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Potential roles of adenosine deaminase-2 in diabetic retinopathy

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ABSTRACT

The early activation of microglia that induces retinal inflammation in DR may serve as a target for therapeutic intervention of DR. Our demonstration that retinal inflammation is attenuated via adenosine receptor A_{2A}AR supports the hypothesis that a mechanism to maintain extracellular concentrations of adenosine important in normal physiology is impaired in DR. Extracellular concentrations of adenosine are regulated by the interplay of equiliberative nucleoside transporter (ENT)s with enzymes of adenosine metabolism including adenosine deaminase-1 (ADA1), adenosine kinase (AK) and CD73. In the vertebrates but not rodents, a macrophage-associated ADA2 is identified. The role of ADA2 is, therefore, understudied as the sequencing probes or antibodies to mouse ADA2 are not available. We identified increased ADA2 expression and activity in human and porcine retinas with diabetes, and in Amadori glycated albumin (AGA)- or hyperglycemia-treated porcine and human microglia. In rodent as well as porcine cells, modulation of TNF-α release is mediated by A_{2A}AR. Quantitative analysis of normal and diabetic porcine retinas reveals that while the expression levels of ADA2, A2AAR, ENT1, TNF-α and MMP9 are increased, the levels of AK are reduced during inflammation as an endogenous protective mechanism. To determine the role of ADA2, we found that AGA induces ADA2 expression, ADA2 activity and TNF-α release, and that TNF-α release is blocked by ADA2-neutralizing antibody or ADA2 siRNA, but not by scrambled siRNA. These results suggest that retinal inflammation in DR is mediated by ADA2, and that the anti-inflammatory activity of A_{2A}AR signaling is impaired in diabetes due to increased ADA2 activity.

1. Introduction

DR is a leading cause of blindness among working-age adults [1]. Treatment options for DR remain limited and with adverse effects [2]. Discovery of new molecular entities with adequate clinical activity for DR remains one of the key research priorities in ophthalmology. Activation of retinal microglia in early diabetes is critical in causing the major complications in DR. Adenosine is elevated at sites of tissue damage resulting from inflammation [3] or hypoxia [4,5]. Adenosine can be formed intracellularly [6,7] and diffuse into the extracellular space via ENTs, or extracellularly from released ATP by ecto-nucleotidases, CD39 and CD73 [8]. Increased adenosine reuptake by ENT allows for adenosine conversion to AMP by adenosine kinase (AK), a predominant pathway for adenosine removal, which leads to inflammation [9]. The pathway for adenosine removal by ADA1 plays only a minor role in regulating adenosinergic function [10]; the major role of ADA1 is to maintain a low level of 2′deoxyadenosine for proper function in immune cells [11]. In addition to ADA1, ADA2 was found in mammals, lower vertebrates and insects as an extracellular enzyme. During inflammation, a disproportionate increase in ADA2 has been found in macrophage-rich tissues including blood [12,13]. The search for a rodent ADA2 gene (also known as cat eye syndrome critical region candidate 1, *CECR1*) by analysis at the critical region (at or near the

Abbreviations: CD73, ecto-5′-nucleotidase; ADA2, adenosine deaminase-2; AK, adenosine kinase; AGA, amadori glycated albumin; ENT1, equilibrative nucleoside transporter 1.

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human chromosome 22 pericentromere) in humans and the region of conserved synteny in mice has not been successful [14–16]. Extracellular adenosine can activate adenosine receptors, which are classified as different subtypes, based on their mechanism of signal transduction [17]. The A_2 receptors stimulate adenylate cyclase through Gs coupling [18]. The increased adenosine at inflamed sites exhibits anti-inflammatory effects to protect against cellular damage through A2AAR [19,20]. We have shown that diabetic A2AAR −/− mice had significantly more cell death, TNF-a release, and ICAM-1 expression compared with diabetic wild-type mice [21]. We have also shown that activation of A2AAR in the stressed retinal microglial cells was the most efficient in mediating TNF-α inhibition [22], and that treatment with the A2AAR agonist resulted in marked decreases in diabetes-induced retinal cell death and TNF-a release [21]. Our recent data demonstrating A2AAR agonist protects against diabetes-induced retinal inflammation suggests that abnormality in adenosine metabolism may contribute to retinal complications in diabetes. By determining the causal relationship between TNF-a release and the expression and activity of ADA2 in porcine models of DR, we present experimental results that suggest that impaired adenosine metabolism in diabetes is at least due to increased expression and activity of ADA2.

2. Materials and methods

2.1. Postmortem eye specimens

Human eyes, 9 non-diabetic and 8 diabetic, obtained from The Georgia Eye Bank (Atlanta, GA) followed the following selection criteria: >50 years old, either insulin requiring diabetes or no diabetes, and no life-support measures. The eyes were enucleated an average of 6.71 ± 0.84 h after death. Aliquots of the same eyes were used in our previous work [23].

2.2. Induction of diabetes

All animal procedures have been approved by the GRU Institutional Animal Care and Use Committee. The procedure of diabetes induction was slightly modified from a previously described procedure [24]. Twelve female Yorkshire pigs weighing 30 kg at arrival were used in the experiment. To six pigs, STZ (50 mg/kg in 0.1 mol/l Na-citrate buffer, pH 4.5) was administered in the ear vein over 1 min each day for 3 days. To the other six pigs, solvent alone was administered. Serum glucose concentrations were measured on a daily basis just prior to injection of STZ, every other day for the next two weeks, and every other week for the entire 12 weeks. The pigs were treated with a subcutaneous injection of short-acting insulin (Normulin, Novo Nordisk) to keep the blood glucose concentration between 350 and 550 mg/dL [25] (Table 1).

Table 1
Diabetic pig blood glucose levels (avg; mg/dl).

Blood glucose mg/dl	
D1	485
D2	412
D3	395
D4	277
D5	358
D6	236
N1	113
N2	75
N3	98
N4	124
N5	115
N6	73

2.3. Western blot analysis

Dissected individual pig retinas were homogenized in RIPA buffer (Upstate, Lake Placid, NY), supplemented with 40 mM NaF, 2 mM Na3VO4, 0.5 mM phenylmethylsulfonyl fluoride and 1:100 (v/v) of proteinase inhibitor cocktail (Sigma). Protein samples of 100 μg were loaded on a gradient gel (4–20%) (Pierce, Rockford, IL), transferred to nitrocellulose membrane and incubated with specific antibodies (Santa Cruz Biotechnology, Santa Cruz, CA), which were detected with a horseradish peroxidase-conjugated antibody and enhanced chemiluminescence (ECL) (Amersham Biosciences, Buckinghamshire, UK). Intensity of immunoreactivity was measured by densitometry.

2.4. Quantitative real-time PCR

Total RNA was isolated from porcine retina using a Promega kit (Promega). Subsequently, cDNAs were generated from 1 μg of total RNA, using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), and subjected to a 40-cycle PCR amplification. The ready-made primer and probe sets were ordered from Applied Biosystems Table 2. Three replicates were run for each gene for each sample in a 96-well plate. 18S RNA was used as the endogenous reference gene.

2.5. RNA interference

Microglial cells were transfected with porcine or control small interfering (si)RNAs Ambion) using HiPerFect (Qiagen) as described by our group [26] per manufacturer's instructions.

2.6. ADA2. activity assay

The ADA2 assay is based on the enzymatic deamination of adenosine at pH6 to inosine, which is converted to hypoxanthine by purine nucleoside phosphorylase (PNP). Hypoxanthine is then converted to uric acid and hydrogen peroxide (H2O2) by xanthine oxidase (XOD). H2O2 is further reacted with N-Ethyl-*N*-(2-hydroxy-3-sulfopropyl)-3-methylaniline (EHSPT) and 4-aminoantipyrine (4-AA) in the presence of peroxidase (POD) to generatequinone dye, which is monitored in a kinetic manner. ADA1 activity is inhibited by ADA1-specific inhibitor EHNA (Diazyme Laboratories, Poway, CA).

Table 2
Primers used for qRT-PCR.

Gene	Primer sequence (5′-3′)	Accessionnumber
TNF-α	AGGAAGAGTTTCCAGCTGGCCC CAACGTGGGCGACGGGCTTA	NM_21402
ADA2	TCGGCACGAGCTCCGAGGAT TCTGGACGTGGCCGAGTGGA	AF384216
MMP-9	GACAGGCAGCTGGCAGAGGAAT GCCGGTTCCAGGGACTGCTT	NM_001038004
A2AAR	CACGCAGAGCTCCATCTTCA ACCAAGCCATTGTACCGGAG	XM_003483462.1
ENT1	CGGGAATTCGATTTCAGTGCCA GCAGGAAGGAGTTGAGGCAG	AJ606303
Cd73	AAGGCTCCACCCTGAAGAAGTA CGTCACGTGAATTCCGCC	XM_001927095.1
AK	CTGTGCATTACTGTACCCTCT CTTCCCGAGAACTCCCGTAT	XM_003359242
18S	TGCATGCTTGCAGGGCGGT GTCCTCGCTGCGGGTGTGGT	AY265350

2.7. Primary retinal microglia cultures from adult pig

Adult Yorkshire pig eyes collected from local slaughter house within one hour post mortem were trimmed to remove attached tissues. Primary cultures of porcine retinal microglial cells were prepared as described previously [27]. Cultures consist of ~95% microglial cells as determined by staining with Iba-1(not shown).

2.8. Human microglial cell culture

The base medium for cell line U937 is ATCC-formulated RPMI-1640 Medium, Catalog No. 30-2001. The complete growth medium contains fetal bovine serum at 10% for use with a 5% CO_2 in air at 37 °C.

2.9. Drug or hypoxia treatment effects on cultured microglial cells

Microglial cells were seeded at a density of 5×10^5 cells/well in a collegen-1-pretreated 12-well tissue culture plate. One day after seeding, the cultured wells were washed with Cellgro Complete (Mediatech, Manassas, VA) and incubated in the same media with various treatments. Cells were pretreated with AR antagonists (all are from Sigma–Aldrich except ZM 241385, which is from Tocris) at the indicated concentrations for 30 min at 37 °C, followed with non-selective AR agonist, adenosine-5′-*N*-ethylcarboxamide (NECA) or vehicle for 30 min at 37 °C. Microglial activation was achieved by addition of Amadori-glycated albumin (AGA; Sigma) with undetectable endotoxin (<0.125 units/mL, 10 EU = 1 ng lipopolysaccharide; Lonza, Basel, Switzerland) [5] to each well at a final concentration of 500 μg/mL at indicated time points [5,8]. After the indicated time course, culture media were collected and assayed for ADA2 activity assay or TNF-α by ELISA. Differentiation of U937 cells to macrophages was achieved by incubation with phorbol 1 2-myristate 13-acetate (PMA, 50 ng/ml) for three days.Microglial cells were placed in 1% oxygen (hypoxic) room air (normoxic) conditions at 37 °C for the indicated time. Both groups were then placed under normoxic conditions for 24 h.

3. Data analysis

The results are expressed as mean ± SD. Differences among experimental groups were evaluated by analysis of variance or *t*-test, and the significance of differences between groups was assessed by the posthoc test (Fisher's PLSD). Significance was defined as $P < 0.05$.

4. Results

4.1. Increased ADA2 expression and activity are identified in human and porcine retinas with diabetes and in AGA- or hyperglycemia-treated microglial cells

An increase in ADA2 expression and activity has been found in the sera from patients with diabetes, suggesting a role of ADA2 in inflammation [28]. However, further study of the role of ADA2 in the animal models of diabetes was hindered because ADA2 gene has not been identified in rodents. To investigate the role of ADA2 in diabetes, we first determined the expression of ADA2 in human donor eyes with diabetes. Using Western analysis, increased expression of ADA2 was identified in the retinas of human donor eyes with diabetes (Fig. 1A). Moreover, increased expression of ADA2 was identified in porcine retinas with 12 weeks of diabetes (Fig. 1B). In the same retinas, expressions of ADA1 and CD73 were found to be unchanged (not shown). Next, ADA2 activity was assayed in the vitreous from the same porcine eyes. The assay was based on the enzymatic deamination of adenosine to inosine in the presence of the ADA1-specific inhibitor EHNA (Diazyme Laboratories, Poway, CA). A >2-fold increase in ADA2 activity was identified in the vitreous from diabetic pigs compared with non-diabetic pigs (Fig. 1C). Retinas from the porcine eyes were also processed for RNA isolation and qRT-PCR analysis, using the expression of MMP9, which is required for inflammatory macrophage migration [29], as a marker for inflammation. As revealed by qRT-PCR analysis, while the expression levels of CD73 remained unchanged, increased expression of TNF-α, MMP9, A2AAR, ENT1 and ADA2, but decreased AK, was identified in porcine retinas with diabetes (Fig. 1D). Further, the effect of hypoxia, hyperglycemia and AGA on ADA2 expression and activity in human microglial cells, U937, were analyzed. As shown (Fig. 1E), ADA2 expression and activity were only detected in PMA-differentiated and hyperglycemia- or AGA-treated, but not in hypoxia-treated cells (not shown).

These results suggest that the alteration of AK expression during inflammation may serve as an endogenous protective mechanism to raise extracellular adenosine levels, and that the up-regulation of all others may be associated with inflammation in the diabetic retina.

4.2. ADA2. is expressed in activated microglia in human and porcine retina with diabetes

During inflammation, a disproportionate increase in ADA2 has been found in macrophage-rich tissues [12,13]. This suggests that ADA2 is involved in inflammation in the macrophages. To determine if ADA2 is involved in inflammation in the activated retinal microglial cells in diabetes, immunolabeling of ADA2 (green) and Iba-1 (red), a marker of activated microglia or macrophages, was made in human and porcine retinas under normal and diabetic conditions. Co-localization of ADA2 and Iba1, as indicated by the merged red and green (yellowish orange, shown on the cell surface in the enlarged inset), is only identified in retinas with diabetes (Fig. 2). It is noted that essentially all the Iba1-labelled cells are also ADA2-labelled. These results suggest that ADA2 up-regulation in the diabetic porcine retina occurs in the activated microglia or macrophages.

4.3. Anti-inflammatory A2AAR-signaling is identified in AGA-activated porcine retinal microglial cells

Adenosine signaling via A2AAR in microglia plays a major role in anti-inflammation in rodent retina during diabetes [21]. To test whether A2AAR has a role in the anti-inflammation function in porcine retinal microglia in diabetes, PRMC were treated with AGA in the presence of a non-selective AR agonist with/without AR-selective antagonists and TNF-α release was determined. Treatment of microglia with AGA, a risk factor for diabetes [26], has been shown to simulate inflammation [30–32]. As shown in Fig. 3A, treatment of porcine retinal microglial cells with AGA triggered a prominent increase in TNF-α release, which was potently inhibited by pre-incubation with 2 μmol/L nonselective AR agonist (adenosine-5′-*N*-ethylcarboxamide (NECA)). When the cells were pretreated with the A1AR antagonist (8-cyclopentyl-1,3-dipropylxanthine (CPX); 0.1 μmol/L), the A2BAR antagonist [8-[4-[((4-cyanophenyl) arbamoylmethyl) oxy]phenyl]-1,3-di(*n*-propyl) xanthine hydrate (MRS 1754); 1 μmol/L], or the A3AR antagonist [3-propyl-6-ethyl-5[(ethylthio)carbonyl]-2-phenyl-4-propyl-3-pyridine-carboxylate; 2-chloro-N6-cyclopentyladenosine, 2-chloro-N6-cyclopentyladenosine (MRS 1523); 10 μmol/L], the inhibitory effect of adenosine-5′-*N*-ethylcarboxamide on TNF-α release was not affected. However, this effect was successfully blocked by the A2AAR antagonist (ZM241385; 0.1 μmol/L). These results identify in pig a

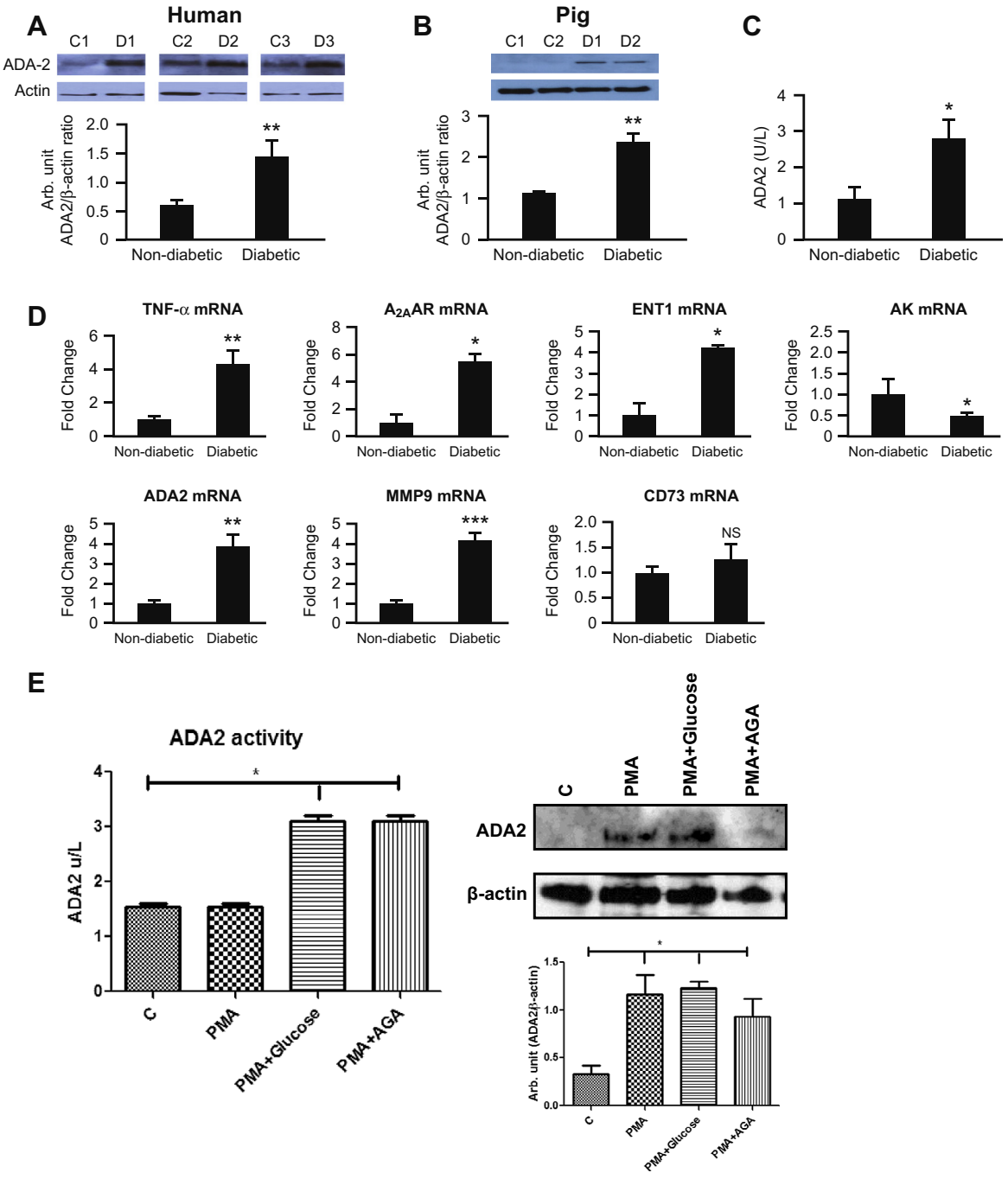

Fig. 1. Expression of ADA2 in retinas with diabetes and in microglial cells. Expression of ADA2 was determined by Western analysis in the retinas of, (A) human donor eyes with and without diabetes; (B) normal porcine eyes and eyes with 12 weeks of diabetes. (C) control or normal; (D) diabetic. Data shown are the mean + SD of four experiments. **Significant compared to normal ($P < 0.005$). (C) ADA2 activity was assayed in the vitreous from the porcine eyes with and without 12 weeks of diabetes. Data shown are the mean + SD of four experiments. *Significant compared to normal ($P < 0.01$). (D) Expression of TNF-α, ADA2, MMP9, A2AAR, ENT1, AK, and CD73 was determined by qRT-PCR analysis in porcine retinas with and without 12 weeks of diabetes. Data shown are the mean + SD of three – six experiments. *Significant compared to normal ($P < 0.01$). (E) Effects of hypoxia (1% oxygen, 8 h), hyperglycemia (35 mM vs. 10 mM) and AGA (500 μg/ml) treatment in ADA2 expression and activity in the un-differentiated and PMA-differentiated U937 cells. Data shown are the mean + SD of three experiments. *Significant compared to normal control ($P < 0.01$).

signaling through A2AAR as a critical control point for TNF-α release in AGA-treated retinal microglial cells.

4.4. AGA-induced TNF-α release in retinal microglia is mediated by ADA2

In light of ADA2′s ability to associate with inflammation in the activated retinal microglia, whether or not ADA2 is involved in AGA-induced TNF-α release was investigated. To determine the causal role of ADA2 in mediating TNF-α release, two approaches were used. First, an ADA2-neutralizing antibody, significantly inhibited AGA-induced TNF-α release nearly in a dose-dependent manner (Fig. 3B). Second, porcine retinal microglial cells were transiently transfected with ADA2 siRNA or scrambled siRNA and treated with AGA as before. Treatment of microglia with AGA enhanced TNF-α release, and this effect was significantly reduced in cells transfected with ADA2 siRNA, but not scrambled siRNA (Fig. 3C). This result was confirmed by decreased ADA2 activity in cells

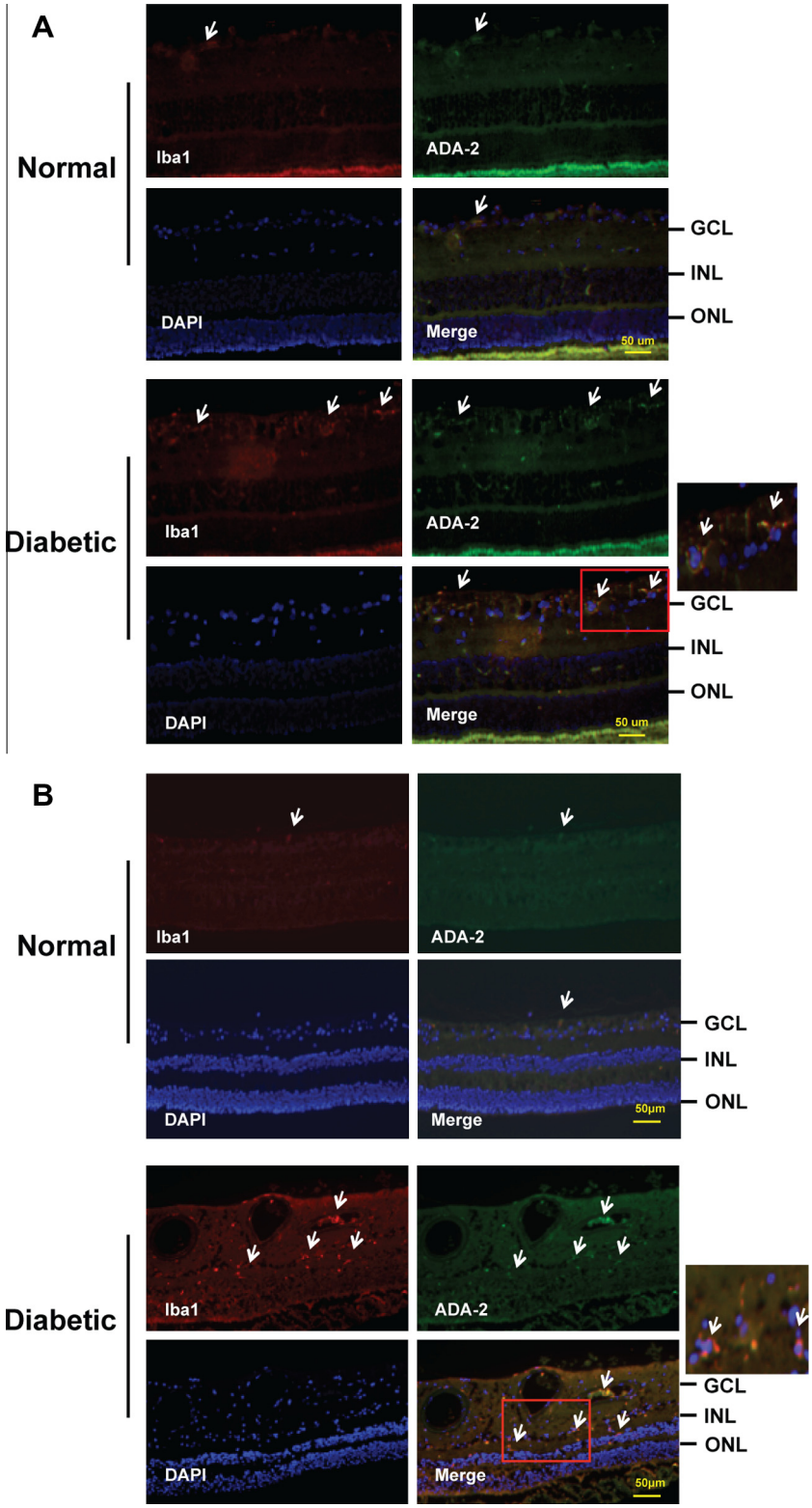

Fig. 2. Localization of ADA2 in activated microglia in human and porcine retina with diabetes Immunolabeling of ADA2 (green) and Iba-1 (red), a marker of activated microglia or macrophages, was made in human and porcine retinas under normal and diabetic conditions. Co-localization of ADA2 and Iba1, as indicated by the merged red and green on the cell surface (enlarged), is only identified in retinas with diabetes.

transfected with ADA2 siRNA, but not scrambled siRNA. This result suggests that ADA2 mediates AGA-induced TNF-α release in retinal microglia.

5. Discussion

During inflammation, increased ADA2 activity has been found in macrophage-rich tissues [12,13]. ADA2 activity is elevated sig-

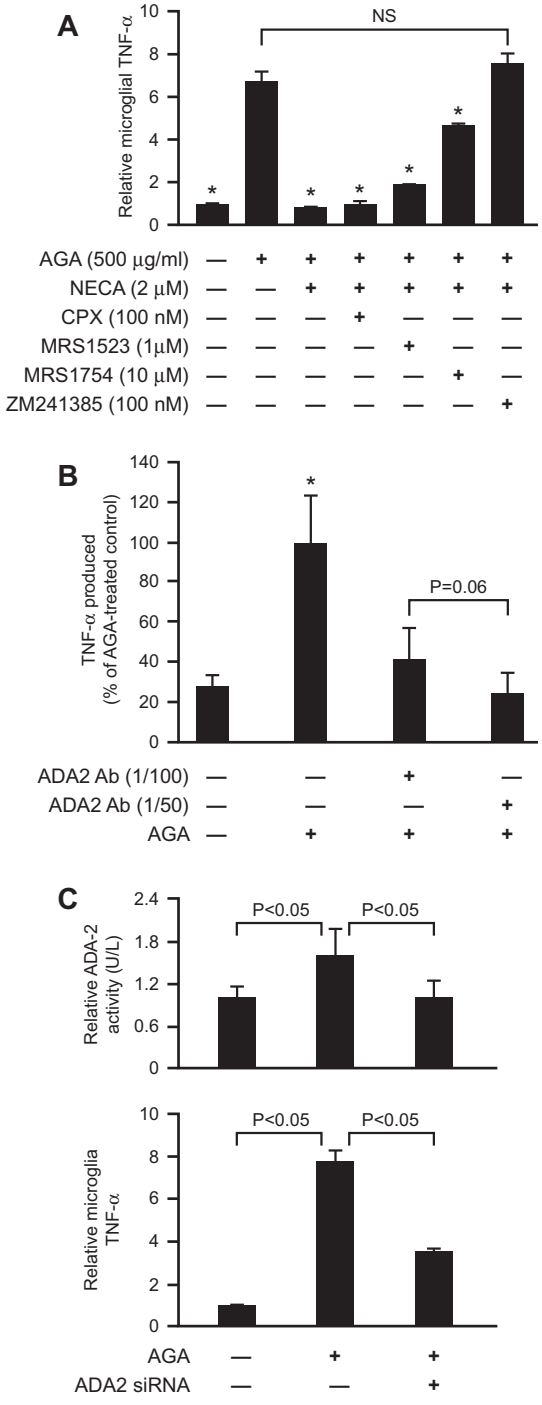

Fig. 3. AGA-induced TNF-α release in the porcine retinal microglia is modulated by A2AAR and mediated by ADA2. (A) Effect of nonselective AR activation on AGA-induced TNF-α release in porcine retinal microglial cells in the presence of AR subtype-selective AR antagonists. TNF-α production was determined in cells treated with AGA in the presence or absence of the nonselective AR agonist adenosine-5′-*N*-ethylcarboxamide (1 μmol/L) and subtype-selective AR antagonists for A1AR (8-cyclopentyl-1,3-dipropylxanthine, 0.1 μmol/L), A2AAR (ZM 241385, 0.1 μmol/L), A2BAR (MRS 1754, 1 μmol/L), and A3AR (MRS 1523, 10 μmol/L). TNF-α levels were determined by ELISA. Data shown are the mean + SD of five experiments. *Significant compared to AGA- treated alone ($P < 0.05$). (B) Isolated primary microglial cells from porcine retina were treated with an ADA2-neutralizing antibody, treated with AGA, and TNF-α release was determined by ELISA. Data shown are the mean + SD of five experiments. *Significant compared to the negative control and to the antibody-treated ($P < 0.05$). (C) Primary microglial cells from porcine retina were transiently transfected with scrambled siRNA or siRNA specific for ADA2. Cells were subsequently exposed to AGA, and media were used for ADA2 activity using an ADA2 activity kit and for TNF-α using ELISA. Data shown are the mean + SD of five experiments.

nificantly in pleural fluids of patients with pulmonary tuberculosis [33], sera from HIV-infected individuals [34,35], and from patients with diabetes [28], making ADA2 activity a convenient marker to improve the diagnosis and follow-up treatment of these disorders. In contrast to ADA1, ADA2 activity for adenosine requires high levels of adenosine and low optimum pH of 6.5, and it shows a weak affinity for 2′deoxyadenosine. This suggests that ADA2 expresses its activity only at conditions that are associated with hypoxia or inflammation [36–38]. However, there has been no study on the activity or expression of ADA2 in the eye during inflammation in diabetes. The present study is the first to identify increased ADA2 expression and activity in the retina and vitreous of porcine model of diabetes, and increased ADA2 expression in the retina of human donor eyes with diabetes. This study is also the first to demonstrate that A2AAR and ENT1 are up-regulated, CD73 remains unchanged, and AK is down-regulated during inflammation. These findings are consistent with previous studies in A2AAR [39], AK [9,10], ENT1 [40], and CD73 [41]. The reduced expression levels of AK may serve as an endogenous protective mechanism poised to raise extracellular adenosine levels. The present study also shows that up-regulated levels of ADA2 are associated with inflammation in diabetes. The elevated ADA2 activity may upgrade retinal inflammation by metabolizing extracellular adenosine, causing local adenosine deficiency, thereby reducing the anti-inflammation activity of the A2AAR signaling. In rodent in which ADA2 is missing, the local adenosine deficiency is likely caused by the up-regulation of ENT1 with increased adenosine re-uptake and metabolism. These results may also be explained by the growth factor-like activity of ADA2, which promotes microglia activation and proliferation of macrophages [42]. Future directions may include efforts to determine the ambient adenosine levels and AK activities in porcine retinas with or without diabetes, as well as efforts to determine the activation and proliferation rate of microglia over-expressing ADA2 compared with the control. Collectively, the experiments in the present and previous studies [21,22,26] provide new insights in understanding the pathogenesis of early features of DR, demonstrating that the accumulation of AGA within the diabetic retina elicits microglial and ADA2 activation, and secretion of TNF-α. More deeply, our in vitro data disclose the molecular mechanisms involved therein, showing that AGA triggers TNF-α release from microglia and its subsequent mechanisms of anti-inflammation involving adenosine. Thus, clinical intervention trials with agents that neutralize AGA and ADA2 individually or in combination might be warranted in modulating early pathological pathways long before the occurrence of vision loss among diabetics.

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References

[1] R. Klein, B.E. Klein, S.E. Moss, M.D. Davis, D.L. DeMets, The Wisconsin epidemiologic study of diabetic retinopathy. II. Prevalence and risk of diabetic retinopathy when age at diagnosis is less than 30 years, Arch. Ophthalmol. 102 (1984) 520–526.
[2] K. Manousaridis, J. Talks, Macular ischaemia: a contraindication for anti-VEGF treatment in retinal vascular disease?, Br. J. Ophthalmol. 96 (2012)179–184.
[3] C. Martin, M. Leone, X. Viviand, M.L. Ayem, R. Guieu, High adenosine plasma concentration as a prognostic index for outcome in patients with septic shock, Crit. Care Med. 28 (2000) 3198–3202.
[4] H.R. Winn, R. Rubio, R.M. Berne, Brain adenosine concentration during hypoxia in rats, Am. J. Physiol. 241 (1981) H235–242.
[5] B. Sperlagh, M. Doda, M. Baranyi, G. Hasko, Ischemic-like condition releases norepinephrine and purines from different sources in superfused rat spleen strips, J. Neuroimmunol. 111 (2000) 45–54.

[6] G.B. Sala-Newby, A.C. Skladanowski, A.C. Newby, The mechanism of adenosine formation in cells. Cloning of cytosolic 5′-nucleotidase-I, J. Biol. Chem. 274 (1999) 17789–17793.
[7] J.L. Palmer, R.H. Abeles, The mechanism of action of S-adenosylhomocysteinase, J. Biol. Chem. 254 (1979) 1217–1226.
[8] P.B. Ernst, J.C. Garrison, L.F. Thompson, Much ado about adenosine: adenosine synthesis and function in regulatory T cell biology, J. Immunol. 185 (2010) 1993–1998.
[9] D. Boison, Adenosine kinase, epilepsy and stroke: mechanisms and therapies, Trends Pharmacol. Sci. 27 (2006) 652–658.
[10] M.A. Pak, H.L. Haas, U.K. Decking, J. Schrader, Inhibition of adenosine kinase increases endogenous adenosine and depresses neuronal activity in hippocampal slices, Neuropharmacology 33 (1994) 1049–1053.
[11] S. Adanin, I.V. Yalovetskiy, B.A. Nardulli, A.D. Sam 2nd, Z.S. Jonjev, W.R. Law, Inhibiting adenosine deaminase modulates the systemic inflammatory response syndrome in endotoxemia and sepsis, Am. J. Physiol. Regul. Integr. Comp. Physiol. 282 (2002) R1324–1332.
[12] M. Gorguner, M. Cerci, I. Gorguner, Determination of adenosine deaminase activity and its isoenzymes for diagnosis of pleural effusions, Respirology 5 (2000) 321–324.
[13] J.P. Ungerer, H.M. Oosthuizen, J.H. Retief, S.H. Bissbort, Significance of adenosine deaminase activity and its isoenzymes in tuberculous effusions, Chest 106 (1994) 33–37.
[14] R. Iijima, T. Kunieda, S. Yamaguchi, H. Kamigaki, I. Fujii-Taira, K. Sekimizu, T. Kubo, S. Natori, K.J. Homma, The extracellular adenosine deaminase growth factor, ADGF/CECR1, plays a role in Xenopus embryogenesis via the adenosine/P1 receptor, J. Biol. Chem. 283 (2008) 2255–2264.
[15] B.A. Conlon, W.R. Law, Macrophages are a source of extracellular adenosine deaminase-2 during inflammatory responses, Clin. Exp. Immunol. 138 (2004) 14–20.
[16] T.K. Footz, P. Brinkman-Mills, G.S. Banting, S.A. Maier, M.A. Riazi, L. Bridgland, S. Hu, B. Birren, S. Minoshima, N. Shimizu, H. Pan, T. Nguyen, F. Fang, Y. Fu, L. Ray, H. Wu, S. Shaull, S. Phan, Z. Yao, F. Chen, A. Huan, P. Hu, Q. Wang, P. Loh, S. Qi, B.A. Roe, H.E. McDermid, Analysis of the cat eye syndrome critical region in humans and the region of conserved synteny in mice: a search for candidate genes at or near the human chromosome 22 pericentromere, Genome Res. 11 (2001) 1053–1070.
[17] M.G. Collis, S.M. Hourani, Adenosine receptor subtypes, Trends Pharmacol. Sci. 14 (1993) 360–366.
[18] B.B. Fredholm, M.P. Abbracchio, G. Burnstock, J.W. Daly, T.K. Harden, K.A. Jacobson, P. Leff, M. Williams, Nomenclature and classification of purinoceptors, Pharmacol. Rev. 46 (1994) 143–156.
[19] G.W. Bong, S. Rosengren, G.S. Firestein, Spinal cord adenosine receptor stimulation in rats inhibits peripheral neutrophil accumulation. The role of Nmethyl-D-aspartate receptors, J. Clin. Invest. 98 (1996) 2779–2785.
[20] V. Ralevic, G. Burnstock, Receptors for purines and pyrimidines, Pharmacol. Rev. 50 (1998) 413–492.
[21] A.S. Ibrahim, M.M. El-Shishtawy, W. Zhang, R.B. Caldwell, G.I. Liou, A(A) adenosine receptor (A(A)AR) as a therapeutic target in diabetic retinopathy, Am. J. Pathol. 178 (2011) 2136–2145.
[22] G.I. Liou, J.A. Auchampach, C.J. Hillard, G. Zhu, B. Yousufzai, S. Mian, S. Khan, Y. Khalifa, Mediation of cannabidiol anti-inflammation in the retina by equilibrative nucleoside transporter and A2A adenosine receptor, Invest. Ophthalmol. Vis. Sci. 49 (2008) 5526–5531.
[23] T.K. Ali, S. Matragoon, B.A. Pillai, G.I. Liou, A.B. El-Remessy, Peroxynitrite mediates retinal neurodegeneration by inhibiting nerve growth factor survival signaling in experimental and human diabetes, Diabetes 57 (2008) 889–898.
[24] R.G. Gerrity, R. Natarajan, J.L. Nadler, T. Kimsey, Diabetes-induced accelerated atherosclerosis in swine, Diabetes 50 (2001) 1654–1665.
[25] P. Velander, C. Theopold, T. Hirsch, O. Bleiziffer, B. Zuhaili, M. Fossum, D. Hoeller, R. Gheerardyn, M. Chen, S. Visovatti, H. Svensson, F. Yao, E. Eriksson, Impaired wound healing in an acute diabetic pig model and the effects of local hyperglycemia, Wound Repair Regen. 16 (2008) 288–293.
[26] A.S. Ibrahim, A.B. El-Remessy, S. Matragoon, W. Zhang, Y. Patel, S. Khan, M.M. Al-Gayyar, M.M. El-Shishtawy, G.I. Liou, Retinal microglial activation and inflammation induced by amadori-glycated albumin in a rat model of diabetes, Diabetes 60 (2011) 1122–1133.
[27] T. Moller, J.J. Contos, D.B. Musante, J. Chun, B.R. Ransom, Expression and function of lysophosphatidic acid receptors in cultured rodent microglial cells, J. Biol. Chem. 276 (2001) 25946–25952.
[28] T. Hoshino, K. Yamada, K. Masuoka, I. Tsuboi, K. Itoh, K. Nonaka, K. Oizumi, Elevated adenosine deaminase activity in the serum of patients with diabetes mellitus, Diabetes Res. Clin. Pract. 25 (1994) 97–102.
[29] Y. Gong, E. Hart, A. Shchurin, J. Hoover-Plow, Inflammatory macrophage migration requires MMP-9 activation by plasminogen in mice, J. Clin. Invest. 118 (2008) 3012–3024.
[30] M.P. Cohen, V.Y. Wu, J.A. Cohen, Glycated albumin stimulates fibronectin and collagen IV production by glomerular endothelial cells under normoglycemic conditions, Biochem. Biophys. Res. Commun. 239 (1997) 91–94.
[31] L.P. Yang, H.L. Sun, L.M. Wu, X.J. Guo, H.L. Dou, M.O. Tso, L. Zhao, S.M. Li, Baicalein reduces inflammatory process in a rodent model of diabetic retinopathy, Invest. Ophthalmol. Vis. Sci. 50 (2009) 2319–2327.
[32] J. Nevado, C. Peiro, S. Vallejo, M. El-Assar, N. Lafuente, N. Matesanz, V. Azcutia, E. Cercas, C.F. Sanchez-Ferrer, L. Rodriguez-Manas, Amadori adducts activate nuclear factor-kappaB-related proinflammatory genes in cultured human peritoneal mesothelial cells, Br. J. Pharmacol. 146 (2005) 268–279.
[33] L. Valdes, A. Pose, E. San Jose, J.M. Martinez Vazquez, Tuberculous pleural effusions, Eur. J. Intern. Med. 14 (2003) 77–88.
[34] S. Chittiprol, P. Satishchandra, R.S. Bhimasenarao, G.R. Rangaswamy, S.V. Sureshbabu, D.K. Subbakrishna, K.S. Satish, A. Desai, V. Ravi, K.T. Shetty, Plasma adenosine deaminase activity among HIV1 Clade C seropositives: relation to CD4 T cell population and antiretroviral therapy, Clin. Chim. Acta 377 (2007) 133–137.
[35] J.G. Niedzwicki, N.M. Kouttab, K.H. Mayer, C.C. Carpenter, R.E. Parks Jr., E. Abushanab, D.R. Abernethy, Plasma adenosine deaminase2: a marker for human immunodeficiency virus infection, J. Acquir. Immune Defic. Syndr. 4 (1991) 178–182.
[36] C. Gakis, A. Cappio-Borlino, G. Pulina, Enzymes (isoenzyme system) as homeostatic mechanisms the isoenzyme (ADA2) of adenosine deaminase of human monocytes-macrophages as a regulator of the 2′deoxyadenosine, Biochem. Mol. Biol. Int. 46 (1998) 487–494.
[37] M.V. Sitkovsky, D. Lukashev, S. Apasov, H. Kojima, M. Koshiba, C. Caldwell, A. Ohta, M. Thiel, Physiological control of immune response and inflammatory tissue damage by hypoxia-inducible factors and adenosine A2A receptors, Annu. Rev. Immunol. 22 (2004) 657–682.
[38] A.V. Zavialov, A. Engstrom, Human ADA2 belongs to a new family of growth factors with adenosine deaminase activity, Biochem. J. 391 (2005) 51–57.
[39] J.M. Duarte, C.R. Oliveira, A.F. Ambrosio, R.A. Cunha, Modification of adenosine A1 and A2A receptor density in the hippocampus of streptozotocin-induced diabetic rats, Neurochem. Int. 48 (2006) 144–150.
[40] G.P. Leung, R.Y. Man, C.M. Tse, D-Glucose upregulates adenosine transport in cultured human aortic smooth muscle cells, Am. J. Physiol. Heart Circ. Physiol. 288 (2005) H2756–2762.
[41] L.F. Thompson, H.K. Eltzschig, J.C. Ibla, C.J. Van De Wiele, R. Resta, J.C. Morote-Garcia, S.P. Colgan, Crucial role for ecto-5′-nucleotidase (CD73) in vascular leakage during hypoxia, J. Exp. Med. 200 (2004) 1395–1405.
[42] A.V. Zavialov, E. Gracia, N. Glaichenhaus, R. Franco, A.V. Zavialov, G. Lauvau, Human adenosine deaminase 2 induces differentiation of monocytes into macrophages and stimulates proliferation of T helper cells and macrophages, J. Leukoc. Biol. 88 (2010) 279–290.