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Developmental endothelial locus-1 modulates platelet-monocyte interactions and instant blood-mediated inflammatory reaction in islet transplantation

Ioannis Kourtzelis[1,*], **Klara Kotlabova**[1,*], **Jong-Hyung Lim**[1], **Ioannis Mitroulis**[1,2], **Anaisa Ferreira**[1], **Lan-Sun Chen**[1], **Bettina Gercken**[1], **Anja Steffen**[3,4], **Elisabeth Kemter**[5], **Anne Klotzsche-von Ameln**[1], **Claudia Waskow**[6], **Kavita Hosur**[7], **Antonios Chatzigeorgiou**[1,2,3], **Barbara Ludwig**[3,4], **Eckhard Wolf**[5], **George Hajishengallis**[7], and **Triantafyllos Chavakis**[1,2,3]

[1]Department of Clinical Pathobiochemistry, Medical Faculty, Technische Universität Dresden, Germany

[2]Institute of Clinical Chemistry and Laboratory Medicine, Medical Faculty, Technische Universität Dresden, Dresden, Germany

[3]Paul Langerhans Institute Dresden of Helmholtz Centre Munich at University Clinic Carl Gustav Carus of TU Dresden and DZD- German Centre for Diabetes Research, Dresden, Germany

[4]Department of Medicine III, Technische Universität Dresden, Dresden, Germany

[5]Chair for Molecular Animal Breeding and Biotechnology, and Laboratory for Functional Genome Analysis, Gene Center, Ludwig-Maximilians-Universität München, Munich, Germany

[6]Institute of Immunology, Medical Faculty, Technische Universität Dresden, Dresden, Germany

[7]University of Pennsylvania, Penn Dental Medicine, Department of Microbiology, Philadelphia, PA 19104, USA

Summary

Platelet-monocyte interactions are strongly implicated in thrombo-inflammatory injury by actively contributing to intravascular inflammation, leukocyte recruitment to inflamed sites, and the amplification of the procoagulant response. Instant blood-mediated inflammatory reaction (IBMIR) represents thrombo-inflammatory injury elicited upon pancreatic islet transplantation (islet-Tx), thereby dramatically affecting transplant survival and function. Developmental endothelial locus-1 (Del-1) is a functionally versatile endothelial cell-derived homeostatic factor with anti-inflammatory properties, but its potential role in IBMIR has not been previously addressed. Here, we establish Del-1 as a novel inhibitor of IBMIR using a whole blood–islet model and a syngeneic murine transplantation model. Indeed, Del-1 pre-treatment of blood before addition of islets diminished coagulation activation and islet damage as assessed by C-peptide release. Mechanistically, Del-1 decreased platelet-monocyte aggregate formation, by specifically blocking the interaction between monocyte Mac-1-integrin and platelet GPIb. Consistently,

Correspondence to: Dr. Ioannis Kourtzelis, Department of Clinical Pathobiochemistry, Medical Faculty, Technische Universität Dresden, Fetscherstraße 74, 01307 Dresden, Germany. Tel.: +49 (0) 351 4586250, Fax: +49 (0) 351 4586301 Iannis.Kourtzelis@uniklinikum-dresden.de.

*I.K. and K.K. contributed equally to this work.

intraportal islet-Tx in transgenic mice with endothelial cell-specific overexpression of Del-1 resulted in a marked decrease of monocytes and platelet-monocyte aggregates in the transplanted tissues, relative to those in wild-type recipients. Our findings reveal a hitherto unknown role of Del-1 in the regulation of platelet-monocyte interplay and the subsequent heterotypic aggregate formation in the context of IBMIR. Therefore, Del-1 may represent a novel approach to prevent or mitigate the adverse reactions mediated through thrombo-inflammatory pathways in islet-Tx and perhaps other inflammatory disorders involving platelet-leukocyte aggregate formation.

Keywords

Instant blood-mediated inflammatory reaction (IBMIR); Developmental endothelial locus 1 (Del-1); coagulation; platelet-monocyte aggregates; islet transplantation; GPIb; Mac-1 integrin

Introduction

The leukocyte adhesion cascade includes a series of adhesive steps that promote the effective recruitment of immune cells into inflamed sites (1,2). In this context, platelet-leukocyte interactions substantially contribute to the cascade and leukocyte recruitment. Platelet-leukocyte adhesion is mediated by P-selectin-dependent interactions (3) and by the binding of the leukocyte β2-integrin Mac-1 to cognate counter-receptors on platelets, predominantly glycoprotein Ib (GPIb) (4–8). Of note, the formation of platelet-leukocyte aggregates amplifies cell activation and adhesiveness and is accompanied by elevated inflammatory gene expression and procoagulant effects. Therefore, platelet-leukocyte aggregates are considered as important mediators of acute and chronic inflammation (5,9–11).

In recent years it has become evident that the leukocyte adhesion cascade is controlled by endogenous negative regulators (1). For example, Del-1 (also known as epidermal growth factor (EGF)-like repeats and discoidin-I-like domains 3; EDIL3) is an endothelial cell-derived anti-inflammatory glycoprotein which controls β2 integrin-dependent leukocyte adhesion (12). Specifically, as previously shown by our group, Del-1 blocks the interaction between the leukocyte integrin αLβ2 (LFA-1) and its endothelial counter-receptor ICAM-1 (13), as well as the binding of the αMβ2 (Mac-1) integrin with its ligand complement fragment iC3b (14). On the basis of its ability to control β2 integrin activities, Del-1 has emerged as a regulator of acute and chronic inflammatory responses in several disease models, including lung inflammation, neuroinflammation and inflammatory bone loss (15–18).

Type 1 diabetes (T1D) results from autoimmune destruction of insulin-producing pancreatic beta cells (19). The cardiovascular complications of T1D may dramatically affect the patients' outcome (20). Transplantation (Tx) of isolated islets of Langerhans represents a possible therapy for T1D (21,22). Due to the large amount of islets required for Tx and the shortage of human donors, the use of islets from different species could be a promising alternative. For instance, the xenogeneic application of pig islets to human diabetic recipients is increasingly considered a feasible therapeutic approach (23). Islet-Tx is usually performed by intraportal delivery to the liver (24). However, the exposure of isolated allogeneic or xenogeneic donor islets to the recipient's circulating blood triggers a rapid

thrombo-inflammatory reaction designated as Instant Blood-Mediated Inflammatory Reaction (IBMIR). The activation of coagulation and complement systems, accompanied by the activation and infiltration of platelets and innate immune cells to the transplantation site constitute major components of IBMIR that dramatically affect the survival and function of the transplanted islets (25). Of note, the IBMIR-associated inflammatory events are more prominent in the setting of islet xeno-Tx, as compared to those observed in allo-Tx (26).

Although substantial progress has been made in combatting the deleterious effects of IBMIR observed after islet-Tx, IBMIR remains a formidable challenge. Therefore, a better understanding of the mechanisms shaping IBMIR and the associated crosstalk between innate immune cells involved is required to improve the therapeutic outcome of islet xeno-Tx. To this end, here we investigated the possible role of the anti-inflammatory molecule Del-1 in islet xeno-Tx by utilizing a xenogeneic human whole-blood porcine islet model in combination with a mouse model of islet-Tx. We demonstrate, in both models, that Del-1 inhibits leukocyte-platelet aggregate formation by blocking the Mac-1–GPIb interaction, thereby decreasing the magnitude of the IBMIR and protecting the islets from damage, thus establishing a novel regulatory role for Del-1 in the islet-Tx setting.

Materials and methods

Del-1 proteins

Del-1–Fc generation and purification has been previously described (15,27). A RGE point mutant of Del-1–Fc in which Glu [E] was substituted for Asp [D] in the RGD motif of the second EGF repeat (Del-1[RGE]-Fc), was constructed by site-directed mutagenesis (Quick change II mutagenesis kit; Agilent). A Del-1 construct lacking the discoidin I-like domains (Del-1[E1-3]-Fc) was generated as follows. The segment of the *EDIL3* cDNA (OriGene) sequence corresponding to the three EGF-like repeats (E1-3) was amplified using appropriately designed primers followed by cloning into the HindIII and KpnI sites of the mammalian expression vector pSecTag2 (Invitrogen), which contains an *N*-terminal secretory tag (murine Igk leader sequence) and a *C*-terminal polyhistidine tag (His-tag). The IgG Fc gene (synthesized by Integrated DNA Technologies) was cloned between the *EDIL3* sequence and His-tag at EcoRI and XhoI sites and in frame with both sequences. Del-1[E1-3]-Fc (predicted to contain the *N*-terminal first 136 a.a. from the total 459 a.a. of the full-length Del-1 sequence) and Del-1[RGE]-Fc were expressed as soluble Fc fusion protein secreted into the culture medium of transfected HEK-293F suspension cells (InVitrogen). Expression and purification by Ni-affinity chromatography was performed as previously described (27). Recombinant human IgG1 Fc protein and recombinant Del-1 were purchased from R&D Systems.

Whole blood-islet model

The whole blood-islet model was developed as previously described (28–30). Briefly, the inner surface of loops consisting of polyvinylchloride (BMS Critical Care, UK) was coated with heparin (Lab Site Heparin Coating kit; Corline Systems AB). Fresh blood samples were collected from healthy donors (upon approval by the Ethics committee of the Technische Dresden). Informed consent was granted by the blood donors; none of them received any

platelet modifying drugs. Blood samples were incubated in coated loops in the presence of porcine islets (200 islets/ml of blood) for 60 min at 37°C with shaking. Isolation of islets from either retired breeders or neonatal piglets was previously described (31,32) and the procedure was approved by the Landesdirektion Sachsen Germany and the Regierung Oberbayern, Germany, respectively. Where indicated, blood was pre-treated with 10 μg/ml of Del-1, Del-1-Fc, Del-1[E1-3]-Fc or Del-1[RGE]-Fc. Pre-treatment of blood with either BSA or Fc protein, respectively, served as a control. After the incubation, blood was collected for the assessment of platelet-monocyte aggregate formation and for the measurement of coagulation parameters and C-peptide levels.

Flow cytometric analysis of platelet-leukocyte aggregates

Blood samples were collected 60 min after incubation in the presence of islets. Staining with antibodies against CD62P (clone AK-4, BD Pharmigen), CD14 (clone MϕP9, BD Pharmigen), CD41 (clone HIP8, BD Pharmigen) was performed, red blood cells were lysed using 1x RBC Lysis buffer (eBioscience) and cells were washed in PBS/5%FBS. Monocytes were identified according to their FSC/SSC characteristics and $CD14^+$ staining, respectively. The percentages of platelet-monocyte aggregates were detected as $CD62P^+CD41^+$ within the respective cell populations.

Measurement of activation of coagulation pathway and C-peptide release

Levels of thrombin-antithrombin (TAT) complexes and the amounts of porcine C-peptide were assessed in plasma isolated from blood samples incubated with islets in the presence or the absence of Del-1 using commercially available ELISA kits (TAT: Sekisui Diagnostics, C-peptide: Mercodia).

Platelet isolation & immobilization

Human blood was collected in trisodium citrate anti-coagulated tubes (0.38% final concentration) and platelet rich plasma (PRP) was obtained by centrifugation at 250g. Platelets were pelleted by centrifugation of PRP at 800g, washed and were resuspended in Tyrode's solution containing 0.1% BSA and 5ug/ml Refludan (Celgene). Platelets (1.5×10^7/well) were added to 96 well microplates (Greiner bio-one) pre-coated with 0.2% gelatin. The degree of platelet activation before and after their immobilization to gelatin was determined using flow cytometry. After 1 h at 37 °C unbound platelets were removed.

Isolation of peripheral blood human monocytes

Peripheral blood mononuclear cells (PBMCs) were isolated using Histopaque 1077 (Sigma-Aldrich) as previously described (33). A negative selection method (Monocyte Isolation Kit II; Miltenyi Biotec) was applied in order to obtain the monocyte population from the PBMCs.

CHO-Mac-1 cell culture

Chinese hamster ovary (CHO) cell lines expressing the human Mac-1 (CHO-Mac-1) or the control neomycin resistance vector (CHO-neo), were maintained under culture as previously described (34,35).

Cell Adhesion Assay

The adhesion of CHO-Mac-1 cells to immobilized ligands (GPIb), or of monocytes and CHO-Mac-1 cells to platelets was carried out as previously described (6,35,36). Briefly, 96 well microplates were coated with GPIb (10μg/ml) overnight at 4°C, or immobilized platelets (1.5×10^7/well), washed with PBS before use and were blocked with 1% BSA in PBS for 1h. Monocytes (10^4/well), platelets (5×10^6/well) or PMA-treated (50ng/ml) CHO cells (2×10^4/well) labeled with BCECF (15 min at 37°C in the dark; Molecular Probes) were washed, and then they were allowed to adhere for 45 min at 37°C to coated plates in serum free conditions. Where indicated, cells were pretreated with Del-1, Del-1-Fc, Del-1[RGE]-Fc, an antibody against CD11b (clone ICRF44, Biolegend) or an isotype control (Biolegend) (10 μg/ml) for 10 min at 37°C. BSA or Fc protein was used as control. The fluorescent intensity was quantified before (cell input) and after washing (adherent cells), using a Synergy HT multi-mode microplate reader (Biotek Instruments). The percentage of adhesion was defined as the fluorescence of adherent cells divided by the fluorescence of the cell input. In the case of the monocyte adhesion to platelets, stained adherent cells were fixed with 4% paraformaldehyde, images were acquired using an Axiovert inverted microscope (Carl Zeiss, Inc., Jena, Germany) and cells were counted.

EC-Del-1 mice and mouse pancreatic islet isolation and islet transplantation

Endothelial cell-specific overexpression of full-length Del-1 (EC-Del-1) was performed in a C57BL/6 background by engaging a Tie2 promoter/enhancer construct (kindly provided by Dr. U. Deutsch, Bern) (37) extensively used to achieve endothelial-specific overexpression in mice (38–40). Stable endothelial-specific overexpression of Del-1 was verified over several generations. Littermate WT mice were used as controls.

Male 10–12 weeks old C57BL/6 mice were used as islet donors. Mice were euthanized by cervical dislocation and a solution of Collagenase XI (0.6 mg/ml, Sigma Aldrich, Munich, Germany) was injected into the common bile duct. Perfused pancreata were collected and digestion was performed in 37°C for 20 min. The digested tissue was washed and islets were purified on discontinuous ficoll gradient (Ficoll 400, Sigma Aldrich, Munich, Germany). Hand-picked islets were cultured overnight in CRML 1066 medium supplied with 10% FBS and 1x PenStrep. Islets were transplanted intraportally as previously described (41). 200 islet equivalents (IEQ) were injected in 100 μl RPMI 1640 containing 5.5 mM glucose to EC-Del-1 mice or WT littermates. Mouse experiments were approved by the Landesdirektion Sachsen, Germany.

Flow cytometric analysis of liver infiltration

Liver was excised and non-parenchymal cells were isolated as previously described (42). Cells were pre-incubated with Fc-blocking antibody (CD16/32 antibody, BD, Heidelberg, Germany) and stained with antibodies against CD45 (clone 30-F11, eBioscience), CD41 (clone MWReg30, eBioscience), Ly6G (clone 1A8, BD Pharmigen), and CD11b (clone M1/70, BD Pharmigen). Hoechst 33258 (Life Technologies) was used to distinguish live from dead cells.

Immunohistochemistry

Paraformaldehyde-fixed liver tissues were embedded in paraffin and cut into 5 μm thick serial sections. The sections were deparaffinized, rehydrated and treated with citrate buffer for antigen retrieval. The first of the serial sections was stained with a rat anti-mouse Ly-6C antibody (1:100, AbD Serotec, Raleigh, NC) while the following section was stained with a guinea pig anti-mouse insulin antibody (1:200, Abcam, Cambridge, UK). Biotinylated rabbit anti-rat antibody from Vectastain ABC Kit (Rat IgG) (1:100, Vector Laboratories, Petersborough, UK) and biotinylated goat anti-guinea pig antibody (1:200, Abcam, Cambridge, UK) were used as secondary antibodies. Horseradish peroxidase was attached to the biotinylated secondary antibodies using Vectastain ABC Kits. Peroxidase substrate AEC Kit (Vector Laboratories, Petersborough, UK) was used for the color development. All sections were counterstained with Mayer's haematoxylin (SAV LP, Flintsbach a. Inn, Germany). Imaging was performed using Axio Observer.Z1 microscope (Carl Zeiss Microscopy GmbH, Jena, Germany).

Real-time PCR analysis

Total RNA from liver or lung tissue obtained from EC-Del-1 or WT littermate mice was isolated using the TRIzol reagent (Molecular Research Center Inc.). cDNA synthesis and real-time PCR were performed as previously described (43). For each sample, the following primer sequences were used: Del-1: (FWD 5-CCTGTGAGATAAGCGAAGC-3, REV 5-GAGCTCGGTGAGTAGATG-3 and the housekeeping gene 18S: FWD 5-GTTCCGACCATAAACGATGCC-3, REV 5-TGGTGGTGCCCTTCCGTCAAT-3. The 2^{-DDCT} method was used to quantify the target gene expression.

Statistical analysis

Wilcoxon matched-pairs test and Student's paired *t*-test were used to assess statistical significance between the analyzed groups in whole blood-islet experiments and adhesion assays, respectively. Unpaired *t*-test was applied for the analysis of the data obtained from the *in vivo* islet-Tx experiments. All data were evaluated with GraphPad Prism (GraphPad Software, Inc., San Diego, CA, USA), and are presented as means ± SE of the means. Statistical significance was set at $p<0.05$.

Results

Del-1 attenuates the thrombo-inflammatory effects occurring during IBMIR in a whole-blood islet model

The coagulation cascade, platelets and infiltration and activation of leukocytes (altogether summarized as thrombo-inflammatory injury) are critical factors in the pathogenesis of IBMIR. Having previously identified Del-1 as an endogenous anti-inflammatory factor interfering with leukocyte β2-integrin-dependent leukocyte recruitment (13,15,44,45), we examined here the impact of Del-1 as a potential inhibitor of IBMIR. To this end, we employed an *ex-vivo* xenogeneic whole-blood model that has been used to simulate the thrombo-inflammatory events occurring early after islet-Tx. The exposure of human blood to isolated porcine islets resulted in pronounced activation of coagulation (Fig. 1A), and in

the formation of aggregates between platelets and monocytes (Fig. 1B). Pre-treatment of blood with Del-1 prior to the addition of the islets, led to significant decrease of the IBMIR parameters as assessed by the measurement of coagulation activation (Fig. 1A) and the platelet-monocyte aggregate formation (Fig. 1B). The protective role of Del-1 in IBMIR was further supported by its ability to decrease islet damage as assessed by the release of C-peptide (Fig. 1C).

The IBMIR-associated leukocyte infiltration is decreased in mice with endothelial Del-1 overexpression

To rigorously test the biological relevance of our findings obtained from the *ex-vivo* whole-blood model, we engaged a mouse model of islet-Tx which enabled us to investigate the *in vivo* role of Del-1 in vascular inflammation. Mice that overexpress Del-1 in the endothelium (EC-Del-1) or WT littermates (Fig. 2A) were transplanted through the portal vein with islets isolated from WT donors. The expression of Del-1 in the liver endothelium is constitutively very low, as compared with lung endothelium (Fig. 2A). However, EC-Del-1 mice displayed substantial Del-1 expression in the liver which was 40-fold higher than in the liver of WT littermate controls (Fig. 2A). Consistent with our *ex vivo* findings, the infiltration of $Ly6G^{-}CD11b^{+}$ cells (monocytes) together with the numbers of platelet-monocyte aggregates were significantly decreased in the liver of the transplanted EC-Del-1 mice, as compared to the WT recipients 24h after Tx (Fig. 2B–C). The presence of monocytes in the proximity of the islets transplanted into the liver was also confirmed by immunohistochemistry (Supplementary Fig. 1).

Del-1 inhibits platelet-monocyte aggregate formation by blocking the Mac-1-GPIb interaction

As Del-1 inhibited platelet-monocyte aggregate formation in the context of IBMIR *ex vivo* and *in vivo* we continued to explore the underlying mechanism of this Del-1 action. Given that the interaction of Mac-1 with GPIb promotes platelet-monocyte aggregate formation and aggravates inflammatory responses (5,11) and our recent report that Del-1 blocks Mac-1–dependent interactions (14), we addressed whether Del-1 could inhibit the Mac-1-GPIb interaction-dependent platelet-monocyte aggregate formation. To this end, we performed a static adhesion assay, whereby isolated monocytes adhered to platelets after immobilization of the latter to gelatin. Platelet immobilization results in their activation, as assessed by upregulation of P-selectin (Supplementary Fig. 2). In this system, platelet-monocyte interaction is largely dependent on the Mac-1-GPIb interaction (6,7). Of interest, pre-treatment with Del-1 resulted in decreased adhesion of monocytes to platelets (Fig. 3A). Moreover, the adhesion of cells transfected with CD11b/CD18 (Mac-1) to either platelets (Fig. 3B) or purified GPIb (Fig. 3C) was significantly decreased in the presence of Del-1. The specificity of the Mac-1-GPIb interaction was further confirmed by inhibiting CD11b (Fig. 3D). Together, these findings demonstrate that the inhibition of Mac-1–GPIb interaction by Del-1 could underlie the inhibitory effect of Del-1 on the platelet-monocyte aggregate formation in the context of IBMIR.

Identification of the domain of Del-1 mediating inhibition of platelet-monocyte aggregate formation

To identify structural components of Del-1 involved in the regulation of platelet-monocyte aggregate formation, we used the whole-blood model and Del-1[E1-3]-Fc, a version of Del-1 lacking the two discoidin I-like domains present at the *C*-terminal part of the protein. Del-1[E1-3]-Fc inhibited platelet-monocyte aggregate formation comparably to full-length Del-1 (Del-1-Fc) (Fig. 4A), thus implicating the *N*-terminal EGF-like domains in this activity. Moreover, Del-1[RGE]-Fc, which has a single amino acid mutation at the RGD motif located in the second EGF-repeat, retained the ability to significantly inhibit platelet-monocyte aggregates (Fig. 4B) and Mac-1-dependent cell adhesion to immobilized GPIb (Fig. 4C).

Discussion

In the clinical setting of islet-Tx, IBMIR comprises several thrombo-inflammatory events including adhesion and activation of leukocytes and platelets and aggregation interactions thereof, as well as the induction of coagulation and thrombus formation, all of which contribute to the destruction of the transplanted islets (25). Therefore, the successful design and application of treatments that attenuate the aforementioned harmful effects is required to increase the survival of islet transplants.

Based on the anti-adhesive and anti-inflammatory properties of the endogenous integrin inhibitor Del-1, that have been documented in inflammatory disease models (15,16), we investigated here a possible novel role of Del-1 on the regulation of IBMIR. To this end, we used a relevant *ex vivo* whole-blood model to mimic IBMIR after islet xeno-Tx and also employed a mouse model of islet-Tx. We demonstrate that Del-1 blocks efficiently the activation of coagulation and the generation of platelet-monocyte aggregates that are triggered after the blood-islet contact. In addition, treatment with Del-1 was associated with decreased islet damage.

The αM sequence P201-K217 of Mac-1 is responsible for GPIb recognition (5) and the Mac-1–GPIb interaction constitutes a major contributor to thrombo-inflammatory injury, consistent with findings by us and other groups that inhibition of this critical interaction can prevent vascular inflammation in the course of several pathologies, including autoimmunity (4,10,11). In this report, we show for the first time that Del-1 can interfere with the Mac-1–GPIb interaction and thereby modulates the adhesion of monocytes to platelets leading to attenuation of IBMIR. Through the same mechanism, Del-1 could also provide protection against other (thrombo)inflammatory disorders associated with platelet-monocyte aggregates, such as atherosclerosis (5,9–11,46).

Of interest, the generation of platelet-leukocyte aggregates is additionally involved in the amplification of coagulation activity and the manifestation of thrombotic events (47–49). Accordingly, the inhibition of platelet-monocyte aggregate formation can lead to decreased activation of coagulation as a result of lower monocyte tissue factor levels and tissue factor activity (48). Therefore, the inhibitory action of Del-1 on coagulation reported here could probably be attributed to the modulation of platelet-leukocyte aggregate formation.

We have also shown that the inhibition of platelet-monocyte aggregate formation is mediated through the *N*-terminal EGF-like 1–3 domains of Del-1. At the same time, the data show that the *C*-terminal discoidin I-like domains are not required for this activity. Our findings also ruled out the involvement of the RGD motif of Del-1 (located in the second EGF-like repeat) in its inhibitory effect on platelet-monocyte aggregation. This indicates that Del-1 did not interfere with the RGD-binding αvβ3 and αIIbβ3 integrins (50) to block platelet-monocyte aggregate formation. The fact that Del-1[RGE]-Fc was capable of blocking the Mac-1-dependent platelet-monocyte interaction is also consistent with the fact that β2 integrins, like Mac-1, bind their ligands in an RGD-independent way (51). Moreover, the localization of the "business part" of Del-1 in the *N*-terminal one-third of the molecule is therapeutically important in the context of IBMIR as it can facilitate drug development especially if future research further confines the inhibitory activity to an even smaller segment or epitope.

The anti-inflammatory role of Del-1 in the course of IBMIR was also demonstrated here in a mouse model of islet-Tx. Using the EC-Del-1 transgenic mice, we found that the overexpression of Del-1 in endothelial cells resulted in decreased monocyte presence and decreased abundance of platelet-monocyte aggregates in transplanted tissues. In summary, the findings of the present study elucidate further the mechanisms underlying the adverse effects observed after islet-Tx and pinpoint a novel role for Del-1 in attenuating IBMIR. Therefore, the use of Del-1 alone or in combination with other already suggested treatment strategies (25) could be a potential therapeutic approach to protect the transplanted islets and thus contribute to achieving normoglycemia after islet-Tx.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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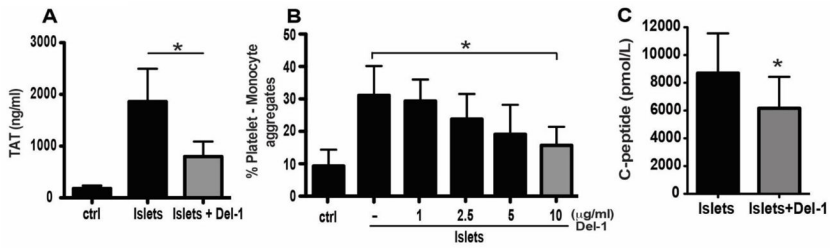

Fig. 1. The effect of Del-1 on IBMIR using a xenogeneic whole-blood islet model

(A) Thrombin-antithrombin complexes (TAT) in plasma isolated from human whole blood that was incubated with porcine islets, in the presence or absence of Del-1 (10 μg/ml), are shown. Incubation of blood in the absence of islets served as control (ctrl). TAT levels were measured using ELISA. Data are mean± SEM; n=10; *: $p< 0.05$. (B) The percentages of platelet-monocyte aggregates after incubation of blood with islets in the absence (−) or presence of increasing concentrations of Del-1. Incubation of blood in the absence of islets served as control (ctrl). Data are presented as mean± SEM; n=9; *$p< 0.05$. (C) The levels of porcine C-peptide in plasma samples, obtained from human blood that was incubated with porcine islets in the presence or absence of Del-1 (10 μg/ml), were assessed by ELISA. Data are mean± SEM; n=8; *: $p< 0.05$.

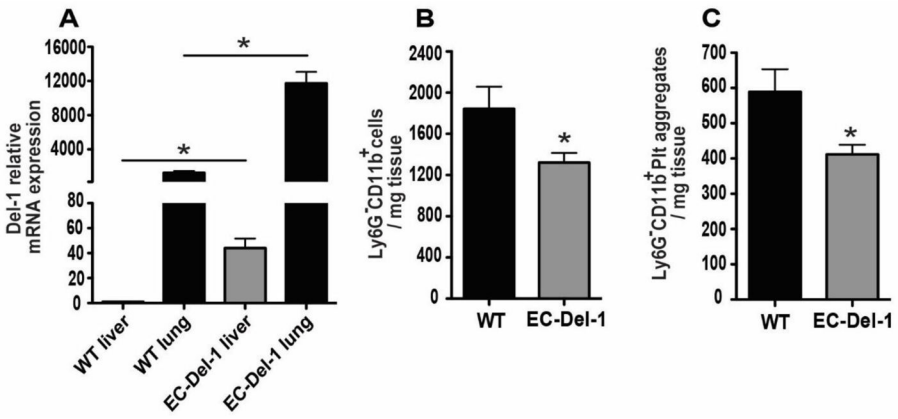

Fig. 2. Endothelial overexpression of Del-1 in mice is associated with decreased monocyte accumulation after islet-Tx

(A) The relative expression levels of Del-1 mRNA in the liver of EC-Del-1 mice or WT littermates or in the lung of WT and EC-Del-1 mice were determined with qPCR. 18S expression was used for normalization. Del-1 expression of WT liver samples was set as 1. Data are presented as mean± SEM; n=5; *p< 0.05. (B, C) Flow cytometric analysis of the liver tissues obtained from EC-Del-1 or WT littermate recipients 24h after intraportal Tx with 200 IEQs from WT mice. Numbers of (B) monocytes ($LyG6^{-}CD11b^{+}$) and (C) platelet-monocyte aggregates ($Plt\text{-}LyG6^{-}CD11b^{+}$) are shown. Cell number is expressed as cells per mg of liver tissue. Data are presented as mean± SEM; n=5–7/group; *p< 0.05.

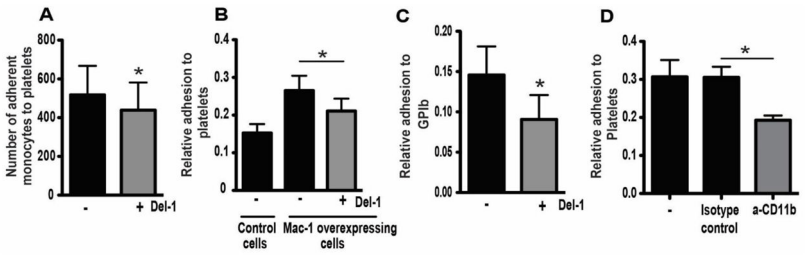

Fig. 3. The Mac-1–GPIb interaction is modulated by Del-1

(A) The adhesion of monocytes to immobilized platelets is shown. Results are presented as the number of adherent monocytes to platelets in the absence (−) or in the presence (+) of Del-1 (10 μg/ml). Data are mean± SEM; n=4; *p< 0.05. (B) The relative adhesion of CHO-Mac1 cells to immobilized platelets is shown. Results are presented as the ratio of fluorescent intensity of the stained adhered cells to the intensity of cell input in the absence (−) or presence (+) of Del-1. CHO-neo cells were used as control (control cells). Data are mean± SEM; n=3; *p< 0.05. (C) The relative adhesion of CHO-Mac1 cells to GPIb is shown. Data are presented as the ratio of fluorescent intensity of the stained adhered CHO-Mac-1 cells to the intensity of cell input in the presence (+) or in the absence (−) of Del-1 (10 μg/ml). Data are mean± SEM; n=3; *p< 0.05. (D) The relative adhesion of CHO-Mac1 cells to immobilized platelets is shown. Results are presented as the ratio of fluorescent intensity of the stained adhered cells to the intensity of cell input in untreated cells (−) and in cells treated with 10 μg/ml of either an inhibitory antibody against CD11b (a-CD11b) or with a control antibody (Isotype control). Data are mean± SEM; n=4; *p< 0.05.

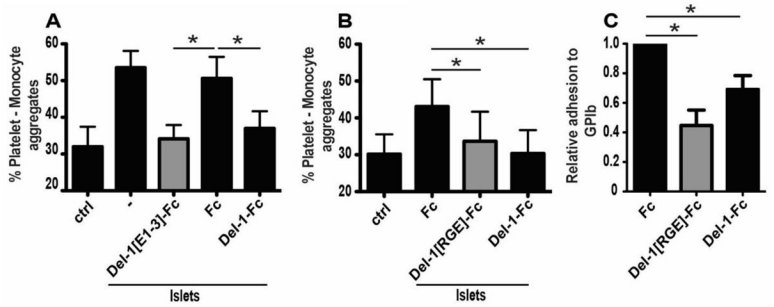

Fig. 4. The role of the specific domains of Del-1 on the inhibition of platelet-monocyte aggregate formation

(A) The percentages of platelet-monocyte aggregates upon exposure of human whole blood to porcine islets in the absence (−) or presence of Del-1-Fc, Fc control or Del-1[E1-3]-Fc (each at 10 μg/ml) are shown. Incubation of blood in the absence of islets served as control (ctrl). Data are presented as mean± SEM; n=7; *p< 0.05. (B) The percentages of platelet-monocyte aggregates after the incubation of whole blood with islets in the presence of Del-1-Fc, Fc control or Del-1[RGE]-Fc (each at 10 μg/ml) are shown. Data are presented as mean± SEM; n=8; *p< 0.05. (C) The relative adhesion of CHO-Mac1 cells to immobilized GPIb is shown. Data are presented as the adhesion to GPIb in the presence of Del-1[RGE]-Fc or Del-1-Fc relative to the adhesion in the presence of the Fc control. The relative adhesion in the presence of the Fc control was set as 1. Data are mean± SEM; n=4; *p< 0.05.

Table 1

Extra table

1. What is known on this topic?
• Platelet-monocyte interactions regulate leukocyte adhesion and recruitment thus enhancing inflammatory responses. • Our group has identified Del-1 as an endogenous negative regulator of inflammatory cell adhesion. • Upon islet-Tx, instant blood-mediated inflammatory reaction (IBMIR) negatively affects the outcome of the graft. • Coagulation together with the activation and infiltration of immune cells and platelets as well as monocyte-platelet interactions constitute major components of IBMIR.
2. What does this paper add?
• Del-1 modulates platelet-monocyte crosstalk by inhibiting the interaction between leukocyte Mac-1 and platelet GPIb. • Del-1 attenuates the IBMIR-associated activation of platelet-monocyte aggregate formation, protects islet function from IBMIR and results in decreased vascular inflammation in the liver of the recipients after intraportal islet-Tx. • The therapeutic use of Del-1 in islet-Tx and other relevant inflammatory models of disease may represent a novel approach to harness the adverse reactions that are mediated through thrombo-inflammatory pathways.