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IL-10 -1082 SNP and IL-10 in primary CNS and vitreoretinal lymphomas

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Abstract

Objectives—Most primary central nervous system lymphomas (PCNSLs) and primary vitreoretinal lymphomas (PVRLs) are B-cell lymphomas that produce high levels of interleukin (IL)-10, which is linked to rapid disease progression. The *IL-10*$_{-1082}$G→A polymorphism (*IL-10* SNP) is associated with improved survival in certain non-CNS lymphoma patients. *PDCD4* is a tumor suppressor gene and upstream regulator of *IL-10*. This study examined the correlation between the *IL-10* SNP, *PDCD4* mRNA expression, and IL-10 expression (at transcript and protein levels) in these lymphoma cells.

Materials and methods—Single-nucleotide polymorphism (SNP)-typing at *IL-10*$_{-1082}$ was performed after micro-dissecting cytospun PVRL cells from 26 specimens. Vitreal IL-10 and IL-6 levels were measured by ELISA. PCNSL cells from 52 paraffin-embedded sections were microdissected and SNP typed on genomic DNA. RT-PCR was performed to analyze expression of *IL-10* and *PDCD4* mRNA. *IL-10*$_{-1082}$ SNP typing was performed on blood samples of 96

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healthy controls. We measured *IL-10*$_{-1082}$ SNP expression in 26 PVRLs and 52 PCNSLs and examined its relationship with IL-10 protein and gene expression, respectively.

Results—More PVRL patients expressed one copy of the *IL-10*$_{-1082}$*G→A* SNP with the GA genotype compared to controls. The frequencies of the three genotypes (AA, AG, GG) significantly differed in PVRL versus controls and in PCNSL versus controls. In PVRLs, the vitreal IL-10/IL-6 ratio was higher in *IL-10*$_{-1082}$ AG and *IL-10*$_{-1082}$ AA patients, compared to *IL-10*$_{-1082}$ GG patients. *IL-10* mRNA expression was higher in *IL-10*$_{-1082}$ AG and *IL-10*$_{-1082}$ AA PCNSLs, compared to *IL-10*$_{-1082}$ GG PCNSLs. No correlation was found between *IL-10* and *PDCD4* expression levels in 37 PCNSL samples.

Conclusions—PVRL and PCNSL patients had similar *IL-10*$_{-1082}$ A allele frequencies, but genotype distributions differed from healthy controls. The findings suggest that the *IL-10*$_{-1082}$ A allele is a risk factor for higher IL-10 levels in PVRLs and PCNSLs. Higher IL-10 levels have been correlated with more aggressive disease in both PVRLs and PCNSLs, making this finding an important and potentially clinically significant observation.

Keywords

Primary vitreoretinal lymphoma; Primary CNS lymphoma; Interleukin-10; Single-nucleotide polymorphism; PDCD4 (program cell death 4)

Introduction

Primary central nervous system lymphomas (PCNSLs) are uncommon extranodal non-Hodgkin's lymphomas (NHLs) that involve only the brain, leptomeninges, spinal cord, and eyes. PCNSL is a rare fatal disease with an incidence of 0.48 in 100,000 persons per year and a 12-month survival of 33 % [1, 2]. The incidence of PCNSL has been increasing over the last 30 years in both immunocompetent and immunodeficient populations [3, 4]. According to the World Health Organization lymphoma classification system, most PCNSLs are diffuse large B-cell lymphomas (DLBCLs) with a heterogeneous population of aggressive malignant B-cells [5, 6]. Primary vitreoretinal lymphoma (PVRL), also known as "primary intraocular lymphoma" (PIOL), is considered a subset of PCNSL that involves the retina, vitreous, and optic nerve head [7–9]. The incidence of PVRL was 0.46 in 100,000 persons per year in the United States from 2004 to 2007 [10], and the median overall survival was 22.5 months [7, 11]. The cell of origin for both PCNSL and PVRL is still unknown. However, it is considered to be a B-cell that has exited the germinal center [12, 13].

PVRLs have been shown to secrete high levels of interleukin-10 (IL-10), and high vitreous IL-10 levels and/or a high vitreous IL-10/IL-6 ratio correspond to a more severe disease course [8, 14–16]. IL-10 levels are also increased in the cerebrospinal fluid and serum of PCNSL patients compared to patients with other CNS tumors and patients who are free of disease [17]. Higher IL-10 levels correlate with radiographic progression of brain lesions in PCNSL, adverse disease features, and poorer prognosis in DLBCL [17, 18]. This is likely due to the stimulatory effect IL-10 has on B-cells [19, 20]. IL-10 also has a strong immunomodulatory effect, and its ability to inhibit inflammation may be a mechanism tumors use to defy immune surveillance and prevent their destruction [21].

Single-nucleotide polymorphisms (SNPs) in the *IL-10* gene promoter have been described to contribute to risk for NHL [22–25]. A well-studied SNP in the *IL-10* promoter is the *IL-10*$_{-1082}$ G →A allele (*IL-10*$_{-1082}$) SNP [26–30]. There is in vitro and in vivo evidence linking the *IL-10*$_{-1082}$ SNP with IL-10 levels [27, 28, 31, 32].

Several human malignancies downregulate *programmed cell death 4* (*PDCD4*), a pro-inflammatory tumor suppressor gene that prevents transformation and inhibits cellular proliferation. The PDCD4 protein inhibits protein synthesis by suppressing translation initiation. PDCD4 is downregulated in different tumors, including lung cancer, hepatocellular carcinoma, breast carcinoma, gliomas, and esophageal carcinoma [33, 34]. Mice deficient in *PDCD4* develop spontaneous lymphomas and have a short life span [35]. It is via NF-κB that *PDCD4* suppresses *IL-10* translation [35, 36].

PCNSLs and PVRLs are extremely rare, and tissue is rarely obtainable for research investigation because of the current modalities of disease treatment. The prevalence of the *IL-10*$_{-1082}$ SNP in PCNSL or PVRL has not been previously reported, and a genotype-phenotype correlation of an *IL-10* SNP with *IL-10* expression in PCNSL cells or vitreal IL-10 levels in PVRL has not previously been documented. The objectives of the present study, based on the availability of the specimens, were to: (a) compare the *IL-10*$_{-1082}$ SNP in PVRL and PCNSL; (b) determine the correlation of the *IL-10*$_{-1082}$ genotype and the vitreous IL-10/IL-6 ratio in PVRLs; (c) examine for an association between the *IL-10*$_{-1082}$ genotype and *IL-10* mRNA levels in PCNSLs; and (d) identify any correlation between *PDCD4* and *IL-10* mRNA levels in PCNSL.

Materials and methods

Sample collection

The study design was approved by the Institutional Review Boards (IRBs) of the National Eye Institute (NEI) and the University of Liverpool. All participants from the NEI and the University of Liverpool signed informed consent forms. All included patients were HIV negative and had no history of transplantation. The Oregon Health & Science University (OHSU) IRB determined that the research did not involve human subjects, and for this reason, consent was not required or obtained at OHSU. The research adhered to the tenets of the Declaration of Helsinki. The clinic-based cases included 27 patients diagnosed with PVRL at the NEI between 1993 and 2009. The cytology slides of the vitrectomy specimens and vitreous cytokine information from these patients were obtained. Slides from a total of 59 paraffin-embedded brain biopsies containing PCNSL were obtained from Oregon Health & Science University and the University of Liverpool. DNA samples of 98 healthy age- and gender-matched subjects were obtained from the NIH blood bank. Study group populations are summarized in Table 1.

Vitrectomy and vitreal cytokine measurement

Vitreous specimens from 27 patients subsequently diagnosed with PVRL were obtained through a standard three-port pars plana vitrectomy [37]. The vitrectomy specimens were delivered for cytopathological analysis according to previously described methods [37]. Vitreous supernatant (100 μl per case) was submitted for IL-10 and IL-6 measurement in pg/ml by ELISA (Quantikine; R&D Systems, Abingdon, UK).

Cytology and histology

The vitreous from the 27 PVRL patients was cytocentrifuged (Wescor, Inc. Cytopro 7620, Logan, Utah) at 443 g for 8 min to concentrate cells and cytospun onto glass slides that were subsequently air dried and stained with Giemsa. Brain biopsy tissue with PCNSL was removed surgically, fixed in 10 % neutral buffered formalin, and embedded in paraffin. Neuropathology was determined on 3- or 5-μm-thick sections stained with hematoxylin and eosin (H&E). CD20 positivity was evaluated using routine immunohistochemistry on the PCNSL cases [9].

Microdissection

Using previously described methods [38], PVRL cells were microdissected from vitreous cytological slides that were stored at 4°C in a refrigerator for months or years, and PCNSL cells were microdissected from the brain pathological slides that were stored at room temperature for one or several years. Briefly, the buffered formalin-fixed paraffin sections of PCNSL were stained with H&E. Fifty-five of the 59 PCNSL cases were deparaffinized, as four PCNSL cases contained insufficient tissue for further analysis. Lymphoma cells were selected by visualization under a light microscope and collected using a 30-gauge needle as previously described [38]. The PVRL cases collected were all characterized to be B-cell lymphomas based on molecular identification of the *IgH* gene rearrangement as previously described [39]. For 27 PVRL cases, lymphoma cells on the Giemsa-stained cytological slides were gently lifted and collected with a 30-gauge needle under a light microscope as previously described [40].

SNP typing

Genomic DNA samples were collected from PVRL cells on the cytospun slides from 27 NEI patients and the PCNSL cells on the pathology slides from 55 cases obtained through OHSU and the University of Liverpool. Ninety-eight blood bank samples from the NIH were also obtained as controls. All samples were amplified using the illustra GenomiPhi V2 DNA Amplification Kit (Amersham Biosciences). Genotyping of rs1800896 was performed (*Taq*man SNP Genotyping Assays C__1747360_10; Applied Biosystems, Foster City, CA). This procedure was successful for 26 PVRL samples, 35 PCNSL cases, and 96 controls.

RNA isolation and quantitative real-time reverse transcriptase polymerase chain reaction

Total RNA from the microdissected cells was isolated using the Paradise™ Sample Quality Assessment Kit (MDS Analytical Technology, Sunnyvale, CA). Universal human RNA was purchased from BD Biosciences (Palo Alto, CA) and used for assay normalization. The cDNA synthesis was performed using SuperScript™ II reverse transcriptase (Invitrogen, Carlsbad, CA). Real-time PCR was performed using a Stratagene Mx3000 Real-Time PCR System and Brilliant SYBR Green QPCR Master Mix (Stratagene, CA). The primers for *IL-10* and *PDCD4* were synthesized by SA Biosciences (Frederick, MD) and supplied as the quantitative RT-PCR Gene Expression Analysis kit. Following PCR, a thermal melt profile was performed for amplicon identification. To determine the Ct, the threshold level of fluorescence was set manually in the early phase of PCR amplification. The $2-\Delta\Delta$Ct analysis method was used to determine relative amounts of product using β-*actin* as an endogenous control. Due to the limited number of microdissected cells, only one common and reliable housekeeping gene, β-*actin*, was selected. The transcript expression was normalized by the level of β-*actin* from the same cDNA sample. The average relative expression due to gene manipulation was again normalized to the transcript level of the universal human RNA and presented graphically. Each sample was analyzed twice. Samples without template and samples without PCR mixtures were used as negative controls.

Statistics

Data were analyzed using GraphPad Prism v5. The χ^2-test was used to detect statistical differences between the genotypic distribution and allelic frequencies of the $IL\text{-}10_{-1082}$ SNP. The non-parametric Wilcoxon *t* test was performed to detect statistical differences between vitreous IL-10 levels, vitreous IL-10/IL-6 ratios, and *IL-10* mRNA transcript expression. When three or more groups were compared, the ANOVA test was used. The spearmen correlation coefficient (*r*) was used to evaluate correlation of *PDCD4* and *IL-10* mRNA expression. Statistical significance was defined as $p<0.05$.

Results

Demography

The PVRL patient cases from the NEI varied in age from 32 to 81 years, with a mean age of 64 years. All patients were HIV negative and did not have a history of transplantation. The PCNSL patient cases from the Oregon Health & Science University and the University of Liverpool varied in age from 20 to 80 years, with a mean age of 56 years. Demographic information was available for all 26 PVRL patients, 52 PCNSL patients, and 96 control patients (Table 1).

Allele and genotype frequencies at *IL-10*$_{-1082}$ in PCNSL and PVRL

Genotyping of the *IL-10* promoter SNP was successful in 26 of 27 PVRL, 35 of 59 PCNSL patients, and 96 of 98 healthy subjects. There was no significant difference in allele frequencies of the *IL-10*$_{-1082}$ A allele or the *IL-10*$_{-1082}$ G allele in patients with PCNSL or PVRL when compared to controls (Table 2). More PVRL patients expressed one copy of the *IL-10*$_{-1082}$G→A SNP with the GA genotype compared to controls (p=0.048), while this was not significant in PCNSL patients (p=0.063). There was no statistical difference in genotype frequencies between PCNSL and PVRL. However, there was a statistically significant difference in the frequencies of the three genotypes (AA, AG, GG) in PVRL versus controls (p=0.0001) and in PCNSL versus controls (p=0.005). Genotypic distributions were in Hardy-Weinberg equilibrium for blood bank controls, PVRLs, and PCNSLs.

PVRL *IL-10*$_{-1082}$ A allele and higher vitreous IL-10/IL-6 ratios

Lymphoma cells from the cytospun slides of 27 PVRL cases were microdissected. A representative image of PVRL cells on a cytospun slide is shown in Fig. 1a. SNP typing was successful in 26 cases. The vitreous IL-10/IL-6 ratio was 6.4±2.6 in patients with *IL-10*$_{-1082}$ GG and 23.5±7.1 in patients with *IL-10*$_{-1082}$ AG or *IL-10*$_{-1082}$ AA (p =0.04), as shown in Fig. 2. Vitreous IL-10 levels (pg/ml) were 724±593 in *IL-10*$_{-1082}$ GG patients and 1,794±603 in *IL-10*$_{-1082}$ AG, and *IL-10*$_{-1082}$ AA patients (p>0.05). PVRLs with at least one *IL-10*$_{-1082}$ A allele have higher vitreous IL-10 levels.

PCNSL *IL-10*$_{-1082}$ A allele and higher *IL-10* mRNA levels

Of the 59 PCNSL cases (Fig. 1b), four cases had insufficient tissue for microdissection. Fifty-five cases were microdissected, and total RNA was extracted from microdissected samples and reverse-transcribed to cDNA. RT-PCR for *IL-10* mRNA was successful for 52 cases. Due to technical difficulty of SNP genotype assay on the paraffin-embed, microdissected cells, only 35 of the 55 PCNSL cases were successfully SNP typed. The mean relative expression of the *IL-10* transcript was 0.002 in PCNSL cases carrying *IL-10*$_{-1082}$ GG and 9.2 for cases carrying *IL-10*$_{-1082}$ AG or *IL-10*$_{-1082}$ AA (p=0.019), as shown in Fig. 3. PCNSLs with at least one *IL-10*$_{-1082}$ A allele have higher *IL-10* mRNA levels (Fig. 3b).

Expression of *PDCD4* and *IL-10* in PCNSLs

RT-PCR for *IL-10* and *PDCD4* mRNA was successful in 52 microdissected PCNSL cases. *IL-10* and *PDCD4* mRNA expression levels were compared in the same cases. When *PDCD4* mRNA levels were plotted against *IL-10* mRNA levels (Fig. 4), a weak positive correlation was found: r^2=0.36, with 95 % confidence interval of 0.093 to 0.58 (p=0.008).

Discussion

IL-10 gene promoter polymorphisms have been considered risk factors for NHL [22–25]. There is some evidence suggesting that the *IL-10*_1082 A allele is a risk allele in lymphoma patients [26, 41]. One group observed that the frequency of the *IL-10*_1082 AA allele was higher in patients with aggressive systemic lymphomas compared to controls [26]. In another study, the frequency of the *IL-10*_1082 G allele was higher in patients with DLBCL compared to controls [41]. The *IL-10*_1082 A allele has been shown to confer greater overall survival in patients with multiple myeloma and chronic lymphocytic leukemia [27], and the G allele has been associated with a better survival in melanoma patients [42]. Genotype-phenotype studies have measured serum IL-10 levels and *IL-10*_1082 SNP expression in vitro after endotoxin stimulation in autoimmune disease and Hodgkin's lymphoma [27–29, 31, 32, 43]. To our knowledge, this is the first study providing a genotype-phenotype correlation for an *IL-10* SNP in PCNSL or PVRL.

In this study, we measured the expression of the *IL-10*_1082 SNP, *IL-10*, and *PDCD4* in the same PCNSL cases, and therefore were able to make a potential genotype-phenotype correlation. In the PVRL cases, we correlated *IL-10*_1082 genotype and vitreous IL-10 levels, which are considered to be a relatively sensitive and specific biomarker of lymphoma cell IL-10 production [14, 44, 45]. Recently, elevation of IL-10 levels in the vitreous has been repeatedly associated with poorer prognosis [16, 46, 47]. A direct relationship cannot be predicted between vitreous IL-10 levels and CSF IL-10 levels, as PVRL cells secrete IL-10 directly into the vitreous, while PCNSL cells may secrete IL-10 remotely from CSF. However, this is a potential area of investigation in future prospective clinical trials such as the International Extranodal Lymphoma Study Group (IELSG) trail in Europe.

This study demonstrated a similar *IL-10*_1082 genotype distribution in PVRLs and PCSNLs, which differed significantly from that observed in healthy controls. Compared to controls, more PVRLs were found to have one copy of the *IL-10*_1082 SNP. However, allelic frequency of the *IL-10*_1082 A and *IL-10*_1082 G alleles was similar in PVRLs, PCNSLs, and controls, supporting evidence of the genetic resemblance of these to non-Hodgkin's lymphomas [48]. This finding differs from a previous study that demonstrates an increased prevalence of the *IL-10*_1082 G allele in DLBCL patients. In our genotype-phenotype study, we noted that PVRLs with at least one copy of the *IL-10*_1082 A allele had a significantly higher vitreous IL-10/IL-6 ratio than PVRLs that were *IL-10*_1082 GG. This finding was replicated for PCNSLs, in which we found a significant association between the *IL-10*_1082 A allele and higher *IL-10* mRNA levels. A dose effect of the *IL-10*_1082 A allele seems to be present in both PVRL and PCNSL. Elevated IL-10 levels have been associated with more aggressive disease in PVRL, PCNSL, and NHL [16, 17, 49–51]. It is possible that the presence of the *IL-10*_1082 SNP might predict a worse prognosis for PVRL and PCNSL patients; however, mortality data is necessary to confirm this hypothesis.

DLBCLs may have low *PDCD4* levels and higher *IL-10* levels. In turn, *PDCD4* expression has not been previously reported in PCNSL or PVRL [35, 36, 52]. The tumor suppressor PDCD4 is a pro-inflammatory protein that promotes activation of the transcription factor NF-κB and suppresses interleukin-10 (IL-10). Given that *PDCD4* suppresses *IL-10*, we expected a negative correlation between *PDCD4* and *IL-10* transcript levels. However, no such relationship was found in 52 PCSNLs. Other factors such as conserved non-coding sequences 9 (CNS-9) may regulate *IL-10* in PCNSL [53, 54].

Despite the rarity of these diseases, a large number of PCNSL (n=52) and PVRL (n=26) cases were analyzed. The *IL-10*_1082 A allele may not be a direct risk allele for PVRL or PCNSL. However, our study has shown that PVRL patients are more likely to have the

IL-10$_{-1082}$ SNP than controls and that PVRL and PCNSL patients have an *IL-10*$_{-1082}$ genotype distribution that differs from controls. The *IL-10*$_{-1082}$ A allele is significantly associated with higher *IL-10* mRNA levels in PCNSL and a higher vitreous IL-10/IL-6 ratio in PVRL.

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References

1. O'Neill, BP. Lymphoma of the nervous system. Butterworth-Heinemann; Boston: 2004. Epidemiology of primary CNS lymphoma; p. 1-207.
2. Pulido JS, Vierkant RA, Olson JE, Abrey L, Schiff D, O'Neill BP. Racial differences in primary central nervous system lymphoma incidence and survival rates. Neuro Oncol. 2009; 11:318–322. [PubMed: 19273630]
3. Olson JE, Janney CA, Rao RD, Cerhan JR, Kurtin PJ, Schiff D, Kaplan RS, O'Neill BP. The continuing increase in the incidence of primary central nervous system non-Hodgkin lymphoma: a surveillance, epidemiology, and end results analysis. Cancer. 2002; 95:1504–1510. [PubMed: 12237919]
4. Schabet M. Epidemiology of primary CNS lymphoma. J Neurooncol. 1999; 43:199–201. [PubMed: 10563423]
5. Banchereau, J.; Schmitt, D. Advances in experimental medicine and biology. Vol. 2. Plenum Press; New York: 1995. Dendritic cells in fundamental and clinical immunology; p. 1-572.
6. Kleihues, PC.; WK. World Health Organization classification of tumors. Lyon; France: 2000. Pathology and genetics of tumours of the nervous system; p. 1-314.
7. Chan, CC.; Gonzales, JA. Primary intraocular lymphoma. World Scientific Publishing Co Pte Ltd; Hackensack, NJ: 2007. p. 1-200.
8. Chan CC, Rubenstein JL, Coupland SE, Davis JL, Harbour JW, Johnston PB, Cassoux N, Touitou V, Smith JR, Batchelor TT, Pulido JS. Primary vitreoretinal lymphoma: a report from an international primary central nervous system lymphoma collaborative group symposium. Oncologist. 2011; 16:1589–1599. [PubMed: 22045784]
9. Coupland SE, Damato B. Understanding intraocular lymphoma. Clin Experiment Ophthalmol. 2008; 36:564–578. [PubMed: 18954321]
10. Fast stats: Statistics stratified by cancer site. National Cancer Institute. Surveillance, Epidemiology, and End Results;
11. Faia LJ, Chan CC. Primary intraocular lymphoma. Arch Pathol Lab Med. 2009; 133:1228–1232. [PubMed: 19653715]
12. Kuppers R. Mechanisms of B-cell lymphoma pathogenesis. Nat Rev Cancer. 2005; 5:251–262. [PubMed: 15803153]
13. Lenz G, Wright GW, Emre NC, Kohlhammer H, Dave SS, Davis RE, Carty S, Lam LT, Shaffer AL, Xiao W, Powell J, Rosenwald A, Ott G, Muller-Hermelink HK, Gascoyne RD, Connors JM, Campo E, Jaffe ES, Delabie J, Smeland EB, Rimsza LM, Fisher RI, Weisenburger DD, Chan WC, Staudt LM. Molecular subtypes of diffuse large B-cell lymphoma arise by distinct genetic pathways. Proc Natl Acad Sci USA. 2008; 105:13520–13525. [PubMed: 18765795]
14. Cassoux N, Giron A, Bodaghi B, Tran TH, Baudet S, Davy F, Chan CC, Lehoang P, Merle-Beral H. Il-10 measurement in aqueous humor for screening patients with suspicion of primary intraocular lymphoma. Invest Ophthalmol Vis Sci. 2007; 48:3253–3259. [PubMed: 17591896]
15. Chan CC, Whitcup SM, Solomon D, Nussenblatt RB. Interleukin-10 in the vitreous of patients with primary intraocular lymphoma. Am J Ophthalmol. 1995; 120:671–673. [PubMed: 7485372]

16. Sen HN, Chan CC, Byrnes G, Fariss RN, Nussenblatt RB, Buggage RR. Intravitreal methotrexate resistance in a patient with primary intraocular lymphoma. Ocul Immunol Inflamm. 2008; 16:29–33. [PubMed: 18379939]

17. Salmaggi A, Eoli M, Corsini E, Gelati M, Frigerio S, Silvani A, Boiardi A. Cerebrospinal fluid interleukin-10 levels in primary central nervous system lymphoma: a possible marker of response to treatment? Ann Neurol. 2000; 47:137–138. [PubMed: 10632117]

18. Lech-Maranda E, Bienvenu J, Michallet AS, Houot R, Robak T, Coiffier B, Salles G. Elevated IL-10 plasma levels correlate with poor prognosis in diffuse large B-cell lymphoma. Eur Cytokine Netw. 2006; 17:60–66. [PubMed: 16613764]

19. Banchereau J, Briere F, Liu YJ, Rousset F. Molecular control of B lymphocyte growth and differentiation. Stem Cells. 1994; 12:278–288. [PubMed: 7521239]

20. Rousset F, Garcia E, Defrance T, Peronne C, Vezzio N, Hsu DH, Kastelein R, Moore KW, Banchereau J. Interleukin-10 is a potent growth and differentiation factor for activated human B lymphocytes. Proc Natl Acad Sci USA. 1992; 89:1890–1893. [PubMed: 1371884]

21. Petersson M, Charo J, Salazar-Onfray F, Noffz G, Mohaupt M, Qin Z, Klein G, Blankenstein T, Kiessling R. Constitutive IL-10 production accounts for the high NK sensitivity, low MHC class I expression, and poor transporter associated with antigen processing (TAP)-1/2 function in the prototype NK target YAC-1. J Immunol. 1998; 161:2099–2105. [PubMed: 9725200]

22. Lan Q, Zheng T, Rothman N, Zhang Y, Wang SS, Shen M, Berndt SI, Zahm SH, Holford TR, Leaderer B, Yeager M, Welch R, Boyle P, Zhang B, Zou K, Zhu Y, Chanock S. Cytokine polymorphisms in the Th1/Th2 pathway and susceptibility to non-Hodgkin lymphoma. Blood. 2006; 107:4101–4108. [PubMed: 16449530]

23. Lech-Maranda E, Baseggio L, Bienvenu J, Charlot C, Berger F, Rigal D, Warzocha K, Coiffier B, Salles G. Interleukin-10 gene promoter polymorphisms influence the clinical outcome of diffuse large B-cell lymphoma. Blood. 2004; 103:3529–3534. [PubMed: 14701701]

24. Purdue MP, Lan Q, Kricker A, Grulich AE, Vajdic CM, Turner J, Whitby D, Chanock S, Rothman N, Armstrong BK. Polymorphisms in immune function genes and risk of non-Hodgkin lymphoma: findings from the New South Wales non-Hodgkin Lymphoma Study. Carcinogenesis. 2007; 28:704–712. [PubMed: 17056605]

25. Rothman N, Skibola CF, Wang SS, Morgan G, Lan Q, Smith MT, Spinelli JJ, Willett E, De Sanjose S, Cocco P, Berndt SI, Brennan P, Brooks-Wilson A, Wacholder S, Becker N, Hartge P, Zheng T, Roman E, Holly EA, Boffetta P, Armstrong B, Cozen W, Linet M, Bosch FX, Ennas MG, Holford TR, Gallagher RP, Rollinson S, Bracci PM, Cerhan JR, Whitby D, Moore PS, Leaderer B, Lai A, Spink C, Davis S, Bosch R, Scarpa A, Zhang Y, Severson RK, Yeager M, Chanock S, Nieters A. Genetic variation in TNF and IL10 and risk of non-Hodgkin lymphoma: a report from the InterLymph Consortium. Lancet Oncol. 2006; 7:27–38. [PubMed: 16389181]

26. Cunningham LM, Chapman C, Dunstan R, Bell MC, Joske DJ. Polymorphisms in the interleukin 10 gene promoter are associated with susceptibility to aggressive non-Hodgkin's lymphoma. Leuk Lymphoma. 2003; 44:251–255. [PubMed: 12688341]

27. Domingo-Domenech E, Benavente Y, Gonzalez-Barca E, Montalban C, Guma J, Bosch R, Wang SS, Lan Q, Whitby D, Fernandez de Sevilla A, Rothman N, de Sanjose S. Impact of interleukin-10 polymorphisms (-1082 and -3575) on the survival of patients with lymphoid neoplasms. Haematologica. 2007; 92:1475–1481. [PubMed: 18024395]

28. Hohaus S, Giachelia M, Massini G, Vannata B, Criscuolo M, Martini M, D'Alo F, Voso MT, Larocca LM, Leone G. Clinical significance of interleukin-10 gene polymorphisms and plasma levels in Hodgkin lymphoma. Leuk Res. 2009; 33:1352–1356. [PubMed: 19201467]

29. Reuss E, Fimmers R, Kruger A, Becker C, Rittner C, Hohler T. Differential regulation of interleukin-10 production by genetic and environmental factors–a twin study. Genes Immun. 2002; 3:407–413. [PubMed: 12424622]

30. Turner DM, Williams DM, Sankaran D, Lazarus M, Sinnott PJ, Hutchinson IV. An investigation of polymorphism in the interleukin-10 gene promoter. Eur J Immunogenet. 1997; 24:1–8. [PubMed: 9043871]

31. Gibson AW, Edberg JC, Wu J, Westendorp RG, Huizinga TW, Kimberly RP. Novel single nucleotide polymorphisms in the distal IL-10 promoter affect IL-10 production and enhance the risk of systemic lupus erythematosus. J Immunol. 2001; 166:3915–3922. [PubMed: 11238636]

32. Kurreeman FA, Schonkeren JJ, Heijmans BT, Toes RE, Huizinga TW. Transcription of the IL10 gene reveals allele-specific regulation at the mRNA level. Hum Mol Genet. 2004; 13:1755–1762. [PubMed: 15198994]
33. Jansen AP, Camalier CE, Stark C, Colburn NH. Characterization of programmed cell death 4 in multiple human cancers reveals a novel enhancer of drug sensitivity. Mol Cancer Ther. 2004; 3:103–110. [PubMed: 14985450]
34. Lankat-Buttgereit B, Goke R. Programmed cell death protein 4 (pdcd4): A novel target for antineoplastic therapy? Biol Cell. 2003; 95:515–519. [PubMed: 14630388]
35. Hilliard A, Hilliard B, Zheng SJ, Sun H, Miwa T, Song W, Goke R, Chen YH. Translational regulation of autoimmune inflammation and lymphoma genesis by programmed cell death 4. J Immunol. 2006; 177:8095–8102. [PubMed: 17114484]
36. Sheedy FJ, Palsson-McDermott E, Hennessy EJ, Martin C, O'Leary JJ, Ruan Q, Johnson DS, Chen Y, O'Neill LA. Negative regulation of TLR4 via targeting of the proinflammatory tumor suppressor PDCD4 by the microRNA miR-21. Nat Immunol. 2010; 11:141–147. [PubMed: 19946272]
37. Gonzales JA, Chan CC. Biopsy techniques and yields in diagnosing primary intraocular lymphoma. Int Ophthalmol. 2007; 27:241–250. [PubMed: 17440686]
38. Shen DF, Zhuang Z, LeHoang P, Boni R, Zheng S, Nussenblatt RB, Chan CC. Utility of microdissection and polymerase chain reaction for the detection of immunoglobulin gene rearrangement and translocation in primary intraocular lymphoma. Ophthalmology. 1998; 105:1664–1669. [PubMed: 9754175]
39. Chan CC. Molecular pathology of primary intraocular lymphoma. Trans Am Ophthalmol Soc. 2003; 101:275–292. [PubMed: 14971583]
40. Levy-Clarke GA, Byrnes GA, Buggage RR, Shen DF, Filie AC, Caruso RC, Nussenblatt RB, Chan CC. Primary intraocular lymphoma diagnosed by fine needle aspiration biopsy of a sub-retinal lesion. Retina. 2001; 21:281–284. [PubMed: 11421029]
41. Lech-Maranda E, Baseggio L, Charlot C, Rigal D, Berger F, Jamroziak K, Warzocha K, Coiffier B, Salles G. Genetic polymorphisms in the proximal IL-10 promoter and susceptibility to non-Hodgkin lymphoma. Leuk Lymphoma. 2007; 48:2235–2238. [PubMed: 17990180]
42. Vuoristo MS. The polymorphisms of interleukin-10 gene influence the prognosis of patients with advanced melanoma. Cancer Genet Cytogenet. 2007; 176:54–57. [PubMed: 17574964]
43. Eskdale J, McNicholl J, Wordsworth P, Jonas B, Huizinga T, Field M, Gallagher G. Interleukin-10 microsatellite polymorphisms and IL-10 locus alleles in rheumatoid arthritis susceptibility. Lancet. 1998; 352:1282–1283. [PubMed: 9788463]
44. Coupland SE, Chan CC, Smith J. Pathophysiology of retinal lymphoma. Ocul Immunol Inflamm. 2009; 17:227–237. [PubMed: 19657975]
45. Wang Y, Shen D, Wang VM, Sen HN, Chan CC. Molecular biomarkers for the diagnosis of primary vitreoretinal lymphoma. Int J Mol Sci. 2011; 12:5684–5697. [PubMed: 22016619]
46. Kawamura H, Yasuda N, Kakinoki M, Sawada T, Sawada O, Ohji M. Interleukin-10 and interleukin-6 in aqueous humor during treatment of vitreoretinal lymphoma with intravitreally injected methotrexate. Ophthalmic Res. 2009; 42:172–174. [PubMed: 19648778]
47. Sou R, Ohguro N, Maeda T, Saishin Y, Tano Y. Treatment of primary intraocular lymphoma with intravitreal methotrexate. Jpn J Ophthalmol. 2008; 52:167–174. [PubMed: 18661266]
48. Coupland SE, Hummel M, Stein H, Willerding G, Jahnke K, Stoltenburg-Didinger G. Demonstration of identical clonal derivation in a case of "oculocerebral" lymphoma. Br J Ophthalmol. 2005; 89:238–239. [PubMed: 15665359]
49. Blay JY, Burdin N, Rousset F, Lenoir G, Biron P, Philip T, Banchereau J, Favrot MC. Serum interleukin-10 in non-Hodgkin's lymphoma: a prognostic factor. Blood. 1993; 82:2169–2174. [PubMed: 8400266]
50. Rajagopal R, Harbour JW. Diagnostic testing and treatment choices in primary vitreoretinal lymphoma. Retina. 2011; 31:435–440. [PubMed: 21336066]
51. Whitcup SM, Stark-Vancs V, Wittes RE, Solomon D, Podgor MJ, Nussenblatt RB, Chan CC. Association of interleukin 10 in the vitreous and cerebrospinal fluid and primary central nervous system lymphoma. Arch Ophthalmol. 1997; 115:1157–1160. [PubMed: 9298057]

52. Allgayer H. Pdcd4, a colon cancer prognostic that is regulated by a microRNA. Crit Rev Oncol Hematol. 2010; 73:185–191. [PubMed: 19836969]

53. Lee CG, Kang KH, So JS, Kwon HK, Son JS, Song MK, Sahoo A, Yi HJ, Hwang KC, Matsuyama T, Yui K, Im SH. A distal cis-regulatory element, CNS-9, controls NFAT1 and IRF4-mediated IL-10 gene activation in T helper cells. Mol Immunol. 2009; 46:613–621. [PubMed: 18962896]

54. Liopeta K, Boubali S, Virgilio L, Thyphronitis G, Mavrothalassitis G, Dimitracopoulos G, Paliogianni F. cAMP regulates IL-10 production by normal human T lymphocytes at multiple levels: a potential role for MEF2. Mol Immunol. 2009; 46:345–354. [PubMed: 19058854]

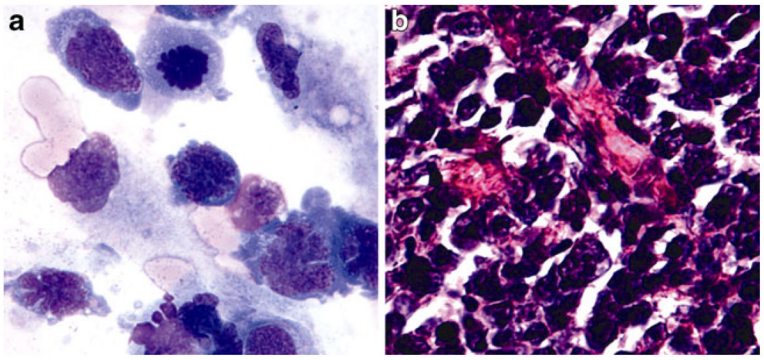

Fig. 1.
Photomicrographs of primary vitreoretinal lymphoma (PVRL) cells and primary central nervous system lymphoma (PCNSL) cells. **a** Retinal lymphoma cells from a cytospun slide (original magnification x640, Giemsa stain). **b** Central nervous system lymphoma cells from a brain biopsy slide (original magnification x400, Giemsa stain)

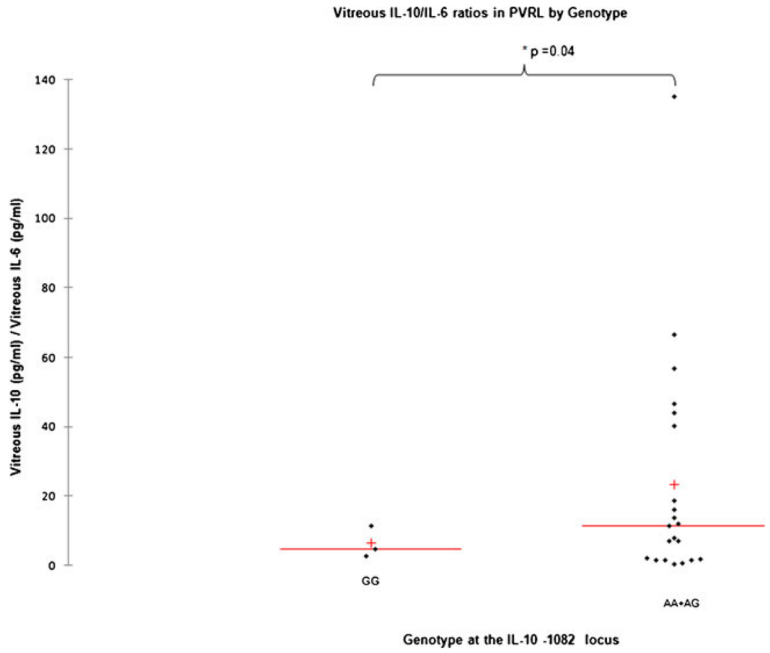

Fig. 2.

Vitreous IL-10/IL-6 ratio compared to the *IL-10*$_{-1082}$ genotype in primary vitreoretinal lymphoma (PVRL) patients. The mean vitreous IL-10/IL-6 ratios (*line*) and medians (*+*) are compared in PVRLs, grouped by genotype at *IL-10*$_{-1082}$. The difference between the two groups is statistically significant

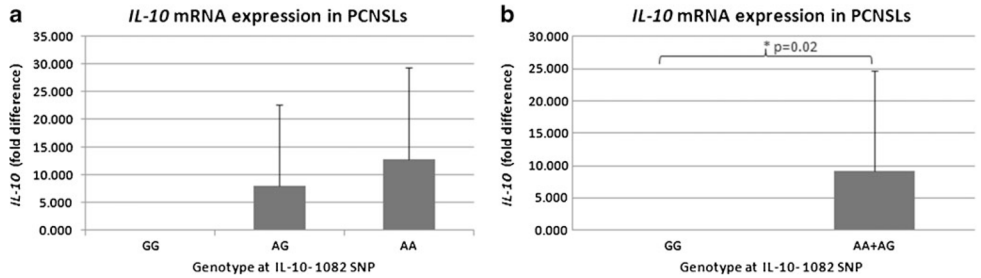

Fig. 3.

IL-10 relative expression levels in primary central nervous system lymphoma (PCNSL) cells. **a** The mean *IL-10* mRNA fold differences ± standard deviations are compared in PCNSL cells grouped by genotype at $IL\text{-}10_{-1082}$. **b** PCNSLs with at least one *IL-10* SNP allele have significantly higher *IL-10* expression

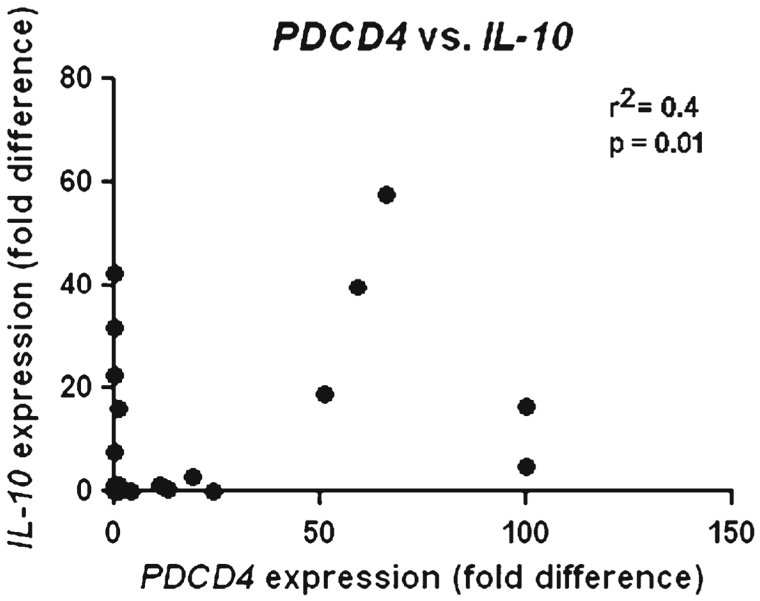

Fig. 4.

Comparison of *PDCD4* and *IL-10* mRNA relative expression. No correlation was detected between *PDCD4* and *IL-10* mRNA expression levels in primary central nervous system lymphoma (PCNSL) cells

Table 1

Demography and clinical information

a) PVRL patients				
Specimen origin (no.)	Age mean (SD)	Gender (♂/♀)	Vitreous IL-10 (pg/ml) mean (SD)	Vitreous IL-10/IL-6 mean (SD)
Eye (*n*=26)	64 (14)	12/14	1,666 (2,632)	37 (82)
b) PCNSL patients				
Specimen origin (no.)	Age mean (SD)	Gender (♂/♀)		
Brain (*n*=52)	56 (15)	34/18		
c) Healthy controls				
Specimen origin (no.)	Age mean (SD)	Gender (♂/♀)		
Peripheral blood (*n*=96)	44 (13)	72/24		

Table 2

Odds ratios and 95 % confidence intervals of primary vitreoretinal lymphoma (PVRL) and primary central nervous system lymphomas (PCNSL) versus unrelated controls at the *IL-10* promoter single-nucleotide polymorphism (SNP)

Sample	IL-10$_{-1082}$ SNP	Control	Case	*p* value	Odds ratio (95 % confidence interval)
PVRL	GG	27 (28.0 %)	4 (15.4 %)		1
	GA	38 (40.0 %)	18 (69.2 %)	0.0480*	3.197 (0.972–10.52)
	AA	31 (32.0 %)	4 (15.4 %)	0.8546	0.871 (0.198–3.823)
	GA+AA	69 (72.0 %)	22 (84.6 %)	0.1856	2.152 (0.678–6.830)
	G	92 (48.0 %)	26 (50.0 %)		1
	A	100 (52.0 %)	26 (50.0 %)	0.7897	1.087 (0.589–2.007)
PCNSL	GG	27 (28.0 %)	6 (17.1 %)		1
	GA	38 (40.0 %)	22 (62.9 %)	0.0630	2.605 (0.931–7.290)
	AA	31 (32.0 %)	7 (20.0 %)	0.9793	1.016 (0.304–3.396)
	GA+AA	69 (72.0 %)	29 (82.9 %)	0.2001	1.891 (0.706–5.067)
	G	92 (48.0 %)	34 (49.0 %)		1
	A	100 (52.0 %)	36 (51.0 %)	0.9252	1.027 (0.594–1.775)

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