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REVIEW ARTICLE

# Macrophage modulation in activation process induced immune thrombocytopenia

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## ABSTRACT

The immune system operates like an orchestra that harmoniously maintains the homeostasis balance while protecting from external or internal pathogens attack. Inflammation is one of the key critical immune defenses to eradicate pathogens and encourage tissue repair and recovery by activating the host's immuneand non-immune cells. As a part of the immune response during inflammation, blood platelets serve various functions; however, their activation and involvement in inflammation can also contribute to pathological conditions, such as thrombosis, which results in myocardial infarction, stroke, and venous thromboembolism. Activated platelets can mobilize and release intracellular granules (alpha and dense granules), which include secondary mediators like chemokine PF4/CXCL4. In contrast to most other chemokines, PF4 participates in several long-term regulatory processes, such as cell differentiation, survival, and proliferation. However, recent findings suggest that PF4 is also responsible for modulating macrophage polarization, which can substantially impact the development of induced immune thrombotic thrombocytopenia. This review aims to explain how PF4 induced vascular problems by modulation of macrophage development during immunological thrombocytopenia. A literature search using the keywords PF4, CXCL4, macrophage M4, platelet macrophage M4, and induced immune thrombocytopeniawas done using the following databases: Google Scholar, ProQuest, ScienceDirect, and Scopus for articlespublished from 2000 to 2023. The literature study was done to find the connection between platelet activation, macrophage modulation, and vascular problems such as atherosclerosis and thromboembolism in induced immune thrombotic thrombocytopenia. Several recent studies on PF4, macrophage modulation, and vaccine-induced thrombotic thrombocytopenia were carefully reviewed. This review concludes that macrophage polarization modulation is promising in managing vascular problems in patients with induced immune thrombotic thrombocytopenia.

**Keywords:** Macrophage modulation, PF4/CXCL4, thrombocytopenia, vascular problems, inflammation resolution

**Abbreviation :**
CXCL4: Chemokine (C-X-C motif) ligand 4
ICAM: Intracellular adhesion molecules

HCV: Hepatitis C virus
HIT: Heparin induced thrombocytopenia
HIV: Human immunodeficiency virus
IL: interleukin
MHCII: Major histocompatibility complex class II
PF4: Platelet factor 4
TNF: Tumor necrosis factor-alpha
VCAM: Vascular adhesion molecules
VITT: Vaccine induced thrombotic thrombocytopenia

## INTRODUCTION

The immune system is the body's defense mechanism that operates harmoniously like an orchestra. As a part of the innate immune system's defense and balance, macrophage plays a critical role.[1–6] They are widely found in almost every tissue in the body and participate in various physiological and pathological processes. This immune cell is critical in maintaining a balance between protection against infections and tolerance for harmless stimuli. Newly formed monocytes in the bone marrow entered circulation, migrated into the tissues, and became macrophages.[7] Since tissue macrophages originated from peripheral blood monocytes, aberrant chemotactic responsiveness of the latter cells may increase vulnerability to viral and other chronic conditions.[8] Chemokine synthesis and receptor expression are also expressed differently by polarized macrophages.

Platelets have been recognized as a critical cell type in atherogenesis, mainly via the release of chemokines.[9-10] Most platelet activity and interactions with other cell types occur inside blood vessels. A recent study has shown that platelets connect with macrophages outside of blood vessels in various forms of cutaneous inflammation, inhibiting the expression of anti-inflammatory markers and boosting the synthesis of pro-inflammatory mediators in the linked macrophages.[11,12] When activated platelets come into contact with microorganisms, they produce platelet factor 4 (PF4/CXCL4). Chemokine (C-X-C motif) ligand 4 also influences monocyte differentiation by inducing a distinct macrophage phenotype called "M4".[13] In the development process of atherosclerotic plaque, M1 macrophages (pro-inflammatory type) had a better phagocytosis effect than the M4 macrophages. (14,15) The M1 macrophages develop in response to TLR4 activation, and the similarly pro-inflammatory M4 macrophages produce in response to CXCL4.[16–18] Additionally, endogenous protein PF4, due to binding with bacteria-induced, will be recognized by anti-PF4/heparin or anti-PF4/polyanion antibodies, resulting in immune complexes critical for antibacterial host defense.[19,20]

A similar process has been seen in response to dengue, influenza, and HIV-1 infections via platelet-derived mediators.[21–24] It may have a role in the antiviral response to SARS-CoV-2 infection since many scientists have discovered anti-PF4/heparin antibodies in COVID-19 patients. M4 macrophages are primarily found in the intima of human atherosclerotic plaques, and their presence is linked with plaque instability,[25] suggesting that they represent a sign of pro-inflammatory activity. In general, macrophage activation may offer a diagnostic and therapeutic target for resolving macrophage dysfunction induced by platelet mediators.[26,27]

In this review paper, a total of 87 articles published in English in the last 24 years (from 2000 to 2023) were retrieved from Science Direct, PubMed, Oxford Academic, NCBI, and Nature databases using the following keywords: PF4 AND CXCL4 AND macrophage M4 AND platelet macrophage M4 AND platelet macrophage M4 induced immune thrombocytopenia. Initially, 122,318 articles were found to match the inclusion criteria (Table 1), but in the end most of those articles were removed due to duplication, failure of access, and irrelevant topic. Finally, this review was written using the 87 articles that met the inclusion criteria to be written into a full paper.

Table 1. Articles found based on selected keywords on different search engines published within year 2000 to 2023

| Keyword(s) | Search engines | | | | | |
|---|---|---|---|---|---|---|
| | Science Direct | PubMed | NCBI (PMC) | Nature | Google Scholar | Oxford Academic |
| PF4 | 1159 | 1981 | 7460 | 574 | 755 | 945 |
| CXCL4 | 123 | 429 | 3812 | 302 | 299 | 311 |
| PF4 AND CXCL4 | 123 | 134 | 1185 | 95 | 7 | 82 |
| Macrophage M4 | 37 | 112 | 4491 | 373 | 1 | 467 |
| PF4 AND CXCL4 AND macrophage M4 | 2 | 2 | 44 | 2 | 0 | 2 |
| Platelet macrophage M4 | 2 | 8 | 1097 | 114 | 0 | 195 |
| PF4 AND CXCL4 AND macrophage M4 AND platelet macrophage M4 AND induced immune thrombocytopenia | 1365 | 3318 | 45560 | 2199 | 700 | 6461 |
| platelet macrophage M4 AND induced immune thrombocytopenia | 0 | 0 | 6 | 0 | 0 | 0 |
| CXCL4 and platelet macrophage M4 | 0 | 0 | 125 | 47 | 0 | 81 |
| TOTAL | 2813 | 45736 | 5990 | 175 | 63893 | 3711 |

This review aims to explain how platelet chemokine PF4 induced vascular problems by modulation of macrophage development during immunological thrombocytopenia. First, the inflammation mechanism as the body's response to infection and the vital role of inflammation mediators such as platelets during infection will be explained. Next, the focus will be on the chemokines PF4 as a pro-inflammatory signal produced by activated platelets that affect macrophage polarization modulation. The adverse effect of plaque formation in atherosclerosis caused by overexpression of pro-inflammatory macrophage will be mentioned. Next, the review will focus on cases of induced immune thrombotic thrombocytopenia, which potentially causes lethal blood clotting similar to atherosclerosis. Then, a potential approach to clinical therapy by controlling macrophage polarization modulation will be discussed to prevent atherosclerosis caused by induced immune thrombotic thrombocytopenia.

**Inflammation and inflammation resolution**

The inflammatory process is essential for the immunological defense system to operate correctly[6] and is particularly helpful when immune responses are short and restricted to a specific area.[28] When the control of inflammatory responses is impaired, processes that harm tissues and organs may occur, a characteristic of most chronic illnesses.[29] Inflammation begins with tissue injury, whether physical, chemical, or biological. As a result, cytokines and chemo-attractants are released into the bloodstream, attracting immune cells to the damaged tissue. These signals for recruitment may originate directly from disturbed cells, mast cells, or macrophages. Following that, immune cells dock and initiate the diapedesis process by upregulating the release of intracellular and vascular adhesion molecules (ICAM, VCAM) and selectin on damaged tissues. Once in the extravascular space, these immune cells continue fighting the cause of tissue injury by sending other signals to attract more cells and secreting chemicals that change vasodilation, platelet activity, and fibrinolytic activity, resulting in the characteristic symptoms of inflammation.[30]

These procedures intend to address the underlying cause of injury and heal the damaged tissue. Unfortunately, many of the signals that initiate the inflammatory response are persistent and, rather than addressing the underlying injury, culminate in a continuous cycle of tissue destruction (Figure 1). Both resident and recruited macrophages are responsible for tissue healing in response to tissue damage.[31–34] They contribute to pathological remodeling in acute and chronic infections and illnesses when this equilibrium is disturbed. Along with structural changes to the tissue, mitochondrial dysfunction in structural cells and immune cells, including macrophages, impairs macrophage's capacity to execute their usual activities, resulting in a persistent low-grade inflammatory state.[35–37]

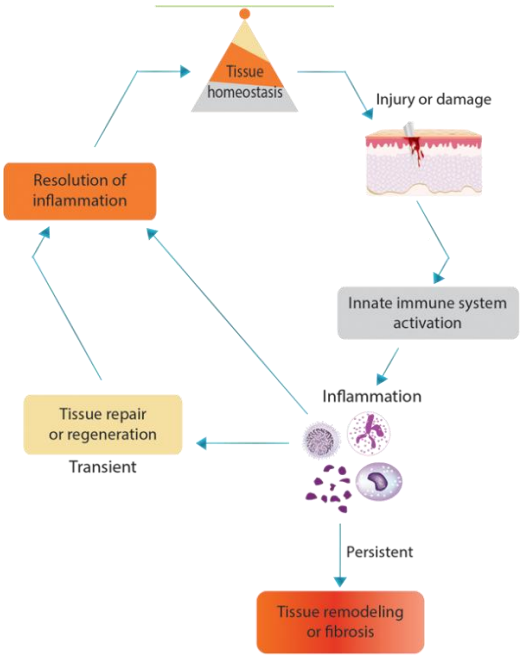


**Figure 1**. The immune system's mechanism to maintain tissue homeostasis during injury or damage

Tissue injury results in the recruitment and activation of immune cells, including platelets. The evolving of thrombus and recruited off neutrophils need to be removed, and the executed inflammatory or hemostatic reaction has to be stopped and resolved for tissue homeostasis.

Platelets contribute to the resolution of inflammation by multiple mechanisms, including interaction and modification of macrophages.[38]

**Mediators of inflammation**

Over the past several decades, many cells, cytokines, and genes implicated in the inflammatory process have been discovered.While only a few have been related to measurableclinical outcomes, many mediator pathways have been identified as anti-inflammatory therapy targets. Between cells, pro-inflammatory signals are conveyed through a network of cytokines and chemokines. While most of these molecules are produced by immune cells, some are produced by non-immune cells, such as the chemokine PF4 (platelet factor 4), also known as CXCL4 (chemokine C-X-C ligand 4) produced by activated platelets (Figure 2).[39,40] Apart from platelets, smooth muscle cells, microglia, macrophages, and T cells also express CXCL4.[41–44] Since the amounts released by these cells are much less than those generated by platelets, the physiological role of "non-platelet CXCL4" is unclear and may be limited to local activities. Other significant pro-inflammatory biomarkers include tumor necrosis factor (TNF-α), interleukin 1 (IL1), and interleukin 6 (IL6).

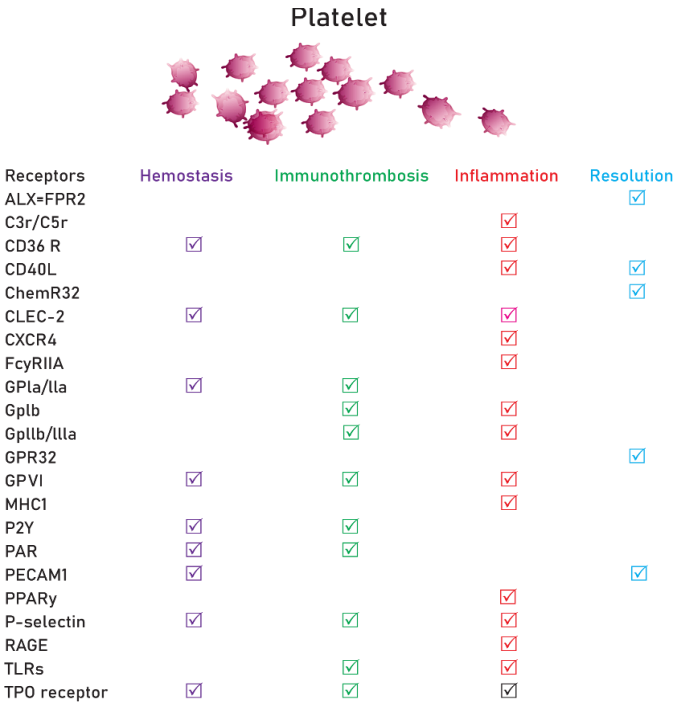


**Figure 2**. The different platelet's membrane receptors responsible for different roles of blood platelets during inflammation (i.e., hemostasis, immunothrombosis, and inflammation) and its resolution [45]

**Monocyte/macrophage activation and differentiation**

When monocytes enter a tissue, they develop into various macrophages with phenotypic and functional characteristics that vary according to the kind of tissue. Platelet activation was involved in induced immune thrombotic thrombocytopenia, which led to the modulation of monocyte/macrophage in the development of inflammation and the healing process. Many changes were occurring in activated macrophages. Platelets and platelet-derived microparticles regulate monocyte/macrophage activity in ways that are not entirely understood, partly due to platelets' release of soluble substances.[46,47] Activated platelets release a variety of alpha-granule-derived cytokines, chemokines, and growth factors that have the potential to modulate macrophage pro- or anti-inflammatory responses.[48]

Macrophages can undergo differentiation through diverse gene expression or polarization. In general, macrophages can be divided into three main groups: naïve (Mφ; also called M0), which readily differentiate into the other two phenotypes: pro-inflammatory (M1) and anti-inflammatory (M2).[20,49–53] The three main groups can be further divided into sub-phenotype groups, such as regulatory type (Mreg), oxidative (Mox), hematophage type (Mha), M3, and M4. The different types of macrophages resulting from the polarization process may contribute differently during the development of atherosclerosis (Figure 3). The functional states of M1 and M2 macrophages exist as two extremes on a continuum. Increased or decreased quantities of cytokines in their tissue milieu tilt the balance one way or the other. Although classified as the M1 type, the "M4" seems to have a unique character compared to the M1, known as classically-activated macrophages, or the M2, also known as alternatively activated macrophages.

Erbel et al.[54] investigated the atherosclerotic plaque of coronary arteries in patients with heart allograft transplants. They found that unstable plaques were associated with much higher M4 macrophages in the intima and the adventitia than stable plaques.

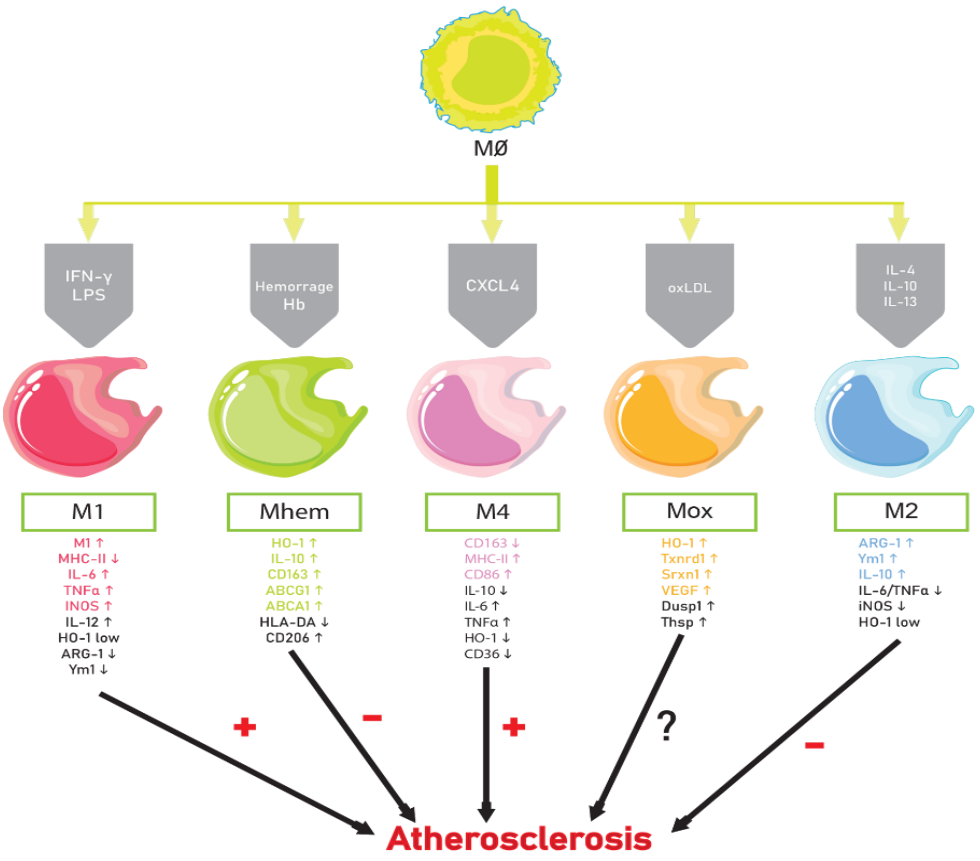


**Figure 3.** Macrophage polarization in atherosclerosis event.
M4 macrophage induces different chemokine and cytokine regulation (low expression of CD163, IL10, and CD36) and an increased expression of MHCII, CD86, IL6, and TNFα).[55]

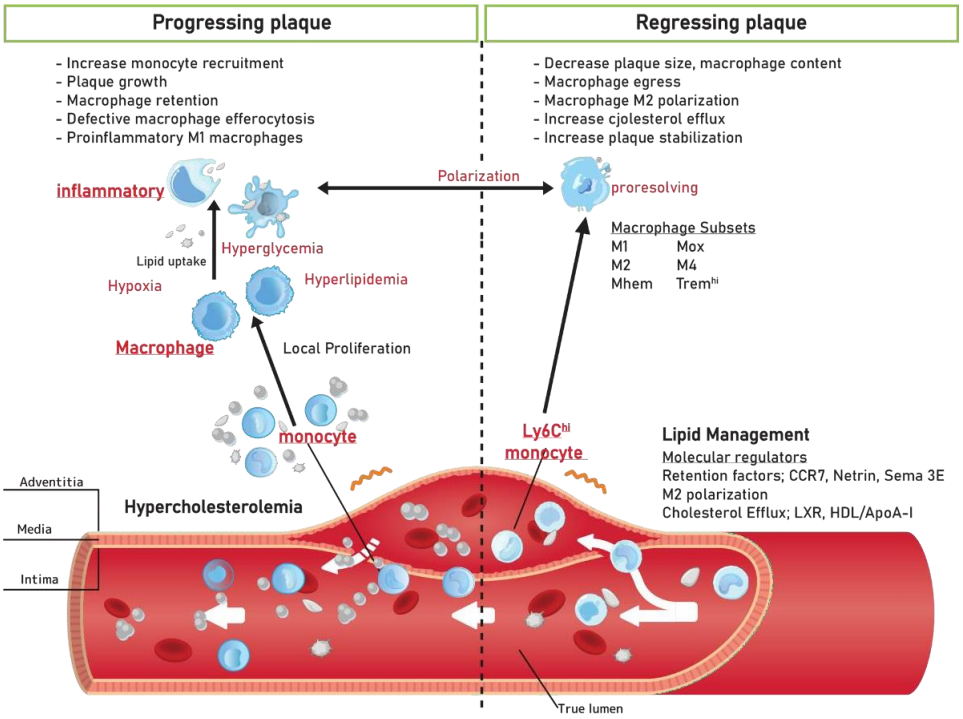


**Figure 4**. Mechanisms involved in plaque progression and regression that were influenced by macrophage modulation[59]

**Immunomodulatory effects of platelet and monocyte/macrophage interaction**

As enucleated discoid-shaped cells descended from megakaryocytes, thrombocytes (platelets) are no longer seen exclusively as critical actors in thrombosis and hemostasis.[56] When platelets interact with other immune mediators (for example, neutrophils, monocytes, and lymphocytes), platelet granulocyte or platelet-leukocyte aggregates form, resulting in further inflammation.[49,50,57] Chemokine (C-X-C motif) ligand 4 is one of many chemokines produced by platelet alpha granules upon activation of protein kinase c (PKC), which results in promoting monocyte differentiation and survival [58] and also promotes macrophage differentiation into the "M4" subtype.[59] M4 macrophages are classified as pro-inflammatory macrophages (M1 classtype), characterized by limited phagocytosis activity, low CD163 expression, and a future propensity for plaque instability that may lead to other cardiovascular problems (Figure 4).[54,59] CD163 expression is linked with an inability of the atheroprotective enzyme heme oxygenase-1 to be increased in response to Hb–Hp complexes, and polarization toward the M4 phenotype seems to be irreversible.

Another function of M4 macrophages was examined in samples of leprosy skin lesions from tuberculoid leprosy patients infected with *Mycobacterium leprae*. M4 was more highly expressed in individuals with lepromatous illness[60], suggesting that this subpopulation is less efficient at removing the bacillus and is, therefore, linked with developing the disease to one of the multibacillary forms. In other studies, platelets were found to convert circulating monocytes in human peripheral blood to IL10-producing regulatory monocytes.[61] The anti-inflammatory cytokine IL10 exerts its effects by blocking the synthesis of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF) in monocytes/macrophages.[62–65]

**Biomarkers of macrophage differentiation**

Because many of the proteins regulating macrophage activity are intracellular, extracellular labelling is inadequate. Numerous essential proteins involved in M1 and M2 functional states vary across mouse models and humans, making biomarker translation from pre-clinical research to clinical use difficult or impossible in some cases. The search for the CXCL4 receptor has been lengthy and has yielded only limited results. The cytochemical combination markers of CD68,

S100A8, and MMP7 reliably identify M4 macrophages in vitro and in vivo within atherosclerotic lesions.[66] CXCL4 seems to link to chondroitin sulphate in neutrophil cells,[67] while it binds to CXCR3B in microvascular endothelial cells. Another study indicated that CXCL4's chemotactic activity was induced by its interaction with an unidentified core protein in the chondroitin sulphate proteoglycan. This interaction seems to have promoted macrophage differentiation.[68]

**Generality and triggering factors of immune thrombocytopenia**

Immune thrombocytopenia (ITP) is an autoimmune disease marked by a low blood platelet count. [69] In this condition, blood platelets formed in megakaryocytes of bone marrow were inadequately produced or destroyed by T cells and/or antibodies, eventually leading to an increase in bleeding risk.[70-71] The incidence of ITP as a primary disease is rare, with unknown initiating factors. However, ITP can also be a secondary disease, followed by other diseases, such as lupus, immune deficiencies, and viral infections (e.g., HIV, HCV, Eppstein Barr Virus, COVID-19). In this case, an infection can trigger the auto-immunity responses against platelets[69] due to the similarity of platelet glycoprotein and the viral protein.[72]

Symptoms of ITP in patients may include petechiae, purpura, and bleeding (gastrointestinal tract, urinary tract, oral cavities). Patients diagnosed with primary ITP has IgG autoantibodies that target and bind platelets and various antigens, such as glycoproteins. Platelets were then recognized by phagocytes and destroyed in the spleen,[73] contributing to a low blood platelet count.

Thrombosis itself is considered a pathological deviation from hemostasis where a blood clot (thrombus) is formed as a part of the innate immune system that involves blood coagulation and platelet activation to prevent further blood loss in the vascular injury.[56] Immunothrombosis is a process that involves the immune system recognizing, containing, and destroying the harmful pathogen.

In vaccine-induced thrombosis thrombocytopenia (VITT), the administration of adeno-viral vaccine induced immunological response of antibodies against platelet factor CXCL4 and triggered thrombotic event through cascading platelet signals.[74,75] This symptom is similar to heparin-induced thrombocytopenia (HIT), where heparin, an anti-coagulant, also binds to CXCL4.[76] The heparin-CXCL complex triggers other platelets, leading to thrombus or blood clot formation and low blood platelet count.[77]

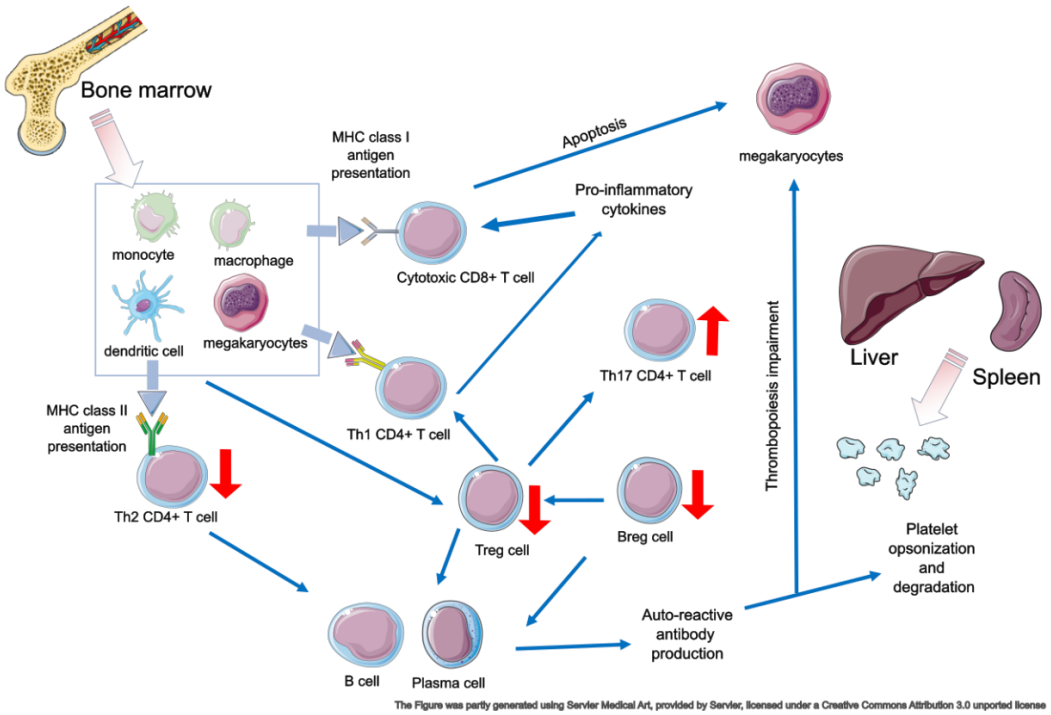


**Figure 5.** Patophysiological mechanism of induced immune thrombocytopenia.[73]

**Mechanisms leading to thrombocytopenia during ITP**

Few steps are involved in the general mechanism of thrombocytopenia during ITP (Figure 5). First, the autoimmune response against megakaryocytes in the bone marrow and lack of thrombopoietin-receptor agonist (TPO-RAs) resulted in inappropriate platelet production. Second, peripheral destruction of platelets involves multiple cells. Abnormal regulation of plasma and B cells produced antibodies that bind platelets and megakaryocytes. These complexes were then impaired and destructed in the spleen and liver. Regulatory T cells (Tregs) and regulatory B cells (Bregs) were also decreased, contributing to plasma cell survival and imbalance of Th $CD4^+$ T cells. Then, $CD8^+$ T was also activated, further inducing the apoptosis of platelets and megakaryocytes. Thus, bone marrow niche homeostasis is becoming dysfunctional.[73]

Understanding the pathophysiology of induced immune disorders such as ITP can help design treatment alternatives. The existing first-line ITP treatment so far is administering corticosteroids such as dexamethasone or prednisone to inhibit autoantibody production and platelet destruction.[73] Meanwhile, the second-line of treatment is thrombopoietin to stimulate platelet production.[78] However, these treatments showed a higher chance of patient remission or no treatment effects. Therefore, a new alternative therapy is proposed by modulating macrophage polarization. As used in other autoimmune disease treatments, macrophage modulation relies on modulating M1 or M2. In the case of ITP, where polarization M1 (specifically M4) promotes platelet destruction, a possible treatment is to promote macrophage M2 polarization. For instance, a therapy using a low dose of decitabine that causes demethylation of promoter peroxisome proliferator-activated receptor γ (PPARγ) and enhances the binding affinity of primer KLF4, contributing to macrophage polarization towards M2.[78]

**Potentially therapeutic naturally occurring compound for macrophage modulation**

Various studies showed potential clinical applications of macrophage modulation. For instance, there were studies involving potential herbal compounds that might be used as feasible therapeutic methods for treating associated diseases. Although there is currently little research on regulating putative herbal active ingredients for M4 macrophage modulation, many studies have shown their capacity and prospective impacts on pro-inflammatory (M1) and anti-inflammatory (M2) macrophages. The 1,3,6,7-tetrahydroxy-8-prenylxanthone from the fruit hull of mangosteen (*Garcinia mangostana*) showed promising effects in upregulating the M2 macrophage polarization.[79] Another study of Chinese Patent Medicines (CPM) Tongxinluo (TXL) with Panax ginseng showed that TXL helps treat atherosclerosis by preventing macrophage polarization into the M1 type.[80]

**Challenges in clinical application**

While inflammation may have developed as an adaptive response, platelets play an essential role in the physiological, protective integration of hemostasis and inflammation in pathologic thrombo-inflammatory events and diseases.[81–86] Despite significant recent advances in identifying macrophage activities and behaviors in tissue healing, much remains unknown about the role of the M4 macrophage in tissue integration, particularly in light of their current interaction with activation platelets. The regulation of macrophages by PF4 released by activated platelets plays many critical functions in inflammation and resolution and the future development of other vascular disorders such as atherogenesis or autoimmune diseases such as ITP.

## CONCLUSIONS

Macrophages are multifunctional phagocyte cells that play essential roles in the immune system and maintain homeostasis. Depending on the environmental stimuli, macrophages display plasticity to change their phenotype. Due to their multi-use in the human body, the modulation of their phenotype expression/polarization gives a promising approach to treating autoimmune diseases and disorders. Developing diagnostic methods that have high accuracy and enable early identification of any life-threatening health problem to improve survival or avoidable consequences of severe organ damage and lower quality of life face many obstacles.

Additionally, much more research has to be done to identify a particular receptor for CXCL4 inside the cells and determine if M4 macrophages are a viable diagnostic target or therapeutic

intervention in people with atherosclerotic diseases. Until today, there are still unanswered questions. These CXCL4-induced specific M4 macrophages may become a possible research study to understand better how to prevent this irreversible M4 macrophage population from forming to reduce the incidence of atherogenesis. Modulating macrophage polarization showed promising results in treating autoimmune diseases such as induced immune thrombotic thrombocytopenia, where antibodies destroy platelets, or low platelet production in bone marrow resulted in low platelet count and high bleeding risk. Among multiple causes of ITP, vaccine-induced thrombocytopenia can be treated by modulating macrophage polarization towards anti-inflammatory M2. Further studies are needed to understand the mechanisms behind each variant of ITP and its multiple predisposing factors; thus, possible treatments can be formulated accordingly.

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### Conflict of interest

Competing interests: No relevant disclosures.

### Author contributions

M – Conception, drafting the article. AB - Critical revision of the article. FCI – Drafting, Final approval of the version to be published. All authors have read and agreed to the published version of the manuscript.

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### Declaration of Use of AI in Scientific Writing

Nothing to declare.

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