Chapter 11
Regulation of Instant Blood Mediated Inflammatory Reaction (IBMIR) in Pancreatic Islet Xeno-Transplantation: Points for Therapeutic Interventions

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Abstract Xeno-transplantation of pancreatic islets represents a promising therapeutic alternative for the treatment of type 1 diabetes mellitus. However, potent innate immune responses induced shortly after the transplantation of donor islets to the recipient, comprising the Instant Blood Mediated Immune Reaction (IBMIR), exert detrimental actions on islet graft function. The coagulation and complement cascades together with the leukocyte and platelet populations are the major players in IBMIR. This innate immune attack affects dramatically islet integrity and leads to significant loss of function of the xenograft. In the present review, we focus on the mechanisms contributing to IBMIR components and address therapeutic intervention approaches to limit IBMIR by administering inhibitors in circulation, by coating the islet surface with inhibitors or by generating transgenic donor animals; these approaches could result in improved xenograft survival.

Keywords Instant blood mediated immune reaction (IBMIR) • Coagulation • Complement • Islet xenotransplantation • Compstatin

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11.1 Introduction

Cell or organ transplantation (Tx) is a promising therapeutic approach for the treatment of patients suffering from end-stage organ deficiency [1, 2]. Type 1 diabetes mellitus (T1DM), which is a disease resulting from an autoimmune reaction, is linked to high morbidity and mortality rates, especially because of its micro- and macrovascular complications. Patients suffering from T1DM can only be treated with exogenous administration of insulin. Allotransplantation of isolated islets of Langerhans or whole pancreas transplantation has been established as a therapeutic option, however, the shortage of appropriate donor islets is a major limiting factor [3, 4].

Transplantation between different species, termed as xenotransplantation (xeno-Tx), may provide a promise to bypass the issue of shortage of human donor organs [5]. Until now, a variety of xenogeneic applications have been described including the transplantation of heart, kidneys, lungs or liver [6]. In the same context, xenogeneic islet transplantation to T1D patients may represent an alternative therapeutic approach to treat T1DM [7, 8], especially due to the unlimited availability of xenografts.

Pigs are currently considered the preferred xenograft donor species for several reasons. This species share physiological similarities with humans, while their low reproduction time together with the high number of progeny are further obvious advantages [9]. Moreover, the major advances in genetics in recent years have yielded the generation of transgenic pigs feasible; these tools are engaged to generate xenogeneic grafts with optimal function and protection from the host immune attack [10–12].

Despite the organ similarities between human and pigs, inter-species incompatibilities give rise to immune and thrombotic reactions that result in the xenograft rejection [13, 14]. Besides rejection reactions that are based on adaptive immunity [15, 16], in the context of islet xeno-Tx, a major potential compromise in graft function may derive from a group of innate immune responses that are termed Instant Blood Mediated Inflammatory Reaction (IBMIR). IBMIR is triggered by the xenogeneic contact between blood and islets and includes a complex interplay between activation of coagulation and the complement system, as well as leukocyte and platelet activation and accumulation (Fig. 11.1), thereby dramatically influencing the function and the survival of the xenograft, thus affecting adversely the outcome of islet xeno-Tx [17, 18]. The present review will focus on the mechanisms and interactions that regulate the pathophysiology of IBMIR, with a special emphasis on innate immunity and will address treatment strategies and points of therapeutic intervention that could ameliorate the adverse responses following islet xeno-Tx.

11.2 The Complement System

Complement system, a major component of immunity, consists of a complex network of soluble and membrane-bound proteins that cooperate in the recognition and elimination of microbial pathogens as well as foreign materials [19]. In recent years, the classical view of the complement system has been extended to include a variety

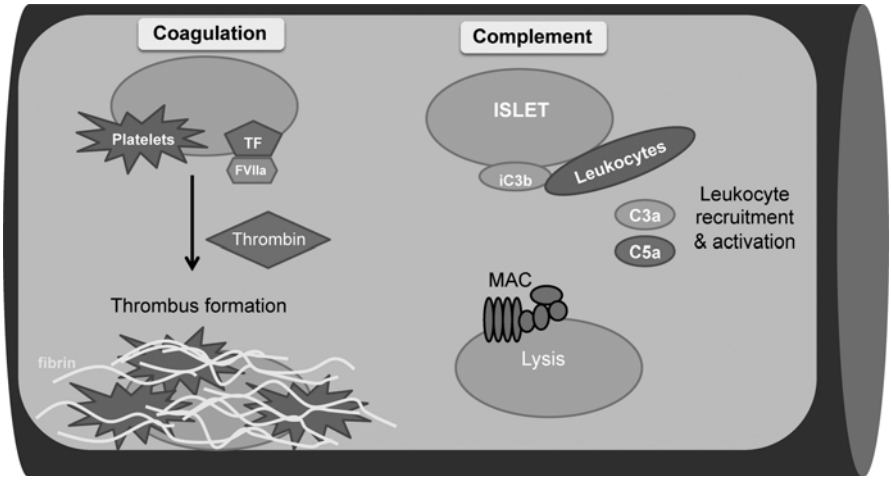

Fig. 11.1 Simplified overview of the key steps occurring during IBMIR in islet xeno-Tx. The xenogeneic contact between blood and islets triggers the activation of the extrinsic pathway of coagulation that is mediated through tissue factor (TF). As a result, the downstream effector thrombin is generated thus leading to fibrin deposition and the entrapment of islets into thrombi. Attachment of platelets to islets further supports the procoagulant effects. Activated fragments of complement (iC3b) are deposited on the islet surface, C3a and C5a anaphylatoxins activate and attract leukocytes and formation of MAC mediates the lysis of islets (*FVIIa*: activated coagulation factor VII, *MAC*: membrane attack complex)

of immune and non-immunological responses, including processes linked with tissue degeneration and regeneration, such as age-related macular degeneration (AMD) [20], liver regeneration [21, 22], and wound healing [23]. Further physiologic and pathophysiologic processes regulated by the complement system include angiogenesis [24], the mobilization of stem cells [25–27], lipid metabolism and inflammation in metabolic organs leading to insulin resistance and diabetes [28–30].

Impaired or excessive complement activation has been associated with the adverse effects observed after biomaterial applications in clinical practice [31, 32], in neurological disorders [33] and several autoimmune diseases [34, 35].

Activation of the complement system occurs via three different loops (termed the classical, lectin and alternative pathways) depending on the nature of the initial trigger. Irrespective of the activation loop, all pathways converge to the cleavage of C3 by C3 convertases. C3 convertases cleave the central component C3 to the anaphylatoxin C3a and C3b [19], the primary function of which, as well as of its split product iC3b is opsonophagocytosis. Moreover, iC3b can bind to the complement receptors CR3 and CR4 and lead to immune cell adhesion and cell activation [36, 37]. In addition, since activation of complement is associated with proteolytic cleavage of its components, proteases represent a further "non-traditional" pathway of complement activation [38, 39].

The classical pathway (CP) is triggered by antigen-antibody complexes, which are recognized by C1q. A major process in this pathway is the generation of CP C3 convertase C4b2b, which results from the cleavage of C4 to C4a and C4b and in turn the splitting of C2 to C2a and C2b [40]. The activation of the lectin pathway (LP), which is initiated by the binding of mannose-binding lectin (MBL) or ficolins to pathogen surfaces and subsequent involvement of MBL-associated serine proteases MASP-1 and MASP-2, shares significant similarities with that of the CP [41]. Spontaneous C3 hydrolysis to C3(H_2O) accounts for the constitutive and continuous low level of activation of the alternative pathway (AP) [42]. The generated C3b assembles together with factor B and factor D the AP C3 convertase C3bBb [43]. The AP C3 convertase complex is stabilized by the binding of properdin [44–46].

The generation of C3b by C3 cleavage in all three pathways is a major component of C5 convertases that cleave C5 to C5a anaphylatoxin and C5b [47]. C5b participates in the formation of membrane attack complex (MAC) by recruiting the complement components C6, C7, C8 and C9 with a main function to mediate the lysis of pathogens or targeted cells [48].

On the other hand, the C3a and C5a anaphylatoxins are very potent chemoattractants, via interaction with their G-protein-coupled C3a- and C5a-receptors, respectively, and thereby contribute to inflammatory cell recruitment to the site of injury or infection. In addition, C3a and C5a can activate immune cells and upregulate expression and release of inflammatory cytokines and mediators [19, 49].

Several soluble and membrane-bound complement regulators ensure that excessive complement activation is prevented [50]. The membrane-bound regulators of complement activity include the decay accelerating factor (DAF or CD55), the membrane cofactor protein (MCP or CD46), the complement receptor type 1 (CR1 or CD35), and the CD59 (or protectin) [50]. CD55 inhibits C3 and C5 convertases [51], whereas CD46 acts as a cofactor with factor I to promote the cleavage of C3b to iC3b [52]. The complement regulatory role of CD59 is mediated by the blockade of the polymerization of C9, thus interfering with the MAC formation [53]. Membrane complement regulators have been chosen as therapeutic strategies to block the function of complement in the context of IBMIR. For that reason, transgenic pigs that overexpress human CD55 (hCD55), human CD46 (hCD46) or human CD59 (hCD59) have been generated. Islet xenografts from these animals were protected from complement-dependent lysis and displayed better engraftment, as will be outlined in detail under the paragraph "Therapeutic targeting of IBMIR" [54–59]. Soluble regulators of complement activity include factor H that affects negatively the AP, the C1 inhibitor (C1INH) that inhibits serine proteases involved in the activation of CP [60] and the C4 binding protein that targets effectively both CP and LP [50, 61].

11.3 The Coagulation Cascade

The coagulation cascade participates in both hemostasis and thrombosis [62]. Tissue factor of the so-called extrinsic cascade is the central player for coagulation [63] and participates in thrombotic pathologies, including cardiovascular disease

[64, 65], and biomaterial-associated processes [32]. An inflammatory stimulus or endothelial cell activation results in generation of the extrinsic Xase complex consisting of TF and activated factor VII (FVIIa) [66]. The Xase-complex in turn promotes the activation of factor X (FX), which together with activated FVa and Ca^{2+} forms the prothrombinase complex that mediates the conversion of prothrombin to thrombin [67]. Thrombin can activate platelets and cleave fibrinogen to fibrin, thereby resulting in the formation of insoluble fibrin clot [68].

Coagulation and thrombosis participate in acute reactions to islet allo- [69] or xeno-Tx [70]. Notably, the exposure of islets of human or porcine origin to human blood results in the rapid activation of coagulation, as evidenced by up-regulation of TF levels [71] and by generation of high amounts of thrombin [72]. Moreover, islet Tx has been associated with thrombotic manifestations, such as fibrin deposition, and localization of the transplanted islets within thrombi [70]. Endogenous antithrombotic agents are therefore of major importance as potential beneficial modulators of IBMIR. The fine tuning of the coagulation cascade [73] is mediated by antithrombin III (ATIII), which inactivates thrombin, FXa and FIXa [74], the activated protein C (APC), which together with Protein S blocks FVa and FVIIIa [75], the tissue factor pathway inhibitor (TFPI) as well as thrombomodulin (TM). TFPI binds to and inhibits either FXa or the TF/FVIIa complex [76]. The anticoagulant activity of TM is mediated by its binding to thrombin. The TM-thrombin complex further promotes the generation of APC [77]. However, thrombin bound to TM can cleave and activate thrombin-activatable fibrinolysis inhibitor (TAFI) [78] that exerts procoagulant properties by blocking fibrinolysis. In the context of islet xeno-Tx, genetically modified pigs that overexpress hemostasis-regulatory molecules have been generated. To this end, expression of hTFPI [79] protected xenografts and promoted the achievement of normoglycemia after xeno-Tx. Porcine TM has shown to be a poor co-factor for human thrombin and its protective function is therefore lost, which leads to increased coagulation [80]. For that reason, transgenic overexpression of hTM in pigs could avert the thrombotic manifestations observed after islet xeno-Tx [81].

11.3.1 Interactions Between Coagulation and Complement

Several connections between complement and the coagulation systems including their mutual regulation have been suggested [38, 39]. On the one hand, coagulation proteases can cleave complement components, thus providing an additional extrinsic way of complement activation. The coagulation factors FIXa, FXa, FXIa as well as thrombin cleave C3 and C5 and as a result C3a and C5a are generated [38]. TM is capable of negatively regulating the activation of complement system [82]. On the other hand, MASP-2 promotes the activation of coagulation by cleaving prothrombin to thrombin [83], while the complement regulator C1INH can inhibit coagulation factors XIa and XIIa [84]. Of interest, C5a either generated as a result of biomaterial-induced complement activation [32] or in antiphospholipid syndrome [85], induces the up-regulation of TF expression. C5a may also promote

coagulation indirectly by up-regulating plasminogen activator inhibitor-1, thus inhibiting fibrinolysis [86]. Therefore, complement and coagulation should be considered as two closely interacting and mutually regulated systems.

11.4 Leukocyte-Endothelial Interactions

Upon tissue inflammation, infection or injury, the interaction of leukocytes with the activated endothelium ensures a proper host response and provides the platform for the recruitment of immune cells to the site of injury or inflammation [87]. The leukocyte adhesion cascade includes multiple steps, such as rolling, adhesion, crawling and the subsequent leukocyte transmigration [88]. Initially, the rolling of leukocytes is mediated by interaction between the endothelial E- and P-selectins and their ligands CD44 and P-selectin glycoprotein ligand-1 (PSGL-1) [89]. The leukocyte adhesion and crawling to the endothelium takes places via the interaction between adhesion molecules present on the endothelial surface and leukocyte integrins. To this end, the β2 integrins Mac-1 (αMβ2) and LFA-1 (αLβ2) bind to intercellular adhesion molecule-1 and 2 (ICAM-1, 2) [87, 90]. Mac-1 has also specificity for binding to the receptor for advanced glycation end products (RAGE) [91]. Moreover, the β1 integrin VLA-4 (α4β1) binds to the adhesion molecule VCAM-1 [92]. Following this step, the adherent leukocytes transmigrate through the endothelium and accumulate within the inflamed tissues [93, 94].

Leukocyte adhesion and infiltration to the transplanted tissues has been associated with xenograft dysfunction and subsequent rejection [15]. Importantly, many of the interactions between leukocyte integrins and adhesion molecules remain operative in pig to human xeno-Tx settings [16]. Several studies have addressed the capacity of human leukocytes to roll and adhere to porcine endothelial cells (pECs), thus demonstrating the functionality of the selectin- and integrin-dependent interactions between the two species [13, 95, 96]. More specifically, the adhesion of human lymphocytes to pECs was shown to be dependent on LFA-1 and VLA-4 [97] and the adhesion of human monocytes to pECs was prevented with combined inhibition of E-selectin, LFA-1 and VLA-4 [98]. In addition, the inhibition of VLA-4, LFA-1 and Mac-1 resulted in decreased adhesion of human NK cells to pECs [99]. Besides the adhesion step, the human leukocyte transmigration across the porcine endothelium has also been studied [100]. Inhibition of β2 integrins, CD99 [96] or VCAM-1 [100] led to the reduction of the xenogeneic leukocyte transmigration. Of note, the activation of complement system has been associated with the upregulation of selectins [101] and adhesion molecules and the blockade of this system was associated with a dramatic decrease of leukocyte adhesion to pECs in a xenogeneic whole blood model [102].

In the context of islet xeno-Tx, leukocyte-endothelial interactions are however less relevant. In the native pancreas, the islets are highly vascularized and upon enzymatic isolation, islets are disconnected from the donor vasculature. It should be noted that in cultured islets the endothelial cells regress or lose their vascular markers [103]. Furthermore, the detection of endothelial cells is decreased after in vitro

culture of porcine islets [104]. The low levels of remaining islet EC may cause a rather low direct involvement of interactions between recipient leukocytes and EC in porcine islets transplanted to the portal vein [105]. Thus, in contrast to other xeno-Tx settings, leukocyte-endothelial interactions may be less operative in the context of islet xeno-Tx.

The smooth function of the leukocyte adhesion cascade is controlled by endogenous negative regulators [106, 107]. These molecules include Del-1, pentraxin-3, growth differentiation factor-15, galectin-1 and annexin 1 that block at several points the cascade [87]. The integrin inhibitor Del-1 (or epidermal growth factor (EGF)-like repeats and discoidin-I-like domains 3; EDIL3) is an endothelial-derived glycoprotein [90]. Del-1 blocks the interaction between the leukocyte integrin LFA-1 and ICAM-1. As a consequence, the absence of Del-1 can result in increased leukocyte recruitment [108, 109]. In addition, Del-1 inhibits the binding of the complement fragment iC3b to Mac-1 integrin [110], thus further enhancing its anti-inflammatory properties. The inhibitory role of Del-1 on leukocyte recruitment has been suggested by the enhanced severity of chronic inflammatory diseases in Del-1-deficient mice [111, 112]. The relevance of endogenous inhibitors of the leukocyte adhesion cascade in the context of xeno-Tx merits further examination.

11.5 Modulation of IBMIR

IBMIR takes place shortly after transplantation of isolated islets into the portal vein of diabetic recipients [18], or after xeno-transplantation of islets (e.g. from pig) to a different species (e.g. non-human primates). The coagulation cascade, the complement system and innate immune cells together with platelets turn out to be main drivers of the IBMIR (Fig. 11.1) [18].

The contact of host blood with the transplanted islets elicits rapidly a series of thrombo-inflammatory reactions, including upregulation of TF expression [71] and thrombin generation [72]. Moreover, the induction of TAFI further propagates procoagulant effects [113]. Intravascular clotting is induced [56] and thrombi, that entrap the islets, are formed [70]. In parallel, activation of complement CP and AP occurs, anaphylatoxins are generated, resulting in inflammatory cell recruitment to the graft. Moreover, active complement fragments are deposited on grafts, thus promoting the complement-dependent lysis of islets [114]. In addition, platelets and leukocytes infiltrate the site of transplantation and bind to the surface of the islets [72, 115]. As a consequence, the integrity of islet grafts is disrupted leading to an early massive loss of transplanted islets [116, 117]. The acute destruction of a significant proportion of transplanted islets by IBMIR is the major reason that the number of islets required for effective Tx is very high [118]. Interestingly, the extent of islet damage increases with decreased compatibility between the donor and recipient species. Thus, in the context of xeno-Tx, IBMIR becomes more relevant, as the recipient cannot control the IBMIR induced by xeno-Tx due to the incompatibility observed between the regulators and the effector molecules that are present

on the xenograft and on the cells of the recipient, respectively [8]. Moreover, regulatory proteins are rather absent from porcine islets preparations [119]. It is therefore imperative to develop efficient therapeutic options targeting the parameters orchestrating IBMIR [18].

11.5.1 Therapeutic Targeting of IBMIR

In an effort to protect islet xenografts from the harmful effects of IBMIR, several approaches have been followed, such as strategies to inhibit coagulation, complement, leukocyte recruitment or combinations thereof. Such strategies involve the application of soluble inhibitors, the immobilization of inhibitory molecules on the surface of the graft or the generation of donor animals that lack antigenic molecules or overexpress regulatory elements.

In a xenogeneic in vitro whole blood system that simulates IBMIR, administration of a recombinant form of APC, either alone or in combination with the platelet activation inhibitor tirofiban, protected islet viability via reduced coagulation and IBMIR [120]. In islet-Tx in vivo, APC decreased the degree of IBMIR, as assessed by reduced inflammation and coagulation markers and thereby promoted graft viability and function [121].

The glycosaminoglycan LMW-DS (low-molecular weight dextran sulfate) has been reported to inhibit effectively both complement and coagulation cascades [122]. Thereby, LMW-DS attenuates significantly the activation of complement [123] and coagulation cascades, thus affecting the degree and outcome of IBMIR in both in vitro and in vivo models [115, 124, 125]. To further ensure the potent inhibition of IBMIR following islet xeno-Tx, LMW-DS was used in combination with inhibitors of complement, such as compstatin [126, 127].

Generation of thrombin has not only a major impact on thrombotic effects, but can also regulate complement activation [128]. The thrombin inhibitor melagatran blocked the activation of plasmatic coagulation and complement and decreased the activation of leukocytes after the exposure of islets to whole blood, thus suggesting a beneficial role for thrombin inhibition in IBMIR [72].

CD39 (ectonucleoside triphosphate diphosphohydrolase 1; ENTPD1) has also served as a target to minimize IBMIR effects. This molecule plays an important role in the regulation of thromboinflammation by degrading ATP and ADP, thus exerting anti-inflammatory and anti-coagulant properties [129]. Incubation of islets overexpressing CD39 with human blood induced a prolongation in clotting time, thereby suggesting a protective role for CD39 in islet xeno-Tx [130].

The specific inhibition of complement system has been extensively tested in the context of IBMIR. The AP seems to be the predominant complement pathway in the course of IBMIR. More specifically, treatment of isolated islets with factor H, or an antibody against factor B resulted in decreased complement activation upon their exposure to human serum, while C1INH did not block the generation of complement effectors in the same context [131]. The contribution of AP in IBMIR was

further confirmed in a xenogeneic model of islet-Tx, whereby administration of factor H resulted in the blockade of complement and protected the islets from damage [131]. A peptide blocking complement effector C5a, alone [132] or in combination with the synthetic protease inhibitor gabexate mesilate [133] was shown to eliminate the detrimental effects of IBMIR, as coagulation activity was decreased and the islet function was improved. Of note, the suppressive effects of C5a-blocking peptide on both complement and coagulation pathways further support the interplay between these two cascades. Compstatin, a potent peptidic inhibitor that blocks complement system at the point of C3 [134], blocked the binding of active fragments of complement to islets exposed to human plasma, diminished complement activation in fluid phase [123, 126] and protected islets from lysis [114].

In further studies targeting IBMIR, Bennet et al. incubated isolated islets with whole blood in the presence of a soluble form of CR1 (sCR1). They demonstrated that treatment with sCR1 blocked the IBMIR-associated complement activation and protected the islets from damage. Simultaneous inhibition with sCR1 and heparin eliminated IBMIR adverse effects as depicted by the decreased activation of coagulation, complement and leukocytes. Interestingly, the protective role of sCR1 was confirmed in vivo, since administration of the inhibitor supported islet integrity, as assessed by reduced insulin release shortly after Tx [70].

It is worth mentioning that isolated islets can serve as a source of procoagulant factors. TF, the main initiator of coagulation in vivo, was found to be present in isolated islets [69, 71] and its knock-down [135, 136] or its inhibition with specific antibodies [137] has been proven beneficial for the blocking of IBMIR. Interestingly, nicotinamide, a vitamin B derivative, was used to decrease the expression levels of TF and coagulation, thereby ameliorating IBMIR [138] and leading to improved islet function after islet-Tx [139].

Islet xenografts can be assumed as foreign biosurfaces, which exposed to recipient blood trigger vigorous innate immune responses. Therefore, an emerging treatment strategy to eliminate the adverse effects of IBMIR is the coating of inhibitory molecules on the surface of isolated islets, thereby suppressing coagulation and complement systems locally at the site of transplantation.

In this context, heparin has been extensively studied as an inhibitor of IBMIR-associated detrimental effects. Several techniques of heparin immobilization have been introduced [140, 141]. Coating of islets with heparin abrogated the thrombotic manifestations during IBMIR [141] and was associated with increased graft survival [142]. Heparin coating of islets in combination with angiogenic growth factor increased the interaction with co-cultured EC and could be beneficial for islet vascularization [143]. Moreover, sCR1-coated islets displayed less release of insulin upon their exposure to serum, as a result of decreased complement-dependent lysis [144, 145], which led to overall better survival and function post transplantation [146]. The simultaneous immobilization of sCR1 with heparin inhibited IBMIR and further increased the frequency of normoglycemia observed after Tx [147]. The plasminogen activator urokinase has also been immobilized on the islet surface [148, 149], either alone or in combination with soluble thrombomodulin [150] or heparin [151]. Furthermore, administration of liposome carriers with TM

contributed to the achievement of normoglycemia after islet-Tx via decreasing levels of fibrin and immune cell accumulation in the transplanted tissues [152].

During recent years, the design and generation of genetically engineered pigs, either lacking or overexpressing a combination of molecules that can regulate complement and coagulation cascades, has opened new ways for the treatment of IBMIR.

Animals deficient in a1,3-galactosyltransferase, an enzyme promoting the synthesis of the Gal antigen, was a first approach to obtain genetically modified islets [153]. Existing xeno-reactive antibodies of the human recipients can bind to Gal that is present in the donor graft [1] and not in humans [154] and in turn induce rapid immune responses that are responsible for the graft dysfunction and loss [155]. In alternative approaches, the expression of human factors in pig islets, e.g. via adenoviral overexpression of the hCD55 or hCD59 made them less susceptible to complement-dependent lysis [156, 157]. These findings were further confirmed when hCD55-overexpressing islets isolated from transgenic pigs were also protected from lysis [158]. Interestingly, transplantation of islets overexpressing the CRP hCD46 resulted in the achievement of long-term normoglycemia in a xenogeneic model of Tx [57].

To further enhance the protection of xenografts from the innate immune mechanisms elicited during IBMIR, research efforts to generate multi-transgenic animals, which target multiple regulation points of complement and coagulation systems, have been undertaken. Pigs overexpressing a combination of human CRPs, such as hCD46, hCD55 or hCD59 in the presence of Gal deficiency have been generated [55, 56, 58, 59]. In addition, the human anticoagulant proteins TFPI and CD39 were simultaneously introduced to the porcine genome [79]. The use of these animals in models of xenogeneic islet-Tx resulted in protection of islet engraftment thus increasing the possibility to treat T1D-associated hyperglycemia.

11.6 Conclusions

Significant efforts are being undertaken to treat type 1 diabetes by applying islet xeno-Tx. Before that is translated into clinical studies, the adverse effects of IBMIR, which is the main culprit for the early damage and loss of islet xenografts, should be effectively bypassed. To unravel the mechanisms that orchestrate IBMIR, ex vivo whole blood models that simulate IBMIR as well as in vivo Tx models are utilized. The combined therapeutic approaches targeting complement, coagulation or leukocyte activation may ameliorate the IBMIR-related complications and bring the islet xeno-Tx closer to clinical practice.

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