ARVO 2017 Annual Meeting Abstracts

234 Immunological influences on AMD

Monday, May 08, 2017 11:00 AM–12:45 PM
Room 310 Paper Session
Program #/Board # Range: 1623–1628
Organizing Section: Immunology/Microbiology

Program Number: 1623
Presentation Time: 11:00 AM–11:15 AM
Incidence of Age-Related Macular Degeneration in Patients with the Acquired Immune Deficiency Syndrome

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Purpose: Patients with the Acquired Immune Deficiency Syndrome (AIDS) have an estimated 4-fold increased age-adjusted prevalence of intermediate-stage age-related macular degeneration (AMD). We investigated the incidence of intermediate-stage AMD in patients with AIDS.

Methods: Participants in the Longitudinal Study of the Ocular Complications of AIDS (LSOCA) cohort study (all of whom had AIDS and no ocular infections) had follow-up photographs taken at 5 and 10 years after enrollment. Photographs were graded in a masked fashion at a centralized imaging reading center using the Age-Related Eye Disease Study-2 (AREDS-2) scoring system. The main outcome measure was intermediate-stage AMD. The incidence of AMD in LSOCA was compared to the published incidence in an HIV-uninfected cohort, the Multi-Ethnic Study of Atherosclerosis (MESA) study, which used a similar photographic methodology. The comparison was race- and gender-adjusted using Poisson regression.

Results: Follow-up photographs were available on 717 participants in LSOCA. The demographic distribution of the population was: 52% non-Hispanic white, 34% non-Hispanic African American, 13% Hispanic, and 1% Asian; 83% men and 17% women. The mean (+/- standard deviation) age of this population was 44 +/- 8 years. 4.7% of participants developed intermediate-stage AMD during follow-up. The incidence of intermediate-stage AMD was 6.5/1000 person-years (PY). The mean age of the comparator MESA cohort was 61 +/- 9 years. The race- and gender-adjusted relative risk for AMD in the LSOCA cohort compared to the MESA cohort was 1.75 (95% confidence interval 1.16-2.64), P=0.008.

Conclusions: There was an apparent 75% greater incidence of intermediate-stage AMD in patients with AIDS compared to an HIV-uninfected cohort. These data are consistent with with the accentuated activation, and systemic inflammation seen in these patients.

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Program Number: 1624
Presentation Time: 11:15 AM–11:30 AM
Retinal monocyte-derived complement, not systemically derived complement contributes to the early onset of focal retinal degeneration

Haihan Jiao1, Riccardo Natoli1, 2, Nilisha Fernando1, Tanja Racic1, Joshua Chu-Tan1, Krisztina Valter1, 2, Matt Rutar1, Jan Provis1, 2.

1John Curtin School of Medical Research, Australian National University, Canberra, ACT, Australia; 2Australian National University Medical School, Canberra, ACT, Australia.

Purpose: The complement cascade is associated with pathogenesis of retinal dystrophies including age-related macular degeneration (AMD) although the cellular events that initiate the cascade remains unclear. In this study, we aimed to determine the functional significance of C3 derived from local/retinal sources and systemically-derived C3, and compared the findings with C3 localized in human donor eyes.

Methods: Photo-oxidative damage was used for a focal lesion in rat and mouse retinas, according to published protocols (Rutar et al., 2011; Natoli et al., 2016). The effect of C3 inhibition on the focal retinal degeneration was studied using C3–/– mice, and intravitreal C3-specific siRNA in wildtype mice to locally deplete C3, and intraperitoneally administered cobra venom factor CVF to deplete C3 systemically. Animals were assessed for using electoretinogram(ERG), immunohistochemistry, in situ hybridisation, and qRT-PCR. Human retinas were assessed using immunohistochemistry and in situ hybridization for C3

Results: Human retinas from AMD patients with atrophic lesions showed C3 mRNA expressed by retinal monocytes within lesions and at the lesions edge, in the subretinal space, optic nerve head and inner retina. In focal lesions of photo-oxidative damaged rodent retina, complement gene expression increased significantly (P=0.0244), with strong immunoreactivity to C3d in retinal lesions in cells that co-localized the monocytes. C3+ retinas had significantly reduced photoreceptor cell death post damage (P=0.0014), a better preserved photoreceptor layer and improved retinal function compared to wildtypes (P=0.005). Local C3 inhibition using siRNA showed significantly reduced C3 gene expression in retinas post damage (P=0.034), accompanied by reduced C3 deposits in the outer retina, thicker photoreceptor layer and higher ERG responses compared to negative-siRNA controls (P=0.036). Systemic complement depletion by CVF had no effect on complement gene expression and did not mitigate the effects of photooxidative damage on retinal morphology or function (P=0.43)

Conclusions: Local C3 deposition by retinal monocytes, not systemic complement contributes to the progression of retinal degeneration. This study emphasises that targeting local complement specifically on retinal monocytes population could be a potential approach to slow down the progression of retinal dystrophy.

Commercial Relationships: Haihan Jiao, None; Riccardo Natoli, None; Nilisha Fernando, None; Tanja Racic, None; Joshua Chu-Tan, None; Krisztina Valter, None; Matt Rutar, None; Jan Provis, None
Deletion of complement factor H is associated with accumulation of subretinal Iba-1-positive cells and retinal degeneration in aged mice


Purpose: Despite the contrasting roles that complement factor H (Cfh) and complement factor P (Cfp) play in alternative pathway regulation, it has been reported that deletion of Cfp in the Cfh-deficient mouse model of dense deposit disease results in exacerbation, rather than moderation, of the glomerulopathy. In order to investigate the role of Cfh and Cfp in complement-mediated eye pathology, Cfh and Cfp single and double knock-out mice were evaluated.

Methods: Male and female Cfh and Cfp single and double knock-out mice were aged from 2 to 9 months. Animals were assessed by fundus photography, optical coherence tomography (OCT), and scanning laser ophthalmoscopy (SLO). Ocular flatmounts and tissue sections were examined for F4/80- and Iba-1-positive cells. Kidney sections were immunostained for complement and Iba-1. Urine was collected to measure albuminuria as an indicator of renal function. Kidney samples were assessed for cytokine production by multiplex cytokine analysis. The two-tailed unpaired t-test was used for statistical analyses.

Results: Cfh-/- Cfp-/- and Cfh-/- Cfp+ mice exhibited retinal flecks by funduscopy (15/19 and 16/21 mice, respectively) and SLO imaging (7/7 and 8/8 mice, respectively). OCT revealed retinal thinning in a subset of these animals (Cfh-/- Cfp+, 3/8 mice; Cfh-/- Cfp-/-, 3/8 mice) that was confirmed histologically, and subretinal Iba-1-positive cells were identified in both Cfh-/- Cfp+/- mice (4/4) and Cfh-/- Cfp-/- mice (4/4). No eye abnormalities were observed in either littermate control Cfh+/- Cfp+/- mice or Cfh+/- Cfp+/- mice. An increase in inflammatory proteins (IL-1B, p<0.01 and TNFα, p<0.05) and complement deposition (p<0.05) was observed in the kidneys of Cfh-/- Cfp+/- animals. Cfh-/- Cfp+/- mice exhibited an additional increase in inflammatory proteins (IL-1β, p<0.001, and TNFα, p<0.05), complement deposition (p<0.01) in the kidneys, and microalbuminuria, as compared to the Cfh-/- Cfp+/- mice (p<0.01).

Conclusions: Aged Cfh+/- Cfp-/- and Cfh+/- Cfp+/- mice exhibited retinal abnormalities, specifically retinal flecks, atrophy, retinal thinning and accumulation of subretinal Iba-1-positive cells. The ocular pathology was not observed in Cfh-/- Cfp+/- mice. Our results provide evidence that chronic over-activation of complement due to a deficiency in complement factor H recapitulates characteristic features of AMD.

Commercial Relationships: Sha-Mei Liao, Novartis (E); Natasha Buchanan, Novartis (E); John Demirs, Novartis (E); Barrett Leehy, Novartis (E); Casey Lewis, Novartis (E); Junzheng Yang, Novartis (E); Vanessa Davis, Novartis (E); Nan Li, Rangaswamy, Novartis (E); Maura Crowley, Novartis (E); Karen Anderson, Novartis (E); Chad E. Bigelow, Novartis (E); Thaddeus P. Dryja, Novartis (E); Bruce D. Jaffe, Novartis (E)

Plasma level of lipocalin-2 is increased in neovascular age-related macular degeneration, particularly in patients with macular fibrosis

Nan Yang, Judith Lechner, Ruth E. Hogg, Levente Toth, Giuliana Silvestri, Usha Chakravarthy, Mei Chen, Heping Xu.

1Queen’s university Belfast, Center for experimental medicine, Belfast, United Kingdom; 2Queen’s university Belfast, Institute for Health Sciences, Belfast, United Kingdom; 3Royal Hospital, Belfast, United Kingdom.

Purpose: Previously we have shown that the population of circulating neutrophil is increased in neovascular age-related macular degeneration (nAMD). The aim of this study was to investigate the plasma level of neutrophil gelatinase-associated lipocalin (NGAL, or lipocalin-2, LCN2) and metalloprotease 9 (MMP9), a regulatory factor of neutrophil transmembrane migration, and MMP9/LCN2 complex in different types of nAMD.

Methods: Two hundred and thirteen participants older than 50 years of age, including 170 nAMD and 43 controls were enrolled from the retina clinics in Belfast. Clinical information, on gender, hypertension, diabetes, medication, smoking habits, family history of AMD and body mass index (BMI) etc., were collected using a structured questionnaire. AMD subtypes, the presence/absence of macular fibrosis and macular atrophy were evaluated using color and fluorescein images, AF and OCT images. Plasma samples were collected and stored at -80°C for the measurement of LCN2, MMP9, and MMP9/LCN2 using commercial human enzyme-linked immunosorbent assay (ELISA) kits.

Results: The plasma level of LCN2, but not MMP9 or MMP9/LCN2 was significantly higher in nAMD patients compared to that in healthy controls. LCN2 positively correlated with the percentage of circulating neutrophils in nAMD patients but not in controls. Further analysis of different types of nAMD showed significantly higher levels of LCN2 in CNV (n = 170) but not retinal angiomatous proliferation (RAP, n = 32) or polypoidal choroidal vasculopathy (PCV, n = 23) compared to that in controls. nAMD patients with fibrosis (n = 58) had significantly higher levels of LCN2 compared to controls in both univariate (p=0.015) and multivariate (after adjusting for age, p=0.033). There was no significant difference in the levels of LCN2 in patients with and without macular atrophy.

Conclusions: Our results suggest that higher levels of circulating neutrophils that are present in patients with nAMD may be responsible for increased plasma levels of LCN2. Our results also suggest that higher plasma level of LCN2 is associated with macular fibrosis in nAMD. The role of LCN2 in macular fibrosis warrants further investigation.

Commercial Relationships: Nan Yang, None; Judith Lechner, None; Ruth E. Hogg, None; Levente Toth, None; Giuliana Silvestri, None; Usha Chakravarthy, None; Mei Chen, None; Heping Xu, None

Support: Dunhill Medical Trust (R188/0211) and Guide Dogs for the Blind Association UK (2008-5a)

Plasma level of lipocalin-2 is increased in neovascular age-related macular degeneration, particularly in patients with macular fibrosis

Sofia Theodoropoulou, David A. Copland, Jian Liu, Jiahui Wu, Andrew D. Dick.

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Program Number: 1627

Presentation Time: 12:00 PM–12:15 PM

Interleukin 33 attenuates choroidal neovascularization by activating mast cells

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Program Number: 1626

Presentation Time: 11:45 AM–12:00 PM

Plasma level of lipocalin-2 is increased in neovascular age-related macular degeneration, particularly in patients with macular fibrosis

Nan Yang, Judith Lechner, Ruth E. Hogg, Levente Toth, Giuliana Silvestri, Usha Chakravarthy, Mei Chen, Heping Xu.

1Queen’s university Belfast, Center for experimental medicine, Belfast, United Kingdom; 2Queen’s university Belfast, Institute for Health Sciences, Belfast, United Kingdom; 3Royal Hospital, Belfast, United Kingdom.

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Methods: Two hundred and thirteen participants older than 50 years of age, including 170 nAMD and 43 controls were enrolled from the retina clinics in Belfast. Clinical information, on gender, hypertension, diabetes, medication, smoking habits, family history of AMD and body mass index (BMI) etc., were collected using a structured questionnaire. AMD subtypes, the presence/absence of macular fibrosis and macular atrophy were evaluated using color and fluorescein images, AF and OCT images. Plasma samples were collected and stored at -80°C for the measurement of LCN2, MMP9, and MMP9/LCN2 using commercial human enzyme-linked immunosorbent assay (ELISA) kits.

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Conclusions: Our results suggest that higher levels of circulating neutrophils that are present in patients with nAMD may be responsible for increased plasma levels of LCN2. Our results also suggest that higher plasma level of LCN2 is associated with macular fibrosis in nAMD. The role of LCN2 in macular fibrosis warrants further investigation.

Commercial Relationships: Nan Yang, None; Judith Lechner, None; Ruth E. Hogg, None; Levente Toth, None; Giuliana Silvestri, None; Usha Chakravarthy, None; Mei Chen, None; Heping Xu, None

Support: Dunhill Medical Trust (R188/0211) and Guide Dogs for the Blind Association UK (2008-5a)
Purpose: We have reported a protective role of pro-inflammatory cytokine, interleukin 33, in choroidal neovascularization (CNV) formation, by attenuating wound-healing responses. Mast cells are associated with fibrosis and are also known target cells of IL-33, but their role in CNV is not known. Based on our finding that RPE-derived IL-33 could activate bone-marrow-derived mast cells (BMMC), we hypothesized that IL-33 attenuated CNV by activating mast cells.

Methods: Upon treatment, RPE cells (ARPE-19 and B6-RPE07) and bone-marrow-derived mast cells (BMMC) were assayed by RT-PCR and Western Blot and ELISA. Choroidal sprouting assay and laser-induced choroidal neovascularization (CNV) were used as models of ocular angiogenesis. RPE-choroid explants were treated with various doses of IL-33 and mast cell inhibitor ACK2 (anti-c-kit antibody), and assayed by ELISA. CNV was induced in WT and ST2/-/- mice (C57BL/6) by laser photocoagulation (4 lesions per fundus). IL-33 alone or in combination with ACK2 was administered by intravitreal injection. The development of neovascular lesions was assessed by optical coherence tomography and immunofluorescence 7 days post injection. Mast cells (MC) infiltration was evaluated by immunohistochemistry.

Results: Mast cells expressed high levels of ST2, and responded directly to IL-33 to produce many inflammatory cytokines and chemokines in vitro, when cultured with IL-33 rich RPE supernatant. Ex vivo, IL-33 treatment reduced vascular sprouting in RPE-choroidal explants, but this effect was perturbed when administered with mast cells inhibitor ACK2.

In vivo, choroidal MCs that had infiltrated the sites of laser injury and surrounding retina were observed 7 days following laser. Intravitreal IL-33 attenuated CNV formation, and this was accompanied by increased MC infiltration in the neovascular lesions. Conversely, in ST2/-/- mice, similar treatment with IL-33 did not affect CNV size or extent of MC infiltration. Intravitreal administration of ACK2 with IL-33 reversed the anti-angiogenic properties of IL-33.

Conclusions: IL-33/ST2 signaling regulates ocular angiogenesis, influencing tissue remodeling via an IL-33-driven, mast-cell-dependent pathway. Collectively, these data distinguishes pathways for subverting AMD pathology.

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Presentation Time: 12:15 PM–12:30 PM

Pro-Angiogenic Mechanism of Activated Macrophages from Patients with Age-related Macular Degeneration

Purpose: Monocytes/macrophages may exert a pro-angiogenic effect in the context of neovascular age-related macular degeneration (nvAMD). We have previously found a pro-angiogenic effect of polarized M(IFNγ and LPS) human macrophages from nvAMD patients as compared to age-matched controls. This effect was identified in-vivo in the rat model of laser-induced choroidal neovascularization (LI-CNV), with a correlation to the ex-vivo choroid sprouting assay (CSA) findings. We now aim to explore the mechanism which mediates the pro-angiogenic effect of the activated macrophages from nvAMD patients.

Methods: Monocytes were isolated from nvAMD patients and were differentiated into Mo, M(IFNγ and LPS), and M(IL-13+IL-4) macrophages. Protein levels of candidate cytokines for mediation of angiogenic effects were assessed in the macrophages’ cell culture supernatants using ELISA. To assess the macrophages’ pro-angiogenic mechanism, we performed CSA, initially with macrophages’ supernatants, and then with the addition of candidate cytokines which were differentially secreted by the activated macrophages.

Results: A higher CSA sprouting area (SA) was identified following addition of media from M(IFNγ and LPS) cells as compared to non-treated wells (mean of ratios ±SEM 3.62±0.52, P =0.0001; t-test; n=19). Mo and M(IL-13+IL-4) cells’ supernatant had no effect on SA (1.57±0.34, P=0.11; n=19, and 1.55±0.5, p=0.28; n=14, respectively). TNFα (5.4-fold, p=0.0001), VEGFa (1.5-fold, p=0.007), and IL-6 (11.2-fold, p=0.0001) were up-regulated in the pro-angiogenic M(IFNγ and LPS) macrophage phenotype as compared to Mo. Cytokines at their levels in the M(IFNγ and LPS) culture media per ELISA, were added to the CSA culture. VEGFa (0.86±0.08, p=0.16; n=9), and IL1β (0.8±0.2, p=0.34; n=10) showed no effect on the SA as compared to control wells, while TNFα was associated with enlarged SA (1.0±0.2, p=0.01; n=8), and IL-6 (0.64±0.1, p=0.01; n=9) and IL-8 (0.47±0.14, p=0.007; n=8) with decreased SA.

Conclusions: These data further supports the putative role of macrophages and their cytokine products in modulating CNV. It also suggests that factors other than VEGF may also mediate such a pro-angiogenic macrophage effect, while other cytokines may suppress CNV. Thus, targeting multiple cytokines simultaneously may potentially serve as a therapeutic strategy for the disease.

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