

Thrombocyte Involvement in Immune Inflammatory Reactions

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INTRODUCTION

The immune inflammatory reaction is a complex interaction between antigens, leucocytes, thrombocytes, blood vessels, connective tissue and an extensive number of soluble factors. Thrombocytes are cell fragments without nuclei and therefore without the ability to synthesize proteins. Their survival time is short and their possibilities for expressing cell activation are limited. Thrombocytes do, nevertheless, take an active part in both hemostatic and inflammatory reactions. Throughout the last decade an increasing amount of knowledge has become available about the physiological mechanism by which thrombocytes take part in these reactions.

Based on a description of normal thrombocyte structure and physiological characteristics, this review represents contemporary knowledge on thrombocyte involvement in immune inflammatory reactions.

THROMBOCYTE STRUCTURE AND PHYSIOLOGY

Structure

Thrombocytes are small, anuclear blood cells with a normal survival time of 8 to 10 days. They have a diameter of 2-3 μm and an average volume of about 8 μ^3 . The thrombocyte surface is smooth with indentations, into which channels of a surface-connected canalicular system opens. Bundles of microtubules are

deposited round the longest diameter very near the cell surface, which may be responsible for the discoid shape. Close to the surface-connected canalicular system a smooth endoplasmatic reticulum is located, often referred to as "the dense tubular system". Throughout the cytoplasm there are mitochondria, peroxisomes, lysosomes and granules referred to as dense bodies and alpha-granules. Actomyosine contractile protein can hardly be seen on the transmission electron microscopy (TEM) (Fig. 1) because of the dense intracellular matrix.

Surface membrane

The thrombocyte surface is coated by a glucocalyx and the external membrane proteins are mainly glycoproteins bound to the lipid bilayer. The lipid bilayer is composed of phospholipids (70 %), cholesterol (25 %) and lipoproteins (2-5 %) (46).

Arachidonic acid is widely represented in the membrane. Liberation of this fatty acid from the membrane phospholipids, by activation of a specific lipase (phospholipase A₂) is believed to play a major role in the control of thrombocyte prostaglandin metabolism (30).

Several receptors of biological and pharmacological interest, such as alpha-adrenoceptors (17), beta-adrenoceptors (48), receptors to histamine (22), serotonin (40), prostacycline (47) and ADP (33) have been detected on the thrombocyte surface. Antigens belonging to the histocompatibility complex (MHC) class I (HLA-A, B, C) but not of class II (HLA-DR),

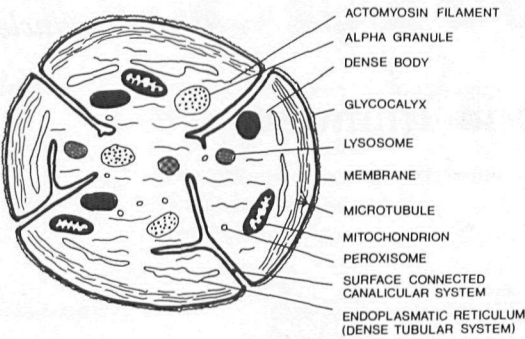


Fig. 1. Structure of the inactive thrombocyte, in the plane of the longest diameter.

are found (24). Fc-receptors (20) as well as binding sites for collagen (3) and fibrinogen (31) are present, although receptors for the two latter components can only be demonstrated on activated thrombocytes.

Granules

Dense granules (Table 1) are easily demonstrated by TEM due to their high amount of Ca^{2+} . They further contain inorganic pyrophosphate, serotonin and ribonucleotides including ATP, ADP, GTP and GDP. Ca^{2+} facilitates the thrombocyte cohesion and ADP is known to accelerate thrombocyte aggregation. Serotonin, which is taken up by thrombocytes from the extensor and stored, is known to amplify aggregation (9).

Alpha-granules (Table 1) contain platelet factor 4 (PF 4) and beta-thromboglobulin, both thrombocyte-specific proteins possessing anti-heparin effect (19). Platelet-derived growth

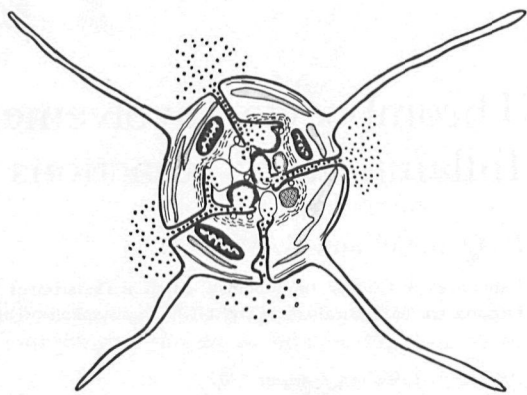


Fig. 2. Structure of the activated thrombocyte.

factor (PDGf) is known as a mitogenic agent for mesenchymal-derived cells (18). Thrombocyte lysosomes (Table 1) are only present in a small number and they contain a number of degradating enzymes including proteases and hydrolases.

Thrombocyte peroxizomes (Table 1) contain catalase, which is believed to play a role in the phagocytic phase and possibly in the decomposition of tissues to which thrombocytes attach (38).

Contractile proteins

Actin and myosine filaments are important for the thrombocyte activation process: actin differs only slightly from the actin in smooth muscle cells. When thrombocytes are activated, actin polymerizes and organizes, forming thin, active filaments. Myosine is structurally different from muscle cell myosine. Phosphorylation of myosine significantly increases contractile force generated by the actin filaments (27). The phosphorylation is Ca^{2+} dependent and does not take place if the cytosol free Ca^{2+} level is low (Fig. 3).

Thrombocyte activation

When thrombocytes are activated they change their shape and form pseudopodia, in a reversible manner (Fig. 2). They can also stick together, thus forming an irreversible aggregate. Moreover, activated thrombocytes secrete

Table 1
Essential content of thrombocyte granules

Dense granule	α -Granule	Lysosome	Peroxi-some
ADP	platelet factor 4	acid hydrolases	catalase
APT	β -thromboglobulin	cathepsins D, E	
Calcium	platelet-derived		
Serotonin	growth factor		
	permeability factor		
	chemostatic factor		
	bacterial factor		
	fibrinogen		

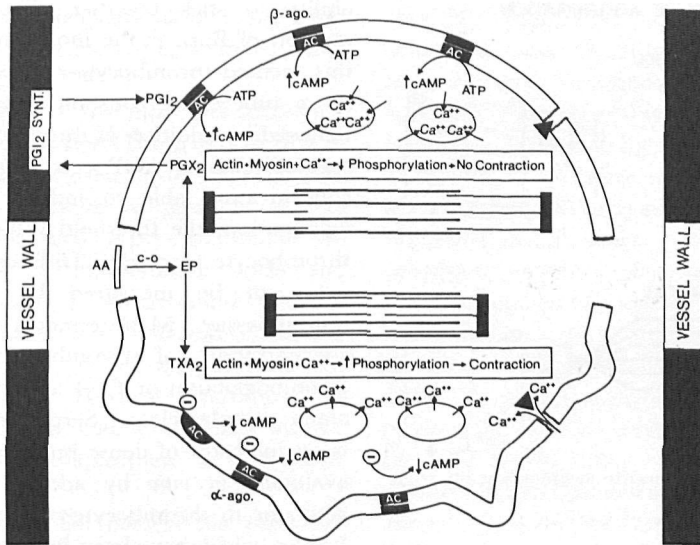


Fig. 3. Essential metabolic pathways involved in the thrombocyte activation process. Inactive thrombocyte at the top. Activated thrombocyte at the bottom.

different products (Fig. 2). Secretion from the dense granules has been referred to as release I and secretion from both dense- and alpha-granules as release II. These two processes may be modified by different mechanisms and do not always lead to irreversible aggregation (14).

Regulation of thrombocyte function

It is accepted that cyclic AMP participates in the regulation of cytosol free Ca^{2+} and thereby modifies thrombocyte function (56). An elevation of intracellular cyclic AMP facilitates the flux of Ca^{2+} into different stores, such as the dense bodies and the dense tubular system (Fig. 3). One of the metabolic pathways modifying thrombocyte cyclic AMP is the arachidonic pathway. Arachidonic acid is hydrolyzed from phospholipids in the thrombocyte membrane and oxidized to endoperoxides (EP) by the cyclooxygenase. Finally, EP are converted to thromboxane, which act as thrombocyte activators (29). Blood vessel endothelium contains prostacycline synthetase which converts thrombocyte-derived EP to prostacycline. Prostacycline inhibits thrombocyte aggregation and release reaction through an activation of the adenylate cyclase system, which converts ATP

to cyclic AMP (11) (Fig. 3). There is also evidence that beta-adrenoceptor stimulation, *in vitro*, results in thrombocyte cyclic AMP formation and that *in vivo* blockade of these receptors enhances aggregation (55). *In vitro* studies further indicate that histamine and adenosine increase thrombocyte cyclic AMP level (22, 33). Components which lower cyclic AMP, e.g. thromboxane and adrenaline, induce thrombocyte activation by increasing the cytosol concentration of free Ca^{2+} (11, 56). The role of cyclic GMP is still obscure. It has been claimed that cyclic GMP might play a role in the regulation of cytosol Ca^{2+} (12). The breakdown of cyclic AMP and cyclic GMP is regulated by phosphodiesterases. A blockade of these enzymes modifies thrombocyte function through interference with the cytosol level of cyclic nucleotide (34).

THROMBOCYTE KINETICS

Thrombocytes are derived from the megakaryocyte in the bone marrow. They circulate for about 9 days in the blood and are taken up by the reticuloendothelial system (RES). About 30 % of the total thrombocyte amount is normally found in the spleen. This pooling takes

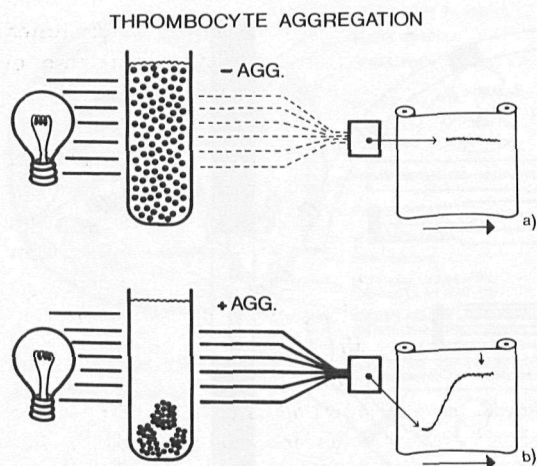


Fig. 4. Principle of turbidometric measurement of thrombocyte aggregation.

place especially along the sinusoidal walls, while the pulp cords only contain a few thrombocytes. The mechanism responsible for a concentration of thrombocytes in these areas is unknown. Hemodynamic principles may explain the high amount of thrombocytes along the sinusoidal walls. Adrenaline is known to mobilize the splenic thrombocyte pool (52).

THROMBOCYTE ANALYSIS

Thrombocytes are usually counted in a coulter counter or directly in a phase contrast microscope. The normal thrombocyte count varies between $120\text{--}450 \times 10^9$ thrombocytes/liter blood. Young thrombocytes are larger and contain more granules than aging thrombocytes. A high number of young thrombocytes indicate an enhanced synthesis, often secondary to an increased thrombocyte consumption.

Thrombocyte survival time is examined by labelling of isolated thrombocytes with radioactive indium. Thrombocytes are then reinfused (autologous thrombocytes), and from blood sample counting, during the following 7 days, thrombocyte survival time can be estimated (44). A low survival time indicates that thrombocytes are consumed somewhere in the organism.

The thrombocyte aggregation test is a very simple *in vitro* test, describing thrombocytes'

ability to stick together. The turbidometric method of Born is the most usual (Fig. 4). In this method thrombocyte-rich plasma is placed in a tube with constant magnetic stirring, followed by addition of thrombocyte activators, e.g. adrenaline, ADP or collagen. The lowest concentration able to induce an irreversible aggregation, the threshold value, indicates the thrombocyte reactivity. Different release products can be measured by use of radioimmunoassay. Measurements of the plasma concentration of thrombocyte-specific beta-thromboglobulin or PF-4 are an *in vivo* test for alpha granule release. Serotonin release, which is an indicator of dense body secretion, can be evaluated *in vitro* by adding a thrombocyte activator to thrombocyte-rich plasma. Thrombocytes which have been hyperactive *in vivo* will show a decrease in serotonin release (10).

The concentration of free Ca^{2+} in thrombocytes can be measured by using the tetraacid Quein 2 (56).

Cyclic AMP, cyclic GMP and the stable metabolites of thromboxane and prostacycline (TXB_2 and 6-keto-PGF_{1 α}) can be determined by radioimmunoassay.

Different receptor binding studies according to the method of Scatchard are routine analyses in some laboratories and so are the determination of membrane-bound immunoglobulins by immunofluorescence techniques.

THROMBOCYTE INVOLVEMENT IN IMMUNE INFLAMMATORY REACTIONS

Although thrombocytes are not, formally speaking, part of the immune system, they may undergo activation during immunoreactions. It has been stated that they are able to act as cellular triggers for the immunoinflammatory response (54) and that they are involved in certain types of chronic inflammation (54). Thrombocytes are then found to accumulate either at the side of local inflammatory lesions or in inflammatory exudates (23, 58). Histopathological evidence for thrombosis in the microcirculation of inflamed tissues can be found in conditions where different im-

munopathogenetic mechanisms are involved, e.g. rheumatoid arthritis (25) and kidney transplantations (42).

The involvement of thrombocytes in inflammatory reactions has been compared to that of the polymorphonuclear leucocytes (PML). Both cell types contain lyzosomal granules able to degranulate. Both cell types accumulate in vessels adjacent to inflammation areas and interact with bacteria, viruses and antigen-antibody complexes. The two cell types are futhermore able to carry out phagocytosis and to migrate *in vitro*. However, thrombocytes exert their pro-inflammatory activities mainly in the intravascular compartment, in contrast to PML.

Thrombocytes do not possess the immuno-active class II major histocompatibility complex (MHC) antigens (HLA-DR). They act in a complex non-specific way by interacting with other blood cells as well as with various soluble inflammatory mediator systems.

The following major functional systems involved in the triggering of thrombocytes in immune inflammatory reactions will be described: immunoglobulins, complement, lymphokines, platelet activating factor (PAF/AGEPC), PML, blood coagulation products, collagen and prostaglandins.

Thrombocytes exert their pro-inflammatory actions as a result of activation. Thrombocyte aggregation, phagocytosis and release reactions are different effector mechanisms exerted by thrombocytes and involved in immune inflammatory reactions, also to be described in the following.

Immunoglobulin-dependent thrombocyte activation

Thrombocyte activation by immunoglobulins (Ig) are either mediated via antigen-antibody or Fc-receptor reactions. Immuno-specific reactions between thrombocyte antigens and Ig are clinically important phenomena in several autoimmune diseases, but only little is known about their significance for local immune inflammatory reactions (53). Studies of sera from patients with rheumatoid arthritis (RA) and systemic lupus erythematosus (SLE) have recently shown

that 24 % and 51 %, respectively, of the tested sera were able to induce *in vitro* release of serotonin from thrombocytes (53). The release-inducing substance was shown to consist of monomer IgG, and its thrombocyte-activating effect was furthermore unaffected by Fc-receptors blocking experiments. It was therefore suggested that thrombocyte-specific IgG might contribute to the pathogenesis of the generalized vasculopathy seen in RA and SLE.

The non-specific interactions between Ig and thrombocytes have been investigated in different *in vitro* experiments (13, 21, 57). *In vivo* observations further indicate that this mechanism of thrombocyte activation is actually involved in the pathogenesis of immune inflammatory reactions (51). It has been demonstrated that heat-aggregated human Ig of all four IgG subclasses causes *in vitro* thrombocyte aggregation and serotonin release (13). IgM, IgA, IgD and IgE were, however, completely inactive in this respect. Insoluble immune complexes (IC) can also mediate thrombocyte activation under *in vitro* conditions (57). Thrombocyte activation by heat-aggregated IgG and IC has been found to take place via Fc-receptors on the cell surface. The exact mechanism by which Fc-receptor stimulation induces thrombocyte activation is not clear. Soluble IC and monomer IgG do not induce thrombocyte activation, but, on the other hand, they have been shown to inhibit the stimulatory effects of unsoluble IC via a blockade of Fc-receptors (7, 21).

The chronic inflammatory connective tissue diseases are often characterized by a deposition of IC in vessel walls. Thrombocyte abnormalities have been demonstrated in several of these diseases. Thrombocytosis is usually observed in active RA (15) and thrombocytopenia may be seen in SLE (36). Both RA and SLE can be accompanied by an increased thrombocyte turnover (4, 15). Enhanced *in vitro* aggregation of circulating thrombocytes has been found in RA. A decreased content of serotonin in circulating thrombocytes has been demonstrated in active RA as well as in SLE, indicating *in vivo* release reactions (59). Various competitive triggering mechanisms may account for the enhanced level of activity in cir-

culating thrombocytes seen in RA and SLE. However, IC-mediated thrombocyte activation in inflammatory lesions is believed to be of pathogenetic importance in these two diseases. Thus, insoluble IgG-containing IC from rheumatoid synovial fluid has been found capable of inducing thrombocyte activation (45). A local consumption of thrombocytes has been documented in SLE nephritis (6).

Complement-dependent thrombocyte activation

Complement constitutes an important pro-inflammatory mediator system closely related to immunological reactions. The classical information about interactions between complement and thrombocytes is derived from the Shwartzman endotoxic shock reaction (49). It is now well established that thrombin-mediated thrombocyte aggregation and release is enhanced by the presence of the terminal complement proteins (41). Activation of complement by thrombin on the thrombocyte surface leads to the production of dimers of the C5b-9 complex. The mechanism of the C5b-9 complex-induced enhancement of thrombocyte activity is not fully understood, but it has been suggested that it is mediated via the arachidonic acid pathway (41).

Activation of complement by IC on the thrombocyte surface, leading to cell lysis, constitutes another mechanism by which the content of storage granules can be released (49).

Lymphokine-dependent thrombocyte activation

Lymphokines are soluble, non-specific factors involved in cell-mediated immune reactions. They are produced by lymphocytes after non-specific stimulation with polyclonal T-cell activators, or after specific stimulation with recall antigen. Lymphokines are known to possess several biological activities related to the immune inflammatory reaction. In 1975 it was found that sensitized mononuclear spleen cells from nephritic New Zealand White rabbits produced a factor with thrombocyte-aggregating activities (TAA), after reexposure to the sensitizing antigen (26). The existence of

human lymphokines with TAA were shortly afterwards suggested, as it was demonstrated that supernatants from Con A-stimulated human lymphocytes contained TAA (8). This was not confirmed in a recent study (39) where the *in vitro* ability of human lymphocytes to produce a factor with TAA was reexamined, following stimulation with a non-specific polyclonal T-cell activator (PHA) as well as specific antigen (PPD). It was therefore concluded that it was most likely that lymphokines with TAA are not involved in the thrombotic processes seen in human cell-mediated immune inflammatory reactions.

PAF/AGEPC-dependent thrombocyte activation

PAF or AGEPC (acetyl-alkyl-glycerol ether analogues of phosphatidyl choline) are abbreviations used to denote a group of related stable neutral lipids which, *inter alia*, cause thrombocyte aggregation and release reactions. A variety of cell types in several species (43) have been found to release PAF/AGEPC. PAF/AGEPC should not be confused with the thrombocyte-aggregating lymphokine (see above) found in rabbits (26).

Immunological generation of PAF/AGEPC has been demonstrated both *in vitro* and *in vivo*. In 1972 it was reported (2) that antigen-induced *in vitro* degranulation of basophils from rabbits immunised with peroxidase yielded a soluble factor which aggregated rabbit thrombocytes. Later *in vitro* experiments have indicated that PAF/AGEPC is likewise released from human monocytes, PML, and basophils upon stimulation, e.g. with IC. However, a considerably higher concentration of PAF/AGEPC is needed to produce *in vitro* thrombocyte aggregation in humans as compared to rabbits (43). Systemic anaphylaxis in rabbits gives rise to immediate increase of PF 4 in plasma (32), indicating that thrombocyte activation is taking place *in vivo*. It is believed that PAF/AGEPC is responsible for the observed thrombocyte activation during immediate hypersensitivity reactions in rabbits (32). The *in vivo* significance of PAF/AGEPC for thrombocyte activation in humans has not yet been proved.

Polymorphonuclear leukocyte (PML)-dependent thrombocyte activation

Thrombocytes have been shown to participate in acute inflammatory reactions at an early stage, often prior to the development of tissue damage (16). A close association in time between PML accumulation and thrombocyte deposition in experimental inflammatory lesions was observed in a recent animal study (16). Various experiments suggested that thrombocytes interact with PML during PML margination along and/or emigration across microvascular walls in inflamed tissues (16). The most likely mediator was hypothesized to be PAF released from activated PML (16). Other studies have demonstrated that PML are able to induce thrombocyte activation via secretion of myeloperoxidase and hydrogen peroxide (5).

It is interesting to note that PML and thrombocytes interact in a two-way manner, as thrombocytes are known to release PML chemotactic factors.

Coagulation-dependent thrombocyte activation

Blood coagulation, and thereby thrombin formation, is an important mechanism for thrombocyte activation. Thromboplastin, which can be liberated from a large number of cell types, is considered the most powerful trigger of blood coagulation. Several substances related to immunological reactions, such as IC, complement split products, and lymphokines, have been found able to induce thromboplastin synthesis in monocytes *in vitro* (28). It has furthermore been shown that monocytes from patients with various rheumatic diseases, characterized by circulating IC, developed a significantly higher thromboplastin activity than did normal monocytes, when cultured *in vitro* without mitogen stimulation (28). It is therefore believed that IC-induced thromboplastin synthesis in monocytes is important for the intravascular coagulation and thrombocyte activation seen in immune inflammatory reactions (28).

Collagen-dependent thrombocyte activation

It is well known that thrombocytes adhere to perivascular tissue and that the interaction involves thrombocyte activation. Collagen is recognized as the tissue component responsible for this type of thrombocyte activation (1). Several levels of the collagen structural organization are involved in the reaction between collagen and specific binding sites on the thrombocyte surface (1). Thrombocyte aggregation and release, induced by collagen, are partly mediated via changes in the concentration of cyclic nucleotides (1).

Collagen-induced thrombocyte activation constitutes an important triggering mechanism of hemostasis. Immune inflammatory lesions often include vascular damage, and thereby exposure of collagen to circulating thrombocytes. Collagen-induced thrombocyte activation is therefore believed to be a significant mechanism by which thrombocytes get involved in immune inflammatory reactions.

Prostaglandin-dependent thrombocyte activation

Prostaglandins (PG) and the related arachidonic acid-derived products are potent local hormones essential for the regulation of a large number of cell functions in health and disease (50). The physiological and pathophysiological roles of PG's are not fully known, but it is believed that they are important factors in the regulation of cyclic nucleotides and transmembranal transport of ions, e.g. calcium. All cells, including thrombocytes, involved in immune inflammatory reactions have the enzymatic capacity to synthesize PG. The PG-thrombocyte interactions are complex, since PG are both produced by and act upon thrombocytes. PGE_2 , PGG_2 , PGH_2 and TxA_2 are known as thrombocyte activators, while PGE_1 , PGD_2 and PGI_2 have the opposite effect.

Thrombocyte aggregation

The ability of thrombocytes to form irreversible aggregates during activation is a quality not only important for hemostasis, but also for certain immune inflammatory reactions. Kidney

allograft rejection is the classic example. Just prior to a rejection episode, there is a decrease in the number of circulating thrombocytes, and a sequestration of thrombocytes in the transplantate (37). Formation of thrombocyte aggregates which obstruct the glomerular capillaries is believed to be an important factor in renal shut-down and rejection (37).

Thrombocyte phagocytosis

As mentioned above thrombocytes show several similarities with PML, including an ability to phagocytose particulate matters (37). Thrombocyte phagocytosis is believed to be involved in immune inflammatory reactions.

During experimental anaphylaxis induced by intravascular antigen-antibody interaction, aggregated thrombocytes in pulmonary vessels have been found to contain phagocytosed IC (35). Release of lysosomal enzymes is believed to take place during the phagocytic process and then to contribute to the inflammatory reaction.

Thrombocyte release reaction

As previously mentioned, thrombocytes can be found in both acute and chronic immune inflammatory reactions. The assortment of release products shows that thrombocytes are equipped to participate actively in different types of inflammatory reactions. It has thus been found that thrombocyte-released products influence both the microvascular-, cellular-, degenerative- and proliferative phase of inflammation. Serotonin, permeability factors, and PG, influence the microvascular phase, chemotactic factors the cellular phase, lysosomal enzymes the degenerative phase, and platelet-derived growth factor, the proliferative phase of inflammation. Some release products (ADP, EP, TxA_2 serotonin and Ca^{2+}) are further known to amplify the thrombocyte activating process.

CLOSING REMARKS

In this review we have only focused on the major functional systems of importance for the

thrombocyte involvement in immune inflammatory reactions. The cooperation between the different types of cells and soluble mediators in these reactions is very complex, and it is difficult to evaluate the relative importance of the different systems modifying thrombocyte function. There may be other systems involved, systems which up till now are unknown.

A full understanding of the structure and function, as well as a knowledge of thrombocyte analyses, are not only of great interest for future research on the subject of thrombosis and hemostasis, but may, in combination with research into the field of immune inflammatory diseases, show fruitful results. The fact that several antiinflammatory drugs influence thrombocyte function might be mentioned, but in the past only slight attention has been given to the clinical implication of this fact.

REFERENCES

1. Beachey, E. H., Chiang, T. M. & Kang, A. H.: Collagen-platelet interaction. *Int. Rev. Connect. Tissue Res.* 8, 1-21, 1979.
2. Benveniste, J., Henson, P. M., & Cochrane, C. G.: Leukocyte-dependent histamine release from rabbit platelets. The role of IgE, basophils and a platelet-activating factor. *J. Exp. Med.* 136, 1356-1377, 1972.
3. Brown, J. A., Jimenez, S. A. & Colman, R. W.: Collagen-induced platelet shape change: The role of collagen quaternary structure. *J. Lab. Clin. Med.* 95, 90-98, 1980.
4. Budman, D. R. & Steinberg, A. D.: Hematologic aspects of systemic lupus erythematosus. *Ann. Intern. Med.* 86, 202-229, 1977.
5. Clark, R. A. & Klebanoff, S. J.: Neutrophil-platelet interaction mediated by myeloperoxidase and hydrogen peroxide. *J. Immunol.* 124, 399-405, 1980.
6. Clark, W. F., Lewis, M. L., Cameron, J. F. & Parsons, V.: Intrarenal platelet consumption in the diffuse proliferative nephritis of systemic lupus erythematosus. *Clin. Sci. Mol. Med.* 49, 247-252, 1975.
7. Clark, W. F., Tevaarwerk, J. M., Moriarity, T. A. T. & Reid, B. D.: Inhibitors of immune complex induced human platelet aggregation and release in plasma. *Thromb. Res.* 28, 681-688, 1982.
8. Coeugnet, E. & Bendixen, G.: Lymphokines and thrombosis. *Acta Allergol.* 31, 94-106, 1976.
9. De Clerch E., David, J. L. & Janssen, P. A. J.: Inhibition of 5-hydroxytryptamine-induced and -amplified human platelet aggregation by ketanserin (R41 468), a selective 5-HT₂-receptor antagonist. *Agents Actions* 12, 388-397, 1982.
10. Fareed, J., Bick, R. L., Squillaci, G., Walenga, J. M.

- & Bermes, E. W.: Molecular markers of hemostatic disorders: Amplifications in the diagnosis and therapeutic management of thrombotic and bleeding disorders. *Clin. Chem.* 29, 1641-1658, 1983.
11. Fritsma, G., Engelman, G. & Yousuf, M.: Control mechanisms in platelet activation. *Am. J. Med. Technol.* 47, 813-818, 1981.
 12. Goldberg, N. D. & Haddox, M. K.: Cyclic GMP metabolism and involvement in biological regulation. *Ann. Rev. Biochem.* 46, 823-896, 1977.
 13. Henson, P. M. & Spiegelberg, H. L.: Release of serotonin from human platelets induced by aggregated immunoglobulins of different classes and subclasses. *J. Clin. Invest.* 52, 1282-1288, 1973.
 14. Holmsen, H.: Biochemistry of the platelet release reaction. CIBA Foundation Symposium, Biochemistry and Pharmacology of Platelets. Elsevier, Amsterdam, 175-205, 1975.
 15. Hutchinson, R. M., Davis, P. & Jayson, M. I. V.: Thrombocytosis in rheumatoid arthritis. *Ann. Rheum. Dis.* 35, 138-142, 1976.
 16. Issekutz, A. C., Ripley, M. & Jackson, J. R.: Role of neutrophils in the deposition of platelets during acute inflammation. *Lab. Invest.* 49, 716-724, 1983.
 17. Kafka, S. M., Tallman, J. F. & Smith, C. C.: Alpha-adrenergic receptors on human platelets. *Life Sci.* 21, 1429-1438, 1977.
 18. Kaplan, K. L., Broekman, M. J., Chernoff, A., Lesnik, G. R. & Drillings, M.: Platelet alpha-granule proteins: Studies on release on subcellular localization. *Blood* 53, 604-618, 1979.
 19. Kaplan, K. L. & Owen, J.: Plasma level of beta-thromboglobulin and platelet factor IV as indices of platelet activation in vivo. *Blood* 57, 199-202, 1981.
 20. Karas, S. P., Rosse, W. F. & Kurland, R. J.: Characterization of the IgG-Fc receptor on human platelets. *Blood* 60, 1277-1282, 1982.
 21. Kekomäki, R. & Myllylä, G.: Effect of normal human serum on the binding of specific antibodies and platelet-unrelated immune complexes to human platelets. *Scand. J. Immunol.* 9, 527-535, 1979.
 22. Klysner, R., Geisler, A., Winther Hansen, K., Skov, P. S. & Norn, S.: Histamine H₂ receptor-mediated cyclic AMP formation in human platelets. *Acta Pharmacol. Toxicol.* 47, 1-4, 1980.
 23. Kravis, T. & Henson, P. M.: Accumulation of platelets at sites of antigen-antibody-mediated injury. *J. Immunol.* 118, 1569-1573, 1977.
 24. Kristensen, T. & Mossin, J.: Competitive inhibition of T-cell mediated lympholysis by platelets. *Tissue Antigens* 19, 321-328, 1982.
 25. Kulka, J. P., Bocking, D., Ropes, M. W. & Bauer, W.: Early joint lesions of rheumatoid arthritis. *Arch. Pathol.* 59, 129-150, 1955.
 26. Lavell, K. J., Ransdell, B. A. & Trygstad, C. W.: Identification of a new platelet aggregating factor released by sensitized leukocytes. *Clin. Immunol. Immunopathol.* 3, 492-502, 1975.
 27. Lebowitz, E. A. & Cooke, R.: Contractile properties of actomyosin from human blood platelets. *J. Biol. Chem.* 253, 5443-5447, 1978.
 28. Lyberg, T., Prydz, H., Baklien, K. & Höyeraal, H. M.: Effect of immune complex-containing sera from patients with rheumatic diseases on thromboplastin activity of monocytes. *Thromb. Res.* 25, 193-202, 1982.
 29. Mancada, S. & Vane, J. R.: Pharmacology and endogenous role of prostaglandin endoperoxides thromboxane A₂ and prostacyclin. *Pharmacol. Rev.* 30, 293-331, 1978.
 30. Marcus, A. J.: The role of lipids in platelet function: with particular reference to the arachidonic acid pathway. *J. Lipid. Res.* 19, 793-826, 1978.
 31. Marguerie, G. A., Plow, E. F. & Edginton, T. S.: Human platelets possess an invisible and soluble receptor specific for fibrinogen. *J. Biol. Chem.* 254, 5357-5363, 1979.
 32. McManus, L. M., Morley, C. A., Levine, S. P. & Pinckard, R. N.: Platelet activating factor (PAF) induced release of platelet factor 4 (PF4) in vitro and during IgE anaphylaxis in the rabbit. *J. Immunol.* 123, 2835-2841, 1979.
 33. Mills, D. C. B. & Smidt, J. B.: The influence on platelet aggregation of drugs that affect the accumulation of adenosine 3',5'-cyclic monophosphate in platelets. *Biochem. J.* 121, 185-196, 1971.
 34. Moncada, S. & Korb, R.: Dipyridamole and other phosphodiesterase inhibitors act as antithrombotic agents by potentiating endogenous prostacyclin. *Lancet* 1, 1286-1289, 1978.
 35. Movat, H. Z., Mustar, J. F., Taichman, N. S. & Uriu-hara, T.: Platelet aggregation and release of ADP, serotonin and histamine associated with phagocytosis of antigen-antibody complexes. *Proc. Soc. Exp. Biol. Med.* 120, 232-237, 1965.
 36. Mulshine, J., Lucas, F. V. & Clough, J. D.: Platelet-bound IgG in systemic lupus erythematosus with and without thrombocytopenia. *J. Immunol. Methods* 45, 275-281, 1981.
 37. Mustard, J. F. & Pakcham, M. A.: Platelet phagocytosis. *Ser. Haematol.* 1, 168-184, 1968.
 38. Niewiarowski, S.: Proteins secreted by the platelets. *Thromb. Haemostas.* 38, 924-938, 1977.
 39. Oxholm, P., Winther, K., Bendtzen, K. & Bendixen, G.: Lack of evidence for human lymphokines with thrombocyte aggregating activity. *Allergy* 39, 618-621, 1984.
 40. Pletscher, A. & Affolter, H.: The 5-hydroxytryptamine receptor of blood platelets. *J. Neural. Transm.* 57, 233-242, 1983.
 41. Polley, M. J. & Nachman, R. L.: Human complement in thrombin-mediated platelet function. Uptake of the C5b-9 complex. *J. Exp. Med.* 150, 633-645, 1979.
 42. Porter, K. A., Dossetor, J. B., Marchioro, T. L., et al.: Human renal transplants. 1. Glomerular changes. *Lab. Invest.* 16, 153-181, 1967.
 43. Poul, W. E.: Fundamental immunology. Raven Press, New York, 1984.
 44. Scheffel, U., McIntyre, P. A., Evatt, B., et al.: Evaluation of "Indium" as a new high photon yield gamma-

- emitting "physiological" platelet label. *Johns Hopkins Med. J.* 140, 285-293, 1977.
45. Shapeleight, C., Valone, F. H., Schur, P. H., Goetzl, E. J. & Austen, K. F.: Platelet-activating activity in synovial fluids of patients with rheumatoid arthritis, gout and non-inflammatory arthropathies. *Arthritis Rheum.* 23, 800-807, 1980.
46. Shattil, S. J. & Cooper, R. A.: Role of membrane lipid composition, organization and fluidity in human platelet function. *Prog. Hemostas. Thromb.* 4, 59-86, 1978.
47. Siegel, A. M., Smith, J. B., Silver, M. J., Nicolaou, K. C. & Ahern, D.: Selective binding site for (^{3} H)-prostaglandin on platelets. *J. Clin. Invest.* 63, 215-220, 1979.
48. Steer, M. L. & Atlas, D.: Demonstration of human platelet beta-adrenergic receptors using ^{125} I-labeled cyano-pindolol and ^{125} I-labeled hydroxybenzyl-pindolol. *Biochim. Biophys. Acta* 686, 240-244, 1982.
49. Sundsmo, J. S. & Fair, D. S.: Relationship among the complement, kinin, coagulation and fibrinolytic systems in the inflammatory reaction. *Clin. Physiol. Biochem.* 1, 225-284, 1983.
50. Trang, L. E.: Prostaglandins and inflammation. *Sem. Arthritis Rheum.* 9, 153-190, 1980.
51. Valone, F. H., Austen, K. F. & Goetzl, E. J.: Identification of the platelet activation activity in rheumatoid synovial fluid as an intermediate molecular weight complex of IgG. *J. Immunol.* 122, 703-709, 1979.
52. Videbæk, Aa., Christensen, B. E. & Jønsson, V.: The spleen in health and disease. FADL, Copenhagen, 1982.
53. Weissbarth, E., Baruth, B., Mielke, H., Liman, W. & Deicher, H.: Platelets as target cells in rheumatoid arthritis and systemic lupus erythematosus: A platelet specific immunoglobulin inducing the release reaction. *Rheumatol. Int.* 2, 67-73, 1982.
54. Willis, A. L.: Platelet aggregation mechanisms and their implications in haemostasis and inflammatory disease. In Vane, J. R. & Ferreira, S. H. (eds.): *Inflammation*, Vol. 50/1, p. 138-205. Springer Verlag, Berlin, 1978.
55. Winther, K., Knudsen, J. B., Glazer, S., Geisler, A. & Klysner, R.: Platelet aggregation and beta-blockers. *Lancet* 1, 224-225, 1982.
56. Yamanishi, J., Kawahara, Y. & Fukuzaki, H.: Effect of cyclic AMP on cytoplasmic free calcium in human platelets stimulated by thrombin: Direct measurement with Quin $_2$. *Thromb. Res.* 32, 183-188, 1983.
57. Yeatts, R. P., Turner, R., Collins, R., Kaufmann, J. & Mashburn, H.: Soluble and insoluble immune complex-platelet interactions in rheumatoid inflammation. *Ann. Rheum. Dis.* 37, 421-427, 1978.
58. Zawilska, K., Giroud, J. P., Timsit, S. & Caen, J. P.: Plaquettes et inflammation. *Pathol. Biol. (Paris)* 21, Suppl. 51-56, 1973.
59. Zeller, J., Weissbarth, E., Baruth, B., Mielke, H. & Deicher, H.: Serotonin content of platelets in inflammatory rheumatic diseases. Correlation with clinical activity. *Arthritis Rheum.* 26, 532-540, 1983.

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