Article title: Why Is Heparin, Despite Being an Anticoagulant, Rarely Associated with Blood Clotting? A Systematic Review

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Introduction

It remains a scientific puzzle as to why heparin, an anticoagulant discovered since the beginning of the twentieth century (McLean, 1916), is associated with blood clotting prior to the inducement of its characteristic low platelets, known as thrombocytopenia, in about 1% to 3% of people who receive heparin (National Library of Medicine, 2013; Warkentin and Greinacher, 2003). This type of blood clotting usually starts 5 to 14 days after starting heparin therapy (National Library of Medicine, 2013) and is incapable of stopping bleeding due to the abnormal concentration and clotting of thrombocytes (platelets) at the site of pseudo-inflammation. This results in a reduction in platelets needed to stop the bleeding in inflammation and has been traced to the outcome of the mechanism of heparin-induced thrombocytopenia (HIT) or other possible but unconnected sites of inflammation (Baroletti and Goldhaber, 2006; Alberta 2021).

The introduction of heparin as an anticoagulant to stop the blood clotting it causes may exacerbate the situation in some patients by causing increased blood clot and low platelets, whereas other anticoagulants, such as danaparoid and lepirudin, may prevent blood clotting and promote the availability of unclotted platelets that can help in the formation of clots in true sites of inflammation (Franchini, 2005). This paper seeks to provide an answer to this behaviour of heparin by analysing the cellular expression of the autoantibodies involved in the mechanism of HIT and its relationship with heparin, as well as by exploring the relationship between heparin and other anticoagulants during the inducement of thrombocytopenia.

Cellular Expression of Autoantibodies

Natural autoantibodies that could react with autoantigens are present in a substantial amount in healthy individuals (Bayry et al., 2014; Mannoor et al., 2012). The autoantigens of natural autoantibodies belonging to IgM, IgG, and IgA classes include a variety of serum proteins, cell surface structures, and intracellular structures (Coutinho et al., 1995). Natural autoantibodies serve as the first line of defence and are expressed mainly by B-1 lymphocytes and marginal zone B cells even in the absence of incoming pathogens, unlike B2 lymphocytes of the adaptive immune response (Palma et al., 2018; Nielsen and Bendtzen, 2012). The role of B-1 cells is evident in older populations with degenerating B-1 cells, as they are prone to age-related diseases, such as microbial infection and atherosclerosis (Rodriguez-zhurbenko, Quach and Hopkins, 2019).

Unfortunately, natural autoantibodies—which are known to be expressed mainly by cells of B-1 and marginal zone B cells—may sometimes cause autoimmune diseases (Griffin and Rothstein), such as systemic lupus erythematosus (SLE), rheumatoid arthritis, and insulin dependent type 1 diabetes, which are often treated by therapeutically reducing the proliferation of the involved B cells to block the expression of such brutal autoantibodies (Nemazee, 2017). Furthermore, the emergence of monstrous autoantibodies is due to the inability of defective genes in some of these B cells to express and confer antibodies with the proteins needed to distinguish between

self and non-self (Nemazee, 2017). These genes known for the proteins they code include adenosine deaminase (ADA), activation-induced cytidine deaminase (AICDA), and Bruton's tyrosine kinase (BTK) (Nemazee, 2017). Thus, genetic disorders are the key players in autoimmune diseases.

By contrast, antibodies of the B-2 origin are specific to antigens and are developed in response to foreign antigen. The recognition of self-antigens by B-2 cells is usually known to have dire consequences, such as destruction or inactivation in the bone marrow (central tolerance). Immature B cells that bind to self will commit suicide (apoptosis) and are cleared from the B-2 cell population (clonal deletion), whereas B-2 cells exposed to soluble antigens in the bone marrow are not physically deleted but become inactive (clonal anergy). Thereafter, mature B cells in contact with the self are checked by nearby Th2 cells and disqualified from expressing antibodies, after which they undergo apoptosis (peripheral tolerance) (Lindsay et al., n.d.). These checkpoints make it difficult for cells of B-2 origin to express antibodies that can bind to the self. Only B-2 cells that qualify differentiate into plasma cells—meaning that they are screened and certified for producing antibodies that cannot bind to self. In addition, these plasma cells die after exhausting their energy in expressing the specific antibodies; however, clonal expansion of an activated B cell ensures the availability of B cells that will keep memory of the pathogen for speedy proliferation during future encounters (Lindsay et al., n.d.).

Heparin and Autoantibodies

Heparin is a naturally occurring anticoagulant primarily synthesized in the endoplasmic reticulum and Golgi apparatus of mast cells to prevent abnormal blood clots in the body (Oduah, Linhardt and Sharfstein, 2016). Due to the anticlotting activity of heparin, it is regarded as a form of treatment for patients experiencing blood clots. Heparin is synthesized pharmaceutically via animal sources such as porcine intestinal mucosa, chicken intestines, salmon (*Salmo salar*) gills and intestines, porcine mucosa, and sheep (ovine) intestines (Meer, Kellenbach and Bos, 2017).

Heparin achieves its anticoagulant effect through an antithrombin-dependent mechanism by inactivating thrombin and activated factor X (Hirsh et al., 2001). However, during thrombocytopenia, heparin binds to one of the clotting factors called platelet factor 4 (PF4) to form heparin-PF4 complex. Then, autoantibodies IgG bind to this complex and simultaneously bind to the Fc receptor on the platelet surface to activate these platelets to release prothrombotic substances (such as thrombin) and PF4, which forms more complexes with heparin for a persistence binding and activation of platelets to create a hypercoagulation state (Nicolas et al., 2020; Cai et al., 2015). The most characteristic clinical feature of HIT is thrombocytopenia due to the consumption of IgG-coated platelets by macrophages and removal by the reticuloendothelial system. The revival of the patient's clotting system relies on the prevention of more clotting as thrombus forms (Nicolas et al., 2020). However, studies have

shown that the activation of PF4 can also occur in the absence of heparin, which can cause non-heparin-induced thrombocytopenia (nHIT) (Nguyen, 2018).

Furthermore, Zheng et al. (2013) reported that the B cells that express the antibodies that recognize the epitopes of the heparin-PF4 complex and bind to them are present in both healthy humans and mice. These antibodies expressed by the B cells have high tolerance for the heparin-PF4 complex and therefore desist from activating platelets. However, a mouse with the same B cells lacking sufficient protein kinase C δ (PKC δ) production spontaneously expressed active IgG antibodies that bind to the heparin-PF4 complex, which forms the pathogenesis of HIT. This finding buttresses the fact that the inability of B-1 cells and zonal B cells to confer antibodies with an adequate amount of proteins due to genetic disorders could cause a breakdown of tolerance. Moreover, if the antibodies were of B-2 cells, the B cells would have died at the checkpoints of B-2 cells for their ability to even recognize self, which is a normal functional process in healthy people (Lindsay et al., n.d.).

The spontaneous expression of these protein-deficient antibodies following the recognition of an autoantigen within a period of 5 days (Zheng et al., 2013; National Library of Medicine, 2013) is a hallmark of natural antibodies that form the first line of defence. This is distinct from an adaptive immune response, as recent studies have shown that the IgG of B-2 cell origin, which are specific to an antigen, was expressed against SARS-CoV-2 28 days or more after infection (Information and Authority, 2020).

The rate of inducement of IgG autoantibodies by the heparin–PF4 complex depends on the PF4: heparin molar ratios. Higher rates of seroconversions occur with greater PF4 content (PHRs >10:1), whereas low PF4 and high heparin concentrations are associated with a decrease in immunogenicity and avidity (low PHRs <1:5) (Khandelwal and Arepally, 2018). This implies that coagulating factors must be upregulated to trigger the spontaneous proliferation of autoantibodies when combined with heparin. This explains why the release of PF4 as a byproduct from anti-heparin PF4 leads to more spontaneity of these autoantibodies and continuous binding to the heparin–PF4 complex.

Furthermore, the factors responsible for the upregulation of coagulating factors includes but are not limited to, birth control pills or hormone replacement therapy, pregnancy, cancer, or having been treated for cancer, and a family history of blood clots. Others include specific conditions, such as Factor V Leiden disease, antiphospholipid syndrome or polycythemia vera that makes clots more, coronavirus disease 2019 (COVID-19), overweight, or obese, a sedentary (or inactive) lifestyle, and smoking cigarettes (Cleveland Clinic, 2020). For example, the oral intake of oestrogen as birth hormone therapy induces inflammation in the liver as it tries to process it, and this triggers the upregulation of clotting factors to a level not observed when the hormones are secreted naturally into the blood. In this case, the severity of the clotting also depends on pre-existing risk factors, such as those listed above (Integrated Medicine Center of Western Colorado, 2021).

The fact that the above risk factors may not induce HIT in the population of people with defective autoantibodies until the administration of heparin suggests that clotting factors, such as PF4, have not reached a threshold at which its reaction with heparin could cause stronger immunogenicity and avidity capable of initiating HIT. This further suggests a direct proportionality between the degree of inflammation and the activation of clotting factors.

Heparin and other anticoagulants

The body is structured in such a way that once there is an injury or bleeding, the body defence mechanism is activated by sending signals responsible for arresting the situation. This can result in the formation of a clot to stop the bleeding; however, when this is not checked, the body can form large clots that could block veins or arteries. To mitigate this, the body sends the body's natural anticoagulant to help in stopping excessive clotting. Hence, while the body has an activating mechanism to arrest bleeding by forming clots, the body is also careful in activating mechanisms that prevent excessive clotting, indicating a balance between both mechanisms to ensure normal life for continual human survival (Dahlbäck, 1991; Walker and Fay 1992; Esmon, 1993).

Natural human anti-coagulants include antithrombin, protein S, and protein C. Protein C is activated by the thrombin thrombomodulin complex and downregulates the clotting cascade via a feedback loop inhibition mechanism by neutralizing factors V and VIII (Dahlbäck, 1991; Walker and Fay 1992; Esmon, 1993). It also releases a plasminogen activator to promote fibrinolysis (Esmon, 1981; De Fouw et al., 1990). A deficiency in any of these anticoagulants could lead to a condition called thrombophilia. This deficiency could be genetic or acquired (NIH, 2021). It is important to note that these anticoagulants act in a way that does not produce large clots to block the circulatory system. Deficiency of the natural anticoagulant in the body could be a result of illness, pregnancy, or vitamin K deficiency (Lipe and Ornstein, 2011). The increase in the risk of bleeding associated with the anticoagulant heparin when existing as a complex with (heparin-PF4 complex) in antiheparin-PF4 complex autoimmune manner is not common with other anticoagulants such as argatroban. Unfractionated heparin is more likely to cause HIT than low-molecular-weight heparin (Warkentin et al., 1995).

HIT results when heparin decreases the platelet blood count to less than 50% (Martel et al., 2005). Unlike heparin, argatroban prevents platelet aggregation and fibrin formation. It also reversibly binds to catalytic thrombin active sites, thereby inhibiting the activation of factors V, VIII, and XIII (Hursting et al., 1997). Argatroban has anticoagulant properties with less systemic toxicity (Bambrath et al., 2013). Another example is the anticoagulant called hirudin (lepirudin), which, unlike heparin, is not inactivated by PF4. Hirudin has the potential to inhibit thrombin bound within clot. This property is particularly important in HIT (Weitz et al., 1990; Weitz and Leslie, 1990). The mechanism of the anticoagulant warafin involves inhibiting vitamin K epoxide reductase, which is an enzyme responsible for the activation of coagulating factors II, VII, IX, and X. Thus, the inhibition of epoxide reductase causes a deficiency of vitamin K, which results in the knockdown of these clotting factors (Lakshmi et al., 2012). Overall, the main aim of any anticoagulant is to downregulate clotting factors by targeting any clotting, and the downregulation of one or a group of clotting factors leads to the downregulation of other

clotting factors in the form of a cascade. Thus, factor IV, which is one of the plasma's clotting factors (PF4), in the clotting cascade is eventually knocked down following the administration of anticoagulants (Harter, Levine, and Henderson, 2015).

Thus, the mechanism by which anticoagulants stop HIT involves the downregulation of PF4, which reduces the expression of autoantibodies of the IgG class via the reduction in immunogenicity and avidity of autoantibodies as the heparin:PF4 ratio is weakened. The inhibition of IgG autoantibodies reduces the probability of having corrupt autoantibodies as well as their avidity towards any available "weak" heparin-PF4 complex. In turn, this prevents the activation of platelets by corrupt autoantibodies to stop the initiation of HIT. Perhaps the reduction of PF4 also creates the availability of free heparin, which returns to its role as an anticoagulant to further prevent HIT.

Conclusion

The moderate formation of heparin-PF4 complex is likely an unrecognised part of the healthy mechanism our body uses to trigger IgG autoantibodies and deploy them as a first line of defence during an inflammation. The molar ratio of the heparin-PF4 complex is crucial and may depend on the severity of the inflammation, which may cause the availability of more PF4 to bind with heparin to form the complex, whereas in the absence of inflammation, the complex formed with lower levels of PF4 has lower immunogenicity. Autoantibodies with the adequate proteins needed for immune tolerance recognize these autoantigens (heparin-PF4 complex) as self, desisting from binding to them and avoiding the initiation of HIT. However, autoantibodies from defective B-1 cells and zonal B cells—whose genes cannot code and confer their antibodies with sufficient protein to distinguish between self and non-self—bind to them. The binding of these defective or corrupt autoantibodies to the autoantigens produces more PF4, which reacts with heparin to form more complexes at stagnant positions in the veins for the continuity of the binding activity. This activity attracts macrophages, after which they are removed by the reticuloendothelial system to cause a shortage of platelets.

However, the immunogenicity and avidity of these defective autoantibodies may not have been so strong if the molar ratio of PF4 to heparin in the binding did not exceed a certain threshold. Factors implicated in causing such upregulation of coagulating factors include PF4, which binds to heparin, SAR-CoV-2 and birth control pills, and hormone replacement therapy. Although anticoagulants are expected to stop this binding activity by downregulating coagulating factors, the addition of anticoagulant heparin in the presence of autoantibodies serves as a building block for the formation of more binding and its characteristic thrombocytopenia. Hence, anticoagulants other than heparin are needed to promote the deactivation of PF4, thereby cutting off the excessive formation of heparin PF4 complex, which also leads to the inhibition of the IgG autoantibodies, as this is the source of their stimulation and attraction. This further implies that the downregulation of anticoagulants due to genetic disorders in anticoagulants secreting cells or other causes such as illnesses could help create favourable conditions for HIT.

The dysregulation of the PF4 threshold to levels that increases the immunogenicity and avidity of corrupt IgG autoantibodies for heparin-PF4 complex in people with defective B cells results in the reduction of platelets.

Recommendations

It is essential to watch for the presence of defective B-1 cells and marginal zone B cells, as well as defective anticoagulant secreting cells, in people with the identified risk factors prior to the administration of heparin or heparin-like compounds found in drugs or vaccines.

REFERENCES

- Alberta 2021. What is heparin induced thrombocytopenia. Available at:
<https://myhealth.alberta.ca/Health/aftercareinformation/pages/conditions.aspx?hwid=abr9773#:~:text=Heparin%20is%20a%20type%20of,clots%20instead%20of%20preventing%20them.>
- Bambrah, R.K., Pham, D.C. and Rana, F. 2013. Argatroban in heparin-induced thrombocytopenia: Rationale for use and place in therapy. *Therapeutic advances in chronic disease*, 4(6), pp.302-304.
- Baroletti, S.A. and Goldhaber, S.Z. 2006. Heparin-induced thrombocytopenia. *Circulation*, 114(8), pp.e355-e356.
- Bayry J, Misra N, Dasgupta S, Lacroix-Desmazes S, Kazatchkine M.D and Kaveri S.V (2005) . Natural autoantibodies: immune homeostasis and therapeutic intervention Available at: <https://doi.org/10.1586/1744666X.1.2.213>
- Cai, Z., Yarovoi, S.V., Zhu, Z., Rauova, L., Hayes, V., Lebedeva, T., Liu, Q., Poncz, M., Arepally, G., Cines, D.B. and Greene, M.I. 2015. Atomic description of the immune complex involved in heparin-induced thrombocytopenia. *Nature communications*, 6(1), pp.1-10.
- Casali, P. and Notkins, A.L. 1989. CD5+ B lymphocytes, polyreactive antibodies and the human B-cell repertoire. *Immunology today*, 10(11), pp.364-368.
- Christiane, S.H. and Hampe, B. 2012. B Cells in autoimmune diseases. *Sci (Cairo)*, 2012(215308), p.215308.
- Cleveland Clinic, Blood clots: risks, symptoms, treatments, prevention (clevelandclinic.org), Available at: Blood Clots: Risks, Symptoms, Treatments, Prevention (clevelandclinic.org)
- Coutinho A., Kazatchkine M D , Avrameas S. 1995 . Natural autoantibodies Available at: DOI: [10.1016/0952-7915\(95\)80053-0](https://doi.org/10.1016/0952-7915(95)80053-0)
- Dahlbäck, B. 1991. Protein S and C4b-binding protein: Components involved in the regulation of the protein C anticoagulant system. *Thrombosis and haemostasis*, 66(07), pp.049-061.

- De Fouw, N.J., Haverkate, F. and Bertina, R.M. 1990. Protein C and fibrinolysis: A link between coagulation and fibrinolysis. In *Fibrinogen, Thrombosis, Coagulation, and Fibrinolysis* (pp. 235-243). Springer, Boston, MA.
- Duan, B. and Morel, L., 2006. Role of B-1a cells in autoimmunity. *Autoimmunity reviews*, 5(6), pp.403-408.
- Elkon, K. and Casali, P. 2008.). *Nat Clin Pract Rheumatol*, 4(9), pp.491-498.
- Esmon, C.T. 1981. Generation of fibrinolytic activity by infusion of activated protein C into dogs. *The Journal of clinical investigation*, 68(5), pp.1221-1228.
- Esmon, C.T. 1993. Molecular events that control the protein C anticoagulant pathway. *Thrombosis and haemostasis*, 70(07), pp.029-035.
- Franchini, M. 2005. Heparin-induced thrombocytopenia: An update. *Thrombosis Journal*, 3, pp. 1–5. doi: 10.1186/1477-9560-3-14.
- Gombart, A.F., Pierre, A. and Maggini, S., 2020. A review of micronutrients and the immune system—working in harmony to reduce the risk of infection. *Nutrients*, 12(1), p.236.
- Harter, K., Levine, M. and Henderson, S. O. 2015. Anticoagulation drug therapy: A review'. doi: 10.5811/westjem.2014.12.22933.
- Hirsh, J., Anand, S.S., Halperin, J.L. and Fuster, V. 2001. Mechanism of action and pharmacology of unfractionated heparin.
- Hursting, M.J., Alford, K.L., Becker, J.P., Brooks, R.L., Joffrion, J.L., Knappenberger, G.D., Kogan, P.W., Kogan, T.P., McKinney, A.A. and Schwarz, R.P. 1997. December. Novastan® (brand of argatroban): a small-molecule, direct thrombin inhibitor. In *Seminars in thrombosis and hemostasis* (Vol. 23, No. 06, pp. 503-516). Thieme Medical Publisher.
- Information, H. and Authority, Q. 2020. Evidence summary of the immune response following infection with SARS- CoV-2 or other human coronaviruses. (August). Available at: [Evidence summary of Immune response following infection with SARS-CoV-2 \(rte.ie\)](https://rte.ie/evidence-summary-of-immune-response-following-infection-with-sars-cov-2)
- Integrated Medicine Center of Western Colorado. 2021. Hormone replacement therapy and the risk of blood clots. Available at: Hormone Replacement Therapy and the Risk of Blood Clots Integrative Medicine Center of Western Colorado: Integrative Medicine Center of Western Colorado (imcwc.com)
- Kelton, J.G., Smith, J.W., Warkentin, T.E., Hayward, C.P., Denomme, G.A. and Horsewood, P. 1994. Immunoglobulin G from patients with heparin-induced thrombocytopenia binds to a complex of heparin and platelet factor 4.
- Khandelwal, S. and Arepally, G. M. 2018. *HHS Public Access*. 116(5), pp. 792–798. doi: 10.1160/TH16-01-0074. Immune.

- Lakshmr R. Anitha S, K Anila N, Roshni P. R. 2012. International journal of pharmaceutical sciences and research available at: <https://ijpsr.com/bft-article/warfarin-resistance-mechanisms-and-management/?view=fulltext>
- Lindsay n.d. Available at: The adaptive immune response: B-lymphocytes and antibodies – Anatomy & Physiology (oregonstate.education)
- Lipe, B. and Ornstein, D.L., 2011. Deficiencies of natural anticoagulants, protein C, protein S, and antithrombin. *Circulation*, 124(14), pp.e365-e368.
- Mannoor, K. et al. 2012 Natural autoantibodies and associated B cells in immunity and autoimmunity. Available at: <https://doi.org/10.3109/08916934.2012.748753>
- Martel, N., Lee, J. and Wells, P.S. 2005. Risk for heparin-induced thrombocytopenia with unfractionated and low-molecular-weight heparin thromboprophylaxis: A meta-analysis. *Blood*, 106(8), pp.2710-2715.
- McLean, J. 1916. The thromboplastic action of cephalin. *American Journal of Physiology-Legacy Content*, 41(2), pp.250-257.
- Meer, J. Van Der, Kellenbach, E. and Bos, L. J. Van Den. 2017. From farm to pharma: An overview of industrial, pp. 1–13. doi: 10.3390/molecules22061025.
- National Library of Medicine. 2013, Evaluating the presence of blood clots in people with heparin-induced thrombocytopenia (HIT) (The HOT Study) (HOT).
- National Library of Medicine. 2013 clinical trial, Available at: <https://clinicaltrials.gov/ct2/show/NCT00594685>
- Nemazee, D. 2017. Mechanisms of central tolerance for B cells, *Nature Reviews Immunology*, 17(5), pp.281–294. doi: 10.1038/nri.2017.19.
- Nguyen, T.H. 2018. Not only heparin but also antibody induces thrombocytopenia. *Thrombocytopenia*, p.33.
- Nielsen, C. H. and Bendtzen, K. 2012. Immunoregulation by naturally occurring and disease-associated autoantibodies binding to cytokines and their role in regulation of T-Cell responses. *Advances in Experimental Medicine and Biology*, 750, pp. 116–132. doi: 10.1007/978-1-4614-3461-0_9.
- NIH. 2021. Rare diseases. Protein C deficiency. Available: Protein C deficiency. Genetic and Rare Diseases Information Center (GARD) – An NCATS Program (nih.gov). accessed: 2021.
- Oduah, E. I., Linhardt, R. J. and Sharfstein, S. T. 2016. Heparin: Past, present, and future. pp. 1 – 12. doi: 10.3390/ph9030038.
- Palma, J. Beata T. Jakub D. and Wiesław D. 2018. Natural antibodies – Facts known and unknown. *Central European Journal of Immunology*, 43(4), pp. 466-475. doi: 10.5114/ceji.2018.81354.

- Rodriguez-zhurbenko, N., Quach, T. D. and Hopkins, T. J. 2019. Human B-1 cells and B-1 cell antibodies change with advancing age. 10(March), pp.1-15. doi: 10.3389/fimmu.2019.00483.
- Vaidyanathan, D., Williams, A., Dordick, J.S., Koffas, M.A. and Linhardt, R.J., 2017. Engineered heparins as new anticoagulant drugs. *Bioengineering & translational medicine*, 2(1), pp.17-30.
- Walker, F.J. and Fay, P.J., 1992. Regulation of blood coagulation by the protein C system. *The FASEB journal*, 6(8), pp.2561-2567.
- Warkentin, T.E. and Greinacher, A., 2003. Heparin-induced thrombocytopenia and cardiac surgery. *The Annals of thoracic surgery*, 76(2), pp.638-648.
- Warkentin, T.E., Levine, M.N., Hirsh, J., Horsewood, P., Roberts, R.S., Gent, M. and Kelton, J.G., 1995. Heparin-induced thrombocytopenia in patients treated with low-molecular-weight heparin or unfractionated heparin. *New England Journal of Medicine*, 332(20), pp.1330-1336.
- Weitz, J.I. and Leslie, B., 1990. Urokinase has direct catalytic activity against fibrinogen and renders it less clottable by thrombin. *The Journal of Clinical Investigation*, 86(1), pp.203-212.
- Wilkerson, W.R. and Sane, D.C., 2002. Aging and thrombosis. In *Seminars in thrombosis and hemostasis* (Vol. 28, No. 06, pp. 555-568). Thieme Medical Publishers.