Antibody-Mediated Retinal Pericyte Injury: Implications for Diabetic Retinopathy

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PURPOSE. To test the hypothesis that autoantibodies against retinal pericytes could develop in diabetic retinopathy, and that these autoantibodies could induce retinal pericyte dysfunction/death via complement.

METHODS. Human primary retinal pericytes cultured in media containing normal (5 mM) or high (30 mM) glucose concentrations were incubated with normal human sera in the presence of a retinal pericyte-reactive antibody, then their viability was assessed by a BCECF-based cytotoxicity assay, and their function was assessed by a T-cell proliferation assay. The pericytes were also analyzed by RT-PCR and flow cytometry to detect CD38, an established diabetes-associated cell surface autoantigen. The potential of the anti-CD38 antibodies in inducing pericyte cellular injury was evaluated using the same cytotoxicity assays. In addition, autoantibody-mediated cytotoxicity in mouse retinal pericytes sensitized by sera from mice with developing diabetic retinopathy or control normal mice were also studied.

RESULTS. Retinal pericyte-reactive antibodies induced cellular damage by activating complement in the serum. The antibody-injured pericytes had reduced efficacy in inhibiting T cells. Hyperglycemic culture conditions rendered pericytes more susceptible to antibody-mediated attack. CD38 was expressed in retinal pericytes, and upregulated by TNF- α and IFN- γ , and anti-CD38 antibodies induced pericyte cytotoxicity. Retinal pericytes sensitized with sera from chronic diabetic mice suffered significantly augmented cytotoxicity compared with those sensitized with sera from the control mice.

CONCLUSIONS. The autoantibody-initiated complement activation could be a mechanism underlying the loss of function, and eventually, death of retinal pericytes in diabetic patients, suggesting that inhibiting complement activation could be a novel therapeutic approach. (*Invest Ophthalmol Vis Sci.* 2012; 53:5520–5526) DOI:10.1167/iovs.12-10010

Pericytes are embedded within the vascular basement membrane of almost all capillaries, and retina capillaries have the highest density of pericytes compared with other tissues.¹ These cells are important regulators of vascular development, stabilization, maturation, and remodeling.^{2,3} Pericytes begin to die relatively early in the course of diabetic retinopathy, and are considered to be integrally involved in the pathogenesis of the retinopathy.⁴ A variety of mechanisms, including oxidative stress,⁵ formation of advanced glycation end-products,⁶ and upregulation of protein kinase C,⁷ have been implicated in pericyte death in diabetes, but the possible contributions of autoantibodies and complement in such cell loss in diabetic retinopathy has not been studied.

Complement is an important part of innate immunity. It serves as a first shield against invading pathogens by assembling membrane attack complexes (MAC; C5b-9) to directly injure/lyse the invading cells, and by recruiting/activating leukocytes to the site of complement activation to promote inflammation.⁸ In addition to directly attacking invading pathogens, complement also functions as an effector mechanism for the humoral immune system. After IgGs/IgMs bind to the target cells, the Fc portion of those antibodies activates complement, therefore assembling MAC to injure/kill the targeted cells. Despite all these benefits, complement is also involved in the pathogenesis of autoimmune diseases where autoantibodies are present. In those cases, self-tissues are injured by excessive complement activation caused by autoantibodies against cell surface antigens, leading to inflammation, apoptosis, and organ function loss.⁹ In this report, using primary human retinal pericytes (RPC) and mice with developing retinopathy, we explored the potential roles of autoantibodies and complement in retinal pericyte dysfunction and cytotoxicity in diabetic retinopathy.

METHODS

Human and Mouse Retinal Pericytes

Most of the studies in this report used human retinal pericytes that were isolated from two sets of eyes of two nondiabetic donors (aged 41 and 72, Cleveland Eye Bank) and characterized as described previously.¹⁰ Primary retinal pericytes were maintained in complete Dulbecco's modified Eagle's medium (DMEM) with 10% fetal bovine serum (FBS; Invitrogen, Grand Island, NY). For culture under hyperglycemic conditions, pericytes were cultured in complete high-glucose DMEM (30 mM glucose; Invitrogen) with 10% FBS for 7 days with daily media change. Retinal pericytes with passage numbers 3 to 5 were used in all the experiments. The ex vivo experiments used mouse retinal pericytes that were isolated from immortomice expressing a temperature-sensitive simian virus (SV), 40 large T antigen (Charles River Laboratory, Wilmington, MA), and characterized as described before.¹¹

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Retinal Pericytes Cell Surface CD38 Expression Detection

The presence of CD38 transcripts in the retinal pericytes was examined by RT-PCR after total RNA isolation with Trizol (Invitrogen), and reverse transcribed with random primers using a first-strand cDNA synthesis kit (Invitrogen). The primers used to amplify a 397-bp CD38 transcript were located on different exons to avoid false-positive results (P1, GTTTCAGAGCTGCCTGTGATGT, and P2, ACCAGCAGGTATGCTGAGTCATGT). The PCR reactions were carried out on a PTC-200 thermal cycler (MJ Research, Waltham, MA) with the following conditions: 94°C, 30 seconds, 58°C, 60 seconds, and 72°C, 60 seconds, 40 cycles. To detect CD38 protein on the cell surface of retinal pericytes, 2×10^5 of cells were cultured with or without 20 ng/mL of TNF- α (PeproTech, Rocky Hill, NJ), 300 U/mL of IFN- γ (PeproTech) or both for 48 hours. After this, the cells were stained with 10 μ g/mL of an anti-CD38 IgG (Clone HIT2; Biolegend, San Diego, CA), or the same concentration of isotype control, following by flow cytometry analysis on a flow cytometer (LSR II; BD Bioscience, San Jose, CA).

Antibody-Mediated Cytotoxicity Assay

An amount of 2×10^5 retinal pericytes were preloaded with 5 μ M of 2',7'-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein acetoxymethyl ester (BCECF-AM; Invitrogen) for 30 minutes at 37°C, then incubated with 2 μ g/mL of anti-HLA ABC IgG (Clone W6/32; Biolegend) or 20 μ g/mL anti-human CD38 IgG (Clone HIT2; Biolegend) in 100 μ L of PBS buffer with 5% BSA for 30 minutes on ice. After washing, these antibody-sensitized pericytes were tested in a standard BCECF release-based cytotoxicity assay. In brief, sensitized pericytes were incubated with different amounts of normal human sera (NHS) or respective complement-depleted sera (Complementtech, Tyler, TX) in 100 μ L of GVB⁺⁺ buffer for another 30 minutes at 37°C. After this, the same volume of supernatants were harvested, and the released BCECF was measured by a fluorescence microtiter plate reader (Molecular Devices, Sunnyvale, CA) with excitation and emission wavelengths of 485 nm and 538 nm. To calculate the percentage of BCECF release (complement-mediated injury), the following equation was used as reported before^{12,13}: percentage of BCECF release = $([A - B]/[C - B]) \times 100\%$, where A represents the mean experimental BCECF release, B represents the mean spontaneous BCECF release, and C represents the mean maximum BCECF released that was induced by incubating cells with 0.1% SDS.

Retinal Pericyte Function Assay

Function of retinal pericytes was assessed based on a T-cell proliferation assay following protocols described before.¹⁴ In brief, freshly prepared human peripheral blood mononuclear cells (PBMCs) (Stem Cell Facility at Case Western Reserve University, Cleveland, OH) were first labeled by incubating them with 0.3 μ M of carboxyfluorescein succinimidyl ester (CFSE) (Invitrogen) at 37°C for 8 minutes. After washing, T cells were activated by incubation with anti-CD3/CD28 Dynabeads (Invitrogen) following manufacturer-provided protocols. The CFSE-labeled, and anti-CD3/CD28-activated cells were then aliquoted into wells of a 96-well plate at a concentration of 0.4×10^6 cells/well, and incubated with different numbers of RPCs (RPC: T-cell ratio: 0, 1:10, 1:20, and 1:40) in triplicates. After 3 days of incubation, T-cell proliferation was assessed by measuring CFSE dilution using flow cytometry, gating on CD4⁺ T cells. The inhibition of T-cell proliferation was also calculated using the following equation: % of inhibition = $(1 - [A - B]/[C - B]) \times 100\%$, where A represents the experimental % of T-cell proliferation, B represents the % of background proliferation (without stimulation), and C represents the % of T-cell proliferation with stimulation and without the inhibition of pericytes.

Ex Vivo Mouse Pericyte Assay

C57BL/6 mice were injected with streptozotocin to induce diabetes, and were maintained diabetic for 10 months following protocols described before.¹⁵ Sera samples were collected from 10 of these chronic diabetic mice and six age-matched control mice. Mouse retinal pericytes were labeled with BCECF and incubated with 30% of sera pooled from the diabetic mice or control mice. After the antibody sensitization, these cells were then incubated with different amounts of NHS and analyzed in the same BCECF-based cytotoxicity assay.

Statistical Analysis

All experiments were repeated at least twice with similar results. The data were analyzed using an independent *t*-test. Probability values less than 0.05 were considered to be significant.

RESULTS

Retinal Pericytes Are Injured by Complement in the Presence of a Reactive Antibody

We first examined whether retinal pericytes could be injured by complement in the presence of a pericyte-reactive antibody using a conventional BCECF leakage-based cytotoxicity assay. Because no anti-human retinal pericyte antibody has been identified, we used an anti-HLA ABC antibody as a model antibody to sensitize the primary retinal pericytes for this proof-of-concept experiment. After incubating the antibody-sensitized retinal pericytes with 5, 10, or 20 μ L of NHS, we assessed cytotoxicity by measuring levels of BCECF that leaked into the culture supernatants. These assays showed that NHS induced BCECF leakage from retinal pericytes in a dose-dependent manner, consistent with a possibility that the antibody-sensitized retinal pericytes were injured by complement (Fig. 1A). To further confirm the role of complement in the antibody-induced pericyte cytotoxicity, we again incubated antibody-sensitized retinal pericytes with 30 μ L of NHS, or complement-depleted human sera (C3-dep or C5-dep), then assessed pericyte cytotoxicity using the same assay. These experiments showed that although NHS induced retinal pericyte cytotoxicity (as reported above), the same amount of C3- or C5- depleted sera had minimal effect on inducing BCECF leakage (Fig. 1B). This indicates that complement plays a primary role in inducing pericyte cytotoxicity in the presence of pericyte-reactive antibodies, and that the terminal pathway of complement activation (starts from C5) is required for NHS to injure pericytes.

Hyperglycemic Conditions Render Retinal Pericytes More Susceptible to Antibody-Mediated Attack

We next asked whether hyperglycemic conditions found in diabetic patients make retinal pericytes more susceptible to the autoantibody-mediated attack. We first cultured retinal pericytes in normal-glucose media (5 mM) or high-glucose media (30 mM) for seven days with daily media change, and then compared the severity of antibody-mediated injury by measuring BCECF leakage in the presence of different amounts of serum. These assays showed that retinal pericytes cultured in the high-glucose media had a greater leak of BCECF than cells cultured in the normal glucose media (Fig. 2), indicating that even seven days of high-glucose conditions is enough to make pericytes more susceptible to antibody-mediated attack.

FIGURE 1. Retinal pericytes are injured by complement in the presence of an antipericyte antibody. Retinal pericytes were incubated with 2 µg/mL of anti-HLA ABC IgG for 30 minutes on ice. After washing, 2×10^5 of the antibody-sensitized retinal pericytes were incubated with 5, 10, and 20 µL NHS (A), 30 µL of NHS or the same amount of complement-depleted sera (B), and the cytotoxicity was assessed by measuring BCECF leakage. Data are mean $\pm$ SEM. * $P < 0.05$.

Antibody-Injured Retinal Pericytes Have Impaired Immunosuppressive Function

We recently demonstrated that retinal pericytes inhibit the proliferation of activated T cells, and protected retinal endothelial cells from inflammation-induced apoptosis.¹⁴ To test whether pericyte-reactive antibodies have any effect on the function of retinal pericytes in the presence of serum, we compared the ability of antibody-sensitized retinal pericytes incubated with or without NHS to inhibit activated T cells using the same CFSE-based T-cell proliferation assay as we have done before.¹⁴ These experiments showed that antibody-sensitized retinal pericytes incubated with NHS had significantly reduced T-cell inhibitory activity compared with those without NHS incubation, indicating that the antibody-injured retinal pericytes had impaired cellular function (Fig. 3).

Autoantigen CD38 Is Expressed in Retinal Pericytes

In the above studies we used an anti-HLA mAb as a model antibody to sensitize the retinal pericytes. Trying to identify clinically associated autoantibodies that could bind to the cell surface of retinal pericytes to activate complement, we examined whether CD38, an established diabetes-associated cell surface autoantigen,¹⁶ is expressed in retinal pericytes by RT-PCR and by flow cytometry. We found that CD38 transcripts were detectable by RT-PCR in the quiescent retinal pericytes (Fig. 4A); however, pericyte cell surface

FIGURE 2. Hyperglycemic culture conditions render retinal pericytes more susceptible to complement-mediated attack. Retinal pericytes were cultured in complete DMEM with normal (5 mM) or high glucose (30 mM) for seven days with daily media change, then the identical numbers of cells were subjected to the same cytotoxicity assays. Data are mean $\pm$ SEM. * $P < 0.05$.

levels of CD38 protein were low (as examined by flow cytometry; not shown) under normal culture conditions. Because local inflammation develops early in diabetes, and inflammatory cytokines TNF- α ^{17,18} and IFN- γ ¹⁹ have been associated with diabetic retinopathy, we then treated the retinal pericytes with TNF- α and/or IFN- γ , and assessed the levels of CD38 protein on retinal pericyte cell surface by flow cytometry again. These studies showed that after 48 hours of treatment, TNF- α alone, but not IFN- γ alone, upregulated CD38 expression on retinal pericytes, and that TNF- α and IFN- γ together had a synergistic effect on upregulating CD38 on the surface of retinal pericytes (Figs. 4B, 4C).

Anti-CD38 IgGs Induce Retinal Pericyte Cytotoxicity

We next examined whether anti-CD38 IgGs could induce retinal pericyte cytotoxicity like the anti-HLA IgGs tested above, and whether inflammatory conditions commonly found in diabetic retinopathy patients could augment the anti-CD38 antibody-induced cytotoxicity. We first cultured retinal pericytes with or without TNF- α /IFN- γ , then incubated them with the same amount of anti-CD38 IgGs. After this, we incubated the antibody-sensitized pericytes with different amounts of NHS, and assessed their cytotoxicity by measuring levels of leaked BCECF. These assays showed that similar to what was discovered in studies using the anti-HLA model antibodies, anti-CD38 IgGs induced retinal pericyte cytotoxicity, and that inflammatory cytokines TNF- α /IFN- γ significantly augmented the cellular injury (Fig. 5).

Sera from Chronic Diabetic Mice Induce Retinal Pericyte Cytotoxicity

We next tried to verify these in vitro results in vivo by examining whether sera from diabetic mice (with developing retinopathy) could induce retinal pericyte cytotoxicity. We induced diabetes in mice by streptozotocin injection, and maintained the mice diabetic for 10 months. After this, we collected and pooled sera from these diabetic mice and age-matched normal mice, then incubated mouse retinal pericytes with the respective sera to allow any pericyte-reactive antibodies to bind. After washing, we incubated the

A

B

FIGURE 3. Antibody-injured retinal pericytes have impaired immunosuppressive function. Retinal pericytes were sensitized with the antibody, then incubated with 30% NHS or the same volume of PBS for 30 minutes. After this, the same numbers of pericytes were cocultured with CFSE-labeled and activated human PBMCs at different ratios for 72 hours. T-cell proliferation was assessed by measuring CFSE dilution on CD4⁺ T cells by flow cytometry. (A) Original T-cell proliferation data. (B) Calculated inhibition of T-cell proliferation at different ratios of pericytes versus PBMCs. Representative results of three individual experiments.

sensitized pericytes with NHS, and assessed their viability by measuring BCECF leakage. These studies showed that pericytes sensitized with sera from the chronic diabetic mice had markedly increased levels of BCECF leakage after incubation with serum (Fig. 6), indicating that like anti-HLA IgGs and anti-CD38 IgGs tested in the above human pericyte studies, autoantibodies developed in chronically diabetic mice induced significantly increased pericyte cytotoxicity compared with those in the control mice.

DISCUSSION

In this report, we found that retinal pericytes were injured by complement in the presence of an antibody that reacted with pericyte cell surface antigens. Hyperglycemic conditions rendered retinal pericytes more susceptible to antibody-mediated injury, and the injured retinal pericytes had reduced activity to inhibit T cells (as a parameter of pericyte function). CD38, an established cell surface autoantigen in diabetes, was expressed in retinal pericytes and upregulated by TNF- α and

FIGURE 4. Autoantigen CD38 is expressed in retinal pericytes. (A) RT-PCR detection of CD38 transcripts in retinal pericytes. Total RNA from THP-1 cells was used as positive control, and total RNA without reverse transcription was used as negative control. (B, C) TNF- α /IFN- γ upregulate CD38 expression on retinal pericytes. Retinal pericytes were incubated with TNF- α , IFN- γ , and both for 48 hours, then the CD38 protein levels on the cell surface were assessed by flow cytometry (in log scale) (B), and the mean fluorescence intensity (MFI) of CD38 staining was analyzed (in linear scale) (C). Representative results of five individual experiments.

IFN- γ . Anti-CD38 IgGs also induced pericyte cytotoxicity in the presence of serum, and TNF- α /IFN- γ augmented this effect. Finally, retinal pericytes sensitized with sera from chronic diabetic mice suffered more severe cellular injury in the presence of serum than those sensitized with sera from the control mice.

Evidence suggests that loss of retinal pericytes likely contributes to the development of diabetic retinopathy, so it is important to understand the mechanisms of retinal pericyte death in diabetes. Vascular permeability is increased in diabetes, in which case components in the blood including complement and autoantibodies have access to retinal pericytes. The possibility that autoantibodies might contribute to the development of pericyte loss and other lesions of diabetic retinopathy has been inadequately studied. One group reported that antibodies from diabetic patients cross-reacted

with *bovine* retinal pericytes,^{20,21} but evidence that autoantibodies against *human* retinal pericytes developed in diabetic patients remains absent, and the possible underlying mechanism is unclear. To test the hypothesis that retinal pericyte-reactive antibodies can induce pericyte cytotoxicity through activating complement, we first used an mAb against the HLA, which is present on retinal pericytes. We sensitized human primary retinal pericytes with the anti-HLA antibodies, then incubated the cells with NHS, or complement-depleted sera. These proof-of-concept experiments demonstrated that pericytes can be injured, and their function can be impaired by autoantibodies through activating complement.

CD38 (ADP-ribosyl cyclase/cADPR hydrolase) has been identified as an autoantigen in diabetes patients.¹⁶ CD38 is a cell surface protein that is present on many types of cells, including T and B cells, monocytes, neuronal cells, and islet

FIGURE 5. Anti-CD38 antibodies induce retinal pericyte cytotoxicity. Retinal pericytes were first cultured with or without 20 ng/mL of TNF- α and IFN- γ for 48 hours. After this, 2×10^5 of the respective pericytes were labeled with BCECF and sensitized with anti-CD38 IgGs. The antibody-induced pericyte cytotoxicity was assessed after incubating the sensitized pericytes with different amounts of serum. * $P < 0.05$.

FIGURE 6. Sera from chronic diabetic mice developing diabetic retinopathy induce retinal pericyte cytotoxicity. Sera from chronic diabetic and control mice were pooled and used to sensitize mouse retinal pericytes. After this, cytotoxicity of these sensitized pericytes was assessed using the same BCECF-leakage assays in the presence of different amounts of serum. * $P < 0.05$.

cells.^{22,23} The presence of anti-CD38 autoantibodies was first described in a Japanese cohort of type 2 diabetes patients.²⁴ Studies from several other groups confirmed this result in different ethnic patient populations.^{25,26} Anti-CD38 autoantibodies were also identified in type 1 diabetes and the prevalence of anti-CD38 autoantibodies was found to be higher with longer duration of diabetes.²⁷ Although anti-CD38 autoantibodies have been detected in both type 1 and type 2 diabetes, their potential pathological roles were unclear, and whether CD38 is expressed in retinal pericytes was not studied. Our results provide evidence for the first time that CD38 is present on retinal pericytes and is upregulated under inflammatory conditions. These results, together with the reports that anti-CD38 autoantibodies are identified in diabetic patients, support the concept that autoantibodies against surface antigens on retinal pericytes can develop in chronic diabetic patients.

After binding to self-tissues, autoantibodies can activate complement, which contributes to the pathogenesis of many autoimmune diseases, including myasthenia gravis²⁸ and membranous glomerulonephritis.²⁹ Complement activation products are detectable in retinal vasculatures in diabetic patients³⁰; however, whether the activated complement could be involved in the dysfunction and loss of retinal pericytes were completely unknown. We found that antibody-sensitized pericytes were injured by NHS but not C3- or C5-depleted serum, and that the complement-injured pericytes had reduced efficacy in inhibiting T cells, suggesting that complement could contribute to the dysfunction and loss of retinal pericytes in chronic diabetic patients.

Based on these data, we propose that complement activation contributes to the functional loss and death of retinal pericytes in diabetes. Especially in patients with type 1 diabetes, autoantibodies develop as a result of islet destruction and other autoimmune conditions. Some of these autoantibodies (including anti-CD38 IgGs) recognize cell surface antigens on retinal pericytes. Under chronic inflammatory conditions in these patients, permeability of the retinal vasculature is increased, which allows retinal pericyte-reactive autoantibodies and complement in the blood to access retinal pericytes. Inflammatory cytokines also upregulate autoantigens, including CD38, on retinal pericytes in diabetic patients. Complement is activated by the autoantibodies binding to surface antigens on retinal pericytes, leading to cytotoxicity, reduced activity, and, eventually, retinal pericyte loss. Our data also suggest that controlling complement activation in diabetic patients could be a new approach for preserving the function and preventing the loss of retinal pericytes in diabetes, which could help to prevent the development of diabetic retinopathy.

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References

1. Sims DE. The pericyte—a review. *Tissue Cell*. 1986;18:153–174.
2. Darland DC, Massingham IJ, Smith SR, Piek E, Saint-Geniez M, D'Amore PA. Pericyte production of cell-associated VEGF is differentiation-dependent and is associated with endothelial survival. *Dev Biol*. 2003;264:275–288.
3. Antonelli-Orlidge A, Saunders KB, Smith SR, D'Amore PA. An activated form of transforming growth factor beta is produced by cocultures of endothelial cells and pericytes. *Proc Natl Acad Sci U S A*. 1989;86:4544–4548.
4. Yafai Y, Iandiev I, Wiedemann P, Reichenbach A, Eichler W. Retinal endothelial angiogenic activity: effects of hypoxia and glial (Müller) cells. *Microcirculation*. 2004;11:577–586.
5. Liu B, Bhat M, Padival AK, Smith DG, Nagaraj RH. Effect of dicarbonyl modification of fibronectin on retinal capillary pericytes. *Invest Ophthalmol Vis Sci*. 2004;45:1983–1995.
6. Denis U, Lecomte M, Paget C, Ruggiero D, Wiernsperger N, Lagarde M. Advanced glycation end-products induce apoptosis of bovine retinal pericytes in culture: involvement of diacylglycerol/ceramide production and oxidative stress induction. *Free Radic Biol Med*. 2002;33:236–247.
7. Pomero F, Allione A, Beltramo E, et al. Effects of protein kinase C inhibition and activation on proliferation and apoptosis of bovine retinal pericytes. *Diabetologia*. 2003;46:416–419.
8. Ricklin D, Hajishengallis G, Yang K, Lambris JD. Complement: a key system for immune surveillance and homeostasis. *Nat Immunol*. 2010;11:785–797.
9. Daha NA, Banda NK, Roos A, et al. Complement activation by (auto-) antibodies. *Mol Immunol*. 2011;48:1656–1665.
10. Miller AG, Smith DG, Bhat M, Nagaraj RH. Glyoxalase I is critical for human retinal capillary pericyte survival under hyperglycemic conditions. *J Biol Chem*. 2006;281:11864–11871.
11. Scheef EA, Sorenson CM, Sheibani N. Attenuation of proliferation and migration of retinal pericytes in the absence of thrombospondin-1. *Am J Physiol Cell Physiol*. 2009;296:C724–734.
12. Quigg RJ, Holers VM, Morgan BP, Sneed AE 3rd. Crry and CD59 regulate complement in rat glomerular epithelial cells and are inhibited by the nephritogenic antibody of passive Heymann nephritis. *J Immunol*. 1995;154:3437–3443.
13. Quigg RJ, Nicholson-Weller A, Cybulsky AV, Badalamenti J, Salant DJ. Decay accelerating factor regulates complement activation on glomerular epithelial cells. *J Immunol*. 1989;142:877–882.
14. Tu Z, Li Y, Smith DS, et al. Retinal pericytes inhibit activated T cell proliferation. *Invest Ophthalmol Vis Sci*. 2011;52:9005–9010.
15. Kern TS, Tang J, Berkowitz BA. Validation of structural and functional lesions of diabetic retinopathy in mice. *Mol Vis*. 2010;16:2121–2131.
16. Mallone R, Perin PC. Anti-CD38 autoantibodies in type 2 diabetes. *Diabetes Metab Res Rev*. 2006;22:284–294.
17. Limb GA, Chignell AH, Green W, LeRoy F, Dumonde DC. Distribution of TNF alpha and its reactive vascular adhesion molecules in fibrovascular membranes of proliferative diabetic retinopathy. *Br J Ophthalmol*. 1996;80:168–173.
18. Limb GA, Soomro H, Janikoun S, Hollifield RD, Shilling J. Evidence for control of tumour necrosis factor-alpha (TNF-alpha) activity by TNF receptors in patients with proliferative diabetic retinopathy. *Clin Exp Immunol*. 1999;115:409–414.
19. Johnsen-Soriano S, Sancho-Tello M, Arnal E, et al. IL-2 and IFN-gamma in the retina of diabetic rats. *Graefes Arch Clin Exp Ophthalmol*. 2010;48:985–990.
20. Attawia MA, Nayak RC. Circulating antipericyte autoantibodies in diabetic retinopathy. *Retina*. 1999;19:390–400.
21. Nayak RC, Agardh CD, Kwok MG, Stjernquist H, Farthing-Nayak PJ, Agardh E. Circulating anti-pericyte autoantibodies are present in Type 2 diabetic patients and are associated with non-proliferative retinopathy. *Diabetologia*. 2003;46:511–513.
22. Terhorst C, van Agthoven A, LeClair K, Snow P, Reinherz E, Schlossman S. Biochemical studies of the human thymocyte cell-surface antigens T6, T9 and T10. *Cell*. 1981;23:771–780.
23. Jackson DG, Bell JL. Isolation of a cDNA encoding the human CD38 (T10) molecule, a cell surface glycoprotein with an unusual discontinuous pattern of expression during lymphocyte differentiation. *J Immunol*. 1990;144:2811–2815.
24. Ikehata F, Satoh J, Nata K, et al. Autoantibodies against CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) that impair

- glucose-induced insulin secretion in noninsulin-dependent diabetes patients. *J Clin Invest.* 1998;102:395-401.
25. Pupilli C, Giannini S, Marchetti P, et al. Autoantibodies to CD38 (ADP-ribosyl cyclase/cyclic ADP-ribose hydrolase) in Caucasian patients with diabetes: effects on insulin release from human islets. *Diabetes.* 1999;48:2309-2315.
 26. Mallone R, Ortolan E, Baj G, et al. Autoantibody response to CD38 in Caucasian patients with type 1 and type 2 diabetes: immunological and genetic characterization. *Diabetes.* 2001;50:752-762.
 27. Mallone R, Ortolan E, Pinach S, et al. Anti-CD38 autoantibodies: characterisation in new-onset type I diabetes and latent autoimmune diabetes of the adult (LADA) and comparison with other islet autoantibodies. *Diabetologia.* 2002;45:1667-1677.
 28. Lin F, Kaminski HJ, Conti-Fine BM, Wang W, Richmonds C, Medof ME. Markedly enhanced susceptibility to experimental autoimmune myasthenia gravis in the absence of decay-accelerating factor protection. *J Clin Invest.* 2002;110:1269-1274.
 29. Molina H. The murine complement regulator Crry: new insights into the immunobiology of complement regulation. *Cell Mol Life Sci.* 2002;59:220-229.
 30. Zhang J, Gerhardinger C, Lorenzi M. Early complement activation and decreased levels of glycosylphosphatidylinositol-anchored complement inhibitors in human and experimental diabetic retinopathy. *Diabetes.* 2002;51:3499-3504.