

Varied Immune Response to FVIII: Presence of Proteolytic Antibodies Directed to Factor VIII in Different Human Pathologies

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Abstract The versatility of antibodies is demonstrated by the various functions that they mediate such as neutralization, agglutination, fixation of the complement and its activation, and activation of effector cells. In addition to this plethora of functions, antibodies are capable of expressing enzymatic activity. Antibodies with catalytic

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function are a result of the productive interplay between the highly evolved machinery of the immune system and the chemical framework used to induce them (antigens). Catalytic antibodies are immunoglobulins with an ability to catalyze the reactions involving the antigen for which they are specific. Catalytic immunoglobulins of the IgM and IgG isotypes have been detected in the serum of healthy donors. In addition, catalytic immunoglobulins of the IgA isotype have been detected in the milk of healthy mothers. Conversely, antigen-specific hydrolytic antibodies have been reported in a number of inflammatory, autoimmune, and neoplastic disorders. The pathophysiological occurrence and relevance of catalytic antibodies remains a debated issue. Through the description of the hydrolysis of coagulation factor VIII as model target antigen, we propose that catalytic antibodies directed to the coagulation factor VIII may play a beneficial or a deleterious role depending on the immuno-inflammatory condition under which they occur.

Keywords Factor VIII · Anti-FVIII antibodies ·
Catalytic antibody

Introduction

Antibodies to factor VIII (FVIII) have first been described in patients with congenital hemophilia A as a consequence of the therapeutic treatment. These antibodies inhibit the procoagulant activity of therapeutic FVIII. Anti-FVIII antibodies have also been documented to be present in non-hemophilic individuals and in healthy donors (with no clinical implications). The development of anti-FVIII antibodies has enormous implications in the day-to-day

management of the patients, in the clinical outcome and lays a heavy socioeconomic burden; it, thus, represents the major pitfall in the treatment of the patients. Research in the last 30 years has concentrated on understanding the nature and mechanisms of action of FVIII inhibitors. A more recently described mechanism is the inactivation of FVIII by IgG through active hydrolysis of the FVIII molecule. Antibodies expressing such an activity are termed in general as *antibody-enzymes* (abzymes) or catalytic antibodies. The present review is a concise summary of the knowledge accumulated on the occurrence of anti-FVIII antibodies, and especially the subtype that hydrolyses the coagulation FVIII, and presents the current understanding on their mechanisms at play that may hallmark their role in the pathophysiology.

The occurrence of anti-FVIII antibodies

Anti-FVIII alloantibodies in patients with hemophilia A

Antibodies directed against FVIII arise in the form of alloantibodies in approximately 20% to 24% of the patients with hemophilia A [1, 2] following repeated therapeutic infusions of exogenous FVIII. Anti-FVIII antibodies inhibit the procoagulant activity of FVIII and are also known as FVIII inhibitors. The frequency of the presence of such inhibitors is up to 30% in patients with severe hemophilia A [3]. The alloimmune response exposes the patients to a higher risk of mortality [4]. A number of factors have been identified in the recent years that may be responsible for the occurrence of alloantibody inhibitor and have been discussed in detail by Ghosh et al. in this issue. The studies of the neutralization kinetics by the anti-FVIII antibodies to FVIII has allowed the identification of two types of antibodies: the antibodies that follow type I kinetics of inhibition of FVIII are capable of completely inhibiting the activity of exogenous FVIII, and the antibodies that follow type II kinetics of inhibition of FVIII never completely inhibit the procoagulant activity of FVIII [5, 6].

Anti-FVIII autoantibodies in patients with acquired hemophilia

The spontaneous development of autoantibodies directed against FVIII in non-hemophilia patients is an extremely rare event [7, 8]. FVIII inhibitors develop in about one per million individuals each year [9, 10]. These autoantibodies, arising predominantly in older individuals, occur in association with autoimmune disorders, lymphoproliferative disorders, solid tumors, and the postpartum state. Almost half of the patients develop autoantibodies spontaneously without an underlying medical condition. Anti-FVIII autoantibodies are

detected by their ability to inhibit FVIII procoagulant activity in plasma, and are referred to as FVIII inhibitors. The anti-FVIII autoantibodies are largely of the IgG4 subclass, with a predominant presence of κ light chains [11, 12]. However, monoclonal IgM and IgA autoantibodies to FVIII have also been described in patients who suffer from acquired hemophilia and have underlying benign or malignant lymphoproliferative disorders and paraproteinemias [13, 14]. The most common complaints are bleeding into the skin or muscles, hematuria, hematemesis or melena and prolonged postpartum or postoperative bleeding [15, 16]. The inactivation of FVIII resulting from this interaction is, however, very different between anti-FVIII autoantibodies and alloantibodies. As described in the previous section, in fact, whereas alloantibodies usually follow type I kinetics of FVIII inhibition, autoantibodies often inactivate FVIII activity incompletely following type II kinetics, and some residual FVIII can be assayed in the patient's plasma [17, 18]. Thus, the Bethesda assay, which quantifies the in vitro inhibitor titer, may underestimate the in vivo inhibitor potency due to the complex, nonlinear reaction kinetics and complicate therapeutic choices and monitoring.

Anti-FVIII autoantibodies in healthy donors

Autoantibodies to FVIII also occur naturally and can be found in the plasma of healthy individuals. The existence of natural anti-FVIII autoantibodies was first described by the work of Algiman et al. [19]. The work demonstrated that 17% of the decompartmented plasma samples tested contained detectable FVIII-neutralizing activity that varied between 0.4 and 2 Bethesda units. A Bethesda unit is the measure of the dilution of plasma that results in 50% inhibition of the activity of FVIII. The prevalence of such activity was equal between men and women. Interestingly, no correlation was found between the titer of FVIII inhibitors, measured by a functional assay, and the amount of anti-FVIII IgG determined by ELISA. The lack of correlation could be due to the presence of anti-FVIII antibodies recognizing epitopes located outside the functional epitopes on the FVIII molecule as demonstrated in patients with hemophilia A [20] and/or to the presence of anti-FVIII antibodies of non-IgG isotype, as found in patients with acquired hemophilia associated with lymphoproliferative disorders [14]. Under physiological conditions, no inhibition of endogenous FVIII by natural anti-FVIII autoantibodies has been measured in plasma. This is due to the presence in normal plasma of natural antiidiotypic antibodies that neutralize potentially inhibitory natural anti-FVIII IgG [21, 22]. Interestingly, antiidiotypic antibodies from healthy donors have been shown to be able to neutralize the inhibitory activity of anti-FVIII antibodies found in patients with acquired hemophilia and congenital

hemophilia A [23, 24]. Taken together, the results indicate that anti-FVIII antibodies with inhibitor properties are not only found in alloimmunized patients with hemophilia A and patients with acquired hemophilia but also within the IgG of healthy individuals.

Mechanisms of inactivation of FVIII

Under physiological conditions, spontaneous dissociation of the A2 domain from the activated heterodimer and by proteolytic cleavage by activated protein C or activated factor X results in the inactivation of FVIII. In contrast, prior to the physiological inactivation, in patients with hemophilia, most FVIII inhibitors described to date inhibit the procoagulant activity of FVIII by steric hindrance, thereby preventing the functional association of FVIII with its interacting partners in the coagulation cascade. Such inhibitors of FVIII then are specific only to the functional epitopes of FVIII. In addition, several anti-FVIII antibodies that arise during the alloimmune response of the patient's immune system against therapeutically administered FVIII recognize domains of FVIII, which are not relevant to its activity [25]. Such anti-FVIII antibodies, which are not detected in inhibitor assays, may be speculated to participate in the reduced half-life of therapeutic FVIII upon infusion in some patients with undetectable levels of functional inhibitors [20]. In the recent years, we have demonstrated a new mechanism of inactivation of the FVIII molecule, i.e., catalytic IgG-mediated hydrolysis of the FVIII molecule.

Physiopathological occurrence of catalytic antibodies

Catalytic antibodies under physiological conditions

Catalytic antibodies are generated spontaneously by the immune system, in the absence of deliberate immunization. Thus, catalytic IgG and IgM have been documented in the serum of healthy individuals [26–28], and antibodies with protein kinase and deoxyribonuclease activities have been detected in the milk of healthy mothers [29, 30]. Recently, Wentworth et al. have demonstrated that all antibodies are potentially endowed with bactericidal activity. However, such an activity is also shared with other non-immunoglobulin molecules such as chicken ovalbumin and β -galactosidase [31]. Nonetheless, these observations suggest that “naturally occurring” catalytic antibodies may participate directly in the elimination of the biochemical wastes that are released by the metabolism of the organism [32] and point towards an intrinsic protective role of antibodies under physiological conditions; a role that is independent on the capacity of antibodies to neutralize circulating exogenous

antigens to facilitate their endocytosis by antigen presenting cells and participate in their elimination from the organism.

Catalytic antibodies under pathological conditions

The first example of catalytic antibodies under pathological condition was reported in bronchial asthma patients and the antibodies were able to cleave the vasoactive intestinal peptide. Since then, DNA- and RNA-hydrolyzing antibodies have been isolated from the serum of patients with different systemic autoimmune manifestations: systemic lupus erythematosus, scleroderma, rheumatoid arthritis or multiple sclerosis [33–35]. Proteolytic antibodies specific for thyroglobulin [36] have been reported in patients with thyroiditis. Levels of IgG with amidase activity were found to be higher in patients with sepsis [37]. Catalytic antibodies to coagulation factors such as factor VIII (FVIII) in patients with hemophilia A [38, 39] and acquired hemophilia [40] and to FVIII and factor IX in patients with renal graft transplant [41] were documented. Prothrombin appears as another procoagulant factor that is targeted by catalytic antibodies [42].

The role of catalytic antibodies found in pathology remains elusive. Examples where catalytic antibodies have been suggested or demonstrated to be detrimental are as follows. (1) Antiplatelet membrane GPIIb/IIIa IgG antibodies have been documented in “immunologic thrombocytopenia” that is associated with HIV-1 infection. Interestingly, the anti-GPIIb/IIIa IgG had a deleterious effect and caused platelet fragmentation in vitro in the absence of complement, and in vivo in both wild-type and C3-deficient mice [43]. (2) A fraction of antibodies purified from the sera of humans with multiple sclerosis and from the sera of mice with induced experimental allergic encephalomyelitis is capable of site-specific degradation of myelin basic protein (MBP). The presence of epitope-specific antibody-mediated degradation of MBP suggests a mechanistic explanation for the slow development of neurodegeneration that is associated with multiple sclerosis [44–46]. (3) Two monoclonal antibody light chains isolated from patients with multiple myeloma were capable of hydrolyzing IEGR-methylcoumarinamide, a peptide substrate corresponding to the residues 268–271 of prothrombin [42] (Table 1). The clinical significance of the prothrombin activating light chains is unknown as the clinical data concerning the patients was not available. It is nevertheless intricate to determine whether the occurrence of catalytic antibodies is a manifestation of the underlying pathological condition or is responsible for the identified clinical parameters of the pathology. We propose to discuss this aspect using FVIII as a model target antigen and antibodies directed against the coagulation FVIII (anti-

Table 1 Catalytic antibodies in pathology

Pathology	Targeted antigen
Inflammatory disorders	
Asthma	Vasoactive intestinal peptide
Sepsis	FVIII, FIX
Autoimmune disorders	
Hashimoto's Thyroiditis	Thyroglobulin
Lupus erythematosus	DNA, RNA
Scleroderma	DNA, RNA
Rheumatoid arthritis	DNA, RNA
Multiple sclerosis	Myelin basic protein
Acquired hemophilia	FVIII
Infectious disorder	
Immune thrombocytopenia associated with HIV-1 infection	Platelet GPIIIa
Neoplastic disorders	
Multiple myeloma	Prothrombin
Alloimmune disorders	
Hemophilia A	FVIII
Renal rejection in transplantation	FVIII, FIX

The presence of catalytic antibodies has been reported under many human pathological conditions. The diseases can be separated under five broad categories, i.e., inflammatory, autoimmune, infectious, neoplastic and alloimmune disorders. The different known target antigens in each disease are listed

FVIII antibodies), more particularly, a small subset that has been characterized to be endowed with enzymatic activity.

Pathological versus physiological relevance of FVIII-hydrolyzing antibodies

The ambivalent role of catalytic antibodies is highlighted in the recent years through the documented occurrence of catalytic antibodies of the IgG isotype under four different pathological conditions, all four of which are linked by the central role of the procoagulation FVIII: congenital hemophilia A and acquired hemophilia, which results from insufficient blood coagulation, and sepsis and renal graft transplantation, which share in common a defective control of the coagulation cascade. The presence of FVIII-hydrolyzing IgG may be associated with a deleterious, beneficial, or a neutral role depending on the immuno-inflammatory context in which they are documented.

Catalytic IgG towards FVIII with a deleterious role

We reported on the proteolysis of FVIII by alloantibodies in the plasma of two high responder patients with severe hemophilia A, demonstrating a new mechanism by which FVIII inhibitors may prevent the procoagulant function of FVIII. Hemophilia was the first model where the generation of catalytic antibodies was documented following the

exogenous administration of a ground state antigen. In brief, anti-FVIII antibodies were purified by affinity chromatography on protein G and size-exclusion chromatography in denaturing conditions. Incubation of the iodine-labeled FVIII with purified IgG resulted in the hydrolysis of FVIII, as demonstrated by Western blotting. Interestingly, IgG from the two patients did not cleave human serum albumin or factor IX, thus, providing indirect evidence that the hydrolytic activity of these antibodies is solely directed towards FVIII. In a later study, significant FVIII-hydrolytic activity was detected in the IgG of 13 of 24 patients with severe hemophilia A [38]. Anti-FVIII antibodies in patients' plasma are a composite mixture of catalytic and non-catalytic antibodies. The FVIII-neutralizing activity of FVIII inhibitors in vivo may result from two simultaneous mechanisms: steric hindrance and/or proteolysis of FVIII. A significant and positive correlation was seen between the extent of hydrolysis of FVIII by the purified IgG and the degree of FVIII inhibitory activity measured in the plasma of patients. Thus, the findings suggested that catalytic IgG play a role in the inhibition of FVIII activity by hydrolyzing FVIII in some patients.

The pathological role of FVIII-hydrolyzing IgG was demonstrated with the use of an in vitro modified Bethesda assay. The assay was modified to minimize the inhibitory activity of "classical" FVIII inhibitors, i.e., those that inhibit FVIII by steric hindrance. Accordingly, purified IgG was used at concentrations yielding more than 75% residual FVIII activity after 2 h of incubation with FVIII. FVIII was incubated in the presence of IgG for 16 h instead of only 2 h. In such conditions, the residual FVIII activity decreased in a time-dependent manner when FVIII was incubated with IgG from two patients with FVIII-hydrolyzing IgG, while it remained stable upon incubation of FVIII in the presence of a monoclonal non-catalytic FVIII inhibitor. The half-life of therapeutic FVIII is comprised between 14 to 18 h in patients without inhibitors. The observed correlation between the rate of FVIII hydrolysis and the inhibitory activity measured in plasma [38], as well as the observed kinetics of IgG-mediated FVIII degradation [47] were compatible with a deleterious role of FVIII-hydrolyzing IgG in the patients.

Catalytic IgG towards FVIII with a beneficial role

Natural proteolytic antibodies that occur under physiological conditions [26, 28] have been proposed to participate in the removal of metabolic wastes and protect from bacterial infections [32]. We have described recently the high prevalence of catalytic antibodies in the case of patients with severe sepsis and septic shock [37]. Plasma was collected from 34 patients with severe sepsis or septic shock on the day of diagnosis. IgGs were purified from

plasma and the catalytic activity of IgG was evaluated by their ability to cleave peptide Pro-Phe-Arg-methylcoumarinamide tri-peptide (PFR-MCA), a generic synthetic substrate for kallikrein-like serine proteases (Fig. 1). PFR-MCA and related synthetic substrates have extensively been used in the past to screen for the presence of human catalytic antibodies since they demonstrate selectivity for cleavage at bonds containing basic amino acids. Higher levels of IgG with serine protease-like activity were associated with a favorable outcome from sepsis [37]. This was the first evidence that catalytic antibodies may be associated with the recovery from a disease, thus, strengthening the hypothesis that catalytic antibodies play a protective role against infection. Interestingly, an inverse correlation was documented between the markers of severity of disseminated intravascular coagulation, which are characteristic of sepsis, and the amidase activity of patients' IgG, thus, pointing towards a potential anti-thrombotic implication of catalytic antibodies in sepsis. Indeed, IgG from three of the surviving patients included in the study, hydrolyzed FVIII and/or factor IX.

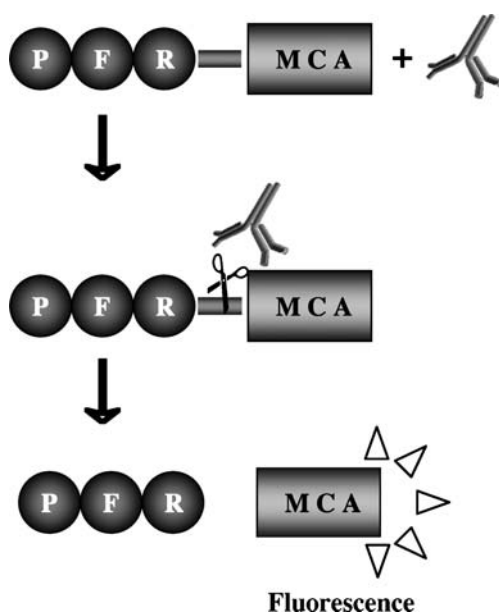


Fig. 1 Schematic representation of the hydrolysis of PFR-MCA by catalytic antibodies. IgG antibodies were mixed with proline-phenylalanine-arginine (PFR-MCA) in 50 mM Tris-HCl, 100 mM glycine, 0.025% Tween 20, and 0.02% NaN₃ (pH 7.7) in white 96-well U-bottom plates and incubated in the dark 24 h at 37°C. Hydrolysis of the PFR-MCA substrate was determined by the fluorescence of the leaving group (aminomethylcoumarin; em 460 nm, ex 365 nm) using a fluorescence spectrophotometer. Fluorescence values were compared with a standard curve of free MCA and the corresponding quantities of released MCA were computed. The background fluorescence measured in the absence of IgG was subtracted from each analysis. The significance of the increase in the hydrolytic activity of patients' IgG as compared with that of IVIg was assessed by an ANOVA and Fischer PLSD post hoc tests

We have characterized the presence of catalytic antibodies in another pathological situation, which is characterized by excessive inflammation and uncontrolled activation of the coagulation [48–51]: chronic rejection in organ transplantation. We obtained in a retrospective manner serum from patients that had undergone renal graft transplant at three different time intervals (at the time of renal transplant, 3 months and 2 years following transplantation). Some patients had developed chronic allograft nephropathy (CAN) following renal transplantation. CAN represents the main cause for the loss of graft following the first year of transplantation [52]. The purified IgG from some of the patients included in the study were able to hydrolyze the coagulation factors VIII and/or IX. PFR-MCA was used as a reliable screening substrate for hydrolytic activity as it is a more quantitative and a sensitive substrate. Interestingly, the IgG from CAN-negative patients demonstrated significantly higher amidase activity compared to that from CAN-positive patients, both 3 months and 2 years post-transplantation. In addition, the catalytic activity measured at 3 months was predictive of CAN, 2 years down the lane. Thus, catalytic IgG present in the serum of CAN-negative patients were associated with a favorable outcome from the nephropathy in renal graft transplant.

Although sepsis and CAN display obviously different pathophysiologies, the two situations share in common a defective control of the coagulation cascade. Indeed, both sepsis and CAN are characterized by an abnormal activation of the endothelium, which is systemic in sepsis [53] and restricted to the endothelium of the graft in transplantation [54]. Activation of the endothelium tilts the coagulation balance towards a hypercoagulatory state. Our studies document that the hydrolytic activity of circulating IgG of some patients with sepsis and renal transplanted patients is directed towards coagulation factors VIII and/or IX. Interestingly, these two factors play a central role in the amplification loop for the generation of thrombin. We have previously demonstrated that IgG-mediated FVIII hydrolysis results in inactivation of the procoagulant activity of FVIII [47]. It is thus tempting to speculate that hydrolytic circulating IgG exert a beneficial role by disrupting the amplification loop of the coagulation cascade therefore counterbalancing the abnormal activation of the endothelium.

Catalytic IgG towards FVIII with a neutral role

Early demonstrations of catalytic antibodies have been documented in association with autoimmune conditions. With the exception of the few diseases described above, the direct pathogenic effect of the catalytic antibodies has not been formerly demonstrated. We investigated for the

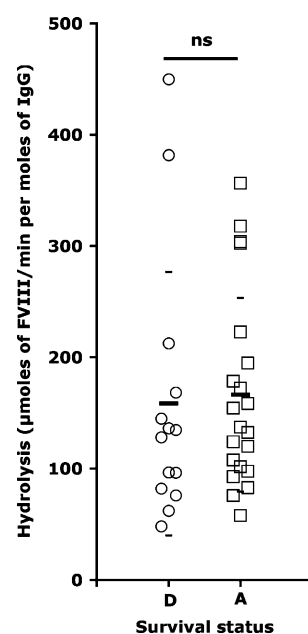
presence of FVIII-hydrolyzing IgG in an autoimmune condition: acquired hemophilia, which is characterized by the presence of autoantibodies against endogenous FVIII [55]. Interestingly, we observed a prevalence of 47% of FVIII-hydrolyzing IgG in patients with acquired hemophilia. Acquired hemophilia is characterized by a complex etiology. Among the patient population in our cohort, acquired hemophilia was idiopathic in 40% of the cases or developed in association with malignancies, autoimmune disorders, reactions to drugs, dermatological disorders, diabetes, or postpartum states. As a result, no single diagnostic parameter for acquired hemophilia has been identified as yet. Accordingly, diagnosis of acquired hemophilia still relies on low levels of circulating FVIII, on the presence of inhibitory anti-FVIII antibodies and on a prolonged activated partial thromboplastin time (aPTT, the test is used to detect abnormalities in blood clotting), together with normal levels of other factors of the intrinsic pathway and of VWF. Furthermore, no prognostic parameter of survival of acquired hemophilia was ever identified: neither the inhibitory titer, the aPTT, the FVIII coagulant activity in plasma, nor the underlying disease was ever associated with the survival or mortality of the patients 1 year following diagnosis. In our study, there was no association between the presence of FVIII-hydrolyzing IgG and the survival or death of the patients 1 year following inclusion into the cohort (Fig. 2). In addition, owing to the restricted number of patients in each of the different disease groups, the occurrence of FVIII-hydrolyzing IgG among patients with acquired hemophilia could not be attributed to a particular underlying pathology.

We did not find a significant correlation between the scored rates of hydrolysis of FVIII and the Bethesda titers

that were measured in the plasma of the patients at the time of diagnosis. These results are in contrast to what was observed in patients with hemophilia A. The discrepancy between these two sets of data may be due to differences in the relative importance of the various mechanisms of FVIII inhibition that coexist in different patients [56–61], i.e., non-proteolytic FVIII inactivation by steric hindrance versus antibody catalyst-mediated FVIII hydrolysis. Alternatively, the difference between the two studies may relate to the different immunological contexts that characterize the development of the anti-FVIII immune response. Indeed, while the development of FVIII inhibitors in patients with congenital hemophilia A is seen as an alloimmune response against an exogenous therapeutic antigen, the occurrence of anti-FVIII autoantibodies is the result of a break of tolerance towards an endogenous protein. Interestingly, a longitudinal study in the case of eight patients for the hydrolysis rates of FVIII and Bethesda titers in plasma resulted in the coevolution of the catalytic activity with the inhibitory titer following similar trends in the case of 6/8 patients.

Taken together, the elevated prevalence of FVIII-hydrolyzing IgG in patients with acquired hemophilia together with the coevolution of the catalytic activity and inhibitory activity in 75% of the cases clearly indicate that the occurrence of FVIII-hydrolyzing IgG is a result of the disease and accompanies the evolution of the pathological anti-FVIII immune response. Conversely, the lack of association between rates of IgG-mediated FVIII hydrolysis and the FVIII inhibitory activity measured in plasma, together with the lack of predictive value of death or survival, suggest that FVIII-hydrolyzing IgG are not detrimental to the patients.

Fig. 2 IgG-mediated hydrolysis of FVIII versus survival status of patients of acquired hemophilia. The survival status from acquired hemophilia was known in the case of 35 patients. Among these, 14 patients were documented as deceased (D, open circles, 158 ± 118.3 $\mu\text{mol}/\text{min}/\text{mol}$) and 21 patients were documented as deceased (A, open squares, 166.1 ± 87.1 $\mu\text{mol}/\text{min}/\text{mol}$), 1 year following diagnosis of acquired hemophilia. The significance between the IgG-mediated activation of FIX and the survival of patients was assessed using the Mann–Whitney nonparametric test. ns no significance



Conclusion

Catalytic antibodies that specifically hydrolyze a large panel of antigens including VIP, DNA, RNA, prothrombin, GPIIIa, thyroglobulin, HIV GP120, and MBP have been documented under pathological conditions. It is unclear if antibodies endowed with catalytic activity are an intrinsic part of the physiological immune system or if the catalytic function of antibodies is gained solely after proper stimulation of the immune system by an appropriate immunogen. Nevertheless, catalytic antibodies are generally attributed with a detrimental role in pathological situations. In this context, FVIII is the first example of a target antigen for proteolytic antibodies. The hydrolysis of FVIII may be neutral, deleterious, or beneficial depending on the pathological context. Firstly, the presence of FVIII-hydrolyzing IgG was documented in patients with acquired hemophilia. The coevolution of the catalytic activity towards FVIII with the inhibitory titers measured in plasma

suggested that catalytic antibodies are associated with acquired hemophilia. Because of the heterogeneous nature of the underlying conditions that are associated with acquired hemophilia, no single diagnostic parameter for acquired hemophilia has been identified as yet. Accordingly, in our study, there was no association between either one of the latter clinical parameters or the presence of FVIII-hydrolyzing IgG measured at the time of diagnosis and the survival/death of the patients 1 year following inclusion. These observations raise the question of the significance of FVIII-related clinical parameters as pertinent markers for disease outcome and warrant the investigation for other relevant markers in the coagulation cascade. Secondly, the kinetics of inactivation of FVIII by FVIII-hydrolyzing IgG described in patients with hemophilia A have been proposed to participate in the inactivation of the therapeutically administered FVIII. This finding demands the understanding of the fine molecular mechanisms by which FVIII-hydrolyzing IgG may function so as to target them with specific neutralizing agents of beneficial significance. Thirdly, the presence of IgG with amidase activity, and, possibly, of FVIII-hydrolyzing IgG in patients with severe sepsis and renal graft transplant correlates with a favorable outcome either for the patients as in the case of sepsis, or for the survival of the graft in the case of patients with renal graft transplantation. This data suggests that the passive administration of catalytic IgG with hydrolytic properties towards FVIII may be of therapeutic value. Care should however be taken to avoid hemorrhagic complications.

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