Coordinating C, Stroke C, Council on Cardiovascular R, Intervention, Council on Clinical C, Council on E, Prevention, Council on A, Thrombosis, Vascular B, Council on C, Critical C, Perioperative, Resuscitation, Council on Cardiovascular N, Council on the Kidney in Cardiovascular D, Council on Cardiovascular S, Anesthesia, Interdisciplinary Council on Quality of C, Outcomes R. Forecasting the future of cardiovascular disease in the United States: a policy statement from the American Heart Association. Circulation 2011;123(8):933-944.

4.   Hansson GK. Inflammation, atherosclerosis, and coronary artery disease. N Engl J Med 2005;352(16):1685-1695.

5.   Swirski FK, Nahrendorf M. Leukocyte behavior in atherosclerosis, myocardial infarction, and heart failure. Science 2013;339(6116):161-166.

6.   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(2):235-248.

7.   Libby P, Hansson GK. Inflammation and immunity in diseases of the arterial tree: players and layers. Circ Res 2015;116(2):307-311.

8.   Ridker PM, Cannon CP, Morrow D, Rifai N, Rose LM, McCabe CH, Pfeffer MA, Braunwald E. C-reactive protein levels and outcomes after statin therapy. N Engl J Med 2005;352(1):20-28.

9.   Nahrendorf M, Swirski FK. Innate immune cells in ischaemic heart disease: does myocardial infarction beget myocardial infarction? Eur Heart J 2016;37(11):868-872.

10.   Dutta P, Courties G, Wei Y, Leuschner F, Gorbatov R, Robbins CS, Iwamoto Y, Thompson B, Carlson AL, Heidt T, Majmudar MD, Lasitschka F, Etzrodt M, Waterman P, Waring MT, Chicoine AT, van der Laan AM, Niessen HW, Piek JJ, Rubin BB, Butany J, Stone JR, Katus HA, Murphy SA, Morrow DA, Sabatine MS, Vinegoni C, Moskowitz MA, Pittet MJ, Libby P, Lin CP, Swirski FK, Weissleder R, Nahrendorf M. Myocardial infarction accelerates atherosclerosis. Nature 2012;487(7407):325-329.

11.   Robbins CS, Hilgendorf I, Weber GF, Theurl I, Iwamoto Y, Figueiredo JL, Gorbatov R, Sukhova GK, Gerhardt LM, Smyth D, Zavitz CC, Shikatani EA, Parsons M, van Rooijen N, Lin HY, Husain M, Libby P, Nahrendorf M, Weissleder R, Swirski FK. Local proliferation dominates lesional macrophage accumulation in atherosclerosis. Nat Med 2013;19(9):1166-1172.

12.   Moore KJ, Tabas I. Macrophages in the pathogenesis of atherosclerosis. Cell 2011;145(3):341-355.

13.   Boring L, Gosling J, Cleary M, Charo IF. Decreased lesion formation in CCR2-/- mice reveals a role for chemokines in the initiation of atherosclerosis. Nature 1998;394(6696):894-897.

14.   Madjid M, Awan I, Willerson JT, Casscells SW. Leukocyte count and coronary heart disease: implications for risk assessment. J Am Coll Cardiol 2004;44(10):1945-1956.

15.   Olivares R, Ducimetiere P, Claude JR. Monocyte count: a risk factor for coronary heart disease? Am J Epidemiol 1993;137(1):49-53.

16.   Taqueti VR, Di Carli MF, Jerosch-Herold M, Sukhova GK, Murthy VL, Folco EJ, Kwong RY, Ozaki CK, Belkin M, Nahrendorf M, Weissleder R, Libby P. Increased microvascularization and vessel permeability associate with active inflammation in human atheromata. Circ Cardiovasc Imaging 2014;7(6):920-929.

17.   Ridker PM, Luscher TF. Anti-inflammatory therapies for cardiovascular disease. Eur Heart J 2014;35(27):1782-1791.

18.   Ridker PM, Thuren T, Zalewski A, Libby P. Interleukin-1beta inhibition and the prevention of recurrent cardiovascular events: rationale and design of the Canakinumab Anti-inflammatory Thrombosis Outcomes Study (CANTOS). Am Heart J 2011;162(4):597-605.

19.   Everett BM, Pradhan AD, Solomon DH, Paynter N, Macfadyen J, Zaharris E, Gupta M, Clearfield M, Libby P, Hasan AA, Glynn RJ, Ridker PM. Rationale and design of the Cardiovascular Inflammation Reduction Trial: a test of the inflammatory hypothesis of atherothrombosis. Am Heart J 2013;166(2):199-207 e115.

20.   Emami H, Singh P, MacNabb M, Vucic E, Lavender Z, Rudd JH, Fayad ZA, Lehrer-Graiwer J, Korsgren M, Figueroa AL, Fredrickson J, Rubin B, Hoffmann U, Truong QA, Min JK, Baruch A, Nasir K, Nahrendorf M, Tawakol A. Splenic metabolic activity predicts risk of future cardiovascular events: demonstration of a cardiosplenic axis in humans. JACC Cardiovasc Imaging 2015;8(2):121-130.

21.   Bernelot Moens SJ, Neele AE, Kroon J, van der Valk FM, Van den Bossche J, Hoeksema MA, Hoogeveen RM, Schnitzler JG, Baccara-Dinet MT, Manvelian G, de Winther MPJ, Stroes ESG. PCSK9 monoclonal antibodies reverse the pro-inflammatory profile of monocytes in familial hypercholesterolaemia. Eur Heart J 2017;38(20):1584-1593.

22.   van der Valk FM, Kroon J, Potters WV, Thurlings RM, Bennink RJ, Verberne HJ, Nederveen AJ, Nieuwdorp M, Mulder WJ, Fayad ZA, van Buul JD, Stroes ES. In vivo imaging of enhanced leukocyte accumulation in atherosclerotic lesions in humans. J Am Coll Cardiol 2014;64(10):1019-1029.

23.   Andersson J, Libby P, Hansson GK. Adaptive immunity and atherosclerosis. Clin Immunol 2010;134(1):33-46.

24.   Hermansson A, Ketelhuth DF, Strodthoff D, Wurm M, Hansson EM, Nicoletti A, Paulsson-Berne G, Hansson GK. Inhibition of T cell response to native low-density lipoprotein reduces atherosclerosis. J Exp Med 2010;207(5):1081-1093.

25.   Tabas I. Macrophage death and defective inflammation resolution in atherosclerosis. Nat Rev Immunol 2010;10(1):36-46.

26.   Libby P, Tabas I, Fredman G, Fisher EA. Inflammation and its resolution as determinants of acute coronary syndromes. Circ Res 2014;114(12):1867-1879.

27.   Fredman G, Tabas I. Boosting Inflammation Resolution in Atherosclerosis: The Next Frontier for Therapy. Am J Pathol 2017;187(6):1211-1221.

28.   Sommerer C, Konstandin M, Dengler T, Schmidt J, Meuer S, Zeier M, Giese T. Pharmacodynamic monitoring of cyclosporine a in renal allograft recipients shows a quantitative relationship between immunosuppression and the occurrence of recurrent infections and malignancies. Transplantation 2006;82(10):1280-1285.

29.   Singh N. Infectious complications in organ transplant recipients with the use of calcineurin-inhibitor agent-based immunosuppressive regimens. Curr Opin Infect Dis 2005;18(4):342-345.

30.   Lahoute C, Herbin O, Mallat Z, Tedgui A. Adaptive immunity in atherosclerosis: mechanisms and future therapeutic targets. Nat Rev Cardiol 2011;8(6):348-358.

31.   Woollard KJ, Geissmann F. Monocytes in atherosclerosis: subsets and functions. Nat Rev Cardiol 2010;7(2):77-86.

32.   Courties G, Herisson F, Sager HB, Heidt T, Ye Y, Wei Y, Sun Y, Severe N, Dutta P, Scharff J, Scadden DT, Weissleder R, Swirski FK, Moskowitz MA, Nahrendorf M. Ischemic stroke activates hematopoietic bone marrow stem cells. Circ Res 2015;116(3):407-417.

33.   Leuschner F, Rauch PJ, Ueno T, Gorbatov R, Marinelli B, Lee WW, Dutta P, Wei Y, Robbins C, Iwamoto Y, Sena B, Chudnovskiy A, Panizzi P, Keliher E, Higgins JM, Libby P, Moskowitz MA, Pittet MJ, Swirski FK, Weissleder R, Nahrendorf M. Rapid monocyte kinetics in acute myocardial infarction are sustained by extramedullary monocytopoiesis. J Exp Med 2012;209(1):123-137.

34.   Giugliano G, Brevetti G, Lanero S, Schiano V, Laurenzano E, Chiariello M. Leukocyte count in peripheral arterial disease: A simple, reliable, inexpensive approach to cardiovascular risk prediction. Atherosclerosis 2010;210(1):288-293.

35.   Bekkering S, Quintin J, Joosten LA, van der Meer JW, 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(8):1731-1738.

36.   Quintin J, Cheng SC, van der Meer JW, Netea MG. Innate immune memory: towards a better understanding of host defense mechanisms. Curr Opin Immunol 2014;29:1-7.

37.   Netea MG, Quintin J, van der Meer JW. Trained immunity: a memory for innate host defense. Cell Host Microbe 2011;9(5):355-361.

38.   Ifrim DC, Quintin J, Joosten LA, Jacobs C, Jansen T, Jacobs L, Gow NA, Williams DL, van der Meer JW, Netea MG. Trained immunity or tolerance: opposing functional programs induced in human monocytes after engagement of various pattern recognition receptors. Clin Vaccine Immunol 2014;21(4):534-545.

39.   Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LA, Jacobs C, van Loenhout J, Xavier RJ, Aaby P, van der Meer JW, van Crevel R, Netea MG. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. J Innate Immun 2014;6(2):152-158.

40.   Kleinnijenhuis J, Quintin J, Preijers F, Joosten LA, Ifrim DC, Saeed S, Jacobs C, van Loenhout J, de Jong D, Stunnenberg HG, Xavier RJ, van der Meer JW, van Crevel R, Netea MG. Bacille Calmette-Guerin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci U S A 2012;109(43):17537-17542.

41.   Medzhitov R, Janeway C, Jr. Innate immune recognition: mechanisms and pathways. Immunol Rev 2000;173:89-97.

42.   Kurtz J. Specific memory within innate immune systems. Trends Immunol 2005;26(4):186-192.

43.   Netea MG, Joosten LA, Latz E, Mills KH, Natoli G, Stunnenberg HG, O'Neill LA, Xavier RJ. Trained immunity: A program of innate immune memory in health and disease. Science 2016;352(6284):aaf1098.

44.   Heinz S, Romanoski CE, Benner C, Glass CK. The selection and function of cell type-specific enhancers. Nat Rev Mol Cell Biol 2015;16(3):144-154.

45.   Hoeksema MA, de Winther MP. Epigenetic Regulation of Monocyte and Macrophage Function. Antioxid Redox Signal 2016;25(14):758-774.

46.   Neele AE, Van den Bossche J, Hoeksema MA, de Winther MP. Epigenetic pathways in macrophages emerge as novel targets in atherosclerosis. Eur J Pharmacol 2015;763(Pt A):79-89.

47.   Baylin SB, Jones PA. A decade of exploring the cancer epigenome - biological and translational implications. Nat Rev Cancer 2011;11(10):726-734.

48.   Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. Cell 2011;144(5):646-674.

49.   Duenas-Gonzalez A, Candelaria M, Perez-Plascencia C, Perez-Cardenas E, de la Cruz-Hernandez E, Herrera LA. Valproic acid as epigenetic cancer drug: preclinical, clinical and transcriptional effects on solid tumors. Cancer Treat Rev 2008;34(3):206-222.

50.   Mann BS, Johnson JR, He K, Sridhara R, Abraham S, Booth BP, Verbois L, Morse DE, Jee JM, Pope S, Harapanhalli RS, Dagher R, Farrell A, Justice R, Pazdur R. Vorinostat for treatment of cutaneous manifestations of advanced primary cutaneous T-cell lymphoma. Clin Cancer Res 2007;13(8):2318-2322.

51.   Wang Z, Patel DJ. Small molecule epigenetic inhibitors targeted to histone lysine methyltransferases and demethylases. Q Rev Biophys 2013;46(4):349-373.

52.   van der Valk FM, Bekkering S, Kroon J, Yeang C, Van den Bossche J, van Buul JD, Ravandi A, Nederveen AJ, Verberne HJ, Scipione C, Nieuwdorp M, Joosten LA, Netea MG, Koschinsky ML, Witztum JL, Tsimikas S, Riksen NP, Stroes ES. Oxidized Phospholipids on Lipoprotein(a) Elicit Arterial Wall Inflammation and an Inflammatory Monocyte Response in Humans. Circulation 2016;134(8):611-624.

53.   van der Valk FM, Kuijk C, Verweij SL, Stiekema LCA, Kaiser Y, Zeerleder S, Nahrendorf M, Voermans C, Stroes ESG. Increased haematopoietic activity in patients with atherosclerosis. Eur Heart J 2017;38(6):425-432.

54.   Rodriguez H, Rafehi H, Bhave M, El-Osta A. Metabolism and chromatin dynamics in health and disease. Mol Aspects Med 2017;54:1-15.

55.   Keating ST, El-Osta A. Epigenetics and metabolism. Circ Res 2015;116(4):715-736.

56.   Montgomery DC, Sorum AW, Guasch L, Nicklaus MC, Meier JL. Metabolic Regulation of Histone Acetyltransferases by Endogenous Acyl-CoA Cofactors. Chem Biol 2015;22(8):1030-1039.

57.   Cheng SC, Quintin J, Cramer RA, Shepardson KM, Saeed S, Kumar V, Giamarellos-Bourboulis EJ, Martens JH, Rao NA, Aghajanirefah A, Manjeri GR, Li Y, Ifrim DC, Arts RJ, van der Veer BM, Deen PM, Logie C, O'Neill LA, Willems P, van de Veerdonk FL, van der Meer JW, Ng A, Joosten LA, Wijmenga C, Stunnenberg HG, Xavier RJ, Netea MG. mTOR- and HIF-1alpha-mediated aerobic glycolysis as metabolic basis for trained immunity. Science 2014;345(6204):1250684.

58.   Arts RJ, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, Kleinnijenhuis J, Lachmandas E, Goncalves LG, Belinha A, Cunha C, Oosting M, Joosten LA, Matarese G, van Crevel R, Netea MG. Immunometabolic Pathways in BCG-Induced Trained Immunity. Cell Rep 2016;17(10):2562-2571.

59.   Arts RJ, Novakovic B, Ter Horst R, Carvalho A, Bekkering S, Lachmandas E, Rodrigues F, Silvestre R, Cheng SC, Wang SY, Habibi E, Goncalves LG, Mesquita I, Cunha C, van Laarhoven A, van de Veerdonk FL, Williams DL, van der Meer JW, Logie C, O'Neill LA, Dinarello CA, Riksen NP, van Crevel R, Clish C, Notebaart RA, Joosten LA, Stunnenberg HG, Xavier RJ, Netea MG. Glutaminolysis and Fumarate Accumulation Integrate Immunometabolic and Epigenetic Programs in Trained Immunity. Cell Metab 2016;24(6):807-819.

60.   Shirai T, Nazarewicz RR, Wallis BB, Yanes RE, Watanabe R, Hilhorst M, Tian L, Harrison DG, Giacomini JC, Assimes TL, Goronzy JJ, Weyand CM. The glycolytic enzyme PKM2 bridges metabolic and inflammatory dysfunction in coronary artery disease. J Exp Med 2016;213(3):337-354.

61.   Bekkering S, van den Munckhof I, Nielen T, Lamfers E, Dinarello C, Rutten J, de Graaf J, Joosten LA, Netea MG, Gomes ME, Riksen NP. Innate immune cell activation and epigenetic remodeling in symptomatic and asymptomatic atherosclerosis in humans in vivo. Atherosclerosis 2016;254:228-236.

62.   Van den Bossche J, O'Neill LA, Menon D. Macrophage Immunometabolism: Where Are We (Going)? Trends Immunol 2017;38(6):395-406.

63.   Bekkering S, Joosten LA, van der Meer JW, Netea MG, Riksen NP. Trained innate immunity and atherosclerosis. Curr Opin Lipidol 2013;24(6):487-492.

64.   Barreto G, Soininen A, Ylinen P, Sandelin J, Konttinen YT, Nordstrom DC, Eklund KK. Soluble biglycan: a potential mediator of cartilage degradation in osteoarthritis. Arthritis Res Ther 2015;17:379.

65.   Senolt L, Grigorian M, Lukanidin E, Simmen B, Michel BA, Pavelka K, Gay RE, Gay S, Neidhart M. S100A4 is expressed at site of invasion in rheumatoid arthritis synovium and modulates production of matrix metalloproteinases. Ann Rheum Dis 2006;65(12):1645-1648.

66.   Kokkola R, Sundberg E, Ulfgren AK, Palmblad K, Li J, Wang H, Ulloa L, Yang H, Yan XJ, Furie R, Chiorazzi N, Tracey KJ, Andersson U, Harris HE. High mobility group box chromosomal protein 1: a novel proinflammatory mediator in synovitis. Arthritis Rheum 2002;46(10):2598-2603.

67.   Chang X, Yamada R, Suzuki A, Kochi Y, Sawada T, Yamamoto K. Citrullination of fibronectin in rheumatoid arthritis synovial tissue. Rheumatology (Oxford) 2005;44(11):1374-1382.

68.   Huang QQ, Koessler RE, Birkett R, Dorfleutner A, Perlman H, Haines GK, 3rd, Stehlik C, Nicchitta CV, Pope RM. Glycoprotein 96 perpetuates the persistent inflammation of rheumatoid arthritis. Arthritis Rheum 2012;64(11):3638-3648.

69.   Hasegawa M, Nakoshi Y, Muraki M, Sudo A, Kinoshita N, Yoshida T, Uchida A. Expression of large tenascin-C splice variants in synovial fluid of patients with rheumatoid arthritis. J Orthop Res 2007;25(5):563-568.

70.   Karouzakis E, Gay RE, Gay S, Neidhart M. Increased recycling of polyamines is associated with global DNA hypomethylation in rheumatoid arthritis synovial fibroblasts. Arthritis Rheum 2012;64(6):1809-1817.

71.   Marnane M, Merwick A, Sheehan OC, Hannon N, Foran P, Grant T, Dolan E, Moroney J, Murphy S, O'Rourke K, O'Malley K, O'Donohoe M, McDonnell C, Noone I, Barry M, Crowe M, Kavanagh E, O'Connell M, Kelly PJ. Carotid plaque inflammation on 18F-fluorodeoxyglucose positron emission tomography predicts early stroke recurrence. Ann Neurol 2012;71(5):709-718.

72.   Bekkering S, Joosten LA, van der Meer JW, Netea MG, Riksen NP. The epigenetic memory of monocytes and macrophages as a novel drug target in atherosclerosis. Clin Ther 2015;37(4):914-923.

73.   Christ A, Bekkering S, Latz E, Riksen NP. Long-term activation of the innate immune system in atherosclerosis. Semin Immunol 2016;28(4):384-393.

74.   Tabas I, Glass CK. Anti-inflammatory therapy in chronic disease: challenges and opportunities. Science 2013;339(6116):166-172.

75.   http://reprogram-horizon2020.eu/.

76.   Rossol M, Kraus S, Pierer M, Baerwald C, Wagner U. The CD14(bright) CD16+ monocyte subset is expanded in rheumatoid arthritis and promotes expansion of the Th17 cell population. Arthritis Rheum 2012;64(3):671-677.

77.   Subramanian S, Tawakol A, Burdo TH, Abbara S, Wei J, Vijayakumar J, Corsini E, Abdelbaky A, Zanni MV, Hoffmann U, Williams KC, Lo J, Grinspoon SK. Arterial inflammation in patients with HIV. JAMA 2012;308(4):379-386.

78.   Huang Q, Ma Y, Adebayo A, Pope RM. Increased macrophage activation mediated through toll-like receptors in rheumatoid arthritis. Arthritis Rheum 2007;56(7):2192-2201.

79.   Edfeldt K, Swedenborg J, Hansson GK, Yan ZQ. Expression of toll-like receptors in human atherosclerotic lesions: a possible pathway for plaque activation. Circulation 2002;105(10):1158-1161.

80.   Wyss CA, Neidhart M, Altwegg L, Spanaus KS, Yonekawa K, Wischnewsky MB, Corti R, Kucher N, Roffi M, Eberli FR, Amann-Vesti B, Gay S, von Eckardstein A, Luscher TF, Maier W. Cellular actors, Toll-like receptors, and local cytokine profile in acute coronary syndromes. Eur Heart J 2010;31(12):1457-1469.

81.   Choi IY, Gerlag DM, Holzinger D, Roth J, Tak PP. From synovial tissue to peripheral blood: myeloid related protein 8/14 is a sensitive biomarker for effective treatment in early drug development in patients with rheumatoid arthritis. PLoS One 2014;9(8):e106253.

82.   Yonekawa K, Neidhart M, Altwegg LA, Wyss CA, Corti R, Vogl T, Grigorian M, Gay S, Luscher TF, Maier W. Myeloid related proteins activate Toll-like receptor 4 in human acute coronary syndromes. Atherosclerosis 2011;218(2):486-492.

83.   Cremers N, Geven E, Blom A, Sloetjes A, DiCeglie I, van Dalen S, van den Bosch M, van der Kraan P, van Lent P. S100A8/A9 increases the mobilization of Ly6C high monocytes to the synovium during experimental osteoarthritis. Osteoarthr Cartil 2017;25:S47-S48.

84.   Angelovich TA, Hearps AC, Jaworowski A. Inflammation-induced foam cell formation in chronic inflammatory disease. Immunol Cell Biol 2015;93(8):683-693.

85.   Pisetsky DS. The origin and properties of extracellular DNA: from PAMP to DAMP. Clin Immunol 2012;144(1):32-40.

86.   Zimmer S, Grebe A, Latz E. Danger signaling in atherosclerosis. Circ Res 2015;116(2):323-340.

87.   Bekkering S, Blok BA, Joosten LA, Riksen NP, van Crevel R, Netea MG. In Vitro Experimental Model of Trained Innate Immunity in Human Primary Monocytes. Clin Vaccine Immunol 2016;23(12):926-933.

88.   Bernelot Moens SJ, van der Valk FM, Strang AC, Kroon J, Smits LP, Kneepkens EL, Verberne HJ, van Buul JD, Nurmohamed MT, Stroes ES. Unexpected arterial wall and cellular inflammation in patients with rheumatoid arthritis in remission using biological therapy: a cross-sectional study. Arthritis Res Ther 2016;18(1):115.

89.   Baghdadi LR, Woodman RJ, Shanahan EM, Mangoni AA. The impact of traditional cardiovascular risk factors on cardiovascular outcomes in patients with rheumatoid arthritis: a systematic review and meta-analysis. PLoS One 2015;10(2):e0117952.

90.   Klein K, Kabala PA, Grabiec AM, Gay RE, Kolling C, Lin LL, Gay S, Tak PP, Prinjha RK, Ospelt C, Reedquist KA. The bromodomain protein inhibitor I-BET151 suppresses expression of inflammatory genes and matrix degrading enzymes in rheumatoid arthritis synovial fibroblasts. Ann Rheum Dis 2016;75(2):422-429.

91.   Kastelein JJ, Akdim F, Stroes ES, Zwinderman AH, Bots ML, Stalenhoef AF, Visseren FL, Sijbrands EJ, Trip MD, Stein EA, Gaudet D, Duivenvoorden R, Veltri EP, Marais AD, de Groot E,

Investigators E. Simvastatin with or without ezetimibe in familial hypercholesterolemia. N Engl J Med 2008;358(14):1431-1443.

92.   Bochem AE, van Wijk DF, Holleboom AG, Duivenvoorden R, Motazacker MM, Dallinga-Thie GM, de Groot E, Kastelein JJ, Nederveen AJ, Hovingh GK, Stroes ES. ABCA1 mutation carriers with low high-density lipoprotein cholesterol are characterized by a larger atherosclerotic burden. Eur Heart J 2013;34(4):286-291.

93.   Tarkin JM, Rudd JH. Techniques for noninvasive molecular imaging of atherosclerotic plaque. Nat Rev Cardiol 2015;12(2):79.

94.   Tawakol A, Migrino RQ, Bashian GG, Bedri S, Vermylen D, Cury RC, Yates D, LaMuraglia GM, Furie K, Houser S, Gewirtz H, Muller JE, Brady TJ, Fischman AJ. In vivo 18F-fluorodeoxyglucose positron emission tomography imaging provides a noninvasive measure of carotid plaque inflammation in patients. J Am Coll Cardiol 2006;48(9):1818-1824.

95.   Masteling MG, Zeebregts CJ, Tio RA, Breek JC, Tietge UJ, de Boer JF, Glaudemans AW, Dierckx RA, Boersma HH, Slart RH. High-resolution imaging of human atherosclerotic carotid plaques with micro 18F-FDG PET scanning exploring plaque vulnerability. J Nucl Cardiol 2011;18(6):1066-1075.

96.   Lehrer-Graiwer J, Singh P, Abdelbaky A, Vucic E, Korsgren M, Baruch A, Fredrickson J, van Bruggen N, Tang MT, Frendeus B, Rudd JH, Hsieh F, Ballantyne CM, Ghoshhajra B, Rosenson RS, Koren M, Roth EM, Duprez DA, Fayad ZA, Tawakol AA. FDG-PET imaging for oxidized LDL in stable atherosclerotic disease: a phase II study of safety, tolerability, and anti-inflammatory activity. JACC Cardiovasc Imaging 2015;8(4):493-494.

97.   Fayad ZA, Mani V, Woodward M, Kallend D, Abt M, Burgess T, Fuster V, Ballantyne CM, Stein EA, Tardif JC, Rudd JH, Farkouh ME, Tawakol A. Safety and efficacy of dalcetrapib on atherosclerotic disease using novel non-invasive multimodality imaging (dal-PLAQUE): a randomised clinical trial. Lancet 2011;378(9802):1547-1559.

98.   Tawakol A, Fayad ZA, Mogg R, Alon A, Klimas MT, Dansky H, Subramanian SS, Abdelbaky A, Rudd JH, Farkouh ME, Nunes IO, Beals CR, Shankar SS. Intensification of statin therapy results in a rapid reduction in atherosclerotic inflammation: results of a multicenter fluorodeoxyglucose-positron emission tomography/computed tomography feasibility study. J Am Coll Cardiol 2013;62(10):909-917.

99.   van Wijk DF, Sjouke B, Figueroa A, Emami H, van der Valk FM, MacNabb MH, Hemphill LC, Schulte DM, Koopman MG, Lobatto ME, Verberne HJ, Fayad ZA, Kastelein JJ, Mulder WJ, Hovingh GK, Tawakol A, Stroes ES. Nonpharmacological lipoprotein apheresis reduces arterial inflammation in familial hypercholesterolemia. J Am Coll Cardiol 2014;64(14):1418-1426.

100.   Paulmier B, Duet M, Khayat R, Pierquet-Ghazzar N, Laissy JP, Maunoury C, Hugonnet F, Sauvaget E, Trinquart L, Faraggi M. Arterial wall uptake of fluorodeoxyglucose on PET imaging in stable cancer disease patients indicates higher risk for cardiovascular events. J Nucl Cardiol 2008;15(2):209-217.

101.   Figueroa AL, Abdelbaky A, Truong QA, Corsini E, MacNabb MH, Lavender ZR, Lawler MA, Grinspoon SK, Brady TJ, Nasir K, Hoffmann U, Tawakol A. Measurement of arterial activity on routine FDG PET/CT images improves prediction of risk of future CV events. JACC Cardiovasc Imaging 2013;6(12):1250-1259.

102.   Davies JR, Rudd JH, Fryer TD, Graves MJ, Clark JC, Kirkpatrick PJ, Gillard JH, Warburton EA, Weissberg PL. Identification of culprit lesions after transient ischemic attack by combined 18F fluorodeoxyglucose positron-emission tomography and high-resolution magnetic resonance imaging. Stroke 2005;36(12):2642-2647.

103.   Rudd JH, Warburton EA, Fryer TD, Jones HA, Clark JC, Antoun N, Johnstrom P, Davenport AP, Kirkpatrick PJ, Arch BN, Pickard JD, Weissberg PL. Imaging atherosclerotic plaque inflammation with [18F]-fluorodeoxyglucose positron emission tomography. Circulation 2002;105(23):2708-2711.

104.   Tawakol A, Singh P, Rudd JH, Soffer J, Cai G, Vucic E, Brannan SP, Tarka EA, Shaddinger BC, Sarov-Blat L, Matthews P, Subramanian S, Farkouh M, Fayad ZA. Effect of treatment for 12 weeks with rilapladib, a lipoprotein-associated phospholipase A2 inhibitor, on arterial inflammation as

assessed with 18F-fluorodeoxyglucose-positron emission tomography imaging. J Am Coll Cardiol 2014;63(1):86-88.

105.   Assmus B, Iwasaki M, Schachinger V, Roexe T, Koyanagi M, Iekushi K, Xu Q, Tonn T, Seifried E, Liebner S, Kranert WT, Grunwald F, Dimmeler S, Zeiher AM. Acute myocardial infarction activates progenitor cells and increases Wnt signalling in the bone marrow. Eur Heart J 2012;33(15):1911-1919.

106.   Wollenweber T, Roentgen P, Schafer A, Schatka I, Zwadlo C, Brunkhorst T, Berding G, Bauersachs J, Bengel FM. Characterizing the inflammatory tissue response to acute myocardial infarction by clinical multimodality noninvasive imaging. Circ Cardiovasc Imaging 2014;7(5):811-818.

107.   Schoors S, De Bock K, Cantelmo AR, Georgiadou M, Ghesquiere B, Cauwenberghs S, Kuchnio A, Wong BW, Quaegebeur A, Goveia J, Bifari F, Wang X, Blanco R, Tembuyser B, Cornelissen I, Bouche A, Vinckier S, Diaz-Moralli S, Gerhardt H, Telang S, Cascante M, Chesney J, Dewerchin M, Carmeliet P. Partial and transient reduction of glycolysis by PFKFB3 blockade reduces pathological angiogenesis. Cell Metab 2014;19(1):37-48.

108.   Ouimet M, Ediriweera HN, Gundra UM, Sheedy FJ, Ramkhelawon B, Hutchison SB, Rinehold K, van Solingen C, Fullerton MD, Cecchini K, Rayner KJ, Steinberg GR, Zamore PD, Fisher EA, Loke P, Moore KJ. MicroRNA-33-dependent regulation of macrophage metabolism directs immune cell polarization in atherosclerosis. J Clin Invest 2015;125(12):4334-4348.

109.   Cao Q, Rong S, Repa JJ, St Clair R, Parks JS, Mishra N. Histone deacetylase 9 represses cholesterol efflux and alternatively activated macrophages in atherosclerosis development. Arterioscler Thromb Vasc Biol 2014;34(9):1871-1879.

110.   Hoeksema MA, Gijbels MJ, Van den Bossche J, van der Velden S, Sijm A, Neele AE, Seijkens T, Stoger JL, Meiler S, Boshuizen MC, Dallinga-Thie GM, Levels JH, Boon L, Mullican SE, Spann NJ, Cleutjens JP, Glass CK, Lazar MA, de Vries CJ, Biessen EA, Daemen MJ, Lutgens E, de Winther MP. Targeting macrophage Histone deacetylase 3 stabilizes atherosclerotic lesions. EMBO Mol Med 2014;6(9):1124-1132.

111.   Gilham D, Wasiak S, Tsujikawa LM, Halliday C, Norek K, Patel RG, Kulikowski E, Johansson J, Sweeney M, Wong NC. RVX-208, a BET-inhibitor for treating atherosclerotic cardiovascular disease, raises ApoA-I/HDL and represses pathways that contribute to cardiovascular disease. Atherosclerosis 2016;247:48-57.

112.   Jahagirdar R, Zhang H, Azhar S, Tobin J, Attwell S, Yu R, Wu J, McLure KG, Hansen HC, Wagner GS, Young PR, Srivastava RA, Wong NC, Johansson J. A novel BET bromodomain inhibitor, RVX-208, shows reduction of atherosclerosis in hyperlipidemic ApoE deficient mice. Atherosclerosis 2014;236(1):91-100.

113.   Brown JD, Lin CY, Duan Q, Griffin G, Federation AJ, Paranal RM, Bair S, Newton G, Lichtman AH, Kung AL, Yang T, Wang H, Luscinskas FW, Croce KJ, Bradner JE, Plutzky J. NF-kappaB directs dynamic super enhancer formation in inflammation and atherogenesis. Mol Cell 2014;56(2):219-231.

114.   Kelly TK, De Carvalho DD, Jones PA. Epigenetic modifications as therapeutic targets. Nat Biotechnol 2010;28(10):1069-1078.

Figure 1

Click here to download Figure Figure 1.tif ⬇

Common pathogenic mechanism

Therapies

Non modifiable
Genome
Gender

Modifiable
CVD risk factors
CID-associated DAMPs

Lipid lowering

Determine susceptibility for

Induction of

HSPCs
↓
Monocytes
↓
Macrophages

Epigenetic modifications in innate immune cells

Epigenetic modulator

Hyperresponsive immune system

Targeted delivery
to immune cells

Chronic inflammatory disease state
Atherosclerotic CVD
CID

Figure 2

Click here to download Figure Figure 2.tif ±