CD4⁺ T cells in atherosclerosis: Regulation by platelets

Nailin Li
Karolinska Institute, Department of Medicine–Solna, Clinical Pharmacology Unit, Karolinska University Hospital–Solna, Stockholm, Sweden

Summary
Atherosclerosis is an inflammatory and thrombotic disease, in which both CD4⁺ T cells and platelets play important roles throughout all stages of atherogenesis. CD4⁺ T cells are the most abundant T cells present in atherosclerotic lesions. They are primarily seen as type 1 T helper (Th1) cells, while the other CD4⁺ T cell subsets Th2, Th17, and regulatory T (Treg) cells are also found in the lesions with lower frequencies. CD4⁺ T effector cells release various cytokines, which exert paracrine or autocrine effects among different CD4⁺ T cell subsets and other lesional cells and subsequently modulate inflammatory processes in the lesions. Platelets are instrumental in thrombosis and haemostasis, but also play important regulatory roles in immune response, inflammation, and angiogenesis. The present review summarizes the current knowledge and/or understanding on how platelets regulate recruitment, activation, differentiation, and cytokine production of different CD4⁺ T cell subsets, as well as impacts of the platelet-CD4⁺ T cell interactions on atherogenesis. The research perspectives of platelet-CD4⁺ T cell interaction in atherosclerosis are also discussed.

Keywords
Atherosclerosis, platelets, CD4⁺ T cells, chemokines, cytokines

Introduction
Atherosclerosis is the primary pathological basis for the ischaemic cardiovascular diseases, which are the leading cause of human mortality and morbidity in developed countries. The classical concepts of atherosclerosis had experienced the hypothesis of passive lipid deposition in the arterial wall and the hypothesis of response-to-injury (1). The latter used to regard atherosclerotic lesions mainly as proliferative lesions of smooth muscle cells together with their secreted extracellular matrix products and accumulated lipids. The intensive research in the field during the last three decades has, however, gradually transformed the modern concept of atherosclerosis into an inflammatory and thrombotic disease (2-6).

Atherosclerosis as an inflammatory disease: Role of CD4⁺ T cells
Immune cells, both from innate and adaptive immunity arms, are present throughout all stages of atherosclerotic lesion development (7-9). Lesional innate immune cells are predominantly monocytes/macrophages, which are accompanied by granulocytes, mast cells, and natural killer cells at lower densities (10). The adaptive immune cells in the lesions are mostly T cells, while a small number of B cells are also present (10). Recognition of the existence and functioning of immune cells in atherosclerotic lesions has eventually given birth to the concept that atherosclerosis is an inflammatory disease (2, 3).

Existence of T cells in the atherosclerotic lesions was first reported some 30 years ago by Hansson et al. (8, 9). T cells can already be detected in the early atherosclerosis lesion of fatty streaks, and their number increases during lesion development, approaching to 10-20% of the lesional cells in advanced human atherosclerotic lesions (9, 11). Most of lesional T cells are T helper (Th) cells, which bear CD3/CD4 antigens and αβ T cell receptors (TCRs), and approximately one third of lesional T cells belongs to cytolytic (Tc) cells (10, 12). CD4⁺ T cells in the lesions are mostly activated, as suggested by their expression of interleukin-2 receptor (IL-2R, CD25) and the MHC class II cell surface receptor HLA-DR. The CD4⁺ T cells are predominately type 1 Th (Th1) cells (10, 13). Other CD4⁺ T cells, including Th2, Th17, and regulatory T (Treg) cells, are also present in the lesions (10, 12).

The importance of CD4⁺ T cells in atherogenesis has been highlighted by earlier animal studies showing that transfer of CD4⁺ T cells (14) aggravates, whilst CD4⁺ T cell deficiency (15) attenuates atherosclerosis in ApoE⁻/⁻ mice. As all major CD4⁺ T cell subsets are found in atherosclerotic lesions, the impact of CD4⁺ T cells on atherogenesis should not be simple.

Th1 cells secrete proinflammatory cytokines, typically interferon γ (IFNγ), interleukin (IL)-2, and tumour necrosis factor (TNF)α (11), which can activate other lesional cells, promote vas-
cular inflammation, and thus augment lesion development. There are increased presence of Th1 cells and deposition of the Th1 cytokine IFNγ in atherosclerotic plaques (16), leading to enhanced atherosclerotic lesion formation (17). Consistently, inhibition of Th1 cell differentiation (18, 19) or deficiency of Th1 cytokine receptor (20) markedly reduced lesion formation. Thus, it is clear that lesional Th1 cells are pro-atherosclerotic.

Th2 cells secrete anti-inflammatory cytokines, e.g. interleukin (IL)-4, IL-5, and IL-10, and enhance antibody production of B cells. Th2 response is largely anti-inflammatory, and was thought to counteract pro-atherosclerotic Th1 response. However, experimental evidence obtained from atherosclerotic mouse models was less than conclusive. There are studies showing that Th2-slanted ApoE^{-/-} mice had a reduced lesion formation as compared to Th1-slanted ApoE^{-/-} mice (21), and that Th2 switch caused by deficiency of the Th1 differentiation-controlling transcription factor T-bet reduced atherosclerotic lesion formation (19). Enhanced Th2 differentiation has also been shown to attenuate lesion formation via a mechanism of IL-5-dependent IgM production (22). However, there is evidence showing that IL-4 deficiency has no effect on atherosclerotic lesion formation (23), or even attenuates lesion formation in a site-specific manner (24) in pro-atherosclerotic LDLR^{-/-} mice fed with a high fat diet. IL-4 deficiency may also reduce lesion formation in ApoE^{-/-} mice (25). Therefore, the impact of Th2 cells on atherogenesis is complex, and may have different manifests with different atherosclerosis models.

Th17 cells are a recently recognised CD4^{+} T cell lineage in atherosclerotic lesions. Th17 cell differentiation is driven by IL-6 and transforming growth factor β (TGFβ), and requires activation of the transcription factor RORyt. Th17 cells secrete its signature cytokine IL-17, as well as other cytokines, e.g. IL-21 and IL-22. IL-17 enhances production of proinflammatory cytokines and chemokines by other cells via activating the transcription factor nuclear factor-kB, and clearly manifests the pro-inflammatory characteristics of Th17 cells in autoimmune diseases (26). The impact of Th17 cell response in atherogenesis is also controversial. Patients with acute coronary syndrome (ACS) have more circulating Th17 cells and elevated plasma levels of the Th17-related cytokines of IL-17, IL-6, and IL-23 (27). Increased Th17 cells and IL-17 synthesis have also been found in the atherosclerotic plaques of ApoE^{-/-} mice (16). IL17 receptor deficiency of haematopoietic cells reduces atherosclerotic lesion formation in LDLR^{-/-} mouse (28). Neutralisation of IL-17 reduced (16, 29), and application of recombinant IL-17 aggavated lesion formation in ApoE^{-/-} mice (16). In contrast, there is also evidence demonstrating that elevated IL-17 production by SOCS3 deficiency, a negative regulator of Th17 differentiation, reduced atherosclerotic lesion size in LDLR^{-/-} mice, and that this athero-protective effect could be mimicked by in vivo administration of IL-17 (30). Moreover, increased Th17 differentiation and IL-17 in human atherosclerotic lesions are associated with a stable plaque phenotype (30).

Treg cells include natural and inducible subtypes, and produce antiinflammatory IL-10 and TGFβ. Treg cells are found in human atherosclerotic lesions in low numbers (31), and are colocalised with both IL-10 and TGFβ expression (32). Treg cell numbers and IL-10 levels in peripheral blood are decreased in patients of ACS (27, 33). Using various mouse models, it has been evident that Treg deficiency is linked to increased atherosclerosis and lesion inflammation (34), and that increased Treg presence and Treg/Th1 ratio reduce lesion formation (32). Furthermore, inhibition of Treg cell production of IL-10 and TGFβ or TGFβ signalling clearly demonstrate atheroprotective effects (35-38). Interestingly, recent studies showed that intravenous or oral administration of CD3 antibody induces a Treg response and thus attenuates atherosclerotic lesion formation (39, 122).

Hence, all major CD4^{+} T cell lineages are present during atherogenesis. It is clear that Th1 response is pro-atherosclerotic, whilst Th2 response is athero-protective. Impacts of Th2 and Th17 responses on atherogenesis remain, however, controversial. As such, anti-atherosclerotic therapeutic developments targeting CD4^{+} T cell-mediated processes should be aimed to hamper Th1 response and/or to enhance Treg cell function.

Atherosclerosis as a thrombotic disease

The engagement of platelets in atherogenesis was recognised more than 60 years ago (40). The contributions of platelet to atherosclerosis have, however, only become established in late 1990s. Activated platelets express various adhesion molecules that mediate their adhesion to endothelial cells, leukocytes, as well as themselves (►Table 1). Platelets can adhere to not only denuded arterial wall (40) but also inflamed endothelium at the sites prone to lesion development (41, 42). Endothelial adhered and leukocyte-conjugated platelets facilitate adhesion and recruitment of leukocytes, including lymphocytes (42-46), and subsequently enhance lesion formation (42, 44). Thus, repeated injections of activated platelets exacerbate atherosclerosis (47), whilst functional blockade or genetic deficiency of platelet adhesion molecules glycoprotein (GP) Iβ (42), GPIIb/IIa (48), and P-selectin (49, 44) reduces lesion formation. Activated platelets also release a number of soluble mediators (►Table 2) that can regulate activation, proliferation, differentiation, and function of CD4^{+} T cells and other lesional cells, and thus regulate atherosclerotic lesion development (5, 50, 51). Clinical data also pinpoint a close connection of platelet hyperactivities with atherosclerotic diseases, such as increased circulating activated platelets (52, 53) and enhanced platelet reactivity (52, 54).

Taken together, platelets are closely involved throughout the different stages of atherogenesis, and can influence the engagements of multiple lesional cells via different mechanisms. The present review will focus on how platelets regulate the engagements of CD4^{+} T cells in atherosclerosis.

Platelet-assisted T cell recruitment into the vessel wall

Atherosclerotic lesions contain a considerable number of T cells. The entry site of these cells from the blood stream – i.e. from the...
arterial blood flow or from microvessels in the arterial wall – is an interesting issue. Leukocytes can adhere to endothelial surface and subendothelial matrix proteins (SEMPs) via self-expressed adhesion molecules under flow conditions with low shear stresses (<1.2 dynes/cm² or 25-50 s⁻¹ when expressed as shear rates) (55, 56). Endothelial-SEMP-adhered platelets can enhance this adhesion, and are absolutely required for leukocyte adhesion at higher levels of shear stress (56-60).

Lymphocytes have a lower adhesion affinity as compared to granulocytes and monocytes (58, 61), but share a similar adhesion procedure with other leukocytes. Their recruitment to the vessel wall follows the sequential processes of tethering, rolling, firm adhesion, and migration, which involve both selectins and integrins. It is clear that platelets can enhance lymphocyte adhesion (58, 62), and that the enhancement is P-selectin-dependent (58). Platelet-expressed P-selectin can substitute L-selectin, restore L-selectin-deficient T cell trafficking into the lymphoid tissue, and reconstitute T cell-mediated immunity (63). These findings indicate that P-selectin-expressing platelets are critical for adhesion and recruitment of activated T cells that lack L-selectin. Giving the early presence of T cells in atherosclerotic lesion development, one might take lymphocyte adhesion at arterial sites as granted. However, earlier studies have only shown sustainable lymphocyte rolling and adhesion under conditions with low shear stresses (<8 dynes/cm² or shear rates<200 s⁻¹) (56, 58, 62, 64). We and others recently showed that human lymphocytes, including CD4⁺ T cells, can adhere on SEMP-coated surfaces and injured vessel wall under arterial flow conditions, and that the enhancement is totally platelet-dependent (46, 65, 66). The latter is evidenced by that all deposited lymphocytes were co-localised with platelets (46), that CD4⁺ is selective among platelet-conjugated cells (46), and that platelet depletion almost abolished CD4⁺ T cell adhesion (66). Platelet-dependent CD4⁺ T cell adhesion involves a number of adhesion molecules of platelets and T cells (▶Table 1). Of note, P-selectin is of key importance for platelet-supported lymphocyte adhesion under arterial flow conditions, because P-selectin blockade produced the most marked reduction of platelet-supported CD4⁺ T cell adhesion in vitro (66), and P-selectin blockade abolished lymphocyte adhesion at the sites of arterial injury in vivo (Li N, unpublished observations). P-selectin is essential but yet insufficient for CD4⁺ T cell recruitment into the arterial vessel wall, and it requires collaboration of integrins, such as GPIb/IX and GPIIb/IIIa, to support firm lymphocyte adhesion in an arterial flow. For instance, GPIIb/IIIa blockade abolishes arterial thrombus formation and subsequently prevents lymphocyte infiltration at the sites of arterial injury (46).

It can thus be assumed that lymphocyte recruitment into atherosclerotic lesions may differ during different atherogenic stages. During the initial phase of arterial injury and lesion formation, i.e. when local microvessels are absent or sparse, lymphocytes are likely recruited from the arterial blood stream. These early recruited cells will have important influences on inflammatory responses, vessel reparation, thrombus reconstruction, and early lesion development. During late phase of atherosclerosis, recruitment of T cells, as well as other leukocytes, likely takes entry into the advanced atherosclerotic lesions through the venules of the plaque vasculature (67).

Th1 cells are the most abundant CD4⁺ T cells in atherosclerotic lesions. The domination of Th1 cells is likely caused by multiple factors. Obviously, Th1 cells often outnumber other CD4⁺ T cell subsets. Our recent work showed that Th1 cell domination over Th2 cell is the major factor accounting for the preferential Th1 cell deposition under arterial flow conditions (66). On the other hand, adhesion properties of different CD4⁺ T cell subsets should also contribute to the Th1 bias. We have shown that adhesion molecule-expression profile and platelet-conjugating capacity of lympho-

<table>
<thead>
<tr>
<th>Platelet ligands</th>
<th>CD4 T cell receptors</th>
<th>Receptor intensity</th>
<th>Effects</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>P-selectin</td>
<td>CD162/PSGL-1, CD15s</td>
<td>+++</td>
<td>conjugation, rolling, tethering, adhesion</td>
<td>(46, 62, 63, 66) (112, 113, 114)</td>
</tr>
<tr>
<td>GPIb/IIa</td>
<td>CD11b/Mac-1*, CD40L</td>
<td>+</td>
<td>conjugation, firm adhesion</td>
<td>(46, 66, 115, 116)</td>
</tr>
<tr>
<td>GPIb</td>
<td>CD11b/Mac-1*</td>
<td>++</td>
<td>rolling, firm adhesion</td>
<td>(117)</td>
</tr>
<tr>
<td>ICAM-1</td>
<td>LFA-1</td>
<td>++</td>
<td>adhesion</td>
<td>(68)</td>
</tr>
<tr>
<td>JAM-3</td>
<td>CD11b/Mac-1</td>
<td>++</td>
<td>adhesion</td>
<td>(118)</td>
</tr>
<tr>
<td>CD40</td>
<td>CD40L</td>
<td>++</td>
<td>conjugation, adhesion</td>
<td>(46, 66, 109, 114)</td>
</tr>
<tr>
<td>CD40L</td>
<td>CD40</td>
<td>++</td>
<td>conjugation, adhesion</td>
<td>(46, 66, 109, 114)</td>
</tr>
</tbody>
</table>

* Bridging via fibrinogen and/or von Willebrand factor. PSGL-1: P-selectin glycoprotein ligand-1; GP: glycoprotein; ICAM-1: intercellar adhesion molecule-1; LFA-1: lymphocyte function-associated antigen-1; JAM-3: junctional adhesion molecule-3; CD40L: CD40 ligand.
hocytes is closely correlated to platelet-supported lymphocyte adhesion (46, 66). Expression of PSGL-1, the principal P-selectin ligand, is higher in Th1 cells than in Th2 and Th17 cells (68), and it may therefore favour Th1 cell recruitment at the lesion.

**Influence of platelets on CD4+ T cell proliferation**

Platelet releasate contains a bunch of mediators with mitogenic effects on diverse cell types, including lymphocytes. Platelet-derived growth factor (PDGF) and stromal cell-derived factor 1α (SDF-1α) are well known to promote proliferation of smooth muscle cells and endothelial progenitor cells. Some of the mediators can, on the other hand, influence CD4+ T cell proliferation (Table 2). Thus, platelet-derived chemokines RANTES and MCP-1 enhance human T cell proliferation in response to anti-CD3 MAb ligation but not phorbol esters (69), in which RANTES augments CD4+ T cell proliferation by sustainably elevating intracellular calcium levels (70). PDGF can also enhance T cell proliferation (71). Thromboxane A2 (TXA2), a major product of phospholipid metabolism in platelets, inhibits CD4+ T cell proliferation (72), but an opposite effect of TXA2 has also been reported (73). Some platelet-derived mediators have more complex effects on CD4+ T cell proliferation. Platelet-activating factor (PAF), another major metabolite of platelet phospholipids, inhibits CD3 ligation-induced T cell proliferation, but enhances CD2-induced T cell proliferation (74). TGFβ alone inhibits CD4+ T cell proliferation by suppressing IL-2R expression and arresting cells in the G0/G1 compartment of the cell cycle (75). When in combination with IL-2, however, TGFβ can enhance CD4+ T cell proliferation (75). The platelet specific chemokine platelet factor 4 (PF4) inhibits anti-CD3/CD28 MAb-induced proliferation of the total CD4+ T cells (76) by suppressing production of the T cell growth factor IL-2 (76, 77), but can selectively stimulate proliferation of Treg cells (77). Therefore, platelets can exert counteracting effects on CD4+ T cell proliferation with different mediators; one platelet-derived mediator can have different effects on different CD4+ T cell subsets; and the final readout of CD4+ T cell proliferation is likely the orchestra effects of multiple platelet-derived mediators. Indeed, we have recently shown that, using a model of human platelet-CD4+ T cell co-culture, platelets decreased CD3/CD28-induced cell proliferation of total CD4+ T cells, but enhanced certain T effector cell differentiation and proliferation (78).

**Platelet regulations on Th1 function**

The primary lesional CD4+ T cells, Th1 cells, are differentiated from naïve CD4 cells upon activation and in the presence of IL-12 and IFNγ. Using various in vitro experimental settings, platelets and platelet-derived mediators have been shown to regulate Th1 cell differentiation and functions in a complex manner. The platelet chemokines RANTES and MCP-1 can enhance IL-2 production of anti-CD3/CD28 MAb-stimulated T cells (69), an activity largely attributed to Th1 cells in that experimental setting. We have also demonstrated that neutralisation of RANTES inhibits Th1 cytokine production of IL-2 and TNFα in CD4+ T cell-platelet co-culture, and that supplementation of RANTES to activated CD4+ T cells elevates Th1 cytokine levels (78). The other platelet-derived chemokine PF4 is, however, associated with more complex stories. With cultured total T cells, PF4 inhibited Th1 cytokine (e.g. IL-2 and IFNγ) production in response to both antigenic stimulation (e.g. tetanus toxoid or a purified protein derivative of tuberculin) and polyclonal stimulation of CD3/CD28 MAbs (76). Such inhibitory effects were, however, absent when T cell culture was supplemented with exogenous IL-2 (76). Another investigation revealed that PF4 exerts distinct effects on different CD4+ T cells, i.e. enhancing Th1 responses in CD25+ cells, but inhibiting Th1 responses in CD25- cells (77). We have recently demonstrated that PF4 enhances Th1 differentiation and cytokine production using a system of autologous human CD4+ T cell-platelet co-culture (78), an effect which should probably be attributed to Th1 effector and memory cells. These distinct manifests of PF4-regulated Th1 responses suggest that caution should be taken when interpreting the effects of PF4 on Th1 cells.

Platelets are rich in TGFβ, which is anti-inflammatory and atheroprotective. Neutralisation of TGFβ (37) or interruption of TGFβ signalling (38, 79) increase inflammatory cellular components and activities in the lesions, and favour the plaque development of an inflammatory/vulnerable phenotype. One major mechanism underlying these manifests is that TGFβ inhibits Th1 differentiation and cytokine production. Thus, T cell-specific TGFβ signalling blockade results in increases of Th1 differentiation and cytokine production (80), as well as lesional Th1 activities (81). TGFβ effect on Th1 response may also be exerted indirectly, such as via enhancement of Th1-inhibiting Treg responses (81) or/and via T cell immunity-regulating dendritic cells (DCs) (82). The latter is evidenced by that TGFβ signalling blockade of DCs markedly enhances Th1 differentiation/cytokine production and atherogenesis (82).

Other platelet-related inflammatory mediators, such as TXA2, PAF, serotonin, and histamine, also display Th1 cell-regulatory effects. Activated platelets produce a significant amount of TXA2, which inhibits Th1 proliferation and cytokine production (72). Activated T cells express PAF receptor (83), and PAF can enhance Th1 cytokine production of cultured T cells (74, 84, 85). Platelets are an important source of histamine (86), apart from mast cells and basophils. Histamine enhances Th1 responses through its action on H1 receptors, which are highly expressed on Th1 cells (87), but inhibits Th2 responses via H2 receptors, which are densely expressed on Th2 cells (87). Furthermore, serotonin is taken up, stored, and released by platelets, and can potentiate platelet activation (88). An effect of serotonin worth noting here is that serotonin is involved in delayed-type hypersensitivity (89), a T cell-mediated defense mechanism involving Th1 responses (e.g. IFNγ production). It has been shown that both antigen-specific and anti-CD3 MAb-stimulated Th1 cytokine (IL-2 and IFNγ) production is inhibited by serotonin 5HT1A receptor antagonism (90), and that 5HT1A blockade inhibits delayed-type hypersensitivity
Table 2: Platelet-derived mediators that regulate CD4 T cell functions.

<table>
<thead>
<tr>
<th>Mediators</th>
<th>Alias</th>
<th>Receptors</th>
<th>Target cells</th>
<th>Effects</th>
<th>Proliferation</th>
<th>Differentiation</th>
<th>CK production</th>
<th>Other effects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Cytokines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-1β</td>
<td>LAF</td>
<td>IL-1RI</td>
<td>Th1</td>
<td>+ (94)</td>
<td>+ (94)</td>
<td>+ (94)</td>
<td>+ (94)</td>
<td>effector cell suppression (106)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>sCD40L</td>
<td>CD154</td>
<td>CD40</td>
<td>CD4 T cell</td>
<td>+ (120)</td>
<td></td>
<td></td>
<td></td>
<td>CD4 T cell substitution GC-Ig format (101)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Chemokines</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PF4</td>
<td>CXCL4</td>
<td>CXCR3A,</td>
<td>CD4 T cell</td>
<td>− (76)</td>
<td>− (76)</td>
<td>− (76)</td>
<td>− (76)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CXCR3B</td>
<td>Th0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th2</td>
<td>+ (77, 78)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treg</td>
<td>+ (77, 78)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RANTES</td>
<td>CCL5</td>
<td>CCR1,</td>
<td>CD4 T cell</td>
<td>+ (69)</td>
<td>+ (69)</td>
<td>+ (69)</td>
<td>+ (69)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>CCR3, CCR5</td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treg</td>
<td>+ (78)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1</td>
<td>CCL2</td>
<td>CCR2</td>
<td>CD4 T cell</td>
<td>+ (69)</td>
<td>+ (69)</td>
<td>+ (69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Growth factors</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TGFβ</td>
<td>TGFβR1/2</td>
<td></td>
<td>CD4 T cell</td>
<td>− (82)</td>
<td>− (82)</td>
<td>− (82)</td>
<td>− (82)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th2</td>
<td>− (80, 82)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th17</td>
<td>+ (81, 82)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Treg</td>
<td>+ (78, 81)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PDGF</td>
<td>PDGFRα/β</td>
<td></td>
<td>CD4 T cell</td>
<td>+ (71)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Others</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TxA2</td>
<td>TP</td>
<td></td>
<td>CD4 T cell</td>
<td>− (72)</td>
<td></td>
<td>− (72)</td>
<td></td>
<td>chemokinesis (72)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAF</td>
<td>PAF R</td>
<td>Th0</td>
<td>CD4 T cell</td>
<td>− (74)</td>
<td>+ (74)</td>
<td>+ (74)</td>
<td>+ (74, 84)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th17</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serotonin</td>
<td>SHT₂A</td>
<td>Th1</td>
<td>+ (90)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Histamine</td>
<td>H1R for Th1, H2R for Th2</td>
<td>Th1</td>
<td>+ (87, 121)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Th2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>− (121)</td>
</tr>
</tbody>
</table>

GC: germinal centre; IL-1β: interleukin-1β; LAF: lymphocyte activation factor; MCP-1: monocyte chemotactic protein-1; NAP-2: neutrophil-activating peptide-2; PAF: platelet activating factor; PDGF: platelet-derived growth factor; PF4: platelet factor 4; RANTES: regulated and normal T cell expressed and secreted; TGFβ: transforming growth factor β; TxA2: thromboxane A2.

(89). Further support comes from the evidence that platelet-derived serotonin contributes to the onset of delayed-type hypersensitivity in mast cell-deficient mice (91). The impacts of these platelet-derived inflammatory mediators on atherosclerotic lesion development have, however, not been well investigated, and it is definitely desirable to further elucidate this.

Platelets are anucleate cells, but retain certain capacities of protein synthesis. Quiescent platelets do not contain pro-IL-1β or ma-
turer IL-1β. Upon activation, however, platelets undergo *de novo* synthesis of IL-1β, and can deposit newly synthesised IL-1β into fibrin nests of thrombi (92, 93). Hence, a thrombus may serve as a reservoir of platelet-derived cytokines and other mediators, and maintain high local concentrations of those mediators to enhance platelet-dependent regulation of T cells in thrombi. It is clear that IL-1β promotes Th1 cell differentiation from naïve CD4+ T cells and the production of Th1 cytokines (94).

Because platelets are armed with multiple regulators of Th1 cells that may exert counteracting effects on Th1 cells, the net effects of platelets on Th1 responses likely depend on the negotiations of multiple platelet-derived regulators. Therefore, it should be stressed that effects of an individual platelet-derived regulator on Th1 cells observed in an *in vitro* experimental setting may not necessarily represent the true impacts of platelets on Th1 responses *in vivo*.

### Platelet-regulated Th17 function

Th17 cells are critical for host defense and autoimmunity by producing IL-17A, IL-17F, and IL-22. Several platelet-derived mediators are known to regulate Th17 differentiation and cytokine production. TGFβ, which is stored and released by platelets in a large amount, orchestrates with IL-6 and IL-21 to promote Th17 differentiation of mouse CD4+ T cells. Notably, TGFβ effect on Th17 differentiation is exerted in a concentration-dependent manner. Low concentrations of TGFβ promote Th17 differentiation, whilst TGFβ at high concentrations enhances Treg differentiation (81). It should, however, be noted that human Th17 differentiation is not entirely identical to that of murine cells. Recent studies showed that Th17 differentiation and the expression of its controlling transcription factor RORγt are actually suppressed by TGFβ in human CD4+ T cells (94, 95). Using a human platelet-CD4+ T cell co-culture, we found that platelets only moderately promote Th17 differentiation and IL-17 production, as compared to the marked enhancements of Treg differentiation and IL-10 production (78). The limited promotion on Th17 differentiation may be partially due to the large amount of TGFβ released from platelets, with a level approximately 50 times higher than that from CD4+ T cells (78). On the other hand, Th17-inhibitory effect of TGFβ may also been counteracted by the Th17-promoting effects of other platelet-derived factors, such as IL-1β.

As already mentioned above, activated platelets can synthesise IL-1β (92). IL-1β can induce RORγt expression and Th17 polarisation in human naive CD4+ T cells (95), which is enhanced by IL-6 (95) but suppressed by TGFβ (94, 95). IL-1β is critical in maintaining the steady status of Th17 cells (96). Moreover, platelet activation leads to PAF synthesis and release, which can promote Th17 differentiation and cytokine production (97, 98). Because activated platelets secrete a large amount of TGFβ and can also synthesise and release IL-1β and PAF, platelet thrombi may thus create a unique microenvironment with counteracting mediators for Th17 polarisation. For CD4+ T cells recruited into a platelet thrombus, it would be interesting to elucidate how the platelet-derived triad modulates Treg-Th17 plasticity and/or stability of Th17 phenotype.

### Platelets and Th2 immune responses

Th2 cells promote humoral immunity by enhancing proliferation and antibody production of B cells. Thus, Th2 response used to be considered as athero-protective. As already discussed above, interventions of Th2 immune responses during atherogenesis are, however, associated with mixed outcomes from different mouse models of atherosclerosis (21, 24). Several platelet-derived mediators also show diverse effects on Th2 response. PAF enhances the Th2 cytokine IL-4 production (99), in which the effect is probably indirect and is exerted via monocytes, as MHC class II blockade abolished the effect (99). Histamine shows, in contrast, an inhibitory effect on Th2 responses via H2 receptors that are densely expressed on Th2 cells (87). TGFβ also seems to be inhibitory for Th2 responses, as signalling blockade of TGFβ enhances not only Th1 but also Th2 responses. Thus, T cell- or DC-specific abrogation of TGFβ signalling increases Th2 cell differentiation and Th2 cytokine production (80, 82), and also results in high Th2 activities in the lesions (38). Our recent study using human platelet-CD4+ T cell co-cultures, however, showed that platelets had little influence on CD3/CD28-induced Th2 polarisation or Th2 cytokine production of IL-4 or IL-5 (78). These observations suggest that cautions should be taken when interpreting the impacts of platelet-Th2 interaction on inflammatory processes in atherosclerosis.

### Platelets and CD4 T cell-dependent humoral immunity

CD4+ T cells are required for robust B cell responses, including germinal centre formation and B cell isotype shifting, which are achieved through ligation and co-stimulatory signalling between CD4+ T cell-expressed CD40L and B cell-expressed CD40 (100). Although humoral immunity is not the theme of the present review, it is still worth noting that the effects of CD4+ T cells on humoral immunity can be enhanced or even substituted by platelets. Platelets can promote CD4+ T cell-dependent germinal centre formation, B cell isotype shifting, as well as IgG production (101), which are most manifest in the presence of a weak immune stimulus and/or a low number of antigen-specific CD4+ T cells (101). Notably, infusion of CD40L-expressing wild-type platelets (102) or platelet-derived microparticles (100) can restore the isotype shifting of B cells in CD40L-deficient mice, in which an immune challenge fails to elicit B cell isotype switch. Infusion of platelet-free supernatant from activated wild-type platelets, which contains soluble CD40L, can also restore isotype switching in the knockout mice (102). These findings indicate that platelets can help CD4+ T cells to foster a prompter and more robust humoral immune response, because platelet activation and CD40L expression take place almost instantly, whilst T cell polarisation takes hours or days. The findings also suggest that platelets may exert their actions in distance and...
Figure 1: Potential regulation of platelets on CD4+ T effector cell responses in atherosclerosis. Platelets enhance CD4+ T (Th) cell adhesion on and recruitment into atherosclerotic lesions. Platelets release various soluble mediators, e.g. platelet factor 4 (PF4), transforming growth factor β (TGFβ), RANTES (regulated and normal T cell expressed and secreted), interleukin-1β (IL-1β), platelet activating factor (PAF), and soluble CD40 ligand (sCD40L). The latter may exert stimulatory (+) or inhibitory (-) effects on different CD4+ T cell subsets: naïve CD4+ T (Th0) cells, type 1 T helper (Th1), Th2, Th17, and regulatory T (Treg) cells. The same platelet-released mediator may have distinct effects on different CD4+ T cell subsets. For instance, TGFβ inhibits Th1 cell proliferation and cytokine production of interferon γ (IFNγ) and tumour necrosis factor α (TNFα), whilst it enhances Treg differentiation and IL-10 production (78).
function. The true effects of platelets on Treg cell function in vivo are likely complex. The net outcome should involve subtle negotiations among the different platelet-derived mediators and with local micro-environments, as the impacts of platelets on CD4+ T cells doted in the lesional tissues and the cells trapped in a platelet thrombus should likely differ. Treg cells are hardly detectable in the normal arterial wall, but can be detected in all developmental stages of atherosclerotic lesions at low frequencies (0.5-5% total lesional T cells and much lower than Th1 cells) (31). The low Treg cell frequency may be due to a reduced adhesion capacity of Treg cells during a prolonged hyperlipidaemia (107) or/and attributed to Treg cell profile of adhesion molecule expression, which determines their capacity of platelet conjugation and platelet-mediated adhesion under arterial flow conditions (46, 66). Interestingly, Treg cells are more frequent in unstable lesions than in stable lesions (31), indicating that there may be an immune suppressing response of Treg cells in the vulnerable lesions. This is in line with a hypothesis that the reduced number of circulating Treg cells in ACS patients may be due to a massive recruitment of Treg cells into the vulnerable and complicated plaques (108). Furthermore, a recent study showed that CD40L deficiency hampered leukocyte conjugation with platelets and leukocyte adhesion under flow, and that infusion of activated wild-type but not CD40L−/− platelets transiently decreases Treg cells in the circulation (109). It is proposed that platelet CD40L-mediated reduction of circulating Treg cells is an important contributing factor to accelerated atherosclerosis in ApoE−/− mice receiving repeated injections of activated platelets (109). Therefore, altered cell adhesion properties of T₁H₁ and T₅REG cells in proatherosclerotic conditions may account for the imbalance between Th1 and Treg cells in the lesions.

Research perspectives

Atherosclerosis is an inflammatory and thrombotic disease, in which both CD4+ T cells and platelets are important players. Platelet-CD4+ T cell interactions are an indispensable part of their engagements in atherogenesis, and platelets can regulate various aspects of CD4+ T cell responses in atherosclerosis (Figure 1). However, our knowledge on the heterotypic cross-talk is still limited, and there is a need to improve our understanding on this regard. First of all, much of current information on platelet-CD4+ T cell cross-talk is derived from in vitro experiments where platelet-derived mediators or platelets themselves were directly added to cultured T cells. Therefore, further work is needed to demonstrate in vivo or physiological relevance of the in vitro findings. More importantly, under in vivo conditions, a good part of CD4+ T cell activation and differentiation takes place in the lymph nodes where platelets are actually absent. Hence, it remains to be shown if and how platelets influence CD4+ T cell activation and differentiation in the lymph nodes. Platelets are the principal source of plasma TGFβ (110) and PF4, and these well-known regulators of CD4+ T cell differentiation should penetrate into lymphatic tissue with ease. Thus, platelet regulation on CD4+ T cell activation in the lymph nodes is likely, but some solid evidence from carefully designed in vivo experiments is needed. While most in vivo studies are performed in various murine models, it is also important to note that human and murine T cells share some similarities but also differ in many aspects of their CD4+ T effector cell responses. Thus, caution should be used when translating the findings from murine models to CD4+ T cell effector responses in humans, and the interpretation should also be made in lights of clinical manifests and findings.

Antiplatelet treatment has been recognised as a cornerstone of secondary prevention of atherosclerotic diseases, while CD4+ T cell intervening approaches, such as adoptive expansion of Treg cells, are under experimental and/or clinical evaluation for future therapeutic applications. In light of the recent findings that platelets markedly promote Treg cell development and cytokine production (77, 78), platelet-enhanced Treg cell differentiation and function may represent a new direction in a current effort to use Treg cell expansion as a therapeutic approach in atherosclerotic disease treatment. Moreover, CD4+ T cell subsets display different cell adhesion properties (111), which can also be affected by hyperlipidaemia (107), and platelets can selectively enhance T cell recruitment under arterial flow conditions (46, 66). It should be of interest to investigate if intervention of platelet-enhanced T cell adhesion may regulate the recruitment of different CD4+ T cell subsets in the lesions, for example during the acute phase after a percutaneous coronary intervention, to alter the ratio of Th1/Th17/Treg presence and subsequently inflammatory processes in atherosclerotic lesions.

Taken together, platelets and CD4+ T cells are both closely involved in atherogenesis. Their heterotypic interactions have attracted more and more research attention. Improved understanding of platelet-CD4+ T cell cross-talk may foster novel strategies in atherosclerotic disease management.

Acknowledgements

The author is grateful to his research co-workers for the fruitful collaboration over the years. The author's research is supported by grants from the Swedish Research Council, the Swedish Heart-Lung Foundation, the Karolinska Institute, the Swedish Society of Medicine, and the Stockholm County Council.

Conflict of Interest

None declared.

References


49. Kuijper PHM, Gallardo Torres HI, van der Linden JAM, et al. Platelet-dependent primary hemostasis promotes selectin- and integrin-mediated neutrophil
adhesion to damaged endothelium under flow conditions. Blood 1996; 87: 3271-3281.
59. Kirton CM, Nash GB. Activated platelets adherent to an intact endothelial cell monolayer bind flowing neutrophils and enable them to transfer to the endothelial surface. J Lab Clin Med 2000; 136: 303-313.
Li: Platelet-CD4+ T cell interactions in atherogenesis


