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Pathophysiology of Retinal Lymphoma

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Abstract

Retinal lymphoma, the most common form of intraocular lymphoma, is a high-grade malignancy, usually of B-cell type, and is associated with a poor prognosis because of frequent central nervous system (CNS) involvement. The neoplastic B-cells of retinal lymphoma have a characteristic morphology and immunophenotype, express certain chemokines and chemokine receptors, and produce interleukins (IL), e.g. IL-10. Together with the cytological features of these tumors, the immunophenotype, presence of immunoglobulin rearrangements, and biochemical profile aid the diagnosis of retinal lymphomas. Immunophenotyping and somatic mutation analysis suggest derivation of most retinal lymphomas from an early post-germinal centre B-cell. Chromosomal translocation data would suggest, however, that a subgroup of these neoplasms may arise from germinal centre B-cells, and these could be associated with a better prognosis. Further investigations, such as gene expression profiling, are required to identify oncogenic pathways potentially involved in retinal lymphoma development, and to identify new prognostic/therapeutic markers for this tumor.

Keywords

retinal lymphoma; vitreoretinal lymphoma; vitreal lymphoma; intraocular lymphoma; IOL; PCNSL; DLBCL

INTRODUCTION

In order to understand the histogenesis of lymphomas and to define distinct lymphoma entities, the current World Health Organization (WHO) classification emphasizes an approach whereby the clinical characteristics are correlated with distinct morphological, immunophenotypical,

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and genotypical features of each neoplasm[1] For each lymphoma entity, it has been attempted to identify or postulate a putative cell of origin. Intraocular lymphomas that originate inside the eye are heterogeneous and have been subsumed for many years under the vague title "primary intraocular lymphoma" or PIOL. In fact, intraocular lymphomas represent several distinct entities, arising in different structures of the eye and have differing clinical courses; therefore, more specific terminology is warranted (Table).[2] In this review, the pathology, molecular biology and biochemical features of the most frequent type of intraocular lymphoma, namely retinal lymphoma, are summarized.

Retinal Lymphoma

Most intraocular lymphomas occur in the retina, usually with vitreous involvement.[2,3] Retinal lymphoma can occur without visible vitreous opacities, but then, conversely, lymphomatous vitreous infiltrates can be seen in the absence of any obvious retinal disease.[4] For ease of reference, these three different clinical variants of retinal and vitreal lymphoma will be described here under the term "retinal" lymphoma.[2,5]

Clinical features—Retinal lymphoma is a high-grade malignant neoplasm, which is often associated with cerebral disease. It may be primary or secondary to CNS lymphoma (CNSL) or may present simultaneously (Table).[6–8] Exceptionally rarely, retinal lymphoma may result from systemic metastatic lymphoma.[9,10] Retinal lymphoma most often affects elderly patients; however, it is also seen in younger individuals, particularly in those who are immunocompromised (e.g. with HIV infection or following organ transplantation). Inexplicably, there has been a clear increase in the incidence of retinal lymphoma worldwide, even in immunocompetent patients.[11]

Retinal lymphoma is usually bilateral (60–90% of patients) but is often relatively asymmetrical and sometimes appears to be unilateral at initial presentation.[2] Signs and symptoms of retinal lymphoma can mimic many other intraocular conditions ("masquerade syndrome"), and therefore, it can often be difficult to diagnose clinically, requiring experience in the interpretation of ophthalmological imaging[12] (Table) (Fig. 1). Confirmation of the clinical suspicion of retinal lymphoma requires ocular fluid or tissue sampling, involving morphological and immunocytological analysis by an experienced pathologist. Adjunct methods, including monoclonality studies using polymerase chain reaction (PCR) and cytokine analysis, are very useful in providing further evidence in support of the diagnosis. These methods will be discussed below.

Approximately 80% of patients with retinal lymphoma subsequently develop lymphomatous involvement of the cerebral parenchyma, spinal cord or meninges (Table). This close association of retinal lymphoma and CNSL is not surprising, considering the embryological origins of the two organs. Whether oculocerebral lymphoma is consequent to direct infiltration along the optic nerve, metastatic spread or multifocal tumour development remains to be clarified. Lymphomatous involvement of the CNS can be focal and/or diffuse but usually occurs in the frontal lobe. There is commonly diffuse, leptomeningeal involvement, and seeding of lymphoma cells into the cerebral spinal fluid (CSF) has been reported in 42% of patients.[13]

Interestingly, retinal lymphoma and CNSL usually disseminate only within the CNS. The reason for this confinement of retinal lymphoma and CNSL is unclear; however, it may be explained to some extent by the chemokine receptor expression pattern of the neoplastic cells.[14,15] As summarised below some data suggest that CNS diffuse large B-cell lymphoma (DLBCL) cells have a differing chemokine receptor profile when compared to their morphological counterparts in systemic DLBCL.[16] A specific chemokine or chemokine

receptor profile of tumor cells of CNSL remains, however, to be validated and identified (see below).[17]

Morphological features of retinal lymphoma—Histologically, the majority of retinal lymphoma are a high-grade B-cell Non-Hodgkin lymphoma (NHL), and can be subtyped as a DLBCL,[7,18,19] according to the WHO lymphoma classification (Table)[1] Rare subtypes have also been described, such as T-cell-rich B-cell lymphoma[20] as well as T-cell lymphoma, not otherwise specified.[10,21–24] Very few cases of primary T-cell retinal lymphomas have been described: compared to the retinal DLBCL, they appear to behave less aggressively, and may or may not be associated with CNS disease.

Retinal lymphomas of DLBCL subtype are characterized by pleomorphic medium-to-large sized cells with minimal cytoplasm, indented or folded nuclei, and prominent, often multiple, nucleoli (Fig. 2). Rarely, atypical mitotic figures can be seen. A background of small reactive lymphocytes, usually T-cells, and scavenger macrophages as well many necrotic cells is a typical feature of these neoplasms.[6–8,25]

Immunophenotypic features—Compared to systemic DLBCL, knowledge about the immunophenotype of retinal lymphoma has developed slowly. This is most likely due to the rarity of this tumor, its subretinal/sub-RPE (retinal pigment epithelium) location and the small amount of tumor material obtained for evaluation via pars plana vitrectomy. Increasingly, however, combined subretinal aspirates and/or chorioretinal biopsies are being performed together with vitreous biopsies to establish or exclude the diagnosis of lymphoma.[26,27] These larger tumor samples have enabled more extensive adjunctive analyses and, thereby, have provided a better understanding of the possible histiogenesis of retinal lymphomas. In particular, they have provided more information to the immunophenotype and genotype of these tumor cells (see below).

Retinal lymphomas are characterized by the following immunohistochemical expression profile: CD79a+,CD20+, BCL-2+, MUM1/IRF4+, BCL-6+(most cases), CD10−/+ (Table) (Fig. 2).[28] Most retinal lymphomas demonstrate a monotypical expression for either a light (typically Ig-Kappa) and/or heavy chain of the immunoglobulin gene (usually IgM, but sometimes combined IgM and IgD). The Ki-67 growth fraction of the neoplastic B-cells is frequently high (i.e., about 90%), reflecting the high-grade malignancy of this lymphoma. Should the neoplastic cells not be monotypical on immunohistochemistry, clonality assessment can be performed on DNA extracted from retinal lymphoma cells using PCR with primers directed against the immunoglobulin heavy and light chains in B-cell lymphomas (IgH-PCR and IgL-PCR, respectively).[29] Rarely, PCR directed against the T-cell receptor (TCR-γ-PCR) is necessary in the case of the suspected intraocular T-cell lymphoma.[10,21]

Genotypic features—Before discussing the genotypic features of retinal lymphoma, it is first necessary to review the developments in our understanding of systemic DLBCL. Until recently, DLBCL represented a large heterogeneous group of lymphomas, which shared some vaguely similar morphological and immunophenotypical features, but which showed considerable variation with respect to clinical course. In 2000, Alizadeh and co-workers used lymphochip complementary DNA microarrays to perform gene expression profiling analysis of untreated *de novo* DLBCL.[30] They subdivided DLBCL into 3 groups, on the basis of genes expressed in a particular stage of the B-cell differentiation pathway or during a particular biologic response.[31,32] These subgroups are: activated B-cell DLBCL (ABC type); germinal centre DLBCL (GCB type); and primary mediastinal (thymic large) B-cell DLBCL (type 3).

The main feature of ABC type DLBCL is the dysregulation of the NF-kB gene, resulting in an uncontrolled proliferation of lymphocytes.[33] Other chromosomal abnormalities of ABC type

DLBCL include gains in chromosome 3q and 18q21–q22, and deletions in 6q21–q22 as well as inactivation of the PRDM1/BLIMP1 gene.[34–36] In contrast, GCB type DLBCL are characterized by the translocation t(14;18)(q32;q21) and a gain in chromosome 12q12[37–39] Type 3 DLBCL have overlapping features of both ABC and GCB-type DLBCL but like ABC DLBCL appear to show constitutive activation of the NF-kB pathway.[40] These findings suggest that the types of DLBCL arise from different stages of normal B-cell development, perhaps representing distinct entities. This molecular subdivision of DLBCL has been shown to have clinical relevance with respect to prognosis.[41,42] Patients with ABC subtype DLBCL have a considerably worse prognosis than those with GCB lymphomas.[41,43]

Gene expression microarray analysis is not yet feasible in routine diagnostic practice, particularly as it requires good quality RNA, which may be difficult to obtain from formalin-fixed material. For this reason, surrogate immunohistochemical markers using algorithms such as the “Hans classifier” have been proposed,[44] to identify GCB-type and ABC subgroups of DLBCL. These include CD10 and BCL-6 as GCB B-cell markers, and MUM1/IRF4 as a non-GCB B-cell marker. Usage of the “Hans classifier” in routine practice and in larger research studies has demonstrated its limitations.[45] Despite this, the Lunenburg Lymphoma Biomarker Consortium have concluded that “semiquantitative immunohistochemistry for prognostic stratification of DLCBL is feasible (if performed) in a reproducible way. . . ”.[45]

When trying to understand the cellular origin of retinal lymphoma and its relationship to either the ABC or GCB types, one can only gather the thin strands of evidence, which are presented in the literature. To date, no gene expression profiling studies have been performed on retinal lymphomas. Therefore, we have to rely on indirect evidence provided by genotypic and immunophenotypic studies, knowing, however, that this evidence is likely to be wanting.

The only chromosomal translocation reported to occur in retinal lymphoma is t(14;18), which involves the *bcl-2* gene, with rearrangements being reported in up to 67% of cases.[29,46] This results in overexpression of BCL-2 protein, a mitochondrial outer membrane protein that protects cells from apoptosis. The presence of this mutation and consequent overexpression of this protein in some retinal lymphoma could suggest that they are DLBCL of GCB type, as this translocation is seen most commonly in this type of DBLCL. The incidence of this particular translocation, however, seems to be considerably higher than in peripheral DLBCL (approx. 20–30%).[47,48] Further studies are needed to validate these data and to determine the exact prevalence in retinal lymphomas.

Possibly contradicting the above data and raising the suggestion that some retinal lymphoma are DLBCL of ABC type are data obtained from sequencing of the variable region of the immunoglobulin heavy chain gene (VH) of retinal lymphoma cells and from their immunophenotyping. Two independent groups of investigators have demonstrated an intermediate to large number of somatic mutations (average 37) in retinal lymphoma cells with no evidence of antigen selection or significant intraclonal heterogeneity.[18,49] Interestingly, a similar high mutation frequency was reported for VH region genes in PCNSL.[50–52] The findings of a high somatic mutations load in the VH genes of retinal lymphoma, together with the tumor cell immunophenotype (MUM1/IRF4+, BCL-6+/−, CD10-), suggest that retinal lymphoma is a DLBCL of ABC subtype, i.e. they are derived from mature B-cells, which have undergone a prolonged interaction in the microenvironment of the germinal center and are either at the late germinal center stage of differentiation or are early post-germinal center B-cells.

Taken together, the genotypic observations suggest that there are possibly at least two different types of retinal lymphoma: i.e. DLBCL of ABC and GCB types. The only way to test this hypothesis is to perform gene expression profiling studies using RNA extracted from retinal

lymphoma cells. To date, such analyses have not been possible, mainly due to the poor quality and quantity of the RNA obtained from retinal lymphoma specimens. Current technologies for RNA amplification and/or new methods that allow expansion of malignant B cell populations[53] and/or amplification of RNA may facilitate future studies. Animal models of retinal lymphoma[54–58] do not allow us to answer this question as yet. Data from gene expression profiling studies of PCNSL provide some information that could be extrapolated with caution to retinal lymphoma. The first gene expression profiling studies of PCNSL by Rubenstein and co-workers[59,60] demonstrated that there was an equal distribution of ABC, GCB and Type 3 DLBCL in this location. Using quantitative reverse transcriptase-polymerase chain reaction (RT-PCR) and hierarchical clustering of gene expression data, Courts et al. revealed two distinct subgroups of PCNSL, which were characterized by significantly different transcriptional levels of BCL10, REL, and IAP-1.[61] However, these subtypes are not identical with the ABC- and GCB-subtype of DLBCL. More recent studies using immunohistochemistry[62,63] and gene expression profiling[64] suggest that PCNSL can be attributed to both ABC and GCB groups.

The Role of IL-10 in Retinal Lymphoma and DLBCL

Biochemical analysis of ocular specimens (e.g. vitreous biopsy or aqueous humor tap) for interleukin ratios may support the diagnosis of retinal lymphoma. Malignant B-cells often express relatively high levels of IL-10 whereas inflammatory cells produce higher levels of IL-6.[65–69] A high IL-10 to IL-6 ratio on ELISA (enzyme-linked immunosorbent assay) may be, therefore, suggestive of B-cell lymphoma.[70,71] However, it is by no means diagnostic of the disease, and as indicated above must be considered in the context of the available cytological, immunohistological and clinical data.

Interleukin-10, originally identified as an immunoregulatory Th2 cytokine able to inhibit Th1 cytokine, is highly homologous to viral IL-10 or BCRF-1, an open reading frame in EBV genome[72] IL-10 is a B-cell autocrine growth factor and can promote B-cell lymphoma development and proliferation.[73] While normal B-lymphocytes produce IL-10,[74] malignant B-cells frequently produce much higher levels of this cytokine. These high levels of IL-10 can promote tumor cell survival in a number of ways. For example, IL-10 can interfere with the immune response against tumor cells through inhibition of Th1 cytokines,.[75] and thus inhibit cytotoxic T-cell effects directed against neoplastic B-cells. In addition, IL-10 enhances Bcl-2 expression on B-cells and prevents apoptosis.[76]

More than 10 years ago, elevations of IL-10 levels were reported to be present in the serum of patients with B-cell lymphomas, such as Burkitt's lymphoma,[77] AIDS-associated lymphomas, [78] and Epstein Barr Virus (EBV)-positive Hodgkin's disease.[79] At a similar time, high IL-10 levels were also detected in vitreous humor samples,[67–71] and later in the aqueous[80] of patients with retinal lymphomas.

Vitreous humor samples are usually obtained by pars plana vitrectomy, and they are often diluted. Thus, the absolute level of IL-10 in vitreous biopsies is difficult to assess. A ratio of IL-10 to IL-6, however, will allow for this dilution effect, as each cytokine will be diluted equally.[70] A ratio of IL-10 to IL-6 greater than 1.0 suggests an intraocular manifestation of a B-cell lymphoma. In a study of 35 retinal lymphoma and 64 uveitic patients, the cutoff made at 1.0 for differential diagnosis was correct when compared with the cytological, immunohistological and clinical findings in 74.7% of cases.[71] This study had a sensitivity of 74.3% and specificity of 75%. This dilution effect is not so problematic with aqueous samples: a cutoff of an absolute value of 50 pg/mL IL-10 in the aqueous has been demonstrated to be associated with a sensitivity of 89% and specificity of 93% for the diagnosis of 51 patients with retinal lymphoma.[80]

Therefore, high IL-10 levels or high IL-10:IL-6 ratios in ocular fluids may be helpful in the diagnosis of retinal lymphoma of DLBCL subtype. It remains to be determined whether they are also useful in the extremely rare retinal lymphomas of T-cell type. Vitreous humor IL-10 and IL-6 levels may be used to predict the responses to chemotherapy in retinal lymphoma.[81,82] Interestingly, both high serum IL-10 and IL-6 levels are reported to correlate with clinical and pathological features and prognosis in patients with peripheral DLBCL.[83] Recently, IL-10 gene polymorphisms have been associated with the risk of development and outcome of DLBCL.[84–86] For example, IL-10-7400DelDel or the haplotype TCA (IL-10-6752T-6208C-3538A) could be a risk factor for poor clinical outcome in patients with aggressive non-Hodgkin's lymphoma.[85] It remains to be determined whether such polymorphisms are significant for the prognosis of retinal or CNSL.

Expression of Chemokines in Retinal Lymphoma and PCNSL

Since the normal CNS contains no lymphoid collections, why retinal lymphoma and PCNSL are confined to the CNS is a perplexing question that remains unanswered. Over 20 years ago, Hochberg andMiller[87] suggested that one explanation for this confined localization might be the "homing" of a malignant clone of B cells to the brain and/or eye. Recently, B cell lymphoid chemokines have been investigated as potential homing signals on retinal and cerebral vascular endothelium.

The lymphoid—or homeostatic—chemokines are chemoattractant cytokines that are expressed in secondary lymphoid organs, where they control cell migration and organize the structure of these organs (reviewed by Cyster)[88] B and T cells express relevant cell surface receptors making them responsive to different chemokines. CCL21 (secondary lymphoid tissue chemokine, SLC), expressed by high endothelial venules, draws naïve CCR7-positive T and B cells into secondary organs. The T cells move to T cell areas in response to the local secretion of CCL21 and CCL19 (EBV-induced molecule 1 ligand chemokine, ELC). Follicular dendritic cells produce CXCL13 (B-cell attracting chemokine 1, BCA-1), which attracts the B cells into follicles. Memory lymphocytes are retained in crypts due to the expression of CXCL12 (stromal derived growth factor 1, SDF-1) and CCL20 (macrophage inflammatory protein-3α, MIP-3α) by crypt epithelium.[89] Although originally described in relation to homeostasis, the lymphoid chemokines have subsequently been implicated in the development of inflammatory diseases and malignancies by multiple groups. In particular, several laboratories have investigated the expression of B cell lymphoid chemokines in retinal lymphoma or PCNSL.[14–17,90]

Brain biopsy specimens are more readily obtained than retinal tissue and have been used for most studies of B cell chemokine expression in PCNSL and retinal lymphoma (Fig. 3). Since genotyping has established that malignant cells from intraocular and intracranial sites in the same patient are identical, it is reasonable to assume that any homing signal(s) expressed in vascular beds of the retina and brain are similar. In one relatively early study of 24 cases of PCNSL,[14] expression of CXCL13 - but not CCL21 or CCL19 - protein was identified in all cases by immunohistochemical testing of brain biopsy material. Expression of CXCL13 by both vascular endothelium and malignant B cells was observed. Interestingly, the vascular endothelium did not appear to synthesize the chemokine, since *in situ* hybridization detected CXCL13 transcript in B cells only. This finding led the authors to postulate that endothelial expression occurred through the process of transcytosis, and to conclude that although expressed in PCNSL, BCA-1 was unlikely to be a primary homing signal. In an independent study of three ocular specimens, CXCL13 expression was detected in the RPE.[90]

Both normal and malignant B cells respond differently to chemokines depending on their status of maturation and chemokine receptor profile. A recent examination of the Ig transcription factors suggests that malignant B cells in retinal lymphoma and PCSNL are mature B cells that

have undergone germinal center reactions[18] This finding suggested that research efforts should be concentrated on the role of CXCL12 and CCL20. In one immunohistochemical analysis of brain biopsy samples from 40 patients,[15] expression of CXCL12 was localized to resident cells, including neurons, meningeal cells and endothelial cells. Interestingly in 80% of the cases of CNSL, expression of CXCL12 by the malignant B cells was observed. In contrast, CCL20 was not detected in the samples. The malignant cells also expressed the CXCL12 receptor, CXCR4 (Fig. 3). The study of the 3 ocular specimens cited in the previous paragraph, also reported that tumor cells expressed CXCR4.[90] However, CXCR4 is also expressed on malignant cells in systemic DLBCL. Consequently, although CXCL12 might attract tumor cells into the CNS, it would be unlikely to be the sole factor responsible for migration.

Perhaps the most interesting observation in PCNSL is the expression of CXCL13 and CXCL12, and their respective receptors, CXCR5 and CXCR4, by the neoplastic B cells: this is of interest since normal B cells are not a source for these B cell chemokines (Fig. 3). This finding has been reported by two independent groups.[14,15,17] The function of tumor-derived CXCL12 and CXCL13 in the pathogenesis of PCNSL can only be speculated. It is possible that they promote anti-tumor responses, or conversely, that they promote neoplastic B-cell proliferation and/or stimulate angiogenesis within the tumor. However, the situation is not straightforward: another group has reported that although cells may express CXCR4 and CXCR5, this expression appears to be restricted to the cytoplasm and nucleus. This cellular distribution would limit the ability of tumor cells to respond directly to the chemokines. To move forward with functional studies that investigate the roles of B cell lymphoid chemokines in retinal lymphoma and PCSNL, it will be necessary to procure large numbers of neoplastic B-cells. A recent publication describes a cell culture system in which human endothelial cells express HIV genes, Vpu and Tat.[53] This system allows outgrowth of CNSL cells from CSF samples of patients with this tumor, and may provide favorable conditions for such studies.

SUMMARY

Retinal lymphoma is a high-grade B-cell malignancy, characterized by typical morphological, immunophenotypical, molecular and biochemical characteristics. Data suggests that the putative cell of origin is either a late germinal-centre or an early post-germinal centre B-cell. It demonstrates a preferential dissemination pattern within the CNS system, exceptionally rarely spreading to the peripheral lymphatic or blood circulation. This may be due to the chemokine and chemokine receptor profile of the neoplastic B-cells; however, genetic and microenvironmental factors may also play a role. Due to the cerebral involvement and to the inherent aggressive nature of retinal lymphoma, patients tend to have a poor prognosis.

In order to better understand the histiogenesis of retinal lymphoma, with a view to identifying biomarkers, signaling pathways, and ultimately improving therapy, additional molecular biological techniques, such as gene expression profiling and array-based comparative genomic hybridisation, are required. These may be applied in conjunction with cell culture or animal models. For this, concentrated efforts are required to collect sufficient tumor material in ocular oncology and uveitis specialist centers, in order to establish Biobanks allowing for collaborative research.

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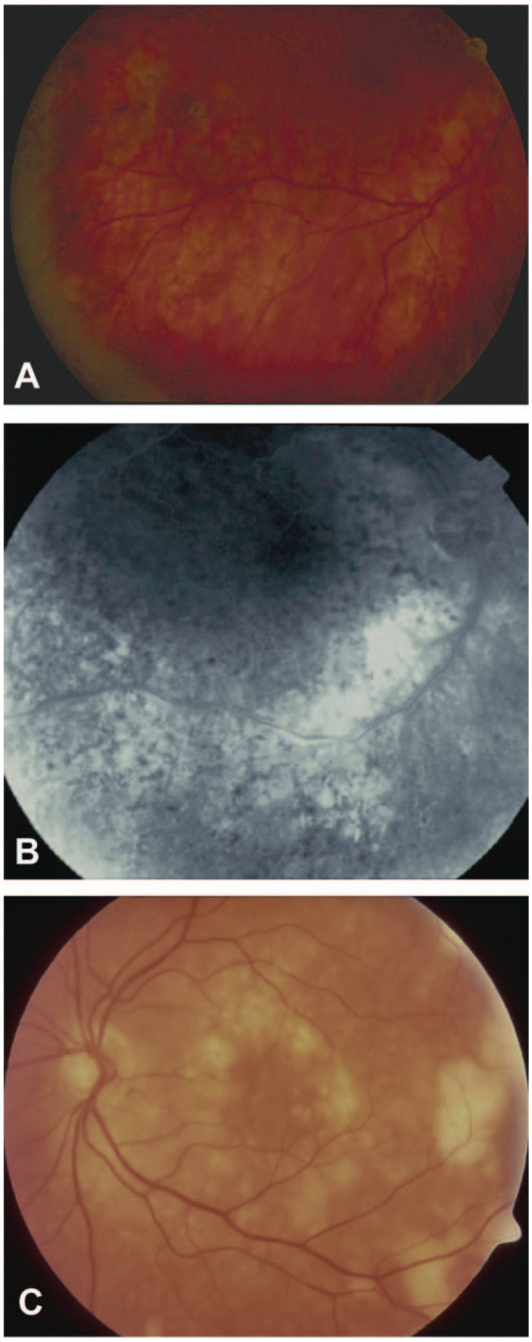

Figure 1.

(**A**) Color fundus photograph of the right eye of a patient with retinal lymphoma, showing some retinal pigment epithelium disturbances. (**B**). Late-phase fluorescein angiogram of the same patient with numerous dark spots (mask effect) corresponding to fresh tumor cells associated with the RPE disturbances. (**C**). Color fundus photograph of another patient, with changes suggestive of retinal lymphoma: subretinal creamy-yellow infiltrates, which appear to coalesce.

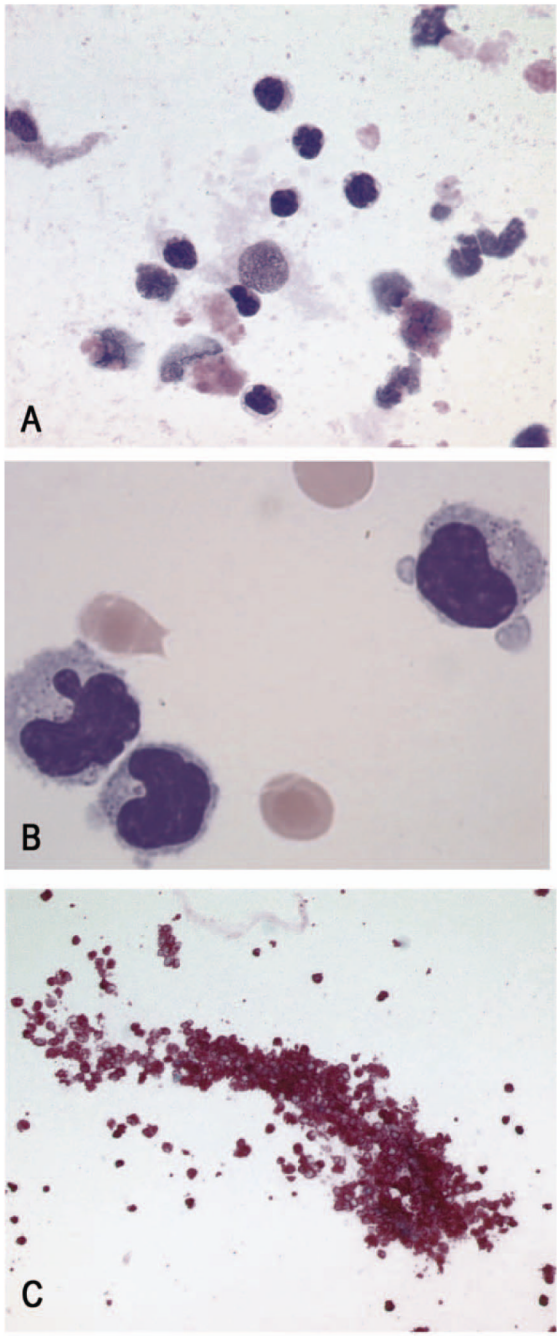

Figure 2.
(**A**) Vitrectomy specimen containing atypical lymphoid cells of a retinal lymphoma on a "dirty" background, consisting of lytic cells and scavenger macrophages (MGG, x60 objective). (**B**) Higher magnification of the atypical cells, showing the folded and irregular nuclei and the condensed chromatin of the lymphoma cells (May Grunwald Giemsa, x 100 objective). (**C**) Immunohistochemical staining using an antibody directed against the B-cell antigen, CD20, demonstrating another vitrectomy specimen of a patient with retinal lymphoma, consisting almost purely of atypical B-lymphocytes (Alkaline Phosphatase-Anti Alkaline Phosphatase (APAAP), x20 objective).

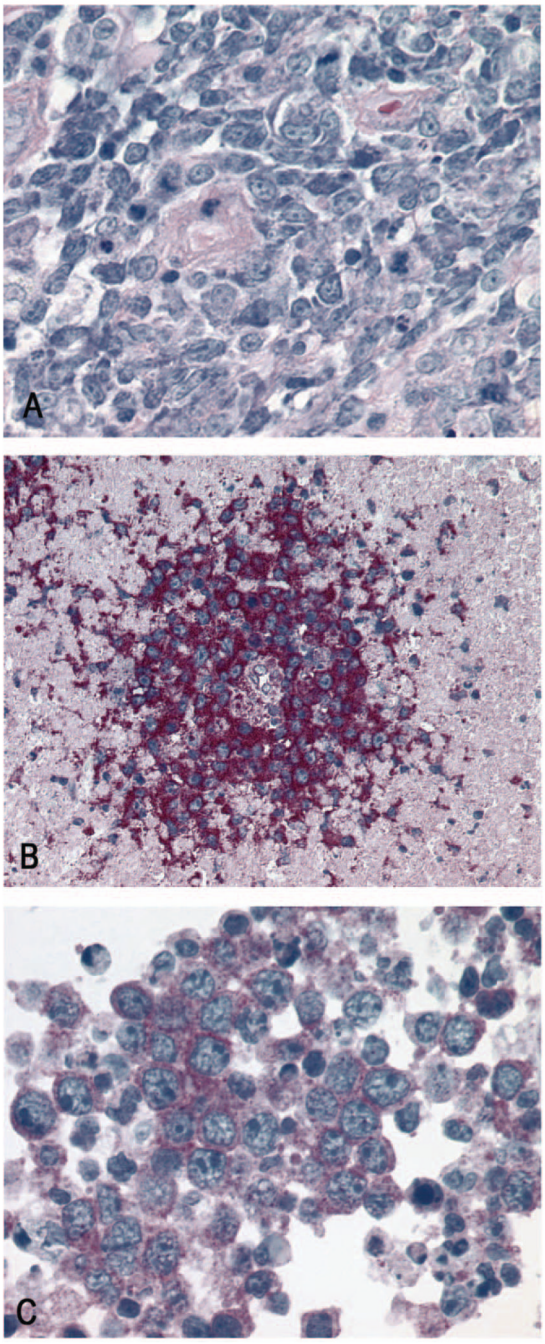

Figure 3.

(**A**) Stereotactic biopsy of a PCNSL, with a dense predominantly perivascular infiltration of medium-sized atypical lymphocytes with minimal cytoplasm, large nuclei and discrete nucleoli. Mitotic figures and scattered apoptotic bodies can be seen (Giemsa, x40 objective). (**B**) Cytoplasmic and possibly membranous positivity of the CNSL cells for CXCR4 (APAAP, x 20 objective). (**C**) Predominantly cytoplasmic immunoreactivity of the CNSL cells for CXCR5 (APAAP, x60 objective).

Table

Summary of the clinical, morphological, immunophenotypical and genotypical features known to date for each of the various intraocular lymphoma subtypes

Intraocular Lymphoma Type	**Retinal**	**Choroidal** ***Primary:***	**Choroidal** ***Secondary:***	**Iridal**		**Ciliary body**
Clinical Features	60–70 years “Floaters” Painless decrease in VA Subretinal infiltrates Often bilateral RPE changes on FA CNS involvement (70–80% of pts)	50–60 years Blurring of vision Metamorphopsia Clear vitreous Diffuse thickening of choroids Usually unilateral Extraocular extension frequent No CNS involvement	Previous history of systemic NHL Decrease in VA Possibly bilateral	Pain Redness Photophobia Pseudohypopyon Usually unilateral Often ultimate systemic dissemination		Raised IOP Ciliary body mass
Most Common Subtype(WHO) Immunoprofile	DLBCL CD79a+ CD20+ PAX5+ BCL2+ BCL6+/− MUM1/IRF4+ CD10−/+ Ki-67 rate: high (>80%)	EMZL CD79a+ CD20+ BCL2+ CD43 +/− IgM + CD5- CD23- CyclinD1- Low Ki-67 rate: 5–15%	Dependent on systemic NHL Dependant on systemic NHL	DLBCL CD20+ Ki-67 rate: high (>80%)	TCL NOS CD3+ CD4+ Ki-67 rate: moderate (40–60%)	EMZL CD79a+ CD20 + BCL-2 + CD43 +/− IgM + CD5- CD23- CyclinD1- Low Ki-67 rate: 5–15%
Genotype	High somatic IgH mutation load Few ongoing somatic mutations Chromosomal translocations: t(14;18)(q31;q21)	Moderate somatic IgH mutation load Few ongoing somatic mutations Chromosomal abnormalities: t(11;18)(q21;q21)	Dependant on systemic NHL	Not known	Not known	Not known
Putative Cell of Origin	2 different types?: a) Early post-germinal centre B cell = DLBCL of ABC type? b) Germinal centre cell = DLBCL of GCB type ?	Post-germinal centre (memory) B cell	Dependant on systemic NHL	Not known	Peripheral T-cell	Post-germinal centre (memory) B cell

Key: DLBCL=diffuse large B-cell lymphoma; EMZL=extranodal marginal zone B-cell lymphoma;NHL=NonHodgkin’s lymphoma;CD=cluster of differentiation; ABC = activated B-cell type; GCB = germinal centre B-cell type; t(N1;N2) = chromosomal translocation between chromosome N1 and N2. WHO = WHO lymphoma classification system.