Enhancement of autoantibody pathogenicity by viral infections in mouse models of anemia and thrombocytopenia☆

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Abstract

Viral infections are involved in the pathogenesis of blood autoimmune diseases such as hemolytic anemia and thrombocytopenia. Although antigenic mimicry has been proposed as a major mechanism by which viruses could trigger the development of such diseases, it is not easy to understand how widely different viruses might induce these blood autoimmune diseases by this sole mechanism. In mice infected with lactate dehydrogenase-elevating virus (LDV), or mouse hepatitis virus, and treated with anti-erythrocyte or anti-platelet monoclonal autoantibodies at a dose insufficient to induce clinical disease by themselves, the infection sharply enhances the pathogenicity of autoantibodies, leading to severe anemia or thrombocytopenia. This effect is observed only with antibodies that induce disease through phagocytosis. Moreover, the phagocytic activity of macrophages from infected mice is increased and the enhancing effect of infection on autoantibody-mediated pathogenicity is strongly suppressed by treatment of mice with clodronate-containing liposomes. Finally, the disease induced by LDV after administration of autoantibodies is largely suppressed in animals deficient for gamma-interferon receptor. Together, these observations suggest that viruses may trigger autoantibody-mediated anemia or thrombocytopenia by activating macrophages through gamma-interferon production, a mechanism that may account for the pathogenic similarities of multiple infectious agents.
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Contents

1. Viruses and blood autoimmune diseases

Involvement of viruses in the pathogenesis of autoimmune diseases mediated by autoantibodies, including blood diseases such as hemolytic anemia and thrombocytopenia, has been reported both in patients and in experimental models [1]. In children, acute immune thrombocytopenic purpura (ITP) often follows a viral infection [2]. Interestingly, the causative infection may be due to very different viruses, such as influenza virus, rubella virus, mumps, parvovirus, cytomegalovirus, Epstein–Barr virus, and varicella zoster virus. Autoantibodies that are secreted in the course of the infection opsonize platelets, leading to their destruction through phagocytosis by cells of the reticuloendothelial system, and especially by macrophages. As a result of platelet drop, clinical symptoms including petechiae or, in more severe cases, bleedings, develop. Although it cannot be excluded that other mechanisms, such as polyclonal B cell activation, anti-idiotypic response, alteration of self antigens by the virus, or enhancement of antigen presentation might play a role in this virally-triggered disease, molecular mimicry between epitopes shared by platelets and infectious agents has been suggested to mainly explain the initial production of autoantibodies. A spread of this autoimmune response to other platelet determinants that are not necessarily shared with the virus may then be responsible for further development of ITP [3,4]. Pro-inflammatory cytokines, like gamma-interferon (IFN-γ), that are produced at higher levels in ITP patients than in normal individuals may contribute to the full expression of the disease [3,4]. However, whereas the involvement of viruses in the etiology of ITP seems well established, it might be difficult to understand how infectious agents that carry distinct antigenic epitopes may trigger, through molecular mimicry, an autoantibody response that is similarly targeted to platelet determinants. Although a relationship between autoimmune hemolytic anemia (AIHA) and viruses has not been shown as clearly as for ITP, many cases of AIHA have been reported after infection with viruses as diverse as hepatitis A and C viruses, human immunodeficiency virus-1, Epstein–Barr virus, rubella virus and varicella virus. Thus, it may be hypothesized that different viruses trigger these two autoantibody-mediated diseases by similar mechanisms.

2. Animal models of ITP and AIHA

Not so many experimental models of ITP or AIHA have been reported. Injection into mice of polyclonal or monoclonal antibodies raised against platelets or red blood cells (RBC), including switch variants derived from monoclonal autoantibodies, allows the analysis of the different mechanisms involved in the destruction of these target cells [5–7]. Anti-platelet autoantibodies that are spontaneously produced in male (NZW×BXSB)F1 mice lead to thrombocytopenia [8]. However, this thrombocytopenia arises in the context of a complex autoimmune syndrome reminiscent of human systemic autoimmune diseases rather than of organ-specific ITP. Similarly, NZB mice develop an AIHA mediated by anti-erythrocyte autoantibodies that are produced concomitantly with other autoantibodies with diverse specificities, although through distinct mechanisms [9].

Anti-erythrocyte and anti-platelet autoantibody responses have been elicited in mice after immunization with rat RBC or platelets. Administration of

rat erythrocytes to normal mice results in the activation of autoreactive B and T lymphocytes that recognize different shared epitopes, including on RBC band-3 antigen, leading to moderate anemia [10–13]. Immunization of mice infected with LDV results in a faster anti-RBC autoantibody response, without enhancement of anemia [14]. Similarly, production, probably through molecular mimicry, of anti-platelet autoantibodies that react with a 145- to 155-kDa autoantigen follows immunization of some strains of normal mice with rat platelets, but the resulting thrombocytopenia is moderate and transient [15]. Anti-platelet autoantibody production has also been reported after interspecies platelet immunization in marmosets [16].

Few animal models mimic the effect of infections on the pathogenesis of human ITP and AIHA. Mice infected with dengue-2 virus develop a transient thrombocytopenia that may result from the secretion of anti-platelet autoantibodies, together with other consequences of this viral infection, such as paraplegia [17]. Infectious agents that induce polyclonal B lymphocyte activation, such as *Trypanosoma brucei*, trigger the secretion of autoantibodies that can recognize diverse autoantigens, including erythrocytes [18]. Infection of mice with lymphocytic choriomeningitis virus (LCMV) results in anemia that is of central origin in most animals and may depend on natural killer cell and CD8+ T lymphocyte activation and on the secretion of interferon (IFN)-α/β [19–21]. However, in C3HeB/FeJ mice, the Docile strain of LCMV triggers peripheral hemolytic anemia that is correlated with the production of anti-erythrocyte autoantibodies recognizing the band-3 antigen [22–24]. Although this anti-erythrocyte autoantibody production apparently does not result from either molecular mimicry nor from B lymphocyte polyclonal activation [23,25], the mechanisms through which it is induced by the infection remain to be determined.

3. Enhancement of anti-erythrocyte and anti-platelet autoantibody pathogenicity by mouse nidoviruses

The effect of a viral infection on antibody-mediated blood autoimmune disease has been tested in two different models. Hemolytic anemia can be induced into normal mice by administration of monoclonal anti-erythrocyte autoantibodies. Distinct pathogenic mechanisms lead to the disease in normal animals, as some antibodies, including 34-3C and 4C8 IgG2a autoantibodies, induce anemia through phagocytosis of opsonized erythrocytes by cells of the reticulo-endothelial system, while other autoantibodies, such as IgG1 31-9D, trigger agglutination of RBC that are then sequestered in the spleen and liver [26,27]. Moreover, complement may also increase the pathogenicity of some autoantibody isotypes [28]. Mice infected with lactate dehydrogenase-elevating virus (LDV), a mouse arterivirus, display a dramatic enhancement of the anemia induced by the 34-3C IgG2a autoantibody, but

Table 1
Exacerbation of antibody-mediated thrombocytopenia by viral infection

Exp.	Mice[a]	Antibody[b]	Infection	Treatment[c]	Platelet count[d]
1	CBA/Ht	Control	–	–	1183±8
		Control	LDV	–	917±94
		N1	–	–	983±30
		N1	LDV	–	192±42
2	BALB/c	Control	LDV	–	812±26
		Polyclonal	LDV	–	444±69
		Polyclonal F(ab′)2	LDV	–	894±73
3	CBA/Ht	Control	LDV	–	562±75
		Control	LDV	IVIg	481±21
		Polyclonal	LDV	–	38±7
		Polyclonal	LDV	IVIg	375±105
4	CBA/Ht	–	LDV	–	481±84
		Anti-CD41	LDV	PBS-liposomes	88±33
		Anti-CD41	LDV	Clodronate-liposomes	756±122
5	129/Sv	Control	LDV	–	508±74
	G129	Control	LDV	–	533±46
	129/Sv	Anti-CD41	LDV	–	33±8
	G129	Anti-CD41	LDV	–	383±68
6	CBA/Ht	Control	–	–	985±66
		Control	MHV	–	545±55
		Polyclonal	–	–	775±76
		Polyclonal	MHV	–	105±20

[a] G129: IFN-γ receptor-deficient mice on a 129/Sv background.
[b] N1: IgG2a monoclonal anti-platelet autoantibody from a (NZW×BXSB)F1 mouse; polyclonal: rabbit anti-mouse platelet; anti-CD41: monoclonal rat anti-mouse CD41.
[c] IVIg: intravenous total immunoglobulin G.
[d] Platelet/μl$\times 10^{-3}$, mean±SEM for groups of usually 4–5 mice.

not by the 31-9D IgG1 antibody, suggesting that the virus may increase the phagocytosis of opsonized erythrocytes [29].

Similarly, LDV exacerbates the thrombocytopenia induced by administration to normal mice of polyclonal or monoclonal anti-platelet antibodies, including auto-antibodies derived from (NZW×BXSB)F1 mice [30] and anti-CD41 (integrin α_{IIb} chain) antibody (Table 1). Mouse hepatitis virus, a coronavirus, has the same enhancing effect on the pathogenicity of anti-platelet antibodies. As a result of concomitant administration of LDV and of anti-platelet antibodies, mice develop petechiae reminiscent of those that are observed in patients with immune thrombocytopenic purpura [30].

4. Phagocytosis as a key factor in virally exacerbated blood autoimmune diseases

Several lines of evidence point towards phagocytosis as the major mechanism that is involved in the exacerbating effect of viruses on antibody-mediated blood autoimmune diseases (Table 1). First, the F(ab′)$_2$ fragment of anti-platelet polyclonal antibody does not induce thrombocytopenia, even in infected animals,

indicating that the Fc fragment, which is recognized by receptors that may mediate phagocytosis, is required for the antibody pathogenic effect [30]. Second, in vivo treatment with total IgG that block Fc receptors on phagocytic cells (IVIg) [31] or with clodronate-containing liposomes that suppress macrophages [32] and thus inhibit phagocytosis of autoantibody-coated target cells [15,33,34], prevents the development of antibody-mediated thrombocytopenia in LDV-infected mice [30]. Third, histological analysis shows in vivo phagocytosis of a much larger number of erythrocytes in the liver of LDV-infected mice that had received an anti-RBC monoclonal autoantibody than in control animals [29]. Finally, the ability of macrophages from LDV-infected mice to phagocytose ex vivo erythrocytes opsonized by a monoclonal autoantibody is enhanced when compared to that of macrophages obtained from uninfected animals [29].

Macrophage stimulation by cytokines or growth factors such as granulocyte-macrophage colony-stimulating factor (GM-CSF) leads to an enhancement of anemia mediated by anti-erythrocyte monoclonal autoantibody [35]. Viral infections, including with LDV [36], frequently trigger the secretion of IFN-γ, a potent macrophage activator. As interleukin-12 is also
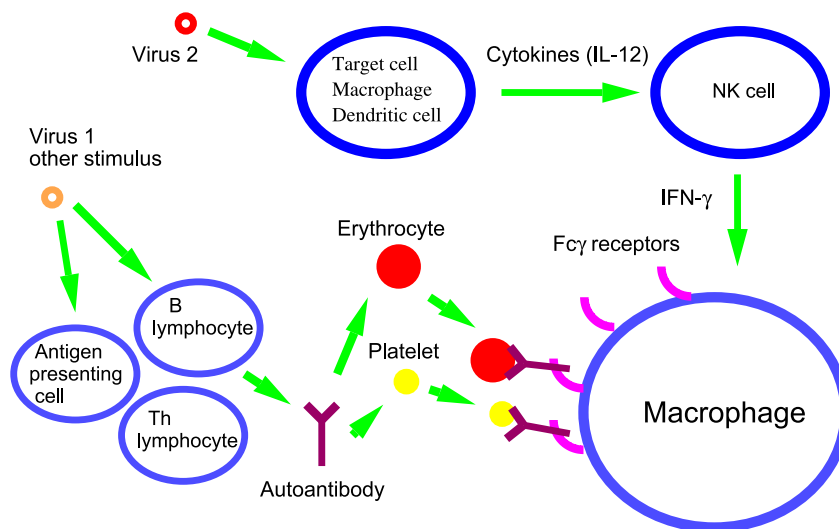

Fig. 1. Hypothesis on the involvement of viral infections in autoantibody-mediated blood autoimmune disease. Anti-platelet or anti-erythrocyte autoantibodies with moderate pathogenicity could be produced in response to a first stimulus of antigen-presenting cells, T helper lymphocytes or B lymphocytes, such as an infection with a first pathogen that may share epitopes with self antigens or that may polyclonally activate lymphocytes. The disease could then be induced when a cascade of cellular and molecular mechanisms, involving cytokine secretion by target cells, macrophages or dendritic cells in response to infection by a second virus, followed by IFN-γ secretion by cells like NK cells, increases the phagocytic activity of macrophages, resulting in an enhanced clearance of autoantibody-coated platelets or RBC.

produced in the course of LDV infection [37], it may be postulated that a cascade of cell activation and cytokine production leads to IFN-γ secretion. Natural killer cells have been identified as a major source for the secretion of this cytokine after infection with this virus [38]. IFN-γ is involved in the exacerbation of autoantibody-mediated hemolytic anemia or thrombocytopenia induced by LDV, as shown by disease prevention in mice deficient for its receptor (G129 mice; [30]; Table 1).

5. Conclusions

From these observations, it may be hypothesized that the development of blood antibody-mediated autoimmune disease can sometimes require two successive events (Fig. 1): a production of autoantibodies with moderate pathogenicity, which remains clinically silent, and that may be induced through B lymphocyte polyclonal activation, antigenic mimicry or other mechanisms by a stimulus like a first infectious agent; and then exacerbation of the pathogenicity of these autoantibodies, leading to disease, by a cascade of events triggered by a second infection with one of many different viruses, that involves secretion of pro-inflammatory cytokines by infected target cells, macrophages or dendritic cells, followed by IFN-γ production by either natural killer cells or T lymphocytes, and finally enhancement of the phagocytic activity of macrophages.

Acknowledgements

Take-home messages

- viral infections increase the pathogenicity of anti-erythrocyte and anti-platelet autoantibodies;
- virally-induced increased autoantibody pathogenicity is mediated through enhanced phagocytic activity of macrophages;
- virally-induced increased phagocytic activity depends on gamma-interferon production.

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Tolerance to pre-pro-insulin in type 1 diabetes

Jaeckel et al. (Nat Immunol 2004;5:1028) report that tolerance to transgenically overexpressed pre-pro-insulin 2 substantially reduced the onset and severity of type 1 diabetes in nonobese diabetic mice. However, some mice still developed type 1 diabetes, supporting the assumption that insulin is a key, but not absolutely essential, autoantigen. The authors suggest that these results are consistent with the idea that the human IDDM2 locus controls susceptibility to type 1 diabetes by regulating intra-thymic pre-pro-insulin expression.